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# 順天堂醫事雜誌

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# JUNTENDO MEDICAL JOURNAL

## 順天堂醫事雑誌



**The History of *Juntendo Medical Journal***

This *Juntendo Medical Journal* has been published under the Japanese name *Juntendo Igaku* (順天堂医学) from 1964 to 2012. However, the origin of *Juntendo Medical Journal* dates back to the oldest medical journal in Japan, *Juntendo Iji Zasshi* (順天堂醫事雑誌), which had been published between 1875 and 1877 (total of 8 issues). Between 1885 and 1886, Juntendo issued a limited release of a research journal titled *Houkoku* [*Juntendo Iji Kenkyukai*] (報告) for a total of 39 issues.

In 1887, *Juntendo Iji Kenkyukai Houkoku* (順天堂醫事研究會報告) was published with the government's approval and we used to regard this as the first issue of *Juntendo Medical Journal*. Since then, *Juntendo Medical Journal* has undergone a series of name changes: *Juntendo Iji Kenkyukai Zasshi* (順天堂醫事研究会雑誌), *Juntendo Igaku Zasshi* (順天堂医学雑誌), and *Juntendo Igaku* (順天堂医学).

Now in commemoration of the 175th anniversary of Juntendo University, starting with the first volume issued in 2013 (Volume 59 Number 1), we return to *Juntendo Medical Journal*'s original Japanese title in 1875-*Juntendo Iji Zasshi* (順天堂醫事雑誌). We also reconsidered the numbering of the journal and set the first issue in 1875 as the initial publication of *Juntendo Medical Journal*. The Volume-Number counting system and the English name *Juntendo Medical Journal* started in 1955 from the January 10 issue. Although this is not our intension, we will retain the Volume-Number counting system to avoid confusion. However, Volume 59 Number 1 will be the 882nd issue, reflecting the sum of all issues to date: 8 issues of *Juntendo Iji Zasshi* (順天堂醫事雑誌), 39 issues of *Houkoku* [*Juntendo Iji Kenkyukai*] (報告) (47 issues combined), and 834 issues from *Juntendo Iji Kenkyukai Houkoku* (順天堂醫事研究會報告) in 1887 to the present.

出典：小川秀興（OGAWA Hideoki, M.D., Ph.D.）：順天堂醫事雑誌（Juntendo Medical Journal）2013；59：6-10.

本誌は昭和39年（1964年）から平成24年（2012年）末まで『順天堂医学』として刊行されてきた．しかし，その起源は明治8年（1875年）から10年（1877年）にかけて発刊された日本最古の医学誌『順天堂醫事雑誌』（計8巻）にある．さらに明治18年（1885年）から19年（1886年）まで，会員限定配本として順天堂醫事研究會の雑誌『報告』（計39集）が発行されている．

その後『順天堂醫事研究會報告』が明治20年（1887年）に官許を受けて公刊されたので，順天堂ではこれを通刊1号としてきた．以来，『順天堂醫事研究会雑誌』，『順天堂医学雑誌』，『順天堂医学』と名称を変更して刊行されてきた．

今般，順天堂が創立175周年を迎える平成25年（2013年）の59巻1号を期して，本来の名称である『順天堂醫事雑誌』と復刻し，その起源である明治8年（1875年）第1巻をもって創刊号（通刊第1号）とすることとした．従来の巻号と欧文誌名は，昭和30年（1955年）1月10日発行のものを1巻1号としており，欧文誌名もこれより付け始めたもので不本意であるが，混乱を避けるためにこれらを継承する．ただし，通刊数は明治8年（1875年）から19年（1886年）にかけて刊行された『順天堂醫事雑誌』8巻分と順天堂醫事研究會の雑誌『報告』39集，計47巻分を通巻834号に加え，59巻1号を通刊882号とした．

出典：小川鼎三，酒井シヅ：順天堂医学　1980；26：414-418.
小川秀興：順天堂醫事雑誌　2013；59：6-10.

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# Introduction

We, organizing committee of the 2nd Congress, International Academy of Sportology, are taking this opportunity to express our hearty thanks to your enormous contributions to the Congress.

Sportology is a newly defined scientific approach to clarify the relationship between sports and health more profoundly. By deepening the respective sciences involved, Sportology simultaneously integrates each of these specializations, creates and presents opportunities where "deepening and integration" are possible and gives back the outcome to medical societies more effective and more efficient academic achievements. In addition, the aim of Sportology is to establish newly academic background for disease prevention and treatment by blending basic and clinical medical sciences, even including philosophy and brain science.

The Inaugural International Academy of Sportology was held on 5th March, 2011, in Tokyo, and finished with a great success. The proceedings of the meeting was published and sent to 700 members of this association, universities and libraries all over the world.

In recent years, considerable progresses in the field of Sportology have been established. So, we took place the **2nd Congress, International Academy of Sportology** on 12 September, 2015, in Tokyo. You will find herewith 69 excellent, innovative research accomplishments, presented at that occasion.

However, there are still a lot of pending issues in the field of Sportology, such as childhood sports and problems in overuse injuries, and so on. It has been recommended that all children have to participate healthy sports at the age as early as 3 years old to make effectively neuro-development, such as, brain and motor function, attention, memory, etc. However, recently with the progresses in brain imaging techniques, neurocognitive impact in adolescents of sport-related concussions are revealed. Thus, how to prevent them are urgent theme for Sportology also.

And the most important pending issue seems to me is "brains and brawn". Do brains and brawn help each other? Or is it pulling of the foot? With the progresses in understanding brain functions in human beings, several minute changes in brain in response to various strength's exercise have been revealed, so most suitable method to activate brain functions in each individual will be established soon.

"Sports" is old, gold, retold! However, "Sportology" is still brand-new! Sportology is requested to prove its real worth urgently. So, please couple your specialty in science to another categories in science, under the banner of Sportology.

Ryuzo Kawamori
Chairman, The 2nd Congress, International Academy of Sportology
Professor & Director, Sportology Center, Juntendo University Faculty of Medicine

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# The Cutting Edge in Brain Science and Sportology

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***Objective***: We aimed to establish a novel approach that enables the prediction of behavioral effects of lesions using neuroimaging data, which is applicable to sportological analyses, particularly in identifying the links between brain activity and behavior related to sports.

***Materials and Methods***: Experimental details are described in our previous reports (Miyamoto *et al*., 2013; Osada *et al*., 2015). Four monkeys (*M. mulatta* and *M. fuscata*) were used. All experimental protocols, animal welfare, and steps for minimizing suffering were in full compliance with the Guidelines for Proper Conduct of Animal Experiments by Science Council of Japan.

Functional images were acquired using a 4.7-T MRI scanner (Biospec47/40, Bruker) during a recognition or temporal-order judgment task. Monkeys performed the tasks while sitting in an MRI-compatible chair. Group analyses of image data were conducted using SPM5.

***Results***: A task-evoked connectivity network was identified based on data from the functional imaging conducted during recognition tasks (Miyamoto *et al*., 2013) and temporal-order judgment tasks (Osada *et al*., 2015). A significant positive correlation was observed between the predicted impact on performance and the betweenness centrality based on task-evoked connectivity network, whereas no correlation was observed between the predicted impact on performance and the betweenness centrality based on anatomical connectivity network.

***Conclusions***: Severity of impairment arising from local brain damage was predicted by network "hubness" of the damaged area in the task-evoked connectivity network. These findings will form the basis for future research that aims to predict the functional brain center for watching or partaking in sports.

***Key words***: sportology, brain science, neuroimaging, network hub, animal model

## Introduction

Sportology is a new integrated scientific approach that aims to clarify the relationship between sports and health[1]. To this end, sportology combines basic and clinical medical sciences, including brain science/neuroscience, and uses cutting-edge methodologies to aid the development of a new scientific approach. In this article, I will review some of our recent attempts to achieve this goal. I will also outline the potential for brain science/neuroscience to contribute to sportology through the development of a new methodology that allows brain imaging data to predict behavioral outcomes.

Sport has an impressive ability to encourage and motivate people, even in their darkest hours. For example, after the catastrophic earthquake and tsunami which devastated Japan on March 11, 2011, the victory of 'Nadeshiko Japan' (Japan women's national football team) in 2011 FIFA Women's World Cup strongly encouraged us, even though most of us only watched it on television. How does sport move us so strongly?

The neuronal and physiological mechanisms by which we are encouraged, moved, and comforted have received much attention in neuroscience. In particular, neuroimaging has identified several brain regions that may contribute to such neuronal

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processes[2) 3)], including, for example, the amygdala, nucleus accumbens, cingulate cortex, orbitofrontal cortex, hypothalamus, and widely distributed cortical areas. However, not all of these areas causally control the neural processes that eventually have impact on behavior (including emotion). Indeed, the severity of the emotional deficit after a lesion in these areas largely depends on which of these identified areas is damaged; lesions in only a fraction of the activated areas actually lead to emotional deficits. Why some areas activated in neuroimaging are "lesion effective" and others are not is currently unknown.

Recently, we developed a novel approach to identify task-related brain networks[4)] and this approach was extended so that it allows behavioral consequences of brain damage to be precisely predicted in primates[5)]. Functional magnetic resonance imaging (fMRI) was used to measure whole-brain activity during the performance of a cognitive task (in our proof-of-concept experiments, we used well-established memory tasks). Task-specific activation was seen in specific areas, and functional connectivity between them formed a hierarchical network centered on a hub. The task-specific "functional hub" in this dynamic network was found to accurately correspond to the well-documented lesion-effective site[6)], and to exclude the neighboring non-lesion-effective sites. Quantitatively, the predicted severity of behavioral impairment was proportional to the network "hubness" of the lesioned area in the functional network, rather than in the anatomical network. The "hubness" was measured by the index of "betweenness centrality," as we will discuss later in this article (Note that the betweenness centrality of a network node is defined as the fraction of shortest paths passing a given node in the network[7)]). These results suggest that the brain areas dynamically shape a hub-centric network and reallocate the lesion-effective site apart from the static anatomical hubs, depending on the specific cognitive demands. This novel approach could thus allow neuroimaging data to be used for the prediction of behavioral outcomes of lesions. This has implications for sportological analyses of behaviors that arise when we play or watch sports.

## Materials and methods

All the experimental protocols, animal welfare, and steps for ameliorating sufferings were in full compliance with the Guidelines for Proper Conduct of Animal Experiments by the Science Council of Japan and with the NIH Guidelines for the Care and Use of Laboratory Animals.

In order to develop this new methodology, we used a well-documented memory system in monkeys[2) 3)]. Experimental details are described in our previous paper by Miyamoto *et al.* (2013) and Osada *et al.* (2015). In this experiment, two monkeys performed a recognition task[4) 8)] or a temporal-order judgment task[6)] modified for fMRI[5)]. In a custom-made MRI-compatible monkey chair (Nakazawa, Tokyo, Japan), each monkey manipulated an optical fiber-based, custom-made three-way joystick with one of its forelimbs. When the monkey became ready, a list of visual stimuli was presented serially (Cue). The stimuli were selected in a pseudorandom order from a pool of 1,200 pictures of natural or artificial objects. The last stimulus was followed by a delay period (Delay). Finally, two stimuli were simultaneously presented (Choice). In the temporal-order judgment task, the choice stimuli were from the studied list and, if the monkey responded by moving the joystick in the direction of the stimulus that had been presented more recently, the monkey received juice drops. In the recognition task, one choice stimuli was from the studied list and the other was a new stimulus and, if the monkey responded by moving the joystick in the direction of the new stimulus, the monkey received juice drops. Incorrect choices resulted in termination of the trial without reward. Trials were separated by an inter-trial interval, during which the screen was black. In the judgment stage of the temporal-order judgment task, two types of trials were performed: (1) trials in which the choice stimulus pair included neither the initial nor last end stimuli in the list (MIDDLE trial) and (2) trials in which the paired stimuli were both the initial and last end stimuli in the list (BOTH-END trial).

Functional images were acquired and processed as described previously[2) 4) 5)]. In brief, the monkeys were scanned in a 4.7-T MRI scanner (Biospec 47/40, Bruker, Ettlingen, Germany) with 100 mT/m

actively shielded gradient coils and a transceiver saddle RF coil (Takashima, Tokyo, Japan). In each session, functional data were acquired using a gradient-echo echo-planar imaging (EPI) sequence (TR = 2.5 s, TE = 20 ms, 1.25 × 1.5 $mm^2$ in-plane resolution, 64 × 96 matrix, slice thickness = 1.5 mm). T2-weighted spin-echo (RARE) images with the same geometry as the EPI were also scanned. In separate sessions, high-resolution T1-weighted structural images were scanned using a 3-D MDEFT sequence (0.5 mm isotropic). High-resolution EPI (32-shot, TR = 2.5 s, TE = 20 ms, 0.625 × 0.75 $mm^2$ in-plane resolution, 128 × 192 matrix, slice thickness = 0.8 mm) was also acquired to serve as the template image for spatial normalization.

The group analysis of the image data was conducted using a random effect model by treating each session's data from both monkeys equally as a random effect. The regions active during temporal-order judgment were identified by comparison of blood-oxygenation-level-dependent (BOLD) signals between the MIDDLE and BOTH-END conditions. Psychophysiological interaction (PPI) was estimated among the activated areas to determine whether the temporal-order retrieval load affects the connectivity among the identified areas. The effect size of the PPI at target ROIs was evaluated as the beta estimate for the PPI predictor averaged across all sessions for the two monkeys.

The topology of the connectivity networks among the activated areas were examined by calculating network metrics[5) 7)]. For each area (node), the network measure of "betweenness centrality" was computed. The betweenness centrality is the fraction of shortest paths passing a given node. In the analysis, the distance from node $A$ to node $B$ ($d_{A->B}$) was calculated as the inverse of the PPI $z$-value from node $A$ to node $B$ ($d_{A->B} = 1/PPI_{A->B}$).

We used Multi-Voxel Pattern Analysis (MVPA) based on Support Vector Machine (SVM) [5) 9)] to determine whether the PPI connectivity pattern encodes behavioral performance. Details are described in Osada *et al.* (2015). In brief, the PPI beta-values (MIDDLE > BOTH-END) for each connection in each session from the high and low performance dataset were analyzed using SVM. SVM discriminated between sessions that belonged to two different classes (high or low performance set), using nonlinear radial basis functions ($f(\mathbf{x},\mathbf{y}) = \exp(-\gamma |\mathbf{x}-\mathbf{y}|^2)$, $\mathbf{x}$, $\mathbf{y}$: training vectors).

To computationally examine whether removal of an area affected prediction accuracy, we conducted an SVM prediction analysis using the node-deleted PPI connectivity patterns. The prediction accuracy of the node-deleted PPI connectivity patterns for each node was estimated and compared with that using all the connectivity patterns. A reduction in prediction accuracy was defined as "predicted impact on performance". The predicted impact on performance was calculated for each node for each monkey. To examine the relationship between betweenness centrality and predicted impact on performance, we performed an ANCOVA on predicted impact on performance after removal of each area (monkey × betweenness centrality). Betweenness centrality and predicted impact on performance after removal of each area were calculated for each monkey and were used for the analysis.

## Results

The activation analysis and PPI analysis revealed a brain network that is engaged during performance of the tasks. Figure-1 shows the task-evoked connectivity network for the temporal-order judgment task. In this figure, only PPIs with $p < 0.01$ (FDR correction) are displayed as directed edges. The diameter of each node represents the betweenness centrality value. These results demonstrate that area 9/46d acted as a hub in the task-evoked connectivity network during the temporal-order judgment task. When we evaluated the betweenness centrality of the areas based on the directed axonal projection pattern (i.e., anatomical network) with the aid of the CoCoMac database[4) 10)], the betweenness centralities of areas 8Ad and LIP were statistically significant, whereas that of area 9/46d was not. Thus, structurally, the cortical areas contributing to this task form a network centered at areas 8Ad and LIP as anatomical hubs. The difference in location between functional and anatomical hubs suggests that there is a dynamic allocation of the functional hub that is dependent on the cognitive demands. Interestingly, the task-specific functional hub in this dynamic task-evoked network accurately corresponded to the well-documented

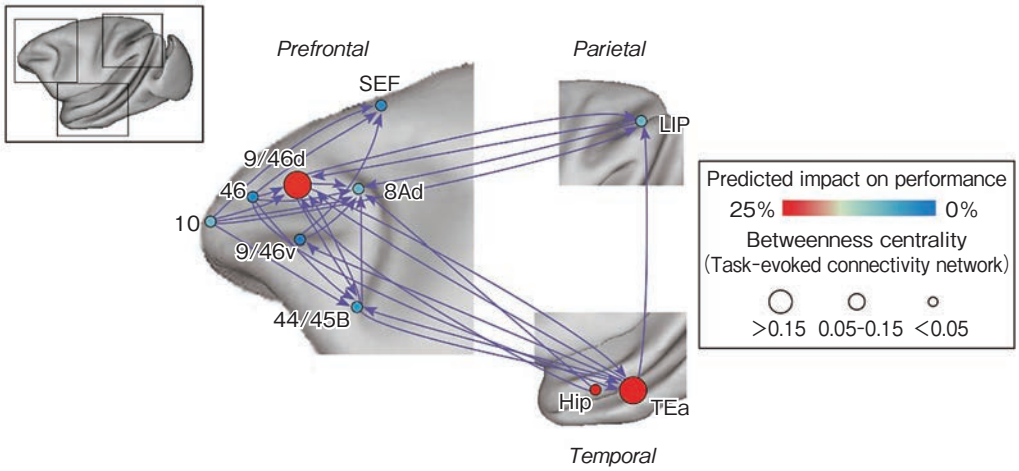


**Figure-1** Activated areas and interareal connections in the temporal-order judgment task
Psychophysiological Interactions (PPI) with $p < 0.01$ (FDR correction) are displayed as directed edges for display purpose. Node color indicates predicted impact on performance. Node diameter represents betweenness centrality. (Modified from Figure-6D of Osada *et al.*, PLoS Biol, 2015; 13: e1002177 [5])

lesion-effective site, and did not include the neighboring non-lesion-effective site (i.e., severe temporal-order memory impairment after lesions in area 9/46d, but not in area 8Ad) [5) 6)].

Figure-1 also shows that a large value of "predicted impact on performance" (%, color coded) was given to area 9/46d ($p = 0.03$, randomization test), indicating a significant performance drop after removal of the 9/46d node. On the other hand, removal of area 8Ad did not cause a significant reduction in prediction accuracy ($p = 0.59$, randomization test). These results indicate that the areal dissociation of lesion-induced impairment was predicted by the node-deleted PPI connectivity pattern.

We thus compared the predicted impact on performance after lesioning and the betweenness centrality based on the task-evoked connectivity network for each area in each individual monkey. An analysis of covariance (ANCOVA) on predicted impact on performance after removal of each area (monkey × betweenness centrality) revealed a significant main effect of betweenness centrality of the removed area ($F(1, 16) = 6.65$, $p = 0.02$). There was no significant main effect of monkey ($F(1, 16) = 0.22$, $p = 0.64$), and no interaction between monkey and betweenness centrality ($F(1, 16) = 0.85$, $p = 0.36$). Importantly, we found a significant positive correlation between the betweenness centrality and the predicted impact on performance ($r = 0.53$, $p = 0.008$) (Figure-2A). These observations indicate that removal of an area with higher betweenness centrality in the task-evoked connectivity network causes a larger reduction in prediction accuracy. On the contrary, no significant correlation was observed between the betweenness centrality based on anatomical connectivity network and the predicted impact on performance ($r = -0.26$, $p = 0.14$) (Figure-2B). These observations suggest that severity of behavioral impairment induced by a focal lesion can be predicted from the task-evoked connectivity network, but not from the anatomical connectivity network.

## Discussion

In this review, I have contrasted the task-evoked connectivity network with the anatomical connectivity network. In particular, I have demonstrated that, when the former is used, the predicted severity of impairment was proportional to the network hubness ("betweenness centrality") of the computationally lesioned area, while it was not when the latter is used. This result could thus represent a novel approach that enables behavioral impacts of lesions to be predicted using neuroimaging data. Most previous investigations of the hub structure of cortical networks have relied on either structural neuroimaging by diffusion tractography (reflecting anatomical connections) or functional

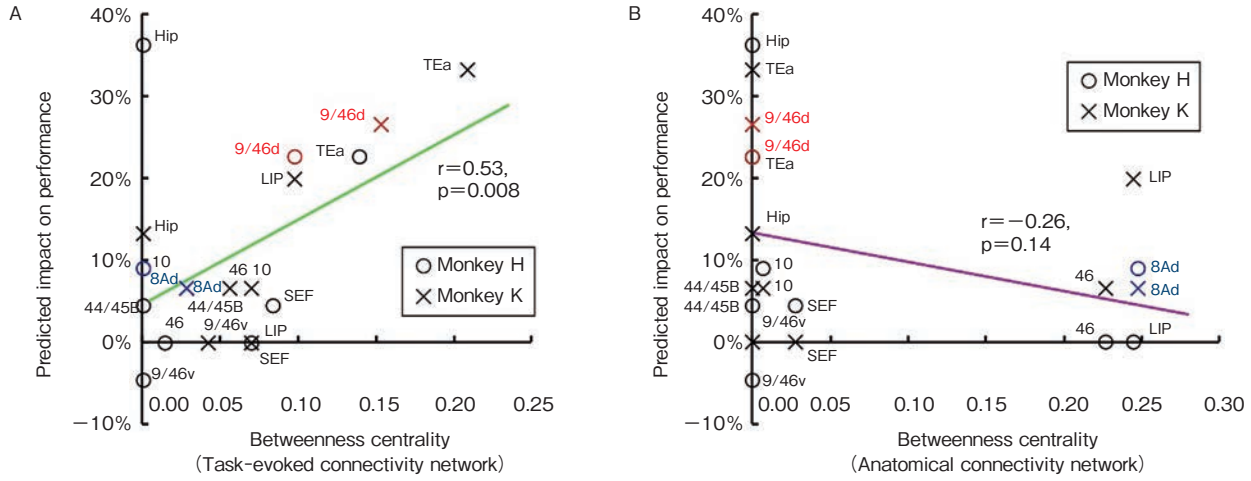


**Figure-2** Relationship of betweenness centrality and predicted behavioral impairment after lesioning
**A.** Betweenness centrality was calculated for each area and for each monkey, based on task-evoked connectivity (horizontal axis). Predicted impact on performance (vertical axis) was also calculated. Each o or x represents a value from each area of each monkey. **B.** Betweenness centrality was calculated based on anatomical connectivity (horizontal axis). Scatter plot is shown as in (**A**). (Modified from Figure-6A & B of Osada *et al.*, PLoS Biol, 2015; 13: e1002177[5])

neuroimaging of spontaneous activity at rest in humans[7]. In addition, the impact of abnormalities in such hubs has been examined in human patients with nonfocal cortical pathologies, such as schizophrenia and Alzheimer's disease[11)-13)]. Thus these previous studies in humans could only investigate brain functions related to general intelligence or general cognitive abilities under the context of static and non-focal brain networks. However, we are usually interested in more specific cognitive abilities and focal brain networks. Indeed, the lesion-effective site is known to vary depending on the cognitive process being engaged[2) 3) 14)] and, as a matter of fact, impairment of a specific function is not always accounted for by structural network hubs. Accordingly, the structural network-based approach is not versatile enough to localize a vulnerable locus for a task-specific cognitive function. Therefore, our novel approach could allow task-evoked fMRI data to be used for the prediction of behavioral impacts of lesions.

I mostly focused in this review on brain functions related to memory tasks, because these are the cutting-edge fields of brain science/neuroscience and we have many previous literatures that attempt to relate brain activity with behavior in this field. When we partake in or watch sports, wider brain areas are incorporated, including subcortical areas such as basal ganglia and cerebellum. Recent advancement of neuroimaging with a high-field MRI scanner at 3T or 7T has just started to make the analysis of the subcortical areas (such as basal ganglia and cerebellum) in humans possible[3) 14) 15)]. I believe that the approach that I reviewed in this article would be also applicable to the data obtained from those wider brain areas and thus applicable to sportological analyses of behavioral effects that arise when we play or watch sports.

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# Selected Issues in Pediatric Sports Medicine Practice in USA

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Participation in sports by children in the United States over the past 3 decades has seen a move from spontaneous and fun activity by children and adolescents to a more organized and competitive activity largely governed by adults and sports governing organizations. With this shift we have also seen a trend in participation in organized sports at younger age, increase in intensity in activity level, and specialization in a single sport from an early age. These and other factors contribute to an increased incidence of sport related overuse injuries in children and adolescents. Another area of intense debate and scrutiny is the long term neurocognitive impact in adolescents of sport-related concussions. Recognition of adverse long term impact of sport related concussions on developing brain has resulted in increased education efforts, prevention strategies, and legislative activity. Screening of young athletes for cardiovascular disease to prevent sudden cardiac death also continues to be a subject of ongoing intense debate in the United States.

***Key words***: concussions, cardiovascular screening, neurodevelopment, sport readiness, neuroimaging

## Sport-related concussions

According to the United States Centers for Disease Control and Prevention the concussion rates in the United States, among 10–19 year olds increased by nearly 100,000 annually in 2009 compared with 2001, concussions represent 6% of all collegiate sports injuries, and 9% of high school athletic injuries[1]. Considering that 50 million American children and adolescents participate in team sports annually head injuries in sports are a major epidemic[1].

Kamitani, *et al* (2013), looked at the epidemiology of catastrophic head and neck injuries in judo in Japan[2]. The authors analyzed the accident reports submitted to the All Japan Judo Federation's System for Compensation for Loss or Damage and found a total of 72 judo injuries (30 head, 19 neck, and 23 other injuries) reported between 2003 and 2010. Kamitani, *et al* (2013) found that, among the reported head injuries, 90% occurred in players younger than 20 years of age[2]. Acute subdural hematoma was found in 94% of head injuries. In players who sustained neck injuries, 18 players had cervical spine injury, 11 of whom had fracture-dislocation of the cervical vertebra. The findings showed that the neck injuries were associated with having more experience and executing offensive maneuvers; whereas, head injuries were associated with age younger than 20 years and with being thrown[2].

According to Kamitani and colleagues (2013), many head injuries occurred when players were thrown to the mat on their back[2]. Among neck injury cases, 60% occurred when a player attempted to throw an opponent, and 40% occurred while a player was being thrown. This was attributed to deficient skills of beginners. It is of interest to note that, severe judo-related head injuries have not been reported in the United States, Canada, Germany, France, or Australia as they are reported in Japan. One reason cited for this is that in Japan, unlike other countries, until 2011, there was no qualification system for judo

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instructors that includes knowledge of safety guidance[2]. In Japan, martial arts have been compulsory in junior high education since 2012. This has raised concern about increased head and neck injuries. Proper education programs and practice have been promoted as possible strategies in preventing catastrophic injuries.

## 1. Current evaluation and management guidelines for concussions

The Zurich consensus statement and the American Academy of Neurology concussion guidelines are the most widely used practice guidelines for evaluation and management of concussion; both have a similar general approach[3)-5)].

The key elements of the Zurich guidelines definition of concussion include the following[3)]:

- Concussion is a brain injury and is defined as a complex pathophysiological process affecting the brain, induced by biomechanical forces.
- Concussion may be caused either by a direct blow to the head, face, neck or elsewhere on the body with an "impulsive" force transmitted to the head.
- Concussion typically results in the rapid onset of short-lived impairment of neurological function that resolves spontaneously.
- In some cases, symptoms and signs may evolve over a number of minutes to hours.
- The acute clinical symptoms largely reflct a functional disturbance rather than a structural injury
- No abnormality is seen on standard structural neuroimaging studies.
- Concussion results in a graded set of clinical symptoms that may or may not involve loss of consciousness.
- Resolution of the clinical and cognitive symptoms typically follows a sequential course. The majority (80-90%) of concussions resolve in a short (7-10 day) period, although the recovery time may be longer in children and adolescents.

The Zurich guidelines stipulate a graduated return to play over a period of 7-10 days in most cases[3)]. For athlete to return to play he or she must be completely symptom free and must have a normal physical and neurological examination. One area that is of special significance here is the concept of cognitive rest[3) 5)]. Many young athletes find cognitive difficulties following concussion. Studies have shown that difficulty in school work include taking standardized tests, and particular difficulty in mathematics and science classes[5)]. Prolonged periods of reading many also aggravate concussion symptoms. Cognitive rest has been shown to improve recovery. The athletes should refrain from television watching, video games or texting. The school should be informed of the concussion and the student athletes be allowed to have lighter school work including assignments and test requirements[5)]. The school work should gradually progress to previous levels and this may takes several weeks to sometime months.

## 2. Modulating factors with special reference to age

Zurich guidelines and others have described factors that may modify the risk of concussion and duration of recovery as shown here[3) 5)-8)]. Although loss of consciousness (LOC) is not a criteria for concussion, prolonged LOC (more than 1 minute) has been shown to increase the duration of recovery[3)]. Young age has been shown in some studies to increase the risk for long term neurocognitive deficits as well as prolong duration of recovery from concussion[3) 6) 8)]. Neurocognitive deficits are of significant concern in those with repeated concussions and concussions that occur close to each other[3) 5)].

Morgan *et al* (2015), looked at the risk factors for post-concussion syndrome (PCS)[7)]. The authors aimed to identify risk factors for PCS development in a cohort of exclusively athletes 9-18 years of age who sustained sport-related concussions (SRC). They identified 40 patients with PCS and matched them by age at injury and sex to SRC control patients. For the purpose of this study, PCS patients were those experiencing persistent symptoms at 3 months after an SRC; whereas, control patients were those with documented resolution of symptoms within 3 weeks of an SRC. Morgan *et al* (2015) found that, in this age-and sex-matched case-control study risk for development of PCS was higher in those with a personal and/or family history of mood disorders, other psychiatric illness, and migraine[7)].

Foley, *et al* (2014) analyzed studies that looked at young age as a modifying factor in sport-related concussion outcome[8)]. They found, based on

multiple empirical studies that younger athletes may take longer to recover from a sports-related concussion (SRC) than their older peers. However, the studies did not indicate that younger athletes were at more risk for prolonged recovery. Although, young age has been shown to be modifying factor in concussion recovery, this study found that, the difference in recovery time was relatively small (a few days) and young age did not predict prolonged recovery.

Lee *et al* (2013) looked at how age affects symptom recovery after sport-related concussion in high school and collegiate athletes and found that there was no statistically significant difference in symptom presence, symptom severity, and total symptoms between the age groups at baseline or at post-concussion testing[6]. There was no statistically significant difference in return to baseline symptom scores between the age groups[6].

**3. Role of neuroimaging**

Numerous neuroimaging studies using advanced dynamic techniques have in recent years shown white matter, neurovascular and neurometabolic abnormalities in athletes who sustained sport related concussions[8)-11)]. Bazarian, *et al* (2014) reported on persistent, long-term white matter changes following sport-related head injuries among college athletes in the United States[11]. Prospective, observational study of college football players was done using diffusion tensor imaging. All subjects underwent diffusion tensor imaging (DTI), physiologic, cognitive, and balance testing at pre-season (Time 1), post-season (Time 2), and after 6-months of no-contact rest (Time 3)[11]. Head impact measures were recorded using helmet-mounted accelerometers. Compared to controls, athletes experienced greater changes in fraction anisotropy (FA) and mean diffusivity (MD) from Time 1 to 2 as well as Time 1 to 3; most differences at Time 2 persisted to Time 3[11].

Dettwiler, *et al* (2014) also reported on persistent difference in patterns of brain activation after sport-related concussion using functional magnetic resonance imaging (fMRI)[10]. Their findings suggest that functional brain activation differences persist at 2 months after following concussion, and recovery of performance on a standard working memory task is comparable to normal controls and normalization of clinical and neuropsychological test results[10]. These results might indicate a delay between neural and behaviorally assessed recovery after support related concussion.

Putukian, *et al* (2014) reported on long-term changes in white matter following sport-related concussion[9]. They used diffusion tensor imaging (DTI) to assess white matter (WM) fiber tract integrity within 2 days, 2 weeks, and 2 months of concussive injury. Investigators found significantly increased radial diffusivity (RD) in athletes who sustained concussion at 2 days, when compared to the 2-week postinjury time point. At the same time fractional anisotropy (FA) was decreased at 2 days and at 2 months postinjury, when compared to healthy controls. At 2 weeks postinjury, no statistical differences between concussed and control athletes were found with regard to either RD or FA. These results support the hypothesis of increased RD and reduced FA within 72 h postinjury, followed by recovery that may extend beyond 2 weeks. RD appears to be a sensitive measure of concussive injury[9].

**4. Concussion prevention and education programs**

As part of prevention and education efforts for concussion, many programs have been developed in the United States; examples of some of them are shown here. Different programs are designed for athletes, their parents, coaches, and physicians. A review by Williamson, and colleagues of these programs note that formal educational programs are effective[12]. Players who have received concussion education are twice as likely to report concussion-type symptoms to their coaches compared with student athletes who have not had such training[12]. College athletes who receive concussion education at the beginning of the season demonstrate increased concussion awareness at the end of the season as a result of this training[12].

An important aspect of concussion prevention in the United States in recent years is the development of laws that require mandatory education, reporting and medical evaluation and clearance of athletes with concussion. This type of action was prompted by severe head injury of a young athlete, Zackery Lystedt, in the state of Washington. Now all states have some form of law that address sport related head injuries in young athletes.

## Cardiovascular screening in young athletes

The incidence of sudden cardiac death or sudden cardiac arrest reported in the United States varies depending on the activity as shown which is higher in US military (1 in 9,000) and low in US marathon racers (1 in 184,000)[13]. Currently in the United States, use of electrocardiogram (ECG) as part of routine cardiovascular screening is not recommended and is a subject of debate[13-18].

The 12-lead ECG obtained at rest on a nontraining day has been considered for screening based on the Italian experience showing marked decrease in sudden cardiac death (SCD) rates after making ECG part of standard screening[18]. The International Olympic Committee and the European Sports Council require an ECG before sports participation. Since 1973, in Japan all first, seventh, and tenth grade students routinely get a screening ECG. An ECG is found to be abnormal in 90% of patients with hypertrophic cardiomyopathy (HCM)[15 18]. Other high risk conditions for sudden cardiac death detectable by ECG include arrhythmogenic right ventricular cardiomyopathy (ARVC), ion channelopathies, dilated cardiomyopathy, and Wolff-Parkinson- White syndrome (WPW)[13 15 18].

The 2010 European Sports Council (ESC) guidelines and the use of Seattle criteria for ECG interpretation in athletes provide support for including resting 12-lead ECG in screening athletes for SCD[13 14 18]. However, differences remain in the recommendations between the American and European cardiology guidelines on whether to use the ECG alone or in conjunction with a standardized history and physical examination before sport participation or heightened physical activity.

Studies in the United States have shown improved cost efficiencies when applying the new ECG criteria provided by the 2010 ESC studies. Studies at several large American colleges have used ECG screening along with echocardiogram (ECHO) for those with abnormal ECG screens[18]. Physicians with appropriate training across specialties could accurately detect abnormal versus normal ECG finding when provided standardized ECG criteria[13 14].

One should first consider which ECG criteria to use. Salient cost factors for different groups or types of ECG screen testing protocols should be considered[18]. In addition to the cost of doing an ECG as a screen, the potential for additional evaluation and patient and family anxiety from false positive findings on ECG should be considered before obtaining an ECG[18].

Although it is not a common practice in the United States to include an ECG in cardiovascular screening of young athletes at present, there is increasing support for use of as part of screening of athletes. A key consideration when using the ECG in athletes is the appropriate interpretation of the ECG findings. Many changes seen in ECG in athletes are result of effect on the heart from exercise training and are considered normal. These changes must be differentiated from truly abnormal findings[13-15].

## Sport and neurodevelopment of the child and adolescent

Over the past 2-3 decades there is an increasing trend for children to start sport as early 5 years of age. The intensity of activity has also increased. There is emphasis on specializing in a single sport. With such a trend studies have shown detrimental effects on child' s psychosocial and emotional development from stress of participation and expectation to excel in sports[19 20]. Also, studies have shown increased physical trauma at young age. One example is injuries to the anterior cruciate ligament in young athletes; also what we call overuse injuries in the young have increased. So the question often arises as to when is a child ready to engage in sport or what we call developmental readiness for sport participation[19 20].

Developmental readiness for sport participation by young children depends on multiple factors[19 20]. These include the context within which sport participation occurs - just for fun or competition; the sociocultural environment - is this something a child wants or parents want, what is the importance of sport participation for the child or the parent? Also how do parents view sport participation by their child? On the other hand genetic factors may also play a role.

Sports readiness refers to the stage at which the child has reached the necessary maturity to learn a given sport- related task[19 20]. In other words, it results from a process in which the child acquires

the required motor, physical, cognitive, social, and adaptive abilities and is ready to meet the demands of a given sport[19) 20)]. Readiness to play and to compete is influenced by biologic, physiologic, psychosocial, and environmental factors.

Participation in sports requires that the child be able to coordinate certain motor, cognitive, and physiologic func-tions, such as movements of the extremities, breathing, thinking, balancing, and many more. The ability to coordinate different actions is influenced by a child's level of thought processing ability, thinking speed, agility, flexibility, strength, and endurance[19) 20)].

Some researchers have proposed that certain critical periods occur, during which sport skills are learned best[19) 20)]. On the other hand, it is not necessary that children learn such skills during these periods. Because multiple factors influence readiness and determine the varying rates of growth and development, athletic talent cannot be reliably identified in children, nor can athletic excellence in children be predicted[19) 20)]. In general, children younger than age 6 do not have the ability to compare their own abilities with those of others; nor do they have the cognitive maturity to understand the competitive nature of sport before age 9[19) 20)]. The ability to fully understand the complex tasks of a given sport may not be fully developed until a child is 12 years of age[19) 20)].

## Conclusion

In recent years in pediatric sports medicine practice many areas have been explored because of increased participation of young children and adolescents in more intense sports. This short update has explored some current issues in sport-related concussions, cardiovascular screening of young athletes and neurodevelopment and sport participation. Sport-related concussion in young athletes can have significant long term neurocognitive implications and prevention and management guidelines should be followed appropriately. In the United States cardiovascular screening of young athletes is largely based on history and physical examination; however, there is an increased attention and practice to include electrocardiogram as part of the screening process. A trend in intense and specialized sport participation among young children has physical and psychological implications for children. Increased overuse injuries have been noted and some studies indicate adverse psychosocial impact in children because of such intense sport participation at very young age. These issues will continue to need more research and attention in coming years.

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# It Is Time to Implement the Sportology Towards the 2020 Games and Beyond

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In order to establish healthy society as legacy of 2020 Games, the Sportology must be promoted and implement concrete actions.

***Key words***: aged society, healthy society, Tokyo 2020 Games, tangible legacies, intangible legacies

## Preface

The concept of the Sportology is the study on Sports through the sciences that contains natural science, social science and culture science, in order to establish a healthy society through Sports. It is time to implement concrete measures to realise a healthy society through Sportology during the preparation phase towards Tokyo 2020 Games and beyond.

The context of this lecture would be the three Es, economy, epidemics and environment which threatens human-beings, the significance of hosting the Olympic and Paralympic Games in 2020, the elements of the bid activities and the importance of the Olympic Legacies which Sportology should be implemented as one of the factors to establish a healthy society.

## The existing state of the world

The three Es which are the economy of the world, epidemics and environment are supposed to threaten human-beings. Very severe economic situation in China, Middle East, Russia and the European Union shows that it is quite critical that it could threaten the livelihood of the world. Epidemics such as Ebola fever, MERS, Dengue fever and especially bird flu are thought to be very dangerous that its virus might be evolved having infectious human to human in the future.

Environment is another key E that endangers human-beings. The climate change caused by the global warming creates extreme temperature changes, floods, draughts, storms and shift in producing area of agricultural products. In this uncertain period around the world, Japan should become a role model at the occasion of the Tokyo 2020 Olympic and Paralympic and leave excellent legacy for the future.

## The significance of hosting the Games

There are two important meanings to host the Games. Needless to say, the first meaning is to deliver the games safely, smoothly, while celebrating world peace, excitement, Olympic Movement and encouraging the people around the world.

The other meaning is to leave tangible and intangible legacies beyond 2020 to help establish a healthy society. Every Olympic legacies are evaluated ten years after the Games to see if the legacies were left effectively to help establish a better society in long term.

## The bid activities

The vision of the bid period was that Tokyo 2020 will bring together dynamic innovation and global inspiration. It will unite the power of the Games

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with the unique values of the Japanese people and the excitement of a city that sets global trends. It will be a unique celebration that will help reinforce and renew the Olympic Values for the new generation. And will contribute to more young people worldwide, sharing the dreams, hopes and benefits of sport.

The new Games vision and the concept of the Games were announced by the Organizing Committee. "Sports has a power to change the world and our future. The Tokyo 1964 Games completely transformed Japan. The Tokyo 2020 Games, as the most innovative in history, will bring positive reform to the world by building on three core concept; Striving for your personal best (Achieving personal best), Accepting one another (unity in diversity), Passing on legacy for the future (connecting to tomorrow)"

The Tokyo 2020 Bid Committee was formed on 7th of Sept. 2011 and the bid activities started officially after all the preparation work had done by the 2020 Bid Strategy Division of Japanese Olympic Committee (JOC) since Nov. 2009 after the 2016 bid defeated by Rio de Janeiro.

The Committee worked with strong team work along the road map until the voting day, essential six bid activities are categorized as follows:

**1. Fund raising**

Taking the advice from the International Olympic Committee (IOC) that the bidding budget should be small, the budget of the 2020 Bid was US$ 75 mil (in case US$1.00=\100-) which was half of the 2016 bid budget. About half of the budget which was $37million was provided by the Tokyo Metropolitan Government (TMG) and the rest, which was 38 mil was raised by the private sector. One difficulty was that the Bid Committee carried over the deficit of 2016 bid which was $7.5 million. Therefore, the Committee had to raise over $45.5 million in total. Fortunately, after the winning of the Liberal Democratic Party (LDP) at the general election of Japan in the end of 2012, the fund raising went quite smoothly reaching the target.

**2. Planning**

Planning team made up excellent comprehensive plan to host the Olympic and Paralympic Games in 2020. There were 14 themes according to the IOC request as follows;

(1) Vision, legacy and communication
(2) Overall concept of the Olympic Games
(3) Political and public support
(4) Legal aspects
(5) Environment
(6) Finance
(7) Marketing
(8) Sport and venues
(9) Paralympic Games
(10) Olympic Village
(11) Games safety, security and medical services
(12) Accommodation
(13) Transport
(14) Media operations

The Candidature File, compiled all the themes which was published and distributed to the IOC and the IOC members, The International Federations and disclosed on the Tokyo Bid Web Site.

**3. Promoting the support rate**

The IOC evaluated the candidate cities by the passion of the citizens desiring to host the Games by a random survey. The first survey showed only 47%. The Bid Committee worked very hard to raise the figures. Unique and powerful promotion was done by the promotion team and the PR team, 58% before London Games, 63% after London Games, making all kinds of promotion such as Ginza Parade to celebrate the medalists which increased the rate to over 70% at the begging of the 2013. The media reports of the IOC Evaluation Commission Visit again raised the rate to 83% and the final stage in August the rate went up to 92% due to heavy media exposures.

**4. Best response to the IOC Evaluation Commission**

The three day evaluation was conducted in early March 2013 by the IOC Evaluation Commission of 15 members which consisted of IOC members, International Federations (IF) and National Olympic Committee (NOC) representatives and experts. There were presentations of the themes followed by question and answer session in the morning. After lunch, there was a venue sight visit by bus. Six months precise preparation was required to for this evaluation. Tokyo obtained very high evaluation in the IOC evaluation report which was published in the end of June 2013.

**5. Presentation**

Every candidate city had equally four chance to make a presentation at international sports conferences in St. Petersburg, twice in Lausanne and a final presentation at the IOC Session in Buenos Aires. The Bid Committee made very careful preparations to set up appropriate themes, selection and appointment of speakers, effective personal training, and rehearsals for every occasions. The bid committee made efforts to convey the themes clearly using easy to understand vocabulary to reach every audiences. It was so nice that the presentation of Team Tokyo was highly evaluated.

**6. Lobbying**

One of the most important element is lobbying in order to obtain support from each IOC members who was eligible to vote at the host city selection. The bid leaders, president of JOC and CEO of the Bid Committee met, talked and tried their best to convince to obtain their support individually at every occasion. Building up friendship, mutual understanding and trust is indispensable for lobbying. At the same time, the Bid Committee was very careful not to infringe the regulations and rules on the bid activities.

## The Legacy

As previously mentioned, there are two meanings for hosting the Olympic and Paralympic Games. Firstly, the Games must be delivered smoothly and safely with sharing excitement, movement, and encouragement as a role model on hosting Games. Secondly, the games must leave valuable legacies beyond 2020. There are tangible and intangible legacies.

**1. The tangible legacies**

a) The permanent sport facilities will be built for the Games, but those venues should not become white elephants. In order to create good legacies, all the facilities must be planned as multi-use and properly filled up with sport events, musical concert and conventions.

b) Barrier free installments

Paralympic Games must be also world showcase for the disabled athletes. The combination of the state of art technology, newly developed raw materials and creativity are expected to develop stunning systems to produce an ideal living for disabled people.

c) Sustainability facilities and so on

No need to build any more infrastructures in the city.

**2. Intangible legacies must be left beyond 2020 continuously for building healthy society such as;**

a) Culture

We must protect and develop Japanese traditional culture in the society and allow people to new creation of cultures through fusion of other culture.

b) Education

Adding creative thinking ability to the traditional learning and memorizing educational way of Japan. The society will keep developing new education for youth but all ages as well.

c) Sustainability

In order to establish sustainable society beyond 2020 as an intangible legacy, the society must work hard to raise awareness of the reality and the severe state of environment and implementation of all the measures we can.

d) International exchange

Japanese are so to say, not good to communicate with foreign people because of language barrier. In order to promote to activate international exchange would be education of foreign language especially English which is most common language people use to communicate internationally. And setting up more occasions of international exchange, promoting for students to study outside of the country, increasing number of exchange students from overseas.

e) Volunteerism

Tokyo 2020 Games is a great chance to establish an ideal principle of the volunteerism to assist to manage the mega event and local event or meetings as well. It is in the Japanese culture to help others without compensation. This volunteer mind makes better communication and mutual understanding amongst citizens. At the same time it is important to set up a clear volunteer management system for recruiting, selecting, allocating, job description and team work building.

f) Tourism

Tourism has been enhanced by Japanese Government as one of the leading industry in the past few

years for welcoming as many visitors for the Tokyo 2020 Games. Clarifying and marketing of all the resources of tourism in each areas should attract tourist from all over the world. Tourist around the wolrd could enjoy Japanese hospitality "Omotenashi".

g) New business

It is estimated that about 70% of young people will establish their own business. Due to longevity and decreasing birthrate, the society's demands will be diversified and different. In order to supply the new different demands, the society must create new business with combination of profession and new technology, health and sustainability beyond 2020 as the legacy.

## It is the time to implement Sportology for the future

In the recent years, Japan has become one of the top longevity society with significant decrease in birthrate. Consequently, citizens have desired better physical and mental health, preventing dementia, happiness, rejoicing and having fun. In order to establish healthy society as legacy of 2020 Games, the Sportology must be promoted and implement concrete actions.

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# Does the Sports Gene Affect Lifestyle-Related Diseases?

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A number of familial and twin studies have assessed the relative contribution of genetic and environmental factors to physical performance or its-related traits, and a significant genetic component has been predicted to affect phenotypes. A twin study indicated that the heritability of athletic status is 66%. Thus, genetic factors appear to be important for determining elite sporting performance. To date, over 200 genes in both nuclear DNA and mitochondrial DNA (mtDNA) have been reported to be associated with physical performance and health-related fitness. However, most studies have been reported in European populations. In the Asian population, only 3 genetic loci have been linked to physical performance, including angiotensin I-converting enzyme and α-actinin-3 genetic polymorphisms and mtDNA polymorphisms (mitochondrial haplogroups). In this review, we discuss the genetics of elite sporting performance, particularly in Asian populations, and their effects on lifestyle-related diseases such as type 2 diabetes and age-related muscle wasting (i.e., sarcopenia).

***Key words***: genetics, physical performance, type 2 diabetes, sarcopenia, Asian

## Introduction

Within the field of sports science, elite sporting performance is understood to be the result of both nature (genetic factors) and nurture (environmental factors) such as physical training[1]. A number of familial and twin studies have assessed the relative contribution of genetic and environmental factors to physical performance or its-related traits (e. g., maximum oxygen consumption, muscle strength, muscle power, and so on) and have estimated that there is a significant genetic component to these phenotypes. De Moor *et al.*[2] examined 4,488 British adult monozygotic and dizygotic female twins and estimated that the heritability of athletic status is 66%. Thus, genetic factors appear to be important for determining elite sporting performance. Aerobic capacity appears to be affected more strongly by maternal inheritance than by paternal inheritance[3) 4)]. This finding indicates that functional differences in maternally inherited mitochondrial DNA (mtDNA)-encoded proteins involved in oxidative phosphorylation affect aerobic performance. Therefore, mtDNA polymorphisms represent promising candidates influencing physical performance.

To date, numerous studies have identified genetic polymorphisms related to physical performance and its-related phenotypes. By 2009, more than 200 genes in both nuclear DNA and mtDNA had been reported to be associated with physical performance and health-related fitness[5]. The relationship between certain genetic polymorphisms and athletic performance was recently been reviewed[6)-8)]. In these reviews, Ahmetov *et al.* indicated that at least 120 genetic variations are linked to elite athlete status based primarily on the candidate gene approach. These included 77 endurance

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performance-related genetic variations and 43 sprint/power-related genetic makers from nuclear DNA and mtDNA. The number of genes associated with physical performance-related phenotypes is expected to increase dramatically with the application of genome-wide methods in elite athlete cohorts; however, few of these studies have been conducted. To date, only one genome-wide association study to identify genes associated with physical performance has been conducted, which was in Russia. Although numerous studies have been indicated an association between physical performance and genetic polymorphisms, most studies have examined the European population. In the Asian population, only 3 genetic loci are known to be linked to physical performance, including the angiotensin I-converting enzyme-ACE) and α-actinin-3 (ACTN3) genetic polymorphisms and mtDNA polymorphisms (mitochondrial haplogroups). In this review, we discuss genetic studies of elite sporting performance, focusing on the Asian population, and their implications in lifestyle-related diseases such as type 2 diabetes and age-related muscle wasting (i.e., sarcopenia).

## Insertion/deletion polymorphism (rs4340) of the ACE gene

ACE plays a critical role in circulatory homeostasis by causing blood vessels to constrict by converting angiotensin I to angiotensin II. This enzyme is also regulated in glucose metabolism by bradykinin, which induces muscle glucose uptake. In addition, local renin-angiotensin systems in a variety of tissues promote cell growth[9]. One of the ACE polymorphisms is a 287-base pair Alu repeat insertion/deletion (I/D) polymorphism (rs4340) in intron 16, which was associated with circulating and tissue ACE levels. Higher ACE activity was associated with the D allele in both Caucasian populations and Asian populations, but not African populations.

A polymorphism in the ACE gene was first reported to impact on human physical performance in 1998[10]. Many case-control association studies have reported that the ACE I/D polymorphism was associated with physical performance; particularly, I allele carriers showed lower ACE serum concentrations and greater success in endurance-related sporting performance[10) 11)]. In contrast, D allele carriers have higher ACE serum concentrations and have greater success in sprint/power-related sporting performance[12)]. Despite the consistency of such findings, data from East Asian populations have revealed conflicting results. Tobina *et al.* reported that the average running speeds of Japanese endurance and marathon runners with the DD + ID genotypes were significantly higher than those with the II genotype[13)]. In addition, Kim *et al.* reported that elite Korean power-oriented athletes with a higher frequency of the II + ID genotype had a higher probability of success in power-oriented sporting events[14)]. Thus, opposite associations were observed between European and Asian populations. We investigated whether such associations differed by sporting event or by ethnicity, focusing on 200 elite Caucasian and 326 elite East Asian (158 Japanese and 168 Taiwanese)

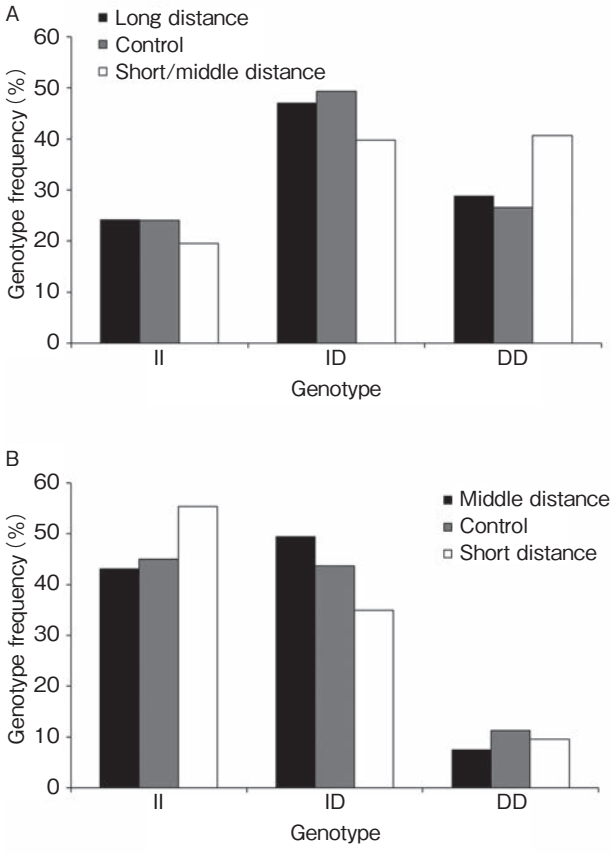


**Figure-1** Genotype frequencies of ACE I/D polymorphism among elite Caucasian swimmers and controls (**A**) and elite East Asian swimmers and controls (**B**).

Modified from Wang G, *et al*: Med Sci Sports Exerc, 2013; 45: 892-900[15)].

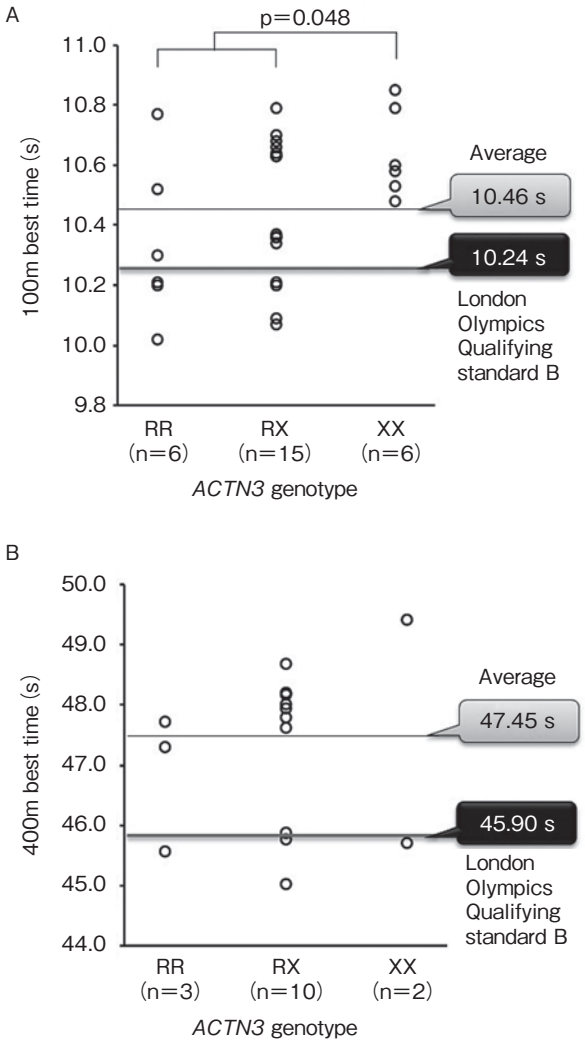


**Figure-2** Associations of ACTN3 R577X polymorphism with 100-m (**A**) and 400-m (**B**) personal best times among male sprinters
*p=0.042.
Modified from Mikami E, *et al*: Int J Sports Med, 2014; 35: 172-177[25].

swimmers[15]. In this study, the D allele was significantly associated with elite short/middle-distance swimmer status in Caucasians (Figure-1). However, the I allele was significantly associated with elite short-distance swimmer status in East Asians (Figure-2). We found that opposite alleles of the ACE I/D polymorphism were associated with elite sprint swimmer status in the Caucasian population and East Asian population. However, Kikuchi *et al.*[16] reported that the DD genotype was associated with power-oriented performance. Further replicate and functional studies are necessary to confirm these findings.

It is well-known that the ACE I/D polymorphism is associated with lifestyle-related diseases in European and Asian populations, as this polymorphism shows the strongest association with circulating ACE activity in European and Asian populations but not African populations. The D allele of the ACE I/D polymorphism is a risk factor for type 2 diabetes in both European and Asian populations[17) 18)]. Thus, physical performance-associated genetic polymorphisms, particularly ACE I/D, may be used to predict lifestyle-related diseases such as type 2 diabetes. This appears to be a reasonable hypothesis for European populations, as the I allele of the ACE I/D polymorphism may be an optimal factor affecting endurance performance because of the high prevalence of mitochondria-rich slow-twitch fibers, which are also associated with the resistance against to type 2 diabetes. However, this has not been confirmed in Asian populations.

## R577X polymorphism (rs1815739) of the ACTN3 gene

α-Actinin-2 and -3 proteins are localized to the Z-disk in the skeletal muscle and help to anchor actin filaments. α-Actinin-2 is expressed in all human skeletal muscle fibers, whereas α-actinin-3 is expressed only in human skeletal muscle fast-twitch fibers[19]. A common genetic variation at codon 577 of the ACTN3 gene causes the amino acid replacement of the arginine (R) with a stop codon (X), i.e., R577X. Homozygosity for the common nonsense polymorphism R577X in the ACTN3 gene results in complete deficiency of α-actinin-3 in fast-twitch muscle fibers. Experiments in knockout mice showed that ACTN3 deficiency affects skeletal muscle function[20].

As described above, more than 200 genetic variants have been associated with physical performance; the most widely studied variant is the ACTN3 R577X polymorphism. Yang *et al.*[21] reported that R allele carriers (RR+RX genotype) of this R577X polymorphism was found more frequently in elite Australian sprint/power athletes than in controls. This finding has been replicated in a broad variety of ethnic groups (See review of Ahmetov *et al.*). In a meta-analysis, Alfred *et al.*[22] found that the ACTN3 RR genotype was more common among European sprint/power athletes

than among controls. Another meta-analysis reported a positive association between the ACTN3 RR+RX genotype and sprint/power athletic status in Europeans, but not in Asian and African populations[23]. Thus, the association between ACTN3 R577X and elite Asian sprint/power athlete status was largely unknown until recently.

We recently investigated the association between the ACTN3 R577X polymorphism and elite Japanese athlete status in a large cohort of Japanese track and field athletes (n = 627 for sprint/power athlete and 430 for endurance athletes) and controls (n = 810)[24]. We found that sprinters with the RR+RX genotype had faster personal best times for the 100-m event than those with the XX genotype (Figure-2A)[25]. Interestingly, all 7 male sprinters who had achieved the London 2012 Olympic qualifying standard for the 100-m event-B standard: 10.24 s) possessed the RR or RX genotypes (Figure-2A), which was not observed in those qualifying for the 400-m event (Figure-2B)[25]. Our results indicates that sprinters with the XX genotype may experience difficulty in the 100-m event and that athletes with the XX genotype transfer may perform better in other events (e.g., long jump or 400-m event). Interestingly, the ACTN3 R577X polymorphism accounted for 12% of the variability in the personal best times for the 100-m run.

It was previously reported that the ACTN3 R577X polymorphism is associated with muscle fiber composition[26] and muscle strength[27,28]. We previously reported that older women with the XX genotype had a smaller thigh-muscle cross-sectional area than those with the RR and RX genotypes, but this was not the case in middle-aged women[29]. This difference appears to be equivalent to 10 years of aging-related muscle wasting. A major characteristic of sarcopenia is the specific loss of type II muscle fibers[30]. Therefore, loss of the ACTN3 protein in type II muscle fiber may be associated with sarcopenia. Delmonico *et al.*[31] conducted a longitudinal study for 5 years and found that the XX genotype had a greater risk of incident lower extremity limitation compared to the RR and RX genotypes in elderly women. Thus, this polymorphism may affect health-related muscle fitness (e.g., sarcopenia) as well as sprint/power performance.

## Haplogroups of mtDNA

Mitochondria are essential to all higher organisms for sustaining life and are extremely important in energy metabolism, providing 36 molecules of ATP per glucose molecule in contrast to the 2 ATP molecules produced by glycolysis. It is reasonable to hypothesize that mitochondria play an important role in determining aerobic performance, as mitochondria supply the majority of cellular ATP through oxidative phosphorylation (OXPHOS), which is the main source of energy for endurance exercise.

Although most DNA is packaged in chromosomes within the nucleus, mitochondria also possess their own circular DNA, designated as mtDNA. The 16,569-base pair human mtDNA contains 13 genes for mitochondrial OXPHOS, as well as 2 rRNA and 22 tRNA genes necessary for protein synthesis within mitochondria. Therefore, the diversity of mtDNA may affect elite endurance athlete status. Since polymorphisms in mtDNA are linked with other mtDNA polymorphisms, many studies have been reported the association between mitochondrial haplogroups, a set of tightly linked mtDNA polymorphisms, and physical performance.

It is important to examine the association between phenotypic traits and mtDNA polymorphisms in terms of population, ethnicity, and/or race, because mtDNA sequences, particularly those in the control region, are known to vary among populations. We sequenced hypervariable sequence 1, a part of control region, and the C > A polymorphism at m. 5178 within the ND2 gene of the mtDNA. We then compared the percent frequency of mitochondrial haplogroups in 141 Japanese Olympians (n = 60 for sprint/power athletes and n = 81 for endurance athletes) with 672 Japanese controls[32]. Subjects were classified into 12 major Japanese mitochondrial haplogroups (F, B, A, N9a, N9b, M7a, M7b, M*, G2, G1, D5, or D4). The distribution of mitochondrial haplogroups in endurance athletes and sprint/power athletes relative to that in the controls is shown in Figure-3. We found that endurance athletes displayed an excess of haplogroup G1. In contrast, sprint/power athletes showed a greater proportion of haplogroup F. We evaluated the detailed characteristics of mtDNA polymorphisms and mitochondrial haplogroups in

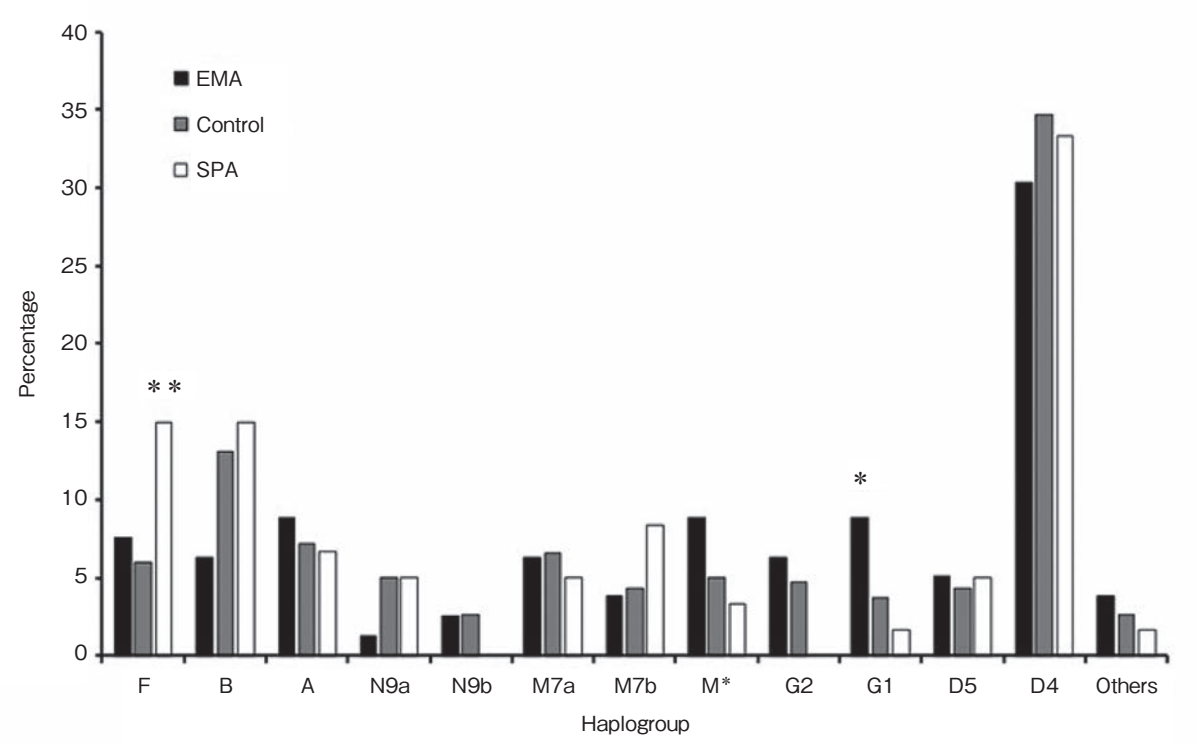


**Figure-3** Mitochondrial haplogroup distribution among endurance/middle power athletes, sprint/power athletes, and controls

Significant differences compared to Controls (gray bars) are indicated by asterisks (*$p<0.05$, **$p<0.01$). For haplogroup analysis, we compared each haplogroup versus the sum of all other haplogroups. P value $<0.05$ was considered statistically significant.
EMA: endurance/middle-power athletes, SPA: sprint/power athletes.
Modified from Mikami E, *et al*: Br J Sports Med, 2011; 45: 1179-1183[32].

elite Japanese athletes and found that some were associated with sprint/power performance as well as endurance performance[33) 34)].

Although numerous studies have reported associations between aerobic performance phenotypes and mitochondrial haplogroups, studies on the associations between "anaerobic" performance phenotypes and mitochondrial haplogroups are limited. Because "anaerobic" capacity relies more heavily upon glycolysis than mitochondrial OXPHOS, mitochondrial haplogroups are not thought to be related to anaerobic capacity. However, we found a positive association between mitochondrial haplogroup F and sprint/power performance in the Japanese population. We also examined the effect of mitochondrial haplogroups on anaerobic performance phenotypes such as muscle power, muscle strength, and muscle mass in 480 healthy Japanese non-athlete adults, and found that macrohaplogroup N was significantly associated with stronger leg extension power and higher vertical jump performance compared with macrohaplogroup M for these Japanese subjects[35)]. Mitochondrial haplogroup F, which is associated with elite Japanese SPA status, is a major component of macrohaplogroup N[36)], which is associated with muscle power in non-athletic Japanese individuals.

We previously reported that mitochondrial haplogroups were associated with type 2 diabetes in Asian populations[37)]. Particularly, mtDNA haplogroup N9a was associated with a decreased risk of type 2 diabetes, whereas haplogroups F and D5 were associated with an increased risk of type 2 diabetes. Thus, mitochondrial haplogroup F was associated with not only sprint/power performance, but also type 2 diabetes. Hwang *et al.*[38)] reported interesting results regarding the association between mitochondrial haplogroups and gene expression profiles in transmitochondrial hybrid cells. Based on the gene expression data from the hybrid cell with haplogroups F and N9a, haplogroup F showed lower gene expression of the oxidative phosphorylation pathway compared with haplogroup N9a. In contrast, haplogroup F showed

greater expression of glycolysis pathway genes compared with N9a. Thus, mitochondrial DNA polymorphisms influence outer mitochondrial functions as well as inner mitochondrial functions.

## Future directions

In this article, we mainly discussed the genetics of sporting performance and its implications on lifestyle-related diseases and/or age-related muscle wasting (i. e., sarcopenia). Particularly, we discussed these findings in Asian (Japanese) subjects. Numerous studies have attempted to identify genetic polymorphisms associated with physical performance, but few studies have been conducted in Asian populations. Therefore, it is necessary to validate the association between the genetic polymorphisms and athletic status, sprint/power performance, and/or endurance performance in the Asian population. Furthermore, subjects in some previous case-control studies were athletes participating in various events, making it difficult to compare the results.

Current studies of athletic performance-associated polymorphisms, which were previously identified through candidate gene-association studies, have revealed the polygenic profile for determining athletic performance[39) 40)]; however, additional studies are needed to explain the genetic component. The strong genetic contributions to physical performance and/or its-related traits indicate the possibility of using genetic approaches to individualize training approaches for enhancing competitive abilities in sports or even to help select appropriate athletic events by using genetic screening for extending the personal limit of athletes (i.e., talent identification and/or talent transfer to other events). Further studies in sports science should include more detailed analyses of genetic polymorphisms detected in both mtDNA and nuclear DNA, including whole genome sequencing for mtDNA and genome-wide association studies for nuclear DNA[41)]. Some polymorphisms, which are found form whole genome wide approach using elite athletes, have a potential to identify novel polymorphisms for determining health-related phenotypes such as type 2 diabetes and/or sarcopenia.

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# Current Status and Future Directions for Cardiac Rehabilitation in Japan

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The prevalence of coronary artery disease (CAD) has increased over the last several decades in Japanese population. Recent epidemiological studies have indicated that the incidence of acute myocardial infarction (MI) has increased, particularly in the urban areas of Japan. Previous data have strongly supported the important role of lifestyle intervention, including physical activity and dietary modification to improve glucose and insulin homeostasis, lipid profiles, and other risk factors for the prevention of CAD. Cardiac rehabilitation (CR) is a comprehensive intervention that includes supervised exercise training, risk factor control, patient education, and psychosocial counseling. CR has been reported to be effective in reducing the risk of cardiovascular events through intensive lifestyle modification. However, the implementation of CR is still low even in academic cardiovascular institutes. In this review, we will discuss the recent status and problems regarding the safety and efficacy of CR and will discuss the future directions of CR in patients with CAD in Japan.

***Key words***: cardiac rehabilitation, cardiovascular disease, diabetes mellitus, elderly patients, frailty

## Introduction

Coronary artery disease (CAD) is a major cause of morbidity and mortality worldwide. Approximately 42,000 Japanese people die of myocardial infarction (MI) every year[1]. Recently, patients with CAD have been treated with medical therapy with lifestyle modification, and coronary revascularization procedures, such as percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG). Although the recent progress of medical therapy and coronary revascularization is remarkable, implementation of cardiac rehabilitation (CR), a powerful methodology for lifestyle modification, appears to be yet unsatisfactory. In this review, we will discuss the recent status, problems, and the future directions of CR in patients with CAD in Japan.

## Establishment of CR

In the 1930s, Mallory *et al* reported the pathological healing process after an acute MI and clearly showed that the period from acute myocardial necrosis to stable scar formation was more than 6 weeks. Therefore, in order to prevent ventricular aneurysm formation, heart failure, rupture and sudden death, bedrest for 6-8 weeks was practiced after acute MI, which, however, induced physical deconditioning and frequent pulmonary thromboembolism. Thus, the likelihood of returning to a normal social life had been relatively low[2]. In 1970s, before the wide utilization of coronary interventions, several studies demonstrated the better prognosis in patients with CAD who participated in a CR program. Thereafter the wide acceptance of PCI, Hambrecht *et al* reported that an exercise-training group with stable angina pectoris had an improved event-free survival at a 12-month follow-up compared with the PCI group[3]. A meta-analysis has shown that CR decreased the all-cause mortality by 20% and cardiac mortality by 25%[4].

Comprehensive CR program for patients with CAD contains exercise and patient education and

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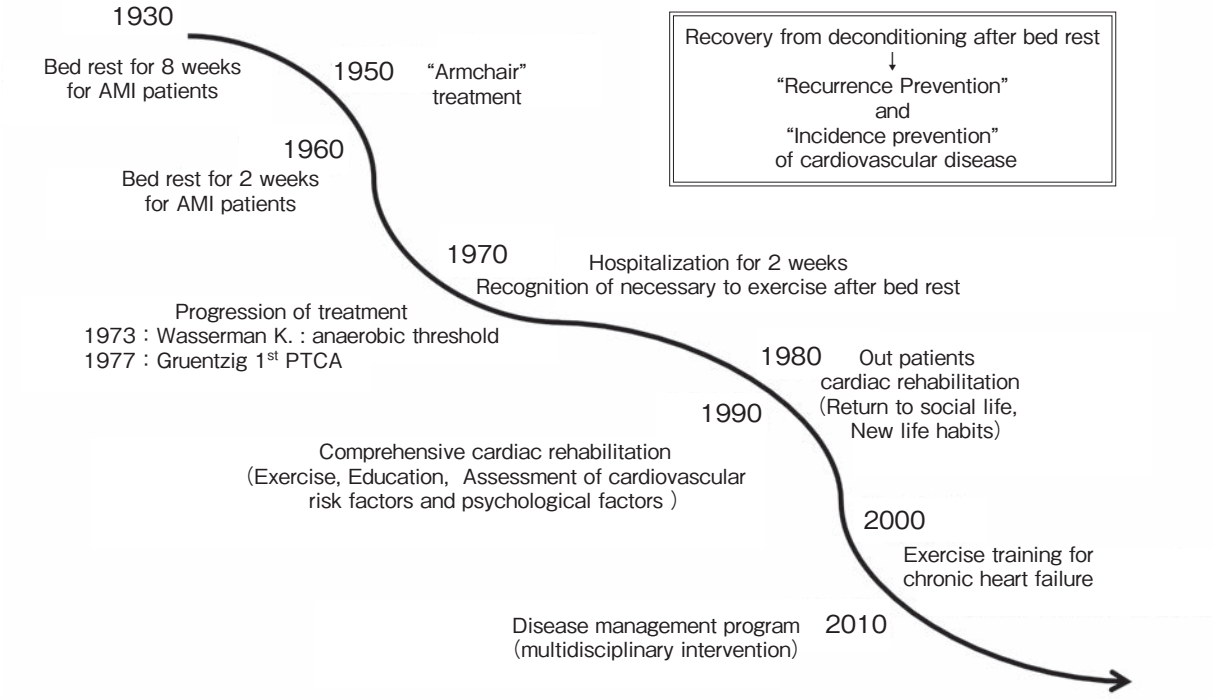


**Figure-1** Current status and directions of CR
CR has been found to improve recurrence prevention and incidence prevention of cardiovascular disease after deconditioning after bed rest.
(Goto Y: J Jpn Coron Assoc, 2015; 21: 58-66. modified)

counseling after ACS, CABG, and heart failure[5]. CR is included as a class I recommendation in most contemporary cardiovascular clinical practice guidelines (Figure-1).

## Trends in risk profiles of patients with coronary artery disease in Japan

Japan is a country with one of the fastest-aging populations. In addition, the prevalence of coronary risk factors in patients with CAD has increased due to the westernization and urbanization of life-styles[6]. We analyzed the data from the Juntendo PCI Registry, which consisted of the information regarding patients who underwent a PCI at Juntendo University Hospital from January 1984 to February 2010. The patients were divided into three groups according to the date of the index PCI procedure [plain old balloon angioplasty (POBA)-era, January 1984-December 1997; bare-metal stents (BMS)-era, January 1998-July 2004; and drug-eluting stents (DES)-era, August 2004-February 2010]. A total of 3,831 patients were examined (POBA-era, n=1,147; BMS-era, n=1,180; DES-era, n=1,504). The mean age was highest during the DES-era. A higher prevalence of diabetes and hypertension was observed in the DES- and BMS-eras than in the POBA-era. These data clearly demonstrated that the patients became older and had higher risk, however, the control of major coronary risk factors has been insufficient and the need for CR is growing particularly among high risk population[7].

## Exercise as vascular medicine

Exercise exerts beneficial effects on blood vessels and suppresses the progression of coronary atherosclerosis. Furthermore, the endothelial function of coronary arteries improved by regular exercise in patients who had MI[8]. Another explanation for atherosclerotic plaque change could be improvements in vascular inflammation. Regular physical exercise has been associated with the decrease in inflammatory markers and ischemic events in patients with CAD[9]. We also found that CR for 6 months ameliorated inflammatory states in patients with metabolic syndrome after CABG[10] and that

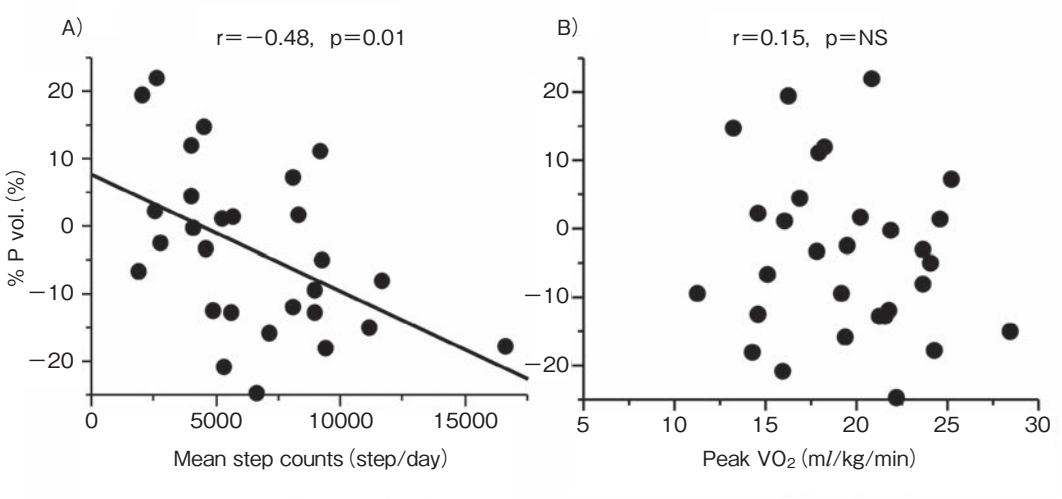


**Figure-2** Correlations between percent change in plaque volume and physical activity (**A**) and exercise tolerance (**B**)
Percent change in plaque volume inversely correlates with physical activity (r=−0.48, p=0.01), but not with exercise tolerance (r=−0.15, p=NS). Reproduced with permission from Nishitani-Yokoyama M, *et al*: Int Heart J, 2015; 56: 597-604 [13].
% P vol, ratio of change in plaque volume.

voluntary exercise improved the progression of endothelial dysfunction and atherosclerotic lesion formation through anti-inflammatory effects in an experimental study [11]. The regression of coronary atherosclerotic lesions has been shown by angiography in patients with CAD who expended an average of 2,200 kcal/week by physical exercise [12]. Recently, we determined the effects of Phase II (PII) comprehensive CR on coronary plaque volume in patients after ACS. We assigned 46 patients with ACS into groups of patients who proceeded with PII-CR (PII-CR; n=21) or those who did not (non-PII-CR; n=25). Although risk factors, muscle strength, and exercise tolerance were improved by PII-CR, plaque regression did not significantly differ between the two study groups. However, a significant correlation between percent change in coronary plaque volume and physical activity was observed (Figure-2). A comprehensive PII-CR program, including frequent supervised exercise sessions and an agenda that encouraged increased physical daily activities, may reduce plaque volume in patients after ACS [13].

### Effects of CR in DM patients with CAD

Patients with DM are at a 2-4 times higher risk of developing CAD and mortality due to CAD compared with patients without DM [14) 15]. The benefits of revascularization are less, whereas the risks and complications are higher than those in patients without DM. Previous studies have also reported a high incidence of bypass graft dysfunction and high mortality even in patients with DM who underwent CABG [16].

Improvement in peak $VO_2$ after CR decreased cardiovascular morbidity and mortality in patients with CAD [17]. However, a previous study demonstrated that the presence of DM was a negative factor for the improvement in peak $VO_2$ [18]. Another report showed a significant inverse relationship between fasting blood glucose levels and changes in peak $VO_2$ in CR participants with DM after coronary events [19]. Park *et al* reported that low muscle strength was a predictor of physical limitations and that DM was associated with low skeletal muscle strength and deterioration in quality [20]. We recently reported that muscle strength and exercise tolerance were significantly lower in patients with DM than in patients without DM at the beginning of CR after CABG [21]. We had enrolled 78 consecutive patients who completed a supervised CR for 6 months after CABG (DM group, n=37; non-DM group, n=41). At the beginning of CR, muscle strength and peak $VO_2$ were significantly lower in the DM group than in the non-DM group. At the end of CR, significant improvement in muscle strength and exercise tolerance was observed in both groups. However, muscle strength, peak $VO_2$, thigh circumference, and mid-upper arm muscle

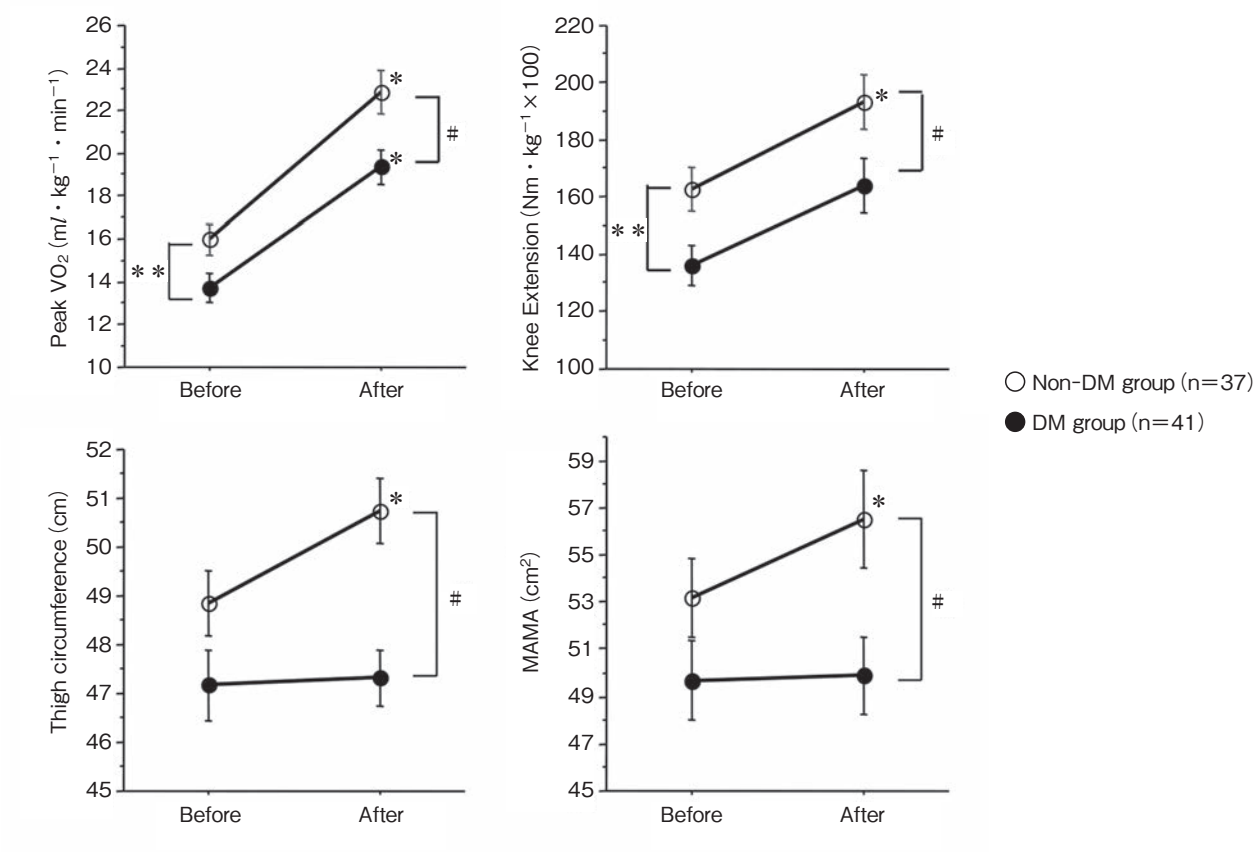


**Figure-3** Effects of CR between the DM and non-DM groups

Exercise tolerance and muscle strength at baseline and after CR in the DM and non-DM groups. At the beginning of CR, muscle strength and peak $VO_2$ were significantly lower in the DM group than in the non-DM group. At the end of CR, significant improvement in muscle strength and exercise tolerance was observed in both groups. However, muscle strength and peak $VO_2$ were significantly lower in the DM group than in the non-DM group. Reproduced with permission from Nishitani M, *et al*: J Cardiol, 2013; 61: 216-221[22].

*$p < 0.05$ compared with at baseline

**$p < 0.05$ compared with the DM group at baseline

#$p < 0.05$ compared with the DM group after 6 months

area (MAMA) were significantly lower in the DM group than in the non-DM group (Figure-3). In addition, no significant improvement in thigh circumference and MAMA was observed in the DM group. At the end of CR, thigh circumference and MAMA correlated with muscle strength. The percent changes in muscle strength were significantly correlated with those in MAMA and hemoglobin A1c. These data suggest that improvements in muscle strength are influenced by changes in muscle mass and high glucose levels in patients with DM undergoing CR after CABG[22]. A CR program, including muscle mass intervention and blood glucose control, may improve deterioration in exercise tolerance in patients with DM after CABG. Armstrong *et al* reported that patients with DM who completed CR derived similar apparent reductions in mortality and hospitalization compared with patients without DM[23].

## Efficacy of CR in elderly patients with stable CAD: Juntendo Cardiac Rehabilitation Program (J-CARP) study

As described above, the rate of aging is the highest in Japan. The MIYAGI-AMI Registry Study demonstrated that the overall age-adjusted incidence of acute MI (/100,000 persons/year) markedly increased by 3.6-fold, from 7.4 in 1979 to 27.0 in 2008 ($p < 0.001$). The number of elderly acute MI patients in Japan has dramatically increased over the past 30 years, particularly that of ≥80 year-old patients, of both sexes (both $p < 0.001$)[24].

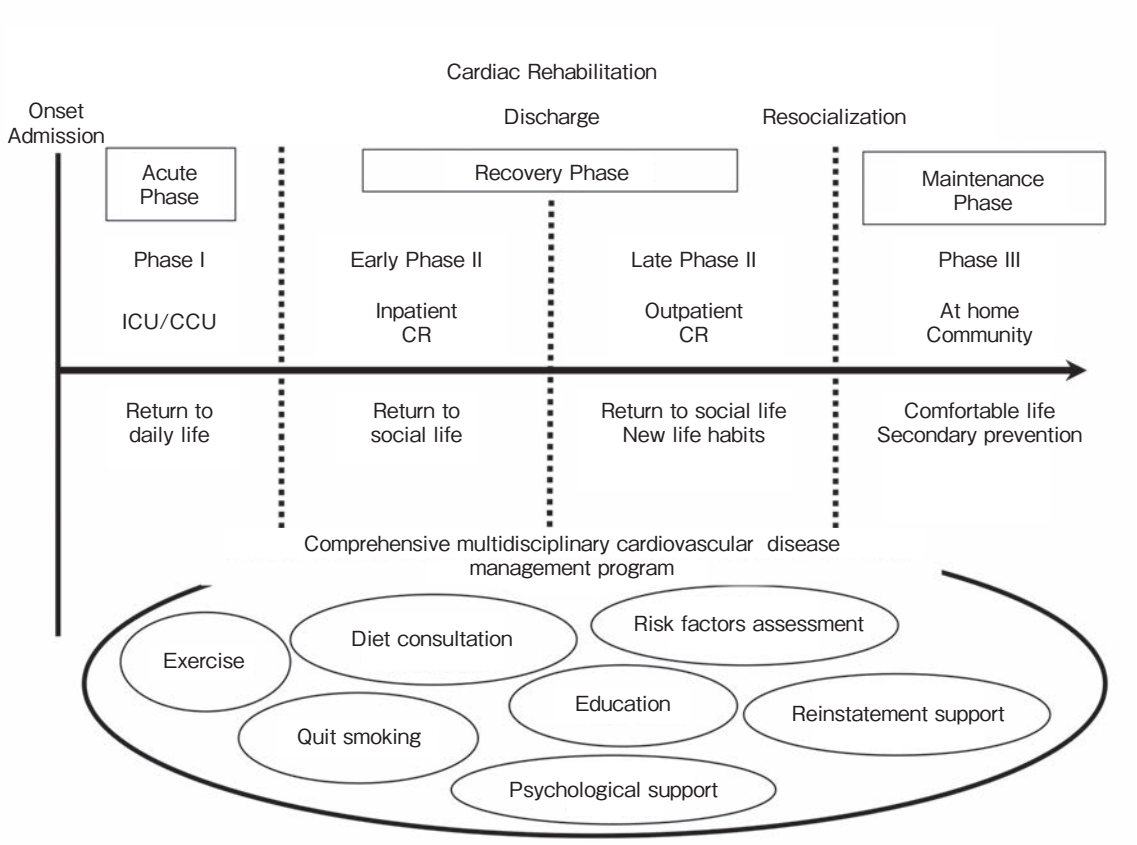


**Figure-4** CR program: Phase and components
CR programs are performed in three stages: acute stage (phase I), subacute stage (phase II), and chronic stage (phase III).

Elderly patients have a 2- to 3-fold higher incidence of acute MI compared with younger persons. They also have more complications, resulting in prolonged hospital stays with lower physical function and substantially higher fatality rates after CAD events. Because of this high rate of mortality and morbidity, primary and secondary prevention strategies are important approaches not only to improve survival but also to maintain the active social life of the elderly population. The benefits of CR have been reported in elderly and in younger patients. A recent report suggested that elderly patients have not been referred to or vigorously encouraged to attend these programs[25].

In general, CR programs are performed in three stages: acute stage (phase I), subacute stage (phase II), and chronic stage (phase III) (Figure-4). In Japan, most CR programs have been performed in phase I and some in phase II[26], however, phase III CR programs have not been performed often because they were not covered by Japanese health insurance until March 2006. We previously reported a phase III CR program called the Juntendo Cardiac Rehabilitation Program (J-CARP). Thirty-four male patients CAD (> 65 years old) were randomly assigned to an intervention group (n = 18) or a control group (n = 16). The intervention group participated in a phase III CR program consisting of exercise training, diet therapy, and weekly counseling for 6 months. In the control group, usual outpatient care was provided. In the intervention group, body mass index, waist size, and fat weight significantly decreased, the peak $VO_2$ and anaerobic threshold $VO_2$ were maintained, and muscle strength significantly improved. In the control group, all parameters were unchanged except for peak $VO_2$, which significantly decreased. In the intervention group, serum total cholesterol levels significantly decreased after CR. However, high-density lipoprotein- cholesterol and apoA-I levels also decreased. In the control group, no significant change in lipid profile was observed[27]. Validated questionnaires were obtained to evaluate the health-related quality of life (QOL) using the

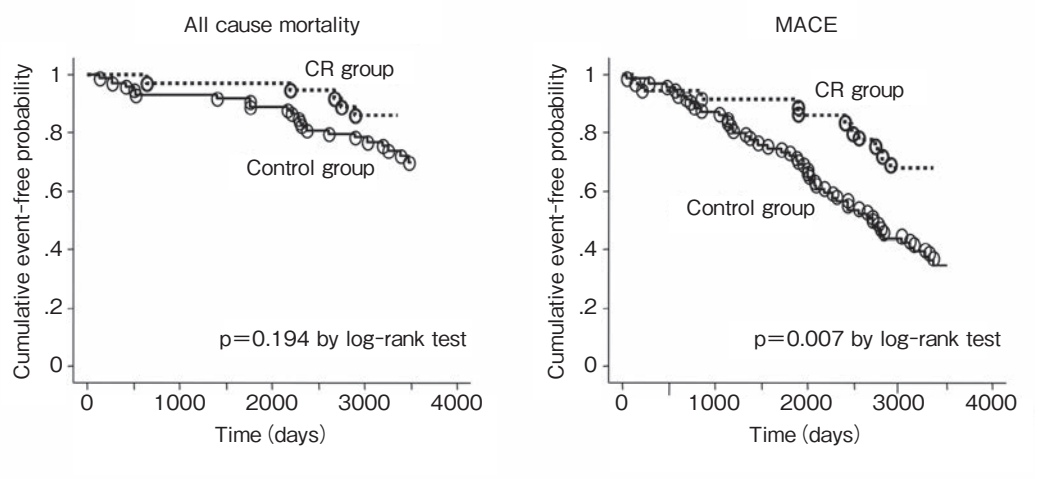


**Figure-5** Effects of phase III CR on mortality and cardiovascular events in elderly patients with stable CAD
Kaplan-Meier curves for all-cause mortality and major cardiovascular events in the CR group and control group. CR, cardiac rehabilitation; MACE, major adverse cardiovascular events combined with cardiovascular death, acute coronary syndrome, refractory ischemia requiring percutaneous coronary intervention or coronary artery bypass grafting, congestive heart failure, and stroke. Reproduced with permission from Onishi T, *et al*: Circ J, 2010; 74: 709-714 [29].

medical outcome study 36-item short-form health survey (SF-36), State-Trait Anxiety Inventory questionnaire (STAI) and self-rating depression scale (SDS) at baseline and after 6 months. At baseline, the scores of SF-36, except for general health, STAI, and SDS were not different in either group. After 6 months, in the intervention group, the scores of bodily pain, general health, vitality, and mental health of SF-36 improved significantly compared with the baseline levels. The state anxiety scores also significantly improved ($p < 0.01$), but SDS depression scores did not improve. In the control group, none of the parameters significantly changed [28].

In the next study, we assessed the effects of phase III comprehensive CR on the morbidity and mortality of elderly male Japanese patients with CAD. The 111 elderly male patients with CAD (≥65 years), including 37 subjects that participated in supervised CR for 6 months and 74 age-matched controls, were analyzed. The patients were followed for up to 3,500 days until the occurrence of death or one of the following major adverse cardiovascular events (MACE): cardiovascular death, ACS, refractory angina requiring revascularization, admission for congestive heart failure, or stroke. All-cause mortality tended to be lower in the CR group than in the control group (14% vs 28%, $p = 0.081$). MACE incidence was significantly lower in the CR group than in the control group (30% vs 62%, $p = 0.001$). The multivariate Cox proportional hazard analysis showed that MACE incidence was significantly lower in the CR group than in the control group [adjusted hazard ratio, 0.43 (95% confidence interval, 0.20-0.91), $p = 0.027$] (Figure-5) [29].

The J-CARP study has demonstrated that phase III comprehensive CR program even in elderly patients with stable CAD improved physical fitness, coronary risk factors, QOL, anxiety, and clinical prognosis.

### Emerging risk of cardiovascular disease: frailty

CAD is one of the diseases that leads to a decrease in the QOL of elderly patients and is the leading cause of morbidity and mortality. Frailty was defined as a clinical syndrome in which three or more of the following criteria were present: unintentional weight loss (>4.5 kg in past year), self-reported exhaustion, weakness (grip strength), slow walking speed, and low physical activity. Frailty is an important risk factor for CAD [30]. Sergi *et al* demonstrated that pre-frailty, which could be potentially reversible, was independently associated with a higher risk of older adults to develop CAD. Among the physical domains of pre-frailty, low gait speed appeared to be the best predictor of

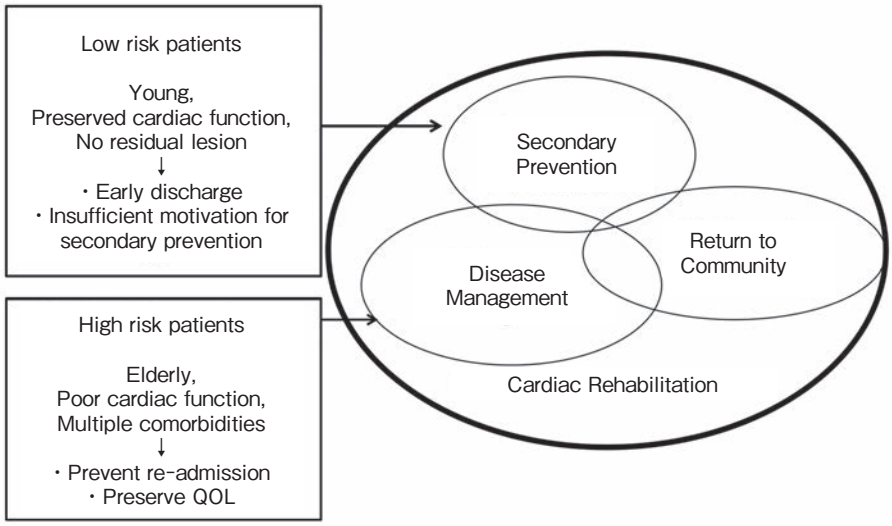


**Figure-6** Bipolarization of patients with cardiovascular diseases
Patients with cardiovascular heart disease have been differentiated into "low-risk patients" and "high-risk patients" in recent years. "Low-risk patients" had insufficient motivation for secondary prevention, however, they had a higher prevalence of multiple coronary risk factors. Secondary prevention and life modification are required. "High-risk patients" needed multidisciplinary strategies to prevent re-hospitalizations and to preserve QOL.
(Goto Y: J Jpn Coron Assoc, 2015; 21: 58-66. Modified)

future CAD[31]. Recent European Society of Cardiology (ESC) guidelines have shown that CR is one of the treatment strategies for subjects with frailty[32].

## Bipolarization of patients with cardiovascular disease in Japan

It has been reported that the patients with CAD have been differentiated into "low-risk patients" and "high-risk patients" based on their clinical backgrounds. "Low-risk patients" have been characterized as young patients with preserved cardiac function and no residual lesions after coronary revascularization. "Low-risk patients" had insufficient motivation for secondary prevention and often discontinued their CR participation because of early hospital discharge and an early return to work. They often have a high prevalence of smoking, dyslipidemia, and multiple coronary risk factors. Therefore, secondary prevention and life modification are strongly required. "High-risk patients" have been characterized as older patients with a high prevalence of heart failure, low cardiac function, and multiple comorbidities (e.g., chronic renal failure, diabetes, anemia, or cerebrovascular disease). They also require long-term disease management to prevent re-admission and to preserve QOL. It is important to participate in a comprehensive CR program after discharge. Individualized programs are often required (Figure-6).

Kamakura *et al* demonstrated that even in "low-risk patients" (<65 years old, successful reperfusion, Killip class I, peak serum creatine kinase < 6,000 U/L, and left ventricular ejection fraction≥40%), active participation (≥20 sessions/3 months) in a PII out-patient CR program has been associated with improved exercise capacity and a better coronary risk factor profile[33].

## Poor implementation of CR in Japan

Recently, the length of hospital stay for patients with acute MI has been substantially shortened, because emergency PCI enables early ambulation. This shortening of hospitalizations has made it difficult to initiate the in-hospital CR program with exercise training and patient education during the in-patient period, but outpatient CR programs do not appear to be widely used.

The Japanese CR Survey demonstrated that the rates of implementation of emergency PCI were very high (92% of Japanese Circulation Society training hospitals) and that the rates of implementation of recovery phase CR were low (20% of the Japanese Circulation Society training hospitals). In addition, the patient education programs (23% of

the Japanese Circulation Society training hospitals) and formulated exercise prescriptions based on exercise testing (16% of the Japanese Circulation Society training hospitals) were poorly implemented. More importantly, only 9% of these hospitals had outpatient CR programs. The nationwide participation rate in outpatient CR after acute MI in Japan was estimated to be only 3.8%–7.6%[27].

The implementation of recovery phase CR, particularly as outpatient CR, is extremely poor in Japan. In addition, patient education programs and exercise prescriptions based on exercise testing have been poorly implemented. The benefits of CR have been established, therefore, it is necessary to improve the spread of recovery-phase and outpatient CR in Japan.

## Conclusions

It is clear that CR improves exercise capacity, QOL, risk factors, and prognosis for patients with CHD. However, the use of outpatient CR programs after hospital discharge remains particularly insufficient in Japan. In addition, it is important to establish CR programs for elderly patients, patients with DM and subjects with frailty. Future studies and social capital supports are required to resolve those problems.

## Disclosures

The authors declare that there are no conflicts of interest.

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# Etiology of Insulin Resistance in Asian Non-Obese Subjects -Juntendo Sportology Center Core Study-

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Obesity and its associated metabolic disorders are rapidly disseminating all over the world. While they became serious health problem, such diseases in Asians shows different features from those in other races such as Caucasian and African. One of the important features is a susceptibility to develop to type 2 diabetes even without excess body mass index. To search for the mechanism underlying this feature is important to find the suitable therapeutic strategy for Asian obesity. Thus, as a core study of the Sportology Center of Juntendo University Graduate School of Medicine, we assessed tissue-specific insulin resistance in Japanese non-obese subjects. In this study, we recruited non-obese and non-diabetic Japanese subjects and measured insulin sensitivity in muscle and liver by a 2-step hyperinsulinemiceuglycemic clamp with glucose tracer and ectopic fat content in muscle and liver by proton magnetic resonance spectroscopy. So far, our data suggests that muscle insulin resistance may play a central role in future onset of the diseases that could make the healthy people to disable people.

***Key words***: insulin sensitivity, euglycemic-hyperinsulinemic clamp, ectopic fat, MRS

### Abbreviations

BMI; body mass index, FABPpm; plasma membrane-associated fatty acid-binding protein, IMCL; intramyocellular lipid content

### Pathophysiology of Asian type 2 diabetes mellitus

Two main defects observed in type 2 diabetes mellitus are insulin resistance and beta cell dysfunction. The combination of these two defects causes decreased effect of insulin that is a sole hormone to reduce blood glucose level, thus, results in hyperglycemia. In each patient with type 2 diabetes mellitus, the different ratio of these two defects seems to present. Accordingly, type 2 diabetes mellitus is considered to be a heterogeneous disease. Given that the onset of most diseases is affected by genetic factors and environmental factors, environmental factors are believed to strongly affect the onset of insulin resistance. The key environmental factor is westernized life style such as high fat intake and less exercise. The exposure of westernized life style causes overnutrition that changes the systemic adiposity. The abnormal adiposity such as enhanced visceral fat accumulation is regarded as the main cause of insulin resistance. Abnormal adiposity with signs of insulin resistance is defined as metabolic syndrome and the metabolic syndrome is regarded as a risk factor for cardiovascular diseases and also for type 2 diabetes. However, the presence of type 2 diabetes mellitus is a stronger risk factor for cardiovascular diseases and also causes a characteristic complication of diabetes such as retinopathy, nephropathy, and neuropathy (Figure-1).

According to the latest report from International Diabetes Federation, the number of the patients with diabetes is increasing. In 2013, there are 382 million people with diabetes mellitus in the world. In addition, whereas mean body mass index (BMI) are variable among the countries, the prevalence rate of

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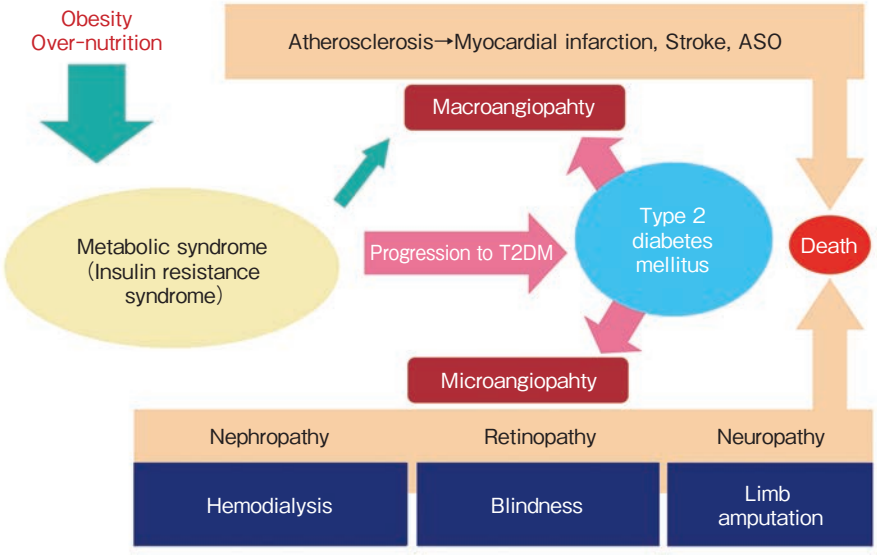


**Figure-1** Natural history of type 2 diabetes mellitus and its complication
This scheme illustrates the natural history of typical type 2 diabetes mellitus. Abbreviation: ASO, atherosclerosis obliterans.

diabetes is similar among the countries[1]. Given that Asian patients with diabetes are relatively lean, Asian is susceptible to develop to type 2 diabetes even with normal body mass index (BMI) ($< 25$ kg/m$^2$)[1)-3)]. Thus, a recent position statement from American Diabetic Association indicated that testing for diabetes should be considered for all Asian American adults who present with a BMI of $\geq 23$ kg/m$^2$)[3]. In addition, Asian also easily developed to metabolic syndrome compared with BMI matched non-hispanic Whites and African Americans[4]. One possible explanation of this feature is that beta cell function in Asian is weaker than that in other races. However, a recent study comparing the beta cell function between Japanese and Caucasian could not find the distinct ethnic difference of beta cell function considering the state of insulin resistance[5].

## Insulin resistance and ectopic fat accumulation

Another possibility is that susceptibility of insulin resistance by overnutrition could be different between Caucasian and Japanese. Overnutrition results in accumulation of excess energy storage in whole body. Adipose tissue, especially subcutaneous adipose tissue is regarded as a suitable place to store the excess energy as a form of triglyceride. However, the accumulation of triglyceride in different places causes different systemic metabolic state. Indeed, increased size of visceral, not subcutaneous adipose tissues is known to cause systemic insulin resistance. In addition, ectopic fat accumulations in muscle and liver are observed in such cases and are considered as a cause of insulin resistance in each organ[6]. These facts suggest that the feature of adiposity may play a key role on the susceptibility for type 2 diabetes in Asian.

Regarding this possibility, Azuma *et al*, compared amount of visceral and liver fat accumulation considering BMI level between Japanese and Caucasian. Intriguingly, while visceral fat accumulation of Japanese is similar to that of Caucasian after

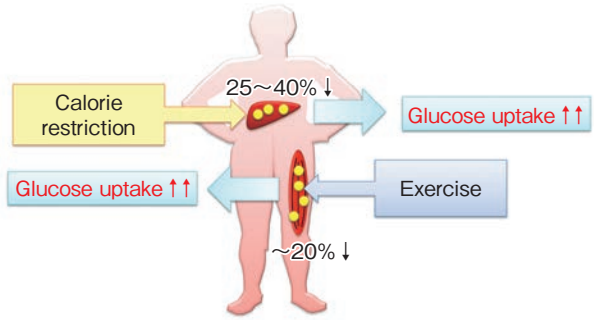


**Figure-2** Effects of calorie restriction and exercise
Even if body weight reduction by diet and exercise is small (2~5%), the reduction of ectopic fat accumulation with the decrease of tissue specific insulin resistance were observed[10) 11)].

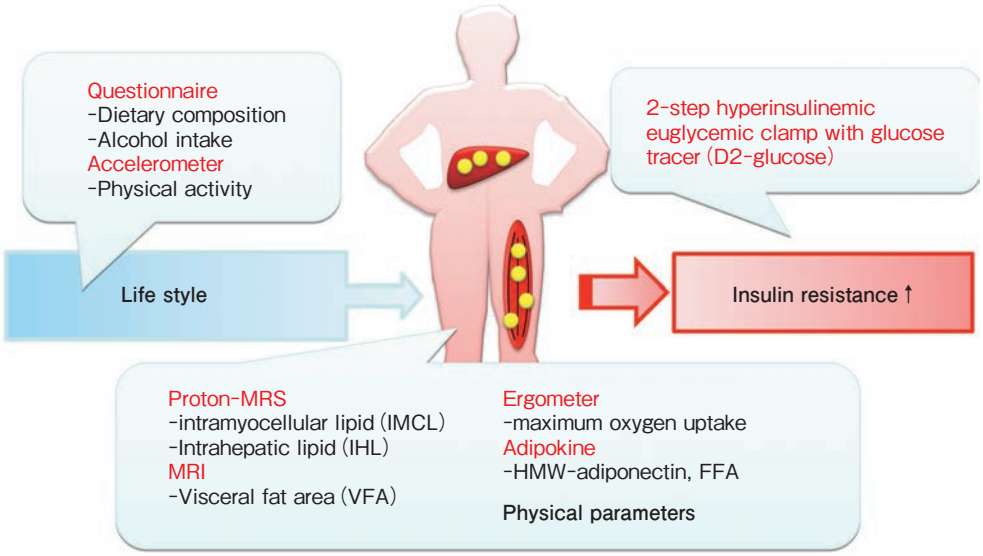


**Figure-3** Outline of Sportology Center Core study

adjustment of BMI level, Japanese easily develop to fatty liver compared with Caucasian[7]. These data suggest that susceptibility for ectopic fat accumulation may play important role in the progression of insulin resistance in Asian with normal BMI level.

Therefore, we have investigated the etiology of ectopic fat accumulation and its relation to insulin resistance in Japanese. The study of high fat diet intervention for normal subjects revealed that high molecular weight-adiponectin and daily physical activity are determinants of intramyocellular lipid content (IMCL) accumulation by a high-fat diet[8]. Similar intervention with the analysis of mRNA expression in muscle sample revealed that IMCL accumulation and impaired insulin sensitivity after high fat diet are closely associated with changes in the expression of genes related to lipid metabolism in muscle[9]. In addition, moderate exercise intervention study in the patients with type 2 diabetes mellitus revealed that exercise decrease IMCL and improve insulin resistance[10]. Furthermore, diet restriction in subjects with impaired glucose tolerance showed that diet therapy decrease intrahepatic accumulation of triglyceride and enhance insulin sensitivity[11]. These data suggest that in Japanese subjects, ectopic fat accumulation seems to be tightly associated with insulin resistance (Figure-2).

## Sportology Center Core study

To investigate the etiology and the role of tissue specific insulin resistance in Japanese non-obese men especially further focusing on ectopic fat accumulation, we planned Sportology Center Core study supported by High Technology Research Center Grant from MEXT. In this study, we recruited subjects who were non-diabetic and aged between 30 and 50 years men and obese men with metabolic syndrome as positive control of metabolic disorders with obesity[12]. In this study, we assessed dietary composition and alcohol intake by questionnaire, physical activity by accelerometer, IMCL, and intrahepatic lipid by proton- magnetic resonance spectroscopy, maximum oxygen uptake by ergometer, visceral fat area by computer tomography and high molecular weight-adiponectin, free fatty acids and other biochemical tests by standard measurement. In addition, we also assessed 2-step hyperinsulinemic euglycemic clamp with glucose tracer. We used the endogenous glucose production suppression at 1$^{st}$ step as an index of hepatic insulin sensitivity and rate of disappearance at 2$^{nd}$ step as an index of muscle insulin sensitivity (Figure-3).

So far, we found the subjects showing various pattern of insulin resistance, even in non-obese, non-diabetic subjects; subjects with neither, or either or both of muscle and liver insulin resistance.

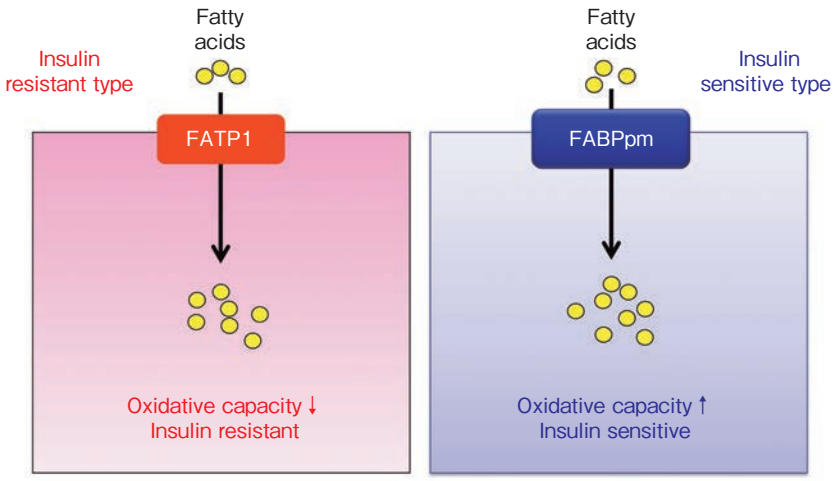


**Figure-4** Difference of fat accumulated muscle between in subjects with insulin resistance and the subjects without insulin resistance
Abbreviation: FABPpm; plasma membrane-associated fatty acid-binding protein, FATP-1; fatty acid transport protein-1.

We found that alcohol intake is one of the major determinants of liver insulin resistance. On the other hand, as the determinants of muscle insulin resistance, not only the factors associated visceral fat accumulation but also, factors related to quality of the muscle, factors associated with systemic microinflammation, and factors associated with daily diet and exercise were identified. These results clearly suggest the different etiology of muscle and liver insulin resistance in Japanese non-obese, non-diabetic subjects.

Regarding the relation between ectopic fat accumulation and tissue specific insulin resistance, liver fat accumulation is associated well with muscle insulin resistance. On the other hand, muscle fat accumulation did not show significant association with muscle insulin resistance. Intriguingly, we found modest association between muscle insulin resistance and muscle fat accumulation by excluding the subjects whose $VO_2$ max is high. Also, we found that some subjects with high muscle fat accumulation show high insulin sensitivity. Thus, we focused the subjects with high fat accumulation in muscle, we compared mRNA expression in muscle between the subjects with insulin resistance and those without insulin resistance. As a result, several lipid oxidation genes in muscle were up-regulated in the subjects without insulin resistance, and this was associated with increased expression of higher plasma membrane-associated fatty acid-binding protein (FABPpm) and decreased expression of fatty acid transport protein-1. Over-expression of FABPpm in culture myocytes increased fatty acid oxidation coupled with the elevated expression of genes related to fatty acid oxidation. These results suggest that the level of FABPpm expression may play an important role in insulin sensitivity in the fat accumulated muscle [13] (Figure-4).

Using the data of Sportology Center Core study, we tried to estimate the outcome of insulin resistance. The presence of insulin resistance in muscle highly associated with cardiovascular risk factors. In addition, it also highly associated with fatty liver that is the well-known basis of liver cancer. Furthermore, our study revealed that insulin resistance is also associated with white matter alteration that may be linked with future onset of dementia [14]. Taken together, muscle insulin resistance may play a central role for future onset of the diseases that could make the healthy people to disable people (Figure-5). Thus, to find the factors that cause insulin resistance and to prevent the onset of insulin resistance seems to be efficient method to prevent the future onset of the disease that makes healthy people disabled.

## Acknowledgments

Sportology Center Core study was mainly

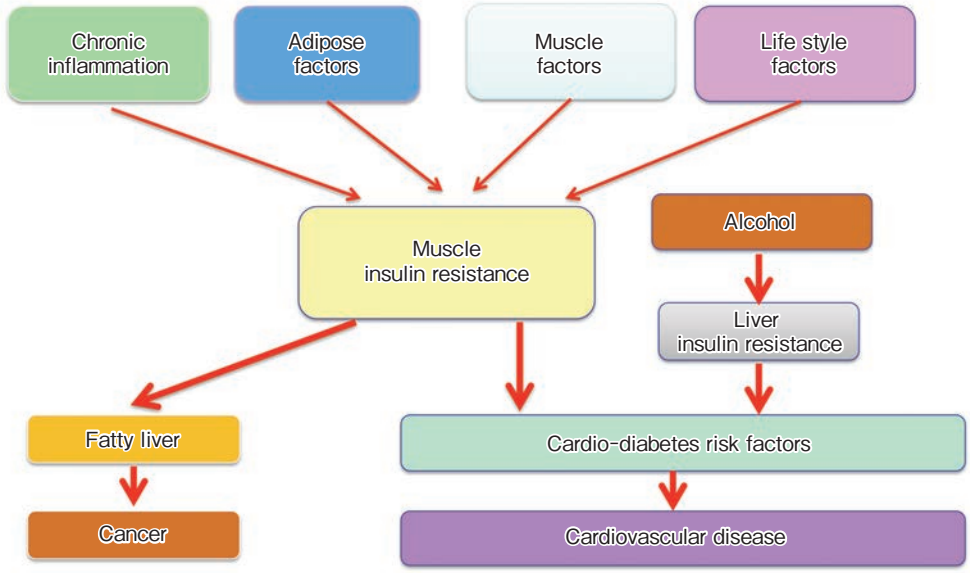


**Figure-5** Factors associated with insulin resistance and consequence of insulin resistance

performed by Minako Kawaguchi, Takahiro Watanabe, Takashi Funayama, Yuko Sakurai, Fumihiko Sato, Kageumi Takeno, and Yoshifumi Tamura from Department of Metabolism & Endocrinology, Juntendo University Graduate School of Medicine, and Saori Kakehi and Ryuzo Kawamori from Sportology Center, Juntendo University Graduate School of Medicine. We thank Mrs. Mutsuko Yoshikawa, Miyuki Iwagami, Naoko Daimaru, Eriko Magoshi, and Emi Miyazawa for their excellent technical assistance.

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## Disclosure

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Proceedings

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# Sportology to Prevent Locomotive Syndrome

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"Locomotive syndrome" is defined as a condition associated with being restricted in one's ability to walk or lead a normal life owing to a dysfunction in one or more of the parts of the locomotion system, including the muscles, bones, joints, cartilage or the intervertebral discs. This syndrome especially refers to those elderly who have come to need nursing care services because of problems with the locomotive organs, or who have conditions which may require them to have such services in the near future. The recent epidemiological studies revealed that the one fourth of the reasons for requiring special assistance or nursing care in elderlies is currently the locomotive disorders. Osteoarthritis of the knee (knee OA) or hip and the spinal canal stenosis due to spondylosis are the three major locomotive disorders those are related to the requiring special assistance or nursing care in elderlies. We are trying to elucidate the mechanistic insight into the pathophysiology of knee OA and osteoporosis by receiving several supports, such as the COI program by a MEXT and the Juntendo Sportology Center under the concept of "Sportology", as we believe to be able to contribute directly the concept of "locomotive syndrome" by these actions.

***Key words***: locomotive syndrome, knee osteoarthritis, osteoporosis, spinal canal stenosis, fragility fracture

## Locomotive syndrome

Japan has been developed to be a leading country for not only the average life span but also the healthy life expectancy worldwide. The healthy life expectancy means the number of years we can expect to live in good health without any special assistance or nursing care. Global healthy life expectancy in Japan (73.4 yo) is no. 1 in worldwide in both female (75.6 yo) and male (71.1 yo)[1]. Therefore, Japan faces a future as the most elderly society humankind has ever known.

The Japanese Orthopaedic Association proposed the concept of "locomotive syndrome" in 2007. It is defined as a condition associated with being restricted in one's ability to walk or lead a normal life owing to a dysfunction in one or more of the parts of the locomotion system, including the muscles, bones, joints, cartilage or the intervertebral discs. This syndrome especially refers to those

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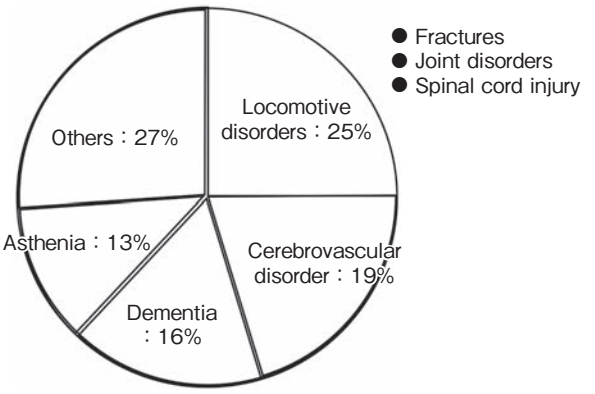


**Figure-1** Causes for nursing care in elderlies in Japan (Annual Health, Labour and Welfare Report 2013-2014. Ministry of Health, Labour and Welfare; 2015[2])

elderly who have come to need nursing care services because of problems with the locomotive organs, or who have conditions which may require them to have such services in the near future.

The recent epidemiological studies revealed that the one fourth of the reasons for requiring special assistance or nursing care in elderlies is currently the locomotive disorders (Figure-1)[2]. The three major locomotive disorders those are related to the requiring special assistance or nursing care in elderlies are the osteoporotic fragility fractures, osteoarthritis of the knee or hip and the spinal canal stenosis due to spondylosis (Figure-2). The prevalence of these disorders is increased dependent upon aging. In addition, it has also been revealed that elderlies are suffering from not only one of these three locomotive disorders but also two or all of these disorders, simultaneously, and the complication rate of these three locomotive disorders in elderlies is increasing depending upon aging (Figure-3)[3]. These locomotive organ disorders worsen as signs go unheeded. Steps need to be taken today to prevent locomotive syndrome and to extend the healthy life expectancy of people living today, so that individuals can continue to be mobile for life (Figure-4).

## Researches for the pathophysiology of the osteoarthritis of the knee (knee OA) under the concept of "Sportology"

Osteoarthritis of the knee (knee OA) is one of the representative age-related chronic motor organ diseases responsible for the locomotive syndrome. OA is an age-related progressive joint disease, which is characterized primarily by cartilage degradation[4]. OA is an increasingly important public health concern, as the prevalence of the disease is increasing with the aging of society. The ideal management of knee OA is illustrated as a sequential, pyramidal approach (Figure-5)[5]. While it has been estimated that there are 25 million people with radiographic knee OA, it has been speculated that eight million have knee pain[6]. Among the patients with painful knee OA, eighty-five thousand cases of total knee arthroplasty (TKA) are currently being performed each year in Japan. For example, the concept of "locomotive syndrome" should therefore be promoted so that the patients with knee OA who either need or don't need TKA can be identified more clearly from the perspective of the locomotive syndrome, and to allow for the earlier identification of patients with symptomatic knee OA to prevent the development of locomotive syndrome by providing adequate pain relief.

For future development of the disease-modifying drugs in knee OA, further studies are required to elucidate precisely the process of the disease progression. Under the concept of "Sportology" to prevent the locomotive syndrome and the support from the Juntendo Sportology Center, we have been tried to elucidate the pathophysiology of knee OA. For instance, we revealed, for the first time, that the degenerative changes, detected by T2 mapping on magnetic resonance imaging (MRI), and the morphological changes, detected on MRI, of the femoral articular cartilage showed a greater degree of deterioration than those of both the tibial- and patellar- articular cartilage in the patients with early stage knee OA (Figure-6)[7] This is considered to be unique and important information for obtaining a better understanding of the disease processes, which has never previously been elucidated using classical radiographs.

We have also investigated the metabolic changes of the articular cartilage in men in early forties without radiographic OA changes and symptom, including pain. The participants of this study were the subjects in the Juntendo Sportology Core study, which was been conducted since 2011. The radiographic medial knee joint space width (JSW) of the subjects showed the normal distribution. The

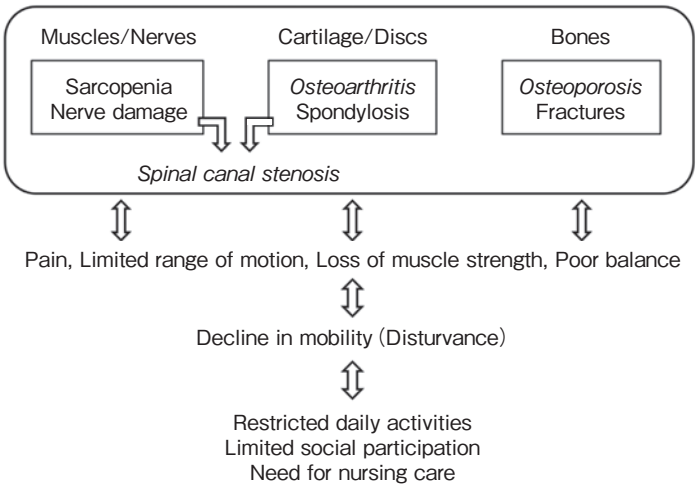


**Figure-2** Locomotive syndrome: A conceptual diagram
(Nakamura K: J Orthop Sci, 2011; 16: 489-491[8])

| | Total | Male | Female |
|---|---|---|---|
| Either one | 44.7 | 21.0 | 26.0 |
| Two among three | 24.7 | 9.9 | 14.8 |
| All three | 5.4 | 1.1 | 4.3 |

(millions)

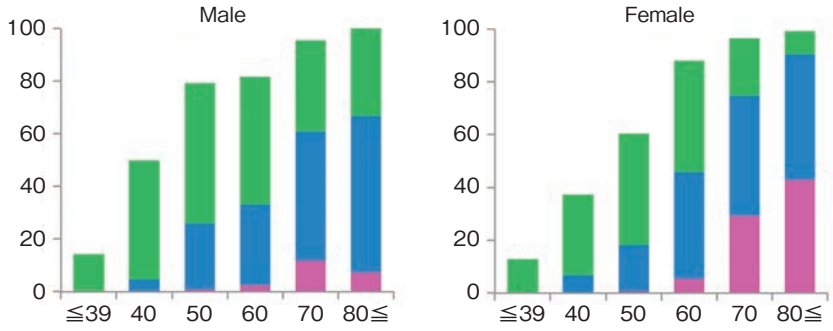


**Figure-3** Prevalence of knee osteoarthritis, lumbar spondylosis and osteoporosis in Japanese men and women
(Yoshimura N, *et al*: J Bone Miner Metab, 2009; 27: 620-628[3])

serum levels of procollagen II C-terminal propeptide (sP II CP ), which is a cartilage synthesis biomarker, and cartilage type II collagen cleavage by collagenase (sC2C) and the urinary levels of cartilage type II collagen C-telopeptide (uCTX-II), cartilage destruction biomarkers, were measured. Among these three cartilage biomarkers, a multiple linear regression analysis with adjustments for age and BMI showed significant positive correlations between the radiographs JSW and the sP II CP level (β: 0.404, p=0.007), suggesting the reduction of cartilage synthesis was associated with the lower JSW, that is one of the typical signs of OA. Thus, the lower cartilage synthesis, rather than cartilage destruction, was associated with the radiographic joint space narrowing of the knee joint in men in early forties without any symptom related to knee OA.

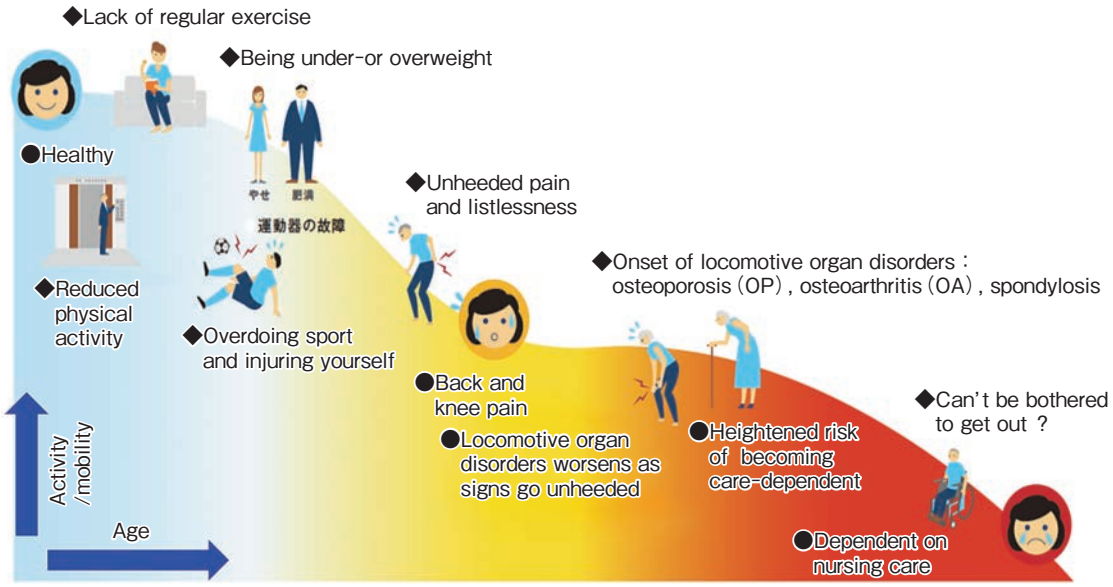


**Figure-4** Relationship between the development of locomotive syndrome and the mobility of people
(Locomotive Syndrome. 2013: Accessed at https://locomo-joa.jp/en/index.pdf.)

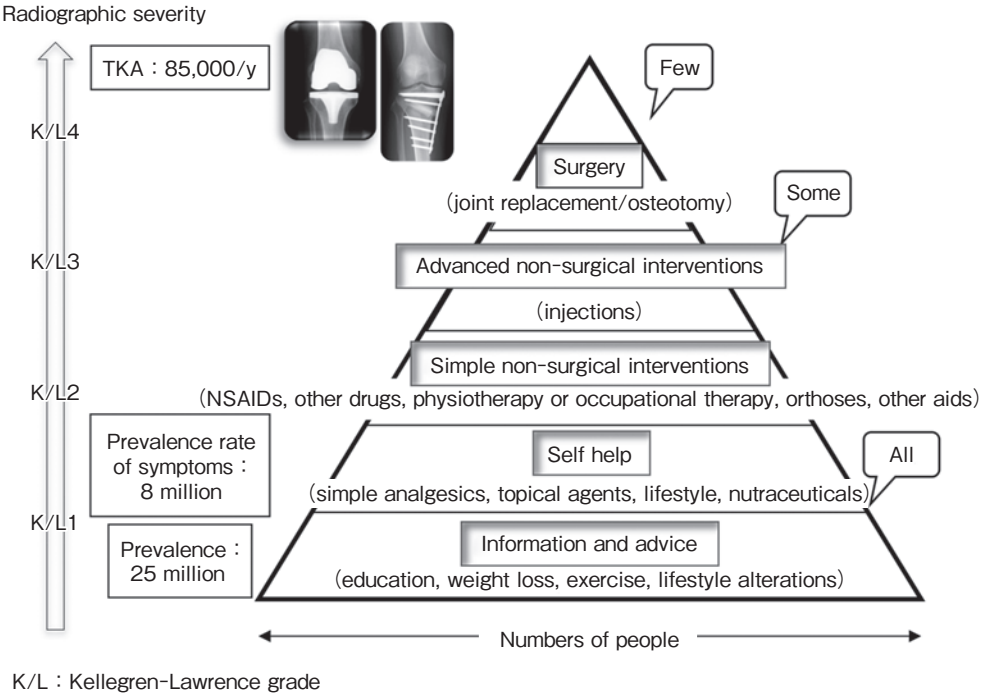


**Figure-5** Principles of the management of knee OA
Suggested sequential, pyramidal approach to disease management.
(Dieppe PA, *et al*: Lancet, 2005; 365: 965-973[5])

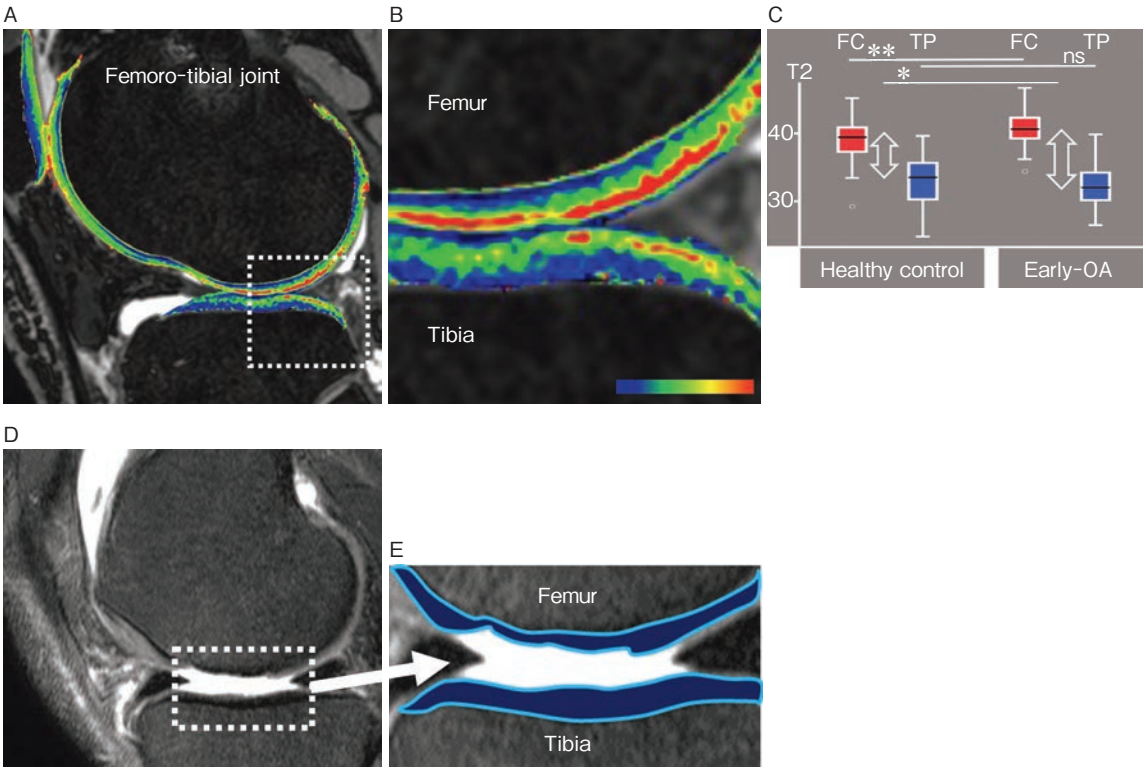


**Figure-6** The early stage OA-induced degenerative and morphological changes between the femoral- and the confronting tibial- or patellar- sides within the knee join were not the same

**A**. T2 mapping image of the sagittal section of the knee joint. **B**. Enlargement of the articular cartilage of (**A**). **C**. Comparison of theT2 levels of articular cartilage in early-stage knee OA : femoral side > tibial side ($p < 0.001$), odd ratio: 3.6 (95%CI: 1.8-6.9, $p < 0.0001$). 3T MRI image of the sagittal section of the knee joint. **D**. Comparison of the cartilage lesions in early-stage knee OA : femoral side > tibial side ($p < 0.001$), odd ratio: 1.9 (95%CI: 1.0-3.5, $p = 0.026$). **E**. Enlargement of the articular cartilage of (**D**).
(Hada S, *et al*: Osteoarthritis Cartilage, 2014; 22: 1583-1589[7])

## Researches for the locomotive syndrome supported by the Center of Innovation (COI) program

We are also supported by the Center of Innovation (COI) program from the Japan Science Technology Agency (JST) and the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan, which is a large-scale and university-industry collaborated R&D platform in which universities concentrate all their powers on innovative research issues and corporations lead the commercialization of their achievements. In the diagnosis and treatment of knee OA, information for gait of patients with knee OA is important. Although the gait of patients with knee OA can be measured using motion capture technology, the devices currently available require a huge space to monitor and scan the patient's motion, making it difficult to use in the clinical setting. Under the support from the COI program and the collaboration with the researchers of both HITACHI Ltd and

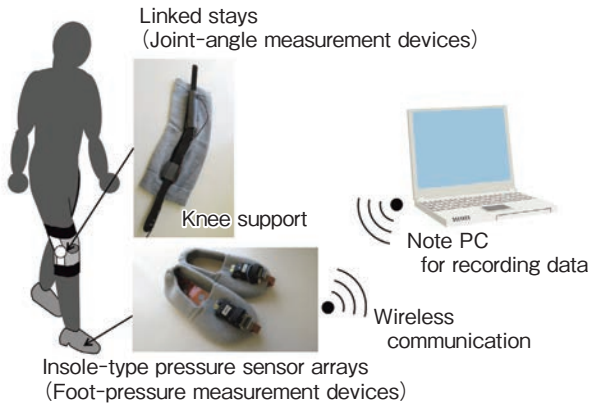


**Figure-7** Novel motion capture technology developed by HITACHI Ltd. to monitor the walking ability of patients with knee OA in the clinical setting
(Kaneko H, *et al*: Juntendo Medical Journal, 2014; 60: 525)

Juntendo University Graduate School of Health and Sports Science, we have established a novel motion capture technology to monitor the walking ability of patients with knee OA in the clinical setting. This device consists of linked stays and insole-type

pressure sensor arrays. The stays have inertial sensors (accelerometers and gyroscopes) to measure the hip joint angles, and potentiometers to measure the knee joint angles. The pressure sensor arrays which are inserted into the shoes measure the balance (center of pressure) of the patients while they walk. These light weight (350 g including shoes) and small ($3 \times 5 \times 2$ cm) devices can measure the gait and send the results to a PC without the need for a video monitoring system (Figure-7). We demonstrated the possibility that the novel miniaturized motion capture technology could monitor the walking ability of patients with knee OA in the clinical setting. We also demonstrated that the range of motion of hip joint and the walking stride were the factors that related early postoperative walking recovery by analyzing gait analysis before and after TKA in end-stage knee OA.

## Sportology to prevent locomotive syndrome

There is an urgent need to improve the understanding of the pathophysiology of the diseases, such as knee OA and osteoporosis, which are responsible for "locomotive syndrome". Further studies are required to facilitate the understanding of the "locomotive syndrome" widely and the concept "Sportology" could be helpful to succeed this proposition.

## Acknowledgement

We deeply appreciate the support by a Center of Innovation (COI) program from the Japan Science Technology Agency (JST) and the Ministry of Education, Culture, Sports, Science and Technology (MEXT) and by a High Technology Research Center Grant from the MEXT.

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# Mechanical Regulation and Maintenance of Organismal Homeostasis – Scientific Basis for Health Promotion by Physical Motility and Exercise

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Mechanical stresses play various different roles in regulating organismal functions, depending on the situation when and where they are borne. Cell signaling related to 'straining' mechanical stresses, such as cell stretching, pressurizing, cytoskeletal tensioning, adhesion to stiff substrate, and high traction force generation, is imperative in the 'constructive' phase of tissues and organs, including development, regeneration and repair. However, once steady state is reached upon completion of tissue/organ formation, straining mechanical stress often causes failure of organismal homeostasis, such as inflammation and cancer. In contrast to such 'detrimental' aspect of straining stress, relaxing mechanical stress contributes to maintenance of homeostasis. Collectively, balance and integration between straining and relaxing mechanical stresses is vital, whose disruption gives rise to diseases, particularly those related to ageing or physical inactivity (Figure-1).

***Key words***: mechanical stress, mechanobiology, organismal homeostasis, relax biology, ageing

## Introduction

All the organisms on earth perceive surrounding mechanical environments and adapt to them by converting physical information into biological signals regulating physiological events. Organismal homeostasis therefore relies on mechanical regulation, for which mechano-sensing followed by mechanically-induced signal transduction, termed mechanotransduction, is responsible.

To date, mechanical 'stress' has often been referred to excessive physical loading that may induce or deteriorate disorder or inflammation of tissues and organs. For example, hypertension (high blood pressure) causes age-related cardiovascular diseases, including cardiac hypertrophy, atherosclerosis and aneurysm. Extravagant mechanical loading on bone and cartilage arising from obesity, extreme physical exercise or joint instability give rise to osteoarthritis and imbalanced bone resorption (osteoporosis or fatigue fracture). Contrary to these excessive loadings, loss of mechanical stress is also detrimental to homeostasis particularly in the case of musculoskeletal organs. For instance, physical inactivity and gravity-free conditions (spaceflight) induce rapid muscle atrophy as well as bone loss. Notably, most of these harmful effects of mechanical stresses involve cell signaling mediated by NF-κB, a transcription factor that plays a key role in regulating inflammatory responses.

Interestingly, physical interventions are typically effective as treatments for the aforementioned mechanical stress-related health problems. Physical therapy, optimal physical exercise and programmed sports activity can redress the mechanically inducible disruption of tissue/organ homeostasis. This clearly indicates the 'beneficial' effects of mechanical stresses on living organisms. However, very little is known concerning the molecular mechanisms behind the positive facets of mechano-sensing and mechanotransduction.

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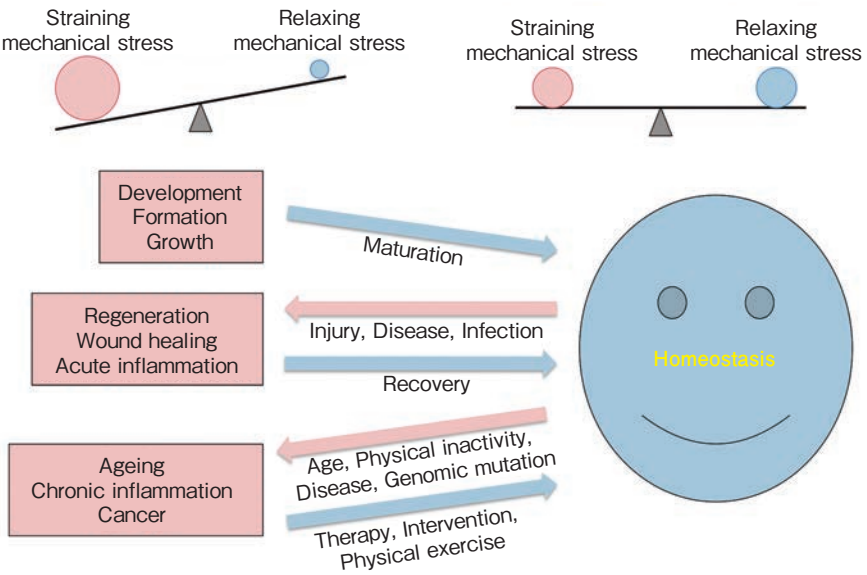


**Figure-1** Roles and implications of straining and relaxing mechanical stresses in organismal homeostasis vary, depending on the phases/situations where they are borne

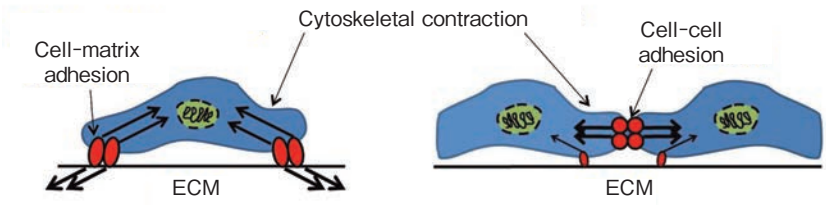


**Figure-2** Mechanical stress is yielded upon cell-matrix or cell-cell adhesion
Cells exert traction force on the substrate (left) or cells (right) to which they adhere. ECM- or neighboring cell-generated counteractive force yields tensile stress.

In this review, beneficial (good) and detrimental (bad) mechanical stresses are distinguished in view of organismal homeostasis. It is also discussed why and how loss of mechanical stresses leads to unfavorable consequences in musculoskeletal systems from mechanobiological perspectives.

## 'Straining' mechanical stress

Cells exert actomyosin-generated traction forces on the extracellular matrix (ECM) or the neighboring cells to which they adhere (Figure-2). These forces yield counteraction, *i.e.* tensile forces from the ECM or neighboring cells. As is evident from Figure-2, cell stretching is transduced over the adhesion complexes to the cytoskeletons, resulting in an increase in cytoskeletal tension. Actomyosin-derived cytoskeletal contractility provides driving forces for cell migration. Thus, cell-generated contractile forces, tension of actin cytoskeletons, adhesion-cytoskeleton linkage, and cell migration are closely interconnected and correlated.

Using the technique called traction force microscopy, we previously demonstrated that traction stress increased with substrate rigidity up to 20 kPa (Figure-3)[1]. Considering that the tissue rigidity is typically below 20 kPa except for bones[2], higher force generation on stiffer substrates appears to be a physiological cell response or function. Cells reportedly respond to hydrostatic pressure appear in a fashion similar to the response to stretching in light of cytoskeletal rearrangement and signal activation[3) 4)]. Collectively, from cell mechano-responsive or mechano-sensitive point of view, it is

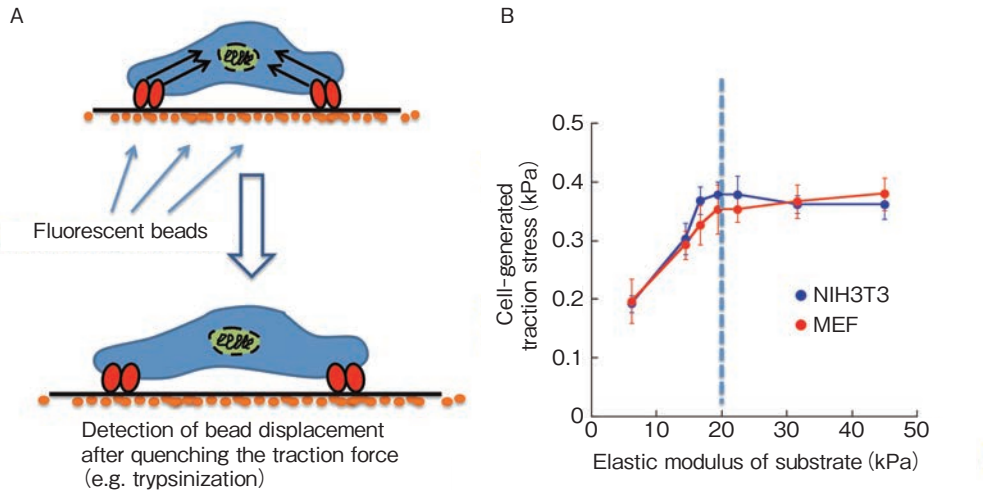


**Figure-3** Cell-generated traction force, which is measurable, increases with substrate stiffness up to 20 kPa
A. Traction force microscope. The displacement of fluorescent beads embedded in elastic substrates with various physical properties is detected using a microscope. Together with the elastic modulus of the substrate, cell-generated force can be calculated.
B. Traction force generated by NIH3T3 cells (NIH3T3) or mouse embryonic fibroblasts (MEF) increased with substrate stiffness up to 20 kPa (cited from Yip AK, *et al*: Biophys J, 2013; 104: 19-29[1]).

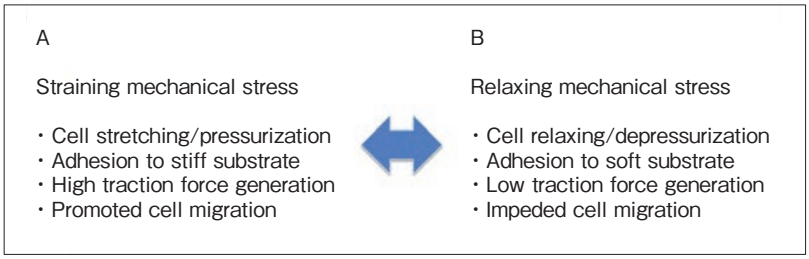


**Figure-4** Straining and relaxing mechanical stresses in light of mechano-sensing and mechanotransduction
A. Straining mechanical stress
B. Relaxing mechanical stress

reasonable to sum up the implications or significance of the following mechanical stimulation/perturbation/situation and physical environments as 'straining mechanical stress' (Figure-4).

- cell stretching
- applying hydrostatic pressure (pressurization)
- cytoskeletal tensioning
- adhesion to stiff(er) substrates
- high(er) traction force generation
- promoted cell migration (including efficient cytoskeleton-adhesion force transmission)

With regard to cell signaling pathways responsible for these straining mechanical stresses. MAP kinase[5) 6)], tyrosine phosphorylation[7) 8)], and YAP/TAZ[9)] have been reported.

## Relaxing mechanical stress

The following 'relaxing' mechanical stresses are contrary to the aforementioned straining ones (Figure-4).

- cell relaxing (but not cell compression)
- reducing hydrostatic pressure (depressurization)
- cytoskeletal relaxing
- adhesion to soft(er) substrate
- low(er) traction force generation
- impeded cell migration (including inefficient cytoskeleton-adhesion force transmission)

## Straining and relaxing (mixed or combined) mechanical stress

Shear stress has a facet of cell relaxation facet as it decreases actomyosin contractility; however, it increases the tension of intermediate filaments (vimentin)[10]. Furthermore, shear stress reportedly activates straining-related signaling molecules such as NF-κB and MAP kinases[11], as well as relaxation-related ones including eNOS[12]. Therefore, shear stress appears to be a mixture or combination of straining and relaxing mechanical stress.

Cyclic application of straining mechanical stress is also a mixture or combination of straining and relaxing. For example, 100 times cyclic cell stretching always relaxes cells 100 times, but not a simple accumulation or summation of stretching. In the situation where relaxing has more evident effect compared with straining, the consequence of cyclic straining originates from relaxing. Furthermore, severing of actin filaments by cofilin after cessation of straining[13] implies that relaxation can be brought about even after single stretching. Consistently, both cell-generated traction force and intercellular tension have recently been reported to decrease after a single bout of cell stretching when compared with pre-stretching[14]. Considering that physiological mechanical stresses are mostly cyclic or transient, straining and relaxing mechanical stresses listed in Figure-4 are likely to be mixture or combined *in vivo*, excluding substrate (or matrix) stiffness.

## Environments and situation of straining and relaxing mechanical stress

### 1. Situation where straining mechanical stress is borne

Actomyosin contraction provides the driving force for active movement and deformation that cells undergo during the process of organismal development and the tissue/organ morphogenesis[15]. Inhibition of Rho kinase, which leads to a decrease in the myosin contractile activity, as well as adhesion to soft substrate allow stem cells to remain undifferentiated and pluripotent[16) 17]. Furthermore, inhibition of nonmuscle myosin II eliminates substrate elasticity-directed lineage specification of mesenchymal stem cells[18]. These findings indicate that straining mechanical stress plays an important role in cell differentiation.

In addition, straining mechanical stress is critically involved in regeneration and wound healing, in which cell proliferation/differentiation and tissue formation are important as in the case with development[19].

### 2. Situation where relaxing mechanical stress makes sense

When a particular space of tissues becomes crowded with newly proliferated cells, they come to contact with each other, consequently leading to a situation of relaxing mechanical stress in terms of decreased cytoskeletal tension. When cells reach their destination, they stop migrating. In the process of wound healing, tentatively formed scar, which is typically hard, is replaced with normal tissues (much softer than scar) and then the homeostasis is established and maintained. These represent the transitions from non-steady states (growth, development, repair, and regeneration) to steady conditions, during which the relaxing mechanical stress becomes dominant over the straining mechanical stress.

Apparently, signals related to relaxing mechanical stress are opposite of those induced by straining mechanical stress. The examples of these relax-related signals are as follows.

- decreased MAP kinase (ERK, JNK, p38) activity in relaxed cells[5]
- decreased Src activity/tyrosine phosphorylation[16] and YAP/TAZ activity[9] in cells adhering to soft substrates

## Diseases caused by abnormal mechanical stress

### 1. Stretching or increased hydrostatic pressure

Hypertension (high blood pressure), which exerts a large magnitude of stretching or pressure on cardiac/arterial walls (endothelium and cardiac/smooth muscle), causes cardiac (muscle) hypertrophy, atherosclerosis, and aneurysm. Overweight (obesity) and joint instability (*e.g.* post ligamentous injury), which exert excessive pressure or stretching, cause osteoarthritis. Increased hydrostatic pressure on nervous tissues relates to severe neural disorder, such as brain damage by

intracranial hypertension, compressive myelopathy, and constrictive peripheral neuropathy.

**2. Increased cytoskeletal tension (increased cytoskeletal contractility)**

Increased cell tension caused by angiotensin, endothelin, oxidative stress, and thrombus formation (platelet contraction) leads to endothelial dysfunction such as increased vascular permeability, impaired antithrombotic function, and enhanced inflammatory response. Increased contractility of cardiac muscle or vascular smooth muscle cells is an obvious trigger of high blood pressure (hypertension).

**3. Adhesion to stiff substrate (stiffening of extracellular matrix)**

The terms 'sclerosis' and 'cirrhosis' refer to stiffening; therefore many diseases related to these words, including hippocampal sclerosis, amyotrophic lateral sclerosis, atherosclerosis, liver cirrhosis, glomerulosclerosis, multiple sclerosis, nodular sclerosis, primary sclerosing cholangitis, may involve straining mechanical stress. In addition, matrix elasticity has been shown to be an important factor for the invasiveness of cancer cells[20].

## Straining mechanical stresses play different roles in regulating organismal functions, depending on the time, phase, stage and situation – straining can induce or enhance inflammation and ageing

As discussed above, straining mechanical stress normally contributes to non-steady or 'constructive' phase, such as the development of organisms and the differentiation/formation/regeneration/repair of cells, tissues and organs. However, if straining mechanical stress is borne even after a steady state is reached, it often causes failure of organismal homeostasis, *i.e.* diseases. As is the case with such straining mechanical stress, inflammatory processes have beneficial effects in non-steady states (*e. g.* acute infection) whereas they often have harmful influences in steady states (*e. g.* chronic inflammation). Indeed, NF-κB, a transcription factor that plays a key role in regulating inflammatory responses is reportedly activated by the above-listed straining mechanical stresses (Figure-4A). NF-κB activation has also been reported to be involved in the above-mentioned straining mechanical stress-related diseases, *i. e.* 'sclerosis' and 'cirrhosis'.

## Anti-inflammatory effects of relaxing mechanical stress

Suppose that straining mechanical stress has pro-inflammatory effects as stated above, it is reasonable to postulate that relaxing mechanical stress may suppress inflammation. Although there are very limited experimental findings concerning relaxing mechanical stress, inhibition of myosin II[21) 22)] or Rho kinase[23)], both of which decrease actomyosin contractility, reportedly has anti-inflammatory effects. Furthermore, adhesion to softer substrates has been reported to down-regulate the NF-κB activity as well as the expression of pro-inflammatory cytokines[24)].

## Beneficial and detrimental mechanical stresses in light of health promotion and organismal homeostasis

In a steady state, such as post-maturation, straining mechanical stress is detrimental in terms of induction or enhancement of inflammation or ageing. To the contrary, application of relaxing mechanical stress, which counteracts straining stress, can be utilized to promote health and to treat diseases.

However, a situation of relaxing mechanical stress cannot be achieved simply by deleting externally applied straining mechanical stress. Loss of mechanical stresses on the musculoskeletal system caused by physical inactivity, joint immobilization, space flight gives rise to health problems, such as decreased bone mass (osteoporosis), joint contracture/osteoarthritis, and muscle atrophy. Because the musculoskeletal systems consist of relatively stiff tissues and organs (bone, cartilage, muscle, tendon), their 'default' situation/setting is detrimental (cell adhesion to stiff substrate). Therefore, 'no mechanical stress' does not produce relaxing condition in the musculoskeletal tissues, but just brings about environments of straining mechanical stress by compelling cells to adhere to stiff substrates. This may underlie the above-mentioned musculoskeletal diseases related to loss of

mechanical stress, most of which involve inflammation[25)26)], and account for the reason why physical activity, such as exercise, is needed to maintain the homeostasis of bones, joints, and muscles.

## How to apply relaxing mechanical stress on human bodies?

Given that relaxing mechanical stress is beneficial in terms of organismal homeostasis, how can we apply it?

Although it may sound paradoxical, straining mechanical stress can be utilized to introduce relaxing. Post-stretching relaxation of muscles and tendons has been demonstrated[27)]. This may not just result from loosening of the tissues caused by plastic deformation (elongation). Post-stretching or -straining relaxation has been shown at cellular and molecular levels[13)14)], suggesting that we can relax cells by applying straining mechanical stress in a regulated manner (mode, magnitude, location, duration, and frequency). Shear stress, which is a mixture of straining and relaxing, can also be utilized to relax cells. ROM (range of motion) exercise to prevent joint contracture or to treat osteoarthritis, which exerts shear on joint cartilage, is an example of therapeutic intervention using shear stress. Weight bearing on legs, which produces long bone deformation, induces flow of interstitial fluid in intraosseous canaliculi, leading to shear stress on osteocytes, the mechanosensory cells in bone[28)].

## Concluding remarks

To date, responses of cells and tissues to mechanical stress have been experimentally tested mainly by exerting straining stimulation. Therefore, the significance of relaxation has been poorly noted or recognized. The molecular mechanism behind the beneficial effects of optimal exercise, physical therapy, massage and Yoga, could be or should be deciphered by examining how relaxing mechanical stress hampers inflammation and ageing.

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# Development of a Small-Molecule AdipoR Agonist AdipoRon as Exercise Mimetics

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In obesity, plasma adiponectin is reduced, which causes insulin resistance and atherosclerosis. We identified its receptors AdipoR1 and R2, and showed that they exert antiaging effects via activation of AMPK/SIRT1 and PPAR-α pathways, respectively, leading to increased mitochondria as well as decreased ectopic fat accumulation, oxidative stress and inflammation. Recently, we identified orally active small-molecule AdipoR agonist AdipoRon, which bound to AdipoR, showed very similar effects to adiponectin in muscle and liver, such as increased exercise endurance and energy expenditure, and ameliorated insulin resistance via AdipoR in obese mice, leading to healthy longevity. Most recently, we determined and reported the crystal structures of human AdipoR, which will facilitate the understanding of novel structure-function relationships and the optimization of AdipoRon as exercise mimetics.

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# Lipid Droplet Formation and Autophagy

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***Objective***: Lipid droplets (LDs) are cytoplasmic inclusions that have a neutral lipid core with a single phospholipid layer, while excess free fatty acids and glucose in plasma are converted to triacylglycerol (TAG) and stored as LDs. However, the mechanism for the generation and growth of LDs in cells is largely unknown. This review will explain the relationship between LD formation and autophagy.

***Results***: LD formation accompanied by accumulation of TAG induced by starvation was largely suppressed in the hepatocytes and cardiac myocytes that cannot execute autophagy. Under starvation conditions, LDs in addition to autophagosomes were abundantly formed in the cytoplasm of these tissue cells, although autophagosomes did not appear in wild-type and autophagy-unable cardiac myocytes. Moreover, LC3 was localized on the surface of LDs and LC3-II (lipidation form) was fractionated to a perilipin-positive lipid fraction from the starved liver.

***Conclusion***: Taken together, these results indicate that the LC3 conjugation system is critically involved in lipid metabolism via LD formation.

***Key words***: lipid droplet, autophagy, LC3, Atg7

## Introduction

It is well known that there are two major pathways for proteolysis in eukaryotic cells: the proteasome and lysosome. Substrates transferred to lysosomes may be taken into heterophagocytosis, endocytosis, or autophagy into heterophagosomes, early endosomes, or autophagosomes. These phagosomes receive lysosomal enzymes via transporting vesicles from the trans-Golgi network or lysosomes and become heterophagolysosomes, late endosomes, or autolysosomes[1)-6)]. Old and unneeded intracellular components containing membranous organelles and part of the cytoplasm are substrates of autophagosomes. Autophagy is now believed to play an essential role in the maintenance of cellular metabolism. In fact, impairment of autophagy causes severe degenerative alterations in various tissue cells[4) 7)-10)].

Neutral lipids, mainly triacylglycerol (TAG) and cholesterol ester (CE), are stored in lipid droplets or lipid bodies[11) 12)]. Excess fatty acids are converted to TAG through lipogenesis under nutrient-rich conditions and stored in lipid droplets, while lipid droplets are degraded to lipids by lypolysis under fasting conditions. Proteins are degraded to produce an amino acid pool that is used in part for energy metabolism through glyconeogenesis under starvation conditions, while free fatty acids (FFAs) from adipocytes are delivered to hepatocytes and cardiac myocytes where they are used as an energy source via β-oxidation[12) 13)].

The microtubule-associated protein 1A/1B light chain 3 (LC3) is analogous to Atg8 in yeast and conjugated with phosphatidylethanolamine (PE). LC3 is synthesized as a precursor form, is processed to expose a glycine residue at its C-terminus and becomes cytosolic LC3-I[14)]. In response to starvation, the cytosolic form of LC3 is converted to the membrane-bound form by covalent conjugation to an amino group of PE through an enzymatic cascade consisting of Atg7 (E1) and Atg3 (E2)[5)].

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We have shown that loss of Atg7 largely suppresses LD formations in hepatocytes and cardiac myocytes 24 hours after the start of starvation, although numerous LDs accumulate in hepatocytes and cardiac myocytes of wild-type mice under the same conditions[15]. Moreover, Atg7-deficient mice specifically in adipose tissue are slim and contained only 20% of the mass of white adipose tissue (WAT) found in wild-type mice[16]. It is interesting that these mutant mice are highly sensitive to insulin that reduces low fed plasma concentrations of FFAs, while they exhibit markedly decreased plasma concentrations of leptin but not adiponectin, and lower plasma concentrations of TAG. LDs, initially considered inert lipid deposits, have gained during the last decade the classification of cytosolic organelles due to their defined composition and the multiplicity of specific cellular functions in which they are involved[17].

Since ubiquitin aggregates have been shown to accumulate in cells and neurons deficient in Atg7[18) 19)], selective autophagy plays an important role in the quality control of cellular components through p62 and NBR1 that have specific domains to bind LC3 and ubiquitin and work as adapter proteins[20) 21)]. Recent studies show that selective autophagy is required to eliminate damaged cellular components including proteins and cytoplasmic organelles such as mitophagy for mitochondria, pexophagy for peroxisomes, lipophagy for lipid droplets, ribophagy for ribosomes, zymophagy for zymogen granules, reticulophagy for endoplasmic reticulum, glycophagy for glycogen granules, and aggrephagy for protein aggregates[22]. At present, it is largely elusive whether autophagy is involved in LD metabolism, although lipophagy may happen to occur in cells.

### Loss of Atg7 suppresses LD formation

It is well know that morphofunctional aspects of hepatocytes differ depending on the location within the liver acinus; Periportal hepatocytes are glycogenic and lypolitic, while perivenous hepatocytes are glycolytic and lipogenic[23) 24)]. Although quantities of cytoplasmic organelles in hepatocytes significantly vary within 24 hours, lysosomes and lipid droplets are more abundant in perivenous hepatocytes than in periportal hepatocytes. Such organization of cytoplasmic organelles is drastically altered in hepatocytes, when animals are starved for 24 hours[25) 26)]; numerous autophagosomes that contain part of the cytoplasm and possess the cisternal or double isolation membranes and autolysosomes appear near bile canaliculi in hepatocytes 24 hours after the start of starvation and those that in some cases contain mitochondria are detected when starvation is extended to 48 hours. In addition to autophagosomes and autolysosomes, LDs accumulate abundantly in the cytoplasm of hepatocytes located in both periportal and perivenous regions of liver acini. By using Atg7-deficient mice specifically in the liver ($Atg7^{flox/flox}$: Albumin-Cre mice), LD formation is confirmed to be normal in hepatocytes at 22 days, or 6 or 8 weeks of age. Positive staining for LDs is sparsely detected in Atg7-deficient hepatocytes under starvation conditions for 12 or 24 hours, although they are abundant in the cytoplasm of the hepatocytes of the control littermate mouse liver. LDs stained by BODIPY were smaller in size and much less in number in hepatocytes deficient in Atg7 than those in control. By electron microscopy, the size of LDs in the control hepatocytes was $1.12 \pm 0.17$ μm (the mean diameter (± SD)) and range to 2.61 μm (diameter). In mutant hepatocytes, however, smaller LDs are mainly detected; the size of smaller LDs ranges to 0.83 μm and the average diameter is $0.19 \pm 0.17$ μm.

Importantly, the total TAG amount in Atg7-deficient liver and control livers is half of that in the control liver. These data indicate that the conjugation system of LC3 by Atg7 is required for the formation of LDs[25].

### LC3 anchors the surface membrane of LDs

Autophagy has been shown to play an important role in normal adipogenesis; inhibition of autophagy by disrupting the *atg7* gene has a unique anti-obesity and insulin sensitization effect[16]. LDs are ubiquitous in eukaryotic cells, while excess free fatty acids and glucose in plasma are converted to TAG and stored as LDs. However, the mechanism for the generation and growth of LDs in cells remains largely elusive. As stated above, Atg7 that mediates the LC3 lipidation and is essential for autophagy is involved in LD formation.

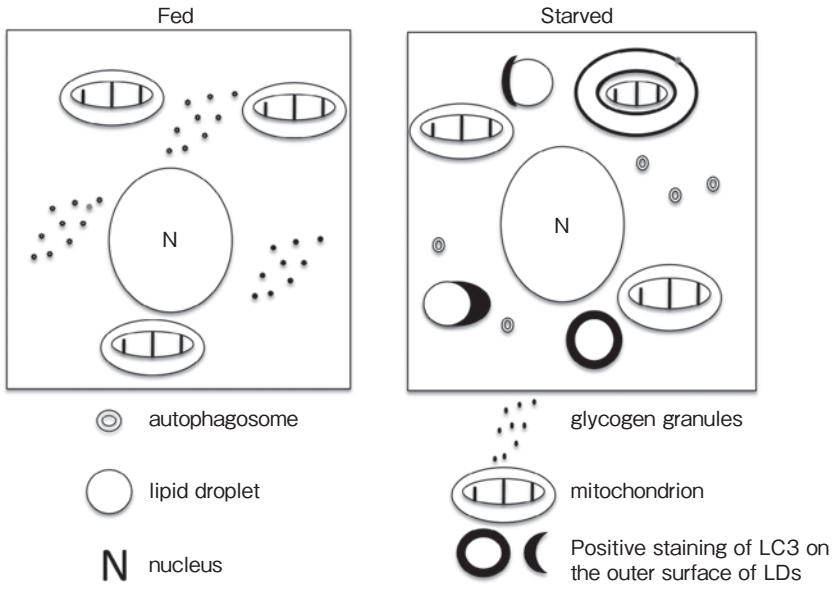


**Figure-1** Localization of LC3 in cells from mice under fed (left panel) and starved (right panel) conditions
When mice are fed, cells start to produce and store glycogen granules, whereas under starved conditions, lipid droplets increase and many autophagic granules with double membranes increase in the cytoplasm. In this situation, the lipidated form of LC3 is attached to the isolation membrane of autophagosomes and the surface membrane of LDs. Starvation is further continued, and mitochondria are enwrapped by the isolation membrane (mitophagy).

LC3 is known to be localized on the surface of the isolation membrane when starvation is induced (Figure-1). Using transgenic (TG) mice of GFP-LC3, GFP-LC3 becomes dot-shaped, cap-shaped and ring-shaped in hepatocytes and cardiac myocytes under starvation conditions[27]. Like GFP-LC3 TG-mice, positive staining for LC3 appears dot-shaped, cap-shaped, and ring-shaped in hepatocytes and cardiac myocytes under starvation conditions[25] (Figure-1). Interestingly, positive staining of LC3 in cardiac myocytes is longitudinally arrayed in parallel to myofilaments. In both hepatocytes and cardiac myocytes, LDs are abundant 24 hours after the onset of starvation. In particular, electron microscopic observations show that no clear-cut autophagosomes are detected in cardiac myocytes, although many large LDs are arranged longitudinally in parallel to the array of myofilaments together with mitochondria. This arrangement of LDs in the cardiac myocytes is very similar to that of LC3-positive granules. To examine the relationship between staining patterns of LC3 and LDs in both hepatocytes and cardiac myocytes, double staining for perilipin with LC3 is performed. The results indicate that perilipin-positive LDs are also immunopositive for LC3 on the surface of LDs in both hepatocytes and cardiac myocytes. In hepatocytes, dotted staining of LC3 is free of staining of LD, but most LD-positive staining is colocalized with LC3 in cardiac myocytes (Figure-1).

In fact, cytosolic LC3 is converted to membrane-bound LC3 (LC3-II) in both hepatocytes and cardiac myocytes 24 hour after the start of starvation. By electron microscopic morphometry, the volume densities of autophagosomes/autolysosomes increase from 0.11% to 0.58 ± 0.10 (mean ± SD, %) at 24 hours and 0.61 ± 0.19 at 48 hours after the onset of starvation, while those of LDs increases from 0.2 ± 0.44 to 18.67 ± 2.44 at 24 hours and 10.89 ± 3.11 at 48 hours. Autophagosomes and autolysosomes, however, are rarely found in cardiac myocytes. The volume densities of LDs are only counted in cardiac myocytes and significantly increase from 0.85 ± 0.19 (mean ± SD, %) to 3.38 ± 0.58 at 24 hours and 2.31 ± 0.35 at 48 hours after the start of starvation. The amounts of TAG in hepatocytes and cardiac myocytes significantly increase after the onset of starvation, whereas the increase in TAG amount is much lower in cardiac myocytes than in hepatocytes and continues until

24 hours. Moreover, LC3 is localized on the surface membrane of LDs and the lipidated form of LC3 is fractionated to a perilipin and ADRP (LD marker)-positive lipid fraction from the starved liver and cardiac myocytes, respectively. In fact, such LDs obtained from the LD fraction are labeled by gold particles showing the antigenicity of LC3[25]. Taken together, these results indicate that the LC3 conjugation system is critically involved in lipid metabolism via LD formation.

## Conclusions

It has been proposed that lipophagy and lypolysis under acidic conditions contribute the breakdown of TAG into FFAs that are used for mitochondrial β-oxidation[28]. Electron microscopic observations, however, show that it would be hard to see the presence of LDs that are enwrapped by double membranes even under starvation conditions. Different from lipophagy, mitophagy can easily be found in hepatocytes if the mice are starved for 48 hours. Hormone-sensitive lipase (HSL) plays a key role in the metabolism of neutral lipids and FFA products, to which other lipases take part in, are used for energy metabolism[29]. In this meaning, it may be important to understand phenotypes of targeted animals of lipases and phospholipases in lysosomes.

According to a model of the trafficking of cytosolic LDs and secreted lipoproteins in mammalian cells including hepatocytes, there is cytosolic LD cycling that may have a constitutive function in cells whereby imported or de novo synthesized fatty acids are buffered in a cytosolic pool of TAG[30]. During this cycling, cytosolic LDs are closely associated with the membrane of ER and some are re-fused with the ER membrane from which ADRP-bound LDs are released into the cytosol. Our data simply show that 1) loss of Atg7 inhibits LD formations in hepatocytes and cardiac myocytes under starvation conditions, and 2) LC3 is localized on the surface of LDs and fractionated into the fraction of ADRP and perilipin. It may be that released LDs repeat fusion and increase in size, but this fusion is a regulated cell-specific process. Although the SNARE system has been reported to be in part involved in this LD fusion, the regulatory mechanism of LD formation remains largely unknown. The surface of LDs is either a hemi-membrane or a phospholipids monolayer containing cholesterol, while oligomelized Atg8 is suggested to be involved in hemifusion of liposomes[31]. When considering that LC3 is localized on the surface of LDs under starvation conditions, oligomerization of LC3 may contribute to the fusion and growth of LDs.

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# Human Brain Mapping of Autonomic Functions

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The hypothalamus contains several sub-nuclei that regulate autonomic functions such as hunger and thirst. The hypothalamus is a small structure of less than 1 cubic centimeter in each hemisphere of the human brain, and very little is known about precise anatomical organization and functional properties of the hypothalamus in the human brain. In this study, in collaboration with departments of endocrinology and radiology, high-resolution fMRI was conducted, while blood glucose level was monitored (Figure-1). Cubic voxels of 1.25 mm were employed, rather than standard high-resolution fMRI of 2 mm cubic voxels, which allowed us to divide the human hypothalamus into several sub-regions using a boundary mapping method based on the resting-state functional connectivity. The defined sub-regions were used to track fMRI signal time-courses when the blood glucose level was manipulated by OGTT. Our preliminary results showed prominent signal changes after OGTT in specific sub-regions in the hypothalamus, and promise future studies of mapping autonomic functions in the human hypothalamus.

**Figure-1** Experiment scene
A subject in a supine position was administered an fMRI scan, while blood glucose level was monitored.

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# Physical Exercise and Dementia

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Approximately 30% of Japanese citizens will be aged 65 or older in 2020, and this ratio will be the highest worldwide. An estimated 4.6 million individuals in Japan and 44 million individuals globally have been diagnosed with dementia. Exercise has been identified as a potential means of preventing or delaying cognitive decline. Several epidemiological studies on older adults demonstrated that gait speed was associated with cognitive function. Randomized control studies on physical exercise interventions also indicated that aerobic exercise is beneficial for cognitive function. In order to determine the effects of short-term treadmill exercise on tau protein modifications, we employed a 3-week treadmill treatment using tau model mice. The results obtained showed increases in oxidative stress, microglial cell proliferation, and C-terminal tau phosphorylation. Previous studies reported that long-term treadmill treatments were beneficial. Therefore, positive compensatory changes may occur with the continuation of treadmill training in mice. The molecular mechanisms underlying the relationship between physical exercise and cognitive function still remain unknown; however, their elucidation may lead to the identification of a novel molecule, which may ultimately result in a cure for dementia.

***Key words***: exercise, walking, dementia, tau

## Introduction

The proportion of elderly citizens in Japan is now the highest worldwide. The aging of Japan has outweighed that in all other nations for a few decades. Approximately 30% of the population will be aged 65 or older in 2020. This proportion will gradually increase and reach 35% in 2040. As a consequence, the percentage of patients with dementia in Japan is also expected to gradually increase. The Hisayama study provided estimates for the percentage of the Japanese population with dementia. Approximately 4.6 million individuals in Japan and 44 million individuals globally were reported to have dementia in 2015. This number will continue to increase in Japan, and will eventually reach 10 million in 2060. Risk factors for dementia need to be identified in an attempt to prevent its development. Several risk factors have been reported for Alzheimer's disease (AD): aging, female gender, the ApoE ε4 gene, a history of brain contusions, cerebrovascular diseases, and depression. Middle-aged individuals with diabetes mellitus (DM), hyperlipidemia, and hypertension are more likely to develop AD in later life. L low physical activity has also been identified as an important risk factor by extensive epidemiological research.

## Physical exercise to prevent dementia

In the care of older adults, the word "frailty" is a term that is often used to describe the general health status. Frailty has recently been associated with an increased risk of poor cognitive outcomes such as AD or mild cognitive impairment (MCI). Frailty is generally characterized by weight loss, fatigue, low physical activity, slowness, and weakness (Table-1). A prospective population-based

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**Table-1** Frailty

| |
|---|
| 1. Weight loss |
| 2. Fatigue |
| 3. Low physical activity |
| 4. Slowness (walking speed) |
| 5. Weakness (grip strength) |

(Gray SL, *et al*: J Gerontol A Biol Sci Med Sci, 2013; 68: 1083-1090[1])

cohort in Seattle reported a relationship between frailty and the incidence of dementia[1]. Participants consisted of approximately 2,600 elderly individuals with normal cognitive function who were followed between 1994 and 2010. Approximately 500 participants developed dementia (448 of which developed AD) over a mean follow-up of 6.5 years. The hazard ratio for frailty is 1.20 for dementia. The hazard ratio for walking speed is the highest among frailty components. The findings of a pooled analysis of 9 cohort studies collected between 1986 and 2000 also revealed that individuals with a high walking speed achieved longer survival[2]. Several large-scale prospective cohort studies have reported a relationship between cognitive function and walking speed (Table-2)[3)-8)]. In older Mexican-American adults without cognitive impairments at baseline, a slow 8-foot walk time was identified as an independent predictor of a Mini-Mental State Examination (MMSE) score decline over a 7-year period although other sociodemographic factors including education, marital status, body mass index, and medical conditions (stroke, heart attack, DM, depression, and hypertension)[3]. In North America, the usual gait speed (m/s) over 6 m was measured at baseline in 2,776 participants[4]. After 5 years, 389 (17.1%) participants exhibited declines in the Digit Symbol Substitution Test (DSST). Participants in the lowest quartile of gait speed were more likely to show declines in DSST than those in the highest quartile (>1.35 m/s). The Sydney Older Persons Study examined the 6-year outcomes of 630 community-dwelling participants aged 75 and older[6]. At baseline, participants were defined as having dementia, being cognitively intact, or having a syndrome possibly representing the preclinical phase of AD, vascular dementia, extrapyramidal dementia, or various combinations of the three. Participants with cognitive impairments in combination with gait and motor slowing were the most likely to develop dementia over the 6-year period (OR 5.6; 95% CI 2.5-12.6).

Walking training has been described as a preventive measure against cognitive decline in the elderly. A previous study reported that walking may increase the size of the hippocampus[9]. In this analysis, 120 older adults were divided into two groups: a walking group and muscle training or stretch group. In the walking group, participants had to walk for 40 minutes 3 times a week. Data were collected after one year. Hippocampal volumes and cognitive test scores were higher in the walking group. Furthermore, blood brain-derived neurotrophic factor (BDNF) levels increased in the walking group. Several studies examined different types of physical training in patients with mild cognitive impairments (Table-3)[10)-15)]. In Western Australia, a randomized controlled trial recruited 311 individuals aged 50 years or older for a 24-week physical activity intervention[11]. Participants were randomly allocated to a home-based physical activity group or education and usual care group. The Alzheimer Disease Assessment Scale Cognitive Subscale (ADAS-Cog) increased by 0.26 points in the intervention group (95% confidence interval, -0.89 to 0.54), but decreased by 1.04 points in the usual care group (95% confidence interval, 0.32 to 1.82). Thirty-seven adults (17 women) with amnestic MCI were randomized either to a high-intensity aerobic exercise or stretching control group[12]. The aerobic group exercised under the supervision of a fitness trainer at 75% to 85% of the heart rate reserve for 45 to 60 min/d, 4d/wk for 6 months. The control group maintained their heart rate at or less than 50% of their heart rate reserve. In women, aerobic exercise improved performance in multiple tests of executive function, increased glucose disposal, and reduced fasting plasma levels of insulin, cortisol, and BDNF. In men, aerobic exercise increased plasma levels of insulin-like growth factor I and had a favorable effect on Trail making test B only. A recent review published by the Cochrane Collaboration concluded that there is promising evidence to show that exercise programs may improve the ability to perform the activities of daily living by individuals with dementia; however, some caution is advised when interpreting these findings[16].

**Table-2** Cohort studies: walking speed and Dementia

| Authors | Subjects | Follow-up | Walking assessments | Results |
|---|---|---|---|---|
| Alfaro-Acha A, *et al*[3] | n=2,070<br>65 years and above<br>MMSE>21 | 7 ys | 8-foot walk | Risk factor for cognitive decline |
| Inzitari M, *et al*[4] | n=2,276<br>73.5 years and above | 5 ys | 6 m walking | OR 1.74<br>Cognitive decline |
| Wang L, *et al*[5] | n=2,288<br>65 years and above<br>MMSE>25 | 6 ys | 10-foot walk | Development of dementia |
| Waite LM, *et al*[6] | n=630<br>75 years and above | 6 ys | 5 m walking | Slow walking speed MCI→AD |
| Atkinson HH, *et al*[7] | n=558<br>65 years and above<br>MMSE>24 | 3 ys | 4 m walking | Cognitive decline and physical ability |
| Marquis S, *et al*[8] | n=108<br>65 years and above<br>MMSE>24 | 6 ys | 30 foots | Prediction of time to onset of persistent cognitive impairment |

**Table-3** Physical activity intervention for MCI

| Source | No. | Patients | Mean age | Exercise | Duration | Outcomes |
|---|---|---|---|---|---|---|
| Lautenshlager NT, *et al*[11] | 170 | MCI & SCI | 69 | Home-based physical activity or an education and usual care | 24 W | Improvement of the ADAS-cog., verbal memory, and CDR |
| Baker LD, *et al*[12] | 33 | MCI | 70 | High-intensity aerobic exercise and stretch | 6 M | Difference of executive function |
| Suzuki T, *et al*[13] | 50 | MCI | 75 | Multicomponent exercise and education | 12 M | Superior improvement of MMSE, logical memory, and verbal fluency |
| Nagamatsu LS, *et al*[14] | 86 | Women with SCI | 75 | Resistance training<br>Aerobic training<br>Balance training | 26 W | Significant difference of executive function |
| Barnes DE, *et al*[15] | 126 | SCI | 73 | A2 × A2 factorial design 4 group | 12 W | Global cognitive score improved |

### Exercise treatments in mice

We previously generated tau transgenic mice, designated as Tg601, that overexpressed wild-type human tau under the calcium/calmodulin-dependent protein kinase IIα (CAMK-IIα) promoter[17]. Tg601 mice expressed the longest form of human tau predominantly in the forebrain. Numerous phosphorylated tau-positive neurons were mainly detected in the cortex, hippocampus, and limbic system. Anxiety behavior decreased in Tg601 mice by the age of 16 months. In spite of the absence of neurofibrillary tangles, a [$^{18}$F] fluoro-2-deoxy-D-glucose PET study revealed that glucose uptake was decreased in young Tg601 mice in the septum only, and this area extended into the hippocampus with old age.

In order to characterize the effects of exercise on the biochemical properties of tau protein, we subjected 17-month-old Tg601 mice to treadmill exercise for 3 weeks (short-term exercise: STE)[18]. Treadmill exercise is regarded as a forced exercise because mice receive an electric shock when they do not run. Mice were sacrificed by cervical dislocation after 3 weeks of exercise training. In order to determine the effects of STE on tau solubility, we prepared different detergent-soluble and -insoluble fractions from brain homogenates, and then performed a western blot analysis using various phosphorylation-dependent and site-specific anti-tau antibodies. The findings obtained showed that the amount of C-terminally phosphorylated tau and insolubility increased after STE. We performed an immunohistochemical analysis using an Iba-1 antibody in order to determine whether STE induced any neuroinflammatory responses in Tg601 mice. Iba-1-positive microglia proliferated after exercise (Figure-1A-D). We

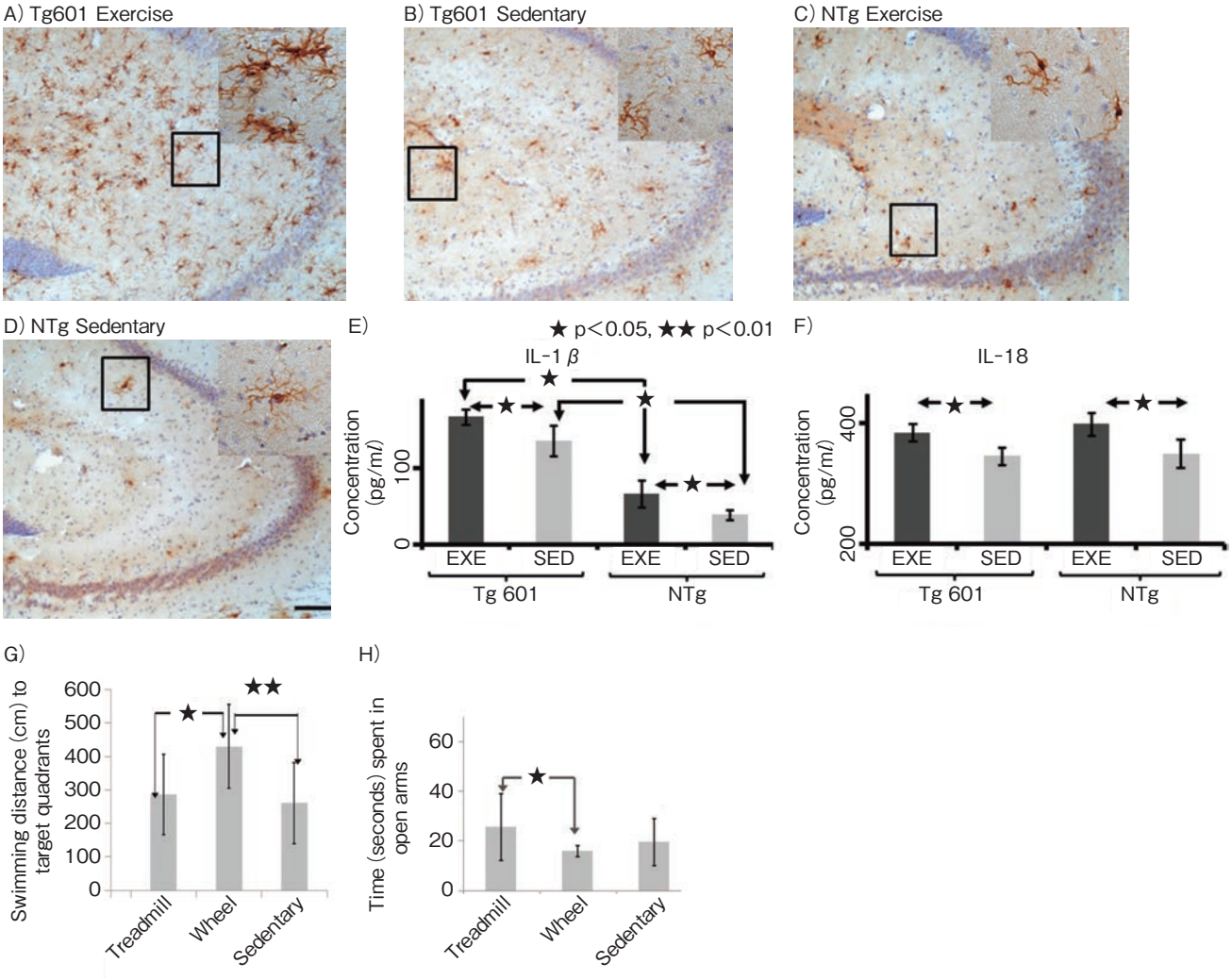


**Figure-1** Inflammation and exercise

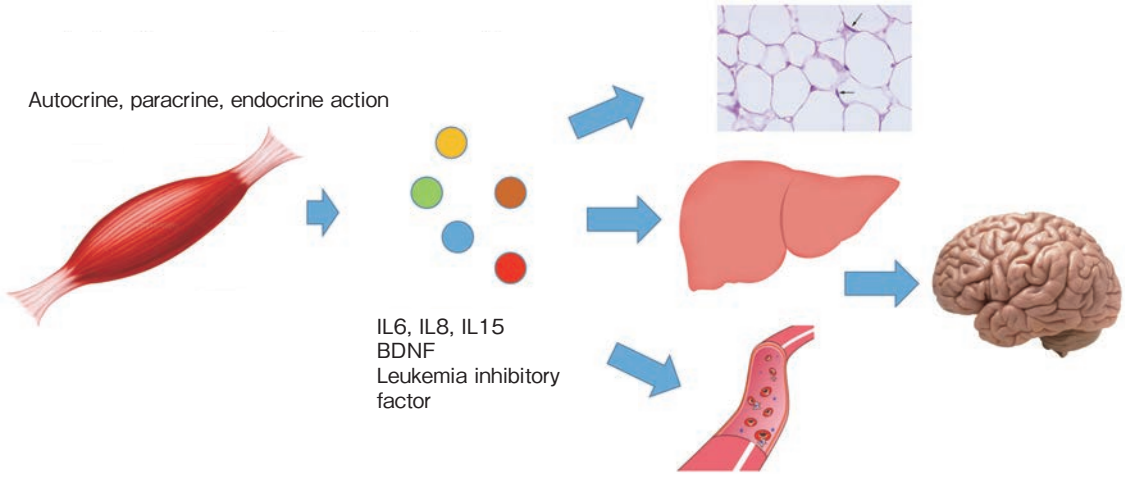


**Figure-2** Skeletal muscle: an endocrine organ
(Pratesi A, *et al*: Clin Cases Miner Bone Metab, 2013; 10: 11-14[24])

measured the levels of inflammatory cytokines including IL-1β, IL-6, IL-18, and TNF-α and those of the chemokines CXCL-1 and CXCL-12 (Figure-1E and F). This analysis also revealed that the levels of the cytokines, IL-1β and IL-18, and chemokines, CXCL-1 and CXCL-12 were higher in exercised groups. Elevations in the levels of the lipid peroxidation markers, 4-hydroxy-*trans*-2-noneal and malondialdehyde indicated the presence of oxidative stress. The beneficial effects of treadmill exercise have always been observed in the case of long-term (> 12 weeks) physical training[19)-23)]; however, our study showed that STE induced completely negative effects. Therefore, we conclude that beneficial compensatory changes may occur with the continuation of treadmill training.

We then subjected 16-month-old normal mice to long-term (5 months) exercise. Mice (n = 20) were divided into 3 groups: voluntary running wheel, forced treadmill, and sedentary groups. In contrast to the treadmill treatment, mice in the voluntary running wheel group ran at their own pace. They played for a while and rested. They played again and then stopped. After the exercise intervention had been completed, behavioral batteries were performed. The wheel group achieved the highest score in the Morris water maze test, which assesses learning ability. The treadmill and sedentary groups had the same scores. The elevated plus maze test, which evaluates anxiety behavior, revealed that mice in the wheel group spent less time in the open arms than those in the wheel group ($p < 0.05$). The Y-maze test, which examines working memory, indicated that the wheel group had a higher number of spontaneous alterations than the treadmill group ($p < 0.05$). These findings indicated that mice in the wheel group showed the best cognitive ability among the three groups. After completing the behavioral tests, we sacrificed animals and removed the brains and soleus skeletal muscles. We are now analyzing these samples using biochemical and histochemical techniques. The role of skeletal muscle as a secretary organ was recently shown to be involved in inflammatory processes (Figure-2)[24)]. Contracting skeletal muscle release myokines, including IL-6, IL-8, IL-15, BDNF, and leukemia inhibitory factor, which function in a hormone-like manner, exerting specific endocrine effects on visceral fat or inhibiting glycogen synthase in the liver. BDNF plays a crucial role in regulating the survival, growth, and maintenance of neurons in the brain. Therefore, we hypothesized that another unknown molecule released by muscle may affect brain function during exercise treatments. We are now analyzing muscle and brain extracts using a protein microarray technique.

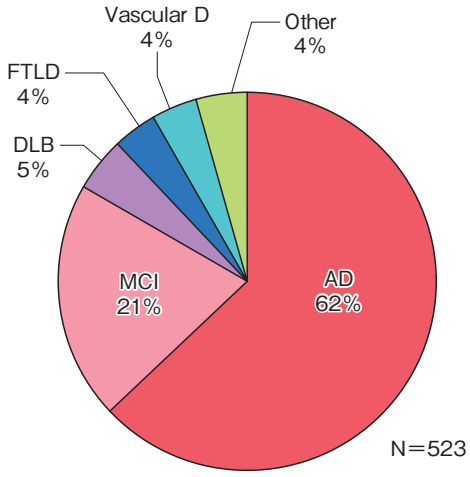


**Figure-3** Patients in my memory clinic

## Patients in our memory clinic

Approximately 500 patients, 60 % of whom have AD and 20 % MCI, regularly visit the memory clinic in our hospital (Figure-3). A 72-year-old man with mild AD visits our memory clinic every 2 months with his wife. He has walked almost every morning for one hour by himself since he retired from his job at the age of 65. His deterioration speed is slower than that of other patients with AD. We speculate that his walking custom may be slowing the deterioration of AD. Therefore, our group has created an original diary, called the "everyday note", for patients to note the number of steps they take every day. We encourage them to walk at least 5,000 steps every day.

## Conclusions

Animal research and studies on the elderly or patients with cognitive decline have indicated that physical exercise is a promising intervention for preventing the development of dementia. Since the underlying mechanisms have not yet been elucidated in detail, neuroscientists are now focusing on

this issue. During the process of examining the mechanisms responsible, scientists may discover novel molecules that will ultimately result in a cure for AD.

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# MR Imaging for Sportology; Non-Invasive Visualization of the Brain and Muscles

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Recent advances of MR imaging allow us to visualize the functional and structural changes of the brain not only in the patients with neurological disorders, but also in normal subjects. Sophisticated MR techniques, such as resting-state functional MR and diffusion MR imaging, can depict subtle brain changes in normal subjects before metabolic syndrome as well as brain changes after a few weeks muscle training. Minimal structural changes in the brain after repeated minor head trauma during sports are now highlighted, because advanced MR techniques can be used to show substantial changes in the brain before neurological decline.

By diffusion tensor imaging (DTI), we can explore microstructure of the tissue in vivo through analysis of water diffusion direction and restriction. DTI was developed mainly in the central nervous system to visualize the white matter tracts and their networks. Plasticity of the brain white matter has been reconfirmed with this technique. Skeletal muscles also have the direction and restriction of water diffusion and can be analyzed by DTI as well.

Recent advances of MR techniques for sportology, especially diffusion MR imaging, will be presented in this paper.

***Key words***: sportology, diffusion tensor imaging, MR imaging

## Introduction

Recent advances of MR imaging (MRI) allow us to evaluate subtle changes of the human brain *in vivo*. Functional MRI can demonstrate local activity changes related to the "task" in the brain. Tasks can be auditory, visual, motor or other complicated procedures. Changes or differences of regional brain volume can be evaluated by the voxel-based morphometry using three-dimensional T1-weighted images of MRI. MR spectroscopy represents a spectrum of several brain metabolites semi-quantitatively. Diffusion tensor imaging (DTI) or diffusion MR analysis can uniquely evaluate the white matter tracts through precise analysis of water diffusion. There are several methods to analyze brain using DTI such as tract-specific analysis (TSA), tract-based special statistics (TBSS), and connectome. In this paper, we would like to present the usefulness of MR imaging for sportology, especially about DTI.

## Quick review of diffusion tensor imaging of the brain

The white matter could not explored precisely, because it was difficult to track the fibers using specimen. DTI is a unique technique to visualize the white matter tract. DTI has been proposed by Basser in 1994[1] and developed rapidly in these years. DTI is a model that used to analyze the diffusion of water molecule *in vivo*, assuming the form of diffusion in one voxel as an ellipsoid (Figure-1). In the brain, diffusion of water molecules is anisotropic due to white matter fibers. Diffusion parallel to the white matter fibers is larger

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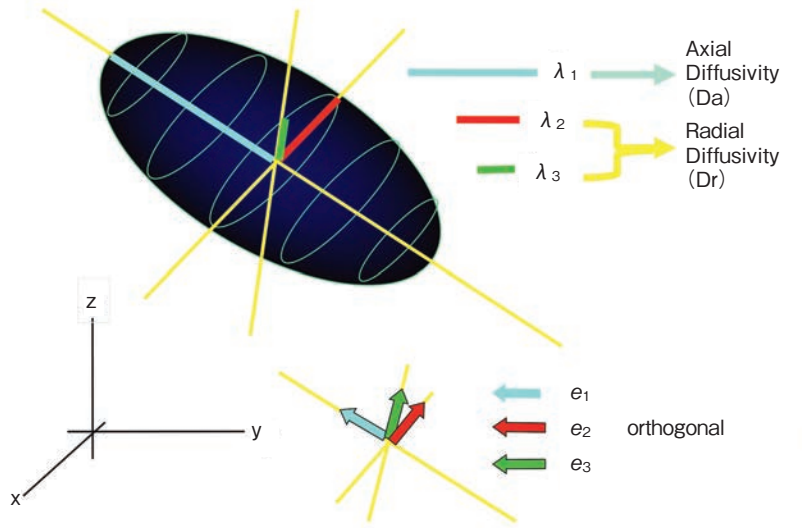


**Figure-1** Diffusion tensor ellipsoid and Lambda 1, 2 and 3

than the one perpendicular to the fibers. The component of diffusion parallel to the white matter is Lambda 1 ($\lambda_1$) or axial diffusivity (Da). The component of diffusion perpendicular to the white matter is Lambda 2 ($\lambda_2$) and 3 ($\lambda_3$) (Lambda 2 and 3 are perpendicular to each other). Average of Lambda 1 and 2 is called radial diffusivity (Dr). The standardized standard deviation of Lambda 1, 2, and 3 is Fractional Anisotropy (FA). When the white matter fibers are densely packed, water molecules move mainly along (parallel to) the fibers, this is due to the myelin and other membranes. The FA value becomes high when the Lambda 1 is large, but Lambda 2 and 3 are small. Decreased FA of the white matter fibers indicates deterioration or degeneration of white matter fibers.

By tracking or chasing the Lambda 1 direction three dimensionally, we can visualize the white matter tracts[2) 3)] (Figure-2, 3). The tracking algorism so called FACT (Fiber Assignment by Continuous Tracking) has been proposed by Mori[2)]. Dense and thick bundles such as the corticospinal tracts, the cingulate, uncinate and fornix are clearly visualized (Figure-3). The tracts without crossing fibers are clearly visualized by DTI. Region of interest (ROI) analysis[4)] and TSA[5) 6)] are developed to quantify the tract integrity measuring parameters within the tract.

Whole brain statistical analysis can be implicated in DTI data. The statistical parametric map (SPM) is widely used for functional MRI analysis and can also be used for DTI analysis. TBSS (tract based special statistics)[7)] is a newly developed technique to analyze DTI and other diffusion MR imaging. Using whole brain statistical analysis, we can explore regional brain abnormalities without the hypothesis of locations (Figure-4).

Non-Gaussian diffusion analysis is now highlighted to analyze precise microstructures using multi-shell (v-value) multi-MPG (>30) MR acquisition[8)].

## Plasticity of brain by training

Changes of the brain after training or exercise that seen by brain MRI had been reported in several conditions. Aerobic exercise training increases brain volume in aging humans[9)]. Volume changes of both gray and white matter, especially on the frontal lobe was observed. Changes of volume in one year had been reported (VOSS HBM). Significant increase of Fractional Anisotropy (FA) of DTI also had been observed.

Plasticity of the brain has been presented by using DTI after training of three-ball juggling[10)]. After 6 week hard training of juggling, increase of whole brain FA, especially at the right parietal lobe, probably related to special recognition, was observed. In addition, after 4 week rest, this increased FA was decreased.

Early plasticity of the brain after learning can be evaluated as well[11)]. Changes of DTI parameters of

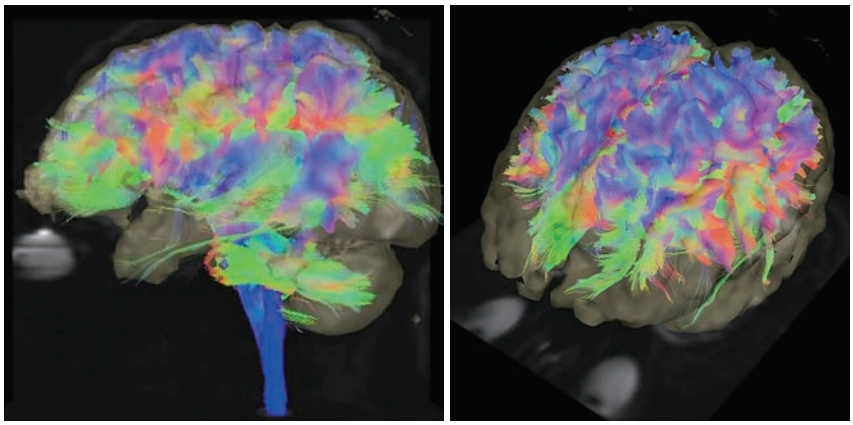

**Figure-2** Diffusion tensor tractography of the whole brain

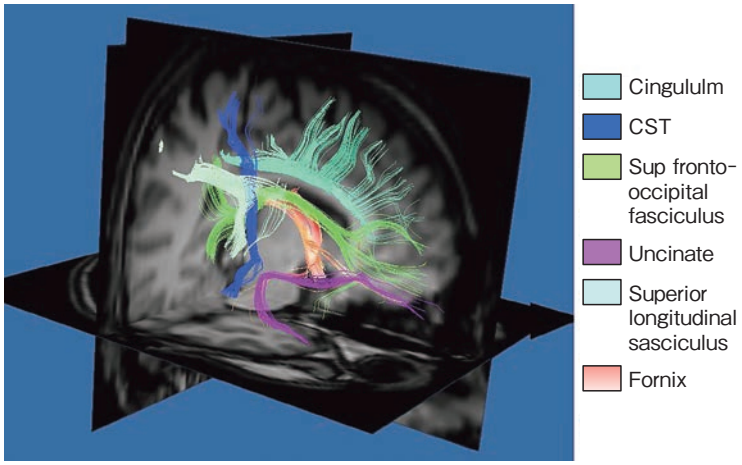


**Figure-3** The limbic system and corticospinal tracts are the tracts that can be visualized consistently by diffusion tensor tractography

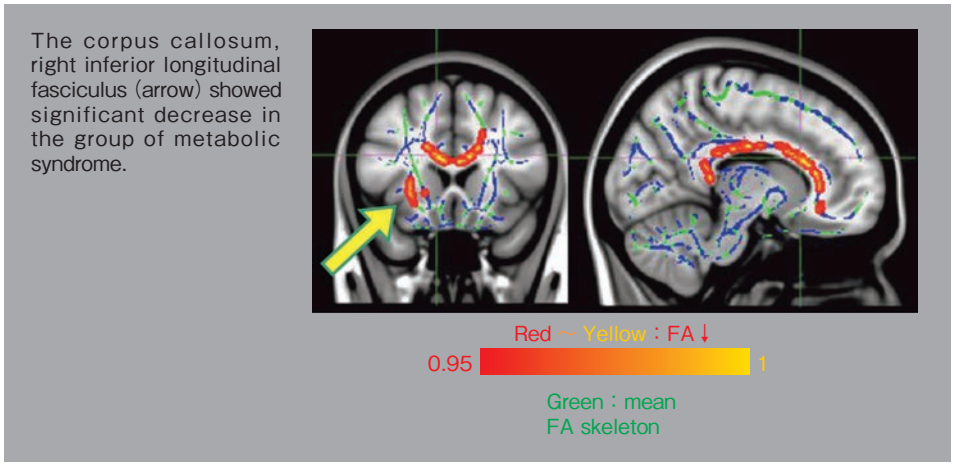


**Figure-4** Brain changes in matabolic syndrome: TBSS analysis. The corpus callosum and external capsule showed significant deterioration. The external capsule was actually the inferior longitudinal fasciculus.
(Shimoji K, *et al*: Diabetes Care, 2013; 36: 696-700 [13])

limbic system (hippocampus and para-hippocampus) were observed after spatial learning and memory task.

### Diffusion tensor imaging of metabolic syndrome

Changes of the brain in individuals with metabolic syndrome have been explored by TBSS[12) 13)] (Figure-4). TBSS revealed a significant decrease of FA in the corpus callosum and the right longitudinal fasciculus. TSA of the right longitudinal fasciculus showed significant correlation between FA of the tract and body-mass index. These changes of the long tract might be related to faint deterioration, possibly related to metabolic syndrome.

### Diffusion tensor imaging and minor trauma

Subtle changes of the minor brain trauma of athletes such as in soccer and American football have been highlighted. National football league in the United States and General Electric had a big project called Head Health Challenges. This project focuses on the development of technologies that can detect early stage mild traumatic brain injuries. Many papers about minor head trauma or concussion have been published recently. It is because of the development of new imaging methods, especially DTI.

Diffusion tensor imaging has been used to evaluate minor trauma due to sports. Subtle changes of repeated heading during soccer have been reported. The quantity of heading prior 12 month and DTI of 37 amateur soccer players were evaluated. "FA at three locations in the temporo-occipital white matter is lower, with a threshold that varied according to location (885-1550 headings per year) ($p < .00001$). Lower levels of FA were also associated with poorer memory scores ($p < .00001$), with a threshold of 1800 headings per year."[14)] Changes of DTI metrics of the individual subjects after sports-related concussion has been reported[15)].

### DTI outside the brain

Diffusion tensor imaging can be used to visualize the complexity of the muscle structures. The bundles of the muscles can be clearly demonstrated using diffusion tensor tractography. Change of apparent diffusion coefficient has been observed even in the Trunk muscles between before and after training[16)].

Change of diffusion tensor parameters after long-distance running (marathon) also has been reported[17)]. Marathon runners were scanned before, 2 days after and 3 weeks after marathon. Change of DTI parameters was observed in different muscles in different degrees at the level of upper leg[17)].

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# Region-Specific Vulnerability of Neuroinflammation, Oxidative Stress and Tau Hyperphosphorylation in Experimental Diabetes

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***Purpose***: Alzheimer disease (AD), involved the abnormal metabolism of β-amyloid and tau, is the major cause of dementia among elderly. Diabetes mellitus (DM) has been identified as a risk factor of AD. Two pathological lesions of AD, Aβ plaques and neurofibrillary tangles, are linked to neuroinflammation and lipid peroxidation, are also induced by abnormal glucose metabolism. Here, we examined the effects of experimental DM in tau transgenic mice Tg 601 (overexpressing wild-type human tau) and analyzed the brain regional difference occurred due to DM in AD.

***Methods***: Hippocampus, midbrain and cerebellum were analyzed from streptozotocin (STZ) injected of Tg601 and non-transgenic (NTg) mice. Immunoblotting and immunohistochemistry (IHC) were performed to assess tau hyperphosphorylation, and IHC to evaluate Ionized calcium binding adaptor molecule-1 (Iba-1) and CD68 positive microglia. Inflammatory cytokines including IL-1β, IL-6, and IL-10 were assayed using multiplexed bead based immunoassay. IL-18 was measured by enzyme linked immunosorbent assay (ELISA) and lipid peroxidation products 4-hydroxy-trans-2-noneal (HNE) and malondialdehyde (MDA) by ELISA and thiobarbituric acid reactive substances assay, respectively.

***Results***: STZ injection induced tau hyperphosphorylation, as detected by AT8 and AT 180 antibodies, in the hippocampus, but not in the cerebellum or midbrain of Tg601 and NTg mice. STZ treatment also elevated the number of Iba1-positive microglial cells, levels of IL-1β, IL-6, IL-10 and IL-18, and lipid peroxidation markers MDA or HNE in the hippocampus of the brain.

***Conclusions***: These results indicated that hyperglycemia-induced tau hyperphosphorylation, neuroinflammation and oxidative stress occurred more severely in the hippocampus than other parts of the brain and could contribute to selective neurodegeneration in human AD.

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# Short-Term Treadmill Exercise Increased Oxidative Stress and Tau Insolubility in Tauopathy Model Mice

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***Background***: Physical exercise has been reported as a preventive measure of Alzheimer's disease, one of the neuropathological hallmarks of which, neurofibrillary tangles, consist of hyperphosphorylated insoluble tau. Long-term treadmill exercise reduced tau hyperphosphorylation: however, it remains unknown whether short-term treadmill exercise alters tau modifications.
***Aim***: Here we attempted to characterize the effects of short-term treadmill exercise on tau solubility and determine its relationship with neuroinflammation using tauopathy model mice (Tg601).
***Results***: 3 weeks of non-shock treadmill exercise in Tg601 and non-transgenic mice markedly increased insoluble tau by increasing the phosphorylation at the tau C-terminal end, particularly at Ser396. The results of immunohistochemical analyses revealed that short-term treadmill exercise increased the number of Ionized calcium binding adaptor molecule-1 (Iba-1) positive microglia in the hippocampus. Elevations in the levels of the lipid peroxidation markers 4-hydroxy-trans-2-noneal and malondialdehyde indicated the presence of oxidative stress.
***Conclusion***: Our results suggested that short-term forced exercise was harmful rather than beneficial to Alzheimer's pathology.

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# Quantitative Analysis of Horizontal Eye Movements and Concentration of Serum and Plasma BDNF Level Before and After Vision Training

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***Purpose***: Eye movements are important factors for dynamic visual acuity (DVA) that refers to the ability to perceive fine details of a moving object. When tracking a moving visual stimulus, we often combine smooth eye movements with catch-up saccades. The velocity of saccade eye movements (up to 500-600 deg/s) is much faster than smooth pursuit (usually less than 50 deg/s). Even though a tracking ability using these two kinds of eye movements is thought to play a critical role in DVA, it is still uncertain whether better DVA is associated with an ability of catch-up saccades. The first purpose of this study was to quantitatively clarify the effects of vision training on DVA and eye movements. Therefore, we repetitively measured the eye movements during DVA training and attempted to determine the effects of visual training on saccade eye movements. The second purpose was to assess the concentration of serum and plasma brain-derived neurotrophic factor (BDNF) level before and after vision training. BDNF is a member of the neurotrophin family of growth factors, which are related to the canonical Nerve Growth Factor. Previous studies have reported that moderate intensity exercise leads to increase in BDNF. However it is unknown whether BDNF level is correlated with eye movements. Therefore, we examined the correlation between BDNF level and eye movement parameters.

***Methods***: DVA was evaluated by a moving visual target (Landolt ring) that was projected on the front screen (HI-10; Kowa, Japan). Eye movements were detected using a video based eye tracking system (Eye Link1000; SR research, Canada). We have performed measuring DVA in five subjects (mean age; 19.0 ± 2.6, age range; 19 to 21 years old) and analyzed saccades eye movements quantitatively using custom analytical software (Matlab; Mathworks Inc., USA). Subjects were seated in front of a screen and put their jaw on a chin supporter of our device to stabilize the head. The subjects were asked to follow the Landolt ring moving across in front of their visual field and judge the direction of the slit. We analyzed saccade latency (msec), peak velocity (deg/sec), error (deg) and a correct response rate (%). The Landolt ring moved either from right to left or left to right with constant speed of 300 deg/s. A total of eight measurements were performed for each subject. We took blood samples from each subject before and after vision training and checked serum and plasma level of BDNF.

***Results***: Our results demonstrated that saccade latency and error showed significant decreases and the correct response rate increased after training for five subjects. Especially for the result of correlation analysis, the correct response rate showed a correlation coefficient of 0.63 ($p=0.021$), indicating a stronger correlation than the other three parameters. In contrast, saccade peak velocity showed different results among subjects. For three subjects, the velocity increased while two subjects showed a decrease in velocity after training. The results of the analysis showed a significant positive correlation of the concentration of plasma BDNF level with velocity and the correct response rate. However, the results showed a significant negative correlation of the concentration of plasma BDNF level with latency and error. It was also revealed that the results of plasma and serum BDNF level were contrary to each other.

***Conclusion***: Our study provided several lines of evidence showing that saccade eye movements, such as latency, error and peak velocity changed after vision training. Since our results showed that visual training improved DVA, better DVA could be associated with the tracking ability using catch-up saccades. We were also able to clarify that the plasma and serum BDNF possibly affected actual eye movements.

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# What We Learned from Brain MR Study from the Sportology Project

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Although it is widely accepted that cerebrovascular events associated with diabetes mellitus adversely affect the brain, it is less well known that diabetes mellitus itself or even prediabetes can also do so. In this presentation, we focus on this issue by using evidence from recent diffusion tensor neuroimaging studies of patients with diabetes mellitus or metabolic syndrome.

First, through the Sportology project, we explored the regional patterns of white matter alteration in subjects with metabolic syndrome. We also investigated whether the degree of white matter alteration was correlated with BMI. Seven middle-aged men with metabolic syndrome and seven without metabolic syndrome underwent diffusion tensor imaging. MRI scans were performed with a 3.0-T unit (Achieva; Philips Medical Systems, Best, the Netherlands). We analyzed the resultant fractional anisotropy (FA) values by using a tractbased spatial statistics technique. We subsequently measured the mean FA values of the right inferior fronto-occipital fasciculus (IFOF) in all subjects by using a tract-specific analysis. We used Pearson's correlation coefficient to evaluate the relationship between BMI and the mean FA value in the right IFOF. In the whole-brain analysis, subjects with metabolic syndrome had significantly lower FA values than control subjects in part of the right external capsule (which is part of the right IFOF), the entire corpus callosum, and part of the deep white matter of the right frontal lobe. In the regional brain analysis, the mean FA value of the right IFOF was $0.41 \pm 0.03$ in subjects with metabolic syndrome and $0.44 \pm 0.05$ in control subjects. A significant negative correlation was observed between BMI and FA values in the right IFOF ($r = -0.56$, $p < 0.04$). These results suggest that there are microstructural changes in the white matter of middle-aged individuals with metabolic syndrome. Our findings add to the increasing body of neuroimaging evidence on white matter alteration in patients with hypertension, diabetes, or metabolic syndrome. Microstructural alterations in the white matter of younger obese individuals may precede brain atrophy or cognitive impairment, or both, in advanced metabolic syndrome.

Second, again through the Sportology project, we explored the regional patterns of white matter alteration in 15 hypertensive middle-aged male participants and 11 normotensive controls by using diffusion kurtosis imaging (DKI) -based whole-brain analysis. DKI data were acquired by use of a single-shot, spin-echo planar imaging sequence. Mean diffusional kurtosis (MDK) values in many brain regions were higher in subjects with hypertension than in control subjects, indicating that there were widespread microstructural changes in the white matter, whereas the conventional diffusion metrics of FA did not differ significantly between subjects with hypertension and normal controls. Moreover, MDK values over the whole brain were significantly and positively correlated with systolic and diastolic blood pressure. This finding suggests that microstructural white matter changes occur in middle-aged men with hypertension, even before the onset of cerebrovascular disease. DKI might therefore be useful as a screening tool for risk of cerebrovascular disease.

DTI is completely noninvasive and is sensitive to white matter pathology in a number of disorders, including metabolic syndrome, in the human brain *in vivo*. Accumulated evidence highlights the need to further elucidate the relationship between metabolic syndrome and other neuronal mechanisms. A clear understanding of these relationships is crucial for managing patients with metabolic syndrome.

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# Voluntary Exercise Preserves Cardiac Function in DCM Model Mice

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***Background***: Dilated cardiomyopathy (DCM) is one of major causes of heart failure (HF), characterized by ventricular dilatation and contractile dysfunction. In addition to HF, about 30–40% of patients with DCM die from premature death with lethal arrhythmia. To date, the exercise is considered to be one of therapy for HF as shown in the guideline for chronic HF (Japanese Society of Cardiology in 2010). However, the effects of exercise on DCM patients are still unclear, because evaluation of exercise therapy in DCM patients is associated with risks of worsening of HF or sudden cardiac death. Investigations with animal models of inherited DCM are necessary. Previously, we have established the method to evaluate the severity of HF using a mouse model of DCM (Sugihara *et al*. PLoS One, 2013). In the course of these studies, we found that voluntary exercise started at young age significantly prolongs survival rate of DCM mice. In this study, we investigated the effects of voluntary exercise on the cardiac function and arrhythmogenesity in DCM model mice.

***Methods***: We used a knock-in mouse model having one of human inherited DCM mutation, TNNT2 ΔK210, which decreases Ca2+ sensitivity of myofilaments (Du *et al*. Circ Res, 2007). Homozygous ΔK210 (below are called DCM mice) and wild type (WT) mice at 1 month-old were housed with a running wheel (diameter=12 cm) every 48 hours or all day long, and daily voluntary running activity was recorded. At 2 month-old, end-diastolic dimension and ejection fraction (EF) were measured by echocardiography. Heart, lung and lower extremity muscle (soleus, plantaris and gastrocnemius muscles) were excised and their weights were measured together with body weight. Gene expressions of major ion channels (Kv1.5, Kv4.2, KChiP2, Nav1.5, Cav1.2, etc.,) were quantified by real time PCR analysis.

***Results and Discussion***: DCM mice died with t1/2 of approximately 70 days as reported previously (Du *et al*, 2007). The average lifespan of the DCM mice who continued running exercise every 48 hours was about 20 days longer than that without exercise. Moreover, systolic cardiac function defined by the average EF was higher in DCM mice with exercise than in those without exercise [0.47 ± 0.10 (n=7) vs. 0.31 ± 0.10 (n=4), $p<0.05$] at 3 month-old. On the other hand, electrical remodeling such as down-regulation of multiple types of K+ channels and accessory subunits has been reported in the DCM mice and closely related to the arrhythmogenesity in them (Suzuki *et al*. PLoS One, 2012). Some of these expression in DCM mice starting exercise at young age were relatively preserved (n=6). We further discuss the relationship between cardiac function, electrical remodeling and leg muscle weights as a measure of exercise intensity.

***Key words***: voluntary exercise, heart failure, lethal arrhythmia, dilated cardiomyopathy

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# Acute Exercise Attenuates Cardiac Dysfunction After Ischemia/Reperfusion in Isolated Rat Heart

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***Purpose***: Regular bouts of endurance exercise can protect the heart against ischemia/reperfusion (I/R) injury. However, the effects of acute exercise immediately before I/R events on myocardial dysfunction remain unclear. This study examined (1) whether a single session of acute exercise reduced cardiac dysfunction after I/R, and (2) whether a single exercise session up-regulated the intracellular signaling pathways involved in cardioprotection in the rat heart.

***Methods***: Male Sprague–Dawley rats were divided into a sedentary control (CON) group and an exercise (EX) group. Rats in the EX group underwent one 30-min session of treadmill running. Following exercise, hearts were excised and subjected to Langendorff perfusion. To evaluate cardiac function during I/R, hearts from both groups were exposed to global ischemia (20 min) followed by reperfusion (45 min). Using western blotting, phosphorylation of Akt, protein kinase C-ε (PKCε) and glycogen synthase kinase 3-β (GSK-3β) in the hearts were analyzed.

***Results***: Cardiac function was significantly higher in the EX group compared with the CON group for 5–20 min after reperfusion ($p<0.05$). Phosphorylation of Akt, PKCε and GSK-3β in hearts of EX rats showed significant increases compared with CON rats ($p<0.05$). Exercise did not change expression levels of heat shock protein 72 in the heart.

***Conclusions***: Acute exercise prior to I/R attenuated cardiac dysfunction in the isolated rat heart. The attenuation might be due to exercise-induced activation of cardioprotective intracellular signaling.

***Key words***: running exercise, Langendorff perfusion, cardiac function, intracellular signaling, heat shock protein

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# The Brain Histaminergic System in Regulating the Cardiovascular System: Implications for Brain Mechanisms Underlying Exercise-Induced Cardiovascular Responses

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A single bout of exercise induces a moderate increase in arterial pressure with marked tachycardia as a result of sympathoexcitation. However, the brain mechanisms underlying cardiovascular regulation during exercise still remain unknown. In this proceeding, we introduce our hypothesis that the brain histaminergic system plays an important role in regulating the cardiovascular system during exercise. The nucleus of the solitary tract (NTS) is one of the ideal brain sites for generating cardiovascular controls during exercise because it is known as a pivotal region which integrates the baroreceptor sensory information with other inputs such as muscle afferents and descending signals from the hypothalamic area. We found that activation of histamine receptor $H_1$ expressed in the NTS neurons induced pressor and tachycardiac responses, and that the pressor response exhibited functional plasticity after long-term daily exercise. These findings suggest that $H_1$ receptors in the NTS are involved in cardiovascular regulation during exercise. Since the NTS receives axons of histaminergic neurons located in the tuberomammillary nucleus (TMN) in the hypothalamus, the functional roles of TMN-NTS pathway have also been investigated. We electrically stimulated the TMN and found pressor and tachycardiac responses. Notably the pressor responses were partially inhibited by cetirizine, a $H_1$ receptor antagonist, microinjected into the NTS whereas we failed to see the inhibitory effects on the heart rate response. Based on all these findings, we postulate that the TMN-NTS pathway has an important role in a central feed forward mechanism underlying pressor responses to exercise.

***Key words***: exercise, blood pressure, nucleus of the solitary tract, tuberomammillary nucleus, histamine

## Typical pattern of cardiovascular responses during exercise and its regulatory mechanisms

The pattern of hemodynamic responses is complicated because it is affected by exercise type, intensity, and time. However, it is generally accepted that a single bout of exercise induces a mild increase in arterial pressure (AP) and marked tachycardia. These cardiovascular responses are mainly due to sympathoexcitation which activates heart function and induces vasoconstriction in the major organs[1) 2)]. These cardiovascular responses are necessary to efficiently supply oxygen-rich blood to organs such as skeletal muscles, which have high metabolic demands. Decreased parasympathetic nerve activity during exercise is also involved in the tachycardiac response. However, accumulating evidence suggests that the role of the sympathetic nervous system in regulating heart rate (HR) is large. Adrenaline secretion induced by adrenal sympathoexcitation also contributes to tachycardia but dilates blood vessels of skeletal muscle via activation of $\beta_2$ adrenergic receptors. This is an important mechanism to maintain high blood flow levels in the active skeletal muscles. The next question relates to how sympathetic outflow is

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centrally regulated during a single bout of exercise. The current understanding of the brain mechanisms underlying sympathoexcitation during exercise involves the following: (i) an ascending neural feedback mechanism from skeletal muscle mechanoreceptors and metaboreceptors[3) 4)]; and (ii) a feedforward (i. e. central command) mechanism which originate from motor control-related brain areas, such as the insular cortex, hypothalamic and mesencephalic locomotor regions[5) 6)]. Although the contribution of both mechanisms may depend on exercise type, time, and intensity, each is capable of 'continuous' increases in sympathetic nerve activity, and consequently, AP and HR. Although many brain areas are likely involved in regulating hemodynamic responses during exercise, we postulate that the nucleus of the solitary tract (NTS) is one of key brain sites for cardiovascular control during exercise. Please also refer to our recent publication introducing potential brain mechanisms underlying cardiovascular control during exercise[7)].

## NTS functions in regulating the cardiovascular system

The NTS, which is located in the dorsomedial aspect of the medulla oblongata, is innervated by visceral inputs from a large number of peripheral receptors within the gastrointestinal, gustatory, pulmonary/respiratory, and cardiovascular systems, which reflexly affect a wealth of autonomic motor outputs, indicating it a vital component for the homeostasis of autonomic function[8)]. With regard to the cardiovascular system, the NTS controls AP and HR to maintain cardiovascular homeostasis. The primary cardiovascular reflex mediated by the NTS is the arterial baroreceptor reflex. Arterial baroreceptors are mechanoreceptors that are located in the aortic arch and the carotid sinuses, and stimulated by stretching of the arterial wall when AP increases. Baroreceptor afferent signals are then sent to the NTS, the primary termination site for the afferents. Second-order NTS glutamatergic neurons excite GABAergic inhibitory neurons in the caudal ventrolateral medulla (CVLM) that project and inhibit rostral ventrolateral medulla (RVLM) glutamatergic neurons, thereby decreasing sympathetic preganglionic neuronal outflow (Figure-1)[9)]. The NTS neurons also excite parasympathetic preganglionic cell bodies located in the nucleus ambiguous. As a result, increased parasympathetic and decreased sympathetic outflows induce bradycardic response, diminished cardiac output, and decreased total peripheral resistance. These responses contribute to normalizing increased AP. Opposite autonomic effects, namely, reduced parasympathetic and increased sympathetic drive, are observed when AP decreases. Thus, the baroreceptor function is required to be modified for continuous increases in sympathetic outflow, AP, and HR under certain physiological conditions such as exercise.

## The NTS is a key brain region in regulating the cardiovascular system during exercise

As discussed above, the NTS is the central termination site for baroreceptor inputs. Importantly the NTS also receives direct projections from spinal dorsal horn neurons, which transmit afferent inputs from skeletal muscle[4) 10)]. And moreover, the NTS also receives numerous inputs from other brain areas, including the dorsomedial hypothalamus: DMH or hypothalamic paraventricular nucleus: PVN[11) 12)], these networks are considered for candidate pathways of the central command) (Figure-1). Therefore, the NTS is considered to be a central site that integrates the descending and ascending inputs while regulating baroreceptor function during exercise. Indeed, strong expression of the c-fos protein, which is generally used as a marker for neuronal activity, was found in the NTS in response to treadmill running in rats (unpublished data by Waki H.), suggesting that the NTS is likely to be one of the cardiovascular centres where activation of local neuronal networks occurs to modulate the baroreceptor reflex during exercise. Our prediction is that some c-fos positive neurones in the NTS are GABAergic inter-neurones which inhibit the NTS barosensitive neurons (Figure-1). Alternatively, those neurones may be the NTS chemosensitive neurones which directly innervate and excite RVLM sympathetic premotor neurons[13)]. Thus, the NTS is capable of continually exciting sympathetic premotor neurons located mainly in the RVLM and this may result in sympathoexcitation during exercise. This scenario needs to be

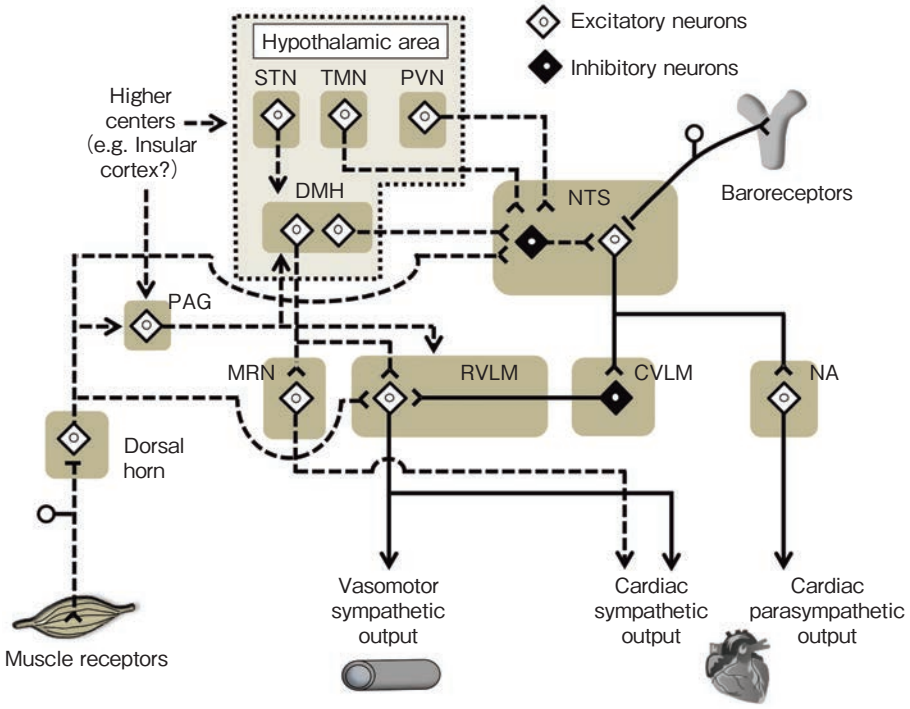


**Figure-1** Hypothetical models to explain the central mechanisms underlying exercise-induced cardiovascular responses
Main brain regions in the hypothalamus and brainstem where can control the cardiovascular system are shown. The current understanding of the brain mechanisms underlying cardiovascular responses during exercise involves an ascending neural feedback mechanism from skeletal muscle receptors and a central command mechanism which may originate from motor control-related brain areas, such as the insular cortex and hypothalamic locomotor regions (subthalamic nucleus: STN). The descending and ascending signals input to the cardiovascular centres located in the medulla oblongata such as the nucleus of the solitary tract (NTS) and the rostral ventrolateral medulla (RVLM). In these central networks, our findings demonstrate that the tuberomammillary nucleus (TMN)-NTS pathway may have an important role in a central feed forward mechanism underlying especially pressor responses to a single bout of exercise (see the text for more details). Note that solid lines represent the central pathways of the baroreceptor reflex arc. PVN: hypothalamic paraventricular nucleus; DMH: dorsomedial hypothalamus; PAG: periaqueductal gray area; MRN: pallidal raphe nucleus; NA: nucleus ambiguous; CVLM: caudal ventrolateral medulla. Figure modified with permissions from Waki H: Phys Fitness Sports Med, 2012; 1: 253-261 [7].

confirmed by further experiments. The next question relates to which mechanisms and brain areas can be involved to control the NTS functions during a single bout of exercise. Our recent findings demonstrate that the posterior hypothalamus - NTS network should be considered for one of the candidate pathways of the central command mechanism.

## Histaminergic system in the NTS

We performed a genome-wide microarray expression analysis using the NTS tissue from exercise-trained animals (over 10 weeks voluntary wheel running) [14]. We screened for genes in the NTS that were differentially expressed between the daily exercised rats and sedentary control rats. Total RNA was extracted from the NTS and Rat Genome Oligo DNA Microarray (Agilent Technologies) followed by a pathway analysis using the KEGG database was performed. We revealed that long-term exercise altered the expression levels of NTS genes that are functionally associated with neuroactive ligand-receptor interactions. One of the genes that belonged to neuroactive ligand-receptor interactions was histamine receptor $H_1$. We further confirmed that the expression level of $H_1$ mRNA was higher than that of the other subtypes and $H_1$ receptor proteins are mainly expressed in the NTS neurons, indicating its important neuronal functions in the NTS. The physiological agonist of $H_1$ receptors, histamine, is a biogenic amine that is found in and released by mast cells and which can induce local immune responses in peripheral organs. Besides these roles, histamine is also contained in neurons in the central nervous system (CNS) [15-19] and central histamine possesses neurotransmitter properties [20-22]. Information regarding

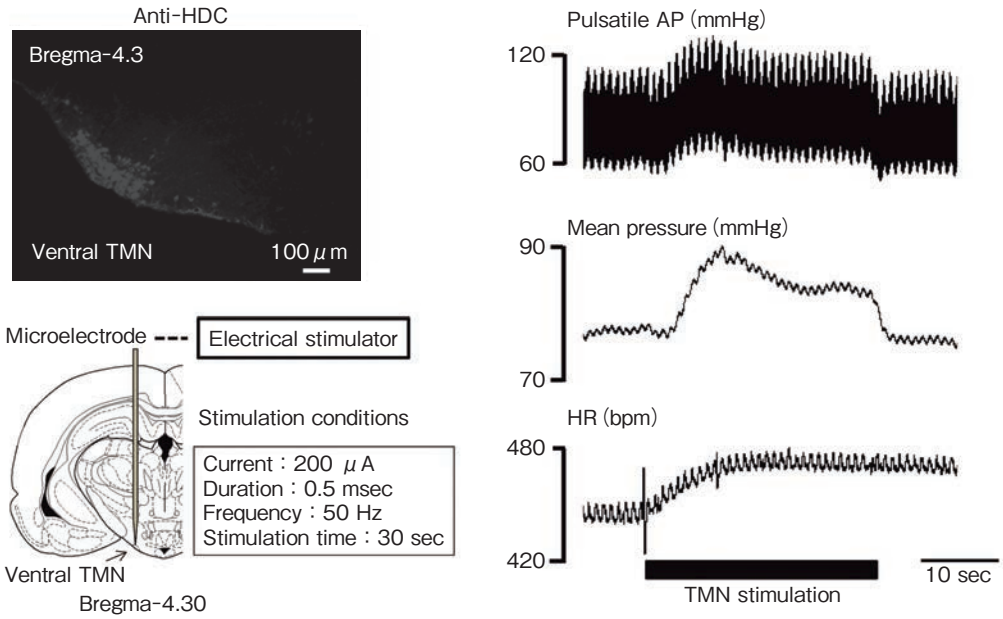


**Figure-2** Pressor and tachycardiac responses during electrical stimulation of the TMN
(Left) Histidine decarboxylase-positive cells (histaminergic cells) were located specifically in the ventral TMN of the posterior hypothalamic region. This area was electrically stimulated by a microelectrode in anesthetized rats.
(Right) Representative recordings illustrating the cardiovascular changes induced by TMN stimulation. Similar to the cardiovascular responses evoked by $H_1$ agonist microinjections into the NTS (see text), pressor and tachycardiac responses were observed.

the physiological role of histamine within the CNS is still emerging. It is an important regulator of the sleep-wake cycle, arousal level, learning, pain sensation, stress responses, fluid balance, food intake and body temperature[15) 23)-25)]. In addition to these functions, it has been reported that central histamine can regulate the cardiovascular system[20) 26)]. Indeed, we found that microinjection of histamine or 2-pyridylethylamine, a $H_1$ receptor specific agonist into the NTS, dose-dependently increased AP and HR[27)]. Most importantly, we confirmed that the pressor response induced by the activation of this receptor in the NTS was increased after long-term daily exercise[14)]. These findings suggest that the histaminergic system may have an important role in regulating the cardiovascular system during a single bout of exercise and its functional plasticity may be involved in exercise training-induced cardiovascular adaptations. The mechanisms underlying the $H_1$ receptor-mediated inhibition of NTS function in regulating AP and HR still need to be elucidated. Considering that the $H_1$ receptor is a member of the G protein-coupled receptor superfamily and excites neurons in most brain regions through activation of the Gq/11-phosphlipase C pathway, histamine in the NTS may activate inhibitory interneurons via $H_1$ receptors, thereby decreasing the excitability of barosensitive neurons in the NTS. The possibility also needs to be considered that the 2$^{nd}$-order chemosensitive neurons in the NTS[13)] are excited by $H_1$ receptors expressed on these neurons and hence, this results in activation of RVLM sympathetic premotor neurons.

## The tuberomammillary nucleus (TMN) - NTS pathway; potential central mechanisms underlying exercise-induced cardiovascular responses

Besides the main medullary cardiovascular centers (i. e. NTS, nucleus ambiguous, CVLM, RVLM), multiple brain areas are predicted to be involved in feedforward and/or feedback mechanisms in regulating the cardiovascular system during exercise. In particular, numerous candidate brain sites are expected to be involved in the central command mechanism. Such brain areas include the insular cortex, hypothalamic locomotor regions (subthalamic nucleus), perifornical region in the hypothalamus, dorsomedial hypothalamus, paraventricular nucleus, mesencephalic locomotor

regions, periaqueductal gray area, medullary raphe (pallidal raphe nucleus) (Figure-1) [4) 7) 10) 11)]. Based on our recent findings, we propose that the tuberomammillary nucleus (TMN) of the posterior hypothalamus may also be an important brain site in exercise-induced cardiovascular responses. The TMN is anatomically located in the hypothalamic defense area, and we confirmed that histamine-immunoreactive neuronal cell bodies are found exclusively in this nucleus as previously reported (Figure-2 left-top) [15) 16) 28) 29)]. However, immunoreactive fibers are observed throughout the cerebral cortex and in parts of other brain regions including the olfactory bulb and tubercle, amygdala, substantia nigra, parabrachial nucleus and the NTS [11) 17) 29)]. Since central histamine is known as an important regulator of arousal level, it is conceivable that the histaminergic system is activated during exercise (i.e. high-arousal condition). We therefore electrically stimulated the TMN in anesthetized rats, and similar to the cardiovascular responses evoked by $H_1$ agonist microinjections into the NTS, pressor and tachycardiac responses were observed (Figure-2 left-bottom and right) [30)]. More importantly the pressor responses were partially inhibited by cetirizine, a $H_1$ receptor antagonist, microinjected into the NTS whereas we failed to see the inhibitory effects on the HR responses [30)]. As previously reported, we also histologically confirmed that the TMN neurons directly project to the NTS by using a retrograde tracer, Fluoro-Gold [30)]. Taken together, these findings demonstrate that the TMN-NTS pathway is likely involved in the central pressor responses presumably under high arousal phase. Our supposition is that the TMN-NTS pathway has an important role especially in a central feed forward mechanism (central command) underlying pressor responses to a single bout of exercise, although this hypothesis needs to be tested in the future.

## Conclusions

During a single bout of exercise, neuronal signals from the central command, mediated by the hypothalamic nuclei including the TMN, and those from the muscle receptors are integrated within the NTS, resulting in sympathoexcitation in most sympathetic fibers, and hence AP and HR increases. This may only partially explain the mechanisms underlying cardiovascular responses during exercise. Since maintaining cardiovascular homeostasis is essential for high exercise performance, further investigations will be required to fully understand the aspects of the central regulatory mechanisms of the cardiovascular system during exercise.

## Acknowledgements

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# Is Watching National Team Matches in World Cup Soccer 2014 on TV Associated with Increasing Ventricular Arrhythmia?

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***Objective***: It has been reported that psychological triggers such as emotional stress, increase the incidence of cardiovascular events. The association between soccer championships and the risk of cardiovascular events is still unclear. World Cup Soccer (WCS) involving the national team might be a strong enough trigger to induce cardiac arrhythmia. However, there are no reports which investigated the relationship between WCS and cardiac arrhythmia in the Japanese population.

***Design***: A multi-center retrospective observational study.

***Methods***: We assessed 25 patients who were evaluated ischemic changes and/or arrhythmia by 24-h Holter electrocardiography in 4 Cardiology Divisions during WCS 2014. The patients were divided into two groups [Watching group (n=7): patients who watched WCS on live-TV on June 20 (Japan vs. Greece) or July 25, 2014 (Japan vs. Colombia), and No-watching group (18 patients)]. Heart rates, arrhythmia, and ischemic changes were evaluated.

***Results***: There were no significant differences of clinical characteristics, frequency of premature atrial contractions, and ischemic changes between the two groups. Although there were no differences in total premature ventricular contractions (PVCs), the frequency of PVCs during matches ($p<0.05$) were significantly higher during live-TV in the Watching group than in the No-watching group. No sustained ventricular tachycardia or fibrillation was observed.

***Conclusions***: A significant association between watching WCS and the frequency of PVCs was observed. These data suggest that emotional stress while watching national team soccer matches may induce stress-related cardiovascular events.

***Key words***: World Cup Soccer, 24-h Holter ECG, arrhythmia, cardiac events, emotional stress

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# Relationships Between Club Activity Stressors, Commitment to Sports, and Resilience in High School Athletes Belonging to School Athletic Clubs

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***Introduction***: School athletic clubs serve as central sites of sports activities for many junior and senior high school students in Japan. School athletic clubs preserve the traditions of pre-war junior and senior high schools, and constitute a sports-education system unique to Japan. Although other countries maintain athletic clubs, only in Japan is there a system, an environment, and leaders (school teachers) under which school athletic clubs seek to win national championships. This system is characterized by the incorporation of sport within school-based education; this arrangement allows students to regularly perform athletic activities. Three million junior and senior high school students spend 700 hours each on athletic club activities annually. These facts indicate a large number of school athletic clubs performing extremely intensive physical training activities. The question thus arises: do school athletic clubs' activities truly cultivate the minds and spirits of young athletes, given the current environment has these problems? In our study, we surveyed athletes belonging to school athletic clubs to examine relationships between concepts related to mental strength and ideas about sports. The authors specifically addressed sport commitment and resilience. Scanlan *et al.* proposed a theory of sport commitment, which describes athletes' persistence in, connection with, and devotion to sports. Research has examined resilience since the 1990s; in Japan, resilience has been discussed in terms of 'ability to recover' and 'restoration of strength'. Resilience has also been considered 'mental strength', which enables one to recover from stress or negative life events, and to recuperate after harm. Additionally, as students may experience a variety of stressors unique to school athletic clubs, stress measures specific to these clubs were used. This study aimed to investigate relationships among these factors.

***Method***: Anonymous questionnaire surveys were administered. Participants were students (n=203) attending private high schools in the Tokyo metropolitan district. Questionnaires were (1) Hagiwara *et al.*'s Commitment to Sports Scale, (2) the Resilience Scale developed by Yamagishi and revised by Oshio and Ishige *et al.*, (3) Shibukura *et al.*'s Stressor Scale for High School Athletic Club Members, and (4) a face sheet inquiring whether the participant is a member of a school athletic club. For follow-up investigation, each participant was issued a password. Data were collected from the 11th to the 15th of May 2015.

***Results***: Factor analysis of Resilience Scale scores extracted five factors that differed slightly from factors reported in previous research: 'positive future orientation and optimism', 'pursuit of novelty', 'emotional adjustment and optimism', 'relationship orientation', and 'metacognition orientation and emotional adjustment'. Factor analysis of scores on the Stressor Scale for High School Athletic Club Members extracted the same five factors as in previous research: 'competitiveness', 'coaches', 'practice time', 'comrades', and 'injury or illness'.

We subsequently internally analysed the athletic club member group. Using the mean score on the Commitment to Sports Scale (M=23.95, SD=5.14), we divided participants into an 'above-mean group' (n=94) and a 'below-mean group' (n=58), and then investigated relationships between the two groups' scores on the Resilience Scale and the Stressor Scale for High School Athletic Club Members. Scores were compared using analysis of variance. Scores on each factor of the Resilience Scale were compared between the above- and belowmean groups. Scores were significantly higher for all factors in the above-mean group ($p < .01$) (Figure-1). Similarly, regarding the Stressor Scale for High School Athletic Club Members, analysis of variance was used to compare the above- and

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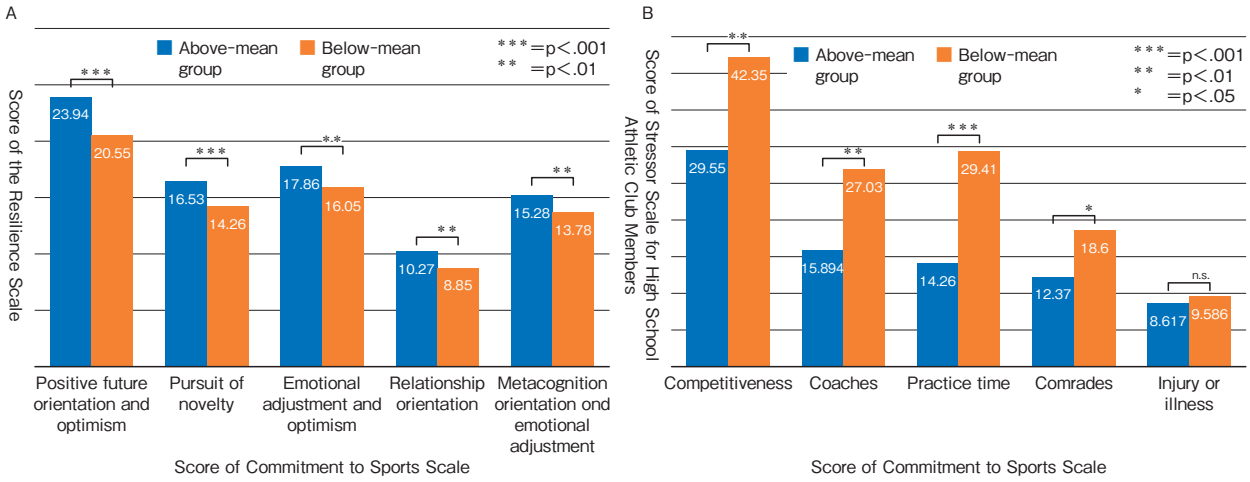


**Figure-1** **A.** Relationship between Score of the Resilience Scale and Score of Commitment to Sports Scale
**B.** Relationship between Score of Stressor Scale for High School Athletic Club Members Score of Commitment to Sports Scale

the below-mean group. Results showed that the below-mean group's scores on four factors—'competitiveness', 'coaches', 'practice time', and 'comrades'—were significantly higher ($p < .01$ or $.05$) (Figure-1).

***Discussions***: Regarding the Commitment to Sports Scale' s above- and below-mean groups, significant differences were observed between these two groups in scores on the Resilience Scale and on the Stressor Scale for High School Athletic Club Members; the above-mean group had significantly higher Resilience Scale scores. This suggests that a persistence in and devotion to sports may strengthen resilience.

Further, regarding the Commitment to Sports Scale, stressor scores for 'competitiveness', 'coaches', 'practice time', and 'comrades' were significantly higher in the below-mean group. We consider this indicates a tendency to find these factors more stressful when commitment to sports is low.

It is of critical interest that opposing tendencies were observed in scores on the Resilience Scale and on the Stressor Scale for High School Athletic Club Members when comparing the above- and below-mean groups. We plan to complete follow-up surveys examining these effects, to further investigate relationships between these scales.

***Conclusions***: The results of our survey of High School athletes who are members of school athletic clubs suggests the following:

- Greater commitment to sports is significantly related to greater resilience.
- Less commitment to sports is significantly related to increased feelings of stress regarding school athletic club activities.

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# Factors Influencing on the Parental Support of Children's Physical Activity

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The objective of this study was to examine the impact of parental support of physical activity and parents' past levels of competitiveness in sports on parenting attitudes. The research subjects were 256 parents of freshman students in a university's Health and Sports Science Department and Medical Department. The time period of this research was November 2014 to January 2015. Questionnaires included the Parental Support Scale, the past levels of competitiveness in sports, and the Parenting Attitude Scale. The exploratory actor analyses were conducted on the Parental Support Scale and the Parenting Attitude Scale, separately. The results revealed that the Parental Support Scale consisted of 3 factors with 4 items each (total of 12 items) and the Parenting Attitude Scale consisted of 2 factors with 4 items each (total of 12 items). The reliabilities and validities of these two scales were also assessed. In the multiple regression analyses, the Parental Support Scale was set as a dependent variable, and the Parenting Attitude Scale and the level of competitiveness in sports were set as independent variables. The analyses were conducted separately on fathers and mothers and separately on male and female children. The path models showed the gender difference between fathers and mothers and difference between male and female children, and both the parenting attitude and the level of competitiveness in sports distinctively influenced on the parental support of children's physical activity. When we considered past levels of competitiveness in sports and parental support of physical activity as potential factors affecting parenting attitudes, the latter factor had an impact among both fathers and mothers, whereas the former factor was significant only among mothers. Parents should increase their emphasis on responsiveness when raising boys and on demandingness when raising girls to contribute to improvement in their parenting attitude.

***Key words***: parenting attitude, parental support of physical activity, past levels of competitiveness in sports, gender

## Introduction

The amount of time that children spend engaging in physical activity has been decreasing, thus hampering their mental and physical health. However, various triggers exist that can be used to encourage children to make exercising a habit and to adopt healthy lifestyles. Furthermore, it is desirable that children begin exercising during childhood, because people who develop good exercise habits at a young age have a high likelihood of maintaining these habits in later years.

It is believed that children's practice of exercise is greatly impacted by their own decision-making processes as well as by the impact of the environment in which they live, i.e., their school, neighbors, friends, and family. In particular, the approach taken by the child's family is considered very influential. Trost *et al.*[1] emphasized the importance of parental support in directly promoting healthy behavior, physical activity, and a sense of self-efficacy in children. Welk[2] identified four aspects of positive parental support: encouragement, involvement, facillitation, and modeling. Konno *et al*[3) 4)]. has

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indicated that these four parenting attitudes impact the shaping of a child's exercise habits from elementary school to college.

So what factors underlie the formation of positive parenting attitude? Taking into account the values, thought processes, and actions of parents, as well as their responses and behavior toward their children, we have focused on the following factors that impact parenting attitude: parents' past levels of competitiveness in sports and their attitude toward educating their children. Among the various ideas regarding parental support of physical activity is a proposal by Baumrind[5]. Treating parental support of physical activity as encompassing the mother's way of thinking about her child and her direct behavior toward her child, she described the two central dimensions as responsiveness and demandingness. However, there is little research in Japan that applies these concepts; rather, most of the existing research has examined the relationship between children's social behavior and self-control, and the relationship between children and parenting attitude has yet to be explored.

Therefore, this study examines the impact of parental support of physical activity and parents' past levels of competitiveness in sports on the basis of their parenting attitude toward their children. Clarifying the relationships between these factors, thereby allowing parents to provide the most desirable form of support, should help more children to develop good habits of physical activity at an early age, thereby preserving their mental and physical health more effectively. The objective of this study was to examine the impact of parental support of physical activity and parents' past levels of competitiveness in sports on parenting attitudes.

## Materials and methods

### 1. Research subjects and time period

The research subjects were 256 parents of freshman students in a university's Health and Sports Science Department and Medical Department. The time period of this research was November 2014 to January 2015.

### 2. Research content

a) Parenting attitude

Referring to the criteria used in previous studies[2) 6)], the authors added additional items, resulting in seven items for each of Welk's four factors, thus producing a total of 28 items; responders were instructed to choose from among four choices. Consistent with Welk[2)], the four areas were encouragement (e.g., praising the child's physical activity and proactively giving feedback), involvement (e.g., playing with the child and teaching the child various ways to play), facillitation (e.g., taking the child to the playground or getting sports and exercise equipment for the child), and modeling (e.g., the parent engaging in physical activity in front of the child).

b) Parental support of physical activity

Referring to the criteria used by Robinson *et al.*[8)] and Nakamichi *et al.*[9)], which were in turn selected from the criteria developed by Baumrind[7)], eight items were constructed for each factor, resulting in a total of 16 items; responders were instructed to choose from among four choices. The two factors were responsiveness (actions that consider the child's intention and desires and that enrich the child's intentions as much as possible by using loving words and physical expression) and demandingness (regardless of the child's intentions, the parent decides what is best for the child and forces that upon the child).

c) Past levels of competitiveness in sports

Information regarding past levels of competitiveness in sports was requested, in terms of middle- and high-school sports activities as well as competitive performance in (1) regional competitions, (2) prefectural competitions, and (3) national competitions.

### 3. Analysis

First, exploratory factor analysis (principal factor method, promax rotation) was conducted regarding parenting attitude and parental support of physical activity. For each item, the standard was set at an eigenvalue of over 1.0 and a factor load of over 0.40, and the items with high loading on multiple factors were eliminated. The analysis was repeatedly conducted in order to assess these items' reliability and validity.

Next, in order to clarify the impact on parenting attitude, parental support of physical activity and past levels of competitiveness in sports were made independent variables, and parenting attitude was

made a dependent variable; multiple regression analysis (stepwise method) was repeatedly conducted. For the analysis, the factor of gender was taken into account: the parents were separated between fathers and mothers and their children between boys and girls.

For statistical processing, SPSS Statistics 22, Amos version 22 was used, and the significance level was less than 5%.

## Results and discussion

### 1. Characteristics of the research sample

The parents included 119 fathers (whose children were 80 boys and 39 girls) and 137 mothers (whose children were 89 boys and 48 girls); the parents' average age was 50.5 ± 3.87 years, with a range from 41 to 61 years (Table-1).

**Table-1** Research subjects

| | Boys | Girls | Total |
|---|---|---|---|
| Fathers | 80 | 39 | 119 |
| Mothers | 89 | 48 | 137 |
| Total | 169 | 87 | 256 |

### 2. Factor analysis

a) Parenting attitude toward the child

A three-factor structure was extracted to define the relationship between parental behavior and parenting attitude (Table-2). The first factor was modeling, the second factor was facillitation, and the third factor was guidance. This factor structure differed from those of Welk[2] and Kinoshita *et al.*[6]. However, each item was appropriate in terms of the content, the interpretation of the factors led to the same substantive conclusions, and in terms of internal consistency, a high coefficient of reliability was achieved. In addition, the degree of suitability for modeling was high at GFI = .929, AGFI = .892, CFI = .954, and RMSEA = .069; thus, it could be determined that the factors' validity was confirmed, and an analysis using this three-factor structure was conducted.

**Table-2** Analysis of survey items related to Welk's four parental support factors

| | No. | Item | Factor loading F1 | F2 | F3 |
|---|---|---|---|---|---|
| Modeling (α = .91) | 18. | Do you yourself engage in exercise and sports, and become a good role model for your child? | .94 | -.13 | .07 |
| | 20. | Has your child seen you engage in exercise or sports? | .91 | .02 | -.09 |
| | 19. | Have you shown your child how to exercise and play? | .76 | .03 | .12 |
| | 13. | In daily life, has your child seen you proactively engaging in physical activities? | .71 | .14 | -.06 |
| Facilitation (α = .77) | 9. | Have you shown understanding for your child's engagement in exercise, sports, and playing outdoors? | -.05 | .73 | .03 |
| | 6. | Have you provided your child with opportunities to play with siblings or neighborhood children? | -.02 | .71 | .05 |
| | 5. | Do you take your child to playgrounds or other places to engage in physical activities? | .14 | .69 | -.16 |
| | 7. | Have you brought exercise equipment to encourage your child's engagement in physical activities (excluding toys such as video games)? | -.03 | .60 | .13 |
| Guidance (α = .75) | 26. | Have you made an effort to encourage your child to participate in a sports team or join a sports club? | -.16 | .04 | .75 |
| | 25. | Have you taught your child the benefits and importance of physical exercise and sports? | .10 | .06 | .65 |
| | 1. | Have you used your words or actions to encourage your child to engage in physical exercise and sports? | .04 | -.07 | .65 |
| | 24. | Have you encouraged your child to engage in physical activity by cutting down the amount of time they spend on the internet or the phone? | .11 | .01 | .51 |
| | | Contribution (%) | 35.92 | 12.16 | 7.14 |
| | | Cumulative Contribution (%) | | 48.08 | 55.22 |
| | | Factor Correlation | F1 | .39 | .45 |
| | | | | F2 | .51 |

**Table-3** Analysis of survey items regarding Baumrind's two factors of parental support of physical activity

| | No. | Item | Factor loading F1 | Factor loading F2 |
|---|---|---|---|---|
| Responsiveness (α=.70) | 2. | I display my affection for my child, such as hugging and saying kind words. | .74 | -.09 |
| | 3. | When my child seems stressed or frustrated, I ask him/her "What's wrong?" | .70 | -.02 |
| | 7. | When my child does something wrong, I ask the reason behind his/her decision to act in such a manner and I discuss how he/she should have acted. | .55 | .06 |
| | 1. | When my child is playing alone and appears bored, I join him/her. | .47 | .02 |
| Demandingness (α=.61) | 10. | I make my child stay quiet in places like libraries and movie theaters. | .03 | .70 |
| | 13. | When my child is playing with a friend and takes a toy away from his/her friend, I make him/her return the toy. | .08 | .62 |
| | 11. | When my child refuses to do something that he/she is supposed to do, I tell him/her to "Do it." | -.05 | .46 |
| | 12. | When out shopping with no plans to buy toys, even if my child says he/she wants a toy and refuses to leave the toy aisle, I do not buy the toy. | -.07 | .44 |
| | | Contribution (%) | 24.31 | 11.56 |
| | | Cumulative Contribution (%) | | 35.88 |
| | | Factor Correlation | F1 | .39 |

b) Parental support of physical activity

For parental support of physical activity, two factors were extracted (Table-3). The first factor was responsiveness and the second factor was demandingness. This factor structure was the same as that in previous research[8], and although the α coefficient was slightly low in terms of internal consistency, a constant coefficient of reliability was achieved. Furthermore, the degree of suitability of the modeling was high at GFI = .969, AGFI = .942, CFI = .949, and RMSEA = .059; thus, the factors' validity was confirmed, and an analysis using this two-factor structure was conducted.

### 3. Multiple regression analysis

Multiple regression analysis was repeatedly conducted with parenting attitude as a dependent variable and parental support of physical activity and past levels of competitiveness in sports as independent variables (Figures-1 and 2). Different path modeling was found for the fathers and mothers; in addition, for boys and girls, further differences in paths were observed. In terms of the five factors extracted from the subscales of parenting attitude and parental support of physical activity, the average value and standard deviation for parents (father, mother) × children (boy, girl) was calculated; for all factors, there was no significant difference between boys and girls. As such, it can be concluded that differences in the path modeling regarding the gender of the parents and children were not significant and were caused by the relationships with the parents.

For the father, a direct path from past levels of competitiveness in sports to parental support of physical activity and parenting attitude was not observed, but a path from parental support of physical activity to parenting attitude was observed. Among this, for the boys, all the subscales from responsiveness to aspects of parenting attitude were significant, and for the girls, the paths from demandingness to modeling and facillitation were significant. For the father, in terms of parenting attitude, there was no impact of past levels of competitiveness in sports, and for parental support of physical activity, the impact differed according to whether the child was a boy or a girl. Specifically, for boys, actions that heightened responsiveness were deemed effective to heighten parenting attitude of support when educating the child; for the girls, actions that heightened demandingness were deemed more effective.

As for the mother, in terms of the path from past levels of competitiveness in sports to parenting attitude, there was a positive path to boys' modeling and guidance, and a negative path to girls' modeling. This path modeling differs from that of the father; in particular, the negative path for the girls was significant, thus signifying the need to pay careful attention to the support provided to girls. For the

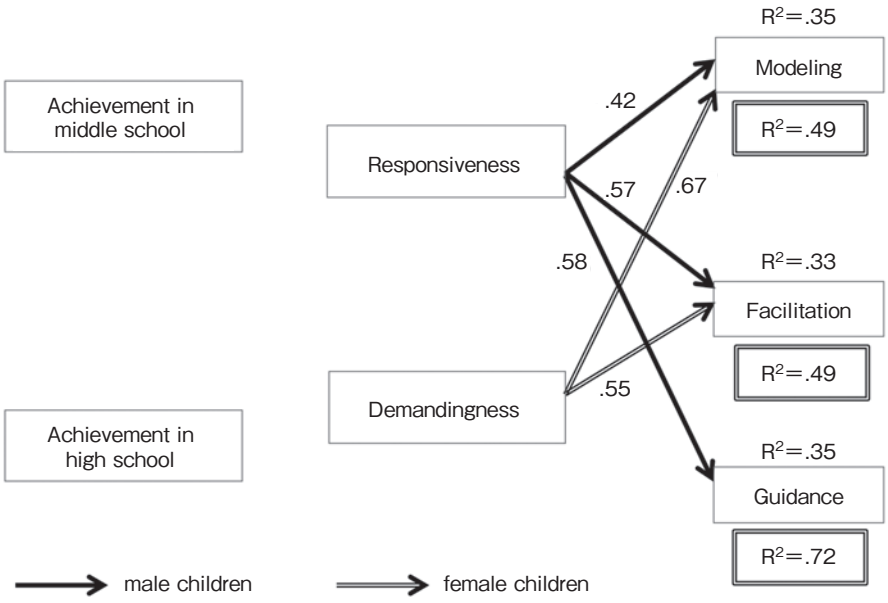


**Figure-1** Path analysis of the parental support (father)

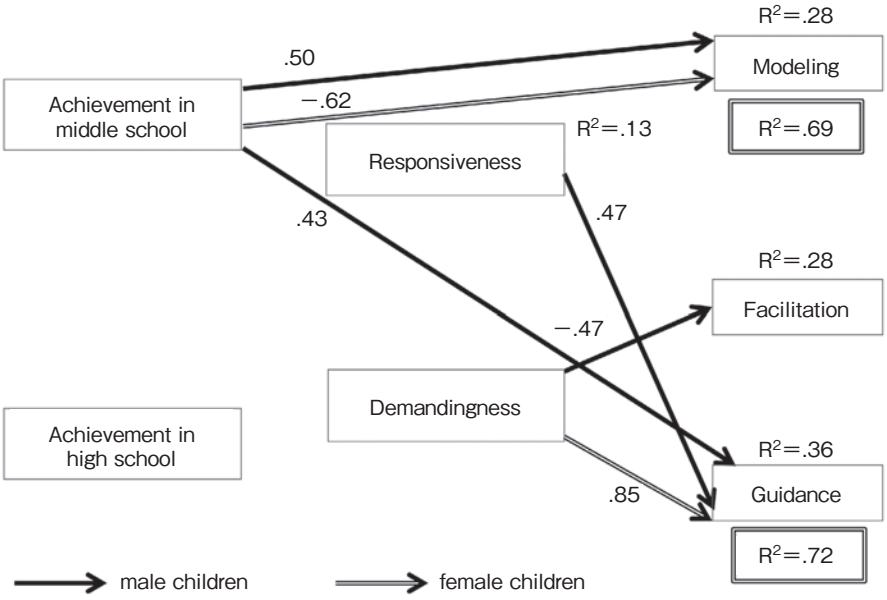


**Figure-2** Path analysis of the parental support (mother)

path from parental support of physical activity to parenting attitude, the positive path from the boys' responsiveness to guidance and the negative paths from the girls' demandingness to guidance and the boys' demandingness to facilitation were significant. The impact of parental support of physical activity on parenting attitude among mothers also differed from that among fathers, and several negative paths were observed; however, it was found that higher levels of responsiveness for boys and demandingness for girls positively impacted parenting attitude. In contrast to the fathers' results, the fact that negative paths could be observed for the mothers and the presence of differences according to the child's gender suggest the possibility that considering the child's gender and paying attention

to the specific factors that contribute to parental support of physical activity could result in a certain degree of improvement in parenting attitude.

In the future, when examining the impact on parenting attitude in addition to assessing the connection of the two factors of responsiveness and demandingness in terms of parental support of physical activity, research should be conducted to assess the connection between authoritative, authoritarian, and permissive parenting and the rise and fall of each factor, as Baumrind demonstrated[5]. Moreover, considering not only the parental support of physical activity but also the children's evaluations of their parents' behavior will lead to a more realistic understanding of the parent–child relationship and provide more information to foster effective parenting attitude.

## Conclusion

When we considered past levels of competitiveness in sports and parental support of physical activity as potential factors affecting parenting attitudes, the latter factor had an impact among both fathers and mothers, whereas the former factor was significant only among mothers. Parents should increase their emphasis on responsiveness when raising boys and on demandingness when raising girls to contribute to improvement in their parenting attitude.

## Acknowledgement

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# Japanese Adolescents Are the Most Physically Fit and Active in East and Southeast Asia

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We compared the level of physical activity, physical fitness, and obesity of adolescents among major metropolitan cities in East and Southeast Asia. A total of 12,588 valid adolescents' data (age 12-15 years) were collected from eight major metropolitan cities in East and Southeast Asia. Analytic items included body mass index (BMI), self-reported moderate to vigorous physical activity (MVPA), and whether or not students engaged in sports club activity. Physical fitness tests included Sit-and-reach Test, Hand Grip test, 1 Minute Sit-up Test and 15 meters Progressive Aerobic Capacity Endurance Run (PACER) Test All measures of Japanese adolescents were significantly ($p<0.05$) greater than the other adolescents. In particular, the effect sizes relating to endurance fitness, and MVPA were much higher than the effect sizes on the other measures. These findings suggested that Japanese adolescents were the most physically fit and active in Asian adolescents.

***Key words***: physical fitness, MVPA, BMI, junior high school students

## Introduction

There are strong evidences showed that lack of physical activity and low levels of physical fitness were important predictors for some chronic diseases including obesity and cardiovascular disease[1]. Some longitudinal studies also indicated that the physical fitness and physical activity levels during adolescence were associated with their disease risk in adulthood[2) 3)]. Some studies suggested that culture, education system, economic and social context may influence children's physical activity, physical fitness, and weight status[4]. Comparing the physical activity, physical fitness, and weight status is very important in physical activity promotion and obesity prevention in the future. In 2012, The Asia-Fit Study was launched in order to compare levels of physical fitness, physical activity and obesity of adolescents among major metropolitan cities in East and Southeast Asia[5]. The study report was summarized in a final report submitted to National University of Singapore-Global Asia Institute by Prof. Stanley Sai-Chuen Hui (The Chinese University of Hong Kong) and our study group. We also reported some conferences[6)-9)].

Focusing on Japanese data in the final report, we compared the level of physical activity, physical fitness, and obesity of adolescents among major metropolitan cities in East and Southeast Asia.

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## Methods

A total of 12,588 valid adolescents' data (age 12-15 years) were collected from eight major metropolitan cities in East and Southeast Asia, including Hong Kong, Shanghai, Tokyo, Seoul, Kuala Lumpur, Taipei, Singapore and Bangkok (Table-1). Analytic items included height, body weight, body mass index (BMI), self-reported moderate to vigorous physical activity (MVPA) (International Physical Activity Questionnaire: IPAQ), and whether or not students engaged in sports club activity (At least two hours activity per week). Physical fitness tests included Sit-and-reach Test (Modified Back Saver Sit-and-reach test)[10], Hand Grip test (Handgrip dynamometer, Takei scientific instruments, Grip-D), 1 Minute Sit-up Test and 15 meters Progressive Aerobic Capacity Endurance Run (PACER) Test[11]. Unpaired t-tests were used to test statistical differences between the means of Japanese adolescents and the other countries' adolescents. Cohen's d effect size was used to indicate the standardized difference between two means. For all analyses, significance was set at an alpha level of $p < 0.05$.

## Results and discussions

Prevalence of sports club participants with two hours or more of physical activity each week in Tokyo (Male: 49.7%, female: 36.7%) was considerably higher than the other cities (Male: 15.7%, female: 10.5%). T-tests, by gender, indicated that all measures of Japanese adolescents were significantly greater than the other adolescents (Table-2). In particular, MVPA, Cardiovascular endurance (PACER), and muscle endurance (1 Minute Sit-up) in Tokyo were higher than the

**Table-1** Sample size and age for each city

| City | Female | Male |
|---|---|---|
| Hong Kong | 13.48 ± 0.99 (N=792) | 13.56 ± 0.96 (N=834) |
| Tokyo | 13.30 ± 0.96 (N=798) | 13.31 ± 0.93 (N=892) |
| Shanghai | 13.97 ± 0.83 (N=785) | 14.05 ± 0.84 (N=814) |
| Taipei | 13.85 ± 0.89 (N=765) | 13.83 ± 0.92 (N=855) |
| Bangkok | 13.99 ± 0.83 (N=556) | 13.91 ± 0.88 (N=562) |
| Kuala Lumpur | 13.71 ± 1.04 (N=755) | 13.77 ± 1.03 (N=758) |
| Seoul | 13.41 ± 0.95 (N=784) | 13.29 ± 1.06 (N=902) |
| Singapore | 13.38 ± 1.22 (N=792) | 13.61 ± 1.19 (N=944) |
| Total | 13.62 ± 1.01 (N=6,027) | 13.65 ± 1.02 (N=6,561) |

Note. Average ± Standard deviation

**Table-2** Comparisons of Japanese adolescents and the other countries' adolescents

| Gender | Country | BMI ($kg/m^2$) | Hand Grip (kg) | Sit and reach (cm) | Sit up (reps) | PACER (reps) | MVPA (min/wk) |
|---|---|---|---|---|---|---|---|
| Male | Japanese | 19.5 ± 3.3* | 55.9 ± 14.9* | 50.7 ± 9.6* | 45.5 ± 12.3* | 72.7 ± 24.5* | 649.8 ± 591.6* |
| | The others | 20.9 ± 4.3* | 58.2 ± 14.8* | 51.7 ± 10.5* | 37.4 ± 10.8* | 45.8 ± 20.8* | 370.7 ± 425.0* |
| Female | Japanese | 19.5 ± 2.9* | 44.9 ± 8.4* | 57.2 ± 10.4* | 36.5 ± 10.7* | 45.2 ± 16.5* | 373.1 ± 424.9* |
| | The others | 20.6 ± 3.9* | 43.6 ± 8.9* | 56.2 ± 10.8* | 28.1 ± 9.8* | 27.6 ± 11.0* | 243.6 ± 299.4* |

Note. Average ± Standard deviation, *: Japanese vs. The others, $p < 0.05$

**Table-3** Effect size (Cohen's *d*) between Japanese adolescents and the other countries' adolescents

| Gender | BMI ($kg/m^2$) | Hand Grip (kg) | Sit and reach (cm) | Sit up (reps) | PACER (reps) | MVPA (min/wk) |
|---|---|---|---|---|---|---|
| Male | 0.32 | 0.16 | 0.10 | 0.74 | 1.26 | 0.62 |
| Female | 0.27 | 0.15 | 0.10 | 0.85 | 1.49 | 0.41 |

other cities (Table-2). The effect sizes on 1 Minute Sit-up (males: 0.74, females: 0.85), PACER (males: 1.26, females: 1.49), and MVPA (males: 0.62, females: 0.41) were much higher than the effect sizes on the other measures (Table-3).

According to a national survey in Japan[12], the rate of participation of sports club conducted outside school is quite low compared with sports club after class in school. Engaging in sports club activity obviously increases physical activity for both males and females. Therefore, our data demonstrated that high participation rate of sport club activity at school played a role in maintaining a high MVPA level among Japanese adolescents. These findings might suggest that promotion of sports club activity in school is one the most important factors to decrease physically unfit, especially in endurance fitness, and inactive adolescents in Asia.

## Conclusions

These findings suggested that Japanese adolescents were the most physically fit and active, especially endurance and MVPA level were much greater than the other countries' adolescents.

## Acknowledge

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Proceedings

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# A Validation Study for Estimating Vertical Stiffness and Leg Stiffness During Running in Children

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***Purpose***: The purpose of this study was to validate for leg and vertical stiffness estimated by using Morin's method.

***Method***: One hundred twenty seven children participated in this study. Each subject sprinted for 50 m. The motion through an interval from 30 m to 40 m was recorded with high-speed camera at 300 Hz from the sagittal plane. The running speed, contact time in stance phase and flight time were measured to estimate maximal force exerted on the foot ($F_{max}$), vertical stiffness ($k'_{vert}$) and leg stiffness ($k'_{leg}$) by Morin's method (2005). In gold standard method (MacMahon and Cheng, 1990), vertical excursion of center of mass and leg spring length variation was calculated from digitized landmarks and ratios of $F_{max}$ to vertical excursion of center of mass and to leg spring length variation were calculated as the vertical stiffness ($k_{vert}$) and leg stiffness ($k_{leg}$).

***Result***: All values are represented in mean value ± standard deviation (SD). $k'_{vert}$ was 15.51 ± 5.97 kN/m, whereas $k_{vert}$ was 17.92 ± 6.59 kN/m. The mean difference of the $k'_{vert}$ between $k_{vert}$ was −2.41 ± 2.62 kN/m. ICC between $k'_{vert}$ and $k_{vert}$ was 0.851 ($p<0.001$). Mean $k'_{leg}$ and $k_{leg}$ was 5.21 ± 1.91 kN/m and 6.81 ± 2.57 kN/m. ICC between $k'_{leg}$ and $k_{leg}$ was 0.642 ($p < 0.001$), while $k'_{leg}$ was highly correlated with $k_{leg}$ ($r = 0.839$). Additionally $k'_{leg}$ was underestimated (−23.6%) to $k_{leg}$.

***Conclusion***: We could conclude that Morin's method might be able to estimate vertical stiffness and leg stiffness in children, although vertical and leg stiffness tended to be underestimated.

***Key words***: accuracy, biomechanics, kinematics, kinetics

## Introduction

In many sports, running ability is required to move quickly the body from one place to another. The time which a person spent time from start to goal in a given distance is generally used to assess the running ability. The time is determined by the distance of the event and by the person's average speed over the distance. The speed at which the person runs is equal to the product of two factors: the stride length and stride frequency. It is, however, a lack of information to assess particularly the running ability for children in development, because running ability for children in development is influenced mainly by the physical fitness as of then[1].

During running, it is known that the leg supported the body behaves like a "spring"[2) 3)]. MacMahon and Cheng (1990) justified spring-mass model consisting of a particle of the body mass and a leg supporting the particle as a linear spring, so called "leg spring" (Figure-1)[3]. A main parameter to describe lower limbs as the leg spring is leg stiffness which is defined as a ratio of peak ground reaction force to the maximal variation of leg spring length during stance phase. Additionally, vertical stiffness is used to describe the vertical motion of the COM during the foot is in contact with the ground. Spring-mass model has been widely used to describe storage and return of elastic energy in

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lower limbs[3)-5)]. There is a possibility to be able to assess running ability for children in development by using leg and vertical stiffness.

Morin *et al* (2005) developed a simple method for estimating vertical stiffness and leg stiffness[6)]. By using this method, we were able to estimate leg and vertical stiffness from running speed, contact time, and flight time. These parameters could be measured by using video camera without using force platform and digitizing manually the anatomical landmarks of the body to calculate position of COM. Morin's method has, therefore, an advantage in the simplicity of the experimental system to measure parameters to estimate leg and vertical stiffness. In the fact, many previous studies employed Morin's method to estimate leg and vertical stiffness[7)-10)].

Validity of Morin's method to estimate leg stiffness and vertical stiffness were tested to compare values of leg and vertical stiffness calculated from measured maximal ground reaction force and leg spring length[6)]. Because the subjects in the validity test were ten young men, the tested validity is delimited in young adults. In our knowledge, it is not validated that vertical stiffness and leg stiffness in children estimated by using Morin's method. To apply Morin's method to children, it is necessary to investigate the validity of the leg and vertical stiffness estimated by using the method. The purpose of this study was to identify validity of Morin's method to estimate leg and vertical stiffness in children during running.


**Figure-1** Spring-mass model (MacMahon and Cheng, 1990)

## Method

### 1. Subjects

A hundred twenty seven children (age 6-12 yrs, height 1.122-1.756 m, body mass 17.1-70.8 kg) participated in this study. The protocol of this study was approved by the Ethics Committee of Department of Sports Science at Juntendo University.

### 2. Data collection

Each subject sprinted for 50 m. The motion through an interval from 30 m to 40 m was recorded with high-speed camera at 300 Hz from the sagittal plane (Figure-2). The running speed, contact time in stance phase and flight time was measured to estimate maximal value of normal force exerted on the foot ($F_{max}$), vertical stiffness ($k'_{vert}$) and leg stiffness ($k'_{leg}$) by Morin's method (2005).

### 3. Estimation of maximal value of normal force $F_{max}$

Maximal value of normal force $F_{max}$ was calculated as follows,

$$F_{max} = mg\left(\frac{t_f}{t_c} + 1\right)$$

where $m$, $g$, $t_f$, $t_c$ represent the body mass, gravitational acceleration, flight time , contact time. The equation of $F_{max}$, which based on a model used by Dalleau *et al.* (2004)[11)], was derived from inputting a half of $t_c$ into sin curve function of time F(t)[12) 13)].

$$F(t) = F_{max} \sin\left(\frac{\pi}{t_c} t\right)$$

The estimated $F_{max}$ was used to calculate leg stiffness and vertical stiffness in Morin's method and reference method.

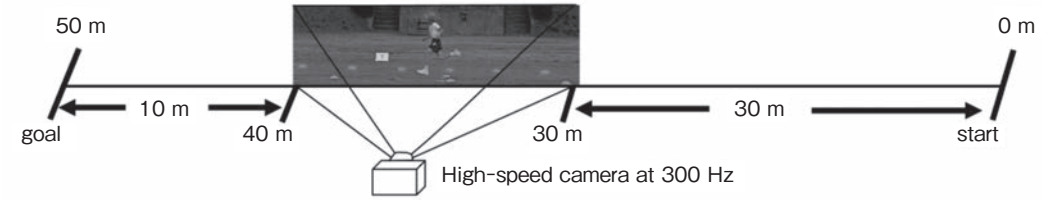


**Figure-2** Experimental set up in the present study

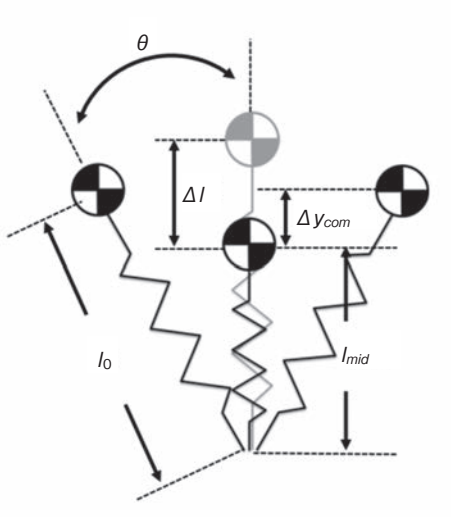


**Figure-3** Spring-mass model and parameters to calculate the leg stiffness and vertical stiffness
$\Delta y_{com}$: Vertical displacement of center of mass, $F_{max}$ : Maximal value of normal ground reaction force, $l_0$ : leg spring length at landing (= height × 0.53), θ : leg tilt angle to vertical axis at landing, $l_{mid}$ : leg spring length at middle of stance phase.

### 4. Morin's method to estimate leg stiffness $k'_{leg}$ and vertical stiffness $k'_{vert}$

Maximal displacement of COM $\Delta y'_{com}$ during stance phase was required to estimate leg stiffness $k'_{leg}$ and vertical stiffness $k'_{vert}$. Therefore, $\Delta y'_{com}$ was estimated from $F_{max}$, $t_c$, and $m$, as follows.

$$\Delta y_c = \frac{F_{max}\, t_c^2}{m\pi^2} + g\frac{t_c^2}{8}$$

Maximal displacement of leg spring length $\Delta l'$ was calculated as follows.

$$\Delta l' = \Delta y'_{com} + l_0 - \sqrt{l_0^2 - (v t_c/2)^2}$$

The $l_0$ represents the leg spring length at landing to the ground calculated from body weight × 0.53[14]. The υ represents the running speed calculated from the 30-40 m interval time.

From the above, follow equations to calculate $k'_{leg}$ and $k'_{vert}$ were derived.

$$k'_{leg} = F_{max}\ \Delta y'^{-1}_{c}$$

$$k'_{vert} = F_{max}\ \Delta l'^{-1}$$

### 5. Reference method to calculate leg stiffness and vertical stiffness

In gold standard method[2], ratio of $F_{max}$ to vertical excursion of COM and to leg spring length variation was calculated as the vertical stiffnes ($k_{vert}$) and leg stiffness ($k_{leg}$). Locations of COM in stance phase were calculated from digitized coordinates and body segment parameters[15]. And, leg spring tilt angle θ at landing was calculated from directional vector $\mathbf{r}=(r_x, r_y)$ form ankle joint to hip joint as follows,

$$\theta = \tan^{-1}\left(\frac{r_x}{r_y}\right)$$

where $r_x$ and $r_y$ represented horizontal and vertical components of the directional vector. This θ is an angle of the directional vector to the vertical axis; therefore the angle means leg spring tilt angle. Maximal displacement of leg spring length in reference method $\Delta l$ was calculated from following equation.

$$\Delta l = \Delta y_{com} + l_0(1 - \cos\theta)$$

From the above, follow equations to calculate $k_{leg}$ and $k_{vert}$ were derived.

$$k_{leg} = F_{max}\ \Delta y_c^{-1}$$

$$k_{vert} = F_{max}\ \Delta l^{-1}$$

### 6. Data analysis

To estimate a bias of leg stiffness and vertical stiffness in Morin's method, $k'_{leg}$ and $k'_{vert}$ were compared to $k_{leg}$ and $k_{vert}$ in reference method. Intra-class correlation coefficients (ICCs) of vertical stiffness and leg stiffness were calculated to assess a validity of the variables estimated by Morin's method relative to gold standard method. The statically significant level was set in 0.05. All values are represented in mean value ± standard deviation (SD).

## Result

Mean $k'_{leg}$ and $k_{leg}$ was 5.21 ± 1.91 kN/m and 6.81 ± 2.57 kN/m. The mean difference of the $k'_{leg}$ between $k_{leg}$ was -1.61 ± 1.42 kN/m. ICC between $k'_{leg}$ and $k_{leg}$ was 0.642 ($p < 0.001$), while $k'_{leg}$ was highly correlated with $k_{leg}$ ($r = 0.839$) (Figure-4).

$k'_{vert}$ was 15.51 ± 5.97 kN/m, whereas $k_{vert}$ was 17.92 ± 6.59 kN/m. The mean difference of the $k'_{vert}$ between $k_{vert}$ was -2.41 ± 2.62 kN/m. ICC between $k'_{vert}$ and $k_{vert}$ was 0.851 ($p < 0.001$) (Figure-5).

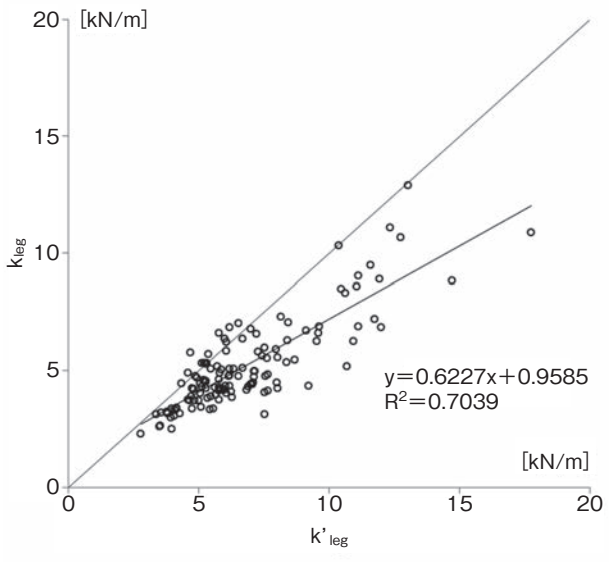


**Figure-4** Relationship between $k'_{leg}$ and $k_{leg}$

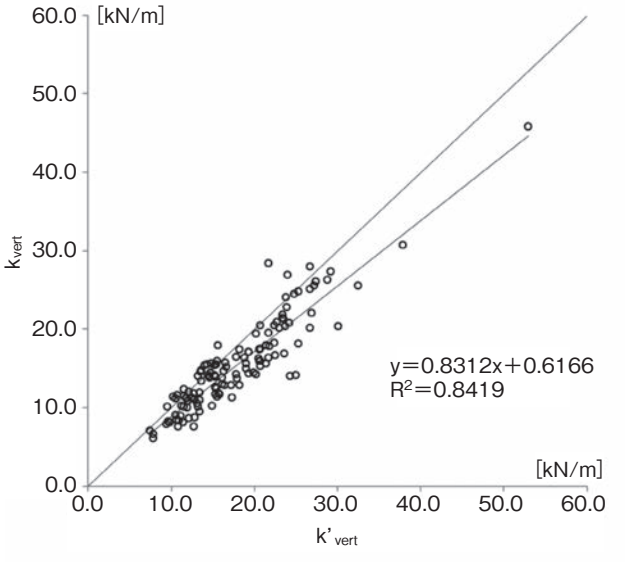


**Figure-5** Relationship between $k'_{vert}$ and $k_{vert}$

**Table-1** Parameters to calculate leg and vertical stiffness

| | Reference method | | Morin's method | | |
|---|---|---|---|---|---|
| | Δl (m) | Δy (m) | Δl' (m) | Δy' (m) | Fmax (N) |
| Mean | 0.123 | 0.057 | 0.167 | 0.049 | 846.4 |
| SD | 0.024 | 0.011 | 0.027 | 0.009 | 259.7 |

## Discussion

In the present study, leg stiffness and vertical stiffness during running were calculated using Morin's method and using reference method. We found that $k'_{leg}$ was highly correlated with $k_{leg}$ (Figure-4). Additionally $k'_{leg}$ was underestimated (-23.6%) to $k_{leg}$. Where mean $k'_{vert}$ demonstrated slightly lower value (-13.4%) relative to mean $k_{vert}$, $k'_{vert}$ was also highly correlated with $k_{vert}$. The tendency of the underestimation for leg stiffness was general agreement with the results reported by Morin *et al* (2005) [6]. However, the biases for $k'_{leg}$ and $k'_{vert}$ were found high in this study. This result represents that the bias of $k'_{leg}$ affected to accuracy of the individual values; however the tendency in the group of children could be validated because of the high relationship between variables in Morin's estimation and gold standard method.

A significance of this study was to test a validity of Morin's method to estimate leg and vertical stiffness for children. Leg and vertical stiffness during running over ground for one hundred twenty seven children showed small value relative to values of leg and vertical stiffness for ten young adults in previous study [6]. 95% continence intervals of $k'_{leg}$ and $k'_{vert}$ in one hundred twenty seven children were ranged from 4.87 to 5.53 kN/m, and from 14.53 to 16.61 kN/m, whereas $k'_{leg}$ and $k'_{vert}$ for ten young adults were ranged from 11.63 to 14.27 kN/m, and from 37.57 to 62.85 kN/m. The leg stiffness and vertical stiffness for children represented about 20-40% to the values for young adults. Morin *et al.* (2005) reported that the values estimated for leg and vertical stiffness highly correlated (leg stiffness: r=0.94; vertical stiffness: r=0.99) with reference values [6]. These results indicated that accuracy of Morin's method for children was lower than for young adults.

The mean values of main parameters to calculate $k_{leg}$, $k'_{leg}$, $k_{vert}$, $k'_{vert}$ were in Table-1. Mean differences between $k_{leg}$ and $k'_{leg}$ were determined from difference between Δl' and Δl. While Δl was calculated from sum of Δy and $l_0(1-\cos\theta)$ in reference method, Δl' was calculated from sum of Δy' and $l_0-\sqrt{l_0^2-(vt_c/2)^2}$. Thus, the determinants of

the differences between $k_{leg}$ and $k'_{leg}$ were a) the difference between $\Delta y'$ and $\Delta y$, and b) the difference between $l_0(1-\cos\theta)$ and $l_0-\sqrt{l_0^2-(vt_c/2)^2}$. The difference between $\Delta y'$ and $\Delta y$ determined 13.2% of the difference between $k_{leg}$ and $k'_{leg}$, whereas the difference between $l_0(1-\cos\theta)$ and $l_0-\sqrt{l_0^2-(vt_c/2)^2}$ determined 86.8% of the difference between $k_{leg}$ and $k'_{leg}$. The main factor of the difference between $k_{leg}$ and $k'_{leg}$, therefore, was the difference between $l_0(1-\cos\theta)$ and $l_0-\sqrt{l_0^2-(vt_c/2)^2}$. Morin *et al.* (2005) analyzed the effects of variations of the different mechanical parameters consisting of the Morin's model on the estimated leg and vertical stiffness[6]. They concluded that contact time was most sensitive parameter in the mechanical parameter. The main factor of the difference between $k_{leg}$ and $k'_{leg}$ in the result of this study included the contact time; therefore it might be affected from sensitivity of contact time.

## Conclusion

We could conclude that Morin's method might be able to estimate vertical stiffness and leg stiffness in children, although vertical and leg stiffness tended to be underestimated. The evidence of this study should be taken care of to interpret correctly the values.

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# The Relationship Between Birth Month, Physical Size, Motor Ability and Physical Activity Evaluated by Kindergarten Teachers Among Japanese Young Children

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***Objective***: The purpose of this study was to examine the relationship between birth month, physical size, motor ability, and physical activity evaluated by kindergarten teachers, of young Japanese children by using structural equation modeling.

***Methods***: We collected the data from 329 children (160 four-year-old children: 76 male and 84 female, and 169 five-year-old children: 85 male and 84 female). Ten teachers in charge of children's classes in the kindergarten were administered questionnaires, and the children's physical size, motor ability, and children's physical activity, as determined by kindergarten teachers, were investigated. Structural equation modeling was used to examine the above-mentioned variables in each grade.

***Results***: The results showed significant goodness of fit in both 4- and 5-year-old children. Specifically, the model in 4-year-old children showed a significant scale, a good fit to the model according to the approximate fit indices (Goodness of Fit Index [GFI]=0.938, Adjusted Goodness of Fit Index [AGFI]=0.894, Comparative Fit Index [CFI]=0.967, and Root Mean Square Error of Approximation [RMSEA]=0.043). The model in 5-year-old children showed a significant scale and a good fit to the model according to the approximate fit indices (Goodness of Fit Index [GFI]=0.938, Adjusted Goodness of Fit Index [AGFI]=0.894, Comparative Fit Index [CFI]=0.936, and Root Mean Square Error of Approximation [RMSEA]=0.043). In both 4- and 5-year-old children, birth month influenced physical activity evaluated by kindergarten teachers, mediating physical size and motor ability.

***Conclusions***: We concluded that the birth months influenced children's physical activity evaluated by kindergarten teachers through physical size and motor ability.

***Key words***: relative age effect, physical size, motor ability, evaluation from teachers

## Introduction

In Japan, various measures have been considered to resolve problems such as decline in physical fitness and reluctance to exercise in children due to their negative attitude to exercise and sport activities[1]. On this basis, the Course of Study for Preschools and Guidelines of Nursing for Nursery Schools in Japan were revised in 2008. In the guidelines, improvement of physical strength and health was indicated as necessary, for which everyday play time and exercises are considered essential[2) 3)]. Moreover, in 2014, "Physical Activity Guideline for Japanese Young Children" was developed as a specific guideline for the types of exercises that were performed and the types of capabilities to be acquired according to the developmental stages in children[4].

In recent years, "relative age effect (RAE)" has become a focus for a positive or negative attitude to exercise or sports among children[5]. RAE is defined as the consequence of relative age differences between individuals within the same cohort, either in school or on sports teams[6]. Children born from April 2 in a year to April 1 in the next year enter schools in the same grade under the Japanese

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school education system. In such a setting, up to 365 days of difference in the growth period between children in the same grade is inevitably associated with a difference in growth and development (observed, in particular, in younger children). Such a difference in relative age not only causes differences in growth and development but also affects learning and proficiency[7)-9)]. Especially, in case of professional athletes (playing soccer, baseball, and basketball) in a sport domain, a large numbers of players with high relative age have a benefit in terms of growth and development[6) 10)].

Although the mechanism underlying RAE is still unclear in previous research, the following is considered: relatively older children experience a greater benefit in terms of physical size, motor ability, and physical activities or sports scenes. This experience fosters self-concept, sport competence and motivation for physical activity. RAE may cause a positive cycle in relatively old children and vice versa[11)]. In other words the relatively old children have a blessed physical size with age, thereby they are able to get the score of motor ability better than relatively young children, and to get the many opportunity for challenges of sports. In this way the relatively old child is able to get an advantage of sports and positive cycle. However, few studies have verified a model of RAE in a sports setting.

In Japan, RAE on physical size, and motor ability, and physical activity evaluated by kindergarten teachers has been reported[5)]. Moreover, motor ability and evaluation by teachers are correlated. In other words, the difference in development caused by birth month (relative age) may be associated either directly or indirectly with physical size and motor ability, and physical activity, as evaluated by kindergarten teachers.

Therefore, the purpose of this study was to examine the relationship between birth month, physical size, motor ability, and physical activity evaluated by kindergarten teachers in young Japanese children by using structural equation modeling.

## Method

### 1. Participants

We collected the data from 329 children (160 four-year-old children: 76 male and 84 female, and 169 five-year-old children: 85 male and 84 female) in July 2011. We conducted a questionnaire investigation with 10 teachers in charge of children's classes in the kindergarten.

### 2. Measurements

We investigated demographic data (sex, age, and grade), children's physical size (body height, the body weight, and sitting height), motor ability (20-m sprint, standing broad jump, and softball throwing), and physical activity evaluated by kindergarten teachers.

a) Demographic data

We asked children's sex, grade, age, birthdate from the kindergarten teachers using a short questionnaire.

b) Physical size

Children's physical size was measured using body height, body weight, and sitting height.

c) Motor ability

Children's motor ability was assessed using 20-m sprint time, the standing broad jump, and softball ball throwing. For the 20-m sprint, we set a 25-m straight alley and created goal lines at 20-m (true goal line) and at 25-m (fake goal line). The measurer stood at the 25-m goal line, and they recorded each child's time from the start cue ("set and go") to the moment children went through at the 20-m line. For the standing broad jump, we set a 3-m tape on the floor and drew a balk line. We instructed the children to jump as far as they could from the balk line, using both their right and left feet at the same time. For the softball throwing, we adopted a relatively smaller official softball (the size of the ball was No.1 for school education). We instructed the children to throw the ball as far as possible, using their dominant hand. Before the measurement trials, we first showed the children how each of these activities were to be performed. Two trials were carried out to assess motor ability, and the best score was used in the analysis. All measurements were performed at around 10:00 a.m..

d) Evaluation of children's physical activity by kindergarten teachers

Children's physical activity, evaluated by kindergarten teachers, was based on children's attitude, motivation, skills, and enjoyment in the physical activity field. We asked the kindergarten teachers to answer the following questions: "How do you feel

about the child's attitude in the physical activity field (children's attitude in the physical activity field)", "How do you feel about the child's motivation in the physical activity field (children's motivation in the physical activity field)", "How do you feel about the child's skill in the physical activity field (children's skill in the physical activity scene)", and "How do you feel about the child's enjoyment in the physical activity field (children's enjoyment in the physical activity field)". Respondents were requested to answer each item on a 5-point scale: "Not good (1 point)" to "Good (5 points)".

### 3. Analysis

Before the analysis, data were checked to adjust for any missing value. Subsequently, we performed a statistical analysis. All statistical analyses were conducted using the SPSS 21.0 for Windows and the AMOS 21.0. In order to examine the relationship between children's birth month, physical size, motor ability and physical activity evaluated by kindergarten teachers, we used structural equation modeling, which is a statistical method to test a conceptual or theoretical model [12]. We assumed that birth month influences physical activity evaluated by kindergarten teachers, reflecting an influence on physical size and motor ability. Figure-1 shows the hypothetical model of this study.

## Results

Figure-2 and Figure-3 shows that the results of structural equation modeling. We obtained significant goodness of fit for both 4- and 5-year-old children. Specifically, the model for 4-year-old children showed a significant scale $\chi^2$ ($\chi^2$=126.19, df=78, p<0.001), a good fit to the model according to the approximate fit indices (Goodness of Fit Index [GFI] = 0.938, Adjusted Goodness of Fit Index [AGFI] = 0.894, Comparative Fit Index [CFI] = 0.967, and Root Mean Square Error of Approximation [RMSEA] = 0.043). Furthermore, the standardized path coefficients for items (the path from birth month to physical size, the path from physical size to motor ability, and the path from motor ability to evaluation) were higher than 0.3 and were significant (Figure-2).

The model for 5-year-old children showed a significant scale $\chi^2$ ($\chi^2$=126.19, df=78, p<0.001), a good fit to the model according to the approximate fit indices (Goodness of Fit Index [GFI] = 0.938, Adjusted Goodness of Fit Index [AGFI] = 0.894, Comparative Fit Index [CFI] = 0.936, and Root Mean Square Error of Approximation [RMSEA] = 0.043). Furthermore, the standardized path coefficients for items (the path from birth month to physical size, the path from physical size to motor ability, and the path from motor ability to evaluation) were higher than 0.3 and were significant (Figure-3). In both 4- and 5-year-old children, birth month influenced children's physical activity evaluated by teachers, mediating physical size and motor ability.

## Discussion

The main goal of this study was to clarify the

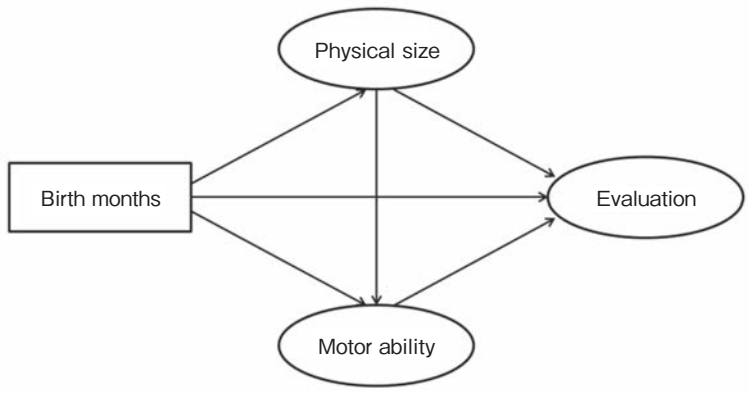


**Figure-1** The structural equation modelling

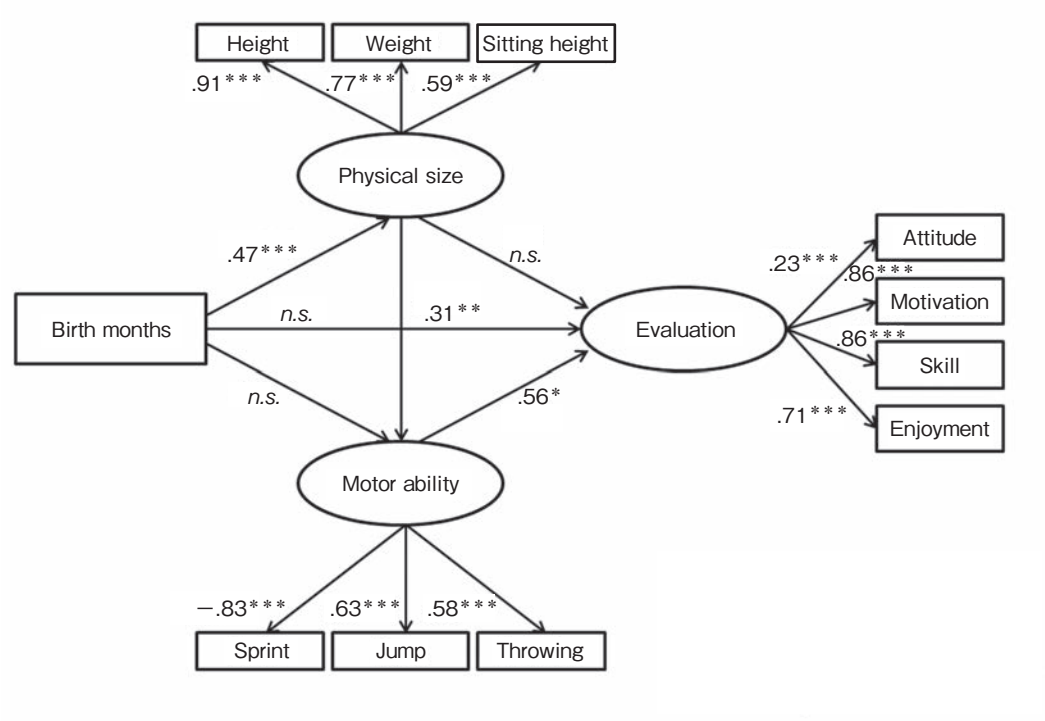


**Figure-2** The structural equation modelling (4-year-old children)
$\chi^2$=126.19, *df*=78, p<0.001, GFI=0.938, AGFI=0.894, CFI=0.967, RMSEA=0.043
*p<.05., **p<.01., ***p<.001., *n.s.*: not significant

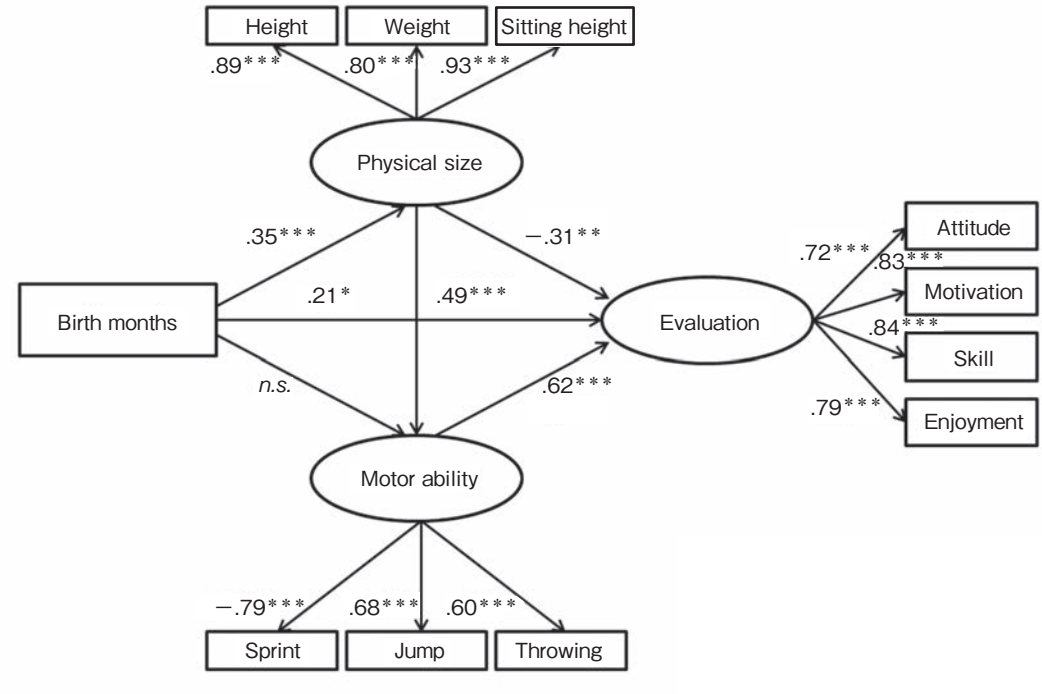


**Figure-3** The structural equation modelling (5-year-old children)
$\chi^2$=126.19, *df*=78, p<0.001, GFI=0.938, AGFI=0.894, CFI=0.936, RMSEA=0.043
*p<.05., **p<.01., ***p<.001., *n.s.*: not significant

relationship between children's birth month, physical size, motor ability and physical activity evaluated by kindergarten teachers. Our study had the following four major findings.

First, birth month influenced children's physical activity through physical size and motor ability. These results support the findings that RAE influences young Japanese children's physical size, motor ability, and physical activity evaluated by kindergarten teachers. However, $\chi^2$ values were significant, indicating insufficiency of a fundamental measure of fit. This is because this statistic method is sensitive to a sample size and covariance of the observed variables. Children's measured values varied widely.

Second, children's birth month had a significant effect on physical size. This reflected differences in physical according to relative age. That is, children with a high relative age have better physical sizes.

Third, physical size significantly affected motor ability. These results explain why RAE influences motor ability. Because relatively-older-age children show a benefit in physical development according to relative age, they have better motor ability. However, in 5-year-old children, the effects of physical size on physical activity evaluation are negative, indicating that children with a better physical size may receive low physical activity evaluation. This is because weight varies with physical size. Some children from the 5-year-old class may have been overweight and may not have had good motor ability. Thus, it is important to understand how difference in physical size affects motor ability during sport or exercise.

Finally, children's motor ability significantly affected physical activity evaluation, suggesting that kindergarten teachers evaluated children's attitudes based on motor ability. As stated earlier, there are large differences in physical sizes between children, reflecting differences in physical development according to relative age. Thus, it is important to reduce the possibility of underestimation of physical activity by devising a method of implementation of sport or exercise.

## Conclusions

We conclude that birth months affects children's physical activity through physical size and motor ability. Thus, we propose that teachers in kindergartens should pay attention to differences in physical development according to relative age during evaluation.

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# Physical Activity of Adolescents in a Medium-Sized City in China

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***Objective***: An important strategy for achieving healthier body compositions is to encourage lifetime physical activity (PA) participation in overweight or obese adolescents. The purpose was to compare the association between PA and obesity among adolescents in China, and to identify the factors that influence the PA and obesity.
***Methods***: In 2013, a cross-sectional study was involved 962 Chinese children and adolescents aged 12–16 years (mean age 14.9 ± 0.9). The adolescents were classified as normal weight (NW), overweight (OW), and obese (OB), according to the BMI cut-offs that was determined by an obesity working in China. The adolescents were recruited for anthropometric measurements and to complete a standardized PA questionnaire. Other confounding variables such as the parents' demographic information, and lifestyle factors were also collected through the questionnaire.
***Results***: Nine hundred thirty-seven valid data were collected from the adolescents. The prevalence of obesity was 8.8% (boys: 9.9%, girls: 7.5%) for junior high-school students (JS) and 7.8% (boys: 12.7%, girls: 4.3%) for high-school students (HS). Regardless of the sex and weight status, the probabilities for achieving PA for HS were less than JS. Moreover, the OW and OB groups had significantly less PA, watched more television, or used the computer more often than the NW groups ($p < 0.05$).
***Conclusion***: The adolescents consistently failed to meet the established PA recommendations and supported the age-related declines in PA. In this study, we highlighted the importance of promoting PA to adolescents, especially those who are OW and OB.

***Key words***: physical activity, adolescents, questionnaire, China

## Introduction

Childhood obesity is a pandemic that is associated with physical and psychological morbidities in children. It also contributes to an increase in non-communicable chronic diseases and premature mortality in adulthood[1]. In mainland China, the rate of increase in childhood obesity prevalence exceeds the trends seen in many other countries over the last 2 decades. Satisfactory results in childhood obesity control have been obtained through tactics that stimulate the decrease in sedentary behaviors[2]. According to Santaliestra-Pasias *et al.*[3], children and adolescents spend much of their leisure time with low intensity and low caloric expenditure activities. Thus, spending more time in less vigorous activities, such as watching TV, and using the computer, has contributed to weight gain in adolescents. The main physiopathology of this disorder is the positive balance of energy intake[4].

An important strategy for achieving healthier body compositions is to encourage lifetime physical activity (PA) participation among overweight or obese children and adolescents[5]. PA surveillance in developed countries has focused primarily on sports and exercise either during or before school. Likewise, we know little about the physical inactivity (e. g., television watching, studying, or passive games) of youth in developing countries.

As a developing country, China is currently experiencing rapid changes in health and nutrition that are linked to political and social reforms that

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were initiated more than 20 years ago. Although there are differences among regions, previous reports suggested that the epidemic of childhood overweight and obesity in China has been spreading all over the country since the end of the twentieth century[6]. In order to respond to this situation, the Chinese government released a new policy in 2007, requiring daily 1 h PA to help the children achieve current Chinese PA guidelines (i.e., doing any kind of PA for a total of 60 minutes or more per day)[7]. However, the Chinese National Physical Fitness and Health Surveillance reported in 2010 that only 22.7% of Chinese students aged 9–18 had achieved the goals of the Chinese PA guideline[8]. Moreover, studies of physical activity questionnaires in China mainly focused on people living in the super urban metropolis[9]. There were no related reports involving people from medium-sized cities such as Loudi. Thus, the purpose of this study is to compare the association between PA and obesity in adolescents, and to identify the factors that influence the PA and obesity in South China.

## Method

### 1. Participants

A cross-sectional study was performed involving 962 participants (500 boys and 462 girls), aged 12–16 years, who were recruited from a middle and high school in Loudi (a city in South China). Those students who refused to participate were excluded from the study. Participants were included if they appeared healthy, and without any health problems that might inhibit PA. For participants who had difficulty understanding the questionnaire, the trained interviewer explained it to them. The subjects provided their informed consent to participants in a study protocol, which adhered to the Declaration of Helsinki, and was approved by the ethical committee of the Juntendo University.

### 2. Anthropometrics

All anthropometric data were collected by trained staff and supervised by the school nurse. Height and body weight were taken by using a portable stadiometer, and digital scale (TCS-200-RT; YaoYi, Shanghai, China). Height was measured to the nearest 0.1 cm without shoes, and body weight was measured to the nearest 0.1 kg in light underwear. BMI was calculated using the following standard equation: BMI = weight in kg/height squared in meter. Weight status, i.e., normal weight (NW), overweight (OW) and obese (OB) were determined according to the Working Group on Obesity in China with age- and sex-specific cut-offs[10].

### 3. Questionnaire development

The present study was conducted from September to October 2013. We used a questionnaire to characterize the levels of PA that were performed in the preceding seven days before the questionnaires were administered, which had been translated and adapted to exclude PA. The questionnaire consisted of question about sports and games as well as PA at school, and in their leisure time, including weekends. The Chinese short-form version of the International Physical Activity Questionnaire (IPAQ)[11] was used to measure the level of PA. The short form measures PA across all domains of leisure time, work transportation, and household tasks. It asks the respondents to report the duration (in minutes) and frequency (days) of performing working, moderate to vigorous physical activity (MVPA), for at least 10 minutes per session, during the previous 7 days. The IPAQ short form was considered flexible enough to be used in telephone interviews or for self-administered applications, and adaptable enough to apply across cultures[11].

Other confounding variables such as the health status, self-efficacy, family, and peer support for PA, screen-based behaviors, and sports facilities in the neighborhood, were also collected through the questionnaires. The screen-based behaviors included using the computer and playing electronic games. Children reported the total time that they engaged in these activities during weekdays and weekends in the past week according to 15 items (range 0–15)[12].

### 4. Statistical analysis

Both boys and girls were divided into three groups (NW, OW, and OB). Analyses were conducted by using the SPSS statistical software (SPSS Inc., IBM, USA). The results were given as mean ± SD or n (%), as appropriate. We estimated

**Table-1** Physical characteristics of adolescents by weight status

| Boys | ALL N=482 | | NW N=346 | | OW N=82 | | OB N=54 | |
|---|---|---|---|---|---|---|---|---|
| | JS (N=261) | HS (N=221) | JS (N=199) | HS (N=147) | JS (N=36) | HS (N=46) | JS (N=26) | HS (N=28) |
| Age (yr) | 12.2 ± 0.8 | 15.0 ± 0.8 | 12.2 ± 0.7 | 15.1 ± 0.8 | 12.2 ± 0.8 | 14.7 ± 0.8 | 12.3 ± 0.8 | 15.0 ± 0.8 |
| Height (cm) | 157.6 ± 8.3 | 171.6 ± 5.9 | 156.9 ± 8.1 | 171.6 ± 6.2 | 159.1 ± 8.6 | 171.7 ± 5.0 | 160.8 ± 8.6 | 171.4 ± 6.4 |
| Weight (kg) | 47.0 ± 11.0 | 62.1 ± 11.3 | 42.6 ± 6.8 | 56.1 ± 7.0 | 56.6 ± 7.1 | 69.7 ± 5.2 | 67.4 ± 10.3 | 81.1 ± 8.0 |
| BMI (kg/m$^2$) | 18.8 ± 3.5 | 21.1 ± 3.3 | 17.2 ± 2.0 | 19.0 ± 1.9 | 22.3 ± 1.0 | 23.6 ± 1.2 | 25.9 ± 2.0 | 27.6 ± 1.7 |
| **Girls** | **ALL N=455** | | **NW N=357** | | **OW N=72** | | **OB N=26** | |
| | JS (N=199) | HS (N=256) | JS (N=159) | HS (N=198) | JS (N=25) | HS (N=47) | JS (N=15) | HS (N=11) |
| Age (yr) | 12.0 ± 0.7 | 14.8 ± 0.8 | 12.0 ± 0.7 | 14.9 ± 0.8 | 12.0 ± 0.7 | 14.7 ± 0.6 | 12.1 ± 0.9 | 14.5 ± 0.5 |
| Height (cm) | 155.7 ± 5.5 | 160.3 ± 5.6 | 155.8 ± 5.1 | 160.6 ± 5.6 | 155.8 ± 6.5 | 159.5 ± 5.7 | 155.6 ± 7.7 | 158.9 ± 5.0 |
| Weight (kg) | 44.7 ± 7.5 | 51.6 ± 7.4 | 42.2 ± 5.6 | 49.1 ± 5.5 | 51.6 ± 4.7 | 58.8 ± 5.0 | 58.9 ± 6.9 | 67.6 ± 6.2 |
| BMI (kg/m$^2$) | 18.4 ± 2.7 | 20.1 ± 2.7 | 17.4 ± 1.8 | 19.0 ± 0.8 | 21.2 ± 1.0 | 23.1 ± 1.1 | 24.2 ± 1.4 | 26.7 ± 1.7 |

BMI: body mass index, JS: junior high school, HS: high school, NW: normal weight, OW: overweight, OB: obese, Mean ± S.D.

**Table-2** The prevalence of overweight and obesity for adolescents in both Junior high school (JS) students and High school (HS) students

| Boys | JS | HS |
|---|---|---|
| Overweight | 13.8 | 20.9 |
| Obesity | 9.9 | 12.7 |
| **Girls** | **JS** | **HS** |
| Overweight | 12.6 | 18.4 |
| Obesity | 7.5 | 4.3 |

(%)

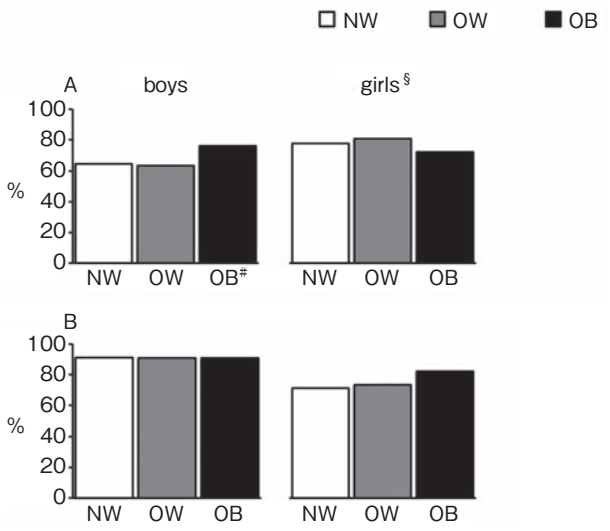


**Figure-1** The prevalence of the inability to achieve the recommended of 60 minutes MVPA by (**A**) Junior high school, (**B**) high school

NW: normal weight; OW: overweight; OB: obese; § vs. boys, # vs. NW, $p<0.05$

the overweight and obesity (%) prevalence among the different groups. Student's t-test and Kruskal-Wallis ANOVA were used to determine significant differences between the weight status and the frequencies of screening time. When the Kruskal-Wallis ANOVA was significant, the Mann-Whitney U test was used in a post hoc analysis to determine significance of differences. A p-value < 0.05 was considered significant.

## Results

### 1. Participant characteristics

The anthropometric characteristics of the sample, such as weight, height, and BMI are shown in Table-1. Nine hundred thirty-seven participants were evaluated in this study, with 482 boys and 455 girls, aged 12-16 years. The prevalence of overweight and obesity were 13.8% and 9.9% as well as 12.6% and 7.5% for the boys and girls' junior high school (JS) participants aged 12 to 13 years, respectively (Table-2). Among high school (HS) participants aged 14 to 16 years, the prevalence of overweight and obesity were 20.9% and 12.7% as well as 18.4% and 4.3% for the boys and girls, respectively. In this sample, the prevalence of OB was higher among the boys ($p<0.05$).

### 2. The probability of failing to achieve the WHO recommendations

The relationship between weight status and the

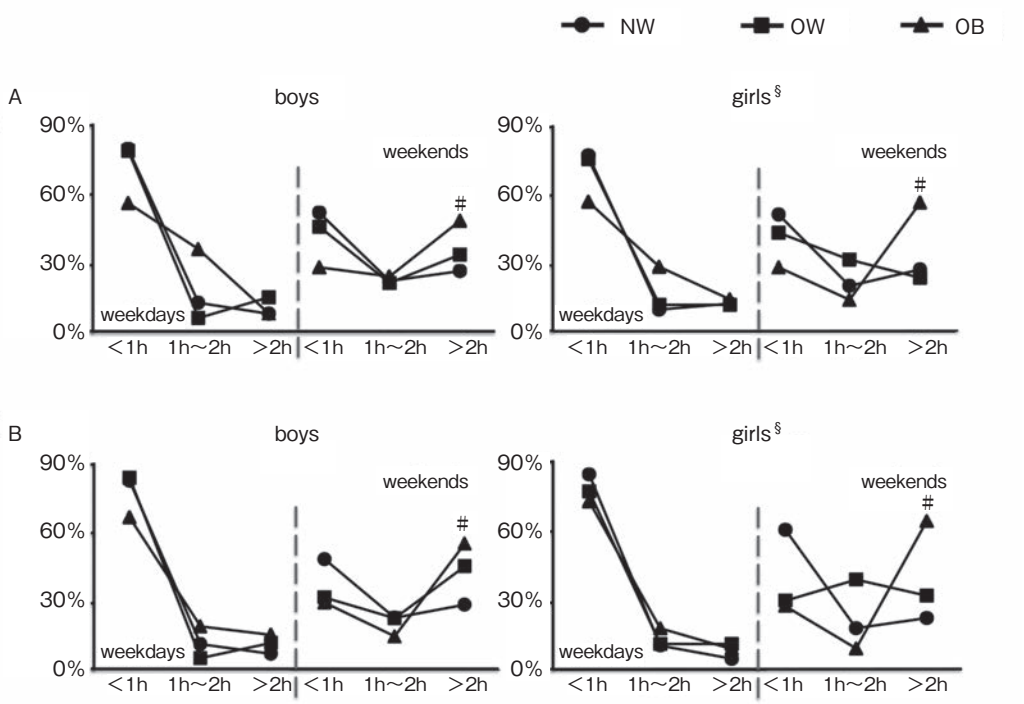


**Figure-2** The relationship between weight status and screen time among adolescents by (**A**) Junior high school, (**B**) high school
NW: normal weight; OW: overweight; OB: obese; § vs. boys, # vs. NW, $p<0.05$

probability of failing to achieve the WHO recommendations (60 min of MVPA per day) for JS and HS participants are shown in Figure-1. The probability of failing to achieve the WHO recommendations for adolescents varied among the student groups. Among the boys JS participants, this was 64.7%, 63.6%, and 76.0% in the NW, OW, and OB groups, respectively. Among the girls JS participants, the probability was 70.7%, 72.7%, and 81.5% in the NW, OW, and OB groups, respectively. The probability of failure was 77.0%, 80.0%, and 71.4% for the NW, OW, and OB boys HS participants, and 91.1%, 90.9% and 90.9% for the NW, OW, and OB girls HS participants, respective. The boys performed significantly more PA compared to the girls ($p < 0.05$). The probabilities of failing to achieve the WHO recommendations were significantly higher in the OW and OB compared to the NW groups for boys, except among the girls ($p < 0.05$).

### 3. The probabilities of screen time

The relationship between weight status and screen time for JS and HS participants are shown in Figure-2. The percentage of screen time of more than 2 h per day for adolescents was 17.1%, 24.2%, and 28.0% for the NW, OW, and OB boys JS participants, respectively. Among the girls JS participants, the probabilities were 20.1%, 18.0%, and 35.7% for the NW, OW, and OB, respectively. For the NW, OW, and OB boys HS participants, the probabilities of screen time were 17.5%, 28.4%, and 35.2%, and 13.6%, 21.6%, and 36.4%, for the NW, OW, and OB girls HS participants, respectively. The boys had significantly less screen time than their girls ($p < 0.05$). The percentage of screen time of more than 2 hours was significantly higher in OB compared to NW groups for the girls' participants ($p < 0.05$). This was not observed in the boys.

## Discussion

This study presents data on the relationship between PA and obesity among adolescents in a medium-sized city in the south of China. Furthermore, there was strong evidence to show that higher the values of BMI, the higher the probabilities of failing to achieve the recommendations, and the prevalence of Chinese youth, especially OW and OB who exceed the recommended screen time

guideline increased significantly.

Some factors that may be associated with the increasing rates of overweight and obesity among adolescents in China have been investigated. Similar to pervious reports, our results also showed that OW and OB adolescents were spending more screen time hours compared to NW adolescents. Chen *et al.*,[13] found that spending more hours watching television and surfing the internet were significantly associated with the increased risk of obesity among the Chinese youth. Moreover, Ahn *et al.*,[14], reported that nutrition transition was accelerating and the result of this trend was a rapid increase in obesity and chronic diseases.

As shown in this study, approximately 60% of adolescents spend more than two hours on screen time per day. In this context, previous studies have shown a direct association between hours spent in front of the screen and weight. This fact may be associated with the lack of parental control over this habit, which results in children desiring the sweets and candy that are shown in TV or computer advertisements.

The results in this study may be due to the high priority that Asian cultures place on education, and the academic pressure to excel is put on the students by their parents and schools. The time spent in sedentary educational activities increased steeply after 3 years of follow-up in both boys and girls, corresponding to the time when students are preparing for junior high school or high school graduation exams, followed by entry into high school or university. During this time, students often spend more time on extracurricular tutoring in addition to homework, including evening classes in private institutions as well as private classes in order to prepare students for the final junior high school or high school examinations.

The lack of association between the screen time and socioeconomic status and income that was observed in this study differs from previous studies. When evaluating screen time among schoolchildren form Niteroi, RJ, Vasconcellos *et al.*,[15], found that this practice was significantly more prevalent among girls. Regarding socioeconomic status, a previous study showed its positive association with screen time. Moreover, according to Keihner *et al.*,[16] lower income adolescents spend more time on activities such as watching TV compared to those belonging to higher income families.

Corroborating these results, Babey *et al.*,[17], also found that adolescents who were more involved in PA spent less time watching TV or using the computer. Furthermore, the association between excess weight and daily hours watching TV that was observed in this study confirms the results reported by Vasconcellos *et al.*,[15] which also found a significant direct association between these variables. Moreover, this study also supports the results reported by Santaliestra-Pasias *et al.*,[3] who found that the reduction of time spent in sedentary behaviors can be used as a strategy to address adolescents obesity.

We recognize some strengths and weaknesses in our study. The study includes a relatively large and diverse sample, a comprehensive measure of the lifestyle environment and physical activity according to the different weight status of the adolescents in China. Limitations include the used of the questionnaire rather than objective assessment tools like the accelerometer to measure physical activity levels. Nonetheless, questionnaires are widely accepted in large-scale surveys with reasonable reliability for analysis. Secondly, the relatively small sample size, as well as the convenience sampling method used in our study, limited the general applicability of the study. Finally, the current research employed cross-sectional data and we therefore cannot draw causal conclusions on the basis of these findings.

## Conclusion

In this survey, the failure of the adolescents to consistently meet established PA recommendations supported the age-related declines in PA. Therefore, it is necessary to stimulate interactive activities and promote more active lifestyles, by reducing the time that young individuals spend in front of the screen time, which contributes to the reduction of PA and excess weight in this population. The interplay between weight statuses, PA, sex, age, and the influence of obesity therein remain inadequately understood and require further research.

## Acknowledgements

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cents and their families who participated in this study. The authors wish to express their sincere appreciation to Mr. Wenquan, Wu and Mr. Liangfu, Zhou for their technical assistance.

## Conflict of Interest

The authors declare that there are no conflicts of interest.

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# The Relationship Between Toe Grip Strength and Physical Fitness in Elementary School Children

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***Introduction***: Toes are the only parts of the body connected to the ground, providing both tactile and pressure information through the plantar afferents, somatic sensation through these organs is also very important for various movements, including standing and walking. In particular, the toes control whole body posture and movement, and can generate propulsive force during walking and prevent forward falls. Toe function has been often represented as toe grip strength in various studies. Some investigators have found that low toe grip strength (TGS) is an important risk factor for falls among elderly individuals (Endo *et al.* 2002). The other side a decrease in children's physical strength and athletic ability has become a problem in recent years. The present study was aimed to reveal the relationship between TGS and physical fitness in elementary school children.

***Methods***: From first to sixth grade 274 primary school children (men=133, women=141) were participated in this study. TGS was measured using a toe grip dynamometer (Takei Scientific Instruments, Japan). Physical performance was evaluated using MEXT's physical fitness tests (Grip strength, sit-up, long seat type anteflexion measurement, sidesteps, multi-stage fitness test, 50-meter run, standing long jump, softball throw), manual muscle test (MMT) (Knee extension torque, knee flexion torque, hip extension torque and Hip flexion torque) and Jumping height (Rebound jump (RJ), squat jump (SQJ), counter movement jump (CMJ)). The correlations for mean TGS between boys and girls were calculated using Peason's correlation coefficient. To assess relationships between mean TGS and the selected parameters, we calculated Pearson's correlation coefficient by sex and conducted a stepwise multiple regression analysis that included physical characteristics, physical fitness and muscle strength as explanatory valuables. For all tests, statistical significance was set at $p<0.05$.

***Results and Discussion***: Toe grip strength was significantly increased after the third grade in comparison to the first grade for both genders ($p<0.01$). It was observed that muscle strength of elementary school children increases with growth. It tended boys higher than girls, however, was not significant gender differences in all of grade. In both genders, TGS was significantly correlated with MEXT's physical fitness tests ($p<0.01$), MMT ($p<0.01$) in all of the items and boys SQJ ($p<0.01$) and CMJ ($p<0.01$) and girls RJ ($p<0.05$). The stepwise multiple regression analysis revealed that boys height ($p<0.01$), knee extension torque ($p<0.01$) girls weight ($p<0.01$), hip flexion/extension torque ($p<0.01$) and both genders grip strength ($p<0.01$), knee flexion torque ($p<0.01$) were associated with TGS. TGS was increased with the growth particularly for the affected height and weight, therefore it was analyzed eliminate the effect of height and weight. The grades were not significantly correlated between the grades for both genders. The stepwise multiple regression analysis revealed that boys knee extension torque and softball throw, girls hip extension torque, standing long jump and grip strength have been selected as the independent variable were associated with both of body weight correction TGS and height correction TGS. Knee extensor strength and hip extensor strength are widening the stride, help to make a walking or running in a large stride. Toe grip strength has been reported to perform the functions push the body forward during walking or moving and by strengthening the power of the walking speed and the running speed to be improved. From these, the TGS stronger person is considered that it is possible to push out to a more forward body and to stabilize the waist.

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# Changes in the Physical Fitness of Taiwanese School Children in Japan: A Cross-Sectional Study

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***Objective***: The purpose of this study was to compare the physical fitness level of Taiwanese elementary and junior high school students in Japan with that of Japanese students.
***Method***: The subjects were Taiwanese school children (T, n=100); specifically, 10 to 14-year-old boys and girls. The evaluation consisted of height, weight, seated height, grip strength, 30-seconds of sit-ups, sit and reach, side steps, a 50 meter dash, standing long jump, softball or handball throw, and a 20 m shuttle run. These data are compared with a research report of physical fitness of children of Kanagawa Prefecture in 2013 (J).
***Results***: T was higher than J in sit-ups (10, 11, 14 years) in boys ($p<0.05$). T was lower than J in the 50 meter dash (12 years), and the 20 m shuttle run (11, 12, 14 years) in boys ($p<0.05$), but the total fitness composite score did not significantly differ between the T and J populations. T was higher than J in sit-ups (10 years) and side steps (10 years) in girls ($p<0.05$). T was lower than J in the 50 meter dash (14 years), standing long jump (14 years), handball throw (13, 14 years), and the 20 m shuttle run (13, 14 years) in girls ($p<0.05$). Furthermore, the total fitness composite score was significantly different between the T and J populations (14 years, $p<0.05$).
***Conclusion***: The physical fitness level of Taiwanese elementary school children is similar to that of Japanese boys and girls, but the physical fitness level of female Taiwanese junior high school students is lower than that of their Japanese counterparts.

***Key words***: overseas Chinese schools in Japan, elementary school, 20 m shuttle run

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# Polymorphism in the CNTF Receptor Gene Is Associated with Elite Japanese Endurance Athlete Status: A Case-Control Study

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***Introduction***: Ciliary neurotrophic factor (CNTF) is a protein that promotes the differentiation and survival of a wide range of neuronal cell types and signals through its receptor (CNTFR) thereby regulating neuronal and muscle growth.
***Purpose***: To examine the association between CNTFR gene polymorphism and elite Japanese endurance-athlete status.
***Methods***: 209 endurance/middle-power athletes: EMA (middle- and long-distance runners) and 814 Japanese controls were genotyped for C/T polymorphism of 3'-UTR of the CNTFR gene by use of TaqMan Genotyping Assay. All athletes were national (n=143) or international (n=66) level and the group included several medalists at the international competitions such as Olympic Games.
***Result***: Genotype distribution of C/T polymorphism in EMA (CC: 40, CT: 48, and TT: 12%) and controls (CC: 52, CT: 40, and TT: 8%) were in Hardy-Weinberg equilibrium. TT+CT genotype frequency was higher in EMA than controls under the T-allele-dominant model (p=0.0015, OR: 1.64 [95% CI: 1.21 - 2.24]). When EMAs were divided into 2 groups: national (N) and international (I) athletes, the TT+CT genotype frequency was higher than controls only in I-EMA under the T-allele-dominant model (p=6e-04, OR: 2.49 [95% CI: 1.45 - 4.28]).
***Conclusion***: The association found between the C/T polymorphism of 3'-UTR of the CNTFR gene and elite Japanese athlete status is of interest and worthy of further study in other elite athlete cohorts.

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# The Relationship Between Alpha-Actinin-3 Gene R577X Polymorphism and Muscle Flexibility

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Muscle flexibility is a component of physical fitness. It is suggested that genetic factor affects individual muscle flexibility, however, the relationship between alpha-actinin-3 gene (*ACTN3*) R577X polymorphism and muscle flexibility is unclear. The purpose of this study was to investigate an association between *ACTN3* R577X polymorphisms and muscle flexibility in Japanese.

In this study, 776 people (208 men and 568 women, 23-88 years old) were included. All subjects answered a questionnaire about exercise habits, and were subjected to a battery of tests to assess their fitness status (including grip strength and sit and reach). Genotyping was performed using the TaqMan approach for the *ACTN3* R577X polymorphism (rs1815739).

The genotype frequencies of the *ACTN3* R577X polymorphism in men (RR, 24.5%; RX, 52.9%; XX, 22.6%) and women (RR, 19.9%; RX, 52.6%; XX, 27.5%) were in the Hardy-Weinberg equilibrium (men, $p = 0.402$; women, $p = 0.160$). In men, there were no differences in age, height, weight, BMI, grip strength, and sit and reach among genotypes. In contrast, the sit and reach flexibility in the RR genotype ($36.1 \pm 0.9$ cm) was significantly lower than that in the RX and XX genotype ($38.9 \pm 0.4$ cm) even after statistically adjusted by age and exercise habit as covariates in women ($p < 0.01$).

In conclusion, *ACTN3* R577X genotype was associated with muscle flexibility assessed by sit and reach test in women. RR genotype had lower muscle flexibility than RX and XX genotype.

***Key words***: alpha-actinin-3, gene, polymorphism, muscle flexibility, sit and reach

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# The Combination of Insulin-Like Growth Factor 2, Alpha-Actinin-3, and Angiotensin-Converting Enzyme Gene Polymorphisms in Judo Athletes: A Pilot Study

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***Objective***: The purpose of this study was to investigate the association between the combination of insulin-like growth factor 2 (*IGF2*), alpha-actinin-3 (*ACTN3*), and angiotensin-converting enzyme (*ACE*) gene polymorphisms and judo status.

***Methods***: We recruited 156 male judo athletes from a top-level university in Japan. These athletes were divided into two groups based on their competitive history: international-level athletes and others. Genomic DNA was extracted from the saliva of each athlete. Genotyping by PCR or PCR-RFLP was used to detect *IGF2* G/A (rs680), *ACTN3* R577X (rs1815739), and *ACE* I/D (rs1799752) polymorphisms. The combined frequencies of the three gene polymorphisms were compared between all judo athletes and controls and between international-level athletes and others.

***Results***: There was a higher frequency of the *IGF2* GG, *ACTN3* RX, and *ACE* II genotype in all judo athletes (10.3%) compared to that of this genotype in the controls (1.2%). Moreover, there was a lower frequency of the *IGF2* AA, *ACTN3* RX, and *ACE* ID genotype in all judo athletes (1.9%) compared to that of this genotype in the controls (8.9%). Two of the 156 judo athletes exhibited the *IGF2* GG, *ACTN3* RX, and *ACE* DD genotype; both of them were international-level athletes.

***Conclusions***: The combination of *IGF2*, *ACTN3*, and *ACE* gene polymorphisms may be associated with the judo status.

***Key words***: genetic polymorphism, judo status, *IGF2*, *ACTN3*, *ACE*

## Introduction

Judo is one of the major sports originating from Japan and requires high absolute and relative levels of maximal strength and muscle power[1]. Judo athletes have to exert a significant amount of muscle strength throughout each match. Over recent years, the number of Japanese competitors who receive winning medals in the Olympic Games and World Championship competitions has markedly declined. To be successful in international competitions, judo athletes must achieve an excellent level of physical fitness and condition through training. However, success in sports also depends upon genetic factors, with 66% heritability for athlete status[2].

To date, at least 206 nuclear genetic markers in the human genome have been associated with physical performance phenotypes as well as physical fitness[3]. Of this group of genes, we specifically focused upon the insulin-like growth factor 2 (*IGF2*) gene, which has been associated with muscle strength[4]. The *IGF2* gene is located on chromosome 11p15.5[5] and plays a role in human muscle development[6]. *IGF2* ApaI polymorphism has resulted in the classification of individuals into the GG, GA, or AA genotype. Previous studies have demonstrated that both arm and leg strength and total body fat-free mass were higher in individuals with the GG+GA genotype compared to those with

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the AA genotype[4]. Recently, we determined that the *IGF2* ApaI polymorphism may be associated with athlete status in judo[7]. In other words, the *IGF2* ApaI polymorphism may represent a genetic marker associated with a predisposition for judo status. However, there have been no other reports associating the *IGF2* ApaI polymorphism with athlete status.

Previous studies have investigated the relationship between athlete status and alpha-actinin-3 (*ACTN3*) and angiotensin-converting enzyme (*ACE*) polymorphisms. The *ACTN3* gene encodes the ACTN3 protein, a sarcomeric protein that is almost exclusively expressed in the fast-twitch (type II) fibers of skeletal muscle and is located on chromosome 11q13.1. A cytosine (C) to thymine (T) transition within exon 16 of the *ACTN3* gene leads to a stop codon, which results in the muscle fibers being devoid of the ACTN3 protein[8]. The homozygosity of the X allele (in other words, an XX genotype) also results in the absence of ACTN3 expression. The XX genotype appears to be far less frequent in elite power athletes compared to endurance athletes or controls[9]. This data suggests that the XX genotype exerts an influence upon certain type of muscle performances. The *ACE* gene is located on chromosome 17q23.3 and contains a polymorphism based on its presence (insertion, I) or absence (deletion, D) within intron 16 of a 287-bp nonsense DNA domain, resulting in three different genotypes (II, ID, and DD). ACE is a key factor in regulating blood pressure in the renin-angiotensin system and acts via the production of angiotensin II. ACE activity is determined by the *ACE* I/D polymorphism. Individuals who are homozygous for the deletion allele (DD genotype) have ACE levels in the serum that are higher than the individuals who are homozygous for the insertion allele (II)[10]. Previous studies have reported associations between the I allele of the *ACE* I/D polymorphism and superior endurance ability of elite athletes[11].

Many previous studies have shown an association between the combination of *ACTN3* and *ACE* gene polymorphisms and athlete status. We hypothesized that the combination of three gene polymorphisms is more effective than that of two gene polymorphisms. In the present study, we specifically considered three genes that have previously been associated with athlete status and/or muscle strength: *IGF2*, *ACTN3*, and *ACE*. Because the *IGF2* gene polymorphism has been related to judo status, it is plausible that the combination of three gene polymorphisms (*IGF2*, *ACTN3*, and *ACE*) may reveal the judo status. Therefore, the purpose of this study was to investigate the association between the combination of *IGF2*, *ACTN3*, and *ACE* gene polymorphisms and judo status.

## Methods

### 1. Subjects

We investigated 156 male judo athletes belonging to the Tokai University Judo Club in Japan. Athletes were divided into three groups based on their results in national or international competitions. Sixteen athletes were classified as "international level" (winners of national championships or participants in international competitions) and the remaining 140 were classified as "others" (members of the University Judo Club). Our controls were individuals of a known genotype from the general population, as reported in previous studies [*IGF2*-Controls, n = 167[12]; *ACTN3*-Controls, n = 1,191[13)-16)]; *ACE*-Controls, n = 5,679[17)-26)]].

The study was approved by the Ethics Committee of Tokai University in Japan and was conducted according to the Declaration of Helsinki. The objectives and methods of the study were explained to all participants, and written informed consent was obtained.

### 2. Genotyping

DNA was extracted from the saliva of all subjects using the QIAamp DNA Mini Kit (QIAGEN, Milano, Italy) in accordance with the manufacturer's protocol. *ACTN3* R577X (rs1815739), *ACE* I/D (rs1799752), and *IGF2* G/A (rs680) polymorphisms were determined by polymerase chain reaction (PCR) or PCR-restriction fragment length polymorphism (RFLP)[27)-29)].

### 3. Statistical analysis

The distribution of genotypes was evaluated for conformity by the Hardy-Weinberg equilibrium using the chi-square test with two degrees of freedom. Levels of significance were set at $p < 0.05$. The frequency of the combination of three gene

polymorphisms was calculated by extrapolating control data from previous studies.

## Results

Genotype frequencies for the *IGF2* ApaI, *ACTN3* R577X, and *ACE* I/D polymorphisms were within the Hardy-Weinberg equilibrium ($p > 0.05$). The genotype frequencies of *IGF2*, *ACTN3*, and *ACE* in Japanese control subjects have been previously published [12)-26)].

Figure-1 shows the combined frequencies of three gene polymorphisms (*IGF2*, *ACTN3*, and *ACE*) in all judo athletes and controls. There was a higher frequency of the *IGF2* GG, *ACTN3* RX, and *ACE* II genotype in all judo athletes (10.3%) compared to that of this genotype in the controls (1.2%). Furthermore, there was a lower frequency of the *IGF2* AA, *ACTN3* RX, and *ACE* ID genotype in all judo athletes (1.9%) compared to that of this genotype in the controls (8.9%). It was also evident (Figure-1) that there were differences between

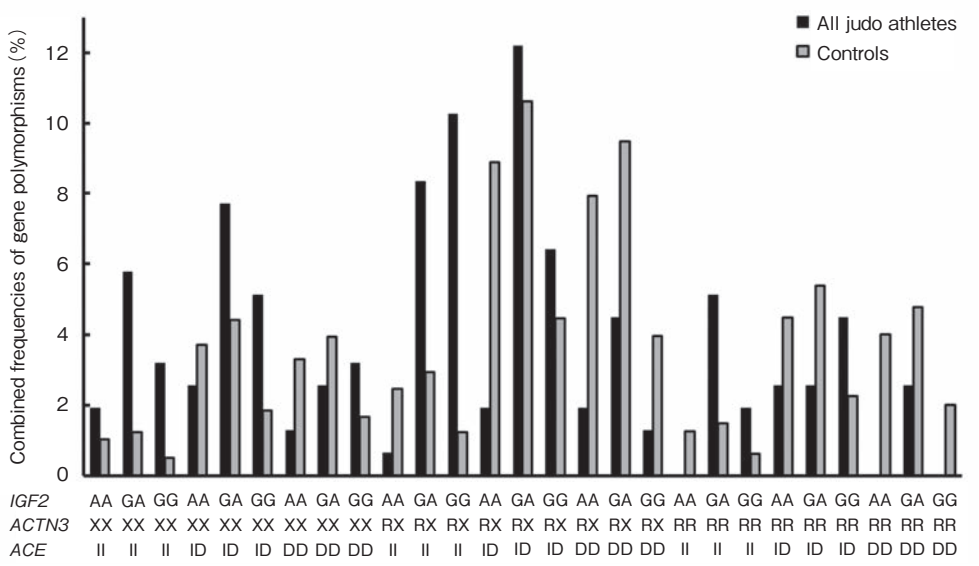


**Figure-1** The frequencies of combination of three gene polymorphisms (*IGF2*, *ACTN3*, and *ACE*) in all judo athletes and controls

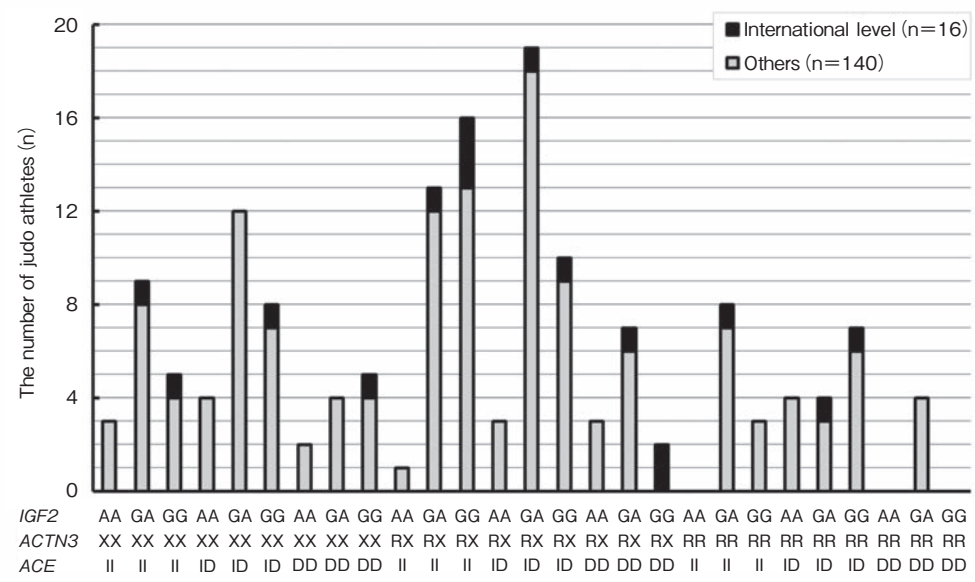


**Figure-2** The number of international-level and other judo athletes exhibiting the combination of three gene polymorphisms (*IGF2*, *ACTN3*, and *ACE*)

the frequencies of judo athletes and controls.

Figure-2 shows the number of athletes exhibiting the combination of three gene polymorphisms among all the judo athletes (international-level athletes and others). Two of the 156 judo athletes exhibited the *IGF2* GG, *ACTN3* RX, and *ACE* DD genotype; both of them were international-level athletes. Figure-2 also shows the characteristics of international-level judo athletes.

## Discussion

In the present study, we investigated an association between the combination of three gene polymorphisms (*IGF2*, *ACTN3*, and *ACE*) and judo status. Many previous studies have revealed an association between the combination of two gene polymorphisms (*ACTN3* and *ACE*) and athlete status. However, to date, there have been no reports of any association between the combination of three gene polymorphisms and athlete status.

Our data suggested that the combination of three genes may have an influence on judo athletes. Moreover, two of the 156 judo athletes exhibited the *IGF2* GG, *ACTN3* RX, and *ACE* DD genotype; both of them were international-level athletes. Therefore, the *IGF2* GG, *ACTN3* RX, and *ACE* DD genotype may confer an advantage pertaining to the judo status. Kikuchi *et al.* (2012) reported higher frequencies of the *ACTN3* R allele + *ACE* DD genotype in elite Japanese wrestlers[14]. These earlier results were supported, at least in part, by our current findings, which showed the combination of the *IGF2*, *ACTN3*, and *ACE* genes only in international-level judo athletes. This result shows that the combination of three genes may be used to develop genetic tests for identifying athletic talent.

An important limitation of the present study is that no statistical analysis was performed and it included a small sample size of international-level athletes. Sports performance is a multifactorial phenotype, influenced by both environmental and genetic factors. However, to our knowledge, this study is the first to investigate the association between the combination of three gene polymorphisms and judo status. Consequently, this research could shed new insight into the selection of international judo athletes.

In conclusion, the findings of the present study indicate that the combination of three genes (*IGF2*, *ACTN3*, and *ACE*) may be associated with judo status.

## Conflict of interest

No conflict of interest was declared for this study.

## Acknowledgement

We wish to thank Ms. Moemi Ioka and Ms. Ai Taketomi for their valuable help. We also thank all volunteers for their participation in this study. This work was supported by JSPS KAKENHI [grant numbers 25350783 (to KA) and 24650331 (to SM)]. A Tokai University Supporters Association Research and Study Grant also supported this study. The authors have no conflicts of interest to declare.

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# Long Term Effect of Cardiorespiratory Fitness for a Prevention Against Diabetes

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In Japan, although the prevalence of overweight (BMI≥25), the strongest predisposing factor for type 2 diabetes, is low compared with that in Europe and the United States, the prevalence of type 2 diabetes has increased over the last 15 years. In epidemiological studies of both Japanese and Caucasian population, a high level of cardiorespiratory fitness has been shown to be a protective factor against type 2 diabetes. However, there are no reports focused specifically on athletes that investigate whether high cardiorespiratory fitness at a young age can prevent disease later in life. Therefore, the present study examined the relationship between cardiorespiratory fitness at a young age and the development of type 2 diabetes in Japanese male college athletes using a cohort study.

Between 2007 and 2009, 3,539 male alumni who graduated at the physical education department, were mailed a follow-up questionnaire about their diabetes background and 1,385 male alumni returned it. The cardiorespiratory fitness of male alumni, as measured by 1,500-m endurance run in college (1971-1991) was available for 41% of the respondents. In the present study, we analyzed for 570 male alumni by Cox's proportional hazards models and adjusted for age, year of graduation, BMI, smoking, and sports club participation at college age.

This study covered a 26-year follow-up period (Interquartile Range: IQR: 23-29 years), and median age at the follow-up questionnaire was 49 years (IQR: 45-52 years). At follow-up, 22 men had developed type 2 diabetes. The incidence of type 2 diabetes was inversely correlated with cardiorespiratory fitness, and the low cardiorespiratory fitness level increased the cumulative incidence rate of type 2 diabetes. There were progressively lower age-adjusted relative risks of type 2 diabetes across cardiorespiratory fitness levels ($p=0.01$ for trend). After adjustment for age, year of graduation, BMI, smoking, and sports participation, hazards ratio and 95% CI by category (low, medium, and high) were 1.00 (reference), 0.40 (0.14-1.13) and 0.26 (0.07-1.00) ($p=0.03$ for trend).

We conclude that cardiorespiratory fitness at a young age can predict type 2 diabetes later in life among Japanese male athletes.

*This data was already published at BMC Public Health. And this report was extracted from Someya Y, *et al*: Cardiorespiratory fitness and the incidence of type 2 diabetes: a cohort study of Japanese male athletes. BMC Public Health, 2014; 14: 493.

***Key words***: cardiorespiratory fitness, diabetes, young age, Japanese, athlete

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# Relationship Between Physical Activity During Pregnancy and Mood Changes After Delivery in Japanese Women

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To assess the association between physical activity during pregnancy and mood changes after delivery, we surveyed 107 pregnant women who had a normal vaginal delivery in the Gynecological Department of Juntendo University Hospital. The study participants completed a structured questionnaire four times, viz. at 12, 25, and 36 weeks' gestation and at 1 month after delivery. The first three questionnaires focused on physical exercise activity in the most recent 4 weeks. The forth questionnaire focused on mood changes, assessed by the Edinburgh Postnatal Depression Scale (EPDS), Profile of Mood States (POMS), and Visual Analog Scale fatigue score (VAS). In total, 12.9%, 29.8%, and 34.6% of the participants engaged in physical activity at 12, 25, and 36 weeks' gestation, respectively. By contrast to the observation of decrease in women exercising from the second to third trimester[1], the proportion of participants exercising increased from the 12th to 36th week of gestation in the present study. This discrepancy was probably due to the differences in socio-demographic characteristics of participants in the two studies. There were no differences in EPDS and VAS between women who had been physically active and inactive at 12, 25, or 36 weeks' gestation. The results of the POMS showed that although there were no differences between women who were active and inactive at 12 and 25 weeks' gestation, the "Vigor" scale was significantly higher ($p<0.05$) and the "Fatigue" scale was significantly lower ($p<0.05$) in women who were physically active at 36 weeks' gestation than in women who were inactive at the same time. These results suggest that even if women do not engage in physical activity during the first or second trimester, physical activity during the third trimester may improve the mood after delivery.

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# Study on Determinants of Faultlines and Occupational Stress: Empirical Results of a Pilot Study

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***Objective***: Managing people from different backgrounds has brought great benefits to companies, sport teams, and surgical teams. However, previous research has shown that diversity may have negative effects on performance because of diversity "faultlines", hypothetical dividing lines that may split a group into subgroups. We conducted an empirical study on faultlines for the first time in Japan to investigate the present status of faultlines and to assess the degree to which workers perceive that (certain) attributes (e.g. age, personality, attitude) may have an impact on the emergence of faultlines in Japanese work organizations.

***Participants***: Participants included 132 workers (122 males and 10 females; ages 20–59 years, M＝48.1 years SD ＝± 16.2).

***Methods***: The questionnaire consisted of three sections, the first of which was a face sheet. The second was a newly developed set of items designed to assess the degree to which participants perceived that (certain) attributes might have an impact on the emergence of faultlines. The third explored participant's perception of faultlines at their present workplace.

***Results***: We found that task-related attributes such as specialty and ability/knowledge were great factors affecting faultlines, and that every participant perceived faultlines based on such attributes as specialty, personality, and attitude.

***Conclusion***: These findings provided the first step towards bridging faultlines in diverse teams. However, they also suggested faultlines are more detrimental than previously thought to individuals as well as organizations. Further research is now being conducted with the cooperation of more than 1,000 participants to examine the relationship between faultlines and individual outcomes, such as occupational stress and work-life conflict.

***Key words***: diversity, faultlines, occupational stress, work-life conflict

## Introduction

Developed countries are concerned over the decrease in the labor force population. Especially in Japan, with its falling birthrate, aging society and shrinking population, the Japanese economy will remain at a low growth level over the long term. Moreover, because of the rise in the eligibility age for receiving public pension and the number of young people without work, many companies or organizations in Japan have to face the further utilization of various human resources, including women, foreign people, the elderly, and people with disabilities.

The Ministry of Economy, Trade and Industry states that diversity management is a key strategy for human resources intended as part of management strategies by enterprises to build a competitive advantage. By exhibiting the diverse capabilities of human resources in management, the base for utilization of human resources will be expanded and linked to responses to diverse market needs to make use of various perspectives, and create the innovation that makes use of those differences[1].

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One good example of promotion of diversity management is ANA Holdings Inc. The ANA Group considers itself as a group that truly utilizes diversity and encourages frank discussion and mutual enhancement regardless of race, age, gender, assignment, role values or any other differences, to create a corporate culture that empowers the entire group. In particular, as women make up the majority of employees within ANA, management has made a point of continuously raising utilization of women's varied perspectives, sensibilities and values, and has set numerical targets for such matters as the number of female managers and the ratio of female to male managers (http://www.anahd.co.jp/en/csr/employees/diversity.html).

The spread of diversity management has been expanding to organizations other than companies. In fact, the participation of women in sports and increasing opportunities for women in decision making and leadership roles have been growing within sports since the Brighton Declaration on Women and Sport (http: //www. sportsbiz. bz/womensportinternational/conferences/brighton_declaration.htm), which provides the principles guiding actions intended to increase the involvement of women in sports at all levels and in all functions and roles. Meanwhile in the medical field, team medical care has received increasing attention, which stands together with regard to the treatment of patients, respecting each other's professional specialties, and continually working to improve their specialist skills in order to provide the best medical care that will satisfy patients (http://www.jira-net.or.jp/radiology_japan/rj_065/02.html).

## Theoretical background and research questions

### 1. Diversity as a double edged sword

In the past 40 years research on diversity management has been conducted to examine the complex relationship between diversity and performance. However, the actual findings on the effects of diversity on performance have been relatively inconsistent (Williams & O' Reilly, 1998)[2]. Some studies have found diversity to positively relate to performance, while other studies have found a negative relationship between diversity and organizational outcomes such as late delivery, inadequate productivity, inability to produce innovative results. As portrayed by Milliken & Martins (1996)[3], diversity is a double-edged sword with both positive and negative aspects.

Horwitz & Irwin (2007)[4] suggested several key implications drawn from their meta-analytic endeavor. First, task-related diversity is found to be positively related to both quality and quantity of team performance, while demographic diversity is not significantly related to team performance. On the contrary, the demographic dimensions of diversity may be more likely to be negatively related to performance (Horwitz & Horwitz, 2007; Joshi & Roh, 2009)[4,5].

### 2. Types of diversity

Taxonomy for describing the types of diversity by West *et al.*, (2003) is shown in Table-1[6]. The columns in Table-1 differentiate between relationship-oriented and task-related diversity. Pelled (1996)[7] proposed this categorization based on the relatedness between attributes and task. Joshi & Jackson (2010)[8] later categorized attributes highly related to job as task-related and those less related to job as relation-oriented.

According to Joshi & Jackson (2010)[8], task-oriented diversity refers to the distribution of attributes that are potentially relevant to team work. Organizational tenure, formal credentials and titles, and cognitive abilities are examples of task-oriented diversity. Relation-oriented diversity refers to the distribution of attributes that are instrumental in shaping interpersonal relationships but which typically have no apparent direct implications for task performance; age, gender and personality characteristics are examples of relationships-oriented diversity. In the past ten years, relation-oriented and surface level diversity were collectively known as demographic diversity.

The rows in Table-1 differentiate between readily-detected or surface-level diversity and underlying or deep-level diversity. Taniguchi (2005)[9] explained that surface-level diversity is that which is easily observable or detectable. Age, sex, and ethnicity are examples of surface-level diversity. Deep-level diversity refers to differences among team members on attributes that generally become known only through interaction, such as personality, attitudes, and skills (Joshi & Jackson, 2010)[8].

**Table-1** A scheme for categorizing the personal attributes of individuals

| | Demographic/Relationship-oriented attributes | Information/Task-related attributes |
|---|---|---|
| Readily detected attributes | Sex | Department |
| | Socioeconomics status | Organizational tenure |
| | Race | Formal credentials and titles |
| | Ethnicity | Educational level |
| | Religion | Employment status |
| | Nationality | |
| Underlying attributes | Personality | Task knowledge |
| | Gender | Communication skills |
| | Class identity | Cognitive skils and abilities |
| | Attitude | Physical skills and abilities |
| | Values | Experience |

(Based on West MA, *et al*, eds. International Handbook of Teamwork and Cooperative Working. Chichester: Wiley, 2003 [6])

## 3. Fautlines theory

Previous studies have tried to clarify why some diverse teams contribute to organizations, and some do not. Lau & Murnighan (1998) [10] proposed the term faultlines refer to hypothetical dividing lines that may split a group into subgroups based on one or more attributes. Faultline theory explains how the combination and configuration of the attributes of team members can influence the team's behavior and ultimately its performance. The attributes that drive faultlines can be surface-level or deep-level (Gratton *et al*., 2007) [11]. See "Figure-1".

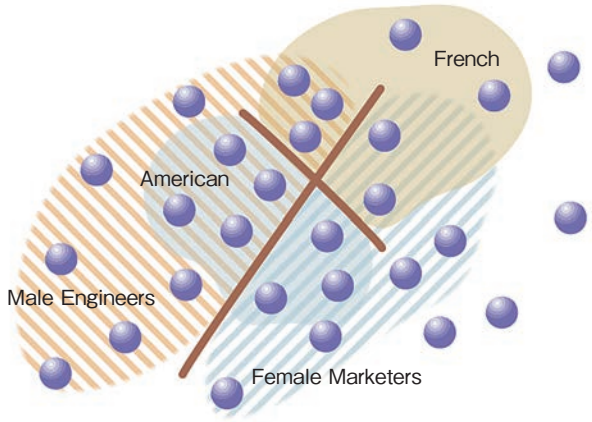


**Figure-1** The emergence of faultlines in a team
(Adopted from Gratton L, *et al*: MIT Sloan Management Review, 2007; 48: 22-29 [11])

Previous studies have also showed a significant negative relationship between faultlines and organizational outcomes such as conflict, miscommunication among members, and turnover. Earley & Mosakowski (2000) [12] reported that faultlines based on nationality harm team identity, group efficacy, team performance, and satisfaction with team's performance. Molleman (2005) [13] indicated that demographic faultlines directly impair the functioning of a team. Rico *et al*., (2007) [14] examined the effects of diversity faultlines stemming from educational background and conscientiousness on team decision quality and social integration and the moderating role of team task autonomy. They found the negative effects of faultlines on performance and social integration.

Jehn & Bezrukova (2010) [15] showed that groups with activated faultlines (members actually perceive subgroups based on the demographic characteristics) are more likely to form coalitions, have high levels of group conflict, and lower levels of satisfaction and group performance than dormant faultline groups.

However, few previous studies have mentioned individual outcomes such as stress and work-life conflict. Strong faultlines can create a fracture in the social fabric of the group. This fracture may become a source of tension and a barrier to the creation of trust, which may be factors having a negative impact on individuals. Therefore we have begun examining the relationship between the individuals' perception of faultlines in the workplace.

**Table-2** A categorization of attributes in this study

| | Demographic/Relationship-oriented attributes | Information/Task-related attributes |
|---|---|---|
| Readily detected attributes | Sex | Specialty |
| | Race | Employment status |
| | Age | Position |
| | | Tenure |
| | | Working plae |
| Underlying attributes | State of health | Work engagement |
| | Blood relationship | Ability/Knowledge |
| | Athletic experience | Learning Style |
| | Attitude | Communication style |
| | Values | |
| | Hometown | |
| | Family composition | |
| | Personality | |

We addressed two research questions in our faultlines study. The first of these was to investigate the present status of faultlines and to explore which attributes will create faultlines in the Japanese workplace (RQ1). The second research question was to assess the relationship between the perception of faultlines, of and occupational stress and work-life conflict (RQ2). Both of them have yet to be empirically investigated in Japan. Most previous studies have mainly been conducted in Europe and America where cultural backgrounds are quite different from Japan. For instance, in general, Japan is a country that has only one race and many Japanese workers associate the term "diversity" with women's participation and empowerment in work organizations.

The ultimate purpose of our research on faultlines is to clarify RQ1 and RQ2. In this paper, we described results of RQ1 for exploring attributes which greatly affected the emergence of faultlines and assessing the degree to which Japanese workers perceived that attributes might have an impact on the emergence of faultlines.

## Methods

### 1. Participants

Data were collected from 132 workers on streets in the center of Tokyo, Japan through an anonymous questionnaire. All the participants were Japanese workers who ranged in age 20-59 (M = 48.1 years SD = ± 16.2), 96.0% were male, 96.9% were full-time workers, and 59.5% were in administrative posts. The participants had a mean tenure of 131.8 months (SD = ± 114.6). Participants' functional backgrounds were sales/clerk (75%), systems engineer/engineer (9.8%), marketing (6.8%), specialist (6.1%), and others (8.8%). By industry, the proportions were as follows: services (16.7%), financing business (18.2%), information & communication (9.8%), manufacturing (8.3%), transportation (8.3%), wholesale trade (6.1%), real estate (5.3%), medical/pharmaceutical (3.8%), government (3.8%), electricity (3.8%), education, (3.1%), construction (2.3%), others (10.5%).

### 2. Measures

The questionnaire consisted of three sections, the first of which was a face sheet (e.g. gender, age, place of residence, married status, tenure, family composition, industry, occupation, size of organizations/enterprises, tenure, position, average working hours per day, employment status). The second section was a newly developed set of items designed to assess the degree to which participants perceived that attributes might have an impact on the emergence of faultlines (e.g. "How much do you think the differences between generations will affect the emergence of faultlines?"), which was measured with four 4-point Likert questions, anchored from 1 = "Not at all" to 4 = "Extremely

**Table-3** The status of the emergence of faultlines

| Attributes | Appeared (n) | Not Appeared (n) | Appeared (%) | Not Appeared (%) |
|---|---|---|---|---|
| age | 42 | 88 | 32.3 | 67.7 |
| sex | 47 | 84 | 35.9 | 64.1 |
| race | 8 | 123 | 6.1 | 93.9 |
| family composition | 13 | 118 | 9.9 | 90.1 |
| hometown | 32 | 98 | 24.6 | 75.4 |
| blood relationship | 26 | 105 | 19.8 | 80.2 |
| tenure | 57 | 74 | 43.5 | 56.5 |
| position | 61 | 69 | 46.9 | 53.1 |
| employment status | 51 | 80 | 38.9 | 61.1 |
| attitude | 68 | 63 | 51.9 | 48.1 |
| communication style | 49 | 82 | 37.4 | 62.6 |
| work location | 22 | 109 | 16.8 | 83.2 |
| personality | 71 | 60 | 54.2 | 45.8 |
| ability/knowledge | 64 | 67 | 48.9 | 51.1 |
| values | 39 | 90 | 30.2 | 69.8 |
| learning style | 39 | 91 | 30.0 | 70.0 |
| specialty | 75 | 56 | 57.3 | 42.7 |
| athletic experiences | 22 | 109 | 16.8 | 83.2 |
| state of health | 10 | 121 | 7.6 | 92.4 |
| work engagement | 55 | 76 | 42.0 | 58.0 |

much." The third section explored participant's perception of faultlines in the present workplace (e.g. "Is there a hypothetical line that may split your team/organization into subgroups based on the difference in generation?"), which was measured with Yes/No questions. All of attributes in the second and third section are listed in Table-2.

## Results

Table-3 displays descriptive statistics in the present status of the emergence of faultlines. Attributes as follow had a high level of impact on the emergence of faultlines; specialty (57.3%), personality (54.2%), attitude (51.9%), ability/knowledge (48.9%), position (46.9%), most of which were categorized into information/task-related attributes. On the other hand, demographic/relationship-oriented attributes such as race and state of health had a low level of impact on faultlines; race (6.1%), state of health (7.6%), family composition (9.9%), athletic experiences (16.8%).

From the perspective of the impact on faultlines, the most influential factors were information/task-related attributes, to wit: attitude (2.63), specialty (2.54), ability/knowledge (2.50), personality (2.41), work engagement (2.32). In contrast to those results, demographic/relationship-oriented attributes had a low level of impact; family composition (1.42), state of health (1.42), athletic experiences (1.52), race (1.66), hometown (1.73). See Figure-2.

## Discussion

In this study, we have found which attributes will have an impact on the emergence of faultlines, and also clarified the actual situation of faultlines at the worksite for the first time in Japan. The results of this study suggested that task-related and deeper level attributes such as ability/knowledge and personality might have a influence on faultlines. However, this result was different from our hypotheses and the results of some previous research on faultlines. Most previous studies have shown that demographic attributes are likely to have an influence on faultlines. The result in this study might result from the bias that most of Japanese participants have not faced demographic diversity (i.e. diversity in nationality, ethnicity, race, religion and sexual orientation and so on).

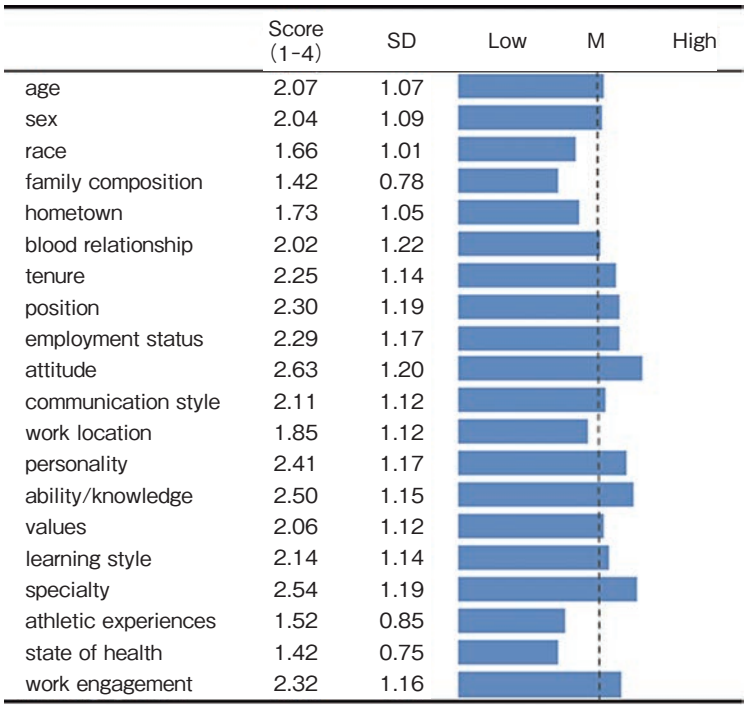


**Figure-2** The impact of attributes on faultlines

## Limitations and future research

One of the limitations of this study was the small sample size. In addition, we could not verify the differences between males and females because of the deviation in the number of female participants. Furthermore, we employed a brief attribute set that consisted of the twenty attributes as shown in Table-1 under consideration of the burden on participants. According to our study, it will be necessary to examine more than forty attributes to obtain further evidence for the validity and reliability. Clearly, further research is needed to test the application of our findings when other types of team diversity are considered, and to unravel how different attributes could enhance the emergence of faultlines. In addition, not only must the various attributes be considered, but also the influence of faultlines on individual outcomes, such as on occupational stress and work-life conflict (RQ2).

This pilot study on diversity faultlines is a first step in this direction. An empirical study is being conducted with the cooperation of more than 1,000 participants from over thirty kinds of industries and occupations all over Japan to examine the relationship between faultlines and individual outcomes, namely, occupational stress and work-life conflict among members.

## Conflict of interest

None declared.

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Proceedings

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# Relationship Between Assertion Types and Communication Channels of Nursing Organization at University Hospital: Communication Data from Electronic Sensors

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The purpose of this study is to obtain a perspective of the communication objectively and analyze the relationship between assertion types and interpersonal communications in a nursing organization of a university hospital. To promote the communications, assertive communication which enables self-expression attracts attention recently. However, in the previous studies of the assertion and communication in the context of nursing organization, it cannot be regarded that the study of this field was clarified scientifically although a few studies exist to clarify the relation with practical communication. Therefore, this study visualizes the communication channels scientifically with electronic sensors and is to clarify how the index influences them with the type of assertion. Participants of this study were 26 nurses in a maternity ward of a university hospital. Measures of this study were electronic sensors and questionnaires. The electronic badges, developed by MIT and applied by Health High-Technologies Corporation in Japan, are used for measuring the communication channels and social signaling behavior of nursing organizations in university hospitals. The results were the communication time of the nurses who had high assertive scores was concentrated in the middle area and the communication time of the nurses who had high passive aggressive scores was relatively low. In the communication signaling, one characteristic was if we analyze the communication time of connecting each nurse, we can see that the nurses with high assertive scores and the nurses with low assertive scores spend a lot of time communicating within their own group. The results of these characteristics show that there is a connection between assertion types and the amount of communication time and the connection of communication channels.

***Key words***: assertion type, interpersonal communication channel, electronic sensor, nursing organization, wearable sensing device

## Introduction

Chester Barnard stressed the importance of communications for the activation of the organization. In his theory of organization study, communication occupies a central place [1]. The quality of the communication is the key to success of the organizational vitalization. In the medical circumstances, communication is directly connected with the health of patients. To promote the communications in the nursing organization, assertive communication which enables self-expression has attracted attention recently. Assertive is one type of assertion and there are four types; assertive (AS), non-assertive (NA), aggressive (AG) and passive-aggressive (PA). The concept of assertion was developed in America in the 1970s period of self-expression and more recently DeGiovanni

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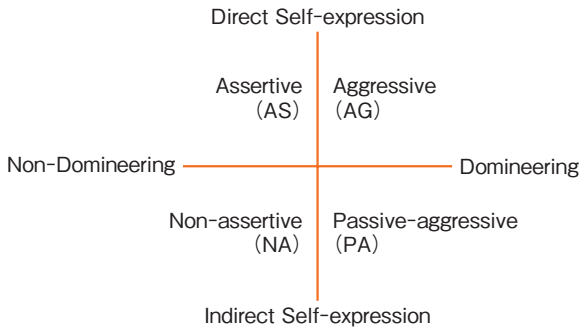


**Figure-1** Two dimensional model (DeGovanni. I. S)

advocated a two dimensional model[2] (e. g. Figure-1). According to Hiraki N, assertive communication is difficult for Japanese nurses. This occurs in the following context; Hiraki N mentions that Japanese nurses has a strong feeling of wanting to be useful to other people and putting the rights of patients before their own as well as having a desire to be sympathetic and kind. Therefore the Japanese nurses think that it is not good to have negative feelings to patients and they suppress their own feelings[3)-7)]. From such a background, there are many previous studies in conjunction with assertion of Japanese nurses. However, in the previous studies of the assertion and communication in the nursing organization, they mainly used questionnaires, interviews and video cameras. Although few studies exist to clarify the relation with practical communication scientifically[8], there are not enough to acknowledge evidence-based outcomes. Therefore, this study visualizes the communication times and communication channels of the nursing organization scientifically with an electronic sensor which was developed by MIT and applied by Health High-Technologies Corporation in Japan and are used for measuring the communication channels and social signaling behaviors of nursing organizations in university hospitals. Further, we clarified how the index influences them with the type of assertion which obtained the data of questionnaires by "Rathus Assertiveness Schedule: Japanese version[9]". We did a pilot study at a psychiatric ward in university hospital. There is a clear pattern showing in the results. The nurses who had high assertive scores were concentrated in the middle area of interpersonal communication time. On the other hand, the high passive-aggressive group tended to be near the top. Because of these results, we repeated the study in a maternity ward using exactly the same method and evaluation criteria. As the result, we obtained not only similar but also, opposite results as well as new results between assertion types and interpersonal communication.

## Purpose

The purpose of this study is to obtain a perspective of communication scientifically with electronic sensors and analyze the relationship between assertion types and interpersonal communications in nursing organization of university hospital.

## Methods

### 1. Participants

This research was carried out in one unit of a maternity ward consisting of 26 nurses. In addition to these nurses, we recruited the senior nursing officer of the university hospital. Through the informed consent procedure, a total of 26 nurses (midwife = 21, nurse = 5) agreed with this study (response rate = 100%, cover rate = 100%). The 26 participants consisted of three administrative nurses including one nursing divisional manager and two nursing chief managers and twenty-three staff nurses. Moreover, they were full time workers. The mean age was 31 (SD = ± 6.1) yrs. old.

### 2. Materials

This study has two materials, firstiy, electronic sensors, developed by MIT[10) 11)] and applied by Hitachi High-Technologies Corporation in Japan are used for measuring the communication channels and social signaling behavior of a nursing organization in university hospital. In detail, electronic badges capable of detecting face to face interactions, conversations, body movement and physical proximity are introduced in order to measure the organizational communication. Participants put on electronic badges (wearable sensing devices) for measuring the communication time at nursing organizations, including formal and informal scenes (nursing station, birthing room, newborn nursery, consultation rooms, hallway and staff rooms) during working hours (e. g. Figure-2). Second, we carried out a questionnaire survey

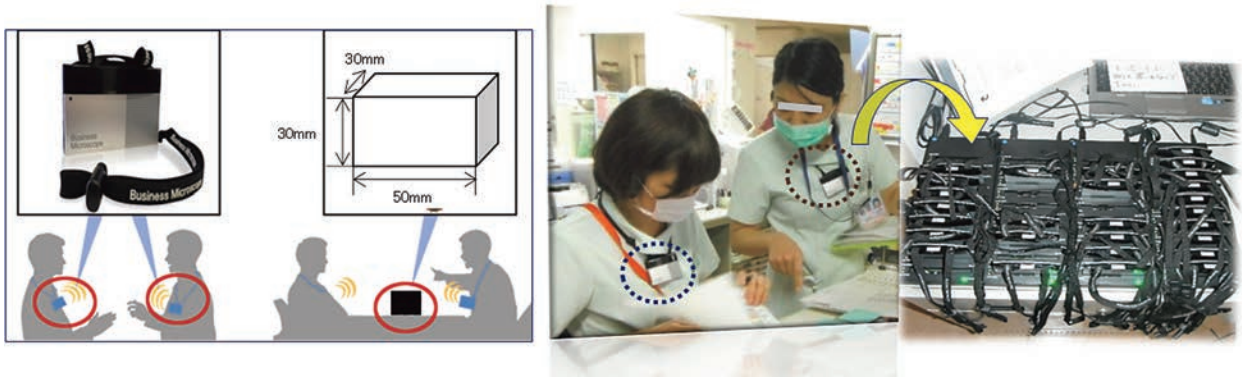


**Figure-2** Investigation method of electronic sensors

which is "Rathus Assertiveness Schedule: Japanese version" at the same time. This scale has four subscales, assertive (AS), non-assertive (NA), aggressive (AG) and passive-aggressive (PA). Factor of each subscale defined as follows[3) 9)], Assertive (AS): It is the expression method where a person can say their opinions and thoughts appropriately according to the situation. It is considered good as the person does not waver and makes sure others understand what they want to communicate (self-affirmation/others affirmation). Non-assertive (NA): This type does not express their opinions and thoughts clearly and they talk in a way that others do not understand. This type of expression has a tendency to result in an unpleasant experience because their thoughts and feelings are not conveyed and there are negative implications (self-abnegation / others affirmation). Aggressive (AG): This type of expression insists on expressing their thoughts and feelings clearly, but they push themselves and are too intense. They are unconcerned about the existence and the behavior of others, and persist in their opinion. This type of person shouts at others and blames them and ignores certain things and is selfish (self-affirmation /others negation). Passive aggressive (PA): This type of self-expression is negative and aggressive, in other words, this type does not attack directly or straight to other people, but is an expression method with bad implications attacking by an indirect method (self-abnegation / others negation).

**3. Survey period**

2 weeks in March of 2015.

**4. Analytical procedure**

1) Divided questionnaire results of each assertion type into 3 levels; high, middle, and low.
2) Used the data of inter personal communication times of 120 min/day or more.
3) Analyzed the relationship between each assertion type and amount of communication time and the interpersonal communication channel.
4) Compared the results with the pilot study results.

## Results

We obtained some characteristics showing that there is a connection between assertion types, the amount of communication time and the connection of communication channels as follows.

**1. Average interpersonal communication time per person/day**

We needed to consider one important thing before analyzing the results about amount communication time. We decided not to include the result of "mid (6)＊ in 3, 4 figures" who has the lowest communication time due to the irregularity of her duty because of her health condition. Bearing this mind, the results show that the communication time of the nurses who had high AS scores was concentrated in the middle area and this was almost identical to the results of the pilot study. However, nurses who had high PA scores were relatively low in this study even though there was the opposite result in the pilot study (e.g. Figure-3, Figure-4).

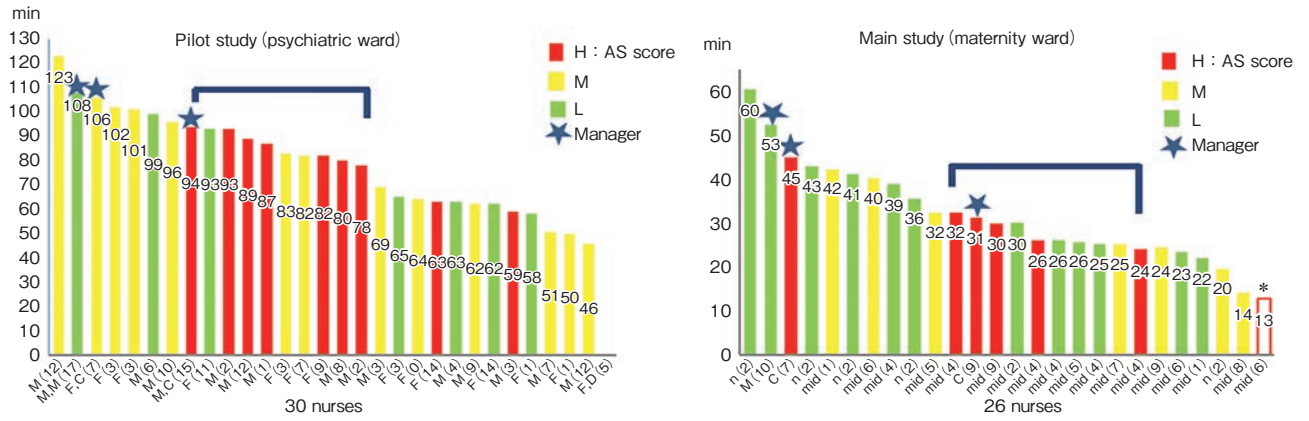


**Figure-3** Average interpersonal communication time per person/day: AS

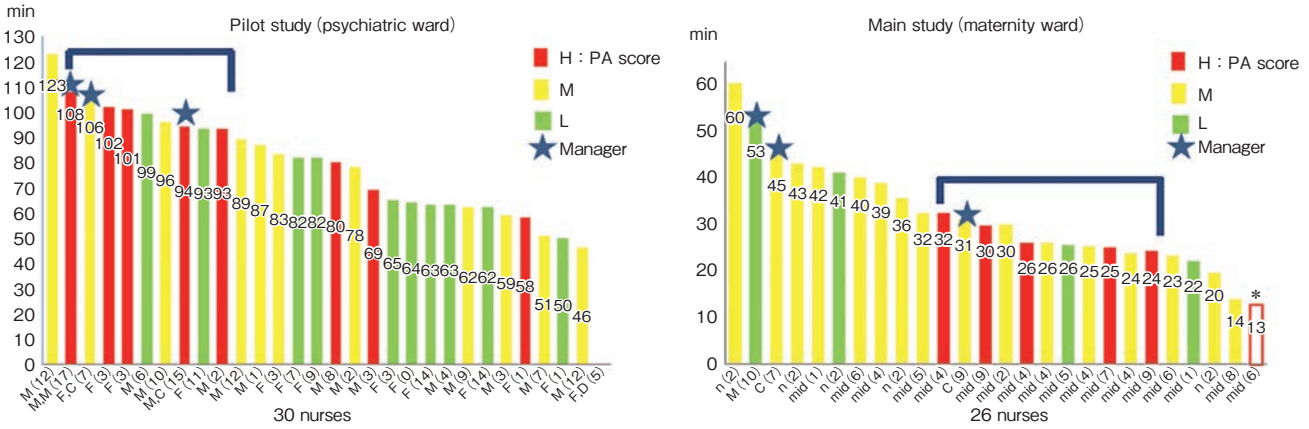


**Figure-4** Average interpersonal communication time per person/day: PA

### 2. Communication Channels

The average time per day (minutes) at which the nurses of the university hospital spent on face to face communication was divided into 30 minutes, 60 minutes, 90 minutes and 120 minutes interval units per day. Communication channels became clearer and more individual. In other words, the longer the time, the clearer the communication channel was. Besides, the key person in the organization became clear in the time unit of 120 minutes (e.g. Figure-5). In the communication channels, one characteristic was if we analyze the communication time of each nurse from the figures, we can see that the nurses with high assertive scores and the nurses with low assertive scores spend a lot of time communicating within their own group (e.g. Figure-6).

## Discussion

As mentioned above, there are four types of assertion and we analyzed them according to each type. It is shown that we cannot categorize the personality clearly in only one type. Each person has a combination of types. However, it can be said in general, each of them has a variety of assertion types individually. In this study, we found the nurses with a high assertive score don't communicate a lot in their nursing organization. There may be a possibility that the nurses with a high assertive score communicate efficiently in their work. Previous study showed that assertion training which can have a confidence and increase communication skills works effectively for Japanese nurses[3) 6) 7)]. Moreover, they reported that the assertive score of the nurse who had training increases[12)]. If the nurses' assertive score increases, it not only lowers

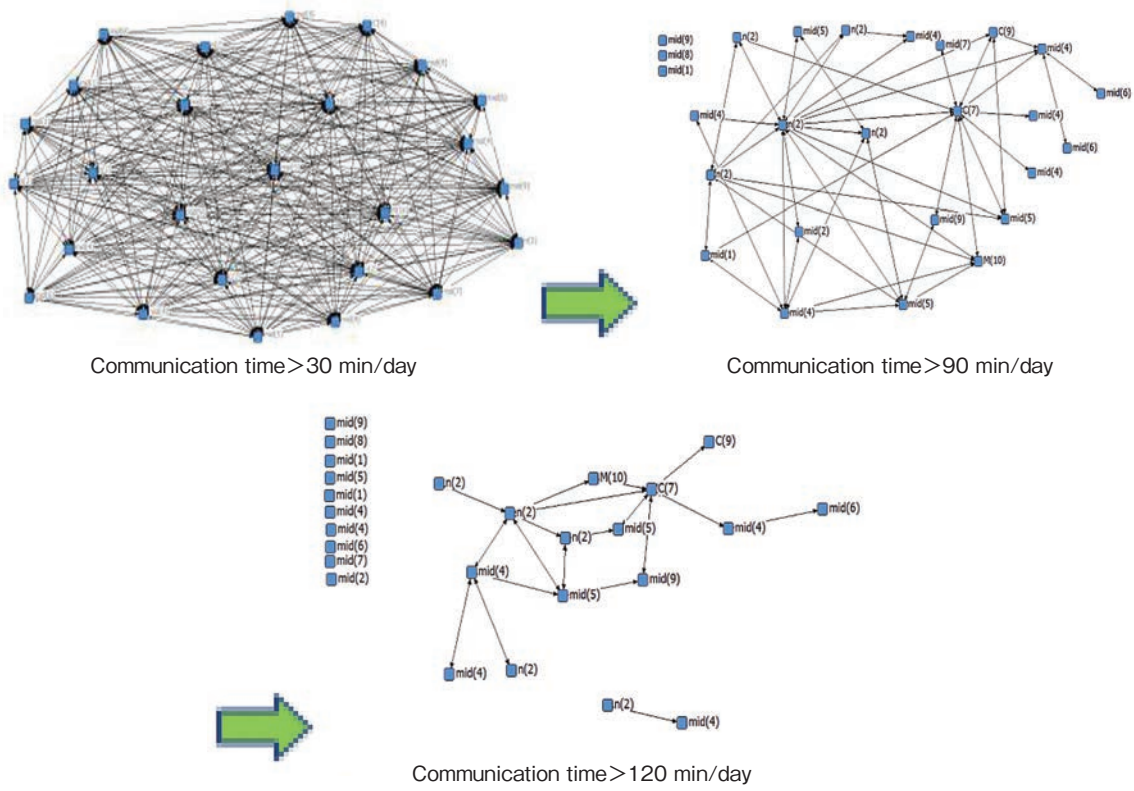


**Figure-5** Interpersonal communication channnel in nursing organization of the maternity ward

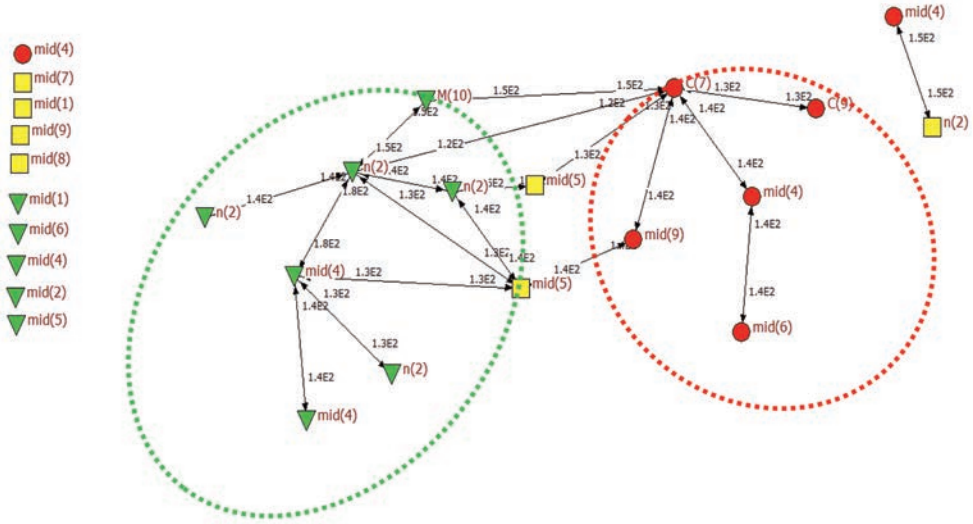

**Figure-6** AS (high middle low) distribute in "communication time＞120 min/1 day"

their stress but also makes more time. In addition, we should point out that one nurse who had a high AS score was the chief of the organization. According to the pilot study, the nursing manager spends much more time on communication than other nurses. This result is similar to the pilot study. This shows that the assertion type is not as influential as the type of job a person does. For example, they receive and in part information, discuss matters, negotiate, etc. Clearly, managers need to spend more time communicating with others. Moreover, the pilot study (psychiatric ward) nurses with high AP scores communicate with others a lot. On the other hand, we found the opposite result in this main study (maternity word). Therefore, we are planning to investigate these differences further using face to face interviews. The limitation of this study is that the implications are from only a quantitative analysis. We should investigate the meaning and causes of these results. It will be necessary to investigate more wards as control groups. The results of this study may give some suggestions for an evaluation of practical communication. Moreover, it is not only for the nursing organization but also other organizations building strong teams.

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# Developing Guidelines for Collecting and Using Feedback in Japanese Fitness Clubs

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The purpose of this study was to develop guidelines for promoting, collecting and using client's feedback in Japanese fitness clubs. Discussions were conducted to develop the guidelines with three business people belonging to three major fitness clubs. Good practices were collected using an interview survey, the guidelines were developed based on good practices. As a result, 7 items and phrases were created for promoting to collect and use client's feedback. The guidelines included 2 concepts. One was to be able to work on immediately and adapt to each work place. The other was to be simple and low cost.

Existing systems of each club to collect client's feedback had a few problems, and was difficult to collect positive feedback. Moreover, each club depended on accidents to collect client's feedback without intention. The guidelines support internationally collecting client's feedback. In addition, each item was decided considering the service record in the fitness industry. Positive feedback was effective for encouraging those who have a short service record. Negative feedback was effective for promoting growth of veterans having a long service record.

The guideline based on good practices was led. The guidelines include, 7 items and phrases considering adjustment for each workplace, ease, and low cost.

***Key words***: resilience, client's feedback, the guideline, fitness clubs

## Introduction

Recently, the importance of the fitness industry is increasing with a desire for a healthy life. However, fitness club employees supporting people's sports are exposed to much stress by complaint handling, emotional labor, sales budget, a shortage of career prospects and others. Hence, resilience is needed for adjusting to stress and overcoming adversities.

Moreover, how to enhance resilience is focused on discipline of business administration. However, most existing resilience training is collective training, which needs very high costs. An approach for enhancing resilience by existing resilience training is difficult for fitness clubs. In addition, it was shown that many Japanese small enterprises and workplaces do not have knowledge and theory for improvement of mental health[1]. Therefore, we decided to develop a tool for simplifying and supporting resilience enhancement with a simple and low cost way by the manager and employees as a self-help effort[2].

The tool includes three perspectives. Firstly, promoting challenge to stretched work with much stress as a trigger for growth. Secondly, constructing a good relationship for promoting to receive much support from associates. Finally, collecting and using feedback from clients[3]. These are shown as important factors in the process for enhancing resilience of fitness club employees[3].

Three improvement areas were decided in the guidelines for supporting resilience enhancement[4]. It was considered that items about promoting challenge and constructing a good relationship are

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able to be applied to other lines of business. However, an improvement area for promoting to collect and use client's feedback is a unique point of fitness clubs requiring a close relationship with clients. That improvement area might not be created, if fitness clubs were not targeted. That is different from other improvement areas. In addition, the paper showing the guidelines for supporting to enhance resilience does not discuss enough about the background [4]. Moreover, the improvement area has a high specialty about fitness club service. Therefore, we showed the process of development and the character of the guidelines for promoting collecting and using client' feedback in this paper.

## Methods

Fieldwork was carried out three times in June of 2014. The purpose of fieldwork was to ascertain the present situation of the system for collecting and managing feedback from clients at branches of three major fitness clubs in Japan. Moreover, we discussed with informants three times about the ways to collect and use feedback from clients.

### 1. Informants

We asked 3 informants. One of them was male, and the others were female. Two informants were regular employees, they were playing the role of manager in a fitness club. Another informant was an owner of a fitness club, who had been instructing simultaneously as a free instructor at a major fitness club for many years. She has experience as an administrator at a major fitness club.

### 2. Data collection

Before fieldwork, we collected good practices about client' s feedback from narrative data collected with semi-structured interviews from October to November in 2012. Discussions were carried out for considering good practices. They were narrated by 13 fitness club employees. Discussions were carried out one to one between the first author and each informant in a meeting room. Each discussion was for 60-90 minutes. Before the discussions, the important narrative about the necessary support from clients in the process of overcoming difficulties was extracted as a good practice. In the discussion, we set the following 3 agendas. 1) Confirming the ways each shop collects feedback from clients. 2) studying good practices about collecting and using client' s feedback, 3) considering contents of the guidelines including select items and determining phrases. The procedure for deciding the items and phrases of the guidelines was follwings. Firstly, items were decided tentatively based on good practices. Secondly, items were decided considering followings, i) possibility, ii) ease, iii) self-help, iv) low cost. Finally, items and phrases were modified based on industrial psychology's basic theory by the research team.

## Result

### 1. Present circumstance

Firstly, we asked informants if client's feedback was important for overcoming adversities from a business person' s point of view. We gained a common answer, they said "Yes". Especially, we had a consensus like the following. "Especially, I think positive feedback plays a significant role when the employee have a short professional career in the fitness industry". However, they felt difficulty in dragging effective feedback intentionally. In addition, we confirmed an existing frame for collecting feedback in each company. As a result, "A lot of feedback had been collected with a sheet of paper, most collected feedback was negative feedback, there was little positive feedback". The answer was a common answer from 3 informants belonging to each company. Under the present circumstance, we obtained approval necessary to collect positive feedback from all informants. We confirmed the need that business people need to have positive feedback, we discussed about how to collect and use client's feedback based on good practices.

### 2. Concepts

We collected many good practices. Good practices were made by a strict selection from the perspective of collecting intentionally and promoting employee's growth with feedback. As a result, we defined 7 items for supporting collection and use of client's feedback. Those included two concepts. One of them was to be able to be worked on immediately and to adapt to each work place. In addition, important things were very simple and need no

**Table-1** The guidelines for collecting and using feedback from clients

| | |
|---|---|
| 1 | **Promoting report of output, admiration for operation, and words of encouragement:**<br>If you received a report or output (change of mind and body, children get the ability to swim) and admiration for operation, you promote feedback directly to the concerned person. If you received warm, welcome growth, and encouraging feedback, you accept honestly. |
| 2 | **Confirming client's real intention of sharp feedback (word, expression, attitude) :**<br>If you received sharp feedback, you confirm the client's real intention of sharp feedback. In many cases, clients have a different intention from their word. The opportunity is a chance for creating a good relationship. |
| 3 | **Sharing of a feedback received personally:**<br>If you received feedback (both positive and negative), you share the feedback with any staff (it is not limited to the administrator). Negative feedback is shared as problem of the organization, it is necessary that the organization work out was to solve the problem. It is not considered a problem of the individual. If the administrators received consultation about negative feedback, it is required to treat the problem as organizational problem and separate it from personal evaluation. |
| 4 | **Treating negative feedback reception staffs received as organizational problem:**<br>Reception staff easily become the contact person to receive negative feedback. Clients have a different intention even when they tell directly negative feedback to reception staff on the surface. It is necessary to treat negative feedback as an organizational problem, separating from personal evaluation on such case. |
| 5 | **Telling positive side of associate to client:**<br>If you are told negative feedback from associates, you have never conformed to a negative campaign. You are required to protect your associates when you were told negative feedback. It is very important to tell clients the positive side of associates. |
| 6 | **Moving out of your position for collecting feedback:**<br>When you go out of your working position (including cleaning, go round to check a locker rom, and helping other sections), communication increases. It is very important to go out of your position. If you do that, you gain many opportunities for joining "Idobatakaigi" (gossip circle or chat) of inter clients. In addition, feedback is collected naturally. |
| 7 | **Using feedback for quantum leap:**<br>There is a lot of feedback which bring significant growth to employees. You can work out how to promote growth with negative feedback. Then, it is desirable to share the challenge based on feedback. |

special cost. As a result, 7 items and each phrase was determined based on these concepts.

### 3. Items and phrase

We discussed contents of the guideline considering the above concepts. As a result of the discussion, the guidelines including 7 items and phrase of each item was led (Table-1). 7 phrases included specific examples based on good practices.

## Discussion

We had discussions based on on-site viewpoints. Because, the previous study about client's feedback was limited[4].

It was found that each company had an existing system with a paper medium for collecting client's feedback. However, it was observed that the existing system might not be functioning enough. The effectiveness was dependent on efforts by each branch. For example, when we visited one shop, the shop had run out of paper for collecting feedback. In one branch, the administrator was not concerned, a part time worker had checked papers on which clients wrote requests. A part time worker gave the paper to the person in charge, but even then the unsolved paper was put aside. Under the present circumstance, there was a question of whether the existing system for collecting feedback was functioning or not. However, one branch had produced some positive results using handling requests from clients. When clients wrote the paper for feedback, most of their requests were about the time schedule of programs and malfunction of facilities. Additionally, this feedback was negative. In this study, we did not need such feedback. We need effective feedback to enhance resilience.

It was shown that client's feedback significantly influence fitness club employees[3]. We found that most of the feedback was not intentionally taken up. About positive feedback, the fitness clubs depend on fortuity and strength of the employee's personal relationship with clients. In this study, we focused on intentional collecting feedback. At first, we considered positive feedback was more important than negative feedback. However, informants

showed that both kinds of feedback was important. The administrator had intended that they utilize positive feedback for employees who have a short service record in the company. The reason for this was that, administrators thought positive feedback was directly linked with the worth and joy of their job. Besides, the administrators thought negative feedback brought opportunities of growth to employees who have a long service record in the company. It showed necessity of a new contrivance for collecting and using feedbacks from clients. Hence, a few items about how to use feedback were included in the guidelines.

The guidelines included 7 items as shown, however we should consider that each branch has a different situation. It is necessary to adapt to each situation flexibly for each branch to be effective. It was explained that responding to local needs is important[2]. In addition, the necessary items were different depending on length of work career and age of individuals. It was required to contrive for adapting situations of each workplace. Moreover, it is necessary that the relation is verified between how to applicate a guideline and the effect applying a guideline.

Finally, we should consider that the content of the guideline depends on the characteristic of the fitness club's workplace. Hence, the way of promoting collection and use of client's feedback has more room for more research. It is needed to refine the content of the guidelines.

## Conclusion

The guidelines based on good practices were led. The guidelines include 7 items and phrases considering adjustment to each workplace, ease, and low cost. In addition, the guidelines showed the tangible practical way to use and collect intentionally.

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# An *In Vitro* Contraction Model in Mouse Primary Cultured Myotubes Using Satellite Cells Originated from the EDL and Soleus Muscles

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***Purpose***: Skeletal muscle cell lines such as mouse C2C12 cells and rat L6 cells often show abnormal characteristics because of repeated-passage cultures and artificial culture conditions. Primary myotubes are considered to retain their *in vivo* properties. Here, satellite cells originating from the extensor digitorum longus (EDL) or soleus muscle were differentiated into primary myotubes and used for an *in vitro* contraction model.

***Materials and Methods***: Satellite cells from the mouse EDL or soleus were isolated by a single-fiber isolation method. We examined the formation of the sarcomere assemblies by α-actininimmunostaining in the differentiated myotubes. We also investigated the contractile characteristics of myotubes stimulated with an electric pulse and insulin induced-glucose uptake. C2C12 myotubes were used for comparison with the primary myotubes.

***Results and Discussion***: The sarcomere assemblies were observed in the primary myotubes but hardly observed in the C2C12 myotubes. The number of myotubes responding to stimulation by the electric pulse was increased in both the C2C12 and primary myotubes, although the movement in the primary myotubes was larger than that in C2C12 myotubes. The glucose uptake stimulated by insulin was significantly increased compared to the basal uptake in the primary myotubes and the C2C12 myotubes. These data suggest that the mouse primary myotubes, with their greater number of sarcomere assemblies and higher level of contractive activity, will be valuable as an *in vitro* contraction model that can be used in place of cell lines or human primary myotubes.

***Key words***: skeletal muscle, primary myotubes, contraction

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# Intramyocellular Lipid Accumulation After High-Fat Diet Is Associated with the Gene Expression Involved in Lipid Metabolism in Skeletal Muscle of Non-Obese Men

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Insulin resistance in skeletal muscle is one of the main features of metabolic syndrome, and it has been associated with lifestyle factors including diet[1) 2)]. Whereas the mechanisms underlying the development of insulin resistance have not been fully elucidated, the accumulation of intramyocellular lipid (IMCL) is recognized as an important determinant of insulin resistance, and is increased by a high-fat diet (HFD)[3) 4)]. The fat content of food is a determinant of the accumulation of IMCL. The effects of HFD on IMCL and insulin sensitivity are highly variable, although, it had shown that a short term (3-day) high-fat diet (HFD) in human increases the IMCL level and impairs insulin sensitivity in skeletal muscle[3) 5)].

The aim of this study was to identify the genes in muscle that are related to this inter-individual variation.

Fifty non-obese healthy men were recruited for this study. Before and after HFD for 3 days, IMCL levels in the tibialis anterior were measured by 1H-magnetic resonance spectroscopy, and peripheral insulin sensitivity was evaluated by glucose infusion rate (GIR) during the euglycemic-hyperinsulinemic clamp. We observed a significant increase in TA-IMCL by HFD. GIR was significantly decreased by HFD. We also observed a negative correlation between changes in TA-IMCL and GIR by HFD ($r=-0.37$, $p<0.01$), Subjects who showed a large increase in IMCL and a large decrease in GIR by HFD were classified as the high-responder (HR), and the subjects who showed a small increase in IMCL and a small decrease in GIR were classified as the low-responder (LR). In 5 subjects in each group, the gene expression profile of the vastus lateralis muscle was analyzed by DNA microarray analyses. Before HFD, gene expression profiles related to lipid metabolism were comparable between the 2 groups. Gene Set Enrichment Analysis demonstrated that 5 gene sets related to lipid metabolism were up-regulated by HFD in the HR group, but not in the LR group. Changes in gene expression patterns were confirmed by qRT-PCR using more samples (LR: n = 9; HR: n = 11). These results suggest that IMCL accumulation/impaired insulin sensitivity after HFD is closely associated with changes in the expression of genes related to lipid metabolism in muscle.

***Key words***: high fat diet, intramyocellular lipid, insulin sensitivity, skeletal muscle

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# Role of Exercise Intensity on Intramyocelluar Lipid Level After Exercise in Subjects with Moderate Insulin Resistance

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It is known that the increased intramyocellular lipid (IMCL) levels observed in obese subjects are closely associated with insulin resistance (IR) in muscle[1]. Interestingly, some reports suggested low intensity exercise (LIE) decreased IMCL and improved IR[2]. On the other hand, a few reports showed vigorous intensity exercise (VIE) improved IR, but increased IMCL level[3) 4)]. This phenomenon is a reminiscent of endurance-trained athletes, who possess a high oxidative capacity and enhanced insulin sensitivity, also have higher IMCL content known as athlete's paradox (AP)[5) 6)]. From these findings, we hypothesized exercise intensity is one of the determinants of AP.

To test this hypothesis, we recruited 20 men with moderate insulin resistance (HOMA-R >1.6) and randomly assigned to LIE (40% $VO_2$ peak) or VIE (70% $VO_2$ peak) group. Each group performed with ergometer for 5 consecutive days. Before and 3-day after completion of protocol, IR was evaluated by glucose clamp. IMCL was measured by $^1$H-MRS. The IMCL was also evaluated immediately after the exercise at day 5. Our preliminary data showed that in VIE group IMCL level was not significantly changed after exercise at day 5. Although IMCL level was decreased at 3-day after last bout of exercise in LIE group, that in VIE group increased about 50% from baseline. Interestingly, insulin resistance was similarly improved in both groups. These data suggested exercise intensity is a determinant of change of IMCL.

Although, changes in IMCL level after exercise were opposite between LIE and VIE, the improvement of insulin resistance was similar. IMCL exists mostly as triacylglycerol (TAG), which may not impair insulin sensitivity in muscle. On the other hand, intramyocellular diacylglycerol (DAG) concentration is considered to induce insulin resistance, which is generally increased in parallel with the amount of intramyocellular TAG. It has been shown that one bout of aerobic exercise increased the expression level of diacylglycerol acyltransferase (DGAT)-1 in muscle and to prevent FFA-induced muscle DAG accumulation and insulin resistance in healthy humans. Thus, we speculated that DAGT1 expression and decreased DAG levels play roles in the mechanisms involved in the athlete's paradox phenomenon seen in VIE group. Future analysis is clearly required to test this hypothesis.

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# Exercise-Induced Transient Increase in IL-6 Stimulates GLUT4 Expression and Enhances Insulin Sensitivity in Mouse Skeletal Muscle

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A single bout of exercise induces transient increase in blood interleukin-6 (IL-6) level in human and rodents, however, the role of exercise-induced IL-6 is poorly understand. Prolonged, chronic increase in IL-6 reflects low-grade inflammation, which decrease insulin sensitivity in adipose tissue, liver and skeletal muscle. On the other hand, acute, short-period of IL-6 enhances insulin sensitivity. Because, the increase in IL-6 after exercise is transient, we hypothesized that transient increase in IL-6 after exercise enhances insulin sensitivity in skeletal muscle. C57BL6J mouse were i.v. injected normal IgG or IL-6 antibody before exercise. Twenty-four hours after a single bout of exercise (treadmill running: 20 m/min, 90 min with 10 degree incline), plantaris muscle was harvested and incubated in oxygenized KRB buffer to measure insulin-stimulated 2-deoxyglucose (2-DG) uptake. Compared with sedentary mouse, insulin-stimulated 2-DG uptake in plantaris muscle was increased 24 h after exercise in IgG-injected mouse, however, the increase induced by exercise was not observed in IL-6 antibody-injected mouse. Concomitant with this results, GLUT4 expression was increased 24 h after exercise in IgG-injected mouse, the increase was canceled in IL-6 antibody-injected mouse. Recombinant mouse IL-6 injection increased GLUT4 expression both fast-twitch plantaris muscle and slow-twitch soleus muscle in C57BL6J mouse. Furthermore, short period incubation of IL-6 (3-12 hours) increased GLUT4 expression in differentiated C2C12 myotubes, however long period (24 h) did not. These results suggests that exercise-induced transient increase in IL-6 affects skeletal muscle in autocrine/paracrine manner, which enhances GLUT4 expression leading to increase insulin sensitivity in skeletal muscle.

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# Potential Usefulness of Intrahepatic Lipid Accumulation and Liver Function Tests to Identify Insulin Resistance Phenotype in Non-Obese Type 2 Diabetes

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Despite low body mass index (BMI), Asian people often develop type 2 diabetes[1)-3)]. In addition to reduced insulin secretion, etiological difference of insulin resistance (IR) between Caucasian and Asian might be involved in this phenomenon[4) 5)]. Previous data demonstrated that non-obese Asians easily develop non-alcoholic fatty liver disease (NAFLD)[6)-8)] which is considered as cause and result of IR[9)-14)]. As well as fat accumulation in liver, liver enzymes, such as alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT), are easily elevated by small increase in BMI within normal limits in Asians[15)]; however those are less observed in other ethnicities[16)]. In addition, both ALT and GGT were correlated to insulin resistance independent of measures of adiposity[17) 18)]. These data suggested that intrahepatic lipid (IHL) accumulation and liver dysfunction could be markers of IR in non-obese type 2 diabetes.

To test this hypothesis, we recruited 16 non-obese (BMI < 25kg/m$^2$) type 2 diabetes (BMI 21.9 ± 2.0 kg/m$^2$, HbA1C 6.8 ± 0.5%, Diet and exercise or take α-glucosidase only). We measured IHL by $^1$H-magnetic resonance spectroscopy (MRS) at overnight fasting state. Total body fat content was measured by using the bioimpedance method. We also evaluated visceral fat and subcutaneous fat area by magnetic resonance imaging (MRI). Then, we performed euglycemic hyperinsulinemic clamp to measure insulin sensitivity (IS) in muscle and liver, respectively. We also measured serum liver function tests, such as AST, ALT and γ-GTP. Based on the upper limit of normal IHL level (4%) in general non-obese Japanese cohort, we divided the subjects into low IHL group (n = 11; 1.3 (0.46–2.39) %) and high IHL group (n = 5; 10.3 (6.26–12.7) %). Our preliminary data showed that compared with low IHL group, high IHL group showed lower muscle IS (6.79 (5.48–7.54) mg/kg/min vs 3.87 (3.84–5.66) mg/kg/min, p = 0.06). Correlation analysis in all subjects revealed that IHL was not significantly correlated to IS in muscle and liver, however, all liver function tests are significantly correlated to both hepatic and muscle IS, respectively.

The present study demonstrated that IHL accumulation and elevated liver enzymes were associated with impaired insulin sensitivity in non-obese Japanese type 2 diabetes. These data suggested the usefulness of those hepatic parameters as marker of impaired insulin sensitivity in non-obese Japanese type 2 diabetes. However, this study is preliminary analysis in small number of subjects; further analysis is clearly required to confirm these relationships.

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# Long-Lasting Effects of Early-Onset Exercise on the Prevention of Obesity and Its Related Lifestyle Diseases

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Decreased physical activity and increased obesity during childhood have recently emerged as significant social problems. Since long-term exposure to risk factors contributes to the development of lifestyle diseases, determining effective early-age prevention strategies is essential. Regular exercise and increased physical activity are well known to prevent obesity and insulin resistance in both animals and humans. However, although physical activity during childhood has a well-known direct effect on child health, the long-term consequences of childhood exercise on adult health and morbidity have not been well studied due to the difficulties of following subjects long-term. To address this, many researchers use rodents or other animal models. Previous studies have suggested that exercise has long-lasting effects on body weight after exercise cessation. In this review, studies examining the long-lasting effects of childhood exercise on obesity and metabolic diseases later in life using animal models are summarized, and the importance of exercise in childhood in preventing obesity and its related comorbidities is highlighted.

***Key words***: early-onset exercise, primordial prevention, long-lasting effect, obesity, metabolic disease

## Introduction

The prevalence of obesity and other lifestyle diseases has dramatically increased in both adults and children in recent decades. According to a 2013 report by the Centers for Disease Control and Prevention in the U.S., 12.7 million children and adolescents aged 2–19 years old were considered obese (http://www.cdc.gov/obesity/data/childhood.html). In Japan, the prevalence of obesity increased threefold in Japanese children and adolescents aged 5–17 between 1978 and 2007[1]. Since long-term exposure to risk factors contributes the development of lifestyle diseases, determining effective early-age prevention strategies is essential. A number of previous studies have demonstrated that overweight children are more likely to be overweight as adults compared to normal weight children, with 40–60% of overweight children becoming overweight adults[2) 3)]. In addition, the Bogalusa heart study suggested that 77% of obese, and presumably sedentary, children remained obese during adulthood[4)]. Therefore, maintaining a normal weight and body fat percentage during childhood may be important for long-term health.

Although the main risk factors of lifestyle diseases include genetic and environmental factors, diet and exercise are commonly recommended for the prevention and amelioration of obesity and lifestyle diseases[5)]. However, the majority of dietary alterations fail in humans, and adults generally regain lost weight in the months and years following diet cessation[6)7)]. Similarly, weight-reduced rats quickly regain lost weight when allowed free

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access to food[8)-11)]. Weight regain in humans and rodents may be partly attributed to the chronic reduction in resting metabolic rate associated with weight loss[12)]. On the other hand, exercise affects energy balance, and those who successfully maintain weight loss for several years have reported maintaining high levels of physical activity[13) 14)]. Although the control of energy balance by exercise and diet is still not well understood, access to a running wheel modulated both food intake and body weight in studies examining rodent obesity[15)]. In addition, in some rodent models, long-lasting effects of exercise on feeding and body weight have been identified[16) 17)]. Overall, these studies suggest that exercise is an effective way to maintain weight loss, and that physical activity levels have a strong influence on obesity and related lifestyle diseases.

Nonetheless, decreased physical activity in childhood has recently emerged as a significant social problem. However, little data exists regarding the effects of physical activity in childhood on adult health and mortality in humans. In addition, while multiple studies have examined the effects of exercise on energy homeostasis in adult rodents[15) 18)], few have examined the effect of early-onset exercise in juvenile animals on the development of obesity[16) 17) 19) 20)]. The mechanisms by which exercise exerts long-term effects are also unknown at present. In this review, studies examining the long-lasting effects of childhood exercise on obesity and metabolic diseases using animal models are summarized, and the importance of exercise in childhood for the prevention of obesity and its related comorbidities is highlighted.

## The importance of physical activity in childhood

Physical inactivity (lack of physical activity) has been identified as leading cause of death, and leads to decreased physical fitness. Convincing evidence suggests that the risk for all-cause and cardiovascular-related disease mortality is increased in the absence of moderate or high levels of cardiorespiratory fitness in adults[21)]. In addition, decreased physical activity in children has emerged as a significant social problem. Maffeis *et al.*[22)] reported that time spent on sedentary activities was positively correlated to body fat percentage in 9 year-old boys, and that 8-10 year-old obese children were more sedentary and spent less time performing non-sedentary activities compared to age-matched non-obese children[23)]. The World Health Organization (WHO) has recommended that children and adolescents aged 5-17 should accumulate at least 60 minutes of moderate- to vigorous-intensity physical activity daily. However, Troiano *et al.*[24)] found that 58% of children aged 6-11 and 92% of adolescents fail to meet these recommendations.

Blair *et al.*[25)] presented three possible models by which enhanced physical activity in childhood may improve health in adults: (1) childhood physical activity influences adult physical activity, which in turn can affect adult health, (2) childhood physical activity has a direct beneficial effect on childhood health, which predicts adult health, and (3) childhood physical activity has a direct beneficial effect on adult health. These models are supported by several other studies. A longitudinal study suggested that high levels of physical activity during childhood were positively correlated to physical activity patterns 21 years later[26)]. Moreover, a similar study revealed that individuals who had high levels of physical activity as adults were more likely to have lower waist circumferences, and were more likely to have been highly active during childhood[27)]. These results suggest that physical activity in childhood and adolescents has an indirect effect on abdominal obesity through the maintenance of physical activity during adulthood. Therefore, establishing high levels of physical activity during childhood, and continuing high levels of physical activity into adulthood may combat the development of lifestyle diseases. Although there have been many epidemiologic studies, including the ones described above, the effects of childhood physical activity on adult health are difficult to establish to due to the challenges of following subjects through their entire lifespans.

## Experimental models by using animal to study the effect of early-onset exercise

To examine whether exercise habits in childhood contribute to health or morbidity in later life, many researchers have used experimental animal models. In the case of rats, they are weaned from the mother at approximately 3 weeks of age, and are used for breeding from 10-12 weeks of age.

Although matching ages between species is difficult, Goto[28] described that a 6-month-old rat is roughly equivalent to a 15-20-year-old human. Therefore, the first of rat's life is roughly equivalent to human children/adolescence, so to examine the effects of early-onset exercise on health during adulthood, animals must engage in exercise during this period.

Experimental models of exercise cessation have often been used to explore how long-lasting the effects of early-onset exercise are and the mechanisms by which long-lasting effects occur. The rodent wheel lock (WL) model was developed by Rhodes *et al.*[29], and involves housing young rats in cages equipped with voluntary running wheels. After a short period of voluntary running (3 to 6 weeks), the wheels are locked, removing the rats' primary source of physical activity. Using this model, a number of researchers have examined how different organ systems in juvenile rats respond to cessation of daily physical activity. Roberts *et al.* recently reviewed the evidence regarding the effects of exercise cessation on various physiological variables, including body weight, glucose and lipid metabolism, insulin resistance, and vascular function using the WL model[30]. However, the longest studies using the WL model have only tracked rats for 173 hours after exercise cessation, and to determine the long-lasting effects of early-onset exercise, animals must be monitored over long periods of time. Several studies have examined the effects of exercise for more than 10 weeks using various models, and these results will be summarized in the next section.

## The long-lasting effects of early-onset exercise on obesity and its related metabolic diseases

In a number of rodent obesity models, early-onset access to a running wheel and the subsequent increase in physical activity normalized body weight in diet-induced obesity (DIO) and type 2 diabetic, Otsuka Long-Evans Tokushima Fatty (OLETF), rats[15) 31) 32)]. OLETF rats represent a well-established animal model of obesity and type 2 diabetes, and are characterized by hyperphagia and obesity, which begin during early childhood[33]. OLETF rats go on to develop hyperglycemia after 18 weeks of age. Since several reports have shown that running activity can prevent obesity in OLETF rats, but not Zucker fatty rats[34], OLETF rats have been used to examine the effects of exercise on the prevention of obesity and metabolic diseases.

In addition, some animal models suggest that early-onset exercise has long-lasting effects on body weight (Table-1). Interestingly, adult rats placed on similar exercise regimens did not sustain their weight loss after exercise cessation[35]. Shima *et al.*[19] reported that exercise had long-lasting preventive effects on obesity and type 2 diabetes development in OLETF rats. Bi *et al.*[18] also demonstrated that the effects of exercise on body weight were long lasting, and that these effects might be mediated by central energy homeostasis regulating pathway, including neuropeptide Y (NPY) signaling in the dorsomedial hypothalamus (DMH). Patterson *et al.*[16] examined the duration of early running wheel activity necessary to produce sustained suppression of body weight and adiposity after exercise cessation, and determined that three weeks of exercise was sufficient to prevent obesity 10 weeks after wheel removal. In contrast, Chao *et al.*[17] examined the effects of a high-fat diet on body weight in OLETF rats that had prior access to running wheels for 4 weeks, and determined that the high-fat diet offset the long-lasting effects of exercise on body weight. In addition, Shindo *et al.*[20] observed the long lasting effects of exercise on weight gain for the longest period, and determined that higher levels of citrate synthase (CS), succinate dehydrogenase (SDH), phosphofructokinase (PFK) activity and uncoupling protein (UCP-3) mRNA in skeletal muscle were found after long-term exercise cessation. Research from our own lab also suggests that the lower body weight and glucose levels are sustained after exercise cessation in OLETF rats compared with sedentary animals. In addition, we observed that exercise completely prevented increase in serum lipid parameters (e.g. triglyceride and total cholesterol), even after cessation (unpublished data).

Taken together, these studies suggest that early-onset exercise has long-lasting effects on the prevention of obesity and its related metabolic diseases through the regulation of central energy homeostasis pathway, and by increasing the activity of enzymes participating in energy metabolism. Further studies are needed to determine the underlying mechanisms by which early-onset exercise prevent obesity and

**Table-1** Study characteristics

| Study (Year) | Method summary | | | Main findings |
|---|---|---|---|---|
| | Animal model | Period of exercise | Period of exercise cessation | |
| Shima *et al.* (1996) [19] | Otsuka Long-Evans Tokushima Fatty (OLETF) rats | 8 weeks (7 to 15 weeks of age) | 13 weeks (15 to 28 weeks of age) | The lower body weight and glucose levels was kept after exercise cessation compared with sedentary animals. Exercise had preventive effects on pancreas fibrosis, at least partly, after exercise cessation. |
| Bi *et al.* (2005) [18] | Otsuka Long-Evans Tokushima Fatty (OLETF) rats | 6 weeks (8 to 14 weeks of age) | 6 weeks (14 to 20 weeks of age) | The lower body weight and glucose levels was kept after exercise cessation compared with sedentary animals. Preventive effects of exercise on weight gain might be mediated by the central energy homeostasis regulating pathway. |
| Patterson *et al.* (2008) [16] | Diet-induced obesity (DIO) rats | 1) 6 weeks (4 to 10 weeks of age) 2) 3 weeks (4 to 7 weeks of age) 3) 2 weeks (4 to 6 weeks of age) | 1) 7 weeks (10 to 17 weeks of age) 2) 10 weeks (7 to 17 weeks of age) 3) 11 weeks (6 to 17 weeks of age) | In exercise animals for more than 3 weeks, the effect of exercise on weight loss was sustained after exercise cessation. In exercise animals for 2 weeks, the body weight was similar with sedentary animal after exercise cessation. |
| Chao *et al.* (2011) [17] | Otsuka Long-Evans Tokushima Fatty (OLETF) rats | 4 weeks (9 to 13 weeks of age) | 8 weeks with regular chow or high-fat diet (13 to 21 weeks of age) | 4 weeks of exercise produced long-lasting effects on body weight in OLETF rats fed a regular chow. High-fat diet offset the long-lasting effects of exercise on prevention of body weight gain. |
| Shindo *et al.* (2014) [20] | OtsukaLong-Evans Tokushima Fatty (OLETF) rats | 14 weeks (5 to 19 weeks of age) | 26 weeks (19 to 45 weeks of age) | Inhibited body weight gain in OLETF rats by prepubertal-onset exercise lasted for a long period after completion of exercise intervention. Higher levels of CS, SDH, PFK activity and UCP-3 mRNA in skeletal muscle were found after long-term exercise cessation. |
| Tsuzuki *et al.* (unpublished data) | Otsuka Long-Evans Tokushima Fatty (OLETF) rats | 10 weeks (5 to 15 weeks of age) | 10 weeks (15 to 25 weeks of age) | The lower body weight and glucose levels were sustained after exercise cessation compared with sedentary animals. Exercise completely prevented increase in serum lipid parameters (e.g. triglyceride and total cholesterol). |

See text and referenced articles for definitions of abbreviations.

lifestyle diseases later in life.

## Summary

Studies on the long-term effects of early-onset exercise and exercise cessation on obesity and lifestyle disease development are difficult to perform in humans. Therefore, animal models provide a translational tool that can be used to identify preventative methods that can be utilized in children to prevent obesity and lifestyle diseases later in life. The information in this review establishes the importance and necessity of physical activity in childhood for the prevention of adult obesity and lifestyle diseases.

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## Conflict of Interest

No conflicts of interest, financial or otherwise, are declared by the authors.

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# Caffeine Increases Contraction-Stimulated 5'-AMP-Activated Protein Kinase Activity and Insulin-Independent Glucose Transport in Rat Skeletal Muscle

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***Objective***: 5'-adenosine monophosphate-activated protein kinase (AMPK) has been identified as a key mediator of contraction-stimulated insulin-independent glucose transport in skeletal muscle. Caffeine acutely stimulates AMPK in resting skeletal muscle, but it is unknown whether caffeine affects AMPK in contracting muscle. In this study, we examined the effect of caffeine stimulation on contraction-stimulated AMPK activity and glucose transport.

***Materials and Methods***: (1) Isolated rat epitrochlearis muscle was preincubated and then incubated in the absence or presence of 3 mM caffeine for 30 min. Electrical stimulation (ES) was used to evoke tetanic contractions during the last 10 min of the incubation period. (2) Rats were given an intraperitoneal injection of caffeine (60 mg/kg body weight) or saline, and the extensor digitorum longus muscle was dissected 15 min later. ES of the sciatic nerve was performed to evoke tetanic contractions for 5 min before dissection.

***Results***: (1) The combination of caffeine plus contraction had additive effects on AMPKα Thr$^{172}$ phosphorylation, α-isoform-specific AMPK activity, and 3-*O*-methylglucose (3MG) transport. Caffeine significantly delayed muscle fatigue during contraction, and the combination of caffeine and contraction additively decreased ATP and phosphocreatine contents. (2) Similar to the findings from isolated muscles incubated *in vitro*, the combination of caffeine plus contraction *in vivo* had additive effects on AMPK phosphorylation, AMPK activity, and 3MG transport.

***Conclusions***: These findings suggest that caffeine and contraction synergistically stimulate AMPK activity and insulin-independent glucose transport, at least in part by decreasing muscle fatigue and thereby promoting energy consumption during contraction.

***Key words***: 5'-AMP-activated protein kinase, muscle contraction, energy deprivation, muscle fatigue, glucose metabolism

## Introduction

Skeletal muscle plays a major role in whole-body glucose metabolism in rodents and humans. Insulin and exercise (muscle contraction) are the physiologically important stimuli of glucose transport, the rate-limiting step in glucose utilization in skeletal muscle. Although both insulin and exercise elicit the translocation of glucose transporter 4 (GLUT4) from intracellular vesicle compartments to the sarcolemma and T-tubules, these stimuli activate specific signaling mechanisms. 5'-adenosine monophosphate-activated protein kinase (AMPK) has been identified as a signaling molecule involved in contraction-stimulated and insulin-independent glucose transport (reviewed in[1) 2)]). AMPK in skeletal muscle has also been implicated in a number of the metabolic effects of exercise such as

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increased insulin sensitivity[3) 4)] and GLUT4 expression[5) 6)], inhibition of acetyl-CoA carboxylase and fatty acid oxidation[7) 8)], modulation of glycogen synthesis[9) 10)], mitochondrial biogenesis via peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α)[11)], activation of sirtuin[12)], and the shift in the metabolic properties toward those of slow oxidative muscle fibers[13)]. These acute and chronic alterations in skeletal muscle suggest that AMPK is a metabolic enhancer that may prevent or delay the development of type 2 diabetes mellitus.

AMPK is a heterotrimer comprising a catalytic α subunit, and regulatory β and γ subunits. There are two different α-isoforms (α1 and α2); α1 is expressed ubiquitously, and α2 is expressed in skeletal muscle, heart, and liver. AMP binding results in the phosphorylation of α $Thr^{172}$, which is essential for kinase activation. Classically, AMPK acts as a signaling intermediary by monitoring cellular energy status[14)]. In isolated rat skeletal muscle incubated *in vitro*, both α1-containing AMPK (AMPKα1) and α2-containing (AMPKα2) are stimulated by energy-decreasing (AMP-increasing) stressors including contraction, hypoxia, chemical inhibition of oxidative phosphorylation, and hyperosmolarity, all of which are potent stimulators of insulin-independent glucose transport[15)].

We reported previously that incubation with caffeine (≥3 mM for ≥15 min) increased AMPKα $Thr^{172}$ phosphorylation and AMPKα1 and α2 activities in isolated rat skeletal muscles and that these effects were accompanied by increased insulin-independent glucose transport[16)]. Caffeine-induced AMPK activation was also accompanied by decreased fuel status; for example, the phosphocreatine content was 23% lower in muscle stimulated with caffeine compared with the control[16)]. These results indicated that caffeine acts directly in skeletal muscle and has similar actions to those of contraction by acutely promoting AMPK activity with energy deprivation in skeletal muscle. It is notable that epidemiological studies have demonstrated that the intake of caffeinated beverages, including coffee and tea, is linked to a reduced risk of type 2 diabetes mellitus[17) 18)].

Caffeine increases force production during contraction by multiple mechanisms such as increased $Ca^{2+}$ release and $Ca^{2+}$ permeability in the sarcoplasmic reticulum, increased $Ca^{2+}$ sensitivity, and slowing of the sarcoplasmic reticulum $Ca^{2+}$ pump (reviewed in[19) 20)]). Many researchers have reported that caffeine increases exercise performance and delays fatigue in rodents[21) 22)] and humans[21) 23)-26)]. These ergogenic actions of caffeine led us to hypothesize that caffeine stimulates AMPK and glucose transport in contracting states by causing profound changes in the cellular energy status in skeletal muscle. To test our hypothesis, we examined the effect of caffeine stimulation on isolated rat skeletal muscle electrically stimulated *in vitro*. We also explored the effect of systemic caffeine administration on contracting skeletal muscle in living rats.

## Materials and methods

### 1. Animals

Male Sprague Dawley rats (150-160 g) were purchased from Shimizu Breeding Laboratories (Kyoto, Japan). Rats were fed a standard diet (Certified Diet MF; Oriental Koubo, Tokyo, Japan) and water *ad libitum*, and fasted overnight before each experiment. Protocols for animal use and euthanasia were approved by the Kyoto University Graduate School of Human and Environmental Studies and Kyoto University Radioisotope Research Center.

### 2. Muscle treatment *in vitro*

Muscles were treated as we described previously[27)] with some modifications. Isolated rat epitrochlearis muscle was preincubated in 7 m*l* of alpha minimum essential medium (αMEM) containing 1% penicillin/streptomycin for 40 min. The muscle was then incubated in 7 m*l* of fresh medium in the absence or presence of 3 mM caffeine for 30 min, 1 mM caffeic acid for 30 min, or 1 mM chlorogenic acid for 30 min. For tetanic contraction, the muscle was stimulated with an electric stimulator (SEN-3401; Nihon Kohden, Tokyo, Japan) during the last 10 min of the incubation period (train rate: 1/min, train duration: 10 s, pulse rate: 100 Hz, pulse duration: 0.1 ms, voltage: 10 V). Force was recorded with a force transducer (TRN001; Kent Scientific, Torrington, CT, USA) and a recorder (U-228-2P-500; Pantos, Kyoto, Japan). Control muscles were preincubated and incubated without contraction. Other muscle samples were used fresh in the 3-*O*-methyl-$_D$-glucose (3MG) transport or

caffeine transport assay, or were immediately frozen in liquid nitrogen and subsequent analysis. All media were gassed with 95% $O_2$/5% $CO_2$ and maintained at 37℃.

### 3. Muscle treatment *in vivo*

Caffeine dissolved in saline was injected intraperitoneally without anesthesia at 60 mg/kg body weight. The injection volume was 2 m*l*/kg body weight. Five minutes after caffeine or saline injection, rats were anaesthetized with intraperitoneal administration of pentobarbital sodium (75 mg/kg body weight), and electrodes (OM209-041; Unique Medical, Tokyo, Japan) were attached to the sciatic nerve on both sides. Fifteen minutes after caffeine or saline injection, the extensor digitorum longus (EDL) muscle was rapidly dissected. The muscle was used fresh to measure 3MG transport activity or other samples were immediately frozen in liquid nitrogen and subsequent analysis. For tetanic contraction, the sciatic nerves were stimulated during the last 5 minutes before dissection (train rate: 1/min, train duration: 10 s, pulse rate: 100 Hz, pulse duration: 0.1 ms, voltage: 2 V) using the SEN-3401 stimulator.

### 4. Analyses

Western blot analysis[27], isoform-specific AMPK activity assay[27], 3MG transport assay[27,28], caffeine transport assay[29], and ATP and PCr assay[16] were performed as we described previously.

### 5. Statistical analysis

Results are presented as mean ± SE. Multiple means were compared using ANOVA followed by post hoc comparisons with Tukey's test. Two means were compared using unpaired Student's *t* test. Differences between groups were considered significant at $p < 0.05$.

## Results and discussion

### 1. Caffeine and contraction additively stimulate AMPK and glucose transport in isolated skeletal muscle

Our previous study demonstrated that maximal activation of AMPK by caffeine is observed with a 30 min incubation at a concentration of 3 mM[16]. We have also demonstrated previously that maximal activation of AMPK by contraction can be induced by 10 repeated 10 s tetanic contractions during 10 min and that there is no further increase in AMPK activity with 15 repeated 10 s tetanic contractions[30]. However, tetanic contraction is not the strongest stimulus of AMPK activity in skeletal muscle. For instance, in incubated rat epitrochlearis muscle, dinitrophenol (0.5 mM for 20 min) increased AMPK activity by 6-fold compared with basal AMPK activity, and 10 tetanic contractions increased AMPKα2 activity only 4-fold[15]. Therefore, even when AMPK activity is increased maximally by contraction in skeletal muscle, it may still be activated further by other stimuli. In the present study, the stimulatory effects of maximally effective caffeine and maximally effective contraction on AMPKα $Thr^{172}$ phosphorylation were partly but significantly additive (Figure-1A). The total AMPK content did not differ between the groups. The caffeine- and contraction-stimulated AMPKα1 and AMPKα2 activities were also significantly additive (Figure-1B). Treatment with 3 mM caffeine for 30 min and contraction increased the rate of 3MG transport by 2.4- and 4.4-fold compared with the basal level, respectively. The caffeine- and contraction-stimulated activity of 3MG transport was significantly additive (5.8-fold compared with the basal level) (Figure-1C). These results suggest that caffeine increases the maximal capacity of contraction-stimulated AMPK activation in skeletal muscle.

### 2. Caffeine and contraction additively decrease ATP and PCr contents in isolated skeletal muscle

To clarify whether the combined effect of caffeine and contraction on AMPK activity is associated with a change in energy status, we measured the muscle contents of ATP and PCr. Treatment with 3 mM caffeine for 30 min and contraction decreased the contents of ATP (Figure-2A) and PCr (Figure-2B). The effects of caffeine and contraction on ATP and PCr were partially additive (Figure-2A and B). Consistent with these findings, caffeine significantly mitigated muscle fatigue during contraction (Figure-2C), in association with an increase in the initial peak force (Figure-2D).

Caffeine can easily pass through the surface membrane of the muscle cell because of its hydro-

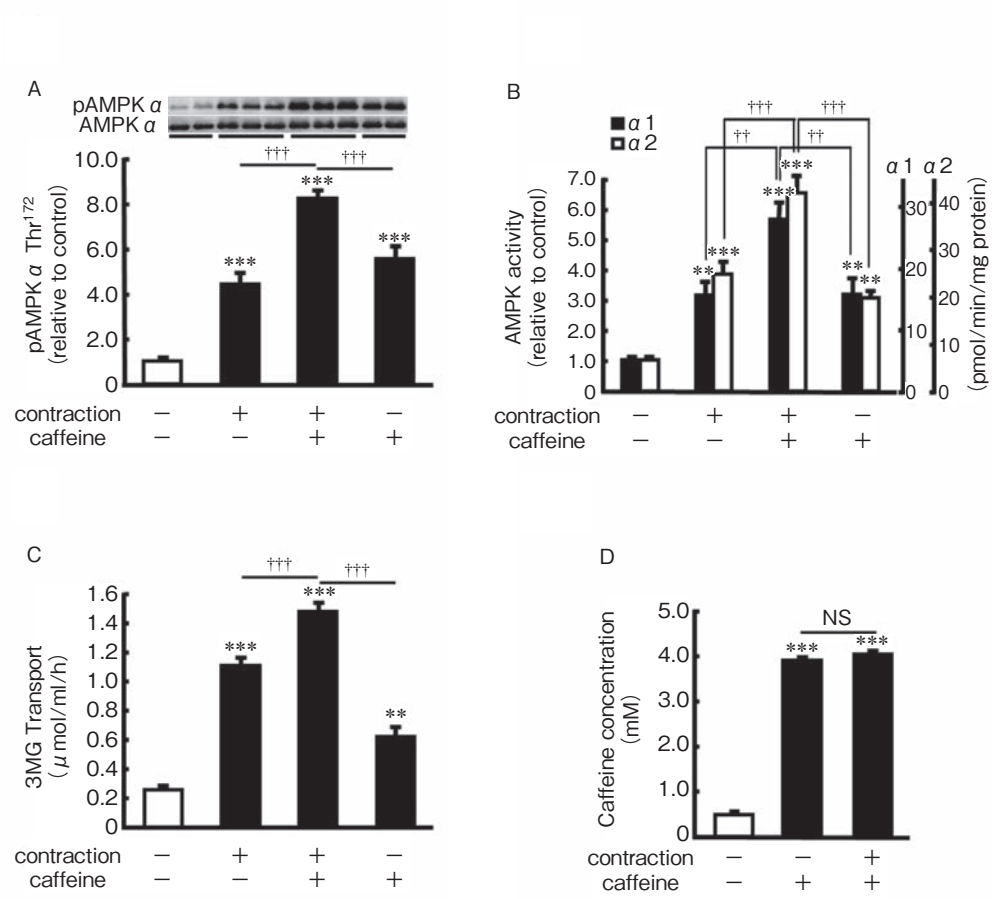


**Figure-1** Effect of caffeine on contraction-stimulated AMPKα Thr$^{172}$ phosphorylation, AMPK activity, and 3-*O*-methyl-D-glucose (3MG) transport activity in incubated rat skeletal muscle

Isolated epitrochlearis muscle was preincubated and incubated for 30 min in the absence (-) or presence (+) of 3 mM caffeine. The muscle was tetanically contracted during the last 10 min of the incubation period and then subjected to Western blot analysis (**A**), an isoform-specific AMPK activity assay (**B**), or, the 3MG transport assay (**C**). Intracellular caffeine concentration was measured after incubation in the presence of 3 mM caffeine for up to 30 min with or without electrical stimulation (**D**).

Values are mean ± SE; n=5-13 per group. $^{**}p<0.01$, $^{***}p<0.001$ vs. control; $^{\dagger\dagger}p<0.01$, $^{\dagger\dagger\dagger}p<0.001$ vs. contraction plus caffeine; NS: not significant.

phobic property[31]. In this study, the intracellular concentration of caffeine reached a maximum by 30 min after the start of the exposure to caffeine and was not affected by contraction (Figure-1D). The ergogenic actions of caffeine may contribute to the decreased muscle fatigue and profound decrease in energy status in contracting skeletal muscle.

In the mechanism of energy reduction by caffeine, Miyazaki *et al.*[32] demonstrated that 1-5 mmol/*l* of caffeine increased oxygen consumption acutely in frog skeletal muscles that were isolated and incubated *in vitro*. Those authors also found that the metabolic enhancement afforded by caffeine was associated with an increase in lactic acid content and decreases in ATP, ADP, and PCr contents in the muscle, without mechanical changes such as contracture formation. Thus, caffeine may act on the muscle energy status via acceleration of the energy supply, rather than via inhibition of mitochondrial function and suppression of ATP production.

### 3. Neither caffeic acid nor chlorogenic acid affects contraction-stimulated AMPKα Thr$^{172}$ phosphorylation in isolated skeletal muscle

In addition to caffeine, caffeic acid and chlorogenic acid, which are the major constituents of coffee, also have antihyperglycemic properties[33)-36)]. We previously reported that caffeic acid, but not chlorogenic acid, acutely promoted AMPK phosphorylation. In that study, the maximal activation of AMPK by caffeic acid was observed at 1 mM after a

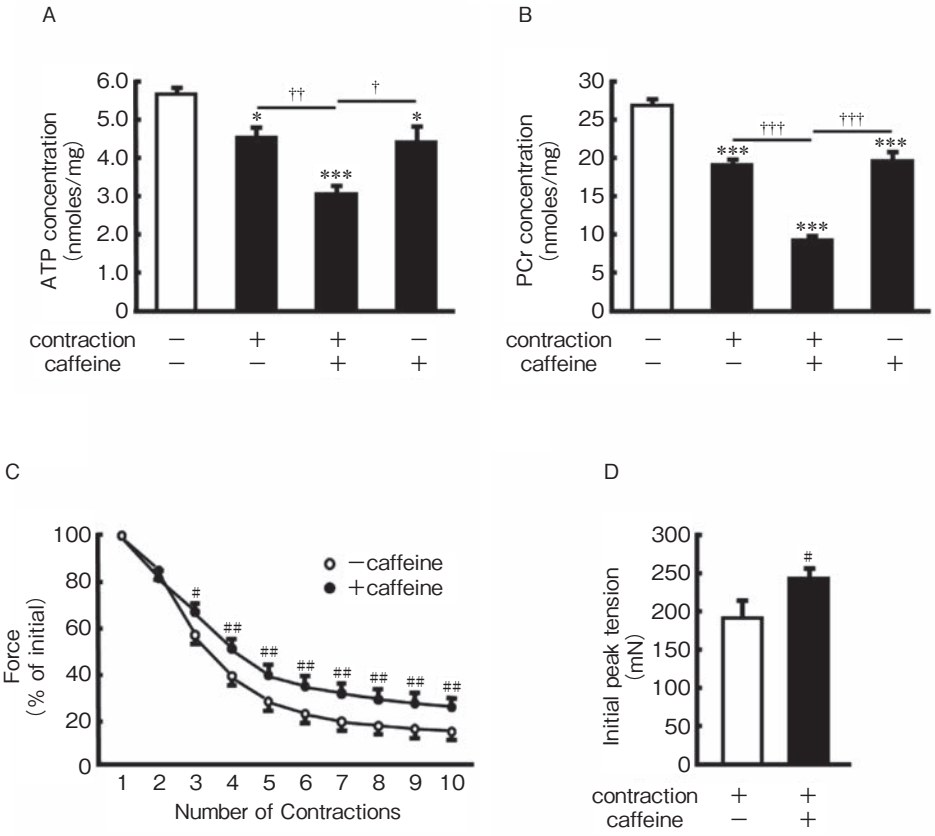


**Figure-2** Effect of caffeine on ATP and PCr contents, and force production during contraction in incubated rat skeletal muscle
Isolated epitrochlearis muscle was preincubated and incubated for 30 min in the absence (−) or presence (+) of 3 mM caffeine. The muscle was tetanically contracted during the last 10 min of the incubation period, after which the concentrations of ATP (**A**) and PCr (**B**) were measured. Peak tension of each contraction (**C**) and the initial peak tension (**D**) were evaluated.
Values are mean ± SE; n = 7–12 per group. $^{*}p < 0.05$, $^{***}p < 0.001$ vs. control; $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$ vs. contraction plus caffeine; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. contraction.

30 min incubation in isolated rat epitrochlearis muscle[37]. In the current study, we examined the effects of caffeic acid and chlorogenic acid on contraction-stimulated AMPK activity in skeletal muscle. AMPKα Thr[172] phosphorylation was increased by caffeic acid (1 mM, 30 min) (Figure-3A), but not by chlorogenic acid (1 mM, 30 min) (Figure-3B). However, unlike caffeine (Figure-1A), incubation with caffeic acid or chlorogenic acid did not affect the contraction-stimulated AMPKα Thr[172] phosphorylation (Figure-3A and B). The total AMPK content did not differ between the groups. These data show clearly that AMPK-activating agents do not necessarily have an additive effect on contraction-stimulated AMPK activity in skeletal muscle.

**4. Intraperitoneal caffeine injection and contraction *in situ* additively activate AMPK and glucose transport in skeletal muscle**

To determine whether caffeine affects contraction-stimulated AMPK activity *in vivo*, we measured the degree of phosphorylation of AMPKα Thr[172] in EDL muscle dissected after intraperitoneal injection of caffeine or saline with or without contraction. AMPK phosphorylation was increased by caffeine and contraction, and the effects of caffeine and contraction were partially additive (Figure-4A). The total AMPK content did not differ between the groups. In the isoform-specific AMPK activity assay, caffeine and contraction increased both AMPKα1 and AMPKα2 activities, and the effects of caffeine and contraction were partially additive (Figure-4B). Caffeine injection

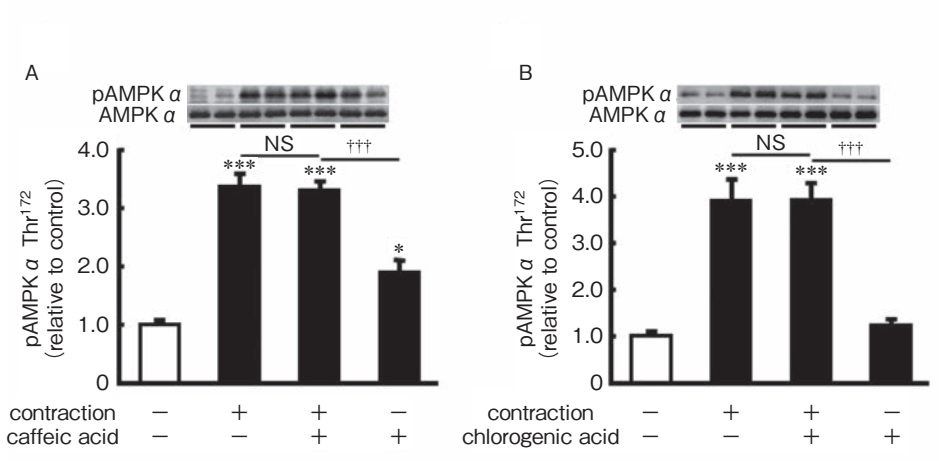


**Figure-3** Effect of caffeic acid and chlorogenic acid on AMPKα Thr$^{172}$ phosphorylation in incubated rat skeletal muscle

Isolated epitrochlearis muscle was preincubated and incubated for 30 min in the absence (−) or presence (+) of 1 mM caffeic acid (**A**) or 1 mM chlorogenic acid (**B**). The muscle was tetanically contracted during the last 10 min of the incubation period and then subjected to Western blot analysis.

Values are mean ± SE; n = 5–12 per group. *$p < 0.05$, ***$p < 0.001$ vs. control; †††$p < 0.001$ vs. contraction plus caffeic acid or chlorogenic acid; NS: not significant.

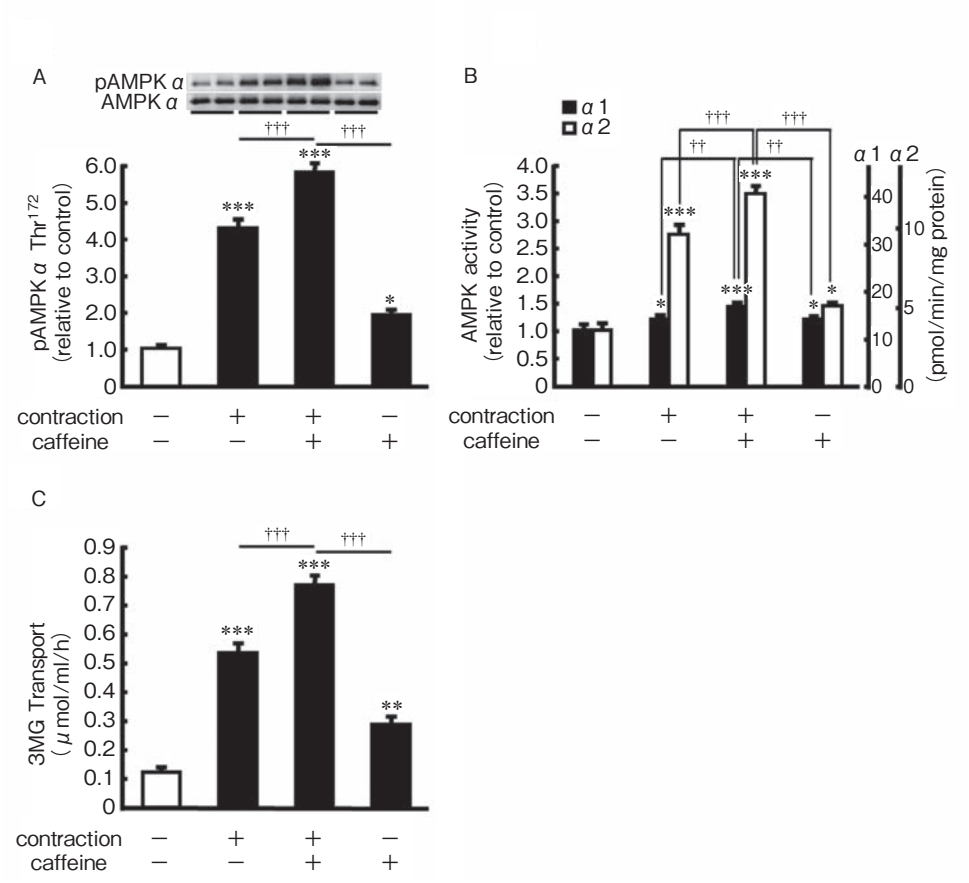


**Figure-4** Effect of intraperitoneal caffeine injection on contraction-stimulated AMPKα Thr$^{172}$ phosphorylation, AMPK activity, and 3MG transport activity in rat skeletal muscle

Caffeine (60 mg/kg) or saline was injected intraperitoneally. Fifteen minutes after injection of caffeine or saline, the EDL was dissected and subjected to Western blot analysis (**A**), isoform-specific AMPK activity assay (**B**), or 3MG transport assay (**C**). Tetanic contraction was elicited by electrical stimulation of the sciatic nerve during the last 5 min before dissection. Representative immunoblots are shown.

Values are mean ± SE; n = 4–11 per group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. control; ††$p < 0.01$, †††$p < 0.001$ vs. contraction plus caffeine.

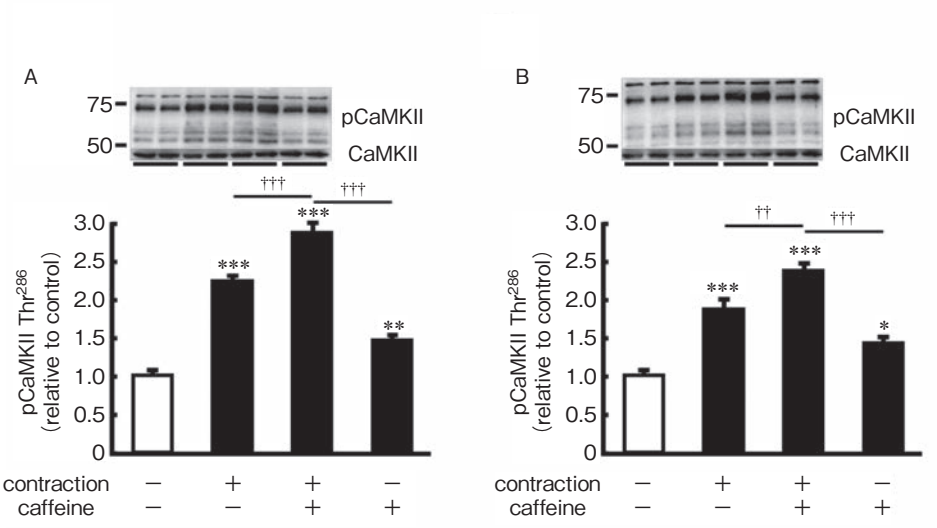


**Figure-5** Effect of caffeine on contraction-stimulated CaMKII Thr$^{286}$ phosphorylation in rat skeletal muscle
Isolated epitrochlearis muscle was preincubated and incubated for 30 min in the absence (−) or presence (+) of 3 mM caffeine. The muscle was tetanically contracted during the last 10 min of the incubation period and then subjected to Western blot analysis (**A**). Caffeine (60 mg/kg) or saline was injected intraperitoneally. Fifteen minutes after injection of caffeine or saline, the EDL was dissected and subjected to Western blot analysis (**B**). Tetanic contraction was elicited by electrical stimulation of the sciatic nerve during the last 5 min before dissection. Representative immunoblots are shown.
Values are mean ± SE; n = 5-11 per group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. control; $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$ vs. contraction plus caffeine.

and contraction also increased 3MG transport by 2.3- and 4.3-fold compared with the saline injection, respectively (Figure-4C). The effects of caffeine and contraction were partially additive (6.2-fold compared with the basal level) (Figure-4C).

**5. Caffeine enhances contraction-stimulated $Ca^{2+}$/calmodulin-dependent protein kinase II (CaMKII) Thr$^{286}$ phosphorylation in skeletal muscle**

$Ca^{2+}$ has been implicated in the activation of glucose transport through signaling pathways involving AMPK[38]. CaMKII has been used as an indicator of elevated cytosolic $Ca^{2+}$ level in skeletal muscle[39]. Our previous study demonstrated that caffeine (3 mM, 15 min) significantly increased CaMKII Thr$^{286}$ phosphorylation in isolated rat epitrochlearis muscle[28], and tetanic contraction significantly increased CaMKII Thr$^{286}$ phosphorylation in isolated rat epitrochlearis muscle[40]. To determine whether caffeine affects contraction-stimulated CaMKII Thr$^{286}$ phosphorylation in skeletal muscle, we measured the degree of phosphorylation of CaMKII Thr$^{286}$. In the present study, CaMKII Thr$^{286}$ phosphorylation was increased by caffeine and contraction, and the effects of caffeine and contraction were partially additive (Figure-5A). Similarly, the combination of caffeine plus contraction *in vivo* had additive effects on CaMKII Thr$^{286}$ phosphorylation (Figure-5B). The total CaMKII content did not differ between the groups. These results suggest that the elevating $Ca^{2+}$ level is partially involved in the additive effects of the combination of caffeine plus contraction on AMPK activity.

In conclusion, our results suggest that caffeine and contraction synergistically stimulate AMPK activity and insulin-independent glucose transport, at least in part by elevating $Ca^{2+}$ level and decreasing muscle fatigue, and thereby promoting energy consumption during contraction. We suggest that the ergogenicity of caffeine may contribute to the enhancement of the effect of exercise-induced promoting glucose metabolism by induction of profound activation of AMPK.

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# The Exercise Therapy Decreases the Serum Interleukin-6 Levels in Patients with Knee Osteoarthritis

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***Purpose***: Exercise is one of the established treatment method for knee osteoarthritis (OA). However, the mechanisms of its action is not fully understood. Although exercise has a muscle strengthening effect that may improve the stability of the knee joint, it has been speculated that exercise may exert anti-inflammatory effect for the joint. The aim of this study was to examine whether exercise had the anti-inflammatory effects for the joint in clinical practice using biomarkers for inflammation.

***Methods***: This study was approved by the Ethics Committee of our university and conducted in accordance with the declaration of Helsinki. The written informed consent for this study participation was obtained from all subjects. A total of one-hundred twenty postmenopausal women with medial type of primary knee OA (K/L2-4) who first visited our outpatient clinic for knee pain were included in the study. Subjects were randomized to either the exercise therapy group or the control group. The patients in the exercise therapy group conducted three different kinds of home exercise and stretching. The patients in the control group took either the oral selective COX2 inhibitor (celecoxib, 200 mg/day for 12 weeks) or the intra-articular injection of hyaluronic acid (HA, high molecular weight 2,700 kDa HA, 5 times with one week interval). Age, body mass index (BMI), Japanese Knee Osteoarthritis Measure (JKOM) score, visual analog scale (VAS) for pain score and serum levels of biomarkers for inflammation (high-sensitivity C-reactive protein [hs-CRP] and interleukin [IL]-6) were evaluated at baseline and 12 weeks after treatment initiation. Values in each group were compared between baseline and 12 weeks using a paired t-test.

***Results***: The sixty-nine of 120 patients were assigned to either the exercise therapy group and the remaining fifty-one patients were to the control group. During the twelve weeks of examination, twelve patients in the exercise therapy group and the eight patients in the control group were withdrawn. No significant differences of the baseline characteristics were observed between the exercise therapy group and the control group. The JKOM score ($p < 0.001$ and $p < 0.001$, respectively) and VAS score ($p < 0.001$ and $p < 0.001$, respectively) at 12 weeks of the patients with both the exercise therapy and control groups were significantly reduced in comparison to those at baseline. The hs-CRP levels remained unchanged in patients with both groups ($p = 0.267$ and $p = 0.137$, respectively). The sIL-6 levels of the patients in the exercise therapy group were significantly decreased ($p = 0.021$) in comparison to those of the patients at baseline, but sIL-6 levels of the patients in the control group were not.

***Conclusions***: The serum IL-6 levels in patients with OA were significantly reduced by the exercise therapy, while sIL-6 levels were unchanged by NSAID, suggesting that exercise therapy may exert anti-inflammatory effects in knee OA.

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# The Effect of Cooling on Muscle Strength and Muscle Cross-Sectional Area During Detraining

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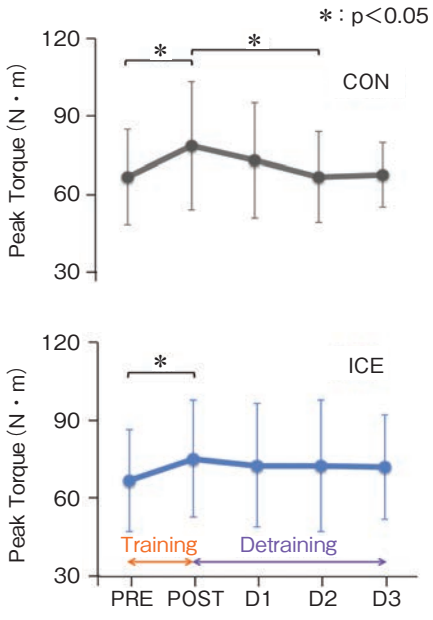
Two weeks of detraining produces 6.4% of muscle atrophy even after long-term strength training. Thus, to maintain the training effect, preventing the muscle weakness and muscle atrophy caused by detraining would be valuable. Exposure to cold stimulus by cold water inhibits muscular atrophy.

The purpose of this study was to investigate the effect of cooling by ice bag on muscle weakness and muscle atrophy caused by detraining.

Six healthy males (23.0 ± 2.5 y/o) performed elbow flexion resistance training three times per week for 6 weeks. After the 6 weeks training, the subjects were instructed to limit upper arm activity within their activity of daily living level during three weeks of detraining period. During the de-training period, one of the arms was cooled at inside of upper arm by an ice bag for 30 min a day (ICE), and the other arms was control condition (CON). The measurements were elbow flexion torques at angular speeds of 60 and 120 deg/s under concentric contraction (CC60, CC120) and isometric contraction (IM), cross-sectional area (CSA) and circumference of the upper arm. The measurements were done, at pre-training (PRE), post-training (POST), after the first week (D1), second week (D2) and third week (D3) of detraining.

IM significantly increased after training in both conditions ($p<0.05$). Significant decreases of IM in CON was observed at D2 (66.6 ± 17.4 N·m) compared to POST (78.8 ± 24.8 N·m) ($p<0.05$). Circumference significantly increased after training in both conditions (CON: 102.1 ± 1.0%, ICE: 101.4 ± 0.7%) ($p<0.05$). Significant decreases of circumference in CON was observed at D3 (29.6 ± 3.4 cm) compared to D2 (29.8 ± 3.5 cm) ($p<0.05$). CSA significantly increased after training in both conditions (CON: 108.5 ± 3.3%, ICE: 107.9 ± 2.2%) ($p<0.05$). CSA significantly decreased after the detraining in both conditions. However, there was no significant difference in all of measurements between CON and ICE.

Only IM increased after the training. It may be because of the fact that the training was composed of isometric contraction. Even though increased the muscle force, circumference and CSA by the training decreased by the detraining. On the other hand, there was no decrease in IM and circumference in ICE during detraining period. This result suggests the possibility that the cooling prevents muscle weakness and muscle atrophy; however, there was no significant difference between both conditions, therefore we consider that the effect of cooling was weak. In conclusion, the cooling for 30 min a day shows possibility that the cooling prevents muscle weakness and muscle atrophy caused by detraining.

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# Morphological Profiles of the Quadriceps Femoris of Varsity Athletes

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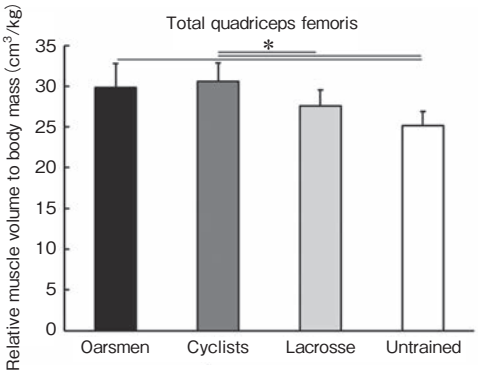
***Introduction***: Sport athletes often show event-related muscular profiles that are possibly associated with their competitive and training activities. Thus, investigation of the muscular profiles in athletes can enlighten the association between muscle-specific hypertrophy and sport-specific movement performance. With respect to the movement of the lower extremities, rowing and pedaling mainly consist of repetitive multi-joint leg extensions (simultaneous extensions of knee and hip joints), which are largely contributed by the quadriceps femoris. On the other hand, several types of joint motions are involved in field sports. It is hypothesized that the quantitative profiles of the quadriceps femoris are similar for the athletes who routinely repeat leg extensions (oarsmen and cyclists) but not for the athletes not regularly performing leg extensions (field sport athletes; such as lacrosse players). This study tested the hypothesis.

***Methods***: T1-weighted MR images of the whole right thigh were obtained from 14 varsity oarsmen, 8 male cyclists, 13 male lacrosse players and 10 untrained men. The anatomical cross-sectional areas (ACSAs) from the origin to insertion of each muscle of the quadriceps femoris (vastuslateralis, vastus medialis, vastus intermedius, and rectus femoris) were measured. The muscle volume of each muscle was determined by summing in-series ACSAs which were multiplied by the slice thickness (1 cm), and the relative muscle volume to body mass (normalized volume) was calculated.

***Results and Discussion***: The normalized total volumes of the quadriceps femoris were significantly greater in the oarsmen and cyclists than in the untrained men, and that of the cyclists was significantly greater than that of the lacrosse players. Likewise, the normalized vastus lateralis volumes of the oarsmen and cyclists were significantly greater than those of the lacrosse players and untrained men, and the normalized volumes of the vastus medialis and vastus intermedius were significantly greater in the oarsmen and cyclists than in the untrained men. In contrast, the normalized rectus femoris volume of the lacrosse players was greatest of the four groups, while no statistical differences were shown among the other three groups. It was made clear that the varsity oarsmen and cyclists had hypertrophied monoarticular vasti (lateralis, medialis, and intermedius), whereas the lacrosse players had hypertrophied biarticular rectus femoris. These results strongly suggest that the varsity athletes demonstrate muscle-specific hypertrophy among the synergistic muscles comprising a muscle group, depending on the sport-specific motions involved during competitive and training activities.

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# The Site-Specific Associations Between the Meniscus Changes and the Osteophyte Formations in Early-Stage Knee Osteoarthritis

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***Purpose***: We recently reported that the degeneration and destruction of femoral articular cartilage and osteophytes showed a greater degree of deterioration than those of the tibial and patellar articular side in early-stage of knee OA using 3TMRI and T2 mapping sequence (Osteoarthritis Cartilage, 2014). In the present study, we focused on the meniscus change and osteophyte formation in early-stage knee osteoarthritis (OA). We examined whether there were any associations between the meniscus changes and the osteophyte formations, and also examined, if so, there were any site-specific differences for the associations in earlystage knee OA by using the magnetic resonance imaging (MRI)-based analyses.

***Method***: A total of 50 patients (mean age 59.7 years) who visited our out-patient clinic for knee pain between May and December 2012 were enrolled in this study. The severity of knee OA was classified by Kellgren-Lawrence (K/L) grading scale based on standing extendedknee X-ray images. All patients showed either K/L grade 0, 1 or 2, and were also performed 3TMRI for the affected knee. Diagnosis of knee OA for the subjects with K/L 0 was conducted using 3TMRI according to the method by Shama *et al* (ARD 2013). Patients who showed less than 174° of femoro-tibial tibial angle (FTA) were excluded from the study. Compartments of the knee joint was divided into 14 places of areas according to WORMS using sagittal and coronal two dimensional (2D) fat suppressed and T2 weighted image fast spin-echo sequence (TR=5,000 ms, TE=70 ms, FOV 160 mm, matrix=384 × 307, Slice thickness=3 mm, turbo-factor=17, Flip-angle=150, scan time=3:00). The severity of osteophyte and meniscus tear were semi-quantitatively evaluated according to the WORMS method. The medial meniscus extrusion distance (MMED) was also measured. Interrelationships between the osteophyte scores in medial femoral condyle (MFC) and medial tibia plateau (MTP) and the medial meniscus (MM) tear and MMED were examined.

***Results***: The patients showed the radiographic OA severities for K/L grade 0 (n=3), 1 (n=27) and 2 (n=20), respectively. Twenty-three of fifty patients were male, while remaining twenty-seven patients were female. No significant differences of FTA were observed between the patients with three different K/L grades. While the MMEDs were not associated with the osteophyte scores in MTP, the MMEDs were associated with the osteophyte scores in MFC ($r=0.39$, $p<0.01$). When the patients were divided into two groups in terms of the MMEDs by the cutline of 3 mm, the osteophyte scores in MFC in group 1 (MMEDs ≧ 3 mm; 3.14) were significantly higher than those in group 2 (MMEDs < 3 mm; 1.50) ($p=0.03$). On the other hand, the MM tear scores were associated with the osteophyte scores in MTP ($r=0.32$, $p=0.03$), while those were not associated with the osteophyte scores in MFC. In addition, when the patients were divided into two groups by the presence or absence of the MM tear, the osteophyte scores in MTP of the patients with MM tear (2.86) were significantly increased in comparison to those of the patients without MM tear (0.64, $p=0.02$).

***Conclusion***: The meniscus changes (MME and meniscus tear) were associated with the osteophyte formation in early-stage of medial knee OA. The associations were site-specific, that is; the MME were associated with the osteophyte in MFC, while the MM tears were associated with osteophyte in MTP.

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# Influences of Hamstring Stretching on Passive Muscle Stiffness Vary Between Hip Flexion and Knee Extension Maneuver: A Pilot Study

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***Introduction***: Muscle injury such as muscle strain frequently occurs in the hamstring, especially in the long head of the biceps femoris (BFl). Pre-exercise stretching has been widely performed as one of the methods for preventing the hamstring muscle strain in sports. It has been reported that less flexibility of the hamstring muscle-tendon unit assessed by passive range of motion (ROM) of the hip joint increases the risk of the hamstring muscle strain. In contrast, according to some recent literature, stretching appears to have no or little protective effect for sport injuries including the hamstring muscle strain. Taken together, no consensus has been reached as to whether stretching exercise can have positive effects for prevention of sports injuries. One of the possible reasons for the discrepancies is due to the joint at which the stretching exercise and flexibility test of the hamstring are performed. Since the hamstring which comprises the BFl, semitendinosus (ST), and semimembranosus (SM) is a biarticular muscle group, the muscles can be stretched by passive hip flexion or knee extension. Nevertheless, no study has examined the substantial effect of each of the two stretching maneuver on passive stiffness of the individual muscles, perhaps because it is impossible from a conventional evaluation of the torque-angle relationship to quantify the passive stiffness of a specific muscle. One of the methods to resolve the problem is to use ultrasound shear wave elastography which can quantify localized tissue stiffness along the principal axis of the probe. As a pilot study, we used this technique and compared passive stiffness of BFl, ST, and SM before and after an acute bout of static stretching by either hip flexion or knee extension.

***Methods***: In one healthy male subject, before and after 5 sets of 90-s stretching, passive lengthening measurements where the knee or hip joint was passively rotated to the maximal range of motion (ROM) were performed. During the passive lengthening, muscle stiffness (defined as shear modulus) of each muscle was measured by using ultrasound shear wave elastography.

***Resuts***: Both stretching maneuvers increased maximal ROM and decreased passive torque at a given hip joint angle. Passive muscle stiffness was prominently reduced in all of BFl, ST, and SM after passive knee extension stretching maneuver, whereas the stretching effect by passive hip flexion maneuver was pronounced in ST and SM, but not in BFl.

***Conclusion***: The present findings suggest that 1) the effects of hamstring stretching on individual passive muscles' stiffness vary between passive knee extension and hip flexion stretching maneuvers, and 2) stretching of the hamstring should be performed by passive knee extension rather than hip flexion which is commonly used as a warm-up exercise. Further research with large number of subjects is required to confirm our conclusion.

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# Hyperventilation-Induced Respiratory Alkalosis Increases the Number of Repetitions Able to Be Performed During Resistance Training

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***Background***: During intermittent high-intensity exercise, the accumulation of hydrogen ions or the resulting fall in intramuscular pH has been considered a major factor contributing to muscle fatigue. Our recent study has shown that reversing the acidic muscle milieu by means of hyperventilation (HV)-induced respiratory alkalosis, implemented during the recovery period of repeated short pedaling sprints, is effective in attenuating the performance decrement associated with fatigue. The present study investigated whether HV-induced respiratory alkalosis could be applied to resistance training, expecting an increased number of repetitions able to be performed with HV.

***Methods***: Eleven power-trained athletes (1RM: 102.5-162.5 kg for bench press and 170.3-246.8 kg for leg press) performed 6 sets of bench press and 6 sets of leg press at 80% 1RM on the same day with and without HV during the last 30-s of 5-min inter-set recovery on 2 separate occasions (protocol A and B). HV was implemented before the 1st, 3rd and 5th sets for protocol A, and before the 2nd, 4th and 6th sets for protocol B ($P_{ET}CO_2$: 15-25 mmHg). Subjects breathed spontaneously for the entire 5-min during the alternate non-HV recovery periods. In each exercise set, lifting was continued until failure with the number of successful repetitions recorded. Wireless electro-goniometers were attached about the elbow and the knee joints to calculate the joint angular velocity per repetition. Blood [$La^-$] and pH were examined to report physiological strains of the exercises, and pH recovery resulting from HV.

***Results***: HV increased blood pH by 0.077 ± 0.024 before the subsequent set ($p < 0.001$). HV either increased (leg press, $p < 0.05$) or maintained (bench press) the repetitions performed compared to the number achieved during the prior non-HV set. Whereas, the repetition number for the non-HV set was always fewer compared to the prior HV set ($p < 0.005$). The sum of repetitions (protocol A + B) was greater for the HV than non-HV sets in both bench press (44 ± 10 vs. 36 ± 10 reps, $p = 0.001$) and leg press (64 ± 9 vs. 50 ± 15 reps, $p < 0.001$). Similarly, the joint angular velocity was enhanced by HV in both bench press and leg press, with the value being either maintained or greater ($p < 0.05$) than the prior non-HV set. For the non-HV set, however, the joint angular velocity was almost always reduced ($p < 0.05$) compared to the prior HV set. After the exercise, the blood [$La^-$] was greater (9.82 ± 2.66 vs. 6.87 ± 1.47 mM, $p < 0.001$) and pH was lower (7.303 ± 0.055 vs. 7.340 ± 0.030, $p < 0.001$) for leg press than bench press.

***Conclusion***: HV countered the reductions of repetitions and joint angular velocities associated with fatigue. These changes reflect a greater training volume and a greater training power output, which may be ultimately translated into a greater performance gain. The ergogenic effect was more pronounced for leg press, which incurred a greater physiological strain than bench press.

***Key words***: fatigue, recovery, performance, power

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# Cartilage Metabolic Status for the Radiographic Medial Knee Joint Space Narrowing in Men in Early Forties Without Knee Pain

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***Objective***: To investigate the cartilage metabolic status which can predict radiographic medial knee joint space narrowing (mJSN) in men in early forties without knee pain.

***Design***: In this prospective study, forty seven healthy male volunteers (41.6 y on average) who didn't have knee pain and experience any traumatic episodes for the knee joints were enrolled.

***Intervention***: A radiograph of both knee were taken and the serum levels of pro-collagen type II C-propeptide (sPIICP) and collagen type II cleavage (C2C) and urinary level of cross-inked type II collagen C-telopeptide (uCTX-II) were measured at the time of study entry. Radiological progression of the knee OA is defined as mJSN greater than 0.3 mm in either of the knees during 3-years of follow up.

***Results***: The fourteen of 47 subjects were involved in the progression (P) group, while the remaining thirty-three subjects were involved in the non-progression (NP) group. No significant differences of the sPIICP, sC2C and uCTX-II in P group were observed in comparison to those in NP group at baseline. The sC2C/sPIICP of P group, but not uCTX-II/sPIICP, was significantly decreased in comparison to those of NP group ($p<0.05$). The age- and BMI- adjusted risk for the progression after 3-years of follow up in the lower tertile (T1) of the sC2C/sPIICP at baseline was significantly higher than that in the higher tertile (T3) [Odds ratio: 9.8 (95%CI: 1.4-67.4)].

***Conclusion***: In a three year prospective study, the lower sC2C/sPIICP was the risk factor for the radiographic mJSN in men in early forties without knee pain.

***Key words***: osteoarthritis, knee joint space width, type II C-propeptide, collagen type II cleavage, type II collagen C-telopeptide

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# AMPK-Mediated Regulation of Protein Degradation Systems in Unloaded Mouse Skeletal Muscle

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***Objective***: The aim of the present study was to investigate the involvement of AMPK in regulating skeletal muscle atrophy during hindlimb unloading.

***Methods***: Transgenic (AMPK-DN) mice expressing a dominant negative mutant of AMPKα1 in the skeletal muscle and their wild-type littermates (WT) mice were randomly divided into two groups: untreated preexperimental control (n=12/group) and unloading (n=12/group) groups. Mice of the unloading group were subjected to continuous hindlimb suspension for 2 weeks.

***Results***: Soleus muscle weight relative to body weight in WT mice was decreased by 30% in response to hindlimb suspension, whereas by 20% in AMPK-DN mice. The expressions of ubiquitinated proteins and MuRF1 mRNA, markers of ubiquitin-proteasome system activation, were upregulated by hindlimb suspension in WT mice, but no changes were observed in AMPK-DN mice. The expression of phosphorylated FoxO3a was decreased by hindlimb suspension in WT mice, but not in AMPK-DN mice. HSP72 expression was higher in AMPK-DN mice compared to WT mice during the experiment, and reduced more in WT mice by hindlimb suspension than AMPK-DN mice.

***Conclusions***: The present study demonstrated that the repression of skeletal muscle AMPK activation suppressed the progress of unloading-induced skeletal muscle atrophy. Our findings suggest that AMPK is involved in adaptation of skeletal muscle mass to atrophic stimuli.

***Key words***: skeletal muscle, muscle atrophy, heat shock protein, ubiquitin-proteasome, autophagy

## Introduction

Skeletal muscle has a large capacity to adapt to various stimuli. For example, aging, poor nutrition, several diseases, and decreased loading such as inactivity, result in skeletal muscle atrophy[1)-3)]. However, the molecular mechanism involved in atrophic process in skeletal muscle is not fully understood.

Several studies have been revealed that 5' AMP-activated protein kinase (AMPK) has a potential role in skeletal muscle mass regulation. Elevated AMPK activity led to diminished capacity for hypertrophy of fast-twitch skeletal muscle in aged rat[4) 5)]. Moreover, impaired hypertrophy of slow-twitch skeletal muscle during overload in diabetic rat was partly attributed to upregulation of the expression of phosphorylated AMPKα Thr$^{172}$ [6)]. A study using a knockout mouse model showed that overload-induced muscle hypertrophy was accelerated in AMPKα1-deficient mice compared to the wild-type mice[7)]. Taken together, AMPK is suggested to involve in the regulation of skeletal muscle mass during hypertrophic conditions.

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However, there are no studies examining a role of AMPK in the regulation of skeletal muscle mass during atrophic conditions. Therefore, the aim of the present study was to investigate the potential function of AMPK in muscle mass adaptation in response to atrophic stimuli. For the purpose, we examined the changes of muscle mass and molecular responses after 2-week hindlimb unloading using transgenic mice that overexpresses muscle-specific dominant-negative mutant of AMPKα1 (AMPK-DN).

## Materials and methods

### 1. Animals

Transgenic (AMPK-DN) mice expressing a dominant negative mutant of AMPKα1 in the skeletal muscle[8] were obtained from JCRB (Japanese Collection of Research Bioresources Cell Bank) Laboratory Animal Resource Bank at NIBIO (National Institute of Biomedical Innovation, Osaka, Japan). Twenty-four male AMPK-DN mice (age: 13.2 ± 3.2 weeks, body weight: 24.4 ± 1.5 g, mean ± SD) and twenty-four their WT mice (age: 13.5 ± 3.5 weeks, body weight: 23.2 ± 2.9 g, mean ± SD) were used. All mice were housed in an animal room maintained at 22-24℃ with a 12:12-h light-dark cycle and fed a standard laboratory diet and water ad libitum. All animal protocols were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health (Bethesda, MD).

### 2. Procedure of hindlimb unloading

Both AMPK-DN and WT mice were randomly divided into two groups: untreated preexperimental control (n=12 in each group) and unloading (n=12 in each group) groups. Mice of the unloading group were subjected to continuous hindlimb suspension for 2 weeks. Hindlimb suspension was performed as described previously[9]. Body weight of each mice was recorded at the end of experiment. Two weeks after the procedure, soleus was dissected from each mice and weighed. Left muscles for real-time RT-PCR analyses and right muscles for western blot analyses and AMPK kinase assay were frozen in liquid nitrogen, and stored at -80℃.

### 3. Western blot analyses

Sample preparation and western blot analyses were performed with some modification of the previously reported method[10) 11)].

### 4. Real-time RT-PCR analyses

Real-time RT-PCR analyses were performed as was described previously[12)].

### 5. Isoform-specific AMPK activity assay

The AMPK activity assay was performed with some modification of the previously reported method[13)].

### 6. Statistical analyses

Values were expressed as mean ± SEM. Statistical significance was analyzed by using two-way analysis of variance (ANOVA) with mice and treatments as main factors. Post hoc analyses were conducted with Tukey-Kramer's test. The differences between groups were considered statistically significant at $p<0.05$.

## Results and discussion

### 1. AMPK activity

The measurement results of AMPK activity and phosphorylation level of ACC Ser[79], a marker of AMPK activity, in soleus muscle are shown in Figure-1. Basal activity of AMPKα1 and AMPKα2 was lower by 20% and 95% in AMPK-DN mice than WT mice, respectively (Figure-1). The predominant reduction of AMPKα2 activity rather than AMPKα1 activity in the transgenic mice expressing inactive α1 mutant was corresponding with the results previously reported[8) 14) 15)]. A recent study reported that skeletal muscle AMPK signaling was up-regulated at the early stage (3 days) of hindlimb unloading and returned to basal state at 7 days in mice[16)]. Correspondingly, our findings showed no activation of AMPK signaling after 2-week hindlimb unloading (Figure-1). Although we did not examine the time-course changes of AMPK activity, it might be that AMPK signaling was temporary activated following hindlimb unloading and returned to basal state at 2 weeks in the present study.

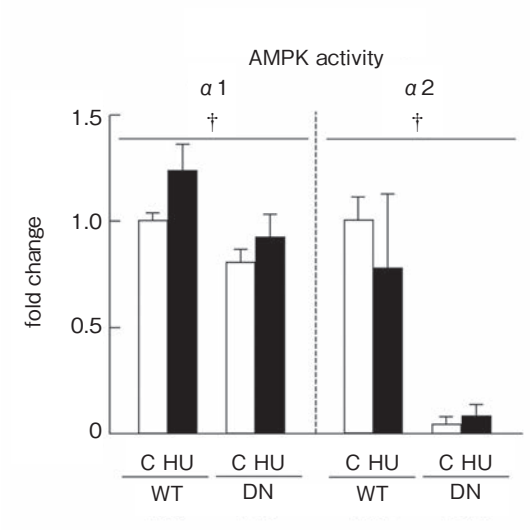


**Figure-1** Changes in the AMPK activity in soleus muscle in response to hindlimb unloading.
Values are mean ± SEM. n = 8 per group. †: post hoc multiple comparisons tests following two-way ANOVA showed that the overall differences were statistically significant between wild-type littermates (WT) mice and mice overexpressed muscle-specific AMPK dominant-negative (DN), ¶: post hoc multiple comparisons tests following two-way ANOVA showed that the overall differences were statistically significant between untreated control (C) and hindlimb unloading (HU) groups. Adapted from Egawa T, *et al*.: Am J Physiol Endocrinol Metab, 2015; 309: E651-662 [40].

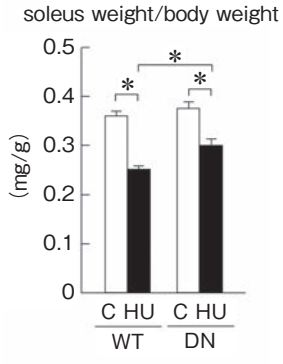


**Figure-2** Changes in the soleus weight in response to hindlimb unloading.
Values are mean ± SEM. n = 6-12 per group. ∗: post hoc simple effects tests following two-way ANOVA showed that the differences were statistically significant between the indicated groups. See Figure-1 for other abbreviations and symbols. Adapted from Egawa T, *et al*.: Am J Physiol Endocrinol Metab, 2015; 309: E651-662 [40].

## 2. Muscle mass

It is well known that diminished loading results in skeletal muscle atrophy. In the present study, when the muscle weight was normalized to body weight to correct for the loss of weight after hindlimb unloading, the relative weight of soleus to body weight were decreased following hindlimb unloading (Figure-2). Notably, soleus muscle was atrophied by ~30% in WT mice during hindlimb unloading, while the deficiency of skeletal muscle AMPKα2 activity weakened the progress of atrophy almost by half (~17%, Figure-2). These data indicate that AMPK, mainly AMPKα2, may be a crucial molecule regulating unloading-induced skeletal muscle atrophy.

## 3. Ubiquitin-proteasome system

Increased protein degradation is contributed to unloading-induced atrophy in skeletal muscle [17]. Ubiquitin-proteasome system is well known as a major protein degradation pathway [18]. The key enzyme in this pathway is E3 ubiquitin ligases, which is responsible for protein ubiquitination. The two muscle-specific ubiquitin ligases, MuRF1 and atrogin-1/MAFbx, have been considered to be master regulators of skeletal muscle atrophy, because these genes are up-regulated in different models of muscle atrophy and have an important role in increasing protein degradation through ubiquitin-proteasome system [19) 20)]. Previous studies have reported that agonist-induced activation of AMPK enhances protein degradation accompanied by increased MuRF1 and atrogin-1/MAFbx mRNA expressions in cultured myotubes [21) 22)]. In addition, we have recently demonstrated that pharmacological activation of AMPK up-regulates MuRF1 mRNA expression and this up-regulation is abolished in AMPK-knockdown cells [23)]. Thus, AMPK appears to be associated with activation of ubiquitin-proteasome system, and it is possible that AMPK regulates protein degradation through ubiquitin-proteasome system during unloading. In the present study, the accumulation of ubiquitinated proteins was observed after hindlimb unloading in WT mice, but not in AMPK-DN mice (Figure-3). MuRF1 mRNA and protein expressions were significantly increased in response to hindlimb unloading by 4.0-fold in WT mice, but not in AMPK-DN mice (Figure-3). These results indicate that the unloading-induced activation of ubiquitin-proteasome system was attenuated in the suppression of AMPK. Therefore, it is suggested that AMPK regulates ubiquitin-proteasome

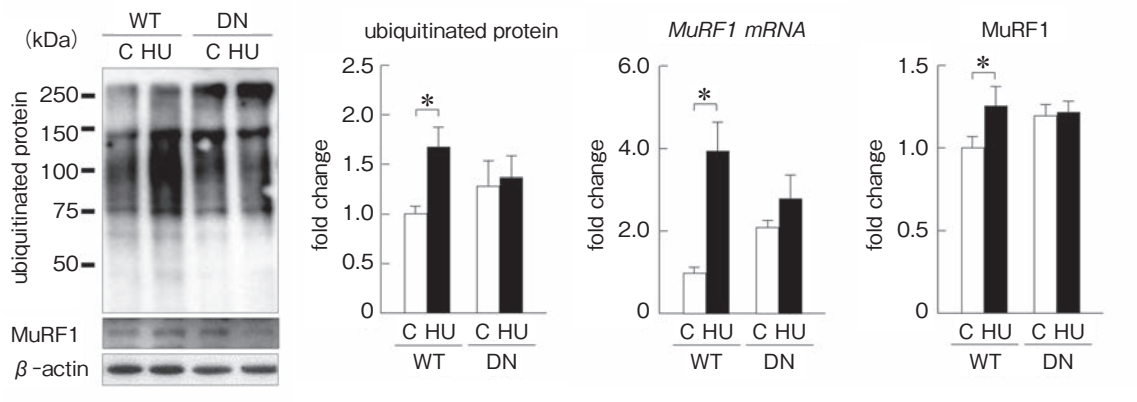


**Figure-3** Changes in the ubiquitin-proteasome system in response to hindlimb unloading.
Representative immunoblots are shown. Values are mean ± SEM. n = 8 per group. See Figure-1 and 2 for abbreviations and symbols. Adapted from Egawa T, *et al.*: Am J Physiol Endocrinol Metab, 2015; 309: E651-662 [40].

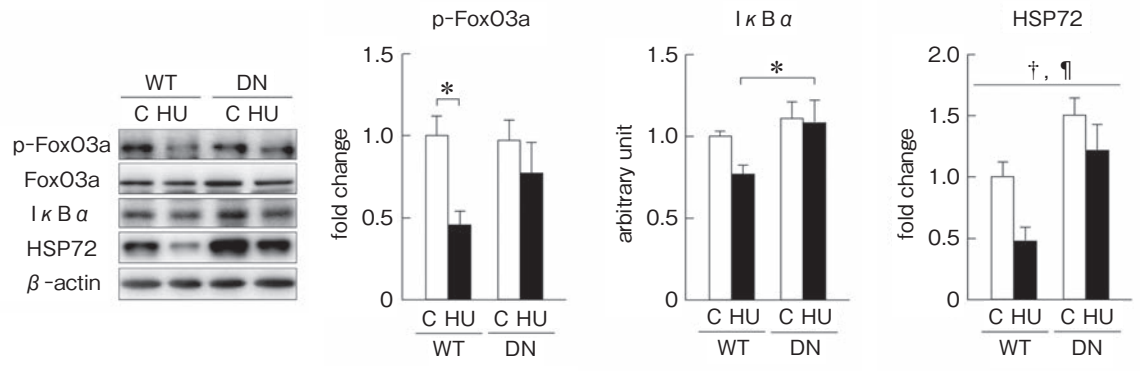


**Figure-4** Changes in the proteins associated with protein degradation in response to hindlimb unloading.
Representative immunoblots are shown. Values are mean ± SEM. n = 8 per group. See Figure-1 and 2 for abbreviations and symbols. Adapted from Egawa T, *et al.*: Am J Physiol Endocrinol Metab, 2015; 309: E651-662 [40].

system-mediated protein degradation during skeletal muscle atrophy in response to unloading.

### 4. Signaling molecules associated with protein degradation systems

Our findings suggest a role of AMPK that regulates unloading-induced skeletal muscle atrophy through modulating protein degradation systems. In this context, there are some possible mechanisms by which AMPK activates protein degradation systems during unloading. FoxOs are transcriptional factors that regulate transcription of genes associated with skeletal muscle homeostasis including skeletal muscle atrophy [24) 25)]. Previous reports have suggested that AMPK-mediated modulation of FoxO3a expression and/or nuclear translocation contributes to activation of ubiquitin-proteasome and autophagy systems in skeletal muscle cells [22) 26) 27)]. Thus, it is possible that AMPK regulates protein degradation systems in unloaded-associated skeletal muscle atrophy through a FoxO3a-dependent mechanism. In the present study, the expression of phosphorylated FoxO3a $Ser^{253}$ was decreased by hindlimb unloading in WT mice, whereas that in AMPK-DN mice was not affected by hindlimb unloading (Figure-4), suggesting that AMPK participates in the activation of FoxO3a during skeletal muscle unloading. Therefore, FoxO3a is a possible molecule related to AMPK-mediated up-regulation of protein degradation systems in response to unloading.

On the other hand, a recent study have suggested that nuclear factor-κB (NF-κB) signaling is more important than FoxO signaling in disuse muscle

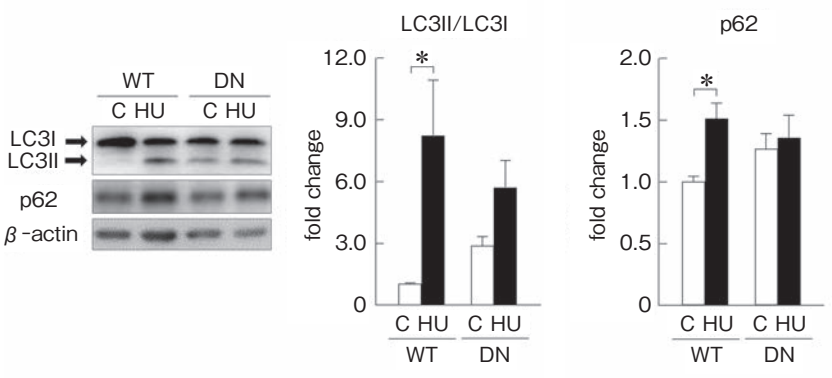


**Figure-5** Changes in the autophagy system in response to hindlimb unloading
Values are mean ± SEM. n=8 per group. See Figure-1 and 2 for abbreviations and symbols. Adapted from Egawa T, *et al*.: Am J Physiol Endocrinol Metab, 2015; 309: E651-662 [40].

atrophy [28], since NF-κB sites, but not FoxO sites, are required for the transcription of MuRF1 during hindlimb unloading. NF-κB is a transcriptional factor that is sequestered in the cytoplasm by a family of inhibitory proteins called IκBα [29]. The IκB kinase complex phosphorylates IκBα, resulting in its degradation, thereby leading to nuclear translocation of NFκB and activation. It has been reported that disruption of NFκB prevents skeletal muscle atrophy induced by hindlimb unloading [30]. In the present study, the expression of IκBα tended to decrease during muscle atrophy in WT mice, and the expression was high in AMPK-DN mice compared to WT mice after hindlimb unloading (Figure-4). These results suggest that AMPK regulates NFκB signaling via the expression of IκBα during unloading-associated muscle atrophy and this might affect the different activation of ubiquitin-proteasome system including MuRF1 expressions.

HSP72 might be another candidate molecule involved in the regulation of AMPK-mediated protein degradation systems during unloading. HSP72 is one of the most prominent member of HSPs family and considered to have an important role in preventing skeletal muscle atrophy [31]. In the present study, it was observed that HSP72 expression in AMPK-DN mice was high and decreased less by unloading compared to WT mice (Figure-4). It has been reported that overexpression of HSP72 in skeletal muscle prevents immobilization-induced atrophy in rat [32]. Furthermore, a molecular mechanism of the resistance to skeletal muscle atrophy by HSP72 seems to be that HSP72 directly prevents FoxO3a activation during unloading [25, 32]. We have also previously demonstrated that AMPK negatively regulates HSP72 expression in skeletal muscle cells and that HSP72 controls AMPK-mediated activation of ubiquitin-proteasome system [23]. Considering these findings, it is suggested that a high expression of HSP72 due to the suppression of AMPK activity is a possible mechanism that attenuates the unloading-induced activation of protein degradation system, partly through FoxO3a deactivation.

### 5. Autophagy system

Autophagy is another important cell proteolytic system that controls protein turnover in skeletal muscle [33]. During autophagosome formation, LC3I is converted to LC3II through lipidation that allows for LC3 to become associated with autophagic vesicles. The presence of LC3 in autophagosomes and the conversion of LC3 to the lower migrating form LC3II have been used as indicators of autophagy activity [34]. Recently, it has been reported that AMPK activation stimulates autophagosome formation in skeletal muscle cells [26], thus a modulation of autophagy process is possible to involve in AMPK-mediated regulation of protein degradation during unloading. In the present study, the relative expression of LC3II to LC3I was increased in response to hindlimb unloading by 8.0-fold in WT mice but by 2.0-fold in AMPK-DN mice (Figure-5). These findings indicate that AMPK mediates autophagosome formation during unloading-induced skeletal muscle atrophy.

The ubiquitin-binding protein p62 which binds to LC3 is preferentially degraded by autophagy [35],

and thus breakdown of p62 is generally used as a marker of autophagy flux[36]. In the present study, accumulation of p62 after hindlimb unloading was also observed in WT mice but not in AMPK-DN mice (Figure-5). This is consistent with the previous findings that p62 mRNA is up-regulated in mouse soleus[37] and gastrocnemius[16] muscle following 3-day hindlimb unloading and that p62 protein is increased by 4-week hindlimb unloading in mouse tibialis anterior and gastrocnemius muscle[38]. Accumulation of p62 generally indicates an impairment of autophagy flux[36], but p62 hyper-expression was also observed in cancer cachexia-induced skeletal muscle atrophy despite the autophagy induction[39]. Although our findings indicate that AMPK modulates the expression of autophagy-related proteins during unloading-induced muscle atrophy, we cannot ascertain whether AMPK-mediated autophagy regulation is associated with the progress of muscle atrophy in response to hindlimb unloading.

## Conclusions

We showed that the suppression of muscle-specific AMPK activity (mainly AMPKα2) partially attenuated unloading-induced atrophy of slow-twitch soleus muscle. The protective effect of muscle atrophy might be attributed to attenuation of the activity of ubiquitin-proteasome-mediated protein degradation. This is supported by the alterations of signaling molecules including FoxO3a, IκBα, and HSP72. Overall, we suggest that AMPK is required for proper adaptation of muscle mass and its related molecules during skeletal muscle unloading.

This article was adapted from Egawa *et al.*[40] with permission by the publisher.

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# Role of Pathogen Sensor on Inactivity-Induced Muscle Atrophy

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***Background***: Cast immobilization can result in skeletal muscle atrophy. Excessive inflammatory response is now considered to be a crucial mechanism for muscle atrophy in cast immobilization. However, the mechanism of muscle atrophy via inflammation induced by immobilization remain incompletely understood. Toll-like receptors (TLR) 4, which are a family of innate cellular pathogen-recognition receptors, have been shown to play critical roles in activation of inflammation. A recent study proposed that TLR4 plays a pathogenic role in muscle atrophy, because TLR4-knockout mice showed both lower sepsis-induced muscle atrophy and ubiquitin ligases activation than wild-type mice. Interestingly, recent study was shown that short-term bed rest induced increased TLR4 expression in the skeletal muscle of healthy older adults. Therefore, increased of TLR4 expression may be an important factor for muscle atrophy and excessive inflammatory response associated with inactivity. We have examined the role of TLR4 in cast immobilization-induced skeletal muscle atrophy using TLR4-defective C3H/HeJ mice.

***Methods***: C3H/HeN mice and C3H/HeJ mice were divided into control and castimmobilization groups. Cast immobilization was imposed for 14 days. Muscle atrophy in the gastrocnemius muscle was evaluated by analyzing the muscle mass and cross-sectional area of the muscle fiber. Gene expressions in the gastrocnemius were evaluated by real time-Reverse Transcriptase –Polymerase Chain Reaction.

***Results***: Cast immobilization resulted in an increase in TLR4 mRNA expression in the gastrocnemius, and in a decrease in muscle mass of C3H/HeN mice. However, no difference in a decrease in gastrocnemius muscle mass, or in the gastrocnemius fiber cross-sectional area at day 14, in the cast immobilized group. Cast immobilization-induced increase in ubiquitin ligase mRNA was not affected by defective TLR4. In control groups, TNF-α mRNA expression in C3H/HeJ mice was lower than that in C3H/HeN mice. However, this variable did not differ between the cast-immobilized groups of the two mouse models at day 14.

***Conclusion***: Our findings in C3H/HeJ mice suggest that TLR4 may not play an essential role in immobilization-induced muscle atrophy.

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# Aging Skeletal Muscle Is Associated with Increased Adipognesis and Impaired Inflammation

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Sarcopenia is the age-related loss of skeletal muscle mass and strength leading to physical frailty, loss of independent daily living, increased lifestyle-related disease, and high health care costs. One concern is that the incidence of sarcopenia is increasing in the world. There is a great deal of interest in strategies to prevent or reverse sarcopenia in our aging population.

Aging muscle consists of fewer myofibers compared to adult muscle and these myofibers show signs of atrophy and increased muscle adiposity. Adiposity in skeletal muscle has been widely recognized as one of the hallmarks of sarcopenia. Adult skeletal muscle has a remarkable regenerative capacity, largely mediated by myogenic stem cells, termed satellite cells (SCs). SCs are located in the plasma membrane of myofibers beneath the basement membrane and are mitotically quiescent in adult muscle. During muscle regeneration, satellite cells are activated, giving rise to myoblasts that proliferate, differentiate and fuse together or fuse to pre-existing muscle fibers to produce fully mature muscle fibers. However, skeletal muscle regeneration is markedly impaired with age. Recently we demonstrated that the number of activated, proliferated, and differentiated SCs was lower in old rats compared with young rats after muscle damage. In addition, old rats exhibited impaired muscle regeneration and increased intermuscular adipocytes post-injury. Our data suggest that impaired regeneration of old skeletal muscle might be attributed to changes in several functions of SCs. We indicated that rat SCs are multipotent cells that can undergo not only myogenic, but also adipogenic differentiation *in vitro* and *in vivo*, and their adipogenic responses increase as a function of age.

Inflammation is an obligatory event in skeletal muscle injury. Recovery of skeletal muscle injuries requires severely injured myofibers to be degraded, phagocytized, and replaced via the migration and maturation of SCs. It is well established that macrophages are the dominant inflammatory cell type during early muscle injury and may contribute to skeletal muscle regeneration by facilitating myofiber repair via the production of inflammatory cytokines, chemokines, and growth factors. Recently we demonstrated that the number of activated macrophages within skeletal muscle was lower in old rats compared with young rats after muscle damage. In addition, our DNA chip data has indicated that the expression of genes including inflammatory cytokines, chemokines, and growth factors was attenuated during the regeneration of aged skeletal muscle. These data suggest that the impaired inflammatory response to muscle damage that occurs with aging may contribute to the impaired muscle regenerative capacity and to increased muscle adiposity, both characteristic of aged muscle. These factors may be underlying causes of sarcopenia.

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# Effects of Treadmill Running on Bone Density and Bone Strength in Young Mice

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***Objective***: To investigate the effects of treadmill running exercise on bone density and bone strength in young mice.

***Methods***: Four-week-old male C57BL/6 mice (n = 10) were assigned to either the control sedentary group (CON, n = 5) or the treadmill running exercise group (TRE, n = 5). Mice in the TRE group were forced to run on a treadmill at a rate of 6–18 m/min, 30 min/day, 5 days/week for 16 weeks. The mice in both groups were euthanized at 20 weeks of age. The right and left lower limbs, skeletal muscle, and white adipose tissue were removed for analysis. Bone density (mg/cm$^3$) was measured in the right limb using microcomputed tomography, and maximal breaking force (N) was determined in the left femur using a three-point bending test as an indicator of bone strength.

***Results***: The TRE and CON groups did not significantly differ with respect to daily food intake during the study period or body weight at 20 weeks of age. Differences in white adipose tissue and skeletal muscle weight of 20-week-old mice in the TRE and CON groups were also not significant. Cortical bone density was significantly higher in the TRE group than in the CON group ($p < 0.05$). However, there were no significant differences in cancellous bone density, total bone density or maximum breaking force between the TRE and CON groups.

***Conclusions***: Treadmill running exercise for 16 weeks can increase cortical bone density of tibia in young mice, but does not improve bone strength in femur.

***Key words***: bone density, bone strength, treadmill running, young mice

## Introduction

In an aging society, people are at higher risk of bone fracture and osteoporosis and would thus benefit from efforts aimed at maintaining lifelong skeletal health. In humans, bone mass increases until adolescence but gradually decreases thereafter. Therefore, measures aimed at increasing peak bone mass during the growing period are likely to be beneficial later in life.

Mechanical stress is imperative for normal bone turnover, which consists of bone formation and resorption. Exercise or physical activity that includes mechanical stress increases bone mass and bone strength, and is therefore critical for normal bone development and bone health. In studies conducted in humans, past physical activity was shown to be beneficial for bone health[1) 2)]. Strope *et al.* demonstrated that physical activity in adolescence (13–18 years) and young adulthood (19–29 years) had positive effects on adult (30–65 years) bone mass[1)]. Exercise training was also shown to improve bone density in children and adults[3)–6)]. However, despite the confirmed effects of exercise in children and young adults with respect to improved bone mass and density during adulthood, whether physical activity and exercise also improve these bone parameters in children is unknown. In animals, various exercise models, including treadmill running, jumping exercise, resistance training,

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and swimming, have been developed to clarify the effect of exercise on bone mass and bone density. Both treadmill running and jumping exercise lead to a gain in bone mass[7)-10)]. Hamrick *et al.* found that treadmill exercise for 4 weeks increased cortical bone in the long bones of 16-week-old female mice[11)]. Huang *et al.* reported that endurance exercise for 8 weeks benefitted the biomaterial quality of bone in growing rats at 15 weeks of age[8)].

Although these studies demonstrated the effects of exercise on bone mass, bone density, and bone turnover, they did not examine bone biomechanical properties. Therefore, in this study we investigated the effects of treadmill running exercise on bone strength and bone density in young mice.

## Materials and methods

### 1. Animals

Four-week-old male C57BL/6 mice (n=10) were purchased from Japan SLC (Hamamatsu, Japan). Mice were maintained in a climate-controlled room at a temperature of 20 ± 1℃ on a 12:12-h light-dark cycle. Both standard rodent chow (Labdiet, EQ5L37, PMI nutrition international) and water were provided *ad libitum* for 16 weeks. After a 1-week acclimatization period, the mice were randomly assigned to either the control sedentary group (CON, n=5) or the treadmill running exercise group (TRE, n=5).

### 2. Exercise training

Exercise training was conducted in accordance with the procedures used in a previous study[11)]. Briefly, mice in the TRE group were forced to run on a motorized treadmill for 30 min/day, 5 days/week for 16 weeks. Exercise intensity was controlled by adjusting the running speed and was increased progressively (6-18 m/min) over the 16-week training period.

### 3. Tissue sampling

All mice were euthanized at 20 weeks of age. Their right and left lower limbs, skeletal muscles (soleus, gastrocnemius, plantaris, anterior tibialis, extensor digitorum longus, and quadriceps), and white adipose tissue were removed and their weights immediately measured using an electronic scale. The right limb was then fixed with buffered 4% paraformaldehyde. Bone mineral density was measured using microcomputed tomography within the following 2 days. The left femur was harvested for testing of bone strength.

### 4. Microcomputed tomography

The bone density (mg/cm$^3$) of the right limb was measured using micro-CT scanning (Latheta LCT-200, HITACHI ALOKA Medical, Tokyo, Japan), performed at a spatial resolution of 48 μm × 48 μm and a slice thickness of 96 μm. All other parameters used followed those recommended in the manufacturer's protocols.

### 5. Bone strength testing

Maximum breaking force (N), as a measure of bone strength, was assessed in the left femur by a three-point bending test (TK-252C, Muromachi, Japan). Briefly, the connective tissue was removed and the bone was attached between two bottom-supporting stands separated by 1.6 mm. The crosshead speed was 2.0 mm/min.

### 6. Statistical analysis

All data are expressed as the mean ± standard deviation (SD). Comparisons among groups were performed using Student's t-test. A p value $< 0.05$ was considered to indicate statistical significance. All analyses were performed using SPSS ver. 18.0 (IBM).

## Results

### 1. Daily food consumption and body weight

The mean (± SD) body weights of the mice in the CON and TRE groups at the start of the experiment were 16.7 ± 1.0 g and 16.4 ± 1.0 g, respectively. At the end of the experiment, they were CON: 24.3 ± 0.9 g and TRE: 24.2 ± 1.1 g. There was no significant difference between the CON and TRE groups either at the start or the end of the experiment.

### 2. White adipose tissue and skeletal muscle weight

White adipose tissue weight was higher in the TRE group than in the CON group (CON: 284.7 ± 22.9 mg and TRE: 331.5 ± 84.4 mg). There was no significant difference between the CON and TRE groups. Differences in skeletal muscle weight were

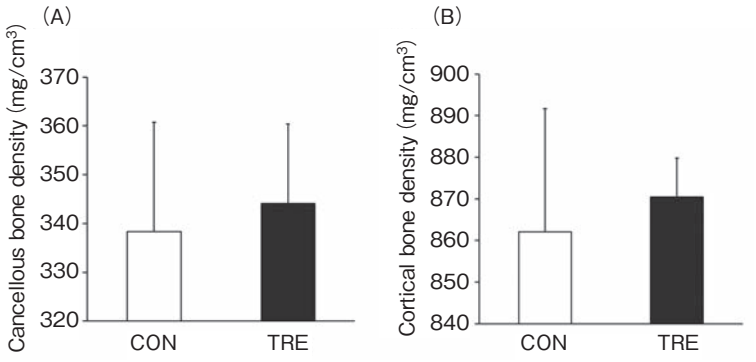


**Figure-1** Cancellous (**A**) and cortical (**B**) bone density of femora measured using micro-CT
Values represent mean ± standard deviation (SD). CON: control sedentary group, TRE: treadmill running exercise group.

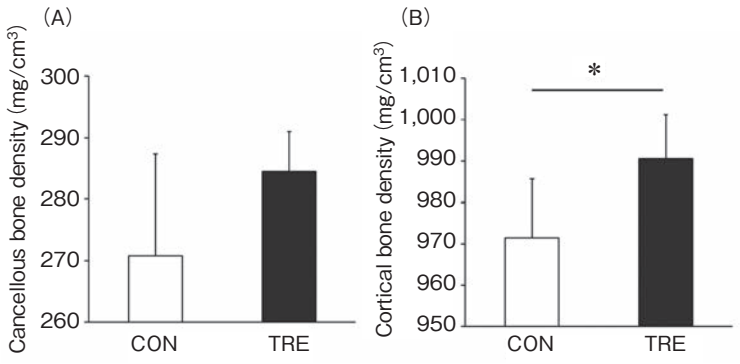


**Figure-2** Cancellous (**A**) and cortical (**B**) bone density of tibiae measured using micro-CT
Values represent mean ± SD. *$p < 0.05$ vs CON.

also not significant: soleus (CON: 8.3 ± 0.6 mg and TRE: 8.3 ± 0.5 mg), gastrocnemius (CON: 124.4 ± 5.6 mg and TRE: 121.0 ± 8.1 mg), plantaris (CON: 17.7 ± 2.3 mg and TRE: 17.9 ± 1.3 mg), anterior tibialis (CON: 49.3 ± 1.5 mg and TRE: 47.1 ± 2.7 mg), extensor digitorum longus (CON: 10.6 ± 0.7 mg and TRE: 10.7 ± 0.4 mg), and quadriceps (CON: 193.2 ± 14.3 mg and TRE: 181.1 ± 8.4 mg). The sum of the muscle weights was therefore 403.5 ± 22.0 mg in the CON group and 386.1 ± 12.8 mg in the TRE group. The difference between groups was not significant.

### 3. Bone density

Figure-1 and 2 shows the bone density at 20 weeks of age in the CON and TRE groups. The difference in cancellous bone density of the tibia was not significantly different between the two groups (CON: 327.5 ± 18.2 mg/cm$^3$ and TRE: 331.6 ± 12.3 mg/cm$^3$). However, cortical bone density of the tibia was significantly higher in the TRE group than in the CON group (904.9 ± 4.9 vs. 884.1 ± 19.7 mg/cm$^3$, respectively, $p < 0.05$). Thus, total bone density of the tibia was significantly higher in the TRE group than in the CON group (594.9 ± 8.0 vs. 576.3 ± 12.6 mg/cm$^3$, respectively, $p < 0.05$). In the femur, cancellous and cortical bone density were not significantly different between the two groups (CON: 327.5 ± 18.2 mg/cm$^3$ and TRE: 331.6 ± 12.3 mg/cm$^3$ at the cancellous, CON: 327.5 ± 18.2 mg/cm$^3$ and TRE: 331.6 ± 12.3 mg/cm$^3$ at the cortical).

### 4. Bone strength

The maximal femoral breaking force is shown in Figure-3. The difference between the CON and TRE groups was not significant (CON: 11.1 ± 1.3 N and TRE: 11.0 ± 0.4 N).

## Discussion

This study investigated the effects of treadmill

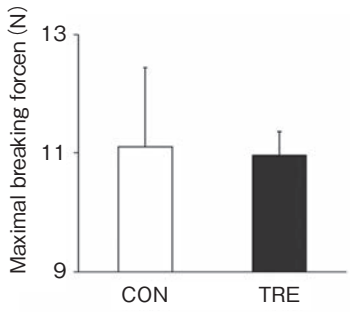


**Figure-3** Maximal breaking force measured using three-point bending test
Values are represent mean ± SD.

running exercise on bone density and bone strength in growing male mice. Whereas treadmill running increased the cortical bone density of the tibia in young mice, there was no significant change in cancellous bone density. The improvement in cortical bone density in response to treadmill running is consistent with the results of previous studies[12) 13)]. In a study using female mice, Hamrick *et al*. demonstrated that cortical thickness increased by 50% at the metaphysis and by 10% at the midshaft in the treadmill running group compared to the non-exercise control group[11)]. However, after 4 weeks of treadmill running, the increase in bone mass was not accompanied by an increase in bone density. By contrast, Joo *et al*. found that 4 weeks of treadmill running exercise increased bone strength in young male rats. The increased bone strength was attributed to alterations of cortical geometry, bone density, and bone microarchitecture[13)]. In another study, endurance treadmill running for 8 weeks did not alter bone mass and bone density in growing male rats[8)]. These inconsistent results may reflect differences in the osteogenic response to exercise, which varies according to loading intensity, frequency, sex, and age[7)].

Bone density, structure, and composition are important determinants of bone strength and, along with bone density and bone mass, should therefore be measured in studies on the effect of exercise on bone health. Indeed, Isaksson *et al*. showed that voluntary wheel running exercise improved both the collagen network of bone and bone strength[14)]. Similarly, Holy and Zérath reported that, in growing rats, short-term voluntary wheel running exercise increased markers of bone formation and improved the histological properties of bone tissue[15)]. In this study, only bone density and bone strength, assessed using microcomputed tomography and a three-point bending test, respectively, were evaluated. Other bone quality parameters were not examined. Nonetheless, our data suggest that, in young mice, 16 weeks of treadmill running exercise do not affect bone strength, but is at least beneficial to site-specific bone density.

## Conclusion

Our study in young mice showed that treadmill running exercise for 16 weeks can increase cortical bone density of the tibia, but does not alter bone strength of the femur.

## Conflict of interest

No conflicts of interest, financial or otherwise, are declared by the authors.

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# *In Vivo* Calcium Regulation in Diabetic Skeletal Muscle: Fiber-Type Specific Effects

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***Introduction***: Type 1 diabetes impairs $Ca^{2+}$ handling in many tissues such as cardiac muscle, platelets, kidney and liver. In skeletal muscle, the diabetic state increases resting intracellular $Ca^{2+}$ ($[Ca^{2+}]_i$) levels and induces activity of $Ca^{2+}$-dependent proteolytic pathways. In skeletal muscle, dysfunctional contractile activity has been linked to impaired $[Ca^{2+}]_i$ regulation. Muscle force production is impaired and fatigability and muscle fragility deteriorate with diabetes. Recently, we succeeded in measuring *in vivo* $[Ca^{2+}]_i$ within the mixed fiber-type rat spinotrapezius muscle[1] following contractions. In this preparation, intramyocyte injection of a high $[Ca^{2+}]$ bolus revealed a depressed $Ca^{2+}$ buffering capability in diabetes which corresponded with the elevated post-contraction $[Ca^{2+}]_i$[2]. Unfortunately, that preparation did not permit resolution of between fiber type effects on the profile of $[Ca^{2+}]_i$ following contraction in Type 1 diabetes which remain to be resolved. We tested the hypotheses that: 1. The rise in resting $[Ca^{2+}]_i$ evident in diabetic rat slow-twitch muscle would be exacerbated in fast-twitch muscle following contraction. 2. These elevated $[Ca^{2+}]_i$ levels would relate to derangement of microvascular $O_2$ pressures ($PmvO_2$) rather than sarcoplasmic reticulum (SR) dysfunction per se.

***Methods***: Adult male Wistar rats were divided randomly into diabetic (DIA: Streptozotocin i.p.) and healthy (CONT) groups. Four weeks later extensor digitorum longus (EDL, predominately type II fibers) and soleus (SOL, predominately type I fibers) muscle contractions were elicited by continuous electrical stimulation (120 s, 100 Hz). $Ca^{2+}$ imaging was achieved using Fura-2 AM *in vivo*. Phosphorescence quenching techniques were used to measure $PmvO_2$.

***Results***: DIA increased fatigability in EDL ($p<0.05$) but not SOL. In recovery, SOL $[Ca^{2+}]_i$ either returned to its resting baseline within 150 s (CONT, $1.00 \pm 0.02$ at 600 s) or was not elevated in recovery at all (DIA, $1.03 \pm 0.02$ at 600 s, $p>0.05$). In recovery, EDL CONT $[Ca^{2+}]_i$ also decreased to values not different from baseline ($1.06 \pm 0.01$, $p>0.05$) at 600 s. In marked contrast, EDL DIA $[Ca^{2+}]_i$ remained elevated for the entire recovery period (i.e., $1.23 \pm 0.03$ at 600 s, $p<0.05$). The inability of $[Ca^{2+}]_i$ 40 to return to baseline in EDL DIA was not associated with any reduction of SERCA1 or SERCA2 protein levels (both increased 30-40%, $p<0.05$). However, $PmvO_2$ recovery kinetics were markedly slowed in EDL such that mean $PmvO_2$ was substantially depressed (CONT, $27.9 \pm 2.0$ vs DIA, $18.4 \pm 2.0$ mmHg, $p<0.05$) and this behavior was associated with the elevated $[Ca^{2+}]_i$. In contrast, this was not the case for SOL ($p>0.05$) in that neither $[Ca^{2+}]_i$ nor $PmvO_2$ were deranged in recovery with DIA.

***Conclusion***: In conclusion, compromised $Ca^{2+}$ buffering and elevated $[Ca^{2+}]_i$ following an *in vivo* fatiguing tetanic contraction occur preferentially in the fast twitch EDL rather than the slow twitch SOL muscle. This process is associated with a substantial reduction in microvascular oxygen pressure that may be responsible, in part, for the dysfunctional $Ca^{2+}$ buffering and greater fatigue. If dysfunction of SERCA1 and SERCA2 does occur it is possible that this relates to oxygen levels ($PmvO_2$) as their protein levels are up- and not down-regulated in EDL. Because the EDL muscle fibers undergo significant diabetes-induced atrophy it is also feasible that the mitochondrial reticulum undergoes damage and its capacity to buffer $[Ca^{2+}]_i$ is degraded. That eventuality remains to be experimentally tested[3].

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# Metabolome and Peptidome Analyses of Autophagic Degradation

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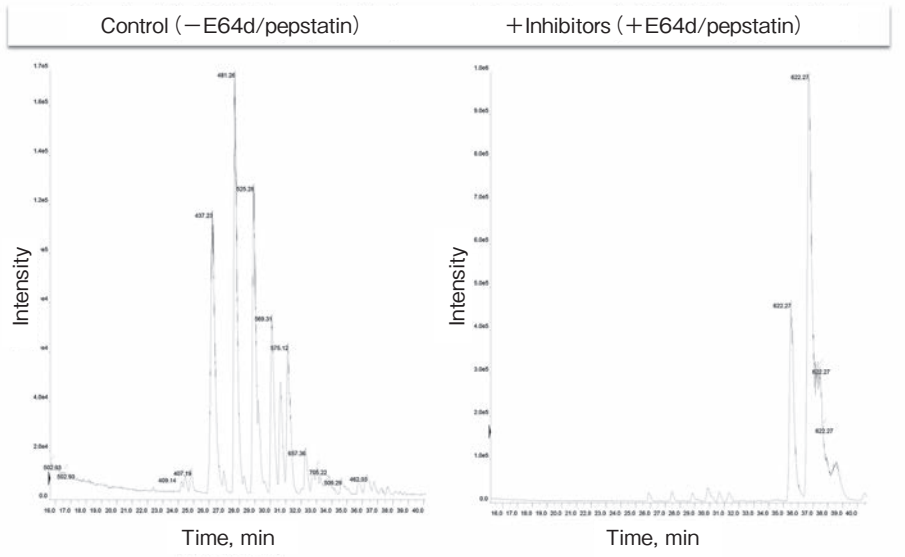
Autophagy is a large-scale degradative system, by which cellular constituents, including cell organelles and cytosolic proteins, are broken down via the lysosomes. Autophagy initiates with the formation of smooth limiting membrane, which extends and sequesters surrounding cytoplasm to form a double-membraned autophagosome. Autophagosome then fuses with the lysosome to mature into autolysosome. It was thought previously that sequestered cytoplasmic components in the lumen were degraded completely by lysosomal hydrolases to their elements, such as amino acids, fatty acids, sugars, etc. However, it has been shown recently that some degradation intermediates or products are discharged to the extracellular milieu. As these compounds derive from the relevant tissues or cells, characterization of these excreted compounds may be beneficial to understand cell type- or tissue type-specific autophagic process.

Using mass spectrometry, we extensively analyzed peptides and low-molecular compounds released from HeLa cells and mouse embryonic fibroblasts (MEF) when they were incubated with Krebs bicarbonate buffer (KRB) (nutrient deprivation conditions). We found that in addition to amino acids significantly more peptides were released from the control cells than those from the cells treated with E64d plus pepstatin A (lysosomal proteinase inhibitors). Identification of these discharged peptides will be reported.

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# Seasonal Changes in Physical Fitness of Adolescent Track and Field Athletes

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***Introduction***: Athletic season is roughly divided into three seasons in Japan, namely the physical training season in winter, the pre-competition season in spring and the competition season between summer and autumn. Therefore, it is generally thought that aims, contents, volume, and programs of workout are different by seasons. For that reason, physical fitness levels of athletes may fluctuate through a year. Thus, the purpose of this study was to identify seasonal changes in physical fitness of adolescent track and field athletes.

***Methods***: Subjects were 11 boys and 7 girls of two different high schools. Tests are performed in each season. We applied assessments of physical fitness: 100 m sprint running, three kinds of vertical jump (squat jump, counter movement jump, and drop jump), single-joint isometric torque (knee extension, knee flexion, dorsiflexion, and plantar flexion), and anthropometric characteristics.

***Results***: Friedman test and post hoc Wilcoxon test revealed that many of physical fitness parameters of boy athletes were higher at the competition season compared with in the other seasons. In girls, only counter movement jump and body weight were significant differences among seasons.

***Discussion***: This study was found that physical fitness of adolescent track and field athlete varied through seasons. Especially, it was trend to improvement of the muscle strength and power output in the competition season.

***Conclusion***: Seasonal changes were found in physical fitness of adolescent track and field athletes. Moreover, sex differences were found in the trends in seasonal changes.

***Key words***: seasonal changes in physical fitness, adolescent athlete, sprint running performance

## Introduction

It could be said necessary to improve physical fitness in order to reach a high level of competition in track and field events. In previous studies of focused on sprint running performance, had reported relationship with leg strength and/or jump performance [1)-3)]. In addition, athletes could be required to decrease amount of body fat as well as increase the muscle mass.

Field tests have been carried out for assessments of physical fitness in the coaching field. In particular, the importance and significance of assessments of physical fitness for adolescent athlete have been pointed out. Barker *et al.*[4)] had indicated that it is important in order to evaluate advantages and weakness, effectiveness of a training programs, and predictive future performance.

We consider to be effective for evaluation of training effects among many significances. Because previous studies had been shown that there are differences in training effect by age. Pesta *et al.*[5)] had found that adolescents were shown higher muscular adaptations of 10-week resistance training compared with adults. And Amigo *et al.*[6)] had reported that response to training and detraining were higher the 16-year group than 14-year and 15-year. Therefore, it would need assessments of physical fitness that take account of the training period.

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Athletic season is roughly divided into three seasons in Japan. There are namely the physical training season in winter, the pre-competition season in spring, and the competition season between summer and autumn. Therefore, it is generally thought that aims, contents, volume, and programs of workout are different by seasons. Although priority of workout vary depending on coaches, characteristics of workout for each season can be shown generally as follows. In the physical training season, workouts are aimed to improve muscle mass, strength, and muscular endurance. Improvement of power output is one of the aim of important in the pre-competition season. In the competition season, a lot of time are spent on technical training and conditioning. These differences might lead seasonal changes in physical fitness of adolescent athletes. In a review of "Seasonal variation in fitness parameters in competitive athletes" by Koutedakis[7], had reported that the swings in fitness variables may be as high as 18% from one season to another. And, it had been mentioned to be depended on performance levels, the type of sports and the fitness parameters in question. However, there is little information about adolescent athletes of track and field events.

There is a limit to evaluate as sprint running predictors, since these were based on cross sectional studies. The longitudinal research of this study will be able to get additional knowledge of previous studies. Evaluating seasonal changes of physical fitness can be utilize as individual workout prescription and coaching. The purpose of this study was to identify seasonal changes in physical fitness of adolescent athletes in track and field. It was hypothesized that physical fitness levels of athletes may fluctuate through a year.

## Methods

### 1. Subjects

Subjects were 11 boys (16.4 ± 0.6 years, 171.9 ± 5.1 cm, 60.8 ± 5.2 kg; at the first test) and 7 girls (16.8 ± 0.5 years, 160.7 ± 3.6 cm, 51.5 ± 4.1 kg; at the first test). All subjects belonged to two different high school's track and field club, and had trained 6 days per week through a year. Participating both athletes and their parents received detailed information about the study, and they gave their written informed consent.

### 2. Testing procedures

Tests were performed three times a year for each seasons; the physical training season in end of November to December, the pre-competition season in March, and the competition season in August. The survey was carried out over a period of two years. We applied assessments of physical fitness: 100 m sprint running, three kinds of vertical jump, single-joint isometric torque, and anthropometric characteristics. Order of tests were random in each of subject and time of measurements.

All subjects performed 100 m sprint running from a block start with spike shoes at athletics track field once. Sprint time (100 m time) was calculated from digital video camera frames (30 Hz).

Jump height of three kinds of vertical jump (squat jump; SQJ, counter movement jump; CMJ, and drop jump from height of 30-50cm; DJ30, DJ40, DJ50) were measured with MULTI JUMP TESTER (PTS-102, DKH Co., Ltd., JAPAN). Figure-1 (A, B, and C) shows examples of each jump modalities. All subjects were instructed to jump as high as possible, and were not allowed to use their arms. The highest score in 2 trials were accepted as the jump scores.

Single-joint isometric torque of knee extension (KE TQ), knee flexion (KF TQ), dorsiflexion (DF TQ), and plantar flexion (PF TQ) of the right side were measured with an isometric dynamometer. KE TQ and KF TQ were measured in sitting position with 90 degrees of their hip and knee joint angles. The attachment pad fixed the lower leg, and set to match the rotation axis of the knee joint center and the torque meter. DF TQ and PF TQ were measured in long sitting position with 90 degrees of their hip and 180 degrees of knee joint angles. And sets of ankle angle were at 110 degrees in measurement of DF TQ, and at 90 degrees in measurement of PF TQ. Then, all subjects performed voluntary isometric contractions as explosively as possible and attempted to maintain the maximum force for 3 seconds. The highest score in 2 trials were accepted as the TQ scores, and relative TQ to body weight was evaluated.

Assessments of anthropometric characteristics were height, body weight, subcutaneous fat thickness, and muscle thickness. Measurements of

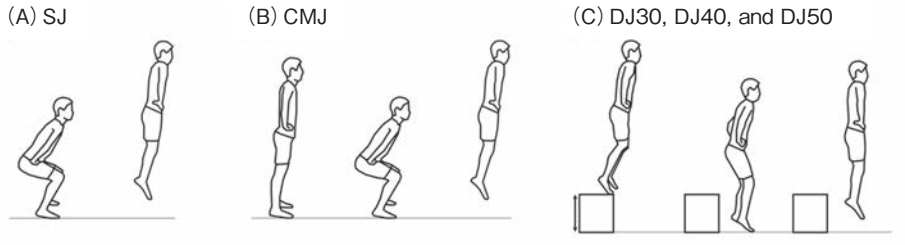


**Figure-1** Examples of vertical jump modalities
A. SJ: squat jump, B. CMJ: counter-movement jump, C. DJ30, DJ40, and DJ50: drop jump from height of 30-50 cm.

subcutaneous fat thickness and muscle thickness at right side were used B-mode ultrasonic apparatus. From these results, percent of body fat (% Body fat) and fat-free mass were estimated from equation by Abe *et al.*[8].

### 3. Statistical analysis

All measurement values are presented as mean ± standard deviation values (mean ± SD). Seasonal changes were assessed with Friedman test and post hoc Wilcoxon test to each sex. A p-value of $<0.05$ was used to indicate statistical significance.

## Results

Table-1 shows descriptive data on physical fitness of boy athletes. There were significant differences among seasons in many of physical fitness parameters of boy athletes. 100 m Sprint running at the competition season was faster compared with in other seasons. Similarly, DJ50, KF TQ, and DF TQ at the competition season was higher. In anthropometric characteristics, body weight at the pre-competition season was higher compared with in other seasons. And fat-free mass at the competition season was higher than the physical training season.

Table-2 shows descriptive data on physical fitness of girl athletes. Girl athletes were found that only CMJ and body weight were significant differences among seasons. CMJ at the physical training season was lower compared with in other seasons. And body weight at the pre-competition season was higher.

## Discussion

This study primarily aimed to identify seasonal changes in physical fitness of adolescent athletes in track and field event. Results of this study indicated that physical fitness were seasonal changes in adolescent track and field athletes of each sex. Moreover, sex differences were seen in the trend. In boy athletes, 100 m sprint running was improved at the competition season. In addition, DJ50, KF TQ, and DF TQ were also increased. On the other hand, seasonal changes of girl athletes were limited in physical fitness parameters.

Sex differences about the trend of seasonal changes would be expected that differed in workouts, trainability and growth. First, workouts were not a little difference between boy and girl athletes. All subjects had completed almost same contents, volume, and programs of workout of their each high school through a year. Next, it is about differences in trainability by sex. In previous studies, sex differences had not found[9]. Relative changes in the strength and muscle hypertrophy that occurred as a 16 -week results of the heavy-resistance training were similar in men and women[9]. Although, this study showed sex differences in an outcome of training in the adolescent track and field athletes. These results may indicate the necessary to divide by sex in training. Lastly, it is about influences of growth for physical fitness. Kanehisa *et al.*[10] had shown that CSA of the lower leg muscles were difference by sex after 13-15 years. And sex differences in DF and PF of strength became apparent in 16-18 year age group. However, they had considered that sex differences in muscle CSA and strength during growth could be accounted for by differences in the lower leg length and muscle mass. In sprint speed improvement, sex

**Table-1** Descriptive data on physical fitness test of boy athletes (Mean ± SD)

| | Test 1 | Test 2 | Test 3 | $p<0.05$ |
|---|---|---|---|---|
| 100 m time (sec.) | 12.2 ± 0.3 | 12.2 ± 0.4 | 11.9 ± 0.4 | Test 1, 2 > Test 3 |
| SJ (cm) | 37.4 ± 4.4 | 38.2 ± 4.0 | 39.3 ± 4.1 | |
| CMJ (cm) | 43.9 ± 3.3 | 43.5 ± 2.7 | 44.2 ± 4.2 | |
| DJ30 (cm) | 34.4 ± 6.2 | 35.2 ± 6.0 | 37.1 ± 4.7 | |
| DJ40 (cm) | 35.2 ± 5.6 | 36.8 ± 5.7 | 39.3 ± 5.5 | |
| DJ50 (cm) | 35.1 ± 5.8 | 36.3 ± 5.7 | 39.9 ± 4.8 | Test 1, 2 < Test 3 |
| KE TQ (Nm/kg) | 2.54 ± 0.31 | 2.55 ± 0.31 | 3.09 ± 0.23 | |
| KF TQ (Nm/kg) | 1.90 ± 0.34 | 1.81 ± 0.29 | 2.20 ± 0.13 | Test 1, 2 < Test 3 |
| DF TQ (Nm/kg) | 0.66 ± 0.00 | 0.65 ± 0.00 | 0.70 ± 0.00 | Test 1, 2 < Test 3 |
| PF TQ (Nm/kg) | 2.84 ± 0.64 | 2.65 ± 0.65 | 3.16 ± 0.34 | |
| Height (cm) | 171.9 ± 5.1 | 172.1 ± 5.1 | 172.0 ± 5.1 | |
| Body weight (kg) | 60.8 ± 5.2 | 62.2 ± 4.9 | 61.0 ± 5.3 | Test 1, 3 < Test 2 |
| %Body fat | 10.3 ± 0.7 | 10.4 ± 0.8 | 10.3 ± 1.0 | |
| Fat-free mass (kg) | 52.6 ± 4.8 | 53.4 ± 4.0 | 53.9 ± 3.9 | Test 1 < Test 3 |

**Table-2** Descriptive data on physical fitness test of males (Mean ± SD)

| | Test 1 | Test 2 | Test 3 | $p<0.05$ |
|---|---|---|---|---|
| 100 m time (sec.) | 13.6 ± 0.6 | 13.7 ± 0.6 | 13.5 ± 0.5 | |
| SJ (cm) | 30.7 ± 1.2 | 32.2 ± 2.5 | 32.1 ± 1.9 | |
| CMJ (cm) | 34.3 ± 3.5 | 37.5 ± 3.4 | 37.3 ± 3.4 | Test 1 < Test 2, 3 |
| DJ30 (cm) | 32.3 ± 3.0 | 33.0 ± 4.3 | 34.8 ± 3.7 | |
| DJ40 (cm) | 32.7 ± 3.4 | 32.9 ± 3.7 | 34.9 ± 3.1 | |
| DJ50 (cm) | 33.4 ± 4.3 | 33.0 ± 3.8 | 34.9 ± 4.0 | |
| KE TQ (Nm/kg) | 2.47 ± 0.26 | 2.44 ± 0.05 | 2.64 ± 0.25 | |
| KF TQ (Nm/kg) | 1.96 ± 0.12 | 1.74 ± 0.30 | 1.96 ± 0.16 | |
| DF TQ (Nm/kg) | 0.54 ± 0.01 | 0.54 ± 0.01 | 0.58 ± 0.00 | |
| PF TQ (Nm/kg) | 2.63 ± 0.37 | 2.55 ± 0.29 | 2.70 ± 0.02 | |
| Height (cm) | 160.7 ± 3.6 | 160.9 ± 3.4 | 161.0 ± 3.7 | |
| Body weight (kg) | 51.5 ± 4.1 | 52.9 ± 4.2 | 50.3 ± 4.6 | Test 1, 3 < Test 2 |
| %Body fat | 15.5 ± 1.6 | 15.9 ± 2.3 | 14.9 ± 1.9 | |
| Fat-free mass (kg) | 40.4 ± 2.8 | 41.2 ± 3.2 | 41.2 ± 2.5 | |

difference was more obvious and reached the significance level after the age of 15[11]. Subjects of this study were the age from 15 to 18, consistent with the age which sex differences were observed in previous study. The trend of seasonal changes by sex may be influence of growth.

Seasonal changes of anthropometric characteristics were similar trend in both boys and girls. Body weight was increased at the pre-competition season, then decreased at the competition season. Moreover, the average value of the fat-free mass at the pre-competition season was higher than the physical training season, and it was kept in the competition season. However, seasonal change of the free-fat mass was not significant difference. These results were suggested that athletes were increasing the muscle mass as well as decreasing amount of body fat towards the competition.

Measured physical fitness of this study have been used as the parameters of sprint running, because the correlation had been found in previous studies[1)-3)]. Alexander[12)] had indicated significant relationships between sprinting times of 100 m and peak torque scores of knee extension and

dorsiflexion in elite sprinters. In addition, Smirniotou *et al.*[1] had been manifested that the best predictors of the 100 m sprint running is probably SQJ or CMJ. In the results of this study, physical fitness were higher when 100 m time was the fastest in all of the seasons. This may be mean that it was similar results with cross sectional studies of previous studies. These findings revalidated that 100 m sprint running is associated with vertical jump performance and leg strength.

This study found that boy athletes improved many of physical fitness parameters at the competition season. These would reflect training effects during season. And it was suggested that workouts in the competition season can be enhance essential physical fitness to the competition for track and field sprint events. All subjects of this study were either sprinter and/or jumper. In general, sprint running trainings increase frequency as well as quantity of intensity during the pre-competition season and the competition season. Markovic *et al.*[13] had reported that sprint running training was caused improvements in leg power output and dynamic athletic performance. Therefore, future research will be necessary to evaluate workouts in more detail by recording, such as contents, volume, and programs.

## Conclusion

Seasonal changes in physical fitness of adolescent Track and Field athletes were found. And sex differences were seen in the trend to seasonal changes.

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# Sprinting Ability with Change of Direction Involving Decision Making in Female Soccer Players

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To investigate the sprinting ability with change of direction (COD) involving decision making in female soccer players to compare it with athletes in a different sports (soccer vs. track-and-field), as well as to compare among competitive levels (Higher performance group [HPG] vs. Lower performance group [LPG]).

Thirty-three university female athletes participated in this study and were divided into 4 groups (n=24: soccer [n = 11: HPG, n = 11: LPG] n = 9: track-and-field). The following protocols were utilized to determine the sprinting ability in a straight line and with COD involved in the decision making. We measured the time from the 20 meter (m) sprint test for the straight sprinting ability. We also conducted the 20 m sprint with cutting movement 90 degree at the 10 m point where athletes needed to make a decision whether they turn right or left according to the light stimulation at 2.5, 5 and 7.5 m using infrared timing gates (Smart-Speed).

The 20 m straight sprint time among track-and-field athletes were significant faster than soccer players ($p < 0.05$). There were no significant differences between soccer players and track-and-field athletes on other measured variables. In the female soccer groups, the 20 m sprint time among did not differ significantly between HPG and LPG. However, all sprint times of COD involving the decision making with HPG were significantly faster than LPG ($p < 0.05$).

Our study suggested that the COD ability with decision making was not correlated to the 20 m sprinting performance in a straight line.

***Key words***: change of direction with decision making, sprint time, soccer, female athletes

## Introduction

An ability to sprint at the high velocity is essential to the game performance in team invasion games, such as soccer[1]. Sprinting constitutes 1–11% of the total distance covered during a soccer corresponding to 0.5–3.0% of the time when the ball is in play[2]. Yet, it is considered critical to the outcome of a game[1]. Previous studies of game analysis for soccer found that repeated short sprints with changes of direction were more often performed during a soccer match than straight sprints[3) 4)]. The studies suggested that the ability to sprint repeatedly and change of direction while sprinting was one of the important physical qualities for soccer players.

Soccer players are required to accelerate, decelerate and change directions throughout the game. These movements are often in response to game cues, such as the movements of the ball and the actions of other players[4) 5)]. Therefore, it is important to evaluate the sprinting ability involved COD that includes reactions to a game-like stimulations with soccer players.

A number of recent studies have developed and evaluated new tests of sprinting involved in soccer specifics, such as decision making and sprinting ability involved change of direction[5)–7)].

Although Hirose[8)] and other previous studies[9) 10)] suggested that developing the ability to change directions while sprinting may improve overall soccer game performance, there are limited studies

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focusing on the topic in female soccer players. While previous studies have examined the straight-sprint speed and the change of direction speed[9) 11)], limited studies has investigated the sprinting and changing direction times involving decision making based on the game situation and cues in female soccer players. More studies on this topic are needed in order for coaches to design the soccer specific training program, so they can improve the ability to sprint and change directions while making decision in the game. Therefore, the purpose of this study was to investigate the sprinting ability with COD involved decision making in female soccer players. We particularly compared them with a different sport and between different competitive levels.

## Methods

### 1. Subjects

Thirty-three university female athletes participated in this study (age: 19.7 ± 1.2 years, height: 161.4 ± 5.3 cm, body mass: 55.7 ± 4.7 kg, body fat: 21.5 ± 5.1%), and were divided into 4 groups (n = 24: soccer [n = 11: Higher performance group (HPG), n = 11: Lower performance group (LPG)] n = 9: track-and-field) (Table-1). In this study, we used track-and-field athletes as a comparison group because playing characteristics is different from soccer. Prior to the investigation, all subjects were fully informed the purpose of this study and procedures, as well as provided the written informed consent. This study was carried out with the approval of the Research Ethics Committee of Juntendo University Graduate School of Health and Sports Science.

### 2. Protocols

a) 20 meter sprint test

20 meter (m) sprint time of subjects was measured using infrared timing gates (Smart-Speed, Fusion Sport Pty Ltd., Queensland, Australia). The subjects were instructed to run as fast as possible along the 20 m distance from a standing start. This test was conducted twice for each subject and best time of two was used for analysis.

b) COD with decision making sprint test

For the COD with decision making sprint time, the subjects completed the 20 m sprint with cutting movement to 90 degree at the 10 m point where they needed to make a decision whether they turn right or left turn according to the light stimulation at 2.5, 5 and 7.5 m using infrared timing gates (Smart-Speed). Figure-1 shows the layout of the decision making sprint test. The subjects started at point A, and sprinted to the point C. They turned at point C, sprinted through the point D. A light stimulation at point D was arranged so the subjects need to make a decision about whether they turned right or left and was initiated when the subjects sprinted through the point B (1: 2.5 m, 2: 5 m, 3: 7.5 m). The subjects were instructed to complete the test as fast as possible. The COD with the decision making sprint test was measured twice for each subjects, and best time of two was used for analysis.

c) Data analysis

For each measurement, the mean and standard deviation of the value were calculated. Comparisons

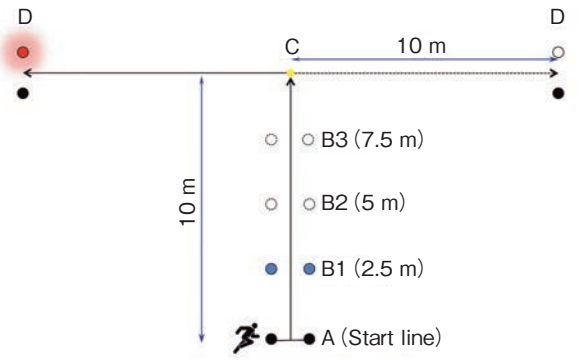


**Figure-1** Layout of the COD with decision making sprint test

**Table-1** Physical characteristics of subjects (Mean ± SD)

| Group | Age (years) | Height (cm) | Body mass (kg) | Body fat (%) |
|---|---|---|---|---|
| Soccer players (n=24) | 19.8 ± 1.3 | 159.7 ± 4.8 | 54.9 ± 4.1 | 22.6 ± 4.6 |
| Higher performance group (n=11) | 20.6 ± 1.3 | 160.8 ± 4.6 | 55.2 ± 5.0 | 20.3 ± 2.6 |
| Lower performance group (n=11) | 19.2 ± 1.0 | 158.9 ± 5.3 | 54.4 ± 3.7 | 23.6 ± 5.1 |
| Track-and-field athletes (n=9) | 19.4 ± 1.0 | 165.8 ± 3.7 | 57.7 ± 5.8 | 18.6 ± 5.5 |

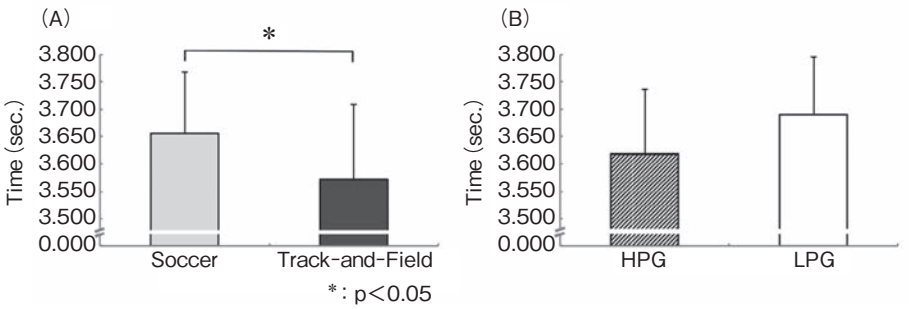


**Figure-2** Result of the 20 m sprint test
A. Soccer players vs. Track-and-field athletes
B. Higher performance group vs. Lower performance group

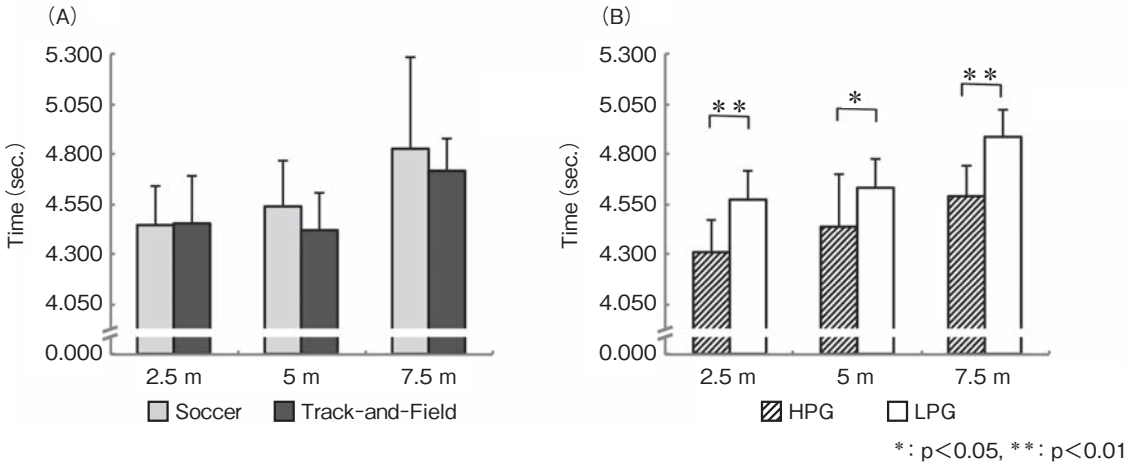


**Figure-3** Results of the COD with the decision making sprint test
A. Soccer players vs. Track-and-field athletes
B. Higher performance group vs. Lower performance group

between soccer players and track-and-field, as well as between HPG and LPG were conducted. T-test was used to compare the groups. The significance level was set at $p < 0.05$.

## Results

Results of the 20 m sprint test are shown in Figure-2A for soccer players and track-and-field athletes. The mean values and standard deviations of the 20 m sprint time in soccer players was 3.656 ± 0.113 seconds (sec), and in track-and-field athletes was 3.572 ± 0.138 sec. The 20 m sprint times of track-and-field athletes were significantly faster than that of soccer players ($p < 0.05$).

Results of the COD with the decision making sprint test are shown in Figure-3A for soccer players and track-and-field athletes. The mean values and standard deviations of the light stimulation point at 2.5, 5 and 7.5 m in soccer players were 4.444 ± 0.915 sec, 4.538 ± 0.226 sec, 4.825 ± 0.462 sec, and in track-and-field athletes were 4.451 ± 0.239 sec, 4.422 ± 0.182 sec, 4.718 ± 0.162 sec, respectively. All sprint times of COD with the decision making did not differ significantly between the soccer players and the track-and-field athletes.

In the female soccer groups, results of the 20 m sprint test are shown in Figure2B for HPG and LPG. The mean values and standard deviations of the 20 m sprint time in HPG was 3.618 ± 0.119 sec, and in LPG was 3.690 ± 0.107 sec. There was no significant difference among female soccer groups on the 20 m sprint times.

Results of the COD with the decision making sprint test are shown in Figure-3B for HPG and

LPG. The mean values and standard deviations of the light stimulation point at 2.5, 5 and 7.5 m in HPG were 4.306 ± 0.168 sec, 4.433 ± 0.266 sec, 4.590 ± 0.151 sec, and in LPG were 4.575 ± 0.139 sec, 4.630 ± 0.146 sec, 4.886 ± 0.139 sec, respectively. All sprint times of COD with the decision making in HPG were significantly faster than LPG ($p<0.05$).

## Discussion

Previous studies have indicated that short-distance sprint ability is essential for achieving the high level performance in soccer[12) 13)]. The purpose of this study was to investigate the sprinting ability with COD involved the decision making in female soccer players and compare them with a different sport and between different competitive levels.

We found that the 20 m sprint times of track-and-field athletes were significantly faster than that of soccer players while other measured variables did not differ significantly between the soccer players and the track-and-field athletes. It was understandable results since the track-and-field athletes specialized the running speed in a straight line and they always trained for the type of sprints. It is unfamiliar for closed skill sports athletes such as track-and-field athletes to sprint with COD and make certain decisions. On the other hand, for open skill sports athletes like soccer players, they are expected to sprint and change directions while making decisions when they are playing games in response to game cues such as an opponent's movement. Therefore, the speed of this movement (i.e., COD with decision making) is influenced by decision making factors, such as the speed of anticipation[14)]. Young[14)] suggested that a player in an open skill sport may have average COD speed but could be very quick if they are highly skilled in the decision making factors.

Therefore, with the better ability to obtain the information and implement into their movements, soccer players demonstrated the better ability to sprint fast and change directions while they are making decisions compared to the track-and-field athletes.

While there was no significant difference on the 20 m sprint times among female soccer groups, all sprint times of COD with decision making in HPG were significantly faster than LPG. These results supported the previous study carried out by Farrow[15)], which reported highly skilled players produced significantly faster movement times and decision times than less skilled players. It was clear that decision-making time had a strong influence on the COD with decision making[16)]. Moreover, this result showed that learning to respond more quickly to a COD stimulus in sport (i.e., game cues) could improve overall game performance[16)]. Thus, COD with the decision making may be one of the useful indicators for the talent among female soccer players.

For the future research, it is important to evaluate the correlations between COD with the decision making sprint time as well as decision making time. In this study, we found that there is no correlation between the COD ability with decision making and the 20 m sprinting performance in a straight line. However, this result may have been depended on the test protocol. Therefore, additional research is needed using various test protocols to measure more accurate correlation.

This study suggested that COD with the decision making while sprinting was one of the important physical qualities for female soccer players. Thus, coaches should include training for this ability of female soccer players as a part of their training program. Moreover, the training program should specifically focus on the COD with decision making.

## Conclusions

This study suggested that the COD ability with decision making was not correlated to the 20m sprinting performance in a straight line.

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# Effects of Different Visual Class on Agility in the Visually Impaired Soccer Players

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The purpose of the present study was to get the reference value of agility of visually impaired soccer players in accordance with the blind class. Twenty one male visually impaired soccer players (B1: n=12, B2: n=4, B3: n=5) and twelve male university soccer players participated in this study voluntarily. Subjects' height, body weight, body mass index, body fat percentage. The agility performance at side step test was evaluated by using two support rope. The kind of the support rope was 3 fulcrums support rope (the weight mobility type=WMT) and 1 fulcrum support rope in (the pendulum mobility type=PMT). The results of there were no difference on the blind class by used WMT method in the side-step test. But there was a difference on the blind class by used PMT method in the side-step test ($p>0.05$). Sighted subjects were higher recorded by used PMT method in blindfold ($p>0.05$). In conclusion, the findings of the present study indicated the reference value of agility ability of each of the blind class. Suggested that they had been relationship in ability to recognize the space involved with pendulum mobility operation. Low vision was suggested that the superior agility performance with spatial perception than blind.

***Key words***: agility performance, visually impaired, blind class, blind, low vision, spatial perception

## Introduction

Agility is generally defined as the ability to change direction of the body rapidly, without losing balance, using a combination of strength, power, and neuromuscular coordination[1)-3)]. Thus, agility is very important in soccer, and the ability of soccer players to produce fast paced variable actions is known to impact soccer performance[4) 5)].

Visually impaired of physical strength is low as compared with the sighted people, especially low agility. Suggested that visually impaired has few opportunities to experience a whole body movement, including the jumping behavior[6)]. In addition, blindness can cause low physical work capacity, posture problems, orientation difficulties, balance problems. Previous studies include information about these disturbances[7)-9)].

In Paralympic sport Football 5-a-side is a game played by athletes with visually impaired and is known worldwide as blind football or blind soccer. This sport had its first national Championship in Spain, in 1986, and first appeared in the Paralympic Games in Athens in 2004[10)]. The athletes can be categorized in three levels: B1 (from no light perception in either eye up to light perception, unable to recognize the shape of a hand at any distance or direction); B2 (from ability to recognize the shape of a hand up to a visual acuity of 20/600 or a visual field of less than 5° in the best eye with the best practical eye correction); B3 (from visual acuity above 20/600-20/200 or a visual field of less than 20° and more than 5° in the best eye with the best correction)[11)]. In football 5-a-side, only B1 athletes can compete.

Side-step test is generally used in the test to

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measure the agility. However, in the test that has been developed for the sighted people, there is a possibility to evaluate lower the agility of the visually impaired [12]. For the visually impaired, in order to adjust the measurement conditions, support rope has been attempted. By using the support ropes, it has been suggested that can accurately measure the agility of the visually impaired [13]. However, there may be characterized in agility depending on the blind class. Investigate the agility ability to another failure class, it is important to report the characteristics of agility in the visually impaired. The purpose of the present study was to get the reference value of agility of visually impaired soccer players in accordance with the blind class.

## Methods

### 1. Subjects

Twenty one male visually impaired soccer players (B1: n=12, B2: n=4, B3: n=5) and twelve male university soccer players (sighted subjects) participated in this study voluntarily. The causes of impaired sight were a congenital disorder in sixteen subjects, an acquired disorder in five subjects. Their mean age, height, body mass and body fat were B1: 24.4 ± 5.6 yrs, 171.1 ± 4.8 cm, 65.0 ± 8.0 kg and 13.8 ± 4.3%, B2: 25.7 ± 4.1 yrs, 171.2 ± 4.0 cm, 64.8 ± 4.6 kg and 12.5 ± 1.2%, B3: 19.8 ± 0.9 yrs, 171.0 ± 6.0 cm, 65.5 ± 5.7 kg and 12.2 ± 1.9% respectively. The study group consisted of active blind football players of the blind football team and all the players were trained for two hours three days per week. Subjects' mean training experience was 2.3 ± 2.5 yrs. Subjects were informed about the possible risks and benefits of the study and gave informed consent to participate in this study.

### 2. Agility performance evaluation (side-step test)

The agility performance of the visually impaired soccer players and university soccer players were evaluated using side-step tests: the weight mobility type (WMT) and the pendulum mobility type (PMT). The number of times was recorded using electronic timing gates (Fusion Sport Smart Speed, Wales, UK) located at the 3 lines. Subjects stood at a center line, then moved 100 cm to the side and touched a line with the closest foot, moved back to the center then jumps 100 cm to the other side, then back to the center. This is one complete cycle. The subjects tried to complete as many cycles as possible in 20 seconds. These tests were performed in an indoor court to eliminate environmental conditions, and visual conditions were the same by using a blindfold. Side-step test each performed twice to measure the good recording.

### 3. Support rope

Two types of support rope were used in the side-step test. The kind of the support rope was 3 fulcrums support rope (the weight mobility type = WMT) and 1 fulcrum support rope (the pendulum mobility type = PMT). WMT method is the way to move the center of gravity of the body to each fulcrum (Figure-1). PMT method is the way to move while fixing the center of gravity of the body on a central fulcrum (Figure-2). Thus, side-step test was conducted respectively with the two

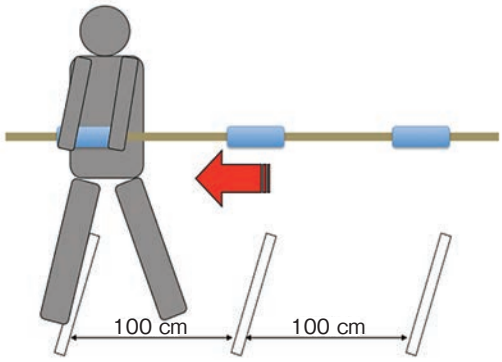


**Figure-1** 3 fulcrums support rope (the weight mobility type=WMT)

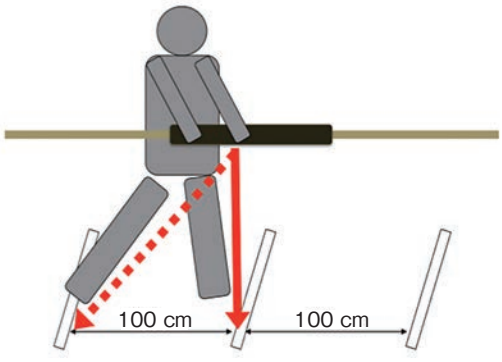


**Figure-2** 1 fulcrum support rope (the pendulum mobility type=PMT)

moving method.

**4. Statistical analyses**

B2 and B3 play a game as "low vision soccer" together at the statistics it was with the same group. The data are reported as means and standard deviations. Repeated-measures ANOVA was used to compare the WMT and PMT performance both within (B1 vs B2・B3 vs Univ) and between the two experimental method (WMT vs PMT) used independent t-test. Then, the relationships between WMT and PMT were evaluated by the Pearson Product Moment Correlation analysis. All analyses were executed in SPSS for Windows version 10.0 and the statistical significance was set at $p<0.05$.

## Results

**1. WMT method (3 fulcrums support rope)**

Record of side-step test by WMT was as follows, B1: 54 ± 7, B2・B3: 56 ± 3 and Univ: 47 ± 4, respectively (Figure-3). Comparison of each group in side-step test by WMT, B2・B3 was significantly higher than Univ ($p<0.05$). There was no significant difference in B1 and B2・B3 within of the WMT method.

**2. PMT method (1 fulcrum support rope)**

Record of side-step test by PMT was as follows, B1: 44 ± 6, B2・B3: 51 ± 3 and Univ: 65 ± 6, respectively (Figure-4). Comparison of each group in side-step test by PMT, B2・B3 was significantly higher than B1 ($p<0.05$), Univ was significantly higher than B1 and B2・B3 ($p<0.05$).

**3. Comparison of WMT and PMT**

B1 and B2・B3 together towards WMT was high recording than PMT, there were a significant difference between the method of only B1 ($p<0.05$). In sighted subjects, the visually impaired was the opposite result (Figure-5). In Univ subjects was higher PMT than WMT, there were a significant difference between the method ($p<0.05$).

**4. Relationship between WMT and PMT**

The correlation between the method of WMT and PMT, B1 ($n=12$): $r=0.5$, B2・B3 ($n=9$): $r=0.2$, Univ ($n=12$): $r=0.01$, respectively. There was no significant relationship of both methods.

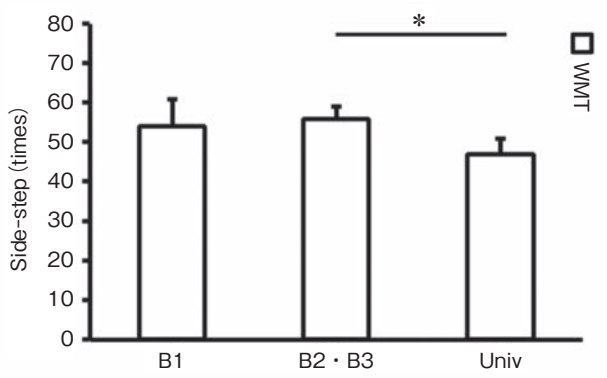


**Figure-3** Comparison of side step test in WMT method
*Significant difference ($p<0.05$).

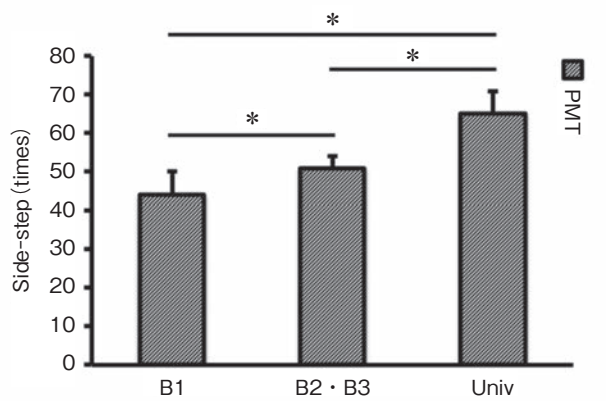


**Figure-4** Comparison of side step test in PMT method
*Significant difference ($p<0.05$).

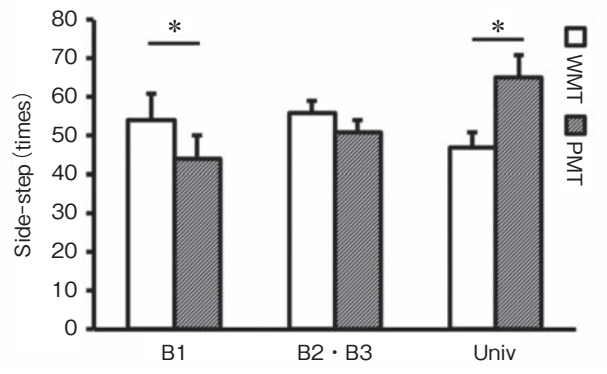


**Figure-5** Comparison of side step test in WMT method and PMT method
* Significant difference ($p<0.05$).

## Discussion

The purpose of the present study was to get the reference value of agility of visually impaired soccer players in accordance with the blind class. Therefore, it was necessary to several methods to evaluate of the agility. Side-step test is generally used in the test to measure the agility. For the visually impaired, in order to adjust the measurement conditions, support rope has been attempted[13]. The authors hypothesized that there may be a feature of the agility by blind class. WMT method that has been used[13], it was added to the PMT method in this study. The cognition is defined as a process for processing information on the basis of reasoning, judgment and memory. Cognitive ability is superior low vision than blind[14]. PMT method was created in order to clarify the differences in the cognition of space in agility. In doing so, it is possible to obtain the characteristics of agility performance in the visually impaired soccer players.

In side-step test using WMT method, the visually impaired was a high recording than sighted subjects. Moreover, there was no significant difference in blind class, WMT method is considered to have characteristics that reduce the influence of blind class. In side-step test using PMT method, the sighted subjects were significantly higher record than the visually impaired. PMT method has been demonstrated to be associated with spatial cognition in previous research[14]. This study was the result to support the previous research. Interestingly, between the blind class, but there was no significant difference in agility performance using the WMT method, there was a significant difference in the PMT method in agility performance. Takato (2013) reported that blind visual experience of spatial perception by the poor, takes time to acquire the space cognitive ability[15]. Therefore low vision (B2・B3), it can be considered to be good in agility with spatial cognition. In addition, PMT method can be expected as a method for measuring the agility with spatial cognition.

Sighted subjects can be in the blindfold, agility performance using the PMT method was significantly higher than the agility performance using WMT method. In addition, it is thought that the influence of visual information is low in PMT method. Thus, agility performance also using the PMT method in the visually impaired is likely to improve. The result of this study, agility performance of visually impaired was not never low clause consideration of the failure.

In blind football corresponds to the changing circumstances, such as the position of the ball and players. It inferred that there has been a growing agility performance throughout the competition.

From that there were no significant relationship to the WMT method and PMT method, there are a uniqueness of both methods. Therefore, to measure the characteristics of agility performance of the visually impaired, suggested that using both measurement methods.

## Conclusion

To the authors' knowledge, this is the first study to report of side-step agility test using WMT method and PMT method in visually impaired soccer players. In this study it was possible to obtain the reference value of agility in visually impaired soccer players. The difference of the blind class was suggested agility with spatial cognition. Therefore, low vision was suggested that the superior agility performance with spatial perception than blind.

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# Impact of Muscular Evaluation by 3D-CT

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***Introduction***: Characteristics of muscular morphology is assumed to be one of the determining factors for its mechanical function and a particularly important evaluation index in the field of sports. Muscle evaluation is generally performed using ultrasound and MRI with major endpoints of thickness and cross sectional area. However, due to its complicated anatomy, a skeletal muscle presents different area depending on its cross section site. Therefore, muscle volume should be used as an index to evaluate the whole muscle. The narrow observable field of view in ultrasound and the long imaging time of an MRI make these methods unsuitable for the evaluation of muscle volume. In contrast, CT allows for short measurement time and clear image over a wide area. It can also selectively extract data from each cross sectional data to build a three-dimensional image (3D-CT) of the target muscle with accurate measurement of the muscle volume. This study was aimed to examine the efficiency and usefulness of the whole muscle extraction by 3D-CT and the measurement of muscle volume.

***Methods***: Cross sectional images of the thigh at slice thickness 1 mm using multi-slice CT were obtained from male athletes specializing in track and field. Quadriceps femoris (QF) and hamstrings (Ham) were selected as target muscles and selectively extracted 10 times using an image analysis work station made by AZE company. We evaluated the whole form, origin, termination and muscle volume of each muscle. As quantitative detail, we calculated the mean, standard deviation, and coefficient of variation (CV) of the volume.

***Results and Discussion***: The mean muscle volume were 2341.3 ± 14.78 $cm^3$ for QF and 1128.3 ± 18.04 $cm^3$ for Ham. CV of QF and Ham were less than 1% and less than 2%, respectively. CT is known to be superior in spatial resolution. Both quadriceps femoris and hamstrings have multiple origins and terminations, all of which were clearly observed in this study. Accurate muscular morphological evaluation is extremely important in elucidating characteristics of the muscular force exertion. This study suggested that 3D-CT is a useful method for evaluating the anatomical basis of the human body in detail. We will further clarify its utility by comparing other muscle volume calculation methods in the future.

**Table-1** The volume, mean value, SD and CV in 10 Trials of QF and Ham

| Volume ($cm^3$) | | QF | Ham |
|---|---|---|---|
| Trials | 1 | 2300 | 1131 |
| | 2 | 2341 | 1105 |
| | 3 | 2343 | 1159 |
| | 4 | 2343 | 1112 |
| | 5 | 2348 | 1117 |
| | 6 | 2344 | 1150 |
| | 7 | 2354 | 1114 |
| | 8 | 2357 | 1117 |
| | 9 | 2344 | 1126 |
| | 10 | 2339 | 1152 |
| Mean | | 2341.3 | 1128.3 |
| SD | | 14.78 | 18.04 |
| CV | | 0.006 | 0.016 |

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# Contribution of Mitochondrial Superoxide and SOD2 Imbalance to the Locomotive Syndrome

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Age-related motor organ failure is the major problem in super-aged society because it is leading to care requirements or bedridden. Although locomotive syndrome is recently defined as the individual state with gait disturbance having functional decline of motor organs; bone, cartilage, intervertebral disc, nerve and skeletal muscle, it has been practically difficult to treat it because the cause is complicatedly multifactorial, such as osteoporosis, osteoarthritis, sarcopenia and so on. Superoxide dismutase 2 (SOD2) is the endogenous mitochondrial antioxidant enzyme which converts superoxide anion to hydrogen peroxide to maintain the reduction-oxidation balance in cells. The physiological role of SOD2 and the pathological role of superoxide in degeneration of motor organs have been investigated in our previous study using tissue-specific SOD2-deficient mice. Here we focused on the effect of mitochondrial superoxide and SOD2 imbalance in the development of the locomotive syndrome.

We generated conditional SOD2-deficient mice crossbreeding *Sod2 flox* mice with dentin matrix acidic phosphoprotein (DMP1) promotor Cre, type2 collagen (Col2) promotor Cre and human skeletal actin (HSA) promotor Cre mice to find tissue-specific phenotypes in osteocyte, chondrocyte and skeletal muscle, respectively. Osteocyte-specific SOD2 deficiency showed both increased expression of sclerostin leading to suppressed bone formation and increased expression of receptor activator of NF-κB ligand (RANKL) causing activated bone resorption. As a result, the mutant mice *in vivo* showed remarkable bone loss in an age-dependent manner, composing increase of disorganized osteocytic canalicular networks and decrease of live osteocytes number. Chondrocyte-specific deletion of SOD2 promoted mitochondrial superoxide overproduction, mitochondrial dysfunction and impaired extracellular matrix homeostasis, leading to spontaneously accelerated cartilage degeneration both during aging and under mechanical loading. Furthermore skeletal muscle-specific SOD2-deficient mice displayed increased selective loss of enzymatic activity in mitochondrial respiratory chain complexes and reduced ATP content in their muscle, leading to severe disturbances in exercise activity.

These findings demonstrate that mitochondrial superoxide in common plays a pivotal role in the development and progression of osteoporosis, osteoarthritis and muscle weakness, so to speak, age-related locomotive dysfunction, and suggest that the regulation of superoxide balance in the local tissue or in the entire body is a promising target for the treatment of locomotive syndrome.

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# Effect of Combined Increased Physical Activity and Walking with Blood Flow Restriction on Leg Muscle Thickness in Older Adults

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***Objective***: To investigate the effect of combined increased walking and stair-climbing, and walking with blood flow restriction (BFR) in working muscles on leg muscle thickness in older adults.
***Methods***: Nineteen older subjects were divided into the following three groups: normal walking and stair-climbing (WS), WS and BFR-walk once a wk (WS-BFR1), and WS and BFR-walk twice a wk (WS-BFR2). All subjects were instructed to walk at a self-selected, faster pace than usual for ≥30 min per session, ≥2-4 days per wk, and climb ≥5 flights of stairs per day, ≥4 days per wk for 11 wk. Additionally, the WS-BFR1 and WS-BFR2 groups performed 20 min of a BFR-walk at a pre-determined exercise intensity of 70-85% of the age-predicted maximum heart rate.
***Results***: Two-way repeated measures analysis of variance showed that the time effects were significant ($p < 0.01$) for muscle thicknesses (MT) of the posterior aspects of the thigh (PT, WS: 8.9%, WS-BFR1: 11.7%, WS-BFR2: 11.8%) and lower leg (PL, WS: 3.0%, WS-BFR1: 1.1%, WS-BFR2: 6.6%). However, there was no significant difference in these values of MT among all the groups. For the 10-m walking time, the main effect of time was significant ($p < 0.05$), but the interaction was not significant.
***Conclusions***: Walking and stair-climbing training can increase MT of the PT and PL, and improve walking performance in older adults, whereas a BFR-walk once or twice a wk may not produce additional training effects.

***Key words***: muscular adaptations, aerobic exercise, occlusion

## Introduction

Skeletal muscle is important for performing activities of daily living, and it has an important role with metabolism. For instance, skeletal muscle is the largest disposal site for ingested glucose[1], and it plays a role in lipid oxidation and immune responses[2) 3)]. The amount of skeletal muscle also decreases with aging, i.e., sarcopenia. This causes disability, falls, and osteoporosis[4)-6)], and it increases the risk of developing a wide range of chronic disorders, including atherosclerosis[7) 8)], insulin resistance, and hyperglycemia[9) 10)]. One of the biggest problems is a reduction of ambulatory ability resulting from the decrease in lower limb muscles. Therefore, strategies to increase or maintain the amount of skeletal muscle, especially in the lower limb, across one's lifespan are important for overall health. High-load resistance training has been primarily recommended as an effective countermeasure against sarcopenia[11) 12)]; in fact, the decline in muscle mass and strength among older adults is at least partly reversible by high-load resistance training[13) 14)]. However, high-load resistance training generally requires a supervised program involving expensive training equipment,

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and high external loads may present a major barrier for older adults.

Recently, Abe *et al.*[15] showed that activities of daily living at moderate and vigorous intensities (i.e., ≥3 Mets) are positively correlated with the triceps surae and tibialis anterior muscle thickness. Additionally, isometric knee flexion strength is positively correlated with the duration of moderate physical activity. Another study reported that 6-month walking training, one of the typical examples of moderate physical activity, increased muscle thickness (MT) in the knee flexors and dorsi flexors, and strength in the knee flexors, dorsi flexors, and plantar flexors; however, there was no significant effect on the knee extensors[16]. Given that age-related changes in MT were a site-specific manner, upper-leg anterior MT especially decreases with age; thus, it is important for older adults to perform other exercise programs to improve muscle size and function in knee extensors as well as walking during daily physical actions. A common example of daily physical action that activates the knee extensors more than walking is stair-climbing[17]. To the best of our knowledge, no study has investigated the hypertrophic effect of stair-climbing.

Physical activities such as walking at moderate to vigorous intensities can induce significant muscle hypertrophy, but the effect is minimal[18]. Muscle hypertrophic adaptations mainly result from exposure to both mechanical and metabolic stresses[19]. Metabolic stress is accumulated by blood flow restriction (BFR) in working muscles even during walking, resulting in an enhanced hypertrophic effect with walking[19]. For example, our studies showed that 20 min of a BFR walk, 4–5 days per wk for 6–10 wk, increased thigh muscle size and strength, whereas these adaptations were not observed after a normal walking program in older adults[20) 21)]. It would be difficult, however, for most older adults to perform a BFR-walk at a high frequency as in previous studies[20) 21)] because a supervised program with a BFR-dedicated device is required.

To develop a more practical training program, we investigated the effect of combined walking and stair-climbing on almost a daily basis in older adults, and whether replacing 1–2 days per wk with a BFR-walk has an additional training effect on leg muscle size in older adults.

## Methods

### 1. Participants

Twenty-six older subjects (age 69 ± 1 years, height 1.63 ± 0.02 m, body weight 64.5 ± 2.0 kg) volunteered to participate in this study. They were recruited through printed advertisements and by word of mouth. None of the subjects had participated in any regular high-load resistance training for at least 1 year. All subjects were informed about the methods, procedures, and risks, and they provided informed consent before participating in this study. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee for Human Experiments of Juntendo University, Japan. Participants were randomly assigned to the following three groups: normal walking and stair-climbing (WS, n = 16, 12 men and 4 women), WS and a BFR-walk once a wk (WS-BFR1, n = 12, 7 men and 5 women), and WS and a BFR-walk twice a wk (WS-BFR2, n = 3, 1 man and 2 women).

### 2. Training program

All subjects were instructed to walk at a self-selected, faster pace than usual for ≥30 min per day, ≥2–4 days per wk (≥4 days per wk in the WS group, ≥3 days per wk in the WS-BFR1 group, and ≥2 days per wk in the WS-BFR2 group), and climb ≥5 flights of stairs per day, ≥4 days per wk for 11 wk. Additionally, both the WS-BFR1 (once a wk) and WS-BFR2 (twice a wk) groups performed 20 min of treadmill walking with BFR at an exercise intensity of 70–85% of the age-predicted maximum heart rate (220 − age). During the BFR-walk session, nylon cuffs (105 mm wide, MT-870 Digital Tourniquet; Mizuho, Tokyo, Japan) were applied tightly at the most proximal portion of both legs. The target pressure was calculated for each subject based on the circumference of the right thigh (33% of the distance from the inguinal crease to the top of the patella) as follows: < 50 cm = 100 mmHg and 50–55 cm = 120 mmHg. This is because arterial occlusion pressure is largely influenced by thigh circumference[22]. The cuff air pressure was released immediately after completing each session.

### 3. MT

MT was measured via B-mode ultrasound using a 5-18 MHz scanning head (Noblus; Aloka, Tokyo, Japan) at the anterior (AT) and posterior (PT) aspects of the right thigh at 50% of the thigh length between the lateral condyle of the femur and the greater trochanter, and at the anterior (AL) and posterior (PL) aspects of the right lower leg at 30% of the lower leg length between the lateral malleolus of the fibula and the lateral condyle of the tibia. Prior to all scans, subjects rested quietly in a seated position for at least 30 min. To avoid the influence of fluid shifts within the muscle, the measurements were performed around the same time. All measurements were performed by the same operator. The ultrasound measurements of MT were performed with subjects in the supine/prone position, with careful attention to ensure that the hip and ankle joint positions, and the distance between both legs were the same before and after the training period. The scanning head, which was coated with a water-soluble transmission gel, was placed on each marked measurement site without depressing the dermal surface. The subcutaneous adipose tissue-muscle interface and the muscle-bone interface were identified on ultrasound images, and the distance between the two interfaces was recorded as the MT. The test-retest (inter-session) reliabilities of the MT measurements were calculated using an intraclass correlation coefficient, standard errors of measurement, and minimal difference. These values were previously determined in 10 young subjects in terms of the AT values, and were 0.999, 0.21 mm, and 0.58 mm, respectively.

### 4. 10-m walking time

Walking performance was evaluated by timing each subject as they walked across a 10-m corridor on a hard-surfaced floor. The width of the corridor was set at 1-m. Subjects performed two timed trials and were encouraged to maintain a straight course. They were asked to walk down the corridor as fast as possible without running. Their times were measured using a digital stopwatch (LC058, Citizen, Tokyo, Japan), and the best time was used for the 10-m walking time.

### 5. Statistical analysis

All results are expressed as means with standard errors. The data of subjects whose adherence rate was ≥80% for both the walking and stair-climbing programs were used for analysis (WS: n=8, 8 men; WS-BFR1: n=8, 6 men and 2 women; WS-BFR2: n = 3, 1 man and 2 women). Statistical analysis featured two-way analysis of variance (ANOVA) with repeated measures [condition (WS, WS-BFR1, and WS-BFR2) × time (PRE and POST)]. All baseline values for all groups were compared using one-way ANOVA. Statistical significance was set at $p<0.05$.

## Results

The mean walking time per day, walking training frequency per wk, number of flights of stairs per day, and stair-climbing training frequency per wk in each group were as follows: 62.8 ± 7.8 min/day, 5.3 ± 0.4 days/wk, 11.2 ± 2.6 flights/day, and 5.1 ± 0.5 days/wk in the WS group; 48.7 ± 5.8 min/day, 4.4 ± 0.4 days/wk, 6.8 ± 0.6 flights/day, and 4.9 ± 0.4 days/wk in the WS-BFR1 group; and 52.0 ± 4.3 min/day, 4.3 ± 0.3 days/wk, 7.7 ± 1.3 flights/day, and 4.4 ± 0.7 days/wk in the WS-BFR2 group, respectively. The mean rates of perceived exertion during walking and stair-climbing training were 12.7 ± 0.5 and 13.4 ± 0.6 in the WS group, 11.7 ± 0.4 and 12.3 ± 0.2 in the WS-BFR1 group, and 10.8 ± 0.2 and 13.0 ± 0.6 in the WS-BFR2 group, respectively. There were no significant differences in these values among all groups. The adherence rates in BFR-walk training were 94% and 100% in the WS-BFR1 and WS-BFR2 groups, respectively. Applying BFR did not increase any relevant side effects such as subcutaneous hemorrhage, numbness, and cerebral anemia. There were no significant differences in body mass and the body mass index after a training program.

Table-1 shows the change of MT and walking performance before and after a 3-month training period. Two-way repeated measures ANOVA showed that the time effects were significant ($p<0.01$) for MT of the PT (WS: 8.9%, WS-BFR1: 11.7%, WS-BFR2: 11.8%) and PL (WS: 3.0%, WS-BFR1: 1.1%, WS-BFR2: 6.6%). Moreover, MT of the AT and AL did not change. A significant

**Table-1** Changes in muscle thicknesses and walking performance after an 11-wk training program

| | WS (n=8) | | WS-BFR1 (n=8) | | WS-BFR2 (n=3) | | P value | | |
|---|---|---|---|---|---|---|---|---|---|
| | PRE | POST | PRE | POST | PRE | POST | Group | Time | Interaction |
| Muscle thicknesses | | | | | | | | | |
| AT, mm | 34.5 (2.2) | 34.0 (1.4) | 33.8 (2.1) | 30.3 (1.3) | 27.1 (1.2) | 28.6 (0.3) | 0.13 | 0.38 | 0.13 |
| PT, mm | 45.6 (1.9) | 49.7 (1.2) | 44.4 (1.9) | 49.6 (1.9) | 42.0 (0.6) | 47.0 (4.2) | 0.59 | <0.01 | 0.87 |
| AL, mm | 27.7 (1.1) | 27.6 (1.0) | 24.0 (1.1) | 25.2 (0.9) | 23.8 (1.8) | 24.2 (1.8) | 0.09 | 0.17 | 0.30 |
| PL, mm | 65.2 (1.7) | 67.2 (1.6) | 62.3 (1.0) | 62.9 (1.3) | 57.7 (3.5) | 61.5 (2.5) | 0.06 | <0.01 | 0.17 |
| Walking performance | | | | | | | | | |
| 10-m WT, sec | 5.07 (0.28) | 5.07 (0.24) | 5.00 (0.22) | 4.66 (0.18) | 5.31 (0.18) | 4.84 (0.26) | 0.69 | <0.05 | 0.20 |

Values are presented as mean (± standard error). AT: anterior aspect of the thigh, PT: posterior aspect of the thigh, AL: anterior aspect of the lower leg, PL: posterior aspect of the lower leg

interaction between the time and group was not observed for these MTs; i.e., there was no significant difference in these MT values among all the groups. For the 10-m walking time, the main effect of time was significant ($p < 0.05$, WS: 0.0%, WS-BFR1: -6.9%, WS-BFR2: -8.9%), but the interaction was not significant.

## Discussion

The major findings of the present study were that 11 wk of walking and stair-climbing training increased MT of the PT and PL, and improved walking performance, whereas additional training effects induced by a BFR-walk once or twice per wk were not observed.

A number of studies have reported the effect of a walking program on body composition and aerobic capacity[23], whereas only a few studies have investigated the influence of walk training on lower body muscle size. Kubo *et al.* demonstrated that MT of the PT increased by 7.6% after a 6-month walking training (45.0 ± 15.6 min/day and 5.4 ± 1.1 days/wk)[16], whereas the present study showed that MT of the PT increased by 8.9% for the WS group following the 11-wk walking program (62.8 ± 7.8 min/day and 5.3 ± 0.4 days/wk). These results suggest that the hypertrophic effects of walking may reach a plateau after about 3 months into the program. In both studies, a significant increase in MT of the AT was not observed. Moreover, two previous studies have demonstrated no significant change in the thigh muscle cross-sectional area (CSA), which was not observed following both the 10-wk and 18-wk walking programs[21,24]. Since the thigh muscle CSA includes both the AT and PT, site-specific muscle hypertrophy may be undetected.

Stair-climbing training was performed in the present study to work the quadriceps femoris muscles more than walking. One study demonstrated that the muscle activation levels of the quadriceps femoris muscles during ascending the stairs were 31.0 ± 10.9% and 28.7 ± 7.6%EMG (%electromyogram, the averaged integrated EMG during the action/that during maximal voluntary isometric contraction) in older men and women, respectively, whereas the activation levels of the triceps surae muscles were 46.9 ± 27.7% and 52.4 ± 34.5 %EMG, respectively[17]. In the present study, MT of the PL increased after the training program, but MT of the AT did not change, which may suggest that stair-climbing training provided enough stimuli to induce muscle hypertrophy to the PL, but not to the AT.

Muscle hypertrophy mainly results from exposure to both metabolic and mechanical stresses[19]. The application of BFR during exercise produces greater and/or faster metabolic fatigue, which would induce greater and/or faster muscle growth[19]. For example, our previous study showed that 20 min of a BFR-walk, 4 days per wk for 10 wk, increased thigh muscle CSA, whereas normal walking did not[21]. To develop a more practical training program (i.e., reduce the training frequency of a BFR-walk), the present study investigated whether a greater hypertrophic effect was observed in the WS-BFR1 and WS-BFR2 groups than in the WS group, but there was no significant difference in the hypertrophic effect among all the

groups. The improvement rate in MT and walking performance tended to be higher in the WS-BFR-2 group than in the WS and WS-BFR1 groups. The training effect with body mass-based training (i.e., walking) depends on the ratio of body mass to lower body strength, which is lower in women than in men; thus, this may explain why a higher percentage of female subjects in the WS-BFR2 group had a higher increasing rate in the present study. Thus, future studies with a more robust experimental design need to investigate the training effect of a BFR-walk with less of a frequency (i.e., 1-2 days per wk).

In conclusion, walking and stair-climbing training can increase MT of the PT and PL, and improve walking performance in older subjects, whereas a BFR-walk once or twice a wk may not produce additional training effects.

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# Effect of Long-Term Training Program Combining Increased Physical Activity and Walking with Blood Flow Restriction on Locomotive Syndrome in the Elderly

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***Objective***: The purpose of this study was to examine the effect of long-term training program combining increased physical activity and walking with blood flow restriction (BFR) on locomotive syndrome in elderly adults.

***Methods***: A total of 13 relatively active subjects (11 men and 2 women) aged 67.2 ± 4.0 years were assigned to the increasing physical activity (walking and climbing stairs) group (CON-W, n=6) or blood flow restriction walk training group (BFR-W, n=7). Both groups performed more than 30-min walking training at a pace faster than usual for at least 3 days and climbed more than 5 flights of stairs per day for at least 4 days per week for 6 months. Additionally, BFR-W group performed a treadmill walking for 20 min once a week at an exercise intensity of 70-85 % of age-predicted maximal heart rate (HRmax=220 - age). Locomotive syndrome risk tests (stand-up test, two-step test, 25-question risk assessment) were performed.

***Results***: There was no main effect of group, time, and interactions between group × time in the two-step test, stand-up test, and 25-question risk assessment.

***Conclusion***: Long-term walking and stair-climbing training programs did not improve the outcomes of locomotive syndrome risk test in physically active elderly subjects.

***Key words***: locomotive syndrome, physical activity, walking, stair-climbing, walking with blood flow restriction

## Introduction

Mobility and skeletal muscle mass imperceptibly decrease with aging[1) 2)]. The Japanese Orthopaedic Association (JOA) has proposed the concept of a "locomotive syndrome"[3)]. Locomotive syndrome refers to conditions for which elderly persons require assistance or care or high-risk conditions for which they may soon require care owing to problems of the locomotive organs such as bones, joints, muscles, and tendons[3)]. The loss of function in locomotive organs leads to walking or self-transportation disabilities. To prevent locomotive syndrome, it is important to increase muscle strength, muscle mass, and bone mass through exercise such as resistance exercise.

Walking is the most popular form of physical activity among adults[4)]. Because of walking is an aerobic exercise and requires light-moderate intensity physical activity (2.0-5.9 Mets), it can be performed safely and easily. Previous studies have shown the effect of walking training on aerobic capacity (maximal oxygen uptake, blood lactate response to submaximal) and body composition[5)], and that walking to work decreased the risk for hypertension and type II diabetes in Japanese

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men[6) 7)]. Recently, Ozaki *et al.*[8)] reviewed the effect of walking training on muscle strength, mass, and function; they indicated that relatively long periods (> 6 months) of walking at moderate to vigorous intensity can increase leg muscle size in older adults. Thus, when older adults perform walking training at moderate to vigorous intensity for a long duration, locomotive organs could be improved. However, to our knowledge, no study has examined the effect of long periods of walking at moderate to vigorous intensity on locomotive syndrome risk test parameters.

Stair climbing is also a safe and easy physical activity for most of us incorporate into our daily lives (home, school, station, etc.). Stair climbing is anti-gravity exercise and classified as a 'vigorous' physical activity (> 6.0 METS), it is expected to improve the muscle strength, especially lower limb muscle. A previous study[9)] reported that home-based moderate intensity bench step exercise program (for a total of 140 min or more every week, for 12 weeks) was found to greatly increase aerobic power (METS at lactate threshold) and leg extension power (watts/kg) in elderly subjects. To our knowledge, few study have examined the effect of stair climbing training in daily routine on muscle strength and locomotive organs.

Recently, low intensity walking combined with blood flow restriction (BFR) has focused on physiological responses, such as oxygen uptake, muscle strength, and muscle mass. Previous studies have reported that BFR walking elicits significant improvements in thigh muscle mass and knee joint strength in both young and elderly men[10)-13)]. Mechanical and metabolic stress are suggested to be involved in the mechanism of improvement of muscle strength and mass[14)]. Walking training combined with BFR (BFR-W) would be more effective for locomotive syndrome than walking training alone.

The purpose of this study was to examine the effect of long-term training program combining increased physical activity and walking with BFR on locomotive syndrome in the elderly adults. We hypothesized that a 6-month training program combining increased physical activity and walking with BFR brought about beneficial effects in the locomotive syndrome risk parameters in elderly adults.

## Materials and methods

### 1. Subjects

We recruited subjects who lived near our university through advertisements in newspapers and word of mouth. Overall, the present study included 18 men and 5 women aged 67.2 ± 4.0 years and with no regular aerobic or resistance training for at least 1 year prior to the start of the present study. Prior to the study, all subjects were provided with an adequate explanation about the study measurements as well as risks; they then voluntarily provided written consent to participate only after completely understanding the explanation. The study protocol was approved by the Research Ethics Review Board of the Juntendo University Graduate School of Health and Sports Science (Tokyo, Japan).

### 2. Study design

Subjects were divided into two groups, walking and stair climbing training group (CON-W, 6 men) and blood flow restriction walk training group (BFR-W, 5 men and 2 women, n = 7). All subjects continued training program for 6 months from December 2014 to May 2015.

### 3. Training program

Both groups performed more than 30 min walking training at a pace faster than usual, and stair climbing more than 5 flights of stairs per day at least 4 days for a week at home (non-supervised). Subjects were instructed to perform walking training more than 4 days for a week in CON-W, and 3 days for a week in BFR. The subjects were instructed to measure their walking training daily (walking time, heart rate, and rating of perceived exertion after walking). Additionally, BFR-W was performed for 20 min on a treadmill at a laboratory once a week under the supervision of exercise trainers. Exercise intensity was set at 70-85 % of age-predicted maximal heart rate (HRmax = 220 - age). During BFR walking period, nylon cuffs (MT-870 Digital Tourniquet; Mizuho, Tokyo, Japan) 105-mm wide were applied tightly at the most proximal portion of both legs. The target pressure was calculated for each subject based on the circumference of the right thigh (33% of the distance from the inguinal crease to the top of the

patella), as follows: <50 cm=100 mmHg; 50–55 cm = 120 mmHg. This is because arterial occlusion pressure is largely influenced by thigh circumference. The cuff air pressure was released immediately upon completion of each session.

**4. Daily physical activity**

Physical activity (the number of steps) was assessed using a small uniaxial accelerometer (wristband type life recorder UW-301, A&D Company Limited). The subjects wore the accelerometer for 24 h, except while bathing, for 7 days before the training program and in the last week. All of the measured variables were averaged over the last 5 days of the measurement period to assess physical activity under free-living conditions.

**5. Measurements**

In the present study, subjects performed locomotive syndrome risk test as follows.

1) Two-step test

Two-step test measures the stride distance to assess walking ability, including muscle strength, balance, and flexibility of the lower limbs. The two-step test was performed using the following procedure: (1) subjects take two long steps as long as possible and then align both feet; (2) the distance of the two steps from subject's toes at the starting line to the point where they stopped. The two-step test score was calculated using the following formula: length of the two steps (cm) ÷ height (cm). The measurements were obtained twice for both legs of each subject, and the higher score was used.

2) Stand-up test

Stand-up test assesses leg strength by having the subject stand up on one or both legs from four seats of different heights 40, 30, 20, and 10 cm. Subjects were instructed to stand up without leaning back to gain momentum and maintain the posture for 3 s. In the present study, if the subject was unable to stand up on one leg (right or left) from a height of 40 cm, then his/her stand-up test was considered failed.

3) A 25-question risk assessment (Locomo 25)

A 25-question risk assessment was developed by Seichi *et al.*[15]. It is a self-administered, comprehensive measure, consisting of 25 items that include questions regarding pain, activities of daily living and mental health during last month. These 25 items are graded on a five-point scale, from 0 point (no impairment) to 4 points (severe impairment) and then added to produce a total score. Thus, a higher score is associated with high risk of developing locomotive syndrome in the future.

Locomotive syndrome risk score was based on the reference value of the Japanese Orthopaedic Association[16]. The prevalence of the indices in locomotive syndrome risk test stage 1 was estimated, including two-step test score <1.3, difficulty with one-leg standing from 40 cm in the stand-up test (either leg), and Locomo 25≥7.

**6. Maximum isometric strength**

Maximum isometric strength of the knee flexion and extension were measured (Biodex System 3 dynamometer; Sakai Medical Instrument, Tokyo, Japan). Each subject was seated on a chair with the hip joint angle positioned at 85° of flexion (0°=full hip extension). The center of rotation of the knee joint was visually aligned with the axis of the dynamometer lever arm and the ankle was firmly strapped to the distal pad of the lever arm. A knee joint angle of 0° corresponded to full knee extension. Subject performed warm-up contractions (4–5 submaximal contractions and 1–2 near-maximal contractions at 180° per second) before the test. they were then instructed to perform maximal isometric knee extension twice for about 5 s at a fixed knee joint angle of 75°, preceded by maximal isokinetic knee extension from 0° to 90°, at 90° and 180° per second. Next, they performed maximal isometric knee flexion three times for about 5 s at a fixed knee joint angle of 30°. We considered each peak torque both knee flexion and extension.

**7. Statistical analysis**

The present study set a criterion for analyzing subjects who had performed more than 80% of both the walking and stair-climbing training programs. Overall, 11 men and 2 women were included in the present study. Microsoft Office Excel 2008 and PASW Statistics 17.0 were used for data processing and statistical analyses, respectively. Statistical analyses were performed by using a two-way

**Table-1** Subjects characteristics at baseline

| Variables | CON-W (n=6) | BFR-W (n=7) |
|---|---|---|
| age (yr) | 67.5 ± 4.8 | 66.9 ± 3.5 |
| height (cm) | 164.3 ± 5.6 | 165.3 ± 5.9 |
| weight (kg) | 66.9 ± 7.3 | 64.4 ± 6.0 |
| BMI (kg/m$^2$) | 24.7 ± 1.8 | 23.6 ± 2.1 |
| muscle mass (kg) | 27.6 ± 2.4 | 25.6 ± 3.4 |
| fat mass (kg) | 17.3 ± 3.5 | 17.7 ± 5.3 |
| %fat | 25.6 ± 2.8 | 27.4 ± 7.7 |
| step/day | 10753.7 ± 4858.5 | 7791.0 ± 2450.2 |
| Two-step | 1.5 ± 0.1 | 1.5 ± 0.1 |
| Stand-up | 5.2 ± 0.4 | 5.2 ± 0.4 |
| Locomo 25 | 1.2 ± 0.4 | 1.4 ± 1.3 |
| Leg extension (Nm) | 184.4 ± 31.1 | 163.2 ± 33.4 |
| Leg flexion (Nm) | 90.8 ± 12.7 | 96.5 ± 21.2 |

analysis of variance (ANOVA) with repeated measures to evaluate the training effects in the locomotive syndrome risk test (Group [CON-W and BFR-W] × Time [0, 3, and 6 months]). All data are shown as the mean ± standard deviation. The cut-off for statistical significance used was $p<0.05$.

## Results

### 1. Baseline data and intervention adherences

The values for the age, body composition (muscle mass and fat mass), locomotive syndrome risk test score (Two-step test, stand-up test and Locomo 25), maximum isometric strength and step count per day were not significantly different between the CON-W and BFR-W groups at baseline (Table-1). Although there were two female subjects in BFR-W, the reference value of the locomotive syndrome risk score was the same for both male and female subjects. Table-2 showed prevalence of indices in the locomotive syndrome risk test (stage 1) between the CON-W and BFR-W groups at baseline. There were three subjects who fulfilled reference value as stage 1 (diagnosed as starting to decline in mobility).

### 2. Effect of the training program on physical activity

During the 6-month training program, the BFR-W group walked for average 46.0 ± 5.2 min per day and 4.2 ± 0.5 days per week, and the CON-W group walked for 64.5 ± 10.5 min per day and 4.6 ± 0.5 days per week. In climbing stairs, the BFR-W group climbed average 6.9 ± 0.7 flights of stairs per day for 4.5 ± 0.5 days per week, and the CON-W group bed 9.1 ± 2.5 flights of stairs per day for over 4.9 ± 0.5 days. There were no significant differences in the walking time and stairs climbed between the CON-W and BFR-W groups.

There was no significant main effect of time in the step counts per day (Figure-1). Moreover, physical activity per day at moderate to vigorous intensity during the 6-month intervention did not increase significantly from that at baseline (data not shown).

**Table-2** Prevalence of indices in the locomotive syndrome risk test (stage 1)

| Variables | CON-W (n=6) | BFR-W (n=7) | Total (n=13) |
|---|---|---|---|
| Two-step<1.3 | 0 (0/6) | 0 (0/7) | 0 (0/13) |
| Stand-up difficulty with one-leg standing from 40 cm (either leg) | 0 (0/6) | 28.5 (2/7) | 15.4 (2/13) |
| Locomo 25>7 | 16.6 (1/6) | 0 (0/7) | 7.7 (1/13) |

two-step test score <1.3, difficulty with one-leg standing from 40 cm in the stand-up test (either leg), and Locomo 25≥7.

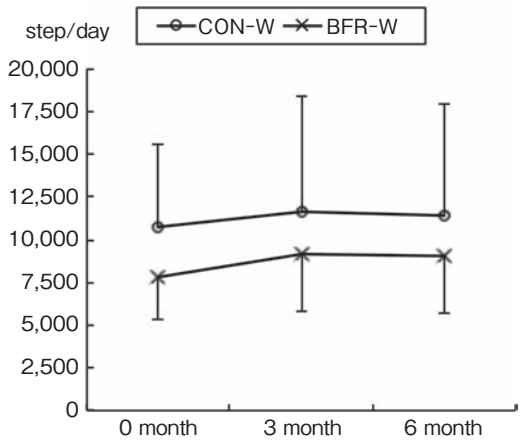


**Figure-1** Changes in step counts per day in CON-W (○) and BFR-W (×) measured at 0, 3, and 6 months
Data are presented as means ± SD.

### 3. Effect of the training program on locomotive syndrome risk test and maximum isometric strength

As shown in Figure-2, there was no significant main effect of group, time, and interactions in the

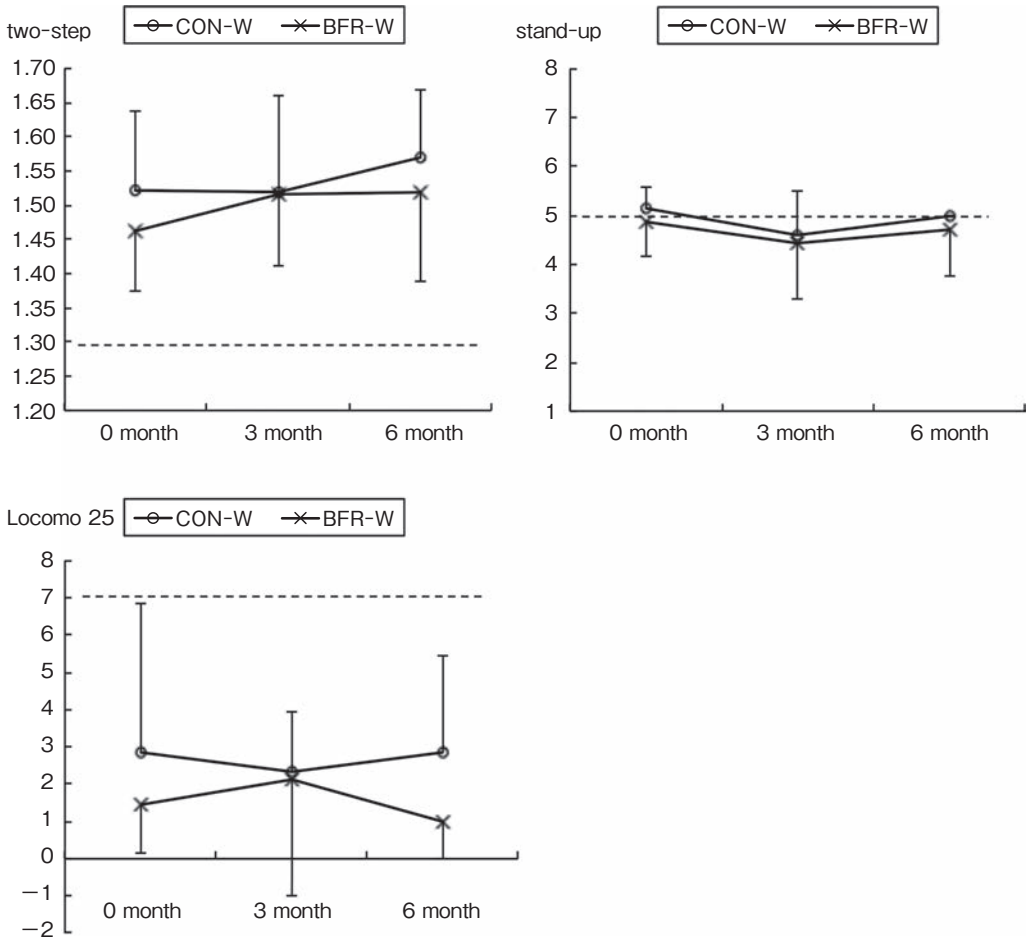


**Figure-2** Changes in locomotive syndrome risk test parameters in CON-W (○) and BFR-W (×) measured at 0, 3, and 6 months

Data are presented as means ± SD.

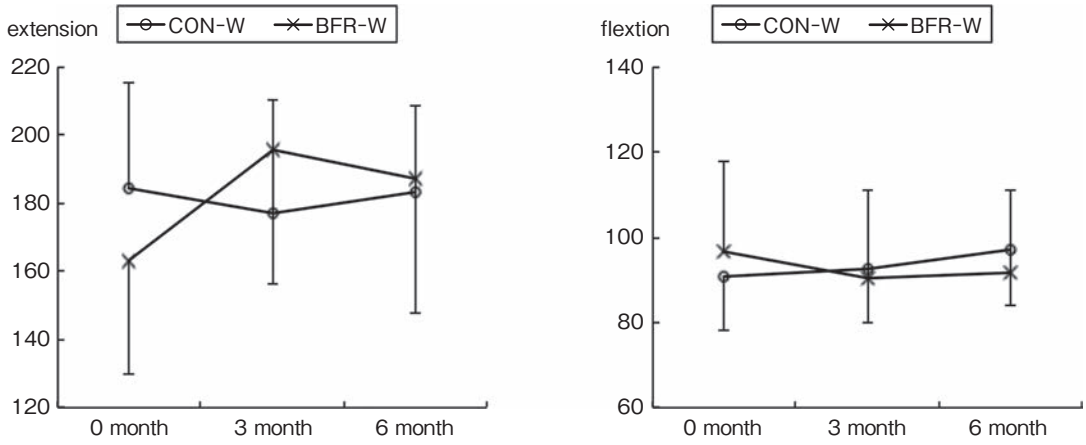


**Figure-3** Changes in maximal isometric strength of the knee flexion and extension in CON-W (○) and BFR-W (×) measured at 0, 3, and 6 months

Data are presented as means ± SD.

locomotive syndrome risk test. Although there was no interaction between group and time, two-step test significantly improved after the 6-month intervention, as shown by paired t-test (1.49 ± 0.10, 1.54 ± 0.11, p = 0.02) when a total of 13 of BFR-W and CON-W was combined. We did not find main effect of group, time, and interactions in maximum isometric strength of the knee flexion and extension (Figure-3).

## Discussion

In this study, we investigated the effect of training program combining increased physical activity and walking with BFR for 6 months on locomotive syndrome in elderly adults. Our results showed that walking and stair-climbing training program did not improve the locomotive syndrome risk test, with no added benefits of BFR. Our hypothesis was that long-term training program combining increased physical activity improves locomotive syndrome risk test parameters. The suggested reasons for no improvement in the locomotive syndrome risk test parameters after by training are as follows.

First, we considered daily physical activity at baseline and during training program. Subjects were instructed to perform walking training for more than 4 days at a faster pace than usual and to climb stairs for more than 4 days. However, there was no significant effect of time on the step count per day and physical activity at moderate to vigorous intensity (Figure-3). Physical activity was assessed averaging over the last 5 days of the measurement period and not every day, but we speculated that the step count per day and physical activity at moderate to vigorous intensity did not increase during training program because of a compensatory decline in these activities during the remainder of the day. In addition, from the data of the National Health and Nutrition Survey in Japan 2013, the average step count of subjects over 60 years was 6,500-6,800 steps per day. In the present study, the average step count in both groups was 7,800-11,000 steps per day, which is higher than the normal range for subjects of the same age (Table-1). Therefore, our training program could not increase their daily physical activity, because of compensatory decline in physical activity during remainder of the day and because the subjects already had physically active life styles.

Second, there were only three subjects who fulfilled the reference value as stage 1 (diagnosed as starting to decline in mobility) in locomotive syndrome risk test parameters at baseline (Table-2). Yoshimura *et al.*[17] indicated that the prevalence of the indices in stage 1 and 2 increased according to age. They estimated that the prevalence of subjects aged > 60 years in stage 1 in Two-step test, Stand-up test, and Locomo 25 was 49.3, 30.7, and 12.0%, respectively. Thus, our subjects originally had an active, not sedentary, lifestyle with a low risk of locomotive syndrome from the point of view of the number of step counts and locomotive syndrome risk test. We speculate that increased physical activity from walking and stair-climbing training program did not improve the results of locomotive syndrome risk test because our subjects were already at a low risk of locomotive syndrome.

In the present study, maximum isometric strength for both knee extension and flexion did not improve compared to the baseline value for either group (CON-W and BFR-W). Some studies[11) 13)] reported that maximum isometric knee extension increased by 8-12% of BFR-W in older subjects. Ozaki *et al.*[13] performed BFR walking with high frequency (4 days per week, 10 weeks, total 40 sessions) in women with sedentary lifestyle. Although the difference in frequency of BFR-W and sex might be responsible for the variability in the training effect, BFR-W once a week for a total of 20 sessions combined with increased physical activity, might be insufficient to elicit additional effect on muscle strength in physically active subjects. But, one subject who fulfilled reference value as stage 1 in Locomo 25 showed improvement in both Locomo 25 and maximum isometric strength from baseline (extension: 133.3 Nm to 145.9 Nm, flexion: 74.3 Nm to 86.8 Nm). Therefore, increase of maximum isometric strength was suggested to be involved in the improvement of locomotive syndrome risk test parameters.

In conclusion, increased physical activity from walking and stair-climbing training program did not improve the results of locomotive syndrome risk test and maximal isometric strength in physically active elderly subjects.

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## Conflict of interests

None of the authors has any conflicts of interest to disclose.

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# Relationship Between Physical Activity and Locomotive Syndrome After a 3-Month Exercise Intervention of Walking and Stair Climbing in Elderly Japanese Individuals

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***Objective***: This study aimed to investigate the relationship between physical activity (PA) and locomotive syndrome after a 3-month exercise intervention of walking and stair climbing in elderly Japanese individuals.
***Design***: Eighteen elderly participants (mean age 68 ± 5 years) were equally assigned to a normal walking and stair climbing (WS) group or a WS and walking with blood flow restriction (WS+BFR) once a week group. Both the groups were instructed to walk at a faster pace than usual for more than 30 min per day and climb more than 5 flights of stairs per day, for more than 4 days per week in the WS group and for more than 3 days per week in the WS+ BFR group for 3 months.
***Methods***: PA was measured using a wristband type acceleration sensor. Locomotive syndrome risk tests (the two-step test, the stand-up test, and 25-question risk assessment) were performed before and after the intervention.
***Results***: No significant difference was found between the groups considering the PA. Step counts over 3 metabolic equivalents (METs) per day increased by exercise intervention ($p=0.014$). Increase in vigorous PA (≥6 METs) was positively correlated with the stand-up test score ($r=0.490$, $p<0.05$), but not the two-step test score and 25-question risk assessment.
***Conclusions***: These results suggest that increase in vigorous PA via walking and stair climbing might lead to improving locomotive syndrome risk in elderly Japanese individuals, regardless of walking with blood flow restriction once a week.

***Key words***: vigorous physical activity, locomotive syndrome risk test, lower limb muscle strength

## Introduction

In Japan, along with the increasing lifespan, the number of elderly individuals who need care has increased as the population ages. Age-related loss of skeletal muscle mass was defined as sarcopenia[1], while the Japanese Orthopedic Association proposed the term "locomotive syndrome" in 2007[2]. Locomotive syndrome represents a high-risk condition in the locomotor apparatus such as the skeletal muscles, bones, joints, cartilage, or intervertebral discs. Recently, tests for the assessment of locomotive syndrome risk were designed by the Japanese Orthopaedic Association (2013)[3]. The tests consist of three subtests (the two-step test, the stand-up test, and the 25-question risk assessment), and the two-step test and the stand-up test, which is able to easily assess the ability of the lower limb muscle, are expected to be useful for screening the need for nursing care. However, most previous studies that focused on locomotive syndrome were performed using the cross-sectional design[4)–9)],

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while only few studies have been performed using the longitudinal design such as an exercise intervention for locomotive syndrome[10) 11)].

Motion in daily activities is restricted by muscle reduction, particularly in the lower limb muscles. Therefore, it is important for individuals to be extremely active in the lower limbs in order to prevent progression of locomotive syndrome and to maintain the lower limb muscle volume and strength. Abe *et al.*[12)] reported that daily physical activity (PA) at moderate and vigorous intensity was associated with a higher muscle thickness in the lower leg and the anterior thigh in women. Moreover, Kubo *et al.*[13)] reported that muscle thickness and strength of the lower limb muscle in elderly individuals increased by moderate walking training. In addition, the muscle activity level is higher in stair climbing than in walking[14)]. Thus, increased PA via walking and stair climbing at moderate or vigorous intensity may prevent or improve the risk of locomotive syndrome in elderly individuals. Although a previous study summarized the evidence for PA via walking or stair climbing as an exercise intervention for metabolic syndrome[15)], the relationship between the changes in PA resulting from exercise intervention of walking and stair climbing and the changes in the test scores for the assessment of locomotive syndrome risk is unknown. Moreover, walking with blood flow restriction in the lower limb muscle increases muscle size and strength[16)]. This method enhances the lower limb muscle function and may lead to an additional beneficial effect for treating locomotive syndrome.

We hypothesized that increased moderate and vigorous PA resulting from walking and stair climbing or walking with blood flow restriction improves locomotive syndrome test scores. The purpose of this study was to investigate the relationship between the changes in PA and the changes in locomotive syndrome risk test scores in elderly Japanese individuals after a 3-month walking and stair climbing intervention program.

## Materials and methods

### 1. Participants

Eighteen elderly participants (age, 68 ± 5 years; height, 1.64 ± 0.08 m; weight, 63.6 ± 2.4 kg) volunteered to participate in this exercise intervention study. They were recruited through printed advertisements. None of the participants had undergone any regular aerobic or resistance training for the previous 1 year. All participants provided informed consent before participating in this study. The participants were randomly assigned into the two following groups: normal walking and stair climbing (WS group; n = 9, 8 men and 1 woman) and a WS and walking with blood flow restriction once a week (WS + BFR group; n = 9, 6 men and 3 women). This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee for Human Experiments of Juntendo University, Japan.

### 2. Exercise intervention program

Both the groups wore an acceleration sensor on the wrist and were instructed to walk at a faster pace than usual for more than 30 min per day, and climb more than 5 flights of stairs per day, for more than 4 days per week in the WS group and for more than 3 days per week in the WS + BFR group. Moreover, the WS + BFR group performed 20 minutes of treadmill walking with blood flow restriction at a pre-determined exercise intensity of 70–85% of the age-predicted maximum heart rate (220 − age). In the WS + BFR group, nylon cuffs (105-mm wide, MT-870 Digital Tourniquet; Mizuho, Tokyo, Japan) were applied tightly at the most proximal portion of both the legs during walking with blood flow restriction. The target pressure was calculated for each participant based on the circumference of the right thigh (33% of the distance from the inguinal crease to the top of the patella) as follows: < 50 cm = 100 mmHg, and 50–55 cm = 120 mmHg. This is because arterial occlusion pressure is largely influenced by the thigh circumference[17)]. The cuff air pressure was released immediately after completing each session.

The acceleration sensor measured PA during the 3-month exercise intervention. Subtests of the locomotive syndrome risk test (the two-step test, the stand-up test, and the 25-question risk assessment) were performed before and after the 3-month exercise intervention.

### 3. Acceleration sensor

A 3-axis wristband-type acceleration sensor

(wristband type life recorder UW-301, A&D Company Limited, Japan) was worn by each participant to evaluate PA for 7 consecutive days. The data criteria were defined as having more than 5 days of wear time in 7 days[18) 19)] and 10 hours of wear time in 24 hours[20) 21)]. The average daily step count and step counts over 3 metabolic equivalents (METs) per day were measured, and PA levels were calculated from the obtained data at each time. PA levels were classified as Ex1 (< 1.1 METs), Ex2 (≥1.1 to <1.5 METs), Ex3 (≥1.5 to <3.0 METs), Ex4 (≥3.0 to <6.0 METs; moderate PA), and Ex5 (≥6 METs; vigorous PA).

**4. Locomotive syndrome risk tests**

Locomotive syndrome risk tests composed of the following three subtests: the two-step test, the stand-up test, and the 25-question risk assessment.

**5. The two-step test**

This test measures the stride length to assess walking ability, including muscular strength, balance, and flexibility of the lower limbs. First, the starting line was decided, and participants were asked to stand behind the line, with the toes of both feet behind it. Second, participants were instructed to take two steps with the maximum possible stride and then align both feet. After the steps, the length of double strides was measured. The formula for calculating the test score was the maximal length of the double stride (cm)/height (cm).

**6. The stand-up test**

This test measures the height of standing to assess leg strength. After preparation of 4 seats of 40, 30, 20, and 10 cm, the participants folded their arms and leg at an angle of 70 degrees to the floor. First, if the participant could stand up from a height of 40 cm on one leg for both the right and left leg, without leaning back to gain momentum, and maintain this position for three seconds, then, the participant passed the height. Second, the participant tried the same thing from lower heights at 10-cm decrements. If the participant was unable to stand up on one leg from the height of 40 cm, the participant was considered to have failed the height and challenge to stand up on both legs from the height of 10 cm.

**7. The 25-question risk assessment**

This test consists of 25 items that include questions regarding body pain and usual daily life for the last one month. These 25 items are graded with a 5-point scale, and then arithmetically added to obtain a total score (0-100 points) A higher score is associated with worse locomotive function.

**8. Statistical analysis**

All statistical analyses were performed using SPSS Version 17.0 (SPSS Inc., Chicago, IL). Data are presented as mean ± standard error. A two-way (group × time) analysis of variance (ANOVA) with repeated measures was used to analyze the PA. The relationship between the changes in PA and the changes in locomotive syndrome risk test scores after a 3-month walking and stair climbing intervention program was analyzed using Pearson's correlation coefficient. A value of $p < 0.05$ was considered significant in all analyses.

## Results

**1. Physical activity**

After the 3-month exercise intervention, the average daily step count (WS: 7,407 ± 1,276 to 9,031 ± 1,587, WS+BFR: 9,412 ± 1,597 to 10,550 ± 1,907; $p = 0.024$, Figure-1) and step count over 3 METs (WS: 3,998 ± 1,240 to 5,700 ± 1,432, WS+BFR: 5,706 ± 1,543 to 6,650 ± 1,759; $p = 0.014$, Figure-2) were significantly increased when compared to baseline in both the WS and WS+BFR groups. There was no significant difference between the groups and no significant group × time interaction considering the average daily step count and the step count over 3 METs per day. Changes in PA levels are shown in Table-1. In comparison with the baseline, Ex1 increased significantly ($p < 0.05$) and Ex3 decreased significantly ($p < 0.01$); no significant changes found in Ex2, Ex4, and Ex5 for both the groups after the 3-month exercise intervention. Moreover, the change in Ex3 was negatively correlated with the change in Ex5 ($r = -0.546$, $p < 0.05$).

**2. Relationship between physical activity and locomotive syndrome risk test scores**

The change in Ex3 after exercise intervention

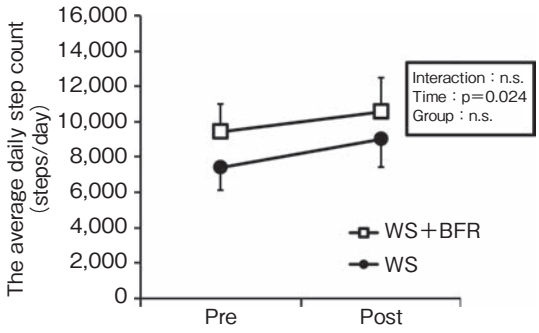


**Figure-1** Changes in the average daily step count before and after 3 month of the walking and stair climbing exercise intervention program (normal walking group: n = 9; walking with blood flow restriction of once a week group: n = 9)
Data are presented as the mean ± standard error.

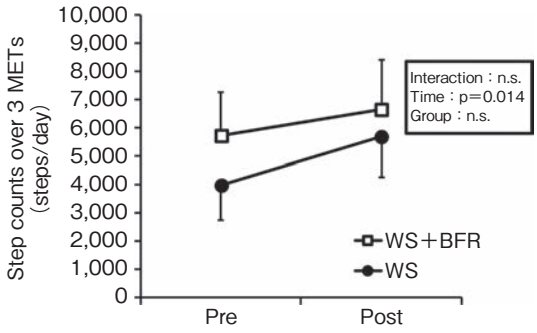


**Figure-2** Changes in the step counts over 3 metabolic equivalents (METs) per day before and after 3 months of the walking and stair climbing exercise intervention program (normal walking group: n = 9; walking with blood flow restriction of once a week group: n = 9)
Data are presented as the mean ± standard error.

**Table-1** Changes in physical activity levels before and after the 3-month exercise intervention of walking and stair climbing

| Physical activity levels | WS (n = 9) | | WS+BFR (n = 9) | | Two-way ANOVA (p-value) | | |
|---|---|---|---|---|---|---|---|
| (min/day) | Pre | Post | Pre | Post | Time | Group | Interaction |
| Ex1 | 584 ± 33.7 | 639 ± 26.3 | 642 ± 43.1 | 673 ± 44.2 | <0.05 | 0.375 | 0.480 |
| Ex2 | 451 ± 37.5 | 443 ± 32.5 | 377 ± 15.7 | 379 ± 21.5 | 0.846 | 0.083 | 0.741 |
| Ex3 | 360 ± 28.8 | 303 ± 28.1 | 352 ± 42.6 | 319 ± 38.4 | <0.01 | 0.938 | 0.233 |
| Ex4 | 37 ± 4.4 | 41 ± 7.5 | 60 ± 12.4 | 59 ± 13.5 | 0.827 | 0.147 | 0.686 |
| EX5 | 7 ± 5.6 | 13 ± 6.1 | 9 ± 5.7 | 11 ± 3.9 | 0.203 | 0.981 | 0.492 |

Two-way analysis of variance (ANOVA) with repeated-measures was used to determine the physical activity levels.
Values are presented as mean ± standard error.
WS, the walking and stair-climbing group (n = 9); WS+BFR, WS and walking with blood flow restriction once a week group (n = 9).
Physical activity levels were classified as Ex1 (< 1.1 metabolic equivalents [METs]), Ex2 (≥1.1 to < 1.5 METs), Ex3 (≥1.5 to < 3.0 METs), Ex4 (≥3.0 to <6.0 METs), and Ex5 (≥6 METs).

was negatively correlated with the changes in the stand-up test score ($r = -0.612$, $p < 0.01$; Figure-3). Meanwhile, the change in Ex5 with exercise intervention was positively correlated with the changes in the stand-up test score ($r = 0.490$, $p < 0.05$), but not the two-step test score and 25-question risk assessment (Figure-4). However, no other PA was significantly correlated with locomotive syndrome risk test scores.

## Discussion

The main findings of this study were that the step count over 3 METs as well as the average daily step count increased through walking and stair climbing intervention and that the increased Ex5 was correlated with the stand-up test score for the assessment of the locomotive syndrome risk.

According to the Compendium of Physical Activities, Ex3 and Ex5 in this study correspond to standing to walking at a slow pace and brisk walking or climbing, respectively[22]. The results of decreases in Ex3 and increases in Ex5 suggest that PA levels increased through exercise intervention. The rectus femoris muscle is a two-joint muscle, crossing both the hip and knee joint, and Ericson *et al.*[23] indicated that the rectus femoris muscle contributed to accelerating the lower limb forward during the start of the swing phase. Abe *et al.*[12] investigated the relationship between PA and muscle thickness in elderly women in a cross-sectional study and reported that a higher muscle mass of the anterior thigh and lower leg was associated with more moderate and vigorous PA. Moreover, Kubo *et al.*[13] reported that muscle thickness and strength of the lower limb muscle increased

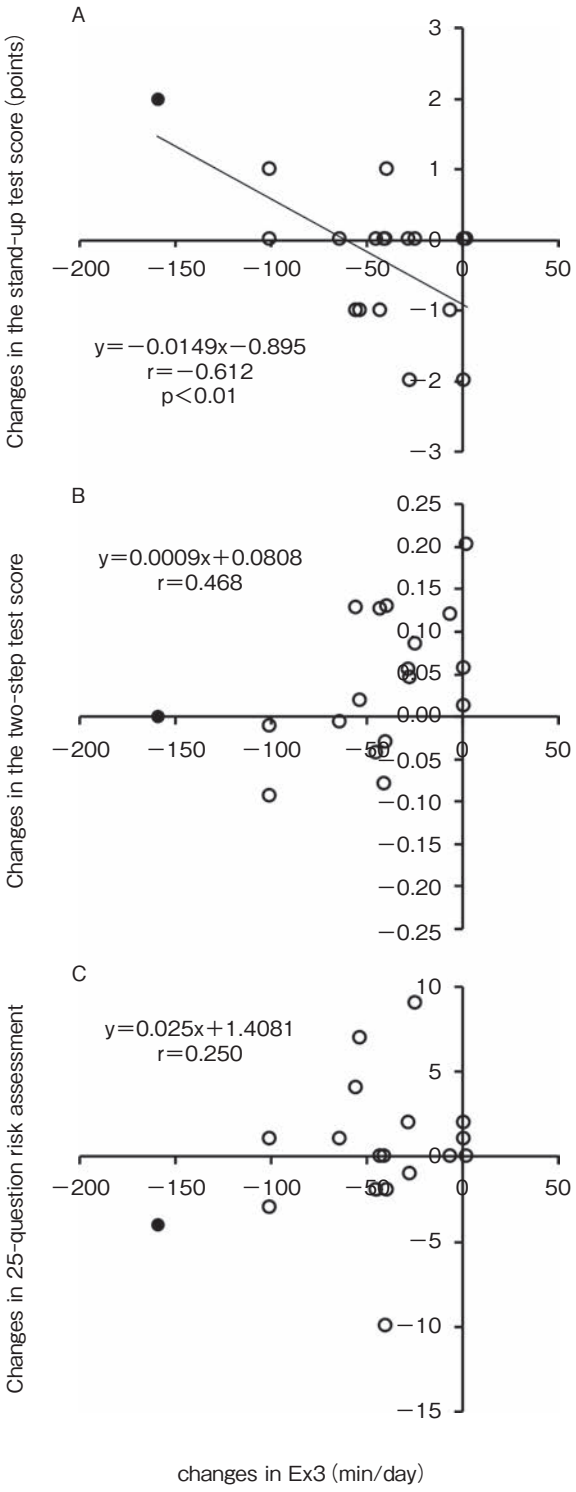


**Figure-3** Relationship between the changes in Ex3 and changes in the score of (**A**) the stand-up test, (**B**) the two-step test and (**C**) 25-question risk assessment for the assessment of locomotive syndrome risk (n=18)

Closed circles represent the participant who was observed the largest changes in vigorous activity.

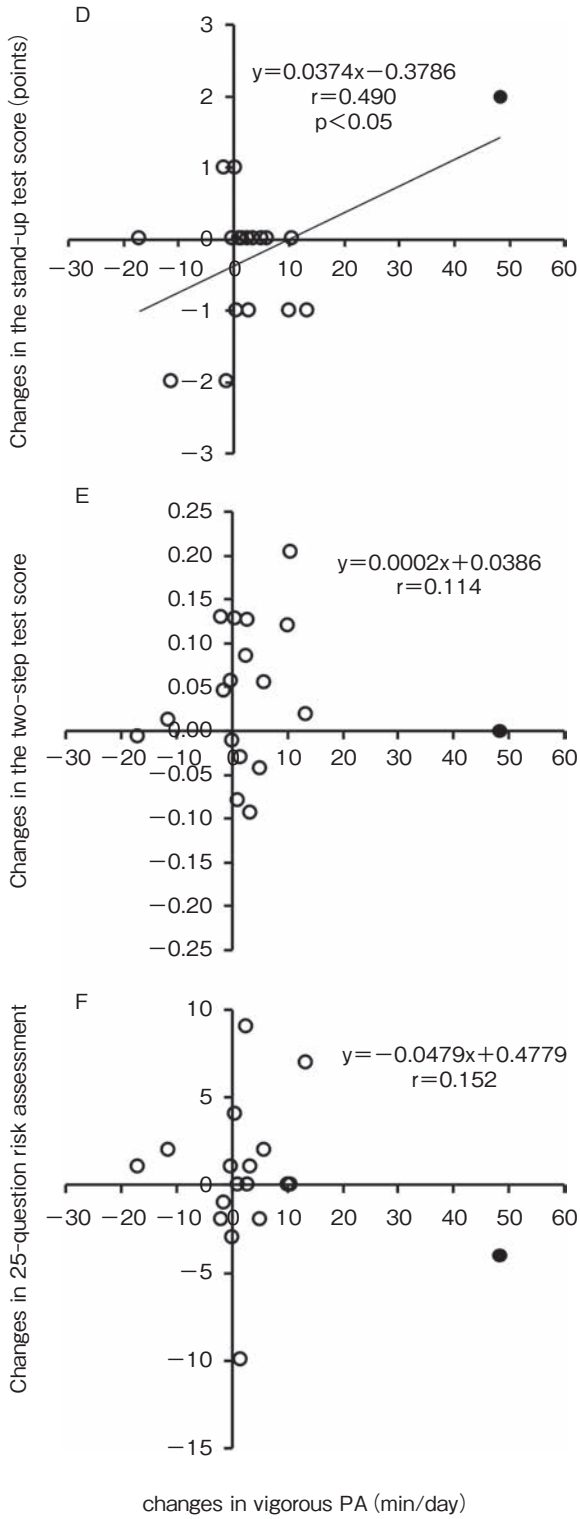


**Figure-4** Relationship between the changes in vigorous PA and changes in the score of (**D**) the stand-up test, (**E**) the two-step test and (**F**) 25-question risk assessment for the assessment of locomotive syndrome risk (n=18)

Closed circles represent the participant who was observed the largest changes in vigorous physical activity.

significantly after 6 months of walking training in elderly individuals. These previous studies indicated that increase in PA at moderate to vigorous intensity may lead to improved locomotive syndrome risk, according to enhancing lower limb muscle function. In fact, a longer Ex5 was correlated with stronger muscle strength of knee extension at 60 degrees per second ($r = 0.780$, $p < 0.01$; unpublished data). Thus, a significant correlation might be found between improvement in the stand-up test score for assessment of the leg strength and the increase in Ex5.

Meanwhile, no significant correlation was observed between the increase in PA and the changes in the two-step test score and the 25-question risk assessment. The relatively high baseline scores on the two-step test score and the 25 question risk assessment may have limited the amount of change possible with the 3-month walking and stair climbing program.

Certain limitations of this study should be mentioned. First, the sample size in this study was small ($n = 18$). Second, owing to the lack of a control group considering the study design, it is unclear if the changes in PA were because of the exercise intervention. Moreover, as we did not sub-classify participants considering only walking and walking and stair climbing, it remains unclear whether walking or stair climbing has a more beneficial effect. Therefore, additional research is need.

In summary, increases in the stand-up test score for the assessment of the locomotive syndrome risk was positively correlated with increases in PA at vigorous intensity after the 3-month exercise intervention of walking and stair climbing in elderly Japanese individuals, regardless of walking with blood flow restriction once a week. These results suggest that walking and stair climbing at a vigorous intensity might lead to improving locomotive syndrome risk.

## Acknowledgement

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## Conflict of interest

No conflicts of interest, financial or otherwise, exist.

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# Locomotive Syndrome Relation to Daily Physical Activity, Physical Function, and Body Composition in Elderly People: A Pilot Study

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***Objective***: To evaluate relationships among locomotive syndrome (LS), daily physical activity (PA), physical function, and body composition measurements in elderly Japanese individuals.

***Materials and Methods***: This study included 39 elderly participants (mean age, 68.4 ± 5.5 years). All participants completed three LS risk assessments, the two-step test, stand-up test, and 25-question geriatric locomotive function scale (GLFS). To assess LS, a stage 1 LS (LS-1) cutoff was applied. Physical function was assessed using the chair stand, sit-up, 10-meter (m) walking time, and 10-m zigzag walking time (ZWT) tests. Additionally, the isometric strength of the knee extensors and flexors were evaluated and body composition measurements were performed. Habitual daily PA (step count and intensity of PA) was evaluated using a three-axis, high-frequency wrist accelerometer.

***Results***: The proportion of LS-1 in this study population was 35.9% (14/39). The number of daily steps, 10-m walking time, 10-m ZWT, knee extension strength, and knee flexion strength were significantly higher among non-LS participants than among LS-1 participants. The 10-m walking time, 10-m ZWT, knee extension strength, and knee flexion strength were significantly higher and the chair stand score tended to be higher among non-LS participants than among LS-1 participants. For all participants, the stand-up test scores correlated with the 10-m walking times, 10-m ZWTs, chair stand scores, sit-up scores, and knee extension strength.

***Conclusions***: LS might be associated with daily PA, physical function, and isometric knee extension strength. Furthermore, the stand-up test is a good index for LS screening in elderly individuals.

***Key words***: locomotive syndrome, daily step count, lower leg muscle strength, walking ability, body composition

## Introduction

Locomotive syndrome (LS) causes difficulties in the ability to stand, walk, run, climb stairs, and perform other physical functions essential to daily life[1) 2)]. Recently, LS evaluation methods have been established[2) 3)]. In 2013, the Japanese Orthopedic Association (JOA) proposed the two-step test, stand-up test, and 25-question geriatric locomotive function scale (GLFS) for assessing the risk of LS[2) 3)]. The two-step test is a screening tool for evaluating horizontal mobility, i.e., walking ability[4)], whereas the stand-up test reflects vertical mobility and assesses leg strength as the participant stands up, using one or both legs, from a specified height. The GLFS assesses a participant's physical condition and lifestyle over the prior month. This self-reported questionnaire has domains covering pain, physical function, basic activities of daily living (ADL), instrumental ADL, and anxiety[5)]. However, whether LS risk tests are associated with locomotive components, such as leg strength and physical

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function, is unknown. The etiology of LS is complex, multifactorial, and poorly evidenced.

LS risk factors may include the decreased levels of daily physical activity (PA) and ADL that occur with aging[6]. To minimize LS, the intensity and duration of daily PA may be important influencing factors[7) 8)]. However, whether there is a relationship between the degree of locomotive component weakness and habitual daily PA is unknown. Similarly, the associated individual physical function and body composition measurements are also unclear. We hypothesized that LS risk is lower in active than in inactive elderly individuals. The purpose of the present study was to evaluate the relationship between LS and daily PA in elderly Japanese individuals. We also evaluated the relationships among LS risk parameters, physical function, and body composition measurements.

## Methods

### 1. Subjects

The study included 39 healthy, elderly Japanese volunteers (25 men, 14 women). Among the 39 participants, 20 had a history of walking 2–7 days/week and two had a history of performing resistance training 2–4 days/week. The mean age of the participants was 68.4 ± 5.5 years (men, 70.0 ± 5.4 years; women, 65.4 ± 4.4 years). All participants were informed of the study methods, procedures, and risks, and each signed an informed consent document before participating in the study. This study was performed according to the Declaration of Helsinki and was approved by the Ethics Committee for Human Experiments of Juntendo University.

### 2. LS risk assessments

a) Two-step test

The participants stood with the toes of both feet behind a starting line. They then took two long steps (as long as possible) and aligned both feet. The length of the two steps from the starting line to the tips of the toes was measured. The two-step test score was calculated using the following formula: length of the two steps (cm) / height (cm).

b) Stand-up test

The participants stood up on one or both legs from a specified height. Seats were placed at the following heights: 40, 30, 20, and 10 cm. The participants stood up from each seat (in descending height order) using both legs and then using one leg. If a participant could stand up without leaning back to gain momentum and maintain the posture for 3 s, he/she was considered to have passed the test at the specified height.

c) The 25-question GLFS

The GLFS is a self-administered, comprehensive questionnaire consisting of 25 items, including questions regarding pain (4), ADL (16), social function (3), and mental health status (2) during the last month. These 25 items are graded using a five-point scale from 0 points (no impairment) to 4 points (severe impairment). The scores are added to produce a total score (minimum = 0, maximum = 100). A higher score is associated with worse locomotive function.

The two-step test, stand-up test, and GLFS were used to assess declines in mobility for each participant The JOA has proposed clinical decision limits for these tools in the assessment of LS risk[2) 3)]. According to the JOA, two stages can be identified depending on the results of the three tools. Stage 1 (LS-1): (1) two-step test score < 1.3, (2) difficulty in standing from a seat at a height of 40 cm using one leg in the stand-up test (either leg), and (3) GLFS score ≥ 7. When a participant meets any of these criteria, the start of mobility decline is diagnosed. Stage 2: (1) two-step test score < 1.1, (2) difficulty in standing from a seat at a height of 20 cm using both legs in the stand-up test, and (3) GLFS score ≥ 16. When a participant meets any of these criteria, progressive mobility decline is diagnosed. In this study, stage 1 was considered the cutoff for assessing LS[2)].

### 3. Anthropometric and body composition measurements

Anthropometric measurements included height (cm), weight (kg), and body mass index (BMI, weight [kg] / height [$m^2$]). Body composition measurements included body fat, muscle mass, waist circumference (WC), and waist-to-hip ratio; these were assessed using a body composition analyzer (Inbody 730, InBody, Seoul, Korea). Muscle mass was corrected for height ($m^2$).

**4. Physical function performances**

Physical function was measured using the chair stand, sit-up, 10-meter (m) walking time, and 10-m zigzag walking time tests. In the chair stand test, the number of times the participants could rise completely from a seated position without using their arms in 30 seconds was determined[9]. In the sit-up test, the participants performed as many bent-knee sit-ups as possible within 30-sec (http://www.mext.go.jp/component/a_menu/sports/detail/__icsFiles/afieldfile/2010/07/30/1295079_04.pdf). The 10-m walking test was performed twice. In this test, the participants walked as fast as possible, without running, in a corridor over a distance of 12 m. A hand-held stopwatch was used to time this test. The stopwatch was started at the 1 m distance and was stopped at 11 m; therefore, a steady-state measurement over 10 m was obtained. Similarly, the 10-m zigzag walking time (ZWT) test was performed twice. The test used a 10-m walkway, according to a previous study[10]. The fastest time of the two trials in both tests was used for analysis.

**5. Maximal isometric strength**

The maximum voluntary isometric strength of the knee extensor and flexor was determined using a dynamometer (Biodex System 4; Biodex Medical Systems, Shirley, NY). During testing, each participant was seated on a chair with the hip joint angle at 85° of flexion (0° = full hip extension). The knee joint's center-of-rotation was visually aligned with the axis of the dynamometer lever arm, and the ankle was firmly strapped to the distal pad of the lever arm. A knee joint angle of 0° corresponded to full knee extension. Several warm-up contractions (4-5 submaximal contractions and 1-2 near-maximal contractions at 180°/s) were performed before testing. Participants were instructed to perform maximal isometric knee extension for about 5 s, at a fixed knee joint angle of 75°. Next, they performed maximal isometric knee flexion for about 5 s, at a fixed knee joint angle of 30°. Two maximal efforts for each isometric measurement were performed, and each peak torque was used for analysis.

**6. Habitual daily PA**

We measured daily PA, throughout November and December 2014, using a three-axis accelerometer (UW-301, A&D, Toshima, Tokyo). The accelerometer was worn continuously, like a wristwatch, for 7-14 days. The participants were requested to wear these devices continuously, except during activities such as dressing and bathing. We considered accelerometer recordings that were taken for a minimum of 5 continuous days, excluding the distribution and collection days; days that the accelerometer was not worn for more than 2 hours were excluded[11]. Activities were classified into 5 levels of intensity, according to the accelerometer data: 1, resting (< 1.1 METs); 2, sitting (1.1-1.4 METs); 3, low PA (LPA, 1.5-2.9 METs); 4, moderate PA (MPA, 3.0-5.9 METs); and 5, vigorous PA (VPA, ≥6 METs). The sum of the time spent in moderate and vigorous PA (MVPA, ≥3 METs) was calculated.

**7. Statistical analysis**

Data are presented as means ± standard deviations. Differences between the non-LS and LS-1 groups were determined using the unpaired Student's t-test. Relationships among the three LS risk parameters, daily PA, and anthropometric and body composition measurements were examined using Pearson's product-moment correlation analysis. All analyses were performed using SPSS ver. 17.0 (IBM, Armonk, NY). A p-value < 0.05 was considered to indicate statistical significance.

## Results

The proportions of two-step test scores < 1.3, difficulty in standing from a seat at a height of 40 cm using one leg in the stand-up test, and GLFS scores ≥7 were 15.4%, 30.8%, and 7.7%, respectively. Overall, the prevalence of LS-1 was 35.9% (14/39; men, 7; women, 7).

The characteristics of the non-LS and LS-1 participants are presented in Table-1. The 10-m walking time, 10-m ZWT, knee extension strength, and knee flexion strength results were significantly higher and the chair stand scores tended to be higher among non-LS participants than among LS-1 participants ($p = 0.042$, $p = 0.003$, $p = 0.013$, $p = 0.041$, and $p = 0.051$, respectively). The mean numbers of steps/day among non-LS and LS-1 participants were 8,782 ± 4,423 and 6,449 ± 1,893, respectively. The mean times spent performing

**Table-1** Anthropometrics, body compositions, LS risk tests, habitual daily PA, and physical function performances for study participants

| Variables | All (n=39) (men=25; women=14) | non-LS (n=25) (men=18; women=7) | LS-1 (n=14) (men=7; women=7) |
|---|---|---|---|
| Age (years) | 68.4 ± 5.5 | 68.2 ± 5.6 | 68.8 ± 5.4 |
| Height (cm) | 161.7 ± 7.9 | 161.6 ± 6.9 | 161.9 ± 9.7 |
| Weight (kg) | 62.5 ± 9.2 | 61.3 ± 8.2 | 64.5 ± 10.8 |
| BMI ($kg/m^2$) | 23.9 ± 3.2 | 23.4 ± 2.3 | 24.7 ± 4.4 |
| Body fat mass (kg) | 16.8 ± 6.3 | 15.4 ± 4.2 | 19.2 ± 8.5 |
| Body fat (%) | 26.6 ± 7.5 | 25.1 ± 5.6 | 29.2 ± 9.6 |
| Muscle mass ($kg/m^2$) | 9.5 ± 1.0 | 9.6 ± 1.0 | 9.3 ± 0.9 |
| WC (cm) | 87.2 ± 9.4 | 85.3 ± 7.3 | 90.5 ± 11.9 |
| W-H rate | 0.86 ± 0.05 | 0.85 ± 0.05 | 0.87 ± 0.06 |
| Two-step test | 1.44 ± 0.11 | 1.48 ± 0.09** | 1.35 ± 0.10 |
| Stand-up test | 4.79 ± 0.92 | 5.32 ± 0.56** | 3.86 ± 0.66 |
| 25-question GLFS | 2.79 ± 2.82 | 2.04 ± 1.84 | 4.14 ± 3.74 |
| Daily Step (step/day) | 7945 ± 3856 | 8782 ± 4423* | 6449 ± 1893 |
| MVPA (min/day) | 50.1 ± 33.3 | 54.2 ± 36.5 | 42.7 ± 26.1 |
| VPA (min/day) | 6.2 ± 13.2 | 8.4 ± 16.0 | 2.3 ± 4.0 |
| 10-m walking time (sec) | 5.44 ± 0.75 | 5.26 ± 0.63* | 5.77 ± 0.86 |
| 10-m ZWT (sec) | 7.15 ± 1.07 | 6.79 ± 0.82** | 7.81 ± 1.19 |
| Chair stand (n) | 23.4 ± 5.3 | 24.7 ± 5.5 | 21.2 ± 4.3 |
| Sit up (n) | 14.3 ± 6.2 | 15.3 ± 6.6 | 12.4 ± 5.1 |
| Knee extension 0°/sec (Nm) | 150.3 ± 46.0 | 162.5 ± 51.3* | 130.0 ± 25.2 |
| Knee extension 0°/sec (Nm/kg) | 2.40 ± 0.64 | 2.63 ± 0.68** | 2.03 ± 0.32 |
| Knee flexion 0°/sec (Nm) | 84.2 ± 25.1 | 90.5 ± 26.2* | 73.4 ± 19.4 |
| Knee flexion 0°/sec (Nm/kg) | 1.34 ± 0.33 | 1.46 ± 0.32** | 1.13 ± 0.24 |

Values are shown as the means ± SD. **$p<0.01$, *$p<0.05$, vs. Females or LS-1.
Note: For non-LS and LS-1, probability value of VPA and chair stand test was 0.078 and 0.051, respectively.

MVPA among non-LS and LS-1 participants were 54.2 ± 36.5 min/day and 42.7 ± 26.1 min/day, respectively. The daily number of steps was significantly higher and the mean time spent performing VPA tended to be higher among non-LS participants than among LS-1 participants (daily steps, $p=0.028$; time spent on VPA, 8.43 ± 16.0 min/day vs. 2.29 ± 4.02 min/day, respectively, $p=0.078$).

The stand-up test scores correlated with 10-m walking times ($r=-0.35$; $p=0.028$), 10-m ZWTs ($r=-0.53$; $p=0.001$), chair stand scores ($r=0.51$; $p=0.001$), sit-up scores ($r=0.42$; $p=0.009$), knee extension strength ($r=0.59$; $p=0.000$), and knee flexion strength ($r=0.46$; $p=0.004$). The two-step test scores correlated with the 10-m ZWTs ($r=-0.43$; $p=0.006$) and chair stand scores ($r=0.51$; $p=0.001$). Further, the GLFS score correlated with knee extension strength ($r=-0.38$; $p=0.018$) and knee flexion strength ($r=-0.44$; $p=0.006$). Also, the stand-up test scores were negatively correlated with BMI ($r=-0.36$; $p=0.023$), body fat ($r=-0.42$; $p=0.007$), percent body fat ($r=-0.34$; $p=0.032$), and WC ($r=-0.47$; $p=0.002$); however, it was not correlated with daily PA parameters (Table-2). The two-step test and GLFS scores were not correlated with anthropometric and body composition measurements or with PA parameters. Knee flexion strength correlated with 10-m walking times ($r=-0.33$; $p=0.045$) and 10-m ZWTs ($r=-0.41$; $p=0.012$).

## Discussion

In the present study, LS-1 was considered as the cutoff for assessing LS. The proportions of partici-

**Table-2** Correlations among LS risk tests, body compositions, habitual daily PA, and physical function performances for study participants

| Variables | Stand-up test | Two-step test | 25-question GLFS | knee extension 0°/sec (Nm) | knee extension 0°/sec (Nm/kg) | knee flexion 0°/sec (Nm) | knee flexion 0°/sec (Nm/kg) |
|---|---|---|---|---|---|---|---|
| Age (years) | −0.29 | −0.15 | −0.04 | 0.08 | 0.06 | −0.00 | −0.02 |
| Height (cm) | 0.03 | −0.15 | −0.24 | 0.56** | 0.37* | 0.61** | 0.44** |
| Weight (kg) | −0.30 | −0.22 | −0.04 | 0.47** | 0.05 | 0.60** | 0.16 |
| BMI ($kg/m^2$) | −0.36* | −0.10 | 0.14 | 0.12 | −0.21 | 0.22 | −0.14 |
| Body fat mass (kg) | −0.42** | 0.04 | 0.17 | −0.05 | −0.35* | 0.06 | −0.27 |
| Body fat (%) | −0.34* | 0.07 | −0.23 | −0.29 | −0.46** | −0.21 | −0.41 |
| Muscle mass ($kg/m^2$) | 0.02 | −0.21 | −0.16 | 0.62** | 0.36* | 0.66** | 0.39 |
| WC (cm) | −0.47** | −0.22 | −0.02 | 0.14 | −0.21 | 0.28 | −0.09 |
| W-H rate | −0.21 | −0.02 | −0.08 | 0.13 | −0.13 | 0.32* | 0.06 |
| Daily Step (steps/day) | 0.29 | −0.03 | −0.15 | 0.11 | 0.22 | 0.02 | 0.12 |
| MVPA (min/day) | 0.24 | 0.03 | −0.08 | 0.19 | 0.06 | −0.07 | 0.06 |
| VPA (min/day) | 0.17 | −0.04 | −0.14 | 0.06 | 0.16 | −0.02 | 0.05 |
| 10-m walking time | −0.35* | −0.31 | 0.21 | −0.20 | −0.12 | −0.38* | −0.33* |
| 10-m ZWT | −0.53** | −0.43** | 0.14 | −0.19 | −0.17 | −0.38* | −0.41* |
| Chair stand | 0.51** | 0.52** | 0.05 | 0.17 | 0.27 | 0.15 | 0.25 |
| Sit up | 0.42** | 0.10 | −0.10 | 0.48** | 0.53** | 0.44** | 0.48 |
| knee extension 0°/sec (Nm) | 0.39* | 0.10 | −0.37* | - | 0.90** | 0.74** | 0.64** |
| knee extension 0°/sec (Nm/kg) | 0.59** | 0.21 | −0.39* | 0.90** | - | 0.55** | 0.63** |
| knee flexion 0°/sec (Nm) | 0.24 | 0.13 | −0.38* | 0.74** | 0.55** | - | 0.88** |
| knee flexion 0°/sec (Nm/kg) | 0.46** | 0.15 | −0.44* | 0.64** | 0.63** | 0.88** | - |

**$p < 0.01$, *$p < 0.05$

pants who scored in the non-LS group in the two-step test, stand-up test, and GLFS were, respectively, 15.4%, 30.8%, and 7.7%. These values are lower than those reported previously for similarly aged people (51.5%, 32.9%, and 17.4%, respectively; 60-69 years) [2].

The present study found that PA was higher among non-LS participants than among LS-1 participants. Additionally, physical functions and isometric strength were higher among non-LS participants than among LS-1 participants, suggesting that the cutoff for defining LS-1 is appropriate for evaluating locomotive weakness in the elderly. Knee extension strength also correlated with the stand-up test score, while knee flexion strength did not correlate with the functional test scores. Further, the stand-up test score was associated with physical function, isometric knee extension strength, and obesity-related parameters, suggesting that the stand-up test score might predict LS better than the two-step test.

We found that the number of daily steps was significantly higher and the mean time spent in VPA tended to be higher among non-LS participants than among LS-1 participants. Previous studies have demonstrated that the intensity of > 3-METs activity may prevent age-related loss of skeletal muscle mass [7) 8)]. For the present participants, the daily step counts (7,944 ± 3,855 steps/day) and MVPA (50.1 ± 33.3 min/day) tended to be higher than those of similarly aged (65.7 ± 6.4 years) participants in a previous study (7,966 ± 3,180 steps/day; 22.5 ± 16.8 min/day) [7], suggesting that the present participants were more active. Thus, exercise intensity and duration may be important factors influencing LS and lower leg muscle mass, in addition to physical and muscle function. However, since we assessed each participant's total muscle mass using a body composition analyzer, further research is needed to assess lower leg muscle mass using dual-energy X-ray absorptiometry.

Abe *et al.* (2012) [10] reported that site-specific thigh muscle loss was inversely correlated with the

ZWT, but not with maximum walking performance. This supports our findings that the 10-m walking times, 10-m ZWTs, knee extension strength, and knee flexion strength were higher among non-LS participants than among LS-1 participants. We also found a significant relationship between the two-step test scores and the 10-m ZWTs. Thus, the ZWT might be appropriate for assessing sarcopenia and LS.

The stand-up test scores correlated with the 10-m walking times, 10-m ZWTs, chair stand scores, sit-up scores, and knee extension strength, suggesting that this test evaluates quadriceps strength. Moreover, this test score was correlated negatively with obesity-related parameters (BMI, body fat, percent body fat, and WC), suggesting that central obesity is associated with LS. Previously, WC was found to correlate better with the GLFS score than other obesity-related parameters (BMI or percent body fat) in elderly women[12], consistent with our findings. Thus, we suggest that the stand-up test may be useful as a morphological and functional (physical and muscle function) assessment in elderly individuals.

In conclusion, LS might be associated with daily PA. Furthermore, physical function and isometric strength might be associated with LS, suggesting that the cutoff for defining LS-1 might be appropriate for evaluating the components of locomotive weakness. Additionally, the stand-up test is a good index for screening LS in elderly individuals.

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# Effect of 6-Month Walking and Stair-Climbing Exercise Program and Walking with Blood Flow Restriction on Body Composition and Hemoglobin A1c Levels in Elderly People

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***Objective***: This study aimed to evaluate the effects of a long-term training program combining increased physical activity and walking with blood flow restriction (BFR) on the body composition and glycated hemoglobin (HbA1c) levels in elderly subjects.

***Methods***: A total of 13 subjects (age, 67 ± 1 years) were assigned to the walking and stair-climbing (WS) group (6 men) or the WS and BFR-walk (BFR) group (5 men and 2 women). Both groups were instructed to complete walking sessions of more than 30 min, at a self-selected, faster pace than usual, and to climb more than 5 flights of stairs per day, for at least four days per week. In addition, the BFR group completed a 20-min treadmill walking session on a weekly basis, for 6 months, at a pre-determined exercise intensity of 70–85% of the age-predicted maximum heart rate and with BFR of 100–120 mmHg applied to both thighs. The measured outcome variables included height, body weight (BW), waist circumference (WC), body mass index (BMI), muscle mass, fat mass, body fat (%Fat), and HbA1c levels, measured at baseline, at the 3-month midpoint of the program, and at the 6-month endpoint.

***Results***: After the 6-month intervention, the BW, WC, BMI, fat mass, %fat, and HbA1c levels were decreased compared to baseline in both the WS and BFR groups ($p < 0.01$), with the muscle mass increasing in both groups ($p < 0.05$).

***Conclusions***: A 6-month walking and stair-climbing exercise program was effective in improving body composition and HbA1c levels in elderly subjects, with no added benefits of BFR.

***Key words***: blood flow restriction, exercise intervention, HbA1c, stair climbing, walking

## Introduction

Obesity and leading an age-related sedentary lifestyle are recognized as major risk factors for the development of several chronic diseases, including type-2 diabetes, cardiovascular diseases, and cancer[1)-5)]. Meanwhile, age-related reduction in skeletal muscle mass may lead to increased risks of impaired glucose control (glycated hemoglobin; HbA1c) and insulin resistance, as well as various other diseases[6)]. Additionally, the consequent decreases in the lower limb muscles lead to reduced ambulatory ability. Therefore, strategies to improve or maintain body composition across one's lifespan are important factors for the general health of elderly people.

Many studies have reported that regular exercise and increased physical activity can improve body composition[7) 8)]. To improve physical fitness and body composition, the American College of Sports Medicine recommends performing physical activity three to five times each week, for 20 to 60 minutes at a time[9)]. As elderly individuals often have multiple diseases that affect their activities of daily life and quality of life, it is important to develop

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practical exercise programs that are effective in increasing muscle mass and/or reducing body fat and obesity-related health risks. Walking is the most popular physical activity for middle-aged and older people; it requires minimal equipment, can be done at any time of day, and can be performed at a self-selected pace. Although walking can produce a significant increase in muscle mass, but the effect is minimal[10]. Evidence indicates that changes in muscle mass require a higher training intensity[11) 12)]. A common example of daily physical activity that has higher exercise intensity than walking is stair-climbing. Stair climbing seems to contribute to improving glucose control in elderly people with type 2 diabetes[13)]. Therefore, a combination of walking and stair-climbing may be more effective for improving body composition (i. e. increasing muscle mass and reducing fat mass) and glucose control in elderly people, compared to only walking. However, the efficacy of this combination on body composition and glucose control in elderly individuals has yet to be evaluated.

Numerous peer-reviewed papers over the past decade have demonstrated that blood flow restriction (BFR) to working muscles during walking enhances the hypertrophic effects thereof[14) 15)]. Abe *et al.*[14)] showed that the mean $VO_2$ was higher in the latter half of a treadmill walking with BFR (5 × 2-min) compared to normal walking at the same speed (75 m/min). The authors speculated this increase in $VO_2$ may be related to an elevation of the serum growth hormone concentration after a single bout of BFR walking[14)]. Further, more recent evidence indicated a greater net metabolic cost ($VO_2$, $V_E$, and $V_E/VO_2$) in 5 × 3-min bouts of treadmill walking with BFR compared to normal walking[16)]. Based on these data, BFR during walking could enhance the effects of normal walking on both increasing muscle mass and decreasing fat mass, which contributes to improving or maintaining body composition and glucose control in elderly people.

With this in mind, our study aimed to evaluate the effects of a long-term training program combining increased physical activity and walking with BFR on the body composition and HbA1c levels in elderly subjects. HbA1c is the most widely used indicator of glucose control in individuals, and can be used to assess overall metabolic control over the preceding 6-8 weeks[17)].

## Materials and methods

### 1. Subjects

Twenty-three individuals, aged > 65 years, who had not undergone resistance training for at least 1 year prior to the start of the study, volunteered to participate in this study and provided signed informed consent. The subjects were allocated to one of two groups: the walking and stair-climbing group with limb BFR (BFR group, 6 men and 3 women) or the walking and stair-climbing only group (WS group, 12 men and 2 women). This study was approved by the Ethics Committee of the Juntendo University (Japan).

### 2. Exercise program intervention

Approximately one week prior to the start of the exercise program, the subjects completed laboratory-based measurements of body composition and HbA1c. All subjects were instructed to walk at a self-selected, faster pace than usual for 30 min consecutively, more than 4 days per week in the WS group and more than 3 days per week in the BFR group, and to climb more than 5 flights of stairs per day, on 4 or more days per week. In addition, subjects in the BFR group attended the lab once a week to complete a 20-min bout of treadmill walking with applied BFR.

### 3. Blood flow restriction

BFR (100-120 mmHg) was performed on a weekly basis over the 6-month duration of the program. BFR was applied during a 20-min bout of treadmill walking at a pre-determined exercise intensity of 70-85% of the age-predicted maximum heart rate (220 - age). During the BFR-walking session, 105-mm wide nylon cuffs (MT-870 Digital Tourniquet; Mizuho, Tokyo, Japan) were applied tightly around the bilateral thighs. as arterial occlusion pressure is largely influenced by thigh circumference, the applied pressure value was calculated for each subject based on the circumference of the right thigh, measured at 33% of the distance from the inguinal crease to the top of the patella: 100 mmHg for a circumference < 50 cm; 120 mmHg for a circumference of 51 to 55 cm. The cuff air pressure was released immediately upon completion of each walking bout.

**4. Body composition and HbA1c measurements**

Height, body weight (BW), waist circumference (WC), body mass index (BMI), muscle mass, fat mass, and percent of body fat (%Fat) were measured for all subjects at baseline (i.e., approximately one week prior to the start of the program), at the 3-month midpoint of the program, and at the termination of the 6-month program. Laboratory-based measurements were performed at approximately the same time of day for all measurement time points to control for diurnal effects on the outcome variables. Body composition was determined by bioelectrical impedance analysis using a body composition analyzer (InBody720; Biospace, Centennial, CO, USA). Blood tests for HbA1c levels were performed after a minimum of 4 hours of fasting.

**5. Statistical analysis**

The data obtained over the 6-month period were analyzed using two-way repeated-measures analysis of variance (ANOVA; Condition × Time). In the current study, subjects who had carried out more than 80% of both the walking and stair-climbing programs were included in the final analysis (WS: n=6, 6 men, BFR: n = 7, 5 men and 2 women). Statistical significance was set at $p < 0.05$. All analyses were performed using Prism software (ver. 6.0; GraphPad Software Inc., San Diego, CA, USA). Data are presented as the mean ± standard error.

## Results

**1. Body weight and body composition**

Changes in body weight and composition over the 6-month exercise program are reported in Table-1. At baseline, there were no significant differences in weight, WC, BMI muscle mass, fat mass, and %fat. For both groups, main effects of exercise time on decreasing body weight, WC, fat mass, %fat, and BMI ($p < 0.01$), as well as on increasing muscle mass ($p < 0.05$), were observed.

**2. Glycated hemoglobin (HbA1c) levels**

Changes in the HbA1c levels over the 6-month exercise program are shown in Figure-1. For both groups, there was a main effect of exercise time on lowering the HbA1c levels ($p < 0.01$).

**3. Training time and frequency**

Over the study period, subjects in the BFR group walked on average 46.0 ± 5.2 min per day, 4.2 ± 0.5 days per week (1-3 months, 45.0 ± 5.2 min per day, 4.7 ± 0.4 days per week; 4-6 months, 47.3 ± 5.4 min per day, 3.8 ± 0.6 days per week), compared to 64.5 ± 10.5 min per day, 4.6 ± 0.5 days per week for the WS group (1-3 months, 61.9 ± 11.2 min per day, 4.9 ± 0.6 days per week; 4-6 months, 72.0 ± 13.6 min per day, 4.3 ± 0.6 days per week). In addition, subjects in the BFR group completed 20 min of treadmill walking with BFR per week. There were no significant differences in the training time and frequency between the groups.

Subjects in the WS group completed a greater absolute number of stairs climbs, with an average 9.1 ± 2.5 flights of stairs completed per day over 4.9 ± 0.5 days per week (1-3 months, 8.7 ± 2.3 flights per day over 5.3 ± 0.7 days per week; 4-6 months, 9.6 ± 2.7 flights per day over 4.4 ± 0.7 days per week), compared to 6.9 ± 0.7 flights of

**Table-1** Effects of 6-months blood flow restriction-walking or control program of physical exercise on metabolic parameters

| | WS (n=6, 6 men) | | | BFR (n=7, 5 men and 2 women) | | | 2-way ANOVA (p) | | |
|---|---|---|---|---|---|---|---|---|---|
| Variable | Baseline | 3M | 6M | Baseline | 3M | 6M | Time | Condition | Interaction |
| Weight (kg) | 66.9 ± 3.0 | 66.4 ± 2.4 | 65.5 ± 2.4 | 64.4 ± 2.3 | 64.3 ± 2.4 | 63.1 ± 2.2 | <0.01 | 0.511 | 0.910 |
| WC (cm) | 89.1 ± 2.1 | 86.1 ± 2.1 | 87.4 ± 2.3 | 88.5 ± 3.1 | 87.8 ± 3.3 | 87.0 ± 3.4 | <0.01 | 0.956 | <0.05 |
| BMI (kg/m$^2$) | 24.7 ± 0.7 | 24.6 ± 0.6 | 24.2 ± 0.7 | 23.6 ± 0.8 | 23.5 ± 0.8 | 23.1 ± 0.9 | <0.01 | 0.327 | 0.949 |
| Muscle mass (kg) | 27.6 ± 1.0 | 28.2 ± 1.0 | 27.9 ± 1.0 | 25.6 ± 1.3 | 25.8 ± 1.2 | 25.8 ± 1.2 | <0.05 | 0.215 | 0.355 |
| Fat mass (kg) | 17.3 ± 1.4 | 15.6 ± 1.3 | 15.2 ± 1.2 | 17.7 ± 2.0 | 17.2 ± 2.3 | 16.1 ± 2.3 | <0.01 | 0.718 | 0.312 |
| % Fat (%) | 25.6 ± 1.1 | 23.4 ± 1.5 | 23.1 ± 1.2 | 27.4 ± 2.9 | 26.5 ± 3.3 | 25.1 ± 3.3 | <0.01 | 0.529 | 0.299 |

Two-factor repeated-measured analysis of covariance was used for the analysis. Values are presented as the mean ± standard error; WS, the walking and stair-climbing group, in which participants performed increased physical activity only (6 men); BFR, the walking group with limb blood flow restriction (BFR) (5 men and 2 women). No significant differences among the groups were observed with regard to any variables at baseline. WC: waist circumference, BMI: body mass index, %Fat: percent of body fat.

stairs completed per day, over 4.5 ± 0.5 days, by subjects in the BFR group (1–3 months, 6.7 ± 0.7 flights per day over 5.1 ± 0.4 days per week; 4–6 months, 7.3 ± 0.8 flights per day over 3.8 ± 0.6 days per week). There were no significant differences in the number of stairs climbs and the frequency between the groups.

## Discussion

In this study, we demonstrated that our 6-month exercise program yielded significant improvements in body weight (−1.4 kg), body compositions (−1.6 cm for WC; −0.53 kg/m$^2$ for BMI; + 0.25 kg for muscle mass; −1.9 kg for fat mass; and −2.38% for %fat), and HbA1c levels (−0.19%) in elderly people. These data are in line with previous studies reporting that increased physical activity significantly decreased BW and WC[7) 8)]. Our data indicate that the combination of increased physical activity due to higher intensity exercise (walk fast and stair climbing) was effective in improving body composition of elderly people, increasing muscle mass and decreasing fat mass.

A meta-analysis by Boulé *et al*.[18)] demonstrated that reductions in the HbA1c levels following a program of aerobic training in diabetic patients were better predicted by exercise intensity than exercise volume. Our exercise program consisted of 4–5 bouts of > 40 min of walking per week, performed at a pace higher than the usual self-selected pace, and climbing of more than 5 flights of stairs per day. Takaishi *et al*. demonstrated that a short bout of stair climbing/descending exercise (12 sets of 21 steps, 18 cm in height) may be a useful method for improving glycemic control, compared with normal walking after a meal, in older people with type 2 diabetes[13)]. Therefore, a combination of walking and stair-climbing may be effective for improving HbA1c levels in elderly people, as compared to only walking. The importance of exercise intensity on glucose control is supported by previous studies that have demonstrated that a combination of aerobic and resistance training is more effective in reducing HbA1c levels than aerobic training or resistance training alone[19) 20)]. Although the reduction in the HbA1c level was small in our study compared with in previous studies that performed a combination of aerobic and resistance

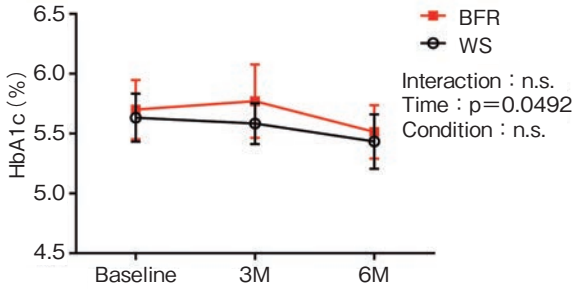


**Figure-1** Changes in the glycated hemoglobin (HbA1c) levels following our 6-month exercise program
The results of two-way ANOVA are displayed. Values are presented as the mean ± standard error. WS: n=6, 6 men; BFR: n=7, 5 men and 2 women.

training, we nonetheless consider that the magnitude of decrease achieved might be effective for reducing the risk of metabolic syndrome, cardiovascular disease, and other age-related diseases.

Recent research has provided evidence of the effects of BFR applied during walking on increasing muscle mass[14) 15)], oxygen consumption during and post-walking[14) 21)], energy expenditure[22)], and the net metabolic cost of walking[16)] in healthy young adults. These data support the notion that walking combined with BFR is more effective for improving the BW, body composition, and HbA1c levels in elderly people than normal walking. However, our BFR walking intervention did not further enhance the effects of the WS program on body composition and HbA1c levels in the present study. This may be related to the fact that the body weight, BMI, and WC of the subjects in the BFR group tended to be lower than those in the subjects in the WS group at baseline, which could have masked the synergistic effect of BFR. It is also possible that only one session of BFR walking per week was insufficient to induce changes in the body weight and composition, as well as in the HbA1c level. The influence of exercise with BFR on obesity and overweight are likely to be multifactorial in nature, and the optimal frequency and intensity have yet to be determined.

The limitations of our study need to be noted when interpreting our results. First and foremost, we did not control for medication use, with the subjects adhering to their usual prescriptions, although we instructed the subjects to not change their medication regimens during the study. As a number of different drugs were used by our subjects, including antihypertensives, antidiabetic

drugs, and anticholesteremic agents, the impact of these prescribed drugs on the blood glucose, serum insulin, and lipid profile could not be determined. Additionally, we did not control for diet; therefore, the effect of intake of fats and carbohydrates on the measured outcome variables could not be evaluated.

## Conclusion

The results of our study indicate that a 6-month walking and stair-climbing exercise program is effective in improving the body composition and HbA1c levels of elderly subjects, with no specific additional benefit provided by including walking with BFR.

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## Conflict of interest

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Proceedings

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# An Outpatient-Based Survey About the Recognition of Locomotive Syndrome in Tokyo: A Survey for 3 Years

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***Background***: In 2007, the Japanese Orthopedic Association (JOA) proposed the term locomotive syndrome (LS) to designate a condition in high-risk groups of patients with musculoskeletal diseases who are highly likely to require nursing care. LS is caused by weakening of the musculoskeletal organs, such as the bones, joints and muscles. Disorders of these organs leads to self-transportation disabilities. These conditions force people suffering from this syndrome to require outside care and support. Therefore, to prevent the decline into disability, patients need to maintain their health, especially their locomotor function. To prevent locomotor dysfunction, the JOA has carried out numerous campaigns to increase the awareness of LS. However, there are no accurate studies regarding the recognition of LS. Therefore, we have started to survey the recognition of LS since 2013 to elucidate the effects and trends of the recognition of LS and the promotion campaigns using orthopedic outpatient cohost.
***Methods***: To investigate the recognition of LS, we conducted a questionnaire survey including both the 25-question Geriatric Locomotive Function Scale (GLFS-25) and/or the "loco-check" in approximate 1,000 orthopedic outpatients at Juntendo University Hospital (Tokyo, Japan) from March to June (for 3 months) since 2013 (for 3 years)
***Results***: We have performed these surveys for 3 years (in 2013, 2014 and 2015). In first year, we surveyed the recognition of LS and found 24.6% of outpatients knew about LS in 2013. In 2014, a total of 26.4% of the patients knew about the concept of LS, which was increased 1.8% in comparison to our survey in 2013. With regard to the prevalence of LS in orthopedic outpatients, 60.5% (734 of 1,027 people who answered the questions) were classified into the LS high-risk group as determined using the GLFS-25. The prevalence of LS was 54.9% in males and 64.3% in females. We also resurveyed the recognition of LS and the prevalence of LS in 2015, and we are analyzing these acquired data in 2015 as on-going study.
***Conclusion***: We investigated the recognition of LS and the prevalence of LS using an outpatient cohort from the Tokyo area. This study demonstrated that the recognition of LS is a little bit increasing. Our outpatient-based survey is therefore considered to positively help obtain a better understanding of the effects and trends of promoting the concept of LS.

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# Acute Changes in Blood Lactate Concentration, Muscle Thickness, and Strength After Walking with Blood Flow Restriction in Older Adults

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***Objective***: The purpose of this study was to investigate whether blood flow restriction (BFR) walking results in an acute increase in muscle size and decrease in maximal strength as well as metabolic accumulation in older adults.

***Methods***: Ten older men and 8 older women (mean ± standard error; age, 68 ± 1 years; height, 161.8 ± 1.8 cm; weight, 60.7 ± 8.5 kg) walked for 20 minutes with BFR at a pre-determined speed (3–4 km/h). Muscle thickness (MT) was measured at the following four sites: knee extensors (KE) and flexors (KF) at 50% of the thigh length and dorsi flexors (DF) and plantar flexors (PF) at 30% of the lower leg length. The maximal voluntary isometric strength of KE and blood lactate concentrations were measured. All parameters were measured before and immediately after the exercise session.

***Results***: MT increased at all sites after BFR walking (KE: pre 28.9 ± 1.0 mm, post 32.3 ± 0.9 mm; KF: pre 53.3 ± 1.2 mm, post 55.2 ± 1.4 mm; DF: pre 23.9 ± 0.6 mm, post 24.7 ± 0.5 mm; PF: pre 61.0 ± 1.1 mm, post 63.8 ± 1.0 mm). The maximal voluntary isometric strength of KE decreased after BFR walking (pre, 144 ± 9.5 Nm; post, 136.5 ± 8.7 Nm). Blood lactate concentration was significantly elevated after BFR walking (pre, 1.3 ± 0.1 mol/*l*; post, 1.9 ± 0.2 mol/*l*).

***Conclusion***: BFR walking causes an acute increase in muscle size and decrease in maximal strength as well as metabolic accumulation in older adults.

***Key words***: ultrasonic, muscle cell swelling, metabolic accumulation

## Introduction

Rosenberg first proposed the term "sarcopenia" to describe the decrease in skeletal muscle mass with advancing age[1]. Advanced skeletal muscle loss leads to obesity and osteoporosis, as well as a decrease in quality of life and physical performance in daily living[2]. Therefore, maintaining a healthy amount of skeletal muscle mass is important to prevent disability. Generally, high-intensity resistance training is recommended for preventing sarcopenia[3]. The guidelines of the American College of Sports Medicine recommend lifting weight equivalent to at least 70% of one repetition maximum to maximize muscular hypertrophy in older adults[4].

Recently, blood flow restriction (BFR) has received attention for inducing muscle hypertrophy. Its unique characteristic is that substantial muscle hypertrophy can occur with an intensity as low as 10% of maximal voluntary contraction[5]. Previous studies have found that muscle hypertrophy occurs following walking (about 10% of maximal voluntary contraction[5]) with BFR in

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young[6] and older adults[7]. Furthermore, compared to 6 months of non-BFR walking exercise[8], BFR walking can induce muscle hypertrophy in a very short period (10 weeks)[7]. Therefore, BFR walking may be beneficial to a wider range of the population, promoting muscle hypertrophy at low intensity and in a short time period[9].

The mechanism of the effect of BFR walking on muscle hypertrophy in exercised muscle is not completely understood. However, a previous study by Ozaki *et al.* indicated that the phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) and the dephosphorylation of eukaryotic translation elongation factor 2 (eEF2) after BFR walking in young adults[10] results in the augmentation of the net protein balance[11]. Muscle cell swelling and fatigue (i.e. decreasing muscle strength) caused by accumulation of metabolites[11)-13)] are thought to be associated with muscle hypertrophy as upstream stimulatory factors of ERK1/2 and eEF2. A previous study by Ogawa *et al.* reported that the increase in thigh muscle thickness, an index of muscle cell swelling, was more pronounced with rather than without BFR after walking in young adults[14]. Furthermore, the relative load during walking was decided by the ratio of body weight to leg muscle strength. Muscle strength per body weight is lower in older adults than in young adults[15]. However, the effects of BFR walking on muscle cell swelling and muscle strength in older adults have not been studied so far. We speculate that it induces more muscle cell swelling and muscle strength reduction in older adults than in young adults. Thus, the purpose of this study was to investigate whether BFR walking can elicit acute increase in muscle thickness and decrease in maximal strength as well as metabolic accumulation in older adults.

## Methods

### 1. Subjects

Ten older men and 8 older woman (mean ± standard error; age, 68 ± 1 years; height, 161.8 ± 1.8 cm; weight, 60.7 ± 8.5 kg) participated in this study. The subjects were recruited through printed advertisements. All subjects were informed of the methods, procedures, and risks, and signed an informed consent form before participating in the study. The study was conducted according to the Declaration of Helsinki and was approved by the Ethics Committee for Human Experiments of Juntendo University, Japan.

### 2. BFR walking

The walking exercise consisted of 20 minutes of walking at a pre-determined speed (3-4 km/h) on a motor-driven treadmill. A 105-mm-wide nylon cuff (MT-870 Digital Tourniquet, Mizuho, Tokyo, Japan) was applied tightly at the most proximal portion of both the legs. The pressure was calculated for each subject based on the circumference of the right thigh (33% of the distance from the inguinal crease to the top of the patella), as follows: < 50 cm = 100 mmHg (men, n = 9; women, n = 5); 51-55 cm = 120 mmHg (men, n = 1; women, n = 2); 56-59 cm = 40 mmHg (women, n = 1). This is because arterial occlusion pressure is largely influenced by thigh circumference[16]. The cuff air pressure was released immediately upon completion of each session.

### 3. Muscle thickness

Before and immediately after an exercise session, muscle thickness was measured via B-mode ultrasound using a 5-18 MHz scanning head (Noblus; Aloka, Tokyo, Japan) at the following four sites as an indirect index of muscle cell swelling: the central surface of the knee extensors (KE) and flexors (KF) at 50% of the right thigh length between the lateral condyle of the femur and the greater trochanter, and the central surface of the dorsi flexors (DF) and the medial surface of the plantar flexors (PF) at 30% of the right lower leg length between the lateral malleolus of the fibula and the lateral condyle of the tibia. Prior to all scans, the subjects rested quietly in a seated position for at least 30 min to avoid an influence of fluid shifts within the muscle,. Circumferences were also measured at 50% of the thigh length and 30% of the lower leg length using a tape measure. The same investigator performed all measurements to maximize intrarater reliability. Ultrasound measurements of muscle thickness were performed in the supine/prone position, with careful attention to ensure that the hip and ankle joint positions and the distance between both legs were the same in all the measurements. The scanning head was coated with

a water-soluble transmission gel and placed on each marked measurement site without depressing the dermal surface. The subcutaneous adipose tissue-muscle interface and the muscle-bone interface were identified on the ultrasound images, and the distance between the two interfaces was recorded as the muscle thickness.

**4. Maximal isometric strength**

Maximal isometric strength of knee extensors was measured using a Biodex system 4 dynamometer (Biodex Medical Systems, Shirley, NY) before and immediately after an exercise session to determine the degree of muscle fatigue after BFR walking. During testing, each participant was seated on a chair with the hip joint angle positioned at 85° of flexion (0° = full hip extension). The center of rotation of the knee joint was visually aligned with the axis of the dynamometer lever arm and the ankle was firmly strapped to the distal pad of the lever arm. A knee joint angle of 0° corresponded to full knee extension. Several warm-up contractions (4-5 submaximal contractions and 1-2 near-maximal contractions) were performed before testing. Participants were then instructed to perform maximal isometric knee extension for about 5 s at a fixed knee joint angle of 75°. Two maximal efforts were performed for each isometric measurement peak torque and the highest value was used in data analysis.

**5. Blood lactate concentration**

Prior to testing, subjects rested quietly in a seated position for at least 30 min. Whole blood samples (0.3 μ*l*) were also taken from the fingertip before and immediately after the exercise session. Blood lactate concentration was determined using lactate oxidase enzyme electrode methods in a lactate analyzer (Lactate pro 2, ARKRAY, Kyoto, Japan). The detection limit was 0.5 mmol/*l*.

**6. Statistical analysis**

Results are expressed as mean ± SE for all variables. First, we performed a two-way analysis of variance (ANOVA) with repeated measures [sex (male and female) × time (pre and post)] to declare sex difference among variables. However, there is no significant interaction within all variables. Therefore, two-tailed paired Student's t-test (pre and post) was collectivity performed the value of older men and older woman as one group. The level of significance was set to $p < 0.05$.

## Results

Figure-1 shows changes in blood lactate concentration after BFR walking. Blood lactate concentration was significantly elevated after BFR walking (pre, 1.3 ± 0.1 mol/*l*; post, 1.9 ± 0.2 mol/*l*).

Figure-2 illustrates changes in circumstance and muscle thickness after BFR walking. Thigh and lower leg circumstance were significantly increased after BFR walking (thigh circumstance: pre 47.9 ± 0.8 cm, post 48.9 ± 0.8 cm; lower leg circumstance: pre 35.7 ± 0.5 cm, post 36.3 ± 0.4 cm). Furthermore, muscle thickness in the entire site were also significantly increased after BFR walking (KE: pre 28.9 ± 1.0 mm, post 32.3 ± 0.9 mm; KF: pre 53.3 ± 1.2 mm, post 55.2 ± 1.4 mm; DF: pre 23.9 ± 0.6 mm, post 24.7 ± 0.5 mm; PF: pre 61.0 ± 1.1 mm, post 63.8 ± 1.0 mm).

Changes in the maximal isometric strength of the KE are shown in Figure-3. Maximal isometric strength of knee extensors significantly decreased after BFR walking (pre, 144 ± 9.5 Nm; post, 136.5 ± 8.7 Nm).

## Discussion

The main finding of the present study was that walking with BFR elicited acute increase in muscle thickness and decrease in maximal isometric strength as well as a concomitant increase in blood lactate concentration.

In the present study, blood lactate concentration significantly increased by 1.9mmol/*l*, immediately after BFR walking. Loenekene *et al* reported that blood lactate concentration increased by 2.0mmol/*l* following BFR walking in healthy young adults[17]. There has been previous study that investigating blood lactate concentration after 20 minutes of BFR walking at 4.5km/h in older people, which significantly increased from 1.1mmol/*l* to 1.4mmol/*l* (Ozaki *et al*. 2015 in press). These results show that blood lactate concentration increase after BFR walking regardless age. However, compared to other exercise modality such as low intensity resistance training with BFR, the fold change in

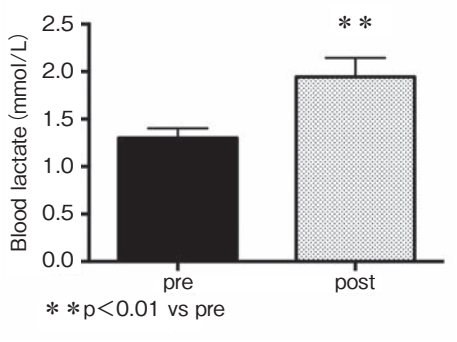


**Figure-1** Changes in blood lactate concentration after blood-flow restriction (BFR) walking
Data are presented as mean ± standard error. Abbreviations: pre, before BFR walking; post, immediately after BFR walking.

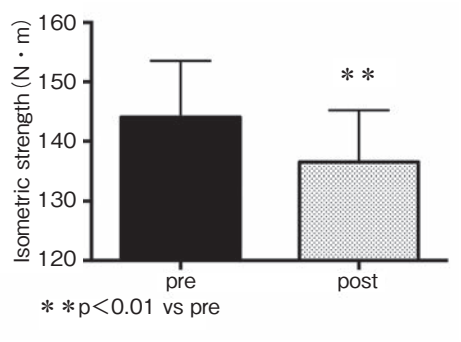


**Figure-3** Changes in maximal isometric strength of knee extensors after blood-flow restriction (BFR) walking
Data are presented as mean ± standard error. Abbreviations: pre, before BFR walking; post, immediately after BFR walking.

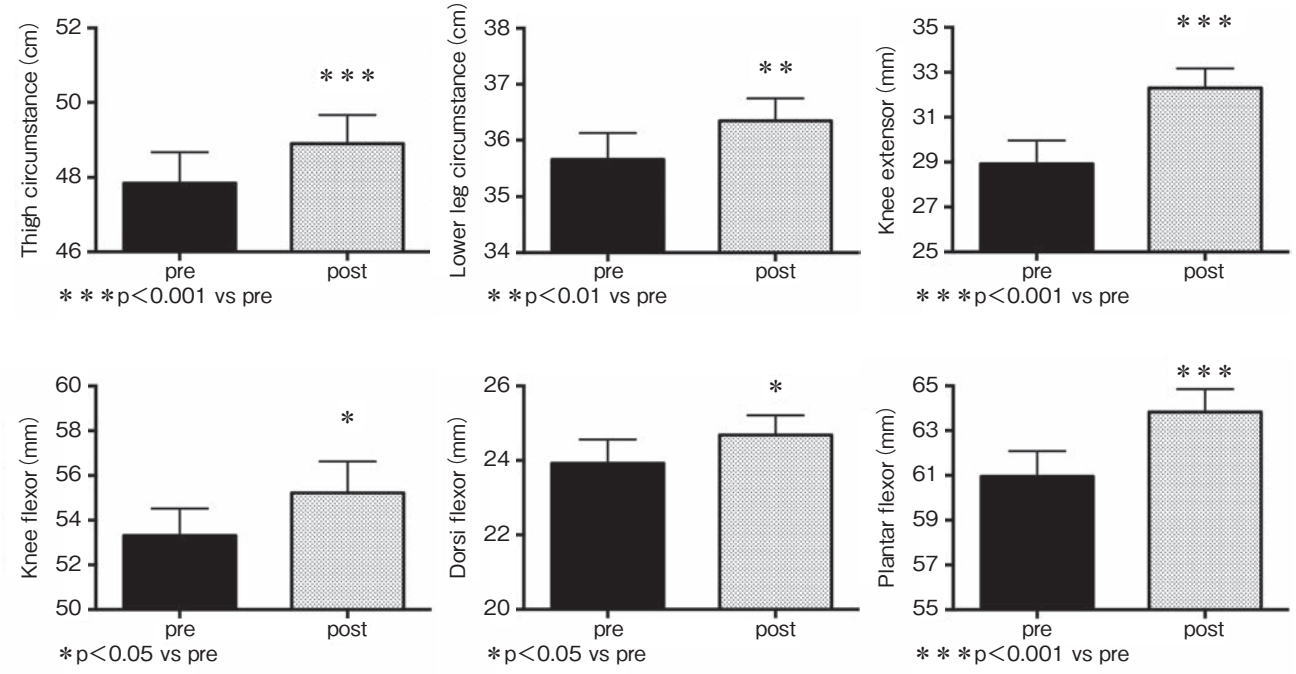


**Figure-2** Changes in circumference and muscle thickness after blood-flow restriction (BFR) walking
Data are presented as mean ± standard error. Abbreviations: pre, before BFR walking; post, immediately after BFR walking.

blood lactate concentration is approximately less than half[18]. The small changes in blood lactate concentration of BFR walking in present study may be attributable to the exercise intensity in addition to differences among exercise modality.

Our results showed that muscle thickness, an index of muscle cell swelling, significantly changed in all evaluated regions following BFR walking in old adults. The percent changes in muscle thickness of KE were approximately 11.7%, which is almost equal to previous study (8.8% and 10.1%, respectively) that investigated the acute increase in muscle thickness following BFR walking in young adults[14]. The precise mechanism for muscle hypertrophy following BFR exercise cannot be ascertained, and it has been speculated that muscle cell swelling plays an important role in gaining training effects. A recent study indicated that acute increase in muscle thickness is correlated with a loss of plasma volume[19], suggesting that the increase in

muscle thickness after BFR exercise reflects primarily a fluid shift from vascular space into exercised muscle. This may occur as a consequence of increase in metabolites such as $Pi^+$ and $H^+$ and decrease in pH during muscle contraction[20) 21)]. Indeed, direct relationships between accumulation of $Pi^+$ and decrease in pH during exercise and muscle hypertrophy following a period of low-intensity resistance exercise with BFR have been reported[22)]. Currently, it is theorized that exercise-induced metabolites mediates muscle protein synthesis via muscle cell swelling, leading to muscle protein signaling, such as through mammalian target of rapamycin and mitogen-activated protein kinase signaling pathway[23)].

Our results showed that maximal isometric strength in older adults decreased significantly by 5.2%, following BFR walking, whereas previous results have shown that maximal isometric strength did not decrease (2.1%) following a bout of walking combined with BFR in young adults[14)]. The relative decrease in the lean body mass and muscle strength compared to body mass in older adults may contribute to the acute decline of muscle strength following BFR walking. Janssen *et al.* reported that age was negatively correlated ($p < 0.01$) with relative skeletal muscle mass (body mass/skeletal muscle mass) in men ($r = -0.50$) and women ($r = -0.24$)[24)]. Furthermore, other study reports show that relative maximum muscle strength (muscle strength/body weight) of KE gradually decreases with age[25)]. This decline would put a load on exercised muscle during BFR walking in older individuals than in young adults. Furthermore, metabolic accumulation was elevated after BFR walking in our study. Several studies have reported that the accumulation of metabolites such as lactate, Pi, $H^+$, and pH causes a decrease in muscle strength[26)-29)]. For example, the rise in whole blood lactate after repeated knee extension and flexion was strongly correlated to the fall in maximal isokinetic strength during exercise ($r = 0.822$)[29)]. Therefore, it is presumed that the accumulated metabolites partly participate in decreasing the muscle strength following BFR walking in older adults.

The limitation of the present study was that there was no control group without BFR. A previous study reported an acute change in muscle thickness in blood-flow restricted quadriceps, but not in non-restricted quadriceps, during and immediately after walking. Thus, we speculate that muscle thickness is not increased after walking in older adults[14)]. Furthermore, even though the magnitude of the decrease in isometric strength after BFR walking is associated with cuff compression pressure[18)], the nylon cuff-inflating device did not apply an initial compressive force. Additional research is needed to address these issues.

In conclusion, our results indicated that an acute increase in muscle thickness and a decrease in muscle strength occur following BFR walking as does metabolic accumulation in older adults.

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## Conflict of interest

No commercial company or manufacturer has any professional relationship with any author involved in this work, and the results of this work will not confer any commercial benefit to any author.

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