Stress Fracture Influences Bone Resorption marker (u-NTX) in Female Long Distance Runners

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In this study, we aim to clarify the influence based on bone resorption markers at onset of stress fracture. Also, we will clarify the state of the bone resorption markers of female long distance runners who have a history of stress fracture and also ones who routinely practices running long distances.

Participants comprised 19 female long distance athletes. The survey period was 2011-2014, and we measured u-NTX as a bone resorption marker at least twice a year, taking the mean ± SD of the periodic measured values without stress fracture as the mean value. Measurements were collected sample when stress fractures developed. 132 u-NTX measurements were taken from 19 participants. As a result, the average was 41.03 ± 12.31 nmolBCE/mmolCRE (25percentile: 33.15, 50percentile: 40.55, 75percentile: 47.95).

In six of the 19 participants, u-NTX could be measured following a stress fracture. The mean value of u-NTX for those participants was 40.16±9.10 nmolBCE/mmolCRE, increasing to 64.08±16.07 nmolBCE/mmol CRE with the stress fracture ($p<0.01$).

The findings showed that, in adult female long distance runners, u-NTX values when there was no stress fracture were within the standard value for mean premenopausal women, but increased when the athletes suffered from a stress fracture.

Introduction

A stress fracture is a break in bone tissue caused by repeated minor external mechanical stress caused by activities such as running that can occasionally lead to a complete fracture. A stress fracture is a serious injury as it takes a long time to completely heal [2, 3] and prevents athletes from training. Many female long distance runners compete while suffering from menstrual disorders; the incidence of stress fractures among such women is much higher than for athletes of other sports [4, 12 16]. To achieve good results through continuous training, it is important to find an indicator for the prevention and early detection of stress fractures in female athletes.

Bone strength is explained by bone density and bone quality (bone metabolism and collagen cross-linking) [21]. It has been reported that low bone density increases the risk of a stress fracture [5, 10, 25]. However, as results based on bone density reflect nutritional condition and mechanical stress over several previous months, they are not suitable for the early detection of stress fractures. In contrast, bone metabolism—bone quality—reflects the condition of bone in a timely manner, and bone metabolism has an effect on subsequent bone density. If the balance of bone resorption and bone formation is maintained (coupling), bone mass is maintained. However, when uncoupling occurs and bone resorption becomes more dominant, bone density decreases. Bone metabolism can be evaluated using bone metabolism markers measured in serum and urine.

The mechanism underlying stress fractures is that repeated mechanical stresses on the bone repeatedly cause microdamage, and as bone repair cannot keep up, bone mass decreases locally [24]. Bone resorption is believed to be accelerated before and after the occurrence of a stress fracture. However, there is insufficient study on bone metabolism during stress fractures. In addition, it was shown that bone resorption is enhanced by continuous running for long periods, such as during a marathon [7, 15]. Thus, long distance runners who repeatedly run may already be suffering from enhanced bone

resorption. In addition, bone resorption marker is high in athletes with a history of stress fracture compared to athletes who do not [27]. From these facts, there is a possibility that the bone resorption marker is elevated when stress fracture develops. However, there is a consideration that bone resorption markers may be elevated with long distance runners practicing on a daily basis and athletes with a history of stress fracture may have an elevated marker even when there is no stress fracture.

In this study, we aim to clarify the influence based on bone resorption markers at onset of stress fracture. Also, we will clarify the state of the bone resorption markers of female long distance runners who have a history of stress fracture and also ones who routinely practices running long distances.

Methods

Participants

Participates consisted of 25 female long distance runners, ages 19 to 34 years old (ave 23.99 ± 4.11). This study was approved by the ethical committee of Juntendo University (21-11). Participants and their team instructors were given explanations of the experiment orally and in writing before written consent was obtained. This study was conducted according to the ethical standards of International Journal of Sports Medicine [13].

Measurement item

Bone metabolism was evaluated noninvasively by measuring type 1 collagen crosslinked N-telopeptide in urine (u-NTX). Participants answered the preliminary questionnaire. The contents of the questionnaire were physical characteristics, experience of irregular menstrual or amenorrhea in the past, or whether they have a past history of stress fracture diagnosed by a doctor. In addition, the same questionnaire was answered each time measurements were taken. We investigated the total distance ran

per month and injury situation.

Measurement methods

Generally, when measuring bone metabolism markers, both bone resorption and formation are taken. But due to the participants being professional athletes, they were uncooperative in collecting blood samples. Therefore, to avoid diurnal and daily variations, the second urine of the morning was sampled for the u-NTX measurement. This was analyzed using ELISA method (Osteomark; Alere Medical Co. Chiba, Japan). To eliminate any effects of the kidney, the creatinine conversion factor was used for the analysis. Results were expressed in nmol bone collagen equivalents (BCE)/mmol creatinine (CRE). All measurements were outsourced to Hoken Kagaku Kenkyujo laboratory.

Measurement period

To measure normal condition, which is condition without stress fracture and when being able to participate in full practices, we measured each athlete's u-NTX 11 times, including three times in 2011, twice in 2012, twice in 2013, and four times in 2014. The measurements were taken at the following months and practice period;

In the April and July 2011 measurements were taken in the regular practice period. In the measurement of April 2012, it was a regular practice period, and October was a performance enhancement practice period. Performance enhancement practice period is pertaining to athletes attending training camp. In the measurement of 2013, both February and October were performance enhancement practice periods. In the measurement of 2014, May and June were regular practices, August was performance enhancement practice period. The u-NTX was taken and assessed by the amount of practice on weekly running distances. As measured values of u-NTX can show considerable variation in an individual, we used the mean value of the measurements obtained during the period without any stress fracture as the normal value. If a stress

fracture occurred during the survey period or before, measurements were obtained during examination at which it was determined that a stress fracture had occurred. Stress fractures were diagnosed using radiographic inspection (i.e. X ray) by orthopedic surgeons. Also, bone metabolism marker was taken at the diagnosis. The onset date of the stress fracture was defined as when the participants felt pain at the injured site. The date of onset and the date of measurement of bone metabolism markers are shown in Table 1.

Exclusion criteria and grouping

Out of 25 participants, 6 participants with u-NTX measurements less than 3 times were excluded from this study; therefore, 19 participants were included in this study (Figure.1). Among them, 6 participants with measurement data of u-NTX when stress fracture occurred were selected as SF group, and other participants were selected as Control group. In the SF group, the values of measurement when stress fracture occurred were compared with the values of measurement without stress fracture. Based on the preliminary questionnaire, participants were grouped into two groups with or without the history of stress fracture, and a comparison was made between the two groups.

Data analysis methods

The measure values were presented as mean ± standard deviation (SD) or median (interquartile rage). To decide a normal value for individual participants, a mean value and SD of measurements without stress fracture of each participant was calculated and used as a “normal value” for each participant.

Wilcoxon signed-rank test was used to compare the difference between the value at the time of stress fracture and the normal value. Unpaired t-test was used to compare the

105 difference between the groups with and without the history of stress fracture. Statical
106 analysis was done using nonparametric Kruskal-Wallis test comparing the difference
107 among the average weekly running distances measuring u-NTX.
108 Furthermore, changes of u-NTX at the time of stress fracture were investigated using
109 the normal values and SD. “Rate of over” was calculated for SF and NSF group using
110 the normal value ± SD of each participant, and the extent of changes of u-NTX values
111 when stress fracture occurred was analyzed. “Rate of over” in the SF group was defined
112 as the rate of participants whose u-NTX values at the time of stress fracture were over
113 1SD, 1.5SD or 2SD of the normal value. “Rate of over” in the NSF group was defined
114 as the rate of participants whose highest u-NTX values were over 1SD, 1.5SD or 2SD
115 of the normal value. Fisher’s exact test was used to compare the difference of “Rate of
116 over” of the two groups.
117 The effect size (ES) and power in post hoc tests were calculated using Gpower
118 software (Version 3.1) [11]. The ES of between 2 groups (with and without the history
119 of stress fracture) and 2 conditions (values at stress fracture and normal value) were
120 calculated using ES (d). The evaluations of the ES strength are: small ($d < 0.04$),
121 moderate ($0,04 \leqq d < 0.80$), large ($d \geqq 0.80$). The ES of among the average weekly
122 running distances measuring u-NTX were calculated using ES (f). The evaluations of
123 the ES strength are: small ($f<0.25$), moderate ($0.25 \leqq f<0.40$), large ($f \geqq 0.40$). The ES
124 between 2 groups (SF group and NSF group) considered as rate of over was calculated
125 using ES (w). The evaluations of the ES strength are: small ($w<0.10$), moderate
126 ($0.10 \leqq w<0.30$), large ($w \geqq 0.50$). α error was set to $p < 0.05$, and β error was set to $(1-\beta)$
127 > 0.80.
128
129
130 **Results**

Their average physical and other characteristics were as follows: height 159.91±6.36 cm, weight 46.13±3.93 kg, body mass index (BMI) 18.02±1.05 kg/m^2, weekly running distance 121.7±49.4 km, and time for 5000m run 15:45.9±23.9. In this study, total of 132 u-NTX measurements were taken from 19 participants. As a result, the average was 41.03 ± 12.31 nmolBCE/mmolCRE (Q1: 33.15,Q2: 40.55, Q3: 47.95).

The weekly running distance when u-NTX was measured is shown in Table2. There was no significant difference in the weekly running distance among measurements (p＝0.36, ES (f)=0.29, 1-β=0.91).

Comparison of u-NTX values between with and without history of stress fracture

Out of the 19 participants, nine had a history of stress fracture (height 159.67±7.55 cm, weight 44.89±4.78 kg, BMI 17.55±0.66) and 10 did not (height 160.14±5.48 cm, weight 47.25±2.78 kg, BMI 18.45±1.19). Although u-NTX values were 36.51±9.84 nmol BCE/mmol CRE for the group with a history of stress fracture and 44.01±8.06 nmol BCE/mmol CRE for the group without, this difference was not statistically significant (p = 0.08, ES (d) = 0.834, 1-β = 0.508).

Comparison of u-NTX value in SF group between measurement with stress fracture and normal value

Data from the time of a stress fracture were available for six participants (Table1). The mean value for u-NTX after a stress fracture was 64.08±16.07 nmol BCE/mmol CRE compared with the mean normal value of 40.16±9.10 nmol BCE/mmol CRE; this difference was statistically significant (p < 0.01, ES (d) = 1.989, 1-β = 0.969) (Figure 2). In addition, in four of these six participants, menstrual condition when stress fracture occurred was irregular or no menstruation.

Changes in u-NTX values at stress fracture

Changes in u-NTX values that were +1.5 SD or more were observed in five out of six (Rate of over: 83%) in the SF group and three out of 13 (Rate of over: 23.1%) in the

NSF group, which represents a significant difference. Changes of +1.5 SD or more were more common in the SF group ($p < 0.05$, ES (w)=1.597, 1-β = 0.616, odds ratio = 16.6). Five out of six (Rate of over: 82%) of the SF group showed a change of +2 SD, a significantly greater proportion than in the NSF group (1/13, Rate of over: 7.7%; $p < 0.01$, ES (w)=2.023, 1-β = 0.786, odds ratio = 60.0) (Table 3).

Discussion

In this study, we regularly measured u-NTX in 19 female long distance runners. For six of these participants, measurements were obtained when a stress fracture occurred. It was found that u-NTX at the time of stress fracture showed a higher value than when there was no stress fracture, indicating enhanced bone resorption.

The underlying mechanism for stress fractures involves repeated mechanical stresses on bones causing repeated microdamage, with which bone repair cannot keep up, leading to a localized reduction in bone mass [24]. In animal experiments, when microdamage accumulates, bone remodeling is locally enhanced to repair the damage, and remodeling space on the bone resorption surface increases [8]. In the present study, although there was a problem that the amount of training was not constant, the mean u-NTX value in multiple measurements obtained during the time without stress fractures was within the standard value for normal premenopausal women of 9.3–54.3 nmol BCE/mmol CRE [17]. In this study, even a history of stress fracture did not lead to increased u-NTX values. The previous study investigated u-NTX values from different sports. The age and u-NTX values of athletes performing high impact sports (basketball and volleyball), medium impact sports (soccer and track) and non-impact sports (swimming) were 19.9±0.3 years old; 72.9±11.4 nmolBCE/mmolCRE, 20.6±0.3 years old; 62.5±7.6 nmolBCE/mmolCRE and 19.4±0.3 years old; 80.0±9.2

183 nmolBCE/mmolCRE, respectively [9]. The value of u-NTX for female cross-country
184 athletes with an average age of 19.8 years similar to the sports category of this study
185 was 62.5 ± 10.3 nmol BCE / mmol CRE [18]. In contrast, the average u-NTX was 41.03
186 ± 12.31 nmolBCE/mmolCRE in the present study. In the previous studies, the average
187 age was 20 years or younger, whereas the participants of this study was 23 years old or
188 older. It is known that the bone metabolism is more active in younger population [19,
189 26].In addition, measurements of u-NTX obtained the day after moderate exercise was
190 reported to be no different from measurements obtained before exercise [28]. We
191 therefore assume that u-NTX would show normal values regardless of the amount of
192 exercise when there is no stress fracture, but with a stress fracture, it would show a high
193 value because of the accumulation of excessive microdamage in adult female long
194 distance runners.
195 We also observed that, when a stress fracture occurs, u-NTX values reach +1.5 SD or
196 more above the normal value. As u-NTX is tested in urine samples, it is a noninvasive
197 bone metabolism marker that does not put too much stress on the athletes. In addition,
198 u-NTX is a superior marker for monitoring [1]. Thus, after three measurements of
199 u-NTX, if the value reaches +1.5 SD or more above the normal value, a stress fracture
200 should be suspected. A detailed early examination could help the early detection of
201 stress fractures.
202 In recent years, tartrate-resistant acid phosphatase isoform-5b (TRACP-5b) has been
203 used as a bone resorption marker for measurements in many studies as it reacts
204 sensitively. In a study that targeted lacrosse players, TRACP-5b measured in athletes
205 with a history of stress fracture was higher than in athletes without stress fracture [27].
206 TRACP-5b also reflects the impact of exercise in particular, reacting sensitively to
207 temporary changes after exercise [20, 23]. As it is a more sensitive marker, it is believed
208 to be able to reflect the effects of exercise performed on the day before or immediately

209 before measurement. In contrast, measurements of u-NTX obtained the day after
210 moderate exercise were reported to be no different from measurements obtained before
211 exercise [28]. Although it was reported that bone metabolism markers are not suitable
212 prediction markers for stress fracture [29], the bone metabolism marker used in the
213 previous study was a serum marker (TRACP-5b, CTX), and u-NTX was not measured.
214 Based on previous studies, TRACP-5b increases when there is a history of stress
215 fracture but it may not be reliable on the onset of stress fracture [27], whereas in our
216 study u-NTX became higher when stress fracture develops which states that there are
217 certain characteristics bone resorption markers. To clarify the characteristics of bone
218 resorption markers, further investigation is necessary in the future.
219 A limitation of this study was that u-NTX was high when stress fractures occurred, but
220 it is unknown whether u-NTX increased prior to the occurrence of stress fracture and
221 how long the u-NTX remains high following stress fracture. A previous study reported
222 high u-NTX values prior to stress fractures [22]. Therefore, by periodically measuring
223 the bone resorption marker to seek if the value is abnormally high, in which we can
224 suspect the occurrence of stress fracture, these tests may be helpful in detection stress
225 fracture in the immature stages. However, as the number of cases was small, and the
226 measurement of u-NTX was more frequent than in the present study, a prospective
227 cohort study is needed to examine whether u-NTX values increase before stress fracture
228 occurs. In addition, based upon having the cooperation of professional athletes
229 participate in this study there is a weakness in this study of not being able to perform
230 the adequate measurements such as collecting blood samples. Therefore, we were
231 unable to examine bone formation. For bone metabolism, the balance between bone
232 formation and bone resorption (coupling) is important, and bone formation markers
233 should therefore be measured and coupling be examined. Also, although intake of
234 calcium and vitamin D is also related to bone density and bone metabolism markers [6,

14], the nutritional condition of our participants is unknown as we did not survey diet in this study. However, all of the athletes were living together in dorms, and breakfast and dinners were provided. Therefore, it is unlikely that there was a significant difference in nutritional status between the athletes, and nutrition probably had little effect on the bone metabolism marker.

The findings of this study showed that, in adult female long distance runners, u-NTX values without stress fracture were within the standard value for normal premenopausal women, but increased when the athletes suffered from a stress fracture. Furthermore, our result showed the possibility that a stress fracture has developed when u-NTX shows a value higher than 1.5 SD from the normal value. From these facts, it was suggested that regular measurement of u-NTX and paying attention to fluctuations could be a convenient and noninvasive indicator of development of stress fracture.

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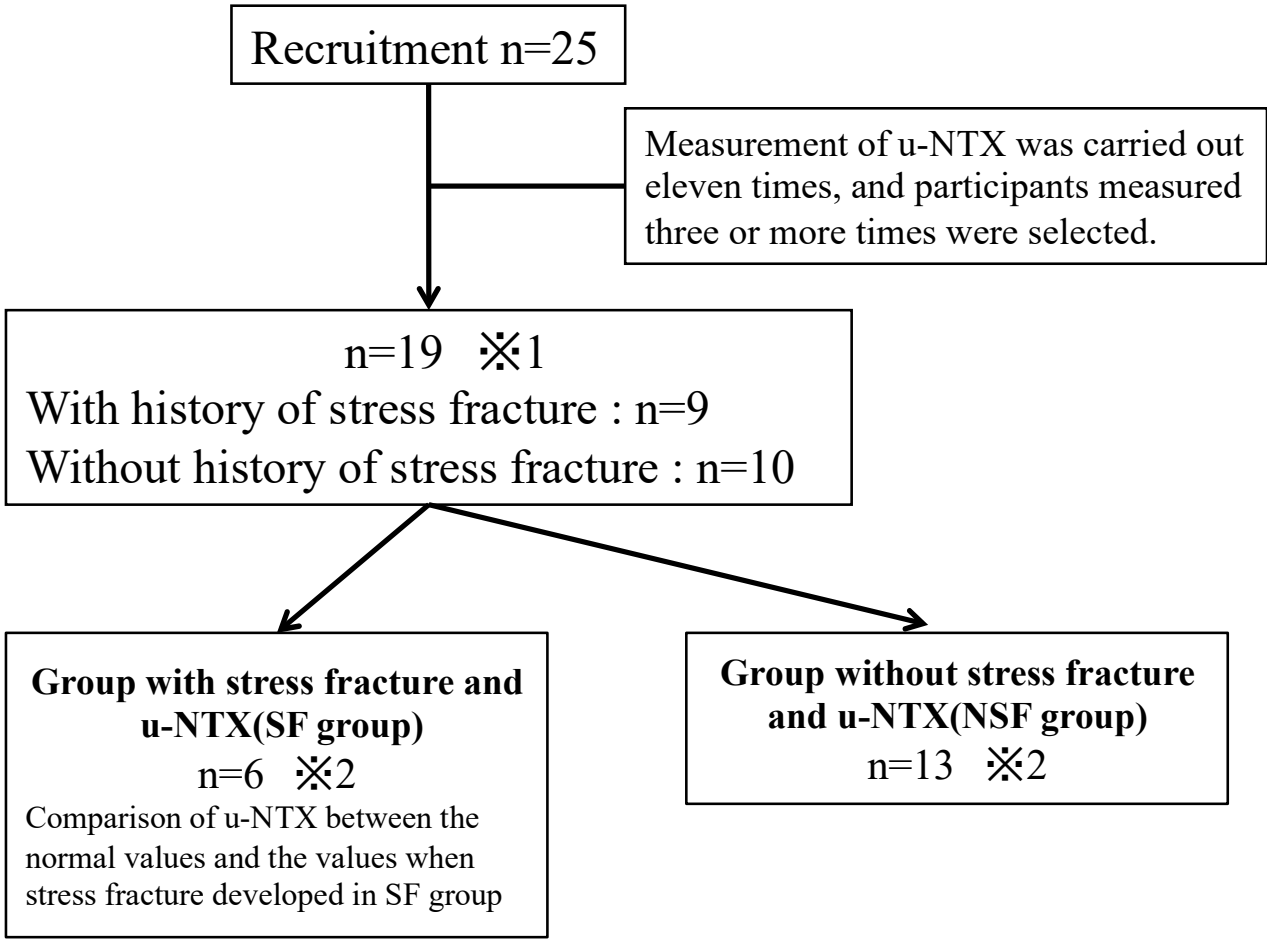


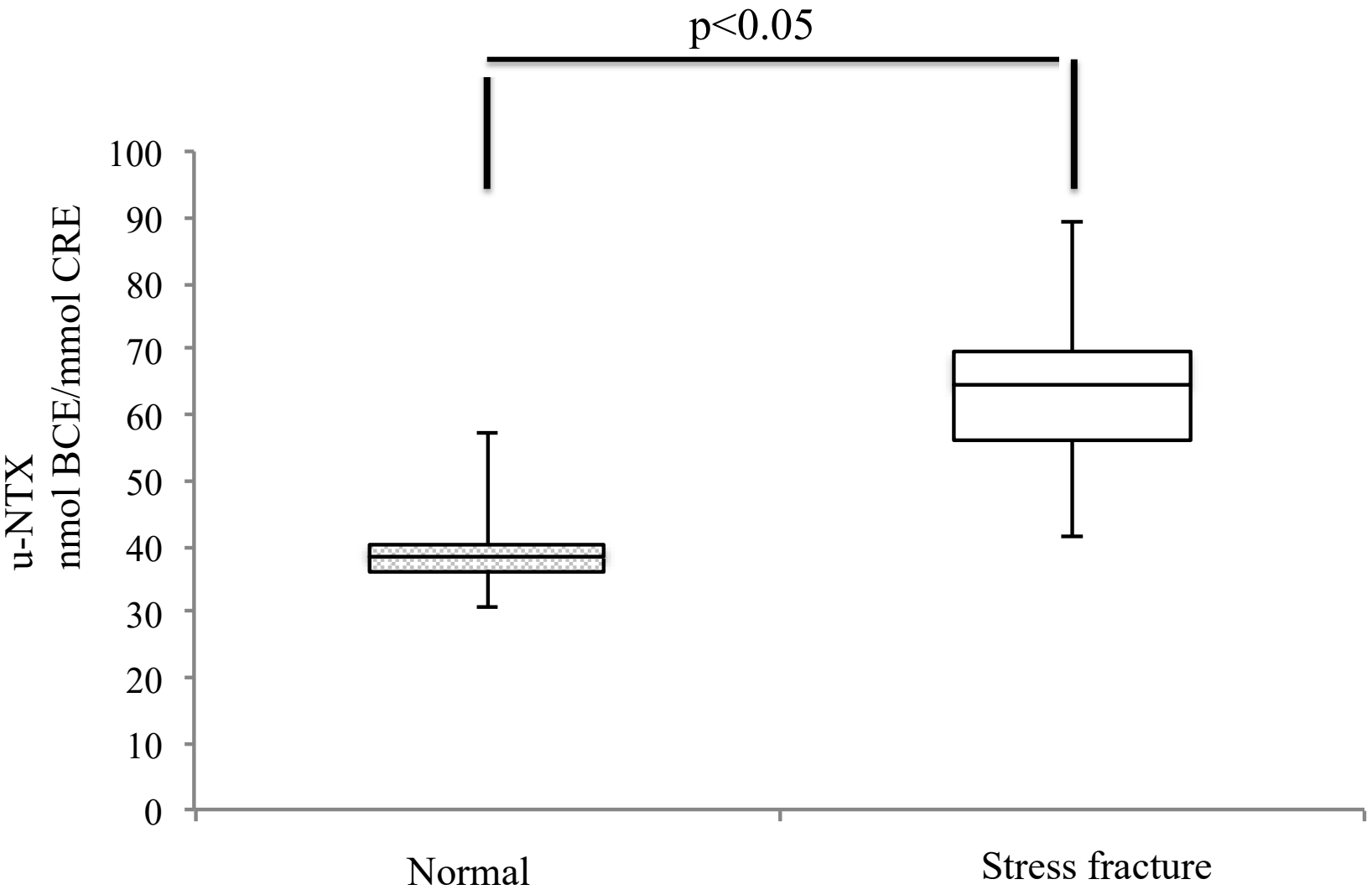
※1 In the measurements of u-NTX, average value ± SD was calculated for each subject and this value was taken as the normal value.

※2 Examination on changes in u-NTX values at stress fracture(comparison of "rate of over" between SF group and NSF group).

"Rate of over" of the SF group : The percentage of participants whose u-NTX value when stress fracture developed was over the normal value + 1 SD, 1.5 SD or 2 SD.

"Rate of over" of the NSF group: The percentage of participants whose highest value in normal value measurement was over normal value + 1 SD, 1.5 SD or 2 SD.

Figure1. Exclusion criteria and grouping



Fgure2. Comparison of u-NTX value in Stress fracture group between measurement with stress fracture and normal value without stress fracture

Table1. Details of stress fracture, Menstruation condition and u-NTX data in SF group

	Location of an injury	date of onest	Urine sample	Normal value	Stress fracture	Urine sample from date of onset	Menstruation situation	Age
A	Left fifth metatarsal bone	Nov. 9 , 2002	Dec. 16, 2002	36.8 ± 14.2	67.9	37 days later	Normal	30
B	Left pubis	May. 14, 2010	June 6 ,2010	35.8 ± 8.6	54.6	19 days later	Irregular menstruation	20
C	Left Medial Tibia	Sept. 2w, 2012	Oct. 30, 2012	40.0 ± 5.2	41.5	6 weeks later	Normal	24
D	Pubic symphysis	Nov. 15, 2011	Dec. 9, 2009	40.3 ± 7.7	70.2	25 days later	Amenorrhea	29
E	5th thoracic vertebra	Mar. 2010	Apr. 20, 2010	30.7 ± 9.9	61.1	3-5weeks later	Primary amenorrhea	22
F	Left proximal one theird of tibia	Late Mar. ,2010	Apr. 21, 2010	57.3 ± 10.3	89.2	3-4 weeks later	Irregular menstruation	21
			Mean	40.2 ± 9.3	64.1			
			SD	9.9 ± 3.0	16.1			

Amenorrhea was defined as a state without menstruation for more than 3 months, and an irregular menstrual was defined as when menstruation does not occur within the regular menstrual cycle (28 - 38 days)

Table2. The mean distance per month indicated by weekly unit when measuring u-NTX

	Weekly running distance (km)		
Date	Mean	±	SD
May, 2011	125.8	±	55.3
Jul., 2011	100.6	±	59.0
Apr., 2012	94.8	±	45.3
Oct., 2012	126.1	±	71.0
Feb., 2013	112.5	±	70.3
Oct., 2013	147.9	±	50.2
May, 2014	127.4	±	29.1
Jun., 2014	119.2	±	31.8
Aug., 2014	120.1	±	43.6
Oct., 2014	137.8	±	32.8

Table3. Changes in u-NTX during stress fracture

	Rate of over(%)			
	SF group (n=6)	NSF group (n=13)	Fisher's exact test	Odds ratio
Normal value＋ 1SD	83.3	84.6	NS	0.9
Normal value＋1.5SD	83.3	23.1	$p<0.05$	16.6
Normal value＋ 2SD	83.3	7.7	$p<0.01$	60.0

SF: stress fracture, NSF: not stress fracture, NS: non-significance

“Rate of over” of the SF group : The percentage of participants whose u-NTX value when stress fracture developed was over the normal value + 1 SD, 1.5 SD or 2 SD.

“Rate of over” of the NSF group: The percentage of participants whose highest value in normal value measurement was over normal value + 1 SD, 1.5 SD or 2 SD.