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The impact of serum testosterone level to reflect age-related multi-organ functions

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Abstract. The aim of this study is to examine the correlation between aging, serum total testosterone and biomarkers of multiple organ functions in men. The participants consisted of 12,547 outpatients, whose serum testosterone level was measured. A multiple regression analysis was conducted to determine whether biomarkers including hemoglobin (Hb), hematocrit (Hct), luteinizing hormone (LH), follicle stimulating hormone (FSH), alkaline phosphatase (ALP), albumin (ALB), creatinine (Cre), aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose (Glu), C-reactive protein (CRP), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) values were associated with serum total testosterone concentration. Significant correlations ($p < 0.05$) were found between total testosterone and Hb, Hct, LH, FSH, ALP, ALB, TG, HDL-C, AST, ALT, Glu, and CRP. In addition, significant correlations ($p < 0.05$) were found between Hb, Hct, LH, FSH, ALP, ALB, TG and HDL-C associated with [age × testosterone]. This large-scale study provided new insights into correlations between serum testosterone and biomarkers associated with age-related diseases, suggesting that testosterone is especially important for maintaining homeostasis in aging males. Thus, hypogonadism in elderly patients may be associated with multiple organ dysfunctions.

Key words: Aging, Biomarkers, Homeostasis, Lifestyle disease, Testosterone

AGING MALES are prone to develop chronic diseases such as obesity, type 2 diabetes, osteoporosis, arthritis, nonalcoholic fatty liver disease, depression, Alzheimer's disease, sarcopenia and erectile dysfunction. Oftentimes the onset of these diseases points to an aging-induced endocrine deregulation rather than lifestyle changes. Interestingly, men's serum testosterone levels have been associated with the onset/worsening of the chronic diseases listed above and seem to decrease almost in parallel with that onset [1].

Data from numerous studies indicate that testosterone seems to play an important role in the regulation of metabolic and cardiovascular mechanisms of the male body. In recent years, the rapidly aging population has become a serious social issue in Japan, leading to an increasing demand for medical care for elderly people. As the population ages, the number of patients with sarcopenia and frailty is also increasing, and the relationship between this increase and low testosterone has also become clear [2]. The purpose of this study is to elucidate a potential association between aging, serum testosterone and several biomarkers for a deeper understanding of how testosterone affects multiple diseases in the aging male population and potentially establish serum testosterone levels as a predictor for those diseases. The controversial question of whether aging itself could cause low serum testosterone levels and the subsequent onset of chronic diseases is especially crucial for an understanding of disease development in the elderly male population.

This is the first large-scale study in Asia to investigate the association between testosterone and serum biomarkers with aging.

Materials and Methods

Subjects

This study was based on data from 12,547 patients whose serum testosterone level was measured at the

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Juntendo University Hospital, Tokyo, between 2008 and 2019. Men under 20 years of age were excluded from our analysis along with female patients, leaving a total of 7,982 subjects. Ages ranged from 20 to 98 years. For each patient, the day with the largest number of test items was extracted, and if there were multiple days, the oldest date was selected. Body mass index (BMI) was measured based on the patients' height and weight. Approval was obtained from the Hospital Research Ethics Board (Approval number: H19-0128).

Blood collection

Blood samples were collected in a fasted state in the morning. Blood for serum hemoglobin (Hb) and hematocrit (Hct) was collected in plain tubes. Blood for serum luteinizing hormone (LH), follicle stimulating hormone (FSH), alkaline phosphatase (ALP), albumin (ALB), creatinine (Cre), aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose (Glu), C-reactive protein (CRP), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) concentrations was collected in plain tubes and then centrifuged at 3,000 rpm for 5 minutes. Blood for serum testosterone was collected in plain tubes and then centrifuged at 3,000 rpm for 10 minutes.

Hb levels were measured by the sodium lauryl sulfate-hemoglobin method using an XE-5000 (Sysmex, Hyogo, Japan). Hct levels were measured by the sheath flow DC method using an XE-5000 (Sysmex). ALB levels were measured by the bromocresol purple method using a Hitachi 7600 or LABOSPECT 008 (Hitachi High-Tech, Tokyo, Japan). Cre, AST, ALT, Glu and ALP levels were measured by the enzymatic method using a Hitachi 7600 or LABOSPECT 008 (Hitachi High-Tech). CRP levels were measured by latex agglutination turbidimetric immunoassay using a Hitachi 7600 or LABOSPECT 008 (Hitachi High-Tech). TG levels were measured by the enzymatic colorimetric method using a Hitachi 7600 or LABOSPECT 008 (Hitachi High-Tech). HDL-C and LDL-C levels were measured by direct method using a Hitachi 7600 or LABOSPECT 008 (Hitachi High-Tech).

Total testosterone levels were measured by chemiluminescent immunoassay using an Architect i2000SR (Abbott Japan, Tokyo, Japan). A Modular EEE or cobas e 801 (Roche Diagnostics, Basel, Switzerland) was used for measuring LH and FSH levels by electrochemiluminescence immunoassay.

Statistical analysis

A multiple regression analysis based on serum total testosterone was performed using biomarkers including Hb, Hct, LH, FSH, ALP, ALB, Cre, AST, ALT, Glu, CRP, TG, HDL-C, and LDL-C values as dependent variables with age and testosterone as explanatory variables. All variables were standardized to reveal standardized partial regression coefficients for age, testosterone, and [age × testosterone]. The advantage of standard values is that different data sets and variables can be compared on the same scale. This facilitates comparison of data expressed in different distributions and units, and allows different data sets to be evaluated on a uniform scale. An interaction effect is the joint effect of testosterone and age on each biomarker. It is assumed that the influence of testosterone on biomarkers is not constant and depends on age. Therefore, in addition to the main effect of testosterone on biomarkers, by considering the interaction effect, in which the influence of testosterone increases or decreases depending on age, we find that the older males are, the stronger the influence of testosterone. This makes it possible to verify whether it becomes stronger (or weaker). Age was treated as a continuous variable. Additionally, the outcome and explanatory variables were standardized to obtain standard partial regression coefficients. Although the mean and variance of testosterone and age differ, this calculation enables us to determine which variable (age, testosterone, and interaction) has a greater effect on each biomarker by simply comparing the standard partial regression coefficients. *P*-values corresponding to the standardized coefficients were calculated to determine statistical significance. The significance level was set at $p < 0.05$. As standardized coefficients were used, any significant association of a biomarker with age, testosterone, and/or [age × testosterone] would be free of other variables' influence, and thus similar to significant correlation in the correlation analysis. In addition, one-way ANOVA with Bonferroni was used for pairwise comparisons of the mean clinical values among the four testosterone groups; Q1: 0.04–2.96 ng/mL, Q2: 2.97–4.5 ng/mL, Q3: 4.6–6.1 ng/mL, Q4: 6.2–30.2 ng/mL. Interquartile range (IQR) indicates a measure of statistical dispersion, which is the spread of the data. Values above or below 1.5 IQR were excluded from this analysis as outliers. The analyses were performed using R software, version 4.1.3 (R Development Core Team, 1993; www.r-project.org).

Results

Basic characteristics

Patient characteristics are presented in Table 1. We aimed to include as many cases as possible without excluding pre-existing diseases and backgrounds. In this analysis, we did not take into account the oral medication status of the patients.

Table 1 Patient background and blood collection result

	Mean ± SD	95% CI	Min–Max
Anthropometric data			
Age (years)	63.4 ± 14.1	63.1–63.7	20.0–98.0
Height (cm)	166.8 ± 14.1	166.6–166.9	111.3–207.0
Body weight (kg)	66.3 ± 12.0	66.0–66.6	21.4–138.9
BMI (kg/m^2)	23.8 ± 3.64	23.70–23.88	12.4–42.9
Hematological and blood biochemical data			
Hb (g/dL)	14.1 ± 1.66	14.1–14.2	5.9–20.1
Hct (%)	41.7 ± 4.57	41.6–41.8	17.7–57.0
ALB (g/dL)	4.2 ± 0.4	4.1–4.2	1.0–5.5
Cre (mg/dL)	0.93 ± 0.83	0.91–0.95	0.15–16.24
AST (IU/L)	25.0 ± 21.2	24.5–25.5	4–1,306
ALT (IU/L)	24.3 ± 49.5	23.2–25.5	1–3,922
Glu (mg/dL)	110.1 ± 33.5	109.3–111	36–505
ALP (IU/L)	234.8 ± 214.3	228.7–240.8	66–6,715
CRP (mg/dL)	0.40 ± 1.50	0.36–0.44	0–38.7
TG (mg/dL)	148.9 ± 109.2	145.4–152.4	25–1,785
HDL-C (mg/dL)	52.4 ± 14.6	51.8–52.9	5–138
LDL-C (mg/dL)	111.1 ± 30.9	109.9–112.2	20–253
Hormonal data			
testosterone (ng/mL)	4.55 ± 2.75	4.49–4.61	0.04–30.2
LH (mIU/mL)	7.46 ± 7.69	7.27–7.65	0.1–132.6
FSH (mIU/mL)	12.45 ± 13.95	12.10–12.79	0.1–168.3

Correlations between testosterone and other laboratory parameters

Given p-values corresponding to standardized coefficients, we found a significant association ($p < 0.05$) between testosterone and each of the following biomarkers: Hb ($p < 0.001$), Hct ($p < 0.001$), LH ($p < 0.001$), FSH ($p = 0.004$), ALP ($p < 0.001$), ALB ($p = 0.004$), TG ($p < 0.001$), HDL-C ($p < 0.001$), AST ($p < 0.001$), ALT ($p < 0.001$), Glu ($p < 0.001$) and CRP ($p < 0.001$). On the other hand, no significant association was found between testosterone and Cre or LDL-C. Each biomarker is used as an objective variable, while age and testosterone are used as explanatory variables. Furthermore, contribution of [age × testosterone] to the following biomarkers was significant ($p < 0.05$): Hb ($p < 0.001$), Hct ($p < 0.001$), LH ($p < 0.001$), FSH ($p = 0.001$), ALP ($p < 0.001$), ALB ($p = 0.046$), TG ($p < 0.001$) and HDL-C ($p = 0.005$). Testosterone positively correlates with Hb, Hct, LH, FSH, and ALB. This correlation increases with age; testosterone negatively correlates with ALP. This correlation increases with age. However, the correlation between testosterone and TG and HDL-C decreases with age. The distribution of total testosterone levels by age group is shown in Fig. 1. The association between each biomarker, testosterone and age is depicted in Fig. 2, with a greater slope indicating a stronger association. Fig. 1 shows a scatter plot of the relationship between each biomarker and testosterone for each age group in 10-year increments. Each variable is standardized and expressed in arbitrary units. Note that standardization is geometrically an operation of translation and scaling, so the shape of the data distribution remains unchanged. We observed varying effects of testosterone depending on the age group. For convenience of illustration, each graph is shown separated, but age is treated as a continuous variable in the regression analysis. The blood data based on the testosterone values divided into quartiles is depicted in Table 2. In the analysis of each biomarker based on testosterone quartiles, statistical significance between groups was observed in Hb, Hct, LH, ALP, ALB, TG, HDL-C, AST, ALT, Glu and CRP (Table 2). A correlation was found between BMI and testosterone with aging. Fig. 3 shows the relationship between BMI and testosterone by age group.

Discussion

Hypogonadal men often display upregulated levels of visceral fat. Indeed, a correlation was found between BMI and serum level of testosterone in this study

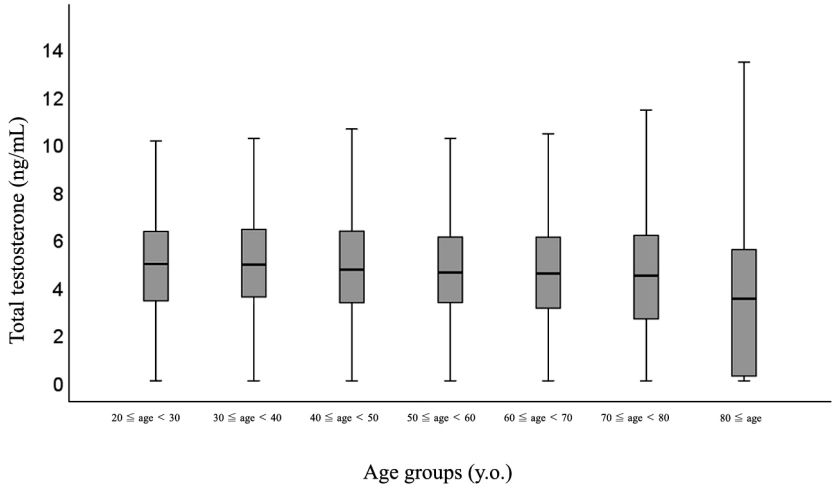

Fig. 1 The distribution of total testosterone by age group
Total testosterone levels have been shown to decline slowly with increasing age.

population. Obesity and metabolic syndrome are known to be associated with decreased testosterone, and the correlation is mutual [3]. Obesity induces increased levels of leptin, insulin, inflammatory cytokines, and estrogen, which are suggested to be associated with secondary hypogonadism [3]. Since testosterone suppresses lipoprotein lipase (LPL), adipocyte hypertrophy occurs in low-testosterone conditions [4]. In addition, testosterone increases the number of β-receptors in adipocytes, suppresses differentiation into adipocytes at the stem cell level, and induces differentiation into muscle cells, so low testosterone results in an increase in adipose tissue [4, 5].

In this study, significant associations ($p < 0.05$) between TG and HDL-C with [age × testosterone] were found, indicating that the correlation between serum testosterone levels and the lipids becomes weaker with age. It has been shown that the TG/HDLC ratio is correlated to cardiovascular diseases (CVD), insulin resistance, and metabolic syndrome [6]. When the TG/HDLC ratio increases, the LDL particles decrease in size, which triggers the fractional esterification rate of the apolipoprotein B-lipoproteins to increase, potentially leading to arteriosclerosis [6]. In our study we observed negative associations between testosterone and TG and positive associations between testosterone and HDL levels, suggesting a protective effect of testosterone with regards to cardiovascular and metabolic diseases. There is a report that HDL-C decreases with aging, but there are also reports that age-related changes in the composition of HDL cause functional impairment [7]. With regard to the effects of testosterone on serum lipids, a positive correlation exists between HDL and circulating T concentrations. HDL particles play a role in immunomodulation, regulation of endothelial cell function, and removal of cholesterol from artery walls through reverse cholesterol transport [8]. Several studies have demonstrated an inverse correlation between T levels and both plasma triglycerides and total cholesterol [9]. In this study, there was a positive correlation between HDL-C and [age × testosterone], and the slope decreased with age, associated with a weaker effect of age-related HDL-C decline.

Low testosterone levels lead to inferior lipolytic responses to catecholamines and elevated LPL in adipose tissue, resulting in impaired triglyceride turnover and body fat accumulation. In addition, the circulating levels of estradiol derived from the aromatization of circulating testosterone is elevated in obese men [10]. The speed of the aromatization of testosterone to estradiol increases with body mass. Serum estradiol levels increase with body mass and affect the hypothalamus, pituitary and testis, resulting in low testosterone levels [11, 12]. It is also known that high triglyceride levels in correlation with low HDL-C increase the risk of CVD. Therefore, testosterone seems to be a crucial factor in the prevention of CVD and should be monitored in individuals with impaired blood lipid levels. Low testosterone has been shown to be associated with a higher TG to HDL-C ratio and increased risk of steatosis [13]. Long-term testosterone replacement therapy has been shown to improve TG and fatty liver index in hypogonadal men [14]. In this study, the association of TG or HDL-C with [age × testosterone] became weaker with aging. It is interesting that the effects of testosterone on either TG or HDL-C manifest in relatively younger generations, since hypogonadism in younger generations would be affected by high TG or low HDL-C, which may lead to CVD events in later life.

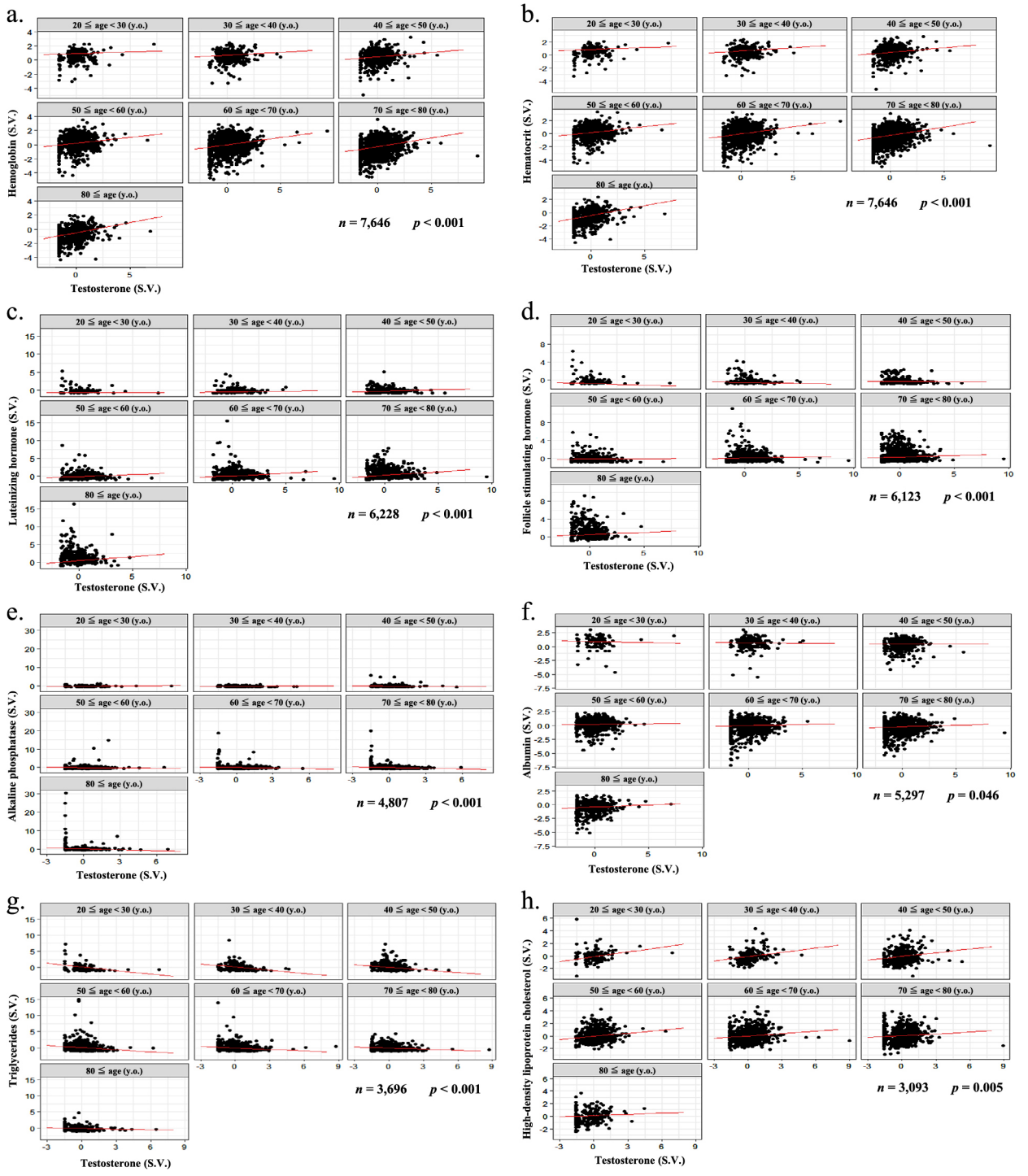

Fig. 2 Associations between each biomarker and [age × testosterone]

"*n*" indicates the number of patients for each biomarker, while "*p*" indicates significance level.
"S.V." indicates standard value.
The red line shows the regression line when assuming the median value of each age group (25 years old for "20 to 30 years old"). A stronger slope with aging is almost synonymous with a stronger correlation. a. Hb, b. Hct, c. LH, d. FSH, e. ALP, f. ALB, g. TG, h. HDL-C.

It is well documented that low levels of serum testosterone are associated with anemia. Testosterone is known to stimulate erythropoiesis in the bone marrow and increase Hb and Hct levels [15, 16]. Low levels of testosterone might be a causal factor for anemia in the aging male and should be monitored in those patients. Testosterone might increase the number of red blood cells through downregulation of hepcidin, triggering an

Table 2 Mean laboratory test values for each testosterone group (Q1–Q4*)

	Q1 (0.04–2.96 ng/mL)	Q2 (2.97–4.5)	Q3 (4.6–6.1)	Q4 (6.2–30.2)
Hb (g/dL)	13.32 ± 1.88	14.36 ± 1.50†	14.40 ± 1.45†	14.38 ± 1.50†
Hct (%)	39.37 ± 5.23	42.32 ± 4.12†	42.48 ± 3.92†	42.39 ± 4.10†
ALB (g/dL)	4.07 ± 0.57	4.21 ± 0.40†	4.19 ± 0.37†	4.16 ± 0.39†
Cre (mg/dL)	0.94 ± 0.78	0.94 ± 0.96	0.94 ± 0.83	0.91 ± 0.77
AST (IU/L)	28.24 ± 36.63	24.23 ± 15.15†	23.49 ± 9.65†	24.12 ± 11.18†
ALT (IU/L)	28.61 ± 93.64	25.05 ± 21.89	22.16 ± 14.78†	21.67 ± 17.50†
Glu (mg/dL)	116.32 ± 39.80	113.18 ± 35.56	106.87 ± 28.52†‡	104.95 ± 28.22†‡
ALP (IU/L)	275.87 ± 356.14	216.32 ± 76.29†	213.44 ± 82.08†	223.93 ± 155.32†
CRP (mg/dL)	0.81 ± 2.52	0.30 ± 0.90†	0.25 ± 0.85†	0.22 ± 0.83†
TG (mg/dL)	165.24 ± 118.54	169.08 ± 134.92	140.18 ± 88.21†‡	121.13 ± 80.38†‡§
HDL-C (mg/dL)	50.77 ± 15.39	49.85 ± 13.25	52.28 ± 13.23‡	56.27 ± 15.58†‡§
LDL-C (mg/dL)	111.40 ± 32.46	109.45 ± 31.16	111.98 ± 30.38	111.20 ± 29.45
LH (mIU/mL)	5.96 ± 10.40	7.71 ± 8.13†	7.85 ± 5.84†	8.16 ± 5.31†
FSH (mIU/mL)	12.53 ± 18.65	12.85 ± 13.69	12.41 ± 12.29	12.03 ± 10.33

* Testosterone levels were divided into quartiles
† $p < 0.01$ *vs.* Q1
‡ $p < 0.01$ *vs.* Q2
§ $p < 0{,}01$ *vs.* Q3

upregulation of iron transport [15]. Furthermore, aromatization of testosterone into estradiol has been shown to stimulate telomerase activity, leading to an upregulation in estrogen receptor α and red blood cell proliferation [15]. Moreover, 5α-reductase of testosterone into dihydrotestosterone is believed to increase erythropoiesis *via* bone marrow. There is also a report that testosterone replacement therapy increases Hct by 5%. Such increases usually occur within the first 48 months of treatment [17]. Even though increases in Hct have been associated with increased risk of cardiovascular diseases in the past, recent research has shown that men with Hct levels > 49.0% had a lower mortality rate compared to men with levels <49.0% [17]. A study showed that 12 months of testosterone treatment significantly increased the Hb levels of patients with unexplained anemia as well as those with anemia from known causes in hypogonadal men [18]. Both Hb and Hct are associated with [age × testosterone]. Thus, hypogonadism in the elderly should receive due attention in daily clinical practice.

LH stimulates the production of testosterone from Leydig cells in the testes. FSH stimulates testicular growth and enhances the production of an androgen-binding protein by the Sertoli cells, which are a component of the testicular tubule necessary for sustaining the maturing sperm cell. Elevated LH and FSH levels suggest primary hypogonadism, whereas low or low-normal LH and FSH levels suggest secondary hypogonadism. Normal LH or FSH levels with low testosterone suggest primary defects in the hypothalamus and/or the pituitary (secondary hypogonadism). One study recorded a longitudinal decrease in serum testosterone while LH and FSH increased in older men [19]. The age-related decline in testosterone is associated with a decrease in testosterone-producing capacity of the aging Leydig cells [20]. Even though the number of Leydig cells does not change with age, the decrease in testosterone production of Leydig cells is due to the relative unresponsiveness of senescent cells to LH [21]. In this study, LH and FSH are associated with [age × testosterone], which indicates that sensitivity of LH and FSH to testosterone in the testis decreases with age.

Most ALP is produced from bone turnover and liver, and highly reflects bone turnover [22]. One study showed that ALP levels were elevated in men with total testosterone levels of <250 ng/dL, suggesting an increase in bone turnover [23]. It is also known that androgens directly and indirectly influence bone health through androgen receptors located on osteoblasts and osteoclasts [23]. In our study, ALP is associated with [age × testosterone], which indicates that older persons' testosterone potentially reflects bone health.

This study was limited by the inclusion of patients with any diseases and health status. A cohort study targeting a healthy population is warranted to further delineate the association between testosterone,

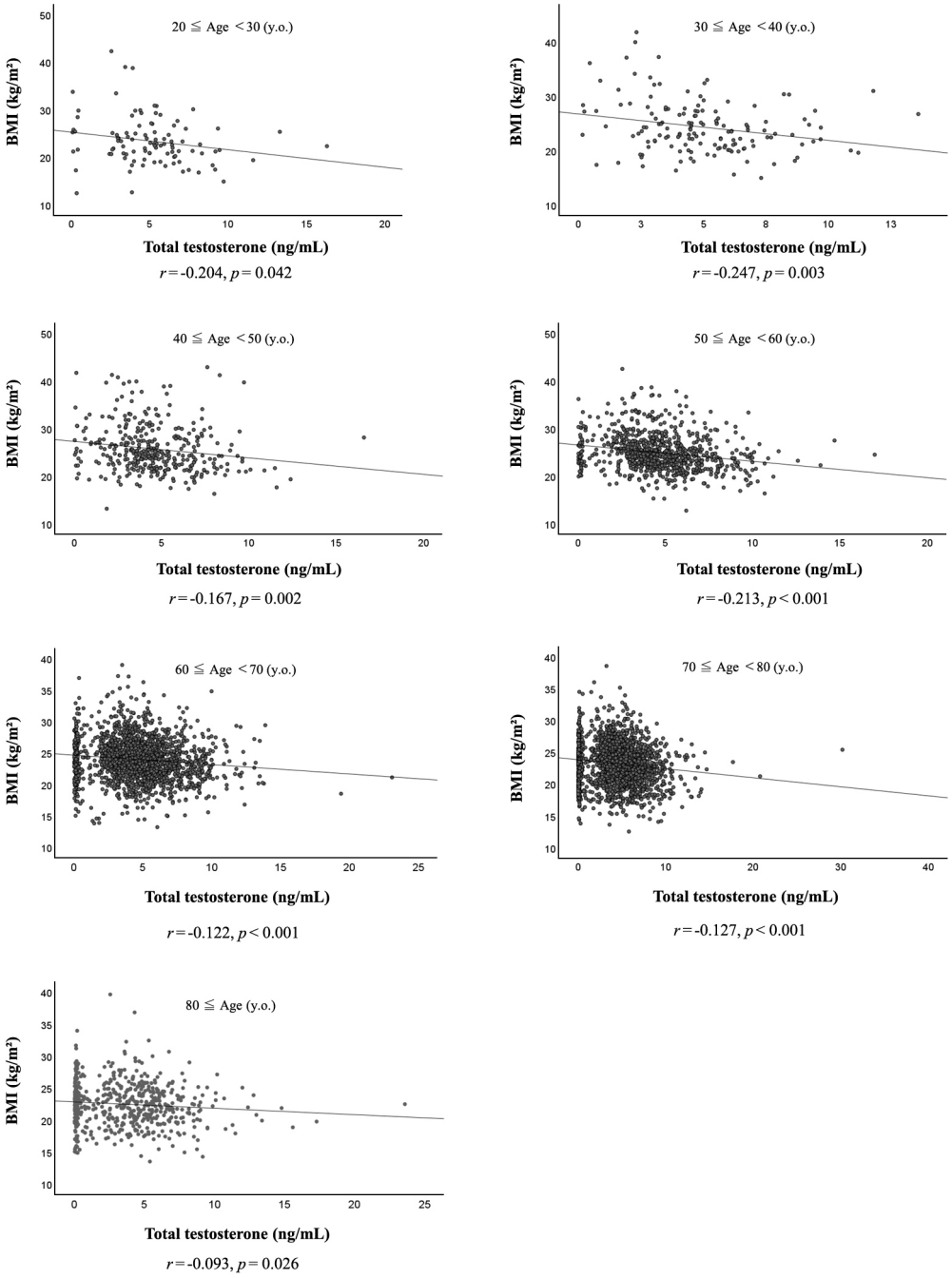

Fig. 3 Association between BMI and testosterone by age group
In all age groups, low testosterone is associated with high BMI.
BMI: Body mass index

biomarkers and aging.

Conclusion

In conclusion, this large-scale study provided new insights into correlations between serum testosterone and biomarkers associated with several age-related diseases. Our study is the first report indicating that the association between serum testosterone and certain biomarkers strengthens with aging.

Each biomarker reflects the function of an organ, and the correlation between testosterone and those biomarkers is considered crucial to understanding organ functions in aging men. Our findings emphasize the importance of including serum testosterone levels in men's health check-ups. The elderly population would

particularly benefit from having testosterone levels monitored to enable rapid and efficient countermeasures to any potential disease onset.

Disclosure

None of the authors have any potential conflicts of interest associated with this research.

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