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Relationship between Low Free Testosterone Levels and Loss of Muscle Mass

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We assessed longitudinal relationships between baseline testosterone and muscle mass changes in Japanese men. Data were collected from community-dwelling 957 adult men who participated in a longitudinal study of ageing biennially from 1997–2010. Appendicular muscle mass (AMM) was measured with dual-energy X-ray absorptiometry at baseline and follow-up examinations. The cut-off point of sarcopenia was defined as a skeletal muscle index (AMM/height^2) < 6.87 kg/m^2. Total testosterone (TT) and free testosterone (FT) were measured with a radioimmunoassay. The calculated FT (cFT) was determined with a formula using albumin, TT, and sex hormone-binding globulin levels. We analyzed 4,187 or 2,010 cumulative data points using generalized estimating equations. Low TT was not associated with sarcopenia. Low cFT (odds ratio = 2.14, 95% confidence interval: 1.06–4.33) and FT (odds ratio = 1.83, 95% confidence interval: 1.04–3.22) were associated with sarcopenia. Low FT may be a predictor of risk for muscle loss in Japanese men.

Sarcopenia is the degenerative loss of skeletal muscle mass and strength associated with ageing[1]. Sarcopenia accelerates the frailty syndrome and leads to deterioration of activities of daily living and quality of life[2,3]. Development of preventative measures for sarcopenia is essential for extending a healthy life expectancy. The European Working Group on Sarcopenia in Older People assumed that muscle loss is a required component of sarcopenia diagnosis and suggested that muscle loss is a symptom of deterioration in muscle strength and physical performances[4]. Estimation of the risks for muscle loss appears to be necessary for developing steps to prevent sarcopenia.

Several cross-sectional studies have reported an association between serum levels of testosterone (T) and muscle mass in men[5,6]. Appendicular muscle mass was correlated with the serum level of free T (FT) in non-Hispanic white men aged 65–97 years[5]. Low appendicular muscle mass was observed in French men in the group with the lowest serum level of FT[6]. Additionally, androgen deprivation therapy for prostate cancer induces a decrease in muscle mass[7]. These studies suggest that the age-related decline in T is a trigger for muscle loss during ageing. Although T is associated with muscle mass, few longitudinal epidemiological studies have been published showing that circulating T levels are associated with prospective decreases in muscle mass[8]. In particular, muscle mass differs among races/ethnicities[9]. Circulating T levels also differ by race/ethnicity or the environment[10–12]. Demonstrating an association between muscle decrease and a decline in T appears to be necessary in each race/ethnicity.

The aim of this study was to determine whether circulating T levels predict muscle loss in middle-aged and elderly Japanese men. We assessed muscle loss with ageing using 10-year follow-up examinations and dual-energy X-ray absorptiometry (DXA) in middle-aged and elderly Japanese men. We also measured serum levels of T and evaluated the association between prospective muscle loss and the T levels in community-living middle-aged and elderly Japanese men using longitudinal analysis.

Results

Table 1 presents the elementary statistics of the participants at baseline according to sarcopenia status. Of the total of 957 men, 249 participants (26.0%) had a diagnosis of sarcopenia at baseline. Participants with sarcopenia were significantly older than those without sarcopenia (each, $p < 0.0001$). Body height ($p = 0.0018$), body weight

Table 1 | The characteristics of participants at the baseline examination. Means ± SE. The p values were obtained using the t-test for continuous data and the chi-square test (Fisher's exact test) for categorical data

	Normal (n = 708)	Sarcopenia (n = 249)	p
Age (years)	58.1 ± 0.4	63.1 ± 0.8	<0.0001
≥60 years (n)	314 (44.4%)	168 (67.5%)	<0.0001
Body height (cm)	164.9 ± 0.2	163.4 ± 0.4	0.0018
Body weight (kg)	65.0 ± 0.3	53.9 ± 0.4	<0.0001
Body mass index (kg/m^2)	23.9 ± 0.1	20.2 ± 0.1	<0.0001
% of body fat	21.8 ± 0.2	20.2 ± 0.3	<0.0001
Appendicular muscle mass (kg)	21.0 ± 0.1	17.2 ± 0.1	<0.0001
Skeletal muscle index (kg/m^2)	7.7 ± 0.02	6.4 ± 0.02	<0.0001
Albumin (mg/ml)	44.4 ± 0.2	44.3 ± 0.2	0.6894
Total testosterone (ng/ml)	5.0 ± 0.06	5.3 ± 0.1	0.0431
Sex hormone binding globulin (nmol/l)†	54.3 ± 1.3	71.4 ± 2.5	<0.0001
Calculated free testosterone (pg/ml)†	78.6 ± 1.1	68.9 ± 1.8	<0.0001
Free testosterone (pg/ml)	13.4 ± 0.1	12.3 ± 0.3	0.0002
Total energy intake (kcal/day)	2363.6 ± 14.2	2170.3 ± 22.7	<0.0001
Total protein intake (g/day)	88.9 ± 0.6	80.9 ± 1.0	<0.0001
Vitamin D intake (μg/day)	10.3 ± 0.2	9.6 ± 0.4	0.1502
Leisure-time physical activity (METs × hour/day)	2.2 ± 0.1	2.2 ± 0.2	0.9518
Current smoker (n)	236 (33.3%)	111 (44.6%)	0.0015
With medical history			
Stroke (n)	26 (3.7%)	10 (4.0%)	0.8063
Heart disease (n)	86 (12.2%)	33 (13.3%)	0.6491
Cancer (n)	17 (2.4%)	13 (5.2%)	0.0281
Diabetes (n)	59 (8.3%)	34 (14.0%)	0.0148
Osteoporosis (n)	4 (0.6%)	10 (4.0%)	<0.0001
Rheumatoid arthritis (n)	41 (5.8%)	18 (7.2%)	0.4171

†cFT and SHBG levels obtained from 327 normal men and 128 men with sarcopenia.

(p < 0.0001), Body mass index (p < 0.0001), and percent of body fat (p < 0.0001) were significantly lower in the sarcopenia group than in the normal group. Appendicular muscle mass (AMM) and skeletal muscle index (SMI) were also significantly lower in the sarcopenia group than in the normal group (each, p < 0.0001). Total T (TT; p = 0.0431) and sex hormone binding globulin (SHBG; p < 0.0001) were significantly higher in the sarcopenia group than in the normal group. cFT and FT were significantly lower in the sarcopenia group than in the normal group (p < 0.0001, p = 0.0002, respectively). Total energy and protein intake were significantly lower in the sarcopenia group than in the normal group (p < 0.0001). No significant differences in albumin, vitamin D intake, and leisure-time physical activity were noted between the normal and sarcopenia groups. The ratio of current smokers (p = 0.0015) in the sarcopenia group was significantly higher than in the normal group. No differences in the ratios of stroke, heart disease, and rheumatoid arthritis history were noted. The ratios of cancer (p = 0.0281), diabetes (p = 0.0148), and osteoporosis (p < 0.0001) history in the sarcopenia group were significantly higher than in the normal group.

Table 2 presents the frequencies according to sarcopenia and the T level status at baseline. No differences in the ratio of sarcopenia between the normal TT group and the low TT group were observed. The ratio of sarcopenia in the low cFT group was significantly higher than that in the normal cFT group (p = 0.0353). The ratio of sarcopenia in the low FT group was also significantly higher than that in the normal FT group (p = 0.0002).

Among the 4,187 cumulative samples, the numbers of samples in the normal and sarcopenia groups were 3,084 (73.7%) and 1,103 (26.3%), respectively. The numbers of participants with low TT in the normal muscle status group (n = 3,084) and the sarcopenia group (n = 1,103) were 141 (4.6%) and 67 (6.1%), respectively (p = 0.0487). The numbers of participants with low FT in the normal muscle status group (n = 3,084) and the sarcopenia group (n = 1,103) were 103 (3.3%) and 87 (7.9%), respectively (p < 0.0001). Among the 2,010 cumulative samples that were analyzed for cFT, the numbers of samples in the normal and sarcopenia groups were 1,460 (72.6%) and 550 (27.4%), respectively. The numbers of participants with low cFT in the normal muscle status group (n = 1,460) and the sarcopenia group (n = 550) were 56 (3.8%) and 40 (7.3%), respectively (p = 0.0013).

The results from the generalized estimating equations (GEE) analyses, controlling for the effects of repeated observations within

Table 2 | The testosterone levels and sarcopenia status at the baseline examination. The p values were obtained using the chi-square test

	Normal (Skeletal muscle index ≥ 6.87 kg/m^2)		Sarcopenia (Skeletal muscle index <6.87 kg/m^2)	
	n	%	n	%
Total testosterone				
Normal (≥2.9 ng/ml)	677	74.5	232	25.5
Low (<2.9 ng/ml)	31	64.6	17	35.4
Calculated free testosterone				
Normal (≥46.3 pg/ml)	313	73.0	116	27.0
Low (<46.3 pg/ml)	14	53.9	12	46.1
Free testosterone				
Normal (≥7.7 pg/ml)	681	75.3	224	24.7
Low (<7.7 pg/ml)	27	51.9	25	48.1

Table 3 | Longitudinal relationships between baseline testosterone levels and sarcopenia. The cumulative data were analyzed with generalized estimating equations. Moderator variables: Crude model: none; Model 1: baseline age; Model 2: age, leisure-time physical activity, nutrition intake (total energy, total protein, vitamin D), medical history (stroke, heart disease, cancer, diabetes, osteoporosis, rheumatoid arthritis), and smoking habit at baseline

	Odds ratio (95% confidence intervals)		
Total testosterone	Normal (≥2.9 ng/ml)	Low (<2.9 ng/ml)	p value
n	3979	208	
Crude model	1.00 (Reference)	1.6178 (0.9486 − 2.7592)	0.0774
Model 1	1.00 (Reference)	1.4790 (0.8606 − 2.5416)	0.1566
Model 2	1.00 (Reference)	1.5717 (0.9004 − 2.7434)	0.1116
Calculated free testosterone	Normal (≥46.3 pg/ml)	Low (<46.3 pg/ml)	p value
n	1914	96	
Crude model	1.00 (Reference)	2.6503 (1.3182 − 5.3285)	0.0062
Model 1	1.00 (Reference)	2.1396 (1.0555 − 4.3370)	0.0349
Model 2	1.00 (Reference)	2.1432 (1.0617 − 4.3262)	0.0334
Free testosterone	Normal (≥7.7 pg/ml)	Low (<7.7 pg/ml)	p value
n	3997	190	
Crude model	1.00 (Reference)	2.8915 (1.7116 − 4.8846)	<0.0001
Model 1	1.00 (Reference)	1.9416 (1.1046 − 3.4129)	0.0211
Model 2	1.00 (Reference)	1.8296 (1.0391 − 3.2215)	0.0364

participants and confounding factors, are presented in Table 3. No significant association of TT levels with sarcopenia was observed in any model. The association of the cFT and FT levels with sarcopenia was significant in all models. The odds ratios of sarcopenia in low cFT participants compared to that in normal cFT participants were 2.65 (95% confidence interval [CI], 1.32–5.33; $p = 0.0062$) in the crude model, 2.14 (95% CI, 1.06–4.34; $p = 0.0349$) in model 1, and 2.14 (95% CI, 1.06–4.33; $p = 0.0334$) in model 2. The odds ratios of sarcopenia in low FT participants compared to that in normal FT participants were 2.89 (95% CI, 1.71–4.88; $p < 0.0001$) in the crude model, 1.94 (95% CI, 1.10–3.41; $p = 0.0211$) in model 1, and 1.83 (95% CI, 1.04–3.22; $p = 0.0364$) in model 2.

Discussion

The etiology of sarcopenia is assumed to be multi-factorial, including factors such as ageing, diseases, nutritional deprivation, and inactivity[4]. Few epidemiologic studies have been published about sarcopenia in Japanese people, and the risk factors for sarcopenia are not understood[9]. In this study, significant associations between muscle loss and FT, regardless of whether FT was calculated or measured, remained after adjustment for age, medical history, nutrition intake, and physical activity. Low FT levels appeared to be independently associated with muscle loss in middle-aged and elderly Japanese men, regardless of these factors. Our result is in line with previous studies that reported a relationship between low FT and low muscle mass in men[5,6]. The observed association between muscle loss and FT in this study appears to have biological plausibility. T stimulates protein synthesis and inhibits protein degradation in muscle cells[13,14]. T also increases satellite cell replication and activation in older men[15]. In this study, no significant association between TT levels and muscle loss were observed. However, recent longitudinal cohort studies have reported that elderly American people with higher baseline TT levels have a low risk of decline in appendicular lean mass[8]. Although a progressive decrease in TT levels with ageing is observed in middle-aged and elderly American men[16,17], the TT levels do not change during ageing in Japanese men[21,22]. The decrease in TT may occur at a later stage when hypogonadism has advanced in Japanese men[21]. FT levels may be a good marker for the loss of muscle mass in Japanese men.

In Japanese men, preventing the decline in FT may prevent the loss of muscle mass during ageing. In this cohort, participants in the low cFT group (<46.3 pg/ml) had approximately a 2.1- to 2.7-fold risk of muscle loss compared to those in the normal cFT group (≥46.3 pg/ml) (Table 3). Participants in the low FT group (<7.7 pg/ml) also had approximately a 1.8- to 2.9-fold risk of muscle loss compared to those in the normal FT group (≥7.7 pg/ml) (Table 3). The serum levels of FT decrease by approximately 50%, from the 20 s through the 70 s in Japanese men[14]. The Japanese Urological Association defined the reference value for androgen replacement therapy as a serum level of 8.5 pg/ml FT as measured with radioimmunoassay (RIA)[21]. Thus, the FT level associated with the risk of muscle loss in this cohort was lower than the reference value for androgen replacement therapy for Japanese men. Improvement in circulating FT levels with appropriate therapies, such as androgen replacement therapy or lifestyle interventions, may reduce the risk of muscle loss during ageing.

The effect of ageing on sarcopenia in Japanese men appear to be large. The prevalence of sarcopenia increased significantly with age[1]. In this study, the baseline age of men in sarcopenia group was statistically older than men in normal group (Table 1). The odds ratio of sarcopenia calculated by the model 1 which were controlled for the baseline age were smaller than those by the crude model (Table 3). The muscle loss might have been affected by the age-related accumulation of the various factors, such as a muscle fiber apoptosis or a mitochondrial dysfunction[4].

Approximately 1% to 2% of T in the blood exists as FT[21]. However, the FT values using RIA are much lower than cFT values[22,23]. In fact, serum FT levels were one-fifth to one-sixth of those of cFT in this study (Table 1). The odds ratio of sarcopenia determined by GEE appeared to have been influenced by these results. The odds ratios of sarcopenia determined by cFT were higher than those of FT, except for in the unadjusted crude model (Table 3). The risk of sarcopenia may be underestimated when FT measured by RIA is an index.

Interestingly, appendicular muscle loss was significantly associated with low levels of FT. These results suggest that a threshold level of FT exists for muscle loss, rather than a dose-response relationship. In the previous cross-sectional and longitudinal studies of French and American men, no dose-response relationships were reported between T and muscle mass[6,8]. A minimal serum level of FT may be needed to preserve muscle mass in men, regardless of race/ethnicity.

This study has significant strengths. The longitudinal design of our analyses lends strength to our inferences. Our study that the same individuals were followed over time provided evidence of a causal association between low level of endogenous FT and the appendicular muscle loss. We adjusted our analyses for potential confounders, including age, physical activities, nutrition intake, medical history, and smoking habit. This is the first population study to evaluate the relationship between sarcopenia and circulating T levels.

This study has several limitations. The first limitation is that the odds ratios of the muscle loss were determined based on serum levels of T at baseline. Although T decreases during ageing, the rate of the decline in T varies depending on different environments and lifestyles among individuals[10,11]. Further studies with longitudinal measurements of T may clarify an association between the decrease in T and muscle loss during ageing. Second, women, who have little T compared with men[18], were not examined in this study. In women, serum FT levels also decrease during ageing[18]. Total lean mass is associated with bioavailable T in postmenopausal women[19]. Further studies are needed to determine the role of androgens in preserving muscle mass in women.

In summary, using the longitudinal design of the cohort, we evaluated the association between loss of muscle mass and decline in FT in community-living, middle-aged and elderly Japanese men with a 10-year follow-up duration. Our data confirm that a low FT level is a significant predictor of a risk for loss of appendicular muscle. The findings in this study may be beneficial for developing methods to prevent sarcopenia in Japanese men.

Methods

Participants. The participants in this study were from the National Institute for Longevity Sciences-Longitudinal Study of Aging (NILS-LSA), which involves ongoing population-based biennial examinations of a cohort of approximately 2,300 persons. The participants in the NILS-LSA were randomly selected from resident registrations and stratified by both decade of age and sex. The NILS-LSA is a comprehensive and interdisciplinary study to observe age-related changes and consists of various gerontological and geriatric measurements, including medical examinations, blood chemical analysis, body composition, anthropometry, nutritional analysis, psychological tests, physical function, and physical activity[20]. Those who did not consent to have blood samples taken and those who did not complete the measurement of muscle mass with DXA were excluded. Participants with a current medical history of Parkinson's disease and androgen preparation users were also excluded. The baseline participants of this study were 957 men aged 40–79 years who completed the first-wave examinations of NILS-LSA between November 1997 and April 2000. Of these, 777 (81.2%) took part in the second-wave examination between April 2000 and May 2002, 689 (72.0%) took part in the third-wave examination between May 2002 and May 2004, 638 (66.7%) participated in the fourth-wave examination between May 2004 and July 2006, 590 (61.7%) took part in the fifth-wave examination between July 2006 and July 2008, and 536 (56.0%) participated in the sixth-wave examination between July 2008 and July 2010. The mean number of repeat visits was 3.2. The total number of visits, including repeat visits, was 4,187; participants from whom the data were derived were 40–88 years of age and took part in the NILS-LSA between November 1997 (the first wave) and July 2010 (the sixth wave).

The study protocol was approved by the Ethics Committee of the National Center for Geriatrics and Gerontology, and written informed consent was obtained from all participants.

Blood sampling and measurement of T. Blood samples were taken between 0800 and 0900 h, separated immediately by centrifugation at 2000 × *g* for 15 min, and sera were frozen and stored in a deep freezer (−80°C). Samples were transferred to the laboratory (SRL Inc., Tokyo, Japan) for TT, FT, SHBG, and albumin measurement.

The serum levels of TT (ng/ml) and FT (pg/ml) were also measured with a RIA using commercially available kits (Diagnostic Products Corporation, Los Angeles, CA, USA). The inter-assay coefficients of variation (CV) were less than 15% for both kits, according to the manufacturer's information. In 455 men who were randomly selected by decade of age at the time of the first-wave examinations, SHBG (nmol/l) was also measured with RIA using a commercially available kit (Diagnostic Products Corporation). The CV was less than 8.5% according to the manufacturer's information. Serum albumin (mg/ml) was measured with nephelometry.

For measuring FT, the detection precision of the equilibrium dialysis was better than that of RIA[21]. However, equilibrium dialysis is not used in Japan, because equilibrium dialysis is difficult to perform, not automated, and largely inaccessible to most clinicians[21]. Thus, the calculated FT (cFT) was derived from serum levels of albumin, TT, and SHBG in 455 male participants[22,23]. In this study, the coefficient of correlation between cFT and FT was 0.80438 ($n = 455$; $p < 0.0001$).

Definition of sarcopenia. Appendicular muscle mass (AMM, kg) and fat mass were assessed with DXA (QDR-4500; Hologic, Bedford, MA, USA). AMM is equal to the appendicular fat-free mass minus bone mineral contents, and is assumed to be an index of the amount of muscle mass.

We used the SMI to evaluate sarcopenia[1]. The SMI was calculated by AMM divided by height squared (kg/m^2). Sarcopenia was defined as muscle mass minus 2 standard deviations below the mean for young adult healthy people[1]. In this study, we set the cut-off point of sarcopenia as SMI $<$ 6.87 kg/m^2. The SMI of 6.87 kg/m^2 was muscle mass minus 2 standard deviations below the mean for young adult healthy people in the Japanese men[9]. Sanada et al.[9] also measured appendicular muscle mass with DXA using the same model (QDR-4500; Hologic) we used in this study. The participants were divided into two groups based on DXA results at baseline and follow-up examinations: the sarcopenia group (SMI $<$ 6.87 kg/m^2) and the normal group (SMI $\geq$ 6.87 kg/m^2).

Other parameters. Body height and weight were measured using a digital scale. Body mass index (kg/m^2) was calculated by weight divided by height squared. Medical history, smoking habit, and use of medications were assessed with questionnaires, which were confirmed by a physician at the medical examinations. All prescribed and non-prescribed medications used during the previous 2 weeks were documented and brought by the participants; the physicians confirmed and coded them. Trained interviewers used a questionnaire and asked the participants about the frequency and exercise intensity (metabolic equivalents: METs) of their physical activity habits during leisure time over the past 12 months[24]. The means per day for leisure-time physical activity (metabolic equivalents; METs × h/day) were calculated. Nutritional intake was assessed with a 3-day diet record[25]. Foods were weighed separately on a scale before cooking or portion sizes were estimated. Participants used a disposable camera to take photographs of meals before and after eating. Registered dietitians used the photographs to complete missing data and telephoned participants to resolve any discrepancies or to obtain further information when necessary. The average over the 3 days for 119 nutrient intake periods was calculated. The means per day for total energy intake (kcal/day), total protein intake (g/day), and vitamin D intake (μg/day) were calculated from the 3-day dietary record.

Statistical analysis. Statistical testing was performed using the Statistical Analysis System release 9.3 (SAS Institute Inc., Cary, NC, USA). A probability level less than 0.05 was considered significant. The results are shown as the means ± standard error (SE). Differences in continuous and class variables between the normal and sarcopenia groups were assessed with t-tests and chi-square tests, respectively. To assess differences in the medical history of osteoporosis between the normal and sarcopenia groups, Fisher's exact test was used because the minimum expected cell size was less than five.

Cumulative data were analyzed using GEE, which take into account the dependency of repeated observations within participants; this is an important feature that is necessary for longitudinal analyses. An additional advantage of GEE is that participants are included regardless of missing values. Thus, participants who were lost to follow-up after early wave examination were also included in the analyses. GEE models were fitted using the GENMOD procedure of SAS. The GENMOD procedure fits generalized linear models. The correlation structure was specified to be autoregressive.

The serum T levels were modeled as dichotomized variables in GEE analyses. In this study, the cut-off values of T were established based on the serum level of FT, because under the current circumstances in Japan, hypogonadism is diagnosed using the serum level of FT. The FT decreases during ageing, whereas the TT levels do not change during ageing in Japanese men[21,22]. In addition, measurement of SHBG cannot be performed for the diagnosis of hypogonadism in Japan, because SHBG measurement is not included in the gonadal function tests covered by health insurance. The participants were divided into two groups based on the serum level of FT in the baseline examination: the low level group ($<$7.7 pg/ml) and the normal level group ($\geq$7.7 pg/ml). The FT of 7.7 pg/ml, which was minus 2 standard deviations below the mean for healthy Japanese men aged 40–49 years, was approximately equal to the 5th percentile of participants in this study[21]. Thus, the cut-off values of TT and cFT were defined as the 5th percentile of serum levels (TT 2.9 ng/ml; cFT 46.3 pg/ml) in participants.

Analyses were carried out with an unadjusted crude model and several adjusted models, controlling for different combinations of confounding variables: age was taken as a moderator variable in model 1; age, leisure-time physical activity, nutrition intake (total energy, total protein, vitamin D), medical history (stroke, heart disease, cancer, diabetes, osteoporosis, rheumatoid arthritis), and smoking habit were considered moderator values in model 2.

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Author contributions

A.Y. designed the study, carried out the statistical analyses and wrote the manuscript. R.O., R.K., I.K. and T.O. participated in data collection and analysis. F.A. and H.S. revised the manuscript and managed the overall project.

Additional information

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