Serum free testosterone and metabolic syndrome in Japanese men

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Abstract. To examine the association between late-onset hypogonadism (LOH) and metabolic syndrome (Mets) or insulin resistance in the Japanese adult male population, we evaluated anthropometric parameters, indices of glucose and lipid metabolism, and hormones related to sexual function in 274 men (mean age: 46.0 ± 11 years) who underwent general health checks. Seventy subjects (25.5 %) were diagnosed as having Mets, while the frequency of LOH was 8.0 %. Glycated hemoglobin was normal in the majority of participants (94.9 %). The serum free testosterone (FT) level was significantly lower in the Mets (+) group than in the Mets (-) group (11.7 ± 4.0 vs. 14.7 ± 4.6 pg/mL, \( p < 0.0001 \)). FT decreased significantly along with an increase in the number of Mets components. Likewise, the number of Mets components showed a significant difference among the eugonadal, borderline, and hypogonadal groups (2.2 ± 1.4, 1.5 ± 1.4, and 0.9 ± 1.1, respectively). After adjustment for age, body mass index (BMI), and waist circumference (WC), FT was still significantly correlated with Mets (standard partial regression coefficient = -0.0971; 95 % confidence interval = -0.1936 ~ -0.0006; \( p = 0.048 \)). A compensatory increase of gonadotropins was not seen in the hypogonadal group. Among Japanese men who were mainly without diabetes, FT was associated with Mets independently of age, BMI, and WC. Mets and insulin resistance may decrease serum testosterone via induction of hypogonadotrophic hypogonadism, and the reduction of testosterone may in turn cause further obesity and insulin resistance, consequently initiating a vicious cycle.

Key words: Free testosterone, Metabolic syndrome

SERUM TESTOSTERONE consists of three components, which are 1) testosterone bound to sex hormone binding globulin, 2) testosterone bound to albumin, and 3) free testosterone. Although free testosterone (FT) is a minor component (2-3 %), it is the only component with hormonal activity [1]. Because albumin-bound testosterone can be easily dissociated, BAT (which is the sum of FT and albumin-bound testosterone) has been widely used as a surrogate measure for active testosterone [2]. Although BAT is thought to be the gold standard for measuring testosterone, the assay involves equilibrium dialysis and is a delicate, expensive, and time-consuming procedure. Therefore, it may not be suitable for population-based studies. Recently, a reliable kit for measurement of FT by immunoassay was developed, and the Japanese Urological Association and the Japanese Association for Men’s Health have verified that FT is strongly correlated with BAT and the decline of FT with aging closely corresponds with that of BAT, and thus have recommended diagnosis of late-onset hypogonadism (LOH) based on FT values [3, 4].

The metabolic syndrome (Mets) is a cluster of visceral obesity, insulin resistance, hypertension, glucose intolerance, and dyslipidemia that substantially increases the risk of type 2 diabetes and cardiovascular disease [5]. Recent studies have suggested that patients with LOH and low levels of testosterone often suffer from the Mets or its components [6, 7]. In
addition, testosterone supplements decrease visceral fat and ameliorate insulin resistance [8]. However, relevant data on the Japanese population are insufficient, especially with regard to non-diabetic subjects. Therefore, we examined FT by direct immunoassay and evaluated its association with Mets and insulin resistance in the Japanese adult male population.

Materials and Methods

Subjects
This study was a cross-sectional survey of 274 Japanese adult men (mean age: 46.0 ± 11 years) who underwent general health checks. Men who had been diagnosed as having diabetes and those being treated with insulin or antidiabetic agents were excluded. The present study was approved by the Ethical Committee of St. Marianna University, and all subjects gave written informed consent.

Anthropometric measurements
All measurements were performed during the morning of a health examination after an overnight fast. Waist circumference (WC) was measured at the umbilicus in the standing position at the end of expiration during normal breathing. Blood pressure was measured in the sitting position after a 5-min rest before breakfast.

Definition of Mets and low serum FT
Mets was diagnosed according to modified National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP-III) guidelines [9], as the presence of three or more of the following: 1) WC ≥ 90 cm; 2) systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg or use of antihypertensive medication; 3) high-density lipoprotein (HDL) cholesterol < 40 mg/dL or lipid medication use; 4) fasting plasma glucose ≥ 110 mg/dL or use of antidiabetic medication, and 5) triglycerides ≥ 150 mg/dL or lipid-lowering therapy. A low serum FT level was defined according to the clinical practice guideline for LOH of the Japanese Urological Association/Japanese Society for Study of the Aging Male [3]. According to this guideline, the FT level for diagnosis of LOH was set at the mean – 2 SD of the level for men in their 20s (8.5 pg/mL).

Measurement of hormone levels
Blood samples were obtained at 8:30 – 9:00 AM after an overnight fast. The blood was immediately centrifuged for 15 min at 4°C, and serum was stored at -80°C until assay. FT was measured with a radioimmunoassay (RIA) kit obtained from Diagnostic Product Co. (Los Angeles, CA, USA), while LH and FSH were measured by using chemiluminescent enzyme immunoassay kits from Abbott Japan Co. (Tokyo, Japan). Insulin was measured with a chemiluminescent enzyme immunoassay kit from Fujirebio Inc. (Tokyo, Japan).

Measurements of other parameters
Glycated hemoglobin (HbA1C), triglycerides, HDL cholesterol, low-density lipoprotein (LDL) cholesterol, and glucose were measured by standard methods. HbA1c results were reported in National glycohemoglobin Standard Program Units (%). Insulin resistance was estimated from the homeostasis model assessment insulin resistance index \([\text{HOMA-R: fasting plasma glucose} \times \text{fasting immunoreactive insulin (IRI)} /405]\) [10].

Statistical analysis
Results are presented as the mean ± SD. The unpaired Student’s \(t\)-test or the \(\chi^2\) test was conducted for inter-group comparisons. Clinical parameters were compared among groups by one-way analysis of variance (ANOVA), and post hoc multiple comparison using Bonferroni’s or Scheffe’s protected least significant difference test. For these analyses, \(p<0.05\) was considered to indicate statistical significance. Multiple regression models were used to investigate the association between testosterone and Mets, and to adjust for potential confounders. The 95% confidence interval (95% CI) was estimated to describe the magnitude of associations. All analyses were performed with the Stat View 5.0 statistical software package (Abacus Concepts, Berkeley, CA, USA).

Results
A clinical profile of the 274 men is presented in Table 1. Subjects who were overweight or obese (BMI ≥ 25 kg/m²), as well as subjects with abdominal obesity (WC ≥ 90 cm), hypertension, abnormal FPG, low HDL cholesterol, and elevated triglycerides accounted for 33.2, 26.3, 53.3, 29.0, 7.0, and 32.0%, respectively. Seventy subjects (25.5%) were diagnosed as having Mets. While 29.0% of subjects showed elevation of FPG, 260 subjects had an HbA1c < 6.2% (94.9%)
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As shown in Fig. 1A, FT was significantly lower in the Mets (+) group than in the Mets (-) group (11.7 ± 4.0 vs. 14.7 ± 4.6 pg/mL, p<0.0001). When FT was stratified by the number of Mets components, it decreased significantly along with an increase in the number of components (15.5 ± 4.7 for 0 component, 14.1 ± 4.4 in 1 or 2, and 11.7 ± 4.0 pg/mL for 3 or more) (Fig. 1B). Similar results were obtained with age-matched groups of subjects in their 40s and 50s (Figs. 1C and 1D). When subjects were classified by FT values into three groups, eugonadal group (FT > 16.8 pg/mL, n=68), a borderline group (FT ≤ 16.8 and ≥ 8.5 pg/mL, n=194), and a hypogonadal group (FT < 8.5 pg/mL, n=22), the number of Mets components increased significantly in the same order (2.2 ± 1.4, 1.5 ± 1.4, and 0.9 ± 1.1, respectively). The result was similar for the age-matched groups of subjects in their 40s and 50s. As shown in Fig. 2, LH and FSH levels were did not differ among the three groups classified by FT levels (Fig. 2A), and the same result was also found in the age-matched groups of subjects in their 40s and 50s (Fig. 2B).

Table 2 summarizes the result of multiple regres-
testosterone levels may have been due to hypogonadotrophic hypogonadism [12]. However, the interaction between testosterone and Mets/insulin resistance in Japanese persons is not fully understood. The present study revealed two main findings in Japanese men who were mostly without diabetes: 1) a significant association of a low serum FT level with Mets that was independent of age, BMI and WC, and 2) lack of significant elevation of the serum LH and FSH levels in the low FT group compared with the normal FT groups, suggesting the presence of hypogonadotrophic hypogonadism. Fukui et al. previously reported that FT was lower in men with type 2 diabetes than in healthy subjects [13]. The present study indicated that a low FT concentration is associated with Mets, as did previous studies of BAT [11]. Thus, measurement of FT by RIA may be a simple and reliable method for analysis, with age, BMI, and HOMA-R being selected as significant correlates of FT. The results of multiple regression analysis of factors related to Mets are shown in Table 3. In addition to the individual components Mets, FT was selected as a significant determinant of this syndrome. After adjustment for age, BMI, and WC, FT was still significantly correlated with Mets (standard partial regression coefficient = -0.0971; 95% CI = -0.1936 ~ -0.0006; p = 0.048).

**Discussion**

Recent cross-sectional and prospective studies have shown significant association of testosterone with type 2 diabetes or Mets [11]. These studies also revealed inappropriately low LH and FSH levels in the subjects with low testosterone levels, so the reduction of testosterone levels may have been due to hypogonadotrophic hypogonadism [12]. However, the interaction between testosterone and Mets/insulin resistance in Japanese persons is not fully understood. The present study revealed two main findings in Japanese men who were mostly without diabetes: 1) a significant association of a low serum FT level with Mets that was independent of age, BMI and WC, and 2) lack of significant elevation of the serum LH and FSH levels in the low FT group compared with the normal FT groups, suggesting the presence of hypogonadotrophic hypogonadism. Fukui et al. previously reported that FT was lower in men with type 2 diabetes than in healthy subjects [13]. The present study indicated that a low FT concentration is associated with Mets, as did previous studies of BAT [11]. Thus, measurement of FT by RIA may be a simple and reliable method for...
assessment of hypogonadism.

The mechanism leading to a decrease of FT in Mets is still unclear. Previous studies have suggested that elevation of plasma leptin associated with obesity inhibits testosterone production by Leydig cells via the leptin receptor [14]. However, there was no compensatory elevation of gonadotrophins in the low FT group in the present study, suggesting that reduction of FT may be due to both primary hypogonadism and hypogonadotrophic hypogonadism. The exact mechanism leading to the occurrence of hypogonadotrophic hypogonadism is also unclear. Dandona et al. compared the prevalence of hypogonadism in age-matched men with type 1 and type 2 diabetes, and reported that only 6% of type 1 patients were hypogonadal compared with 26% of type 2 patients [15]. Interestingly, they observed an inverse correlation of testosterone with BMI in type 1 patients as well as type 2 patients. Thus, hypogonadotropic hypogonadism may not be induced hyperglycemia, but may be related to obesity and insulin resistance. Kahn et al. generated neuron-specific insulin receptor knock-out mice (NIRKO) [16], and observed a 60-90% decrease of LH along with normal or supernormal LH release in response to GnRH stimulation in male mice. These results imply that insulin acts on hypothalamic neurons to facilitate GnRH release and thus promote gonadotrophin secretion. However, it is unclear or not hypothalamic insulin resistance occurs in Mets. It has been reported that CRP is elevated in patients with hypogonadotropic hypogonadism and is inversely correlated to the plasma testosterone concentrations in type 2 diabetic patients, suggesting an important role of inflammation [17]. The inflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-1β have been shown to reduce hypothalamic GnRH and LH secretion in animals and in vitro [18, 19]. Furthermore, high plasma estradiol level derived from insulin resistance and hyperglycemia is associated with inhibition of gonadotrophins via hypothalamic estrogen receptors [20]. Although we did not measure leptin, CRP, and inflammatory cytokines, such reports suggest that Mets or related abnormalities may play a causative role in the reduction of testosterone levels.

A previous study of mouse pluripotent stem cells demonstrated that testosterone regulates body composition by promoting the commitment of mesenchymal cells to the myogenic lineage and inhibiting differentiation toward the adipogenic lineage, suggesting a reciprocal effect of androgens on muscle and fat mass in men [21]. Testosterone also inhibits the differentiation of 3T3-L1 preadipocytes into adipocytes in vitro [22]. Another study showed that testosterone increases lipolysis and adrenoreceptor expression by male rat adipocytes [23]. Furthermore, effects of testosterone on expression of the insulin-receptor substrate (IRS)-1 and glucose transporter (GLUT)-4 genes have been observed in vitro [11]. These studies suggest that testosterone may promote insulin sensitivity and, while deficiency of testosterone induces obesity and insulin resistance. Laaksonen et al. followed 702 middle-aged men without diabetes or Mets at baseline for 11 years, and they observed that the subjects with baseline testosterone levels in the lower quartile had a several-fold higher risk of developing Mets and diabetes after adjustment for age [24]. These reports suggest the possibility that a decline of testosterone may induce insulin resistance or even diabetes per se.

### Table 3: Multiple regression analysis of factors related to metabolic syndrome (Mets) after adjustment for age.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standard partial regression coefficients</th>
<th>Standard error</th>
<th>95% confidence intervals</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free testosterone (pg/mL)</td>
<td>−0.0234</td>
<td>0.01050</td>
<td>(−0.0442 to −0.00278)</td>
<td>0.02639</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.04861</td>
<td>0.00616</td>
<td>(0.036491 to 0.060734)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>0.01426</td>
<td>0.00471</td>
<td>(0.004988 to 0.023539)</td>
<td>0.00271</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>0.02051</td>
<td>0.00677</td>
<td>(0.007181 to 0.033832)</td>
<td>0.00244</td>
</tr>
<tr>
<td>FPG (mg/dL)</td>
<td>0.01168</td>
<td>0.00276</td>
<td>(0.006076 to 0.016951)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>0.00335</td>
<td>0.00051</td>
<td>(0.002343 to 0.004356)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>−0.01248</td>
<td>0.01051</td>
<td>(−0.01944 to −0.00552)</td>
<td>0.00049</td>
</tr>
</tbody>
</table>

Free testosterone (FT) was a still significant explanatory variable for Mets. According to standard partial regression coefficients, the impact of low FT on the presence of Mets was comparable to that of the Mets components themselves. SBP: systolic blood pressure, SBP: diastolic blood pressure, FPG: fasting plasma glucose, TG: triglycerides, HDL: high-density lipoprotein.
As discussed above, Mets and insulin resistance may decrease the serum testosterone level via primary hypogonadism and/or hypogonadotrophic hypogonadism, and a reduction of testosterone may in turn further exacerbate obesity and insulin resistance, consequently initiating a vicious cycle. Several clinical studies have showed a beneficial effect of testosterone supplementation on visceral adiposity, hyperglycemia, and dyslipidemia in hypogonadal men, including those with diabetes or abdominal obesity [25, 26]. However, these were small-scale trials, and the risk of stimulating prostate cancer growth or worsening the symptoms of benign prostatic hypertrophy with testosterone therapy means that it remains controversial [27]. In addition, testosterone has been found to reduce plasma concentration of adiponectin, an insulin-sensitizing and anti-diabetic adipokine [28, 29]. Adiponectin secretion from adipocytes was inhibited by testosterone [29]. Thus, further investigations are necessary for clarification of the pathophysiological mechanisms behind the testosterone-Mets interaction as well as to assess the clinical usefulness of testosterone therapy for patients with Mets or diabetes.

In conclusion, the serum FT level was associated with Mets independently of age, BMI, and WC, while the serum LH and FSH levels of low FT subjects were not higher than those of normal FT subjects.

References

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