

## Low Serum Testosterone Concentration in Middle-aged Men with Type 2 Diabetes

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**Abstract.** Low concentrations of endogenous androgens have been linked with insulin resistance and atherosclerosis. Men with diabetes have been reported to have lower serum testosterone concentration than non-diabetic men; however, there has never been a large study. The aim of this study was to investigate if endogenous androgen concentration is certainly lower in a relatively large number of Japanese patients with type 2 diabetes compared with healthy men, and to identify what factors may be associated with low serum testosterone concentrations in men with type 2 diabetes. Serum free testosterone concentrations were measured in 524 healthy men and in 331 consecutive Japanese men with type 2 diabetes between 40 and 69 years old. In addition, we investigated the relationships between serum free testosterone concentration and luteinizing hormone (LH) concentration as well as major cardiovascular risk factors including age, blood pressure, plasma lipid concentration, glycemic control ( $HbA_{1c}$ ), and BMI. Serum free testosterone concentrations were lower in men with type 2 diabetes than in healthy men in the 40–49 years group ( $10.9 \pm 3.3$  vs.  $14.0 \pm 3.6$  pg/ml,  $P < 0.0001$ ), in the 50–59 years group ( $10.4 \pm 3.2$  vs.  $12.1 \pm 2.9$  pg/ml,  $P < 0.0001$ ), and in the 60–69 years group ( $9.5 \pm 2.6$  vs.  $10.5 \pm 2.9$  pg/ml,  $P = 0.0104$ ). A negative correlation was found between serum free testosterone and LH concentrations ( $r = -0.326$ ,  $P < 0.0001$ ). In conclusion, serum free testosterone concentration is certainly lower in a relatively large number of Japanese patients with type 2 diabetes compared with healthy men with each decade of life between 40 and 69 years old.

**Key words:** Testosterone, Luteinizing Hormone, Type 2 diabetes, Men

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**LOW** concentrations of endogenous androgens have been linked with insulin resistance, which is an important upstream driver for metabolic abnormalities such as hyperglycemia, hypertension, or dyslipidemia, and increased cardiovascular risk [1, 2]. Moreover, results

from the Massachusetts Male Aging Study suggested that low concentrations of testosterone might play a role in the development of insulin resistance and subsequent type 2 diabetes [3]. Keating *et al.* [4] recently demonstrated that androgen deprivation therapy is associated with an increased incidence of diabetes and cardiovascular disease (CVD). On the other hand, administration of testosterone to hypogonadal middle-aged men improved insulin sensitivity and glucose homeostasis [5, 6]. Studies in laboratory animals support the hypothesis that diabetes has a detrimental effect on testicular function; reductions in both Leydig cell num-

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ber and testosterone secretion have been reported [7].

Men with diabetes have been reported to have lower serum testosterone concentrations than non-diabetic men [8, 9]. However, there has never been a large scale study of serum testosterone concentration in men with type 2 diabetes. Previous reports had small numbers of patients and did not investigate much about what factors may be associated with low serum testosterone concentrations in patients with type 2 diabetes. Recently, the Endocrine Society Clinical Practice Guideline demonstrated that men with type 2 diabetes is one of the conditions in which there is a high prevalence of low serum testosterone concentration and in which the guideline recommend measurement of serum testosterone concentration [10].

In this study, we investigated if endogenous androgen concentration is certainly lower in a relatively large number of Japanese patients with type 2 diabetes compared with healthy men, and identified what factors may be associated with low serum testosterone concentrations in men with type 2 diabetes.

## Materials and Methods

### *Subjects*

Serum free testosterone concentrations were measured in 524 healthy men who underwent general health screening tests and in 331 consecutive Japanese men with type 2 diabetes between 40 and 69 years old recruited from outpatient clinics of Kyoto Prefectural University of Medicine and Osaka General Hospital of West Japan Railway Company. The number of subjects in the 40–49 years, 50–59 years, and 60–69 years age group were 235, 169, and 120 among 524 healthy men. The number of subjects in the 40–49 years, 50–59 years, and 60–69 years age group were 39, 173, and 119 among 331 men with type 2 diabetes. In addition, we investigated the relationships between serum free testosterone and luteinizing hormone (LH) concentrations in a subgroup of 154 randomly selected diabetic patients as well as between serum free testosterone concentrations and major cardiovascular risk factors including age, blood pressure, plasma lipid concentration, glycemic control (HbA<sub>1c</sub>), and BMI, severity of diabetic retinopathy, severity of diabetic nephropathy, current treatment for diabetes, presence of hypertension, presence of dyslipidemia, or presence of CVD.

Serum free testosterone concentrations (normal ranges: 14.0 to 40.0 pg/ml) were measured by the Coat-A-Count free and total testosterone kit (Diagnostic Products Corporation, Los Angeles, CA, USA). The intra-assay coefficients of variance (CV) were 10.0, 6.0, and 5.0%, for free testosterone concentrations of 1.87, 11.8, and 38.7 pg/ml, respectively. The inter-assay CV were 21.0, 8.0, and 7.0%, for free testosterone concentrations of 1.38, 11.03, and 37.3 pg/ml, respectively. Serum LH concentrations (normal ranges: 0.79 to 5.72 mIU/ml) were measured by chemiluminescent immunoassay on an ARCHITECT automated analyzer (Abbott Laboratories Diagnostic Division, IL, USA). The intra-assay CV were 5.68, 3.37, and 3.11%, for LH concentrations of 7.37, 24.6, and 102 mIU/ml, respectively. The inter-assay CV were 5.43, 2.60, and 1.64%, for LH concentrations of 6.84, 23.3, and 98.3 mIU/ml, respectively. Plasma total cholesterol, HDL cholesterol, and triglyceride concentrations were assessed using standard enzymatic methods. HbA<sub>1c</sub> was assayed using high-performance liquid chromatography.

Type 2 diabetes was diagnosed according to the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus [11]. Retinopathy was graded as follows: no diabetic retinopathy, NDR; simple diabetic retinopathy, SDR; and proliferative diabetic retinopathy, PDR. Nephropathy was graded as follows: normoalbuminuria, urinary albumin excretion less than 30 mg per gram of creatinine (mg/g Cr); microalbuminuria, 30 to 300 mg/g Cr; or macroalbuminuria, more than 300 mg/g Cr. In patients with type 2 diabetes, mean values for biochemical parameters obtained during the preceding year were used for statistical analysis. Hypertension was defined as blood pressure of at least 140/90 mmHg or use of antihypertensive medication. Dyslipidemia was defined as total cholesterol exceeding 220 mg/dL, triglyceride exceeding 150 mg/dL, or use of antihyperlipidemic medication. CVD was defined as the presence of previous myocardial infarction or cerebral infarction based on the clinical history or physical examination.

Patients were excluded if they had been castrated for the treatment of testicular or prostate cancer, or were taking any medications known to affect sex hormone concentrations (*e.g.*, antiandrogenic agents for prostate cancer). Approval for the study was obtained from the local Research Ethics Committee, and informed consent was obtained from all participants.

### Statistical analysis

Means or frequencies of potential confounding variables were calculated as appropriate. Unpaired Student's *t* tests or analyses of variance (ANOVA) were conducted to assess statistical significance of differences between groups using Stat View software (version 5.0; SAS Institute, Cary, NC). A two-factor ANOVA was used to determine the effect of diabetes adjusted for age on serum free testosterone concentrations. Correlations between serum free testosterone concentration and LH concentration as well as age, blood pressure, serum lipid concentration, and glycemic control (HbA<sub>1c</sub>), BMI, or other variables were examined by linear regression analysis. All continuous variables are presented as the mean  $\pm$  SD. A *P* value  $<0.05$  was considered statistically significant.

### Results

The clinical characteristics of the 331 men with type 2 diabetes enrolled in this study are shown in Table 1. The mean serum free testosterone concentration was  $10.1 \pm 3.0$  pg/ml.

Serum free testosterone concentrations were lower in men with type 2 diabetes than in healthy men in the

40–49 years group ( $10.9 \pm 3.3$  vs.  $14.0 \pm 3.6$  pg/ml,  $P < 0.0001$  after adjustment for age), in the 50–59 years group ( $10.4 \pm 3.2$  vs.  $12.1 \pm 2.9$  pg/ml,  $P < 0.0001$  after adjustment for age), and in the 60–69 years group ( $9.5 \pm 2.6$  vs.  $10.5 \pm 2.9$  pg/ml,  $P = 0.0104$  after adjustment for age; Fig. 1).

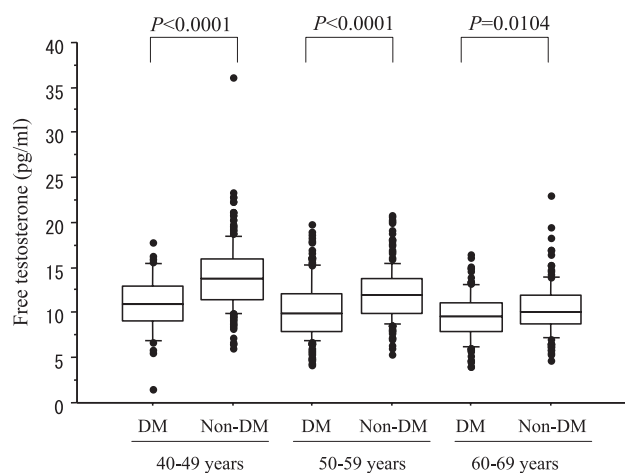
Negative correlations were found between serum free testosterone concentration and age ( $r = -0.214$ ,  $P < 0.0001$ ), and age at onset ( $r = -0.121$ ,  $P = 0.0354$ ). A positive correlation was found between serum free testosterone and total cholesterol concentrations ( $r = 0.140$ ,  $P = 0.0108$ ). No correlations were found between serum free testosterone and duration of diabetes ( $r = -0.036$ ,  $P = 0.5281$ ), BMI ( $r = 0.077$ ,  $P = 0.1718$ ), HbA<sub>1c</sub> ( $r = 0.066$ ,  $P = 0.2328$ ), systolic blood pressure ( $r = -0.008$ ,  $P = 0.8864$ ), diastolic blood pressure ( $r = 0.084$ ,  $P = 0.1343$ ), plasma triglyceride ( $r = 0.030$ ,  $P = 0.5819$ ), and HDL-cholesterol ( $r = 0.021$ ,  $P = 0.7064$ ). A negative correlation was found between serum free testosterone and LH concentrations ( $r = -0.326$ ,  $P < 0.0001$ ; Fig. 2).

Serum free testosterone concentrations did not differ between patients with or without hypertension ( $10.1 \pm 2.9$  vs.  $10.3 \pm 3.3$  pg/ml,  $P = 0.4926$ ), dyslipidemia ( $10.3 \pm 3.1$  vs.  $10.1 \pm 3.0$  pg/ml,  $P = 0.6360$ ), or CVD ( $10.0 \pm 3.4$  vs.  $10.2 \pm 3.0$  pg/ml,  $P = 0.7028$ ). In addition, serum free testosterone concentrations did not differ between patients treated with or without insulin

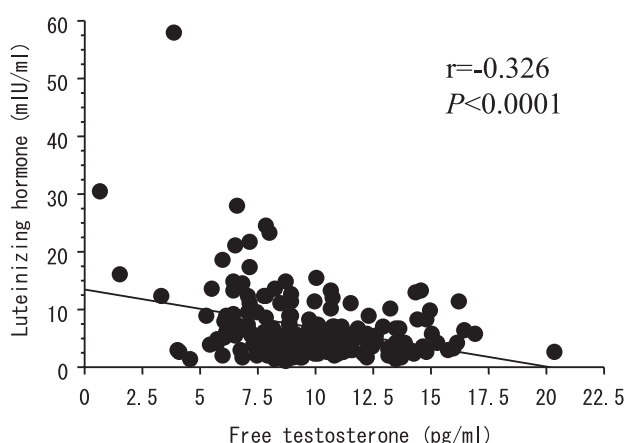
**Table 1.** Clinical characteristics of patients with diabetes

	Mean $\pm$ SD
n	331
Age (years)	$57.5 \pm 6.8$
Age at onset (years)	$46.6 \pm 10.2$
Duration of diabetes (years)	$11.2 \pm 9.4$
BMI (kg/m <sup>2</sup> )	$23.6 \pm 3.2$
HbA <sub>1c</sub> (%)	$7.2 \pm 1.2$
Systolic blood pressure (mm Hg)	$134 \pm 15$
Diastolic blood pressure (mm Hg)	$80 \pm 9$
Total cholesterol (mmol/l)	$5.12 \pm 0.83$
Triglyceride (mmol/l)	$1.71 \pm 0.95$
HDL-cholesterol (mmol/l)	$1.37 \pm 0.39$
Smoking (none/past/current)	67/125/139
Retinopathy (NDR/SDR/PDR)	224/47/60
Nephropathy (normo-/micro-/macroalbuminuria)	209/74/48
Cardiovascular disease (–/+)	294/37
Current treatment (Diet/OHA/Insulin)	40/214/77
Free testosterone (pg/ml)	$10.1 \pm 3.0$

NDR, no diabetic retinopathy; SDR, simple diabetic retinopathy; PDR, proliferative diabetic retinopathy; OHA, oral hypoglycemic agents.



**Fig. 1.** Comparison of serum free testosterone concentrations in men with type 2 diabetes (DM) and in healthy men (Non-DM) with each decade of life between 40 and 69 years old. Data are presented as medians, 25th and 75th percentiles (boxes), and 10th and 90th percentiles (whiskers).



**Fig. 2.** Correlation between serum free testosterone and luteinizing hormone concentrations in men with type 2 diabetes.

( $9.9 \pm 2.9$  vs.  $10.2 \pm 3.1$  pg/ml,  $P = 0.4894$ ). Serum free testosterone concentrations did not differ based on the severity of diabetic nephropathy ( $10.2 \pm 3.2$  vs.  $10.2 \pm 2.5$  vs.  $9.8 \pm 3.2$  pg/ml for patients with normoalbuminuria, microalbuminuria, or macroalbuminuria, respectively). Serum free testosterone concentrations were lower in patients with PDR ( $9.4 \pm 3.1$  pg/ml) than in patients with NDR ( $10.4 \pm 2.9$  pg/ml,  $P = 0.0452$ ), and than in patients with SDR ( $11.1 \pm 3.6$  pg/ml,  $P = 0.0105$ ). Serum free testosterone concentrations did not differ among patients with nonsmoker, past smoker, or current smoker ( $10.4 \pm 3.4$  vs.  $9.9 \pm 2.7$  vs.  $10.5 \pm 3.1$  pg/ml, respectively).

## Discussion

In the present study, we demonstrated that serum free testosterone concentration is significantly lower in a relatively large number of Japanese patients with type 2 diabetes compared with healthy men with each decade of life between 40 and 69 years old.

Moreover, we demonstrated a negative correlation between serum free testosterone and LH concentrations, which indicated hypergonadotropic hypogonadism in men with type 2 diabetes. Ando *et al.* [12] also demonstrated that lower serum free testosterone and higher LH concentrations in men with type 2 diabetes compared with control subjects. The present study is not in line with recent report by Dhindsa *et al.* [13] demonstrating serum LH concentrations correlated positively with serum free testosterone concentra-

tions (hypogonadotropic hypogonadism) in men with type 2 diabetes. A possible explanation is that mean BMI is much higher in their patients compared with ours ( $33.4$  vs.  $23.6$  kg/m<sup>2</sup>). In obese men there is a decrease in free testosterone concentration, resulting from the peripheral conversion of testosterone into estrogen, which decreases the amplitude of LH pulse, promoting central inhibition of androgenic production [14]. It is also possible that the decreased concentrations of testosterone in obese men result from an inhibitory effect of leptin on the hypothalamus-pituitary-gonadal axis [15]. Obesity is associated with increased plasma levels of proinflammatory cytokines such as tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-6, C-reactive protein, and adhesion molecules [16]. TNF- $\alpha$  and IL-1 $\beta$  have been shown to reduce hypothalamic LH secretion in animals and *in vitro* [17, 18]. Dhindsa *et al.* also demonstrated that LH levels were significantly lower in obese men.

We examined a number of possible explanations for the lower serum testosterone concentrations in middle-aged men with type 2 diabetes. In this study, both age-specific and age-adjusted analyses showed that age did not explain the lower serum testosterone concentrations observed in men with type 2 diabetes. Studies in laboratory animals support the hypothesis that diabetes has a detrimental effect on testicular function; reductions in both Leydig cell number and testosterone secretion have been reported [7]. The association between glycemia and reduced testosterone concentration may be an effect of glycemia on the testicular microvasculature. Data from animal studies support the hypothesis that glycemia alters Leydig cell function directly. The inverse association of serum testosterone concentration and plasma glucose concentration was also observed in those without diabetes [1, 2]. BMI, smoking status, or alcohol consumption might influence the difference of serum free testosterone concentration between men with type 2 diabetes and healthy men. However, BMI and smoking status were not associated with serum free testosterone concentration among men with type 2 diabetes. Moreover, treatment for diabetes, hypertension, and hyperlipidemia might influence the results, although serum free testosterone concentration was not different between patients treated with and without insulin. Our study demonstrated a positive correlation between total cholesterol and free testosterone concentrations. Because cholesterol is the immediate biosynthetic precursor of steroids, the re-

duction in cholesterol concentration associated with statin therapy could be the cause of the reductions in hormone.

The cutoff values for serum testosterone levels in diagnosing hypogonadism differ among institutions and research groups. Nakazawa *et al.* [19] regarded a serum free testosterone concentration less than 10 pg/ml as indicating hypogonadism. According to their cutoff value, the prevalence of hypogonadism was 35.9% in the 40–49 years group, 48.0% in the 50–59 years group, and 58.8% in the 60–69 years group in men with type 2 diabetes. Interestingly, there was no significant difference between serum free testosterone concentration in the 50–59 years age group in men with type 2 diabetes ( $10.4 \pm 3.2$  pg/ml) and that in the 60–69 years age group in healthy men ( $10.5 \pm 2.9$  pg/ml).

The present study demonstrated no association between serum free testosterone concentration and the severity of diabetic nephropathy. However, serum free testosterone was lower in patients with progressed diabetic retinopathy. Loss of retinal capillary pericytes, a characteristic change in the early phase of diabetic retinopathy, is considered a key step in retinopathy progression [20]. Brignardello *et al.* [21] demonstrated that by its antioxidant properties, dehydroepiandrosterone (DHEA), which is a weak androgen that contributes to androgenicity mainly after peripheral conversion to testosterone, prevents suppression of retinal capillary pericyte growth caused by excessive glucose.

We compared serum free testosterone rather than total testosterone concentration in men with type 2 diabetes and healthy men because free testosterone concentration is a more sensitive and active marker for insulin resistance or atherosclerosis in previous studies compared with total testosterone. Rhoden *et al.* [22] also demonstrated that subnormal free testosterone concentration had a stronger correlation with the presence of diabetes mellitus than subnormal total testosterone concentration.

Limitation of the present study was that serum free testosterone concentration was measured by radioimmunoassay (RIA), a method that has been criticized for its variability [23]. Unfortunately, equilibrium dialysis, a method considered to be the gold standard for measuring serum free testosterone concentration, was not available. Nevertheless, the present study is unique in comparing serum free testosterone concentration in diabetic and nondiabetic middle-aged men. Despite the limitation of RIA for measuring serum free tes-

tosterone concentration, we believe that the main findings of the present study are valid, considering that the presence of a nondiabetic control group has reduced associated biases. Although, equilibrium dialysis is thought to be the best method for measuring serum free testosterone concentration, the International Society for the Study of the Aging Male (ISSAM) recommended calculated free testosterone as a standard diagnostic indicator for partial androgen deficiency in aging males (PADAM) because equilibrium dialysis is a delicate, expensive, and time-consuming technique and therefore not suitable for population-based or large studies [24]. In Japan, sex hormone binding globulin (SHBG) measurement is uncommon because SHBG is not approved under the medical care system. At present, RIA is the only available measurement for serum free testosterone concentration. Okamura *et al.* [25] demonstrated a significantly positive relationship between serum free testosterone concentration measured by RIA and calculated free testosterone concentration. We believe that serum free testosterone concentration determined by RIA would be useful for identifying hypogonadism for large studies.

Men with diabetes have significantly lower serum testosterone concentration than non-diabetic men. Low concentrations of endogenous androgens have been linked with increased cardiovascular risk. The increased risk for CVD in diabetic men could be partially mediated through low concentrations of testosterone. Increased age is one of the strongest predictors for coronary artery disease. The Telecom Study demonstrated a significant decrease in testosterone concentration with each decade of life [26]. Both mean intima-media thickness and plaque score, determined by carotid ultrasonography, have been proven to be inversely associated with serum free testosterone concentration in our previous study [27].

Hypogonadism is associated with an increase in fat mass, decreased muscle mass, accelerated bone loss, and decreased libido, which would be improved by treatment with testosterone [28]. Moreover, hypogonadism is associated with increased insulin resistance and progression of atherosclerosis [2, 29]. Because men with type 2 diabetes have relative hypogonadism, supplementation with androgen might reduce both insulin resistance and atherosclerosis. Life-style changes could be a logical first-line treatment in many ventrally obese men with andropause. Weight loss is important in prevention of progressive metabolic decompensa-

tion and CVD associated with obesity and the metabolic syndrome. While a number of preparations are available for testosterone replacement, little is known about suitability or efficacy of individual preparations for protection from insulin resistance or atherosclerosis. Long-term trials of testosterone replacement therapy are needed to assess both efficacy and safety.

In conclusion, serum free testosterone concentration is certainly lower in a relatively large number of Japanese patients with type 2 diabetes compared with healthy men. The clinical and physiologic implications of the reduced serum testosterone concentrations in diabetic men merit further investigation.

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