Association Between Serum Testosterone Concentration and Carotid Atherosclerosis in Men With Type 2 Diabetes

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**OBJECTIVE** — There is evidence to suggest that low concentrations of testosterone are associated with an increased risk of cardiovascular disease in men. The aim of this study was to evaluate the relationship between serum testosterone concentration and carotid atherosclerosis as well as major cardiovascular risk factors in men with type 2 diabetes.

**RESEARCH DESIGN AND METHODS** — Serum free and total testosterone concentrations were measured in 253 consecutive men with type 2 diabetes. The relationships between serum testosterone concentration and carotid atherosclerosis, determined by ultrasonographically evaluated intima-media thickness (IMT) and plaque score (PS) in a subgroup of 154 diabetic patients, as well as major cardiovascular risk factors, including age, blood pressure, and lipid concentrations, were evaluated.

**RESULTS** — Inverse correlations were found between free testosterone (F-tes) concentration and IMT and between F-tes concentration and PS (r = −0.266, P = 0.0103) and between F-tes concentration and PS (r = −0.334, P < 0.001). The IMT and PS were significantly greater in patients with lower concentrations of F-tes (<10 pg/ml) than in patients with higher concentrations of F-tes (1.01 ± 0.29 vs. 0.91 ± 0.26 mm, P = 0.038; 4.5 ± 3.8 vs. 2.4 ± 3.2, P = 0.0003; respectively). An inverse correlation was found between serum F-tes concentration and age (r = −0.420, P < 0.0001). A positive correlation was found between serum F-tes and total cholesterol concentrations (r = 0.145, P = 0.0238).

**CONCLUSIONS** — Serum F-tes concentration is inversely associated with carotid atherosclerosis determined by ultrasonographically evaluated IMT and PS in men with type 2 diabetes.

**CONCLUSIONS**

Cardiovascular disease (CVD) is the primary cause of mortality and morbidity in patients with type 2 diabetes, and several risk factors, including smoking, hypertension, and hyperlipidemia, have been shown to accelerate the progression of CVD (1–3). Male sex is an independent risk factor for CVD (4).

Therefore, some researchers regard testosterone as detrimental in terms of the development of CVD. Although the incidence of coronary artery disease increases in women after menopause, postmenopausal women have a lower incidence of coronary artery disease than men of a similar age.

Hyperinsulinemia is a risk factor for CVD (5). Furthermore, there is an association in men between low concentrations of free and total testosterone (T-tes) and hyperinsulinemia (6,7). Compared with men with normal concentrations of T-tes, men with low concentrations of T-tes have a significantly higher BMI, waist-to-hip ratio, systolic blood pressure, fasting and postprandial plasma glucose concentrations, and fasting serum insulin and total cholesterol concentrations. We have previously described six patients in whom glycemic control was worsened, despite increased endogenous insulin secretion, by castration for management of prostate cancer. This finding suggested that decreased concentrations of testosterone might play an important role in insulin resistance (8). In addition, castrated male rats have decreased insulin sensitivity that is improved by low-dose testosterone administration (9). Men with diabetes have significantly lower concentrations of free and T-tes than nondiabetic men (10,11). Moreover, results from the Massachusetts Male Aging Study suggest that low concentrations of testosterone might play a role in the development of insulin resistance and subsequent type 2 diabetes (12).

There is evidence to suggest that low concentrations of testosterone are associated with an increased risk of CVD in men (13). Significant inverse correlations between testosterone concentration and both the presence and the severity of coronary artery disease have been found in coronary angiographic studies to detect atheroma (14,15). A recent study from Denmark (16) determined the relation-
ship between low concentrations of testosterone and acute ischemic stroke. In that study, T-tes concentration was inversely associated with stroke severity, infarct size, and mortality.

To our knowledge, the relationship between serum testosterone concentration and carotid atherosclerosis, determined by ultrasonographically evaluated intima-media thickness (IMT) and plaque score (PS), has never been explored in men with type 2 diabetes. In this study, we evaluated the relationships between serum testosterone concentration and both carotid atherosclerosis and major cardiovascular risk factors in men with type 2 diabetes.

**RESEARCH DESIGN AND METHODS**

**Patients**

Serum free and T-tes concentrations were measured in 253 consecutive men with type 2 diabetes. The relationship between serum testosterone concentration and carotid atherosclerosis, evaluated by carotid ultrasonography, was investigated in a subgroup of 154 randomly selected diabetic patients. In addition, the relationships between serum testosterone concentrations and major cardiovascular risk factors were evaluated, including age, blood pressure, serum lipid concentrations and major cardiovasculogenic agents for prostate cancer). In addition, patients with hepatitis C virus infection were also excluded because this infection has been reported to cause atherosclerosis (18). Approval for the study was obtained from the local Research Ethics Committee, and informed consent was obtained from all participants.

Ultrasonographic measurement of carotid IMT and PS

B-mode ultrasonographic imaging of the carotid artery was performed using high-resolution, real-time ultrasonography with a 7.5-MHz transducer. The examination and image analysis were performed by a trained sonographer who remained unaware of other data. In brief, the right and left carotid arteries were scanned for CVD. Fasting serum C-peptide concentrations were measured in non-insulin-treated patients with type 2 diabetes.

Serum free and T-tes concentrations (normal ranges 14.0–40.0 pg/ml and 2.7–10.7 ng/ml, respectively) were measured by the Coat-A-Count free and T-tes kit (Diagnostic Products, Los Angeles, CA). The intra-assay CVs were 10.0, 6.0, and 5.0% for free testosterone (F-tes) concentrations of 1.87, 11.8, and 38.7 pg/ml, respectively. The interassay CVs were 7.0% for F-tes concentrations of 3.18, 11.03, and 37.3 pg/ml, respectively. The intra-assay CVs were 6.0, 4.0, and 9.0% for T-tes concentrations of 0.32, 2.82, and 8.21 ng/ml, respectively. The interassay CVs were 16.0, 8.0, and 9.0% for T-tes concentrations of 0.32, 2.97, and 7.61 ng/ml, respectively. Plasma total cholesterol, HDL cholesterol, and triglyceride concentrations were assessed using standard enzymatic methods. HbA1c 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 (17). 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 <30 mg/g creatinine (Cr), microalbuminuria, urinary albumin excretion 30–300 mg/g Cr, or macroalbuminuria, urinary albumin excretion >300 mg/g Cr. In patients with type 2 diabetes, mean values for biochemical parameters obtained during the previous year were used for statistical analysis. 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 as treatment for testicular or prostate cancer or if they were taking any medications known to affect sex hormone concentrations (e.g., antiandrogenic agents for prostate cancer). In addition, patients with hepatitis C virus infection were also excluded because this infection has been reported to cause atherosclerosis (18). Approval for the study was obtained from the local Research Ethics Committee, and informed consent was obtained from all participants.

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between T-tes concentration and mean IMT ($r = -0.109$, $P = 0.1797$) or PS ($r = -0.101$, $P = 0.2171$) in men with type 2 diabetes (Fig. 1). Mean IMT and PS were significantly greater in patients with lower concentrations of F-tes ($<10$ pg/ml) than in patients with higher concentrations of F-tes ($1.01 \pm 0.233$, $P = 0.0067$) and systolic blood pressure ($10.7 \pm 0.385$, $P = 0.0097$) were independent determinants of mean IMT. Serum F-tes concentration ($10.7 \pm 0.122$, $P = 0.001$), BMI ($10.7 \pm 0.206$, $P = 0.001$), and systolic blood pressure ($10.2 \pm 0.165$, $P = 0.001$) were independent determinants of PS.

Serum F-tes concentrations were significantly lower in patients treated with insulin than in those treated without insulin ($10.4 \pm 0.49$, $P < 0.0001$). Serum F-tes concentrations did not differ between patients with or without CVD ($10.4 \pm 0.39$, $P = 0.2594$). In addition, serum F-tes concentrations did not differ between patients with or without cerebral infarction ($10.4 \pm 0.39$, $P = 0.1560$) or between patients with or without coronary artery disease ($10.6 \pm 0.39$, $P = 0.824$). Serum T-tes concentrations did not differ between patients with or without CVD ($10.4 \pm 0.17$, $P = 0.5122$), cerebral infarction ($10.4 \pm 0.17$, $P = 0.3806$), or coronary artery disease ($10.4 \pm 0.17$, $P = 0.4092$). Serum F-tes concentrations did not differ based on the severity of diabetic retinopathy ($10.4 \pm 0.17$, $P = 0.0003$, respectively), but were independent determinants of mean IMT and PS, which are early preclinical markers of atherosclerosis.

**CONCLUSIONS** — We evaluated the relationships between serum testosterone concentration and carotid atherosclerosis, determined by ultrasonographically evaluated IMT and PS, as well as major cardiovascular risk factors in men with type 2 diabetes. Serum F-tes concentrations were inversely correlated with mean IMT and PS. Patients with low concentrations of F-tes ($<10$ pg/ml) had greater mean IMT and PSs than those with high concentrations of F-tes. We divided our patients into two subgroups based on a F-tes concentration of 10 pg/ml, which could be the threshold value for testosterone replacement therapy for hypogonadism, although thresholds that have been used to define hypogonadism varied between studies (20,21). Decreasing testosterone concentrations in elderly men are important in development of sexual dysfunction and symptoms such as depression and fatigue. Men with low concentrations of testosterone are candidates for testosterone therapy for prevention of atherosclerosis as well as improvement of libido.

Studies examining the association between low concentrations of testosterone and CVD mortality have been inconclusive. Despite several reports suggesting that low concentrations of testosterone are associated with an increased risk of CVD in men, some investigators have found no significant association between T-tes concentration and the prevalence of CVD (22). Our study demonstrated that serum testosterone concentrations are not significantly different between patients with or without CVD. However, serum F-tes concentrations were significantly correlated with ultrasonographically evaluated mean IMT and PS, which are early preclinical markers of atherosclerosis. In other words, serum F-tes concentration correlated with the severity of atheroscle-

### Table 1 — Clinical characteristics of patients with diabetes

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
<th>Age (years)</th>
<th>Age at onset (years)</th>
<th>Duration of diabetes (years)</th>
<th>BMI (kg/m²)</th>
<th>HbA₁c (%)</th>
<th>Systolic blood pressure (mmHg)</th>
<th>Diastolic blood pressure (mmHg)</th>
<th>Total cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>HDL cholesterol (mg/dl)</th>
<th>F-tes (pg/ml)</th>
<th>T-tes (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>253</td>
<td>62.0 ± 9.9</td>
<td>52.0 ± 10.7</td>
<td>10.2 ± 8.1</td>
<td>23.2 ± 3.2</td>
<td>7.2 ± 1.2</td>
<td>130 ± 15</td>
<td>79 ± 9</td>
<td>201 ± 33</td>
<td>156 ± 114</td>
<td>55 ± 16</td>
<td>10.8 ± 1.6</td>
<td>4.4 ± 4.2</td>
</tr>
</tbody>
</table>

Data are mean ± SD. Ins, insulin; SU, sulfonylurea; a-GI, α-glucosidase inhibitor.

### Table 2 — Correlation between serum testosterone and other variables

<table>
<thead>
<tr>
<th></th>
<th>F-tes</th>
<th></th>
<th>T-tes</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>Age</td>
<td>-0.420</td>
<td>&lt;0.0001</td>
<td>-0.099</td>
<td>0.1295</td>
</tr>
<tr>
<td>Age at onset</td>
<td>-0.289</td>
<td>&lt;0.0001</td>
<td>-0.115</td>
<td>0.0940</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td>-0.165</td>
<td>0.0114</td>
<td>0.008</td>
<td>0.9009</td>
</tr>
<tr>
<td>BMI</td>
<td>0.117</td>
<td>0.0726</td>
<td>-0.206</td>
<td>0.0015</td>
</tr>
<tr>
<td>HbA₁c</td>
<td>-0.122</td>
<td>0.0598</td>
<td>-0.041</td>
<td>0.5308</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>-0.039</td>
<td>0.5453</td>
<td>-0.061</td>
<td>0.3469</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.081</td>
<td>0.2129</td>
<td>-0.069</td>
<td>0.2890</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.145</td>
<td>0.0238</td>
<td>0.024</td>
<td>0.7142</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>0.015</td>
<td>0.8203</td>
<td>-0.148</td>
<td>0.0223</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>-0.067</td>
<td>0.3052</td>
<td>0.020</td>
<td>0.7580</td>
</tr>
<tr>
<td>Fasting serum C-peptide</td>
<td>-0.148</td>
<td>0.0860</td>
<td>-0.145</td>
<td>0.0964</td>
</tr>
</tbody>
</table>
rosis, regardless of the presence of clinical manifestations. In addition, we demonstrated no association between serum F-tes concentration and the severity of diabetic microangiopathy (retinopathy and nephropathy).

Men with central or upper body obesity often have abnormal carbohydrate tolerance, hyperinsulinemia, insulin resistance, and lower concentrations of male sex hormones (23). Low testosterone concentrations precede the development of central obesity (24). Testosterone therapy can reduce intra-abdominal obesity and improve insulin concentrations in older men (25). In keeping with our previous finding that decreased concentrations of testosterone as a result of castration might play an important role in insulin resistance (8,9), an inverse correlation was found, although it did not reach statistical significance, between serum testosterone and fasting serum C-peptide and F-tes concentrations (23,30). In this study, the association between F-tes concentration and the severity of carotid atherosclerosis was greater than that between T-tes concentration and the severity of carotid atherosclerosis.

Our study demonstrated a positive correlation between fasting serum C-peptide and F-tes concentrations. Because cholesterol is the immediate biosynthetic precursor of steroids, the reduction in cholesterol concentration associated with statin therapy could be the cause of the reductions in hormone. In contrast, low concentrations of testosterone might lead to increased concentrations of cholesterol by changing body fat distribution (29).

In previous studies that measured both free and T-tes concentrations, the association between fasting serum C-peptide and F-tes concentrations was as strong or stronger than the relationship between fasting serum C-peptide and T-tes concentrations (23,30). In this study, the association between F-tes concentration and the severity of carotid atherosclerosis was greater than that between T-tes concentration and the severity of carotid atherosclerosis.

Men with diabetes have significantly lower plasma concentrations of free and T-tes than nondiabetic men. The increased risk for CVD in diabetic men could be partially mediated through low concentrations of testosterone. Advanced 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 (31). The decrease in testosterone concentration with age may partly explain the greater risk of CVD with advancing age. Because decreased concentrations of testosterone are responsible for aging, adjusting for age to assess the relationship between testosterone concentration and carotid atherosclerosis can be considered an overadjustment. Then, we have performed multiple regression analysis to assess the combined influence of variables on mean IMT or PS using the following factors: serum F-tes concentration, BMI, systolic blood pressure, total cholesterol concentration, and HbA1c. Both mean IMT and PS have been proven to be inversely associated with serum F-tes concentration in the multivariate analysis.

![Figure 1](image1.png)

**Figure 1**—Correlation between concentration of F-tes and carotid mean IMT (A) and between concentration of F-tes and PS (B), as well as correlation between concentration of T-tes and carotid mean IMT (C) and between concentration of T-tes and PS (D), in men with type 2 diabetes.

![Figure 2](image2.png)

**Figure 2**—Correlation between lower concentrations of F-tes (<10 pg/ml) and carotid mean IMT (A) and between lower concentrations of F-tes and PS (B) in men with type 2 diabetes. Data are presented as medians, 25th and 75th percentiles (boxes), and 10th and 90th percentiles (whiskers).
Serum testosterone concentrations in diabetic patients treated with insulin were significantly lower than in patients treated without insulin, which is in contrast to previous reports that insulin can increase production of testosterone (32, 33). Type 2 diabetic patients treated with insulin might require insulin for improved glycemic control because of insulin resistance due to low concentrations of testosterone. Low concentrations of testosterone may play an important role in the progression of atherosclerosis in patients treated with insulin.

A few prospective clinical trials, some cross-sectional studies, and experimental studies suggest that testosterone has a beneficial effect on the development of atherosclerosis or its clinical manifestations in men (34). Large prospective trials and intervention studies are needed to better assess the metabolic and cardiovascular benefits of testosterone.


References


25. Fukui and Associates