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 ($r = -0.206$, $P = 0.0103$) 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.

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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-

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Abbreviations: Cr, creatinine; CVD, cardiovascular disease; F-tes, free testosterone; IMT, intima-media thickness; NDR, no diabetic retinopathy; PDR, proliferative diabetic retinopathy; PS, plaque score; SDR, simple diabetic retinopathy; T-tes, total testosterone.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

See accompanying editorial, p. 1929.

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 concentration, and glycemic control (HbA_{1c}), BMI, endogenous insulin secretion (fasting serum C-peptide concentration), severity of diabetic retinopathy, severity of diabetic nephropathy, current treatment for diabetes, or presence of 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 21.0, 8.0, and 7.0% for F-tes concentrations of 1.38, 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 0.35, 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. HbA_{1c} 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.

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 measurement of IMT and plaques in both the longitudinal and transverse projections over an arterial segment including 30 mm of the distal common carotid artery, the bifurcation, and 15 mm of the internal carotid artery (19). IMT was measured in the far wall of the vessel as the distance from the leading edge of the lumen-intima interface to the leading edge of the intima-adventitia interface. The average measurement was taken as the mean IMT. We defined a plaque as a visually distinct area with an IMT greater than that of neighboring sites. The PS was determined as the sum of the maximum thicknesses of all plaques measured in millimeters on the near and far walls of the vessels.

Statistical analysis

Data were analyzed using unpaired Student's *t* tests for continuous variables with Stat View software (version 5.0; SAS Institute, Cary, NC). Relationships between the serum testosterone concentration and mean IMT, PS, age, glycemic control, and other variables were examined by linear regression analysis. All continuous variables are presented as means ± SD. Multiple regression analysis was performed to assess the combined influence of variables on mean IMT or PS. To examine the effects of various factors on mean IMT or PS, the following factors were considered as independent variables: serum F-tes concentration, BMI, systolic blood pressure, total cholesterol concentration, and HbA_{1c}. A P value <0.05 was considered statistically significant.

RESULTS — The clinical characteristics of the 253 men with type 2 diabetes enrolled in this study are shown in Table 1. The mean concentrations of T-tes and F-tes were 4.4 ± 1.6 ng/ml and 10.8 ± 4.2 pg/ml, respectively. The mean IMT and PS were 0.96 ± 0.28 mm and 3.5 ± 3.7, respectively, in a subgroup of 154 diabetic patients.

The relationships between serum testosterone concentration and other variables are shown in Table 2. Inverse correlations were found between serum F-tes concentration and age ($r = -0.420$, $P < 0.0001$), age at onset ($r = -0.289$, $P < 0.0001$), and duration of diabetes ($r = -0.165$, $P = 0.0114$). A positive correlation was found between serum F-tes and total cholesterol concentrations ($r = 0.145$, $P = 0.0238$). Inverse correlations were found between serum T-tes concentration and BMI ($r = -0.206$, $P = 0.0015$) and plasma triglyceride concentration ($r = -0.148$, $P = 0.0223$).

Inverse correlations were found between F-tes concentration and mean IMT ($r = -0.206$, $P = 0.0103$) and between F-tes concentration and PS ($r = -0.334$, $P < 0.0001$) in men with type 2 diabetes. No significant correlations were found

Table 1—*Clinical characteristics of patients with diabetes*

Characteristic	
n	253
Age (years)	62.0 ± 9.9
Age at onset (years)	52.0 ± 10.7
Duration of diabetes (years)	10.2 ± 8.1
BMI (kg/m^2)	23.2 ± 3.2
HbA_{1c} (%)	7.2 ± 1.2
Systolic blood pressure (mmHg)	130 ± 15
Diastolic blood pressure (mmHg)	79 ± 9
Total cholesterol (mg/dl)	201 ± 33
Triglyceride (mg/dl)	156 ± 114
HDL cholesterol (mg/dl)	55 ± 16
Retinopathy (NDR/SDR/PDR)	198/35/20
Nephropathy (normoalbuminuria/microalbuminuria/macroalbuminuria)	172/54/27
Current treatment (Ins/SU/a-GI/diet)	40/113/27/73
T-tes (ng/ml)	4.4 ± 1.6
F-tes (pg/ml)	10.8 ± 4.2

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

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 ± 0.29 vs. 0.91 ± 0.26 mm, $P = 0.038$, and 4.5 ± 3.8 vs. 2.4 ± 3.2, $P = 0.0003$, respectively; Fig. 2).

Multiple regression analysis demonstrated that serum F-tes concentration ($\beta = -0.233$, $P = 0.0067$) and systolic blood pressure ($\beta = 0.227$, $P = 0.0097$) were independent determinants of mean IMT. Serum F-tes concentration ($\beta = -0.329$, $P < 0.0001$), BMI ($\beta = -0.225$, $P = 0.0090$), and systolic blood pressure ($\beta = 0.170$, $P = 0.0358$) were independent determinants of PS.

Serum F-tes concentrations were significantly lower in patients treated with insulin than in those treated without insulin (8.2 ± 4.9 vs. 11.3 ± 4.0 pg/ml, $P < 0.0001$). Serum F-tes concentrations did not differ between patients with or without CVD (9.6 ± 3.7 vs. 10.4 ± 4.3 pg/ml, $P = 0.2594$). In addition, serum F-tes concentrations did not differ between patients with or without cerebral infarction (9.5 ± 4.4 vs. 10.9 ± 4.2 pg/ml, $P = 0.1560$) or between patients with or without coronary artery disease (10.6 ± 3.6 vs. 10.8 ± 4.3 pg/ml, $P = 0.824$). Serum T-tes concentrations did not differ between patients with or without CVD (4.5 ± 1.7 vs. 4.2 ± 1.6 ng/ml, $P = 0.5122$), cerebral infarction (4.7 ± 1.7 vs. 4.4 ± 1.6 ng/ml, $P = 0.3806$), or coronary artery disease (4.7 ± 1.7 vs. 4.4 ± 1.6 ng/ml, $P = 0.4092$). Serum F-tes concentrations did not differ based on the severity of diabetic retinopathy (10.8 ± 4.2 vs. 11.0 ± 4.8 vs. 10.5 ± 2.9 pg/ml for patients with NDR, SDR, and PDR, respectively) or based on the severity of diabetic nephropathy (10.9 ± 4.4 vs. 10.7 ± 4.0 vs. 9.9 ± 3.3 pg/ml for patients with normoalbuminuria, microalbuminuria, or macroalbuminuria, respectively).

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 2—*Correlation between serum testosterone and other variables*

	F-tes		T-tes	
	r	*P*	*r*	*P*
Age	−0.420	<0.0001	−0.099	0.1295
Age at onset	−0.289	<0.0001	−0.115	0.0940
Duration of diabetes	−0.165	0.0114	0.008	0.9009
BMI	0.117	0.0726	−0.206	0.0015
HbA_{1c}	−0.122	0.0598	−0.041	0.5308
Systolic blood pressure	−0.039	0.5453	−0.061	0.3469
Diastolic blood pressure	0.081	0.2129	−0.069	0.2890
Total cholesterol	0.145	0.0238	0.024	0.7142
Triglyceride	0.015	0.8203	−0.148	0.0223
HDL cholesterol	−0.067	0.3052	0.020	0.7580
Fasting serum C-peptide	−0.148	0.0860	−0.145	0.0964

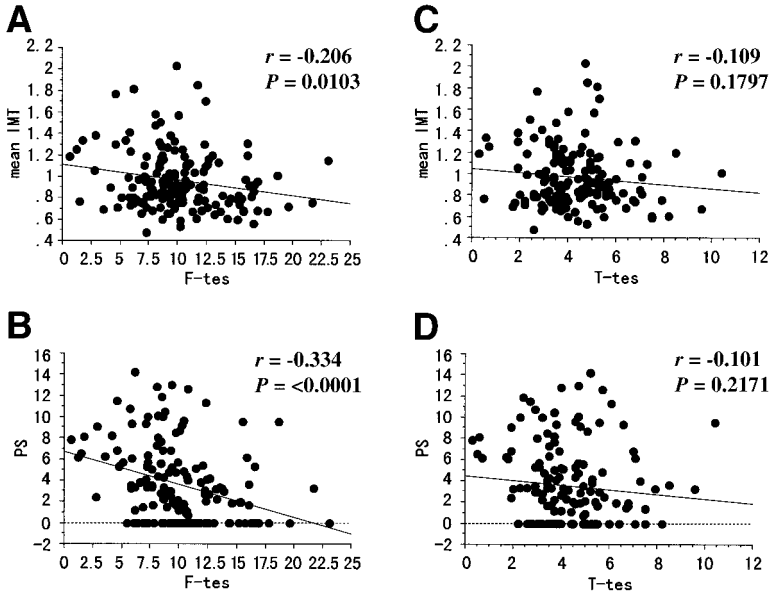

Figure 1—*Correlation between concentration of F-tes and carotid mean IMT* (A) *and between concentration of F-tes and PS* (B)*, as well as correlation between concentration of T-tes and carotid mean IMT* (C) *and between concentration of T-tes and PS* (D)*, in men with type 2 diabetes.*

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 concentrations in men with type 2 diabetes treated without insulin. Testosterone concentration is inversely correlated with procoagulable factors, plasminogen activator inhibitor, and fibrinogen (26). Testosterone has also been shown to act as a vasodilator in both the coronary and systemic circulations in in vitro animal models (27), and short-term intracoronary administration of testosterone, at physiologic concentrations, induces coronary artery dilation and increases coronary blood flow in men with established coronary artery disease (28).

Our study demonstrated a positive correlation between total cholesterol 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 HbA_{1c}. Both mean IMT and PS have been proven to be inversely associated with serum F-tes concentration in the multivariate analysis.

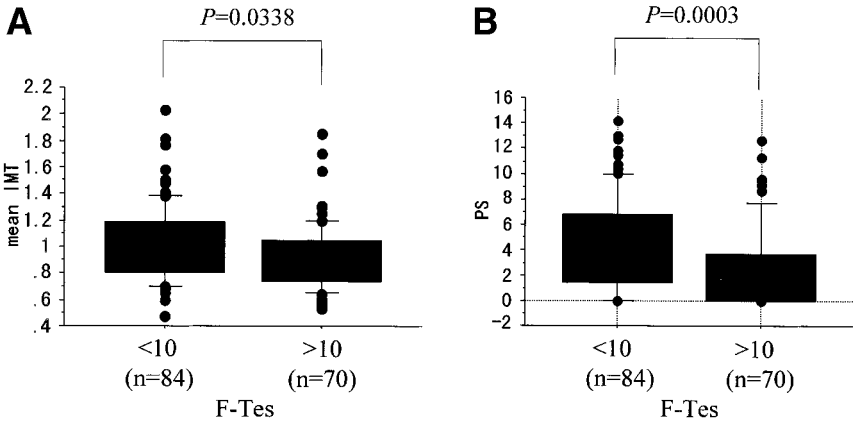

Figure 2—*Correlation between lower concentrations of F-tes (<10 pg/ml) and carotid mean IMT* (A) *and between lower concentrations of F-tes and PS* (B) *in men with type 2 diabetes. Data are presented as medians, 25th and 75th percentiles (boxes), and 10th and 90th percentiles (whiskers).*

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.

In conclusion, serum F-tes concentration is inversely associated with carotid atherosclerosis determined by ultrasonographically evaluated IMT and PS in men with type 2 diabetes.

References

1. Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, Taskinen MR, Groop L: Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care* 24:683–689, 2001
2. Multiple Risk Factor Intervention Trial Research Group: Multiple Risk Factor Intervention Trial: risk factor changes in mortality results. *JAMA* 248:1465–1470, 1982
3. Castelli WP: Lipids, risk factors and ischemic heart disease. *Atherosclerosis* 124:S1–S9, 1996
4. European Atherosclerosis Society Study Group: The recognition and management of hyperlipidaemia in adults: a policy statement of the European Atherosclerosis Society. *Eur Heart J* 9:571–600, 1988
5. Ducimetiere P, Eschwege E, Papoz L, Richard JL, Claude JR, Rosselin G: Relationship of plasma insulin levels to the incidence of myocardial infarction and coronary heart disease mortality in a middle aged population. *Diabetologia* 19:205–210, 1980
6. Simon D, Charles MA, Nahoul K, Orssaud G, Kremski J, Hully V, Joubert E, Papoz L, Eschwege E: Association between plasma total testosterone and cardiovascular risk factors in healthy adult men: the Telecom Study. *J Clin Endocrinol Metab* 82:682–685, 1997
7. Haffner SM, Karhapaa P, Mykkanen L, Laakso M: Insulin resistance, body fat distribution and sex hormones in men. *Diabetes* 43:12–19, 1994
8. Fukui M, Koyama M, Nakagawa Y, Itoh Y, Nakamura N, Kondo M: Castration and diabetes. *Diabetes Care* 23:1032–1033, 2000
9. Holmang A, Bjorntorp P: The effects of testosterone on insulin sensitivity in male rats. *Acta Physiol Scand* 146:505–510, 1992
10. Andersson B, Marin P, Lissner L, Vermeulen A, Bjorntorp P: Testosterone concentrations in women and men with NIDDM. *Diabetes Care* 17:405–411, 1994
11. Barrett-Connor E: Lower endogenous androgen levels and dyslipidaemia in men with NIDDM. *Ann Intern Med* 117:807–811, 1992
12. Stellato RK, Feldman HA, Hamdy O, Horton ES, McKinlay JB: Testosterone, sex hormone-binding globulin, and the development of type 2 diabetes in middle-aged men. *Diabetes Care* 23:490–494, 2000
13. English KM, Steeds R, Jones TH, Channer KS: Testosterone and coronary heart disease:is there a link? *Q J Med* 90:787–791, 1997
14. Chute CG, Baron JA, Plymate SR, Kiel DP, Pavia AT, Lozner EC, O'Keefe T, MacDonald GJ: Sex hormones and coronary artery disease. *Am J Med* 83:853–859, 1987
15. Phillips GB, Pinkernell BH, Jing TY: The association of hypotestosteronaemia with coronary artery disease in men. *Arterioscler Thromb Vasc Biol* 14:701–706, 1994
16. Jeppeson LL, Jorgensen HS, Nakayama H, Raaschou HO, Olsen TS, Winther K: Decreased testosterone in men with acute ischaemic stroke. *Arterioscler Thromb Vasc Biol* 16:749–754, 1996
17. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 25:S5–S20, 2002
18. Ishizaka N, Ishizaka Y, Takahashi E, Tooda E, Hashimoto H, Nagai R, Yamakado M: Association between hepatitis C virus seropositivity, carotid-artery plaque, and intima-media thickening. *Lancet* 359:133–135, 2002
19. Handa N, Matsumoto M, Maeda H, Hougaku H, Ogawa S, Fukunaga R, Yoneda S, Kimura K, Kamada T: Ultrasonic evaluation of early carotid atherosclerosis. *Stroke* 21:1567–1572, 1990
20. Sih R, Morley JE, Kaiser FE, Perry HM 3rd, Patrick P, Ross C: Testosterone replacement in older hypogonadal men: a 12-month randomized controlled trial. *J Clin Endocrinol Metab* 82:1661–1667, 1997
21. Tenover JS: Effects of testosterone supplementation in the aging male. *J Clin Endocrinol Metab* 75:1092–1098, 1992
22. Barrett-Connor E, Khaw KT: Endogenous sex hormones and cardiovascular disease in men. A prospective population based study. *Circulation* 78:539–545, 1988
23. Seidell JC, Bjorntorp P, Sjostrom LS, Kvist H, Sannerstedt R: Visceral fat accumulation in men is positively associated with insulin, glucose, and C-peptide levels, but negatively with testosterone levels. *Metabolism* 39:897–901, 1990
24. Khaw KT, Barrett-Connor E: Lower endogenous androgens predict central adiposity in men. *Ann Epidemiol* 2:675–682, 1992
25. Rebuffe-Scrive M, Marin P, Bjorntorp P: Testosterone administration to middle-aged men decreases abdominal adipose tissue? A preliminary report. *Int J Obesity* 2:675–682, 1992
26. Anderson RA, Ludlam CA, Wu FC: Haemostatic effects of supraphysiological levels of testosterone in normal men. *Thromb Haemost* 74:693–697, 1995
27. Yue P, Chatterjee K, Beale C, Poole-Wilson, Collins P: Testosterone relaxes rabbit coronary arteries and aorta. *Circulation* 91:1154–1160, 1995
28. Webb CM, McNeill JG, Hayward CS, Zeigler D, Collins P: Effects of testosterone on coronary vasomotor regulation in men with coronary heart disease. *Circulation* 100:1690–1696, 1999
29. Brodsky IG, Balagopal P, Nair KS: Effects of testosterone replacement on muscle mass and muscle protein synthesis in hypogonadal men: a clinical research center study. *J Clin Endocrinol Metab* 81:3469–3475, 1996
30. Haffner SM, Valdez RA, Stern MP: Decreased free testosterone and dehydroepiandrosterone sulfate are associated with decreased glucose and insulin concentrations in nondiabetic men. *Metabolism* 43:599–603, 1994
31. Simon D, Preziosi P, Barrett-Connor E, Roger M, Sait-Paul M, Nahoul K, Papoz L: The influence of ageing on plasma sex hormones in men: the Telecom Study. *Am J Epidemiol* 135:783–791, 1992
32. Bebakar WM, Honour JW, Foster D, Liu YL, Jacobs HS: Regulation of testicular function by insulin and transforming growth factor-beta. *Steroids* 55:266–270, 1990
33. Pasquali R, Casimirri F, De Iasio R, Mesini P, Boschi S, Chierici R, Flamia R, Biscotti M, Vicennati V: Insulin regulates testosterone and sex hormone-binding globulin concentrations in adult normal weight and obese men. *J Clin Endocrinol Metab* 80:654–658, 1995
34. Howell S, Shalet S: Testosterone deficiency and replacement. *Horm Res* 56:86–92, 2001