The Association between Serum Testosterone and Risk Factors for Atherosclerosis

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Apolipoprotein B • Atherosclerosis • Hyperlipidemia • Hypogonadism

Introduction

Aging in men is associated with a gradual decline of testosterone levels [1]. A large number of studies have reported an increased risk of atherosclerosis among men with low testosterone levels [2], and serum lipids are essential for atherosclerosis development [3, 4].

The association between testosterone levels and risk factors for atherosclerosis such as obesity, hypertension, diabetes mellitus, and hyperlipidemia has gained increased attention in recent years. In this line, the European Male Aging Study of 2,966 men aged 40–79 years reported a higher body mass index (BMI), waist circumference, systolic pressure, glucose and insulin levels, and lower levels of total cholesterol, high density lipoprotein, and low density lipoprotein in hypogonadal men as compared to eugonadal men [5]. In addition, randomized control studies reported a beneficial effects of testosterone replacement therapy on insulin resistance and lipid metabolism in hypogonadal men with type 2 diabetes [6], and reduction in waist circumference, fasting blood glucose, and biomarkers of atherosclerosis in hypogonadal men with metabolic syndrome [7].

Men from the general population are an ideal group for investigating the associations between testosterone and risk factors for atherosclerosis. However, no sufficient data regarding this association is available from this group of men. Therefore, the aim of the present study is...
to evaluate the associations between serum testosterone levels and risk factors for atherosclerosis including BMI, systolic blood pressure, diastolic blood pressure, blood glucose tests, prothrombotic factors, and lipid levels. We made an effort to include available co-variables known to affect testosterone levels in our adjusted statistical analysis obtained from 119 middle-aged men from the general population.

**Material and Methods**

This present study was based on 119 middle-aged men from the general population from the southern part of Sweden between January 2006 and January 2011 at the Department of Cardiology, Malmö University Hospital. Figure 1 is a flow chart showing the recruiting process of the study population which was described in details elsewhere [8].

Men with past or present history of medical disease including psychological illnesses or those who received prescription of regular medications during the last 6 months prior to inclusion, men younger than 45 years and older than 60 years at the time of inclusion were excluded from the study.

All study subjects underwent a 24-hour ambulatory blood pressure monitoring by means of a portable automatic device (SpaceLabs monitor 90207, Kontron). Systolic and diastolic pressures were measured at 30-minute intervals during daytime hours (7 AM to 9 PM) and at 60-minute intervals during nighttime hours (10 PM to 6 AM). The subjects were instructed to maintain normal daily activities and to follow their normal life schedule. The portable monitor was worn on a belt connected to a standard cuff on the upper left arm. Artifactual readings due to arm motion or other interferences during recording were removed automatically by the recorder. Outliers were removed by the computer in case the diastolic pressure < 20 mmHg, the diastolic pressure < 40 mmHg, or the systolic pressure > 260 mmHg as previously described [9]. Data was given as mean 24-hour systolic and diastolic pressure. For the 2-hour glucose tolerance test, a blood sample was taken 2 hours after oral administration of 75 g of glucose dissolved in 300 ml of water.

Data were also gathered on age, height, weight, BMI, smoking and alcohol consumption. Each man was asked to deliver a blood sample for the analysis of testosterone, fasting blood glucose, HbA1c, Factor VIII, von Willebrand factor antigen (vWF:Ag), von Willebrand factor ristocetin cofactor (vWF:RCO) activity, apolipoprotein A-1 (ApoA-1), apolipoprotein B (ApoB), and ApoB/ApoA-1 ratio. Blood samples were delivered between 7 and 10 AM.

**Fig. 1.** Flow chart of recruitment process of participants in the study.
Table 1. Descriptive statistics of the study population

| Variables                  | n  | Mean ± SD or n (%) | Median (range) |
|----------------------------|----|--------------------|----------------|
| Age, Years                 |    |                    |                |
| < 50                       | 9  | 47 ± 1.0           | 47 (46–49)     |
| > 50                       | 110| 56 ± 3.0           | 57 (50–60)     |
| BMI, kg/m²                 | 119| 26 ± 3.0           | 26 (20–38)     |
| Smoking                    |    |                    |                |
| Never                      | 118| 61 (51%)           | 57 (49%)       |
| Alcohol consumption, cl/week| 119|                    |                |
| ≤ 10                       |    | 56 (47%)           |                |
| 10.1–20                    |    | 41 (35%)           |                |
| 20.1–30                    |    | 18 (15%)           |                |
| > 30                       |    | 4 (3.0%)           |                |
| Systolic blood pressure, mmHg | 108| 125 ± 14           | 74 (57–105)    |
| Diastolic blood pressure, mmHg | 108| 76 ± 9.0           | 5.5 (4.2–7.1)  |
| Fasting blood glucose, mmol/l | 89 | 6.7 ± 1.8          | 6.4 (3.2–12.7) |
| 2-Hour glucose tolerance test, mmol/l | 89 | 4.7 ± 0.68         | 4.5 (4–10)     |
| HbA1c, %                   | 107| 0.99 ± 0.37        | 1.1 (0.42–1.98) |
| Factor VIII, kIE/l         | 119| 1.00 ± 0.40        | 0.96 (0.43–1.88) |
| vWF:Ag, kIE/l              | 119| 0.94 ± 0.35        | 1.1 (0.87–1.40) |
| vWF:RCo, kIE/l             | 119| 1.4 ± 0.20         | 1.37 (0.86–1.93) |
| ApoA-1, g/l                | 108| 1.0 ± 0.25         | 1.0 (0.30–1.64) |
| ApoB/ApoA-1 ratio          | 108| 0.76 ± 0.22        | 0.75 (0.22–1.52) |
| Testosterone, nmol/l       | 108| 15.4 ± 6.0         | 14.6 (0.6–32.3) |

Table 2. Variables representing risk factors for atherosclerosis according to testosterone levels

| Variables                  | Testosterone, nmol/l | P   | ≤ 12 (n = 37) | > 12 (n = 71)     |
|----------------------------|----------------------|-----|--------------|-------------------|
| Age, years                 | 56 ± 4.0             | 0.03| 55 ± 4.0     | 0.01              |
| BMI, kg/m²                 | 28 ± 4.0             |     | 26 ± 3.0     |                  |
| Systolic blood pressure, mmHg | 129 ± 16            | 0.03| 123 ± 12     | 0.03              |
| Diastolic blood pressure, mmHg | 77 ± 11             |     | 75 ± 9.0     | 0.50              |
| Fasting blood glucose, mmol/l | 5.9 ± 0.7           |     | 5.5 ± 0.6    | 0.03              |
| 2-Hour glucose tolerance test, mmol/l | 7.3 ± 1.8      |     | 6.5 ± 1.9    | 0.08              |
| HbA1c, %                   | 4.9 ± 1.0            |     | 4.6 ± 0.3    | 0.10              |
| Factor VIII, kIE/l         | 1.1 ± 0.3            | 0.40| 1.0 ± 0.3    | 0.40              |
| vWF:Ag, kIE/l              | 1.1 ± 0.4            |     | 1.1 ± 0.3    | 0.90              |
| vWF:RCo, kIE/l             | 0.99 ± 0.3           |     | 0.99 ± 0.3   | 0.90              |
| ApoA-1, g/l                | 1.4 ± 0.2            |     | 1.4 ± 0.2    | 0.50              |
| ApoB, g/l                  | 1.1 ± 0.30           |     | 1.0 ± 0.3    | 0.03              |
| ApoB/ApoA-1 ratio          | 0.8 ± 0.2            | 0.03| 0.7 ± 0.2    |                  |

Table 3. Association between BMI, mean systolic blood pressure, fasting blood glucose, 2-hour glucose tolerance test, ApoB, ApoB/ApoA-1 ratio and serum levels of testosterone from 119 middle-aged men

| Variables                  | Testosterone, nmol/l | β  | 95% CI         | P   |
|----------------------------|----------------------|----|----------------|-----|
| BMI, kg/m²                 | -1.80                | 0.03| -3.50          | -0.20|
| Systolic blood pressure, mmHg | -6.00               | 0.07| -12.00         | 0.60|
| Fasting blood glucose, mmol/l | -0.40               | 0.01| -0.70          | -0.10|
| 2-Hour glucose tolerance test, mmol/l | -1.00             | 0.04| -2.00          | -0.02|
| ApoB, g/l                  | -0.20                | 0.02| -0.30          | -0.03|
| ApoB/ApoA-1 ratio          | -0.10                | 0.04| -0.20          | -0.02|

Statistical Analysis

Statistical analyses were performed using the SPSS software, version 16 (SPSS, Inc; Chicago, IL). Men were classified into 2 groups based on testosterone levels: hypogonadal (testosterone ≤ 12 nmol/l), and eugonadal (testosterone > 12 nmol/l). The age of subjects, BMI, mean systolic blood pressure, mean diastolic blood pressure, serum levels of fasting blood glucose, 2-hour glucose tolerance test, HbA1c, Factor VIII, vWF:Ag, vWF:RCo, ApoA-1, ApoB, and ApoB/ApoA-1 ratio were compared between groups using non-parametric Mann-Whitney U test.

Thereafter, the associations between testosterone levels (categorized) and variables that showed significant difference between the 2 groups (BMI, mean systolic blood pressure, fasting blood glucose, 2-hour glucose tolerance test, ApoB, and ApoB/ApoA-1 ratio) were evaluated using multivariate linear regression analysis.
Results

Descriptive statistics of the study population are summarized in table 1. When compared to eugonadal men (testosterone > 12 nmol/l), those who were hypogonadal (testosterone ≤ 12 nmol/l) and were significantly older (56 vs. 55 years, p = 0.03), had greater BMI (28 ± 26 kg/m², p = 0.01). Moreover, mean systolic blood pressure (129 vs. 123 mmHg, p = 0.03), serum levels of fasting blood glucose (5.9 ± 5.5 mmol/l, p = 0.03), 2-hour glucose tolerance test (although closed to statistical significant level) (7.3 ± 15 mmol/l, p = 0.08), ApoB (1.1 ± 1.0 g/l, p = 0.03), ApoB/ApoA-1 ratio (0.8 vs. 0.7, p = 0.03) were significantly higher in hypogonadal men than in eugonadal men, respectively. In contrast, mean diastolic blood pressure, serum levels of Factor VIII, vWF:F:Ag, vWF:RCo, and ApoA-1 did not differ significantly between groups (p > 0.05, table 2).

When we tested the associations between testosterone levels and BMI, mean systolic blood pressure, serum levels of fasting blood glucose, 2-hour glucose tolerance test, ApoB, and ApoB/ApoA-1 ratio using adjusted multivariate analysis regression model, we found a negative significant associations between testosterone levels and BMI (β = -2.00, p = 0.03, 95% CI = -3.50 – -0.20), mean systolic blood pressure although closed to statistical significant level (β = 1.00, p = 0.01, 95% CI = -1.00 – 0.02), ApoB (β = 0.20, p = 0.02, 95% CI = -0.30 – -0.03), 2-hour glucose tolerance test (β = -0.20, p = 0.04, 95% CI = -0.40 – -0.02), ApoB/ApoA-1 ratio (β = -0.10, p = 0.04, 95% CI = -0.30 – -0.02) (table 3).

Discussion

Men with low testosterone levels had significantly higher BMI, mean systolic blood pressure, fasting glucose, 2-hour glucose tolerance test, ApoB, and ApoB/ApoA-1 ratio. This was further confirmed in an adjusted multivariate regression analysis model, where testosterone levels showed negative significant associations with BMI, mean systolic blood pressure, fasting blood glucose, 2-hour glucose tolerance test, ApoB, and ApoB/ ApoA-1 ratio. These results indicate an inverse association between testosterone levels and risk factors for atherosclerosis.

We can confirm previous findings considering low testosterone as a potential risk factor for atherosclerosis [10–15]. Data from a large number of studies support a relationship between low testosterone and obesity [16–19], a finding that explained by the increased activity of aromatase, an enzyme that converts testosterone to estradiol, which located in the visceral fat [20]. The association between testosterone and hypertension is not well understood. Some investigators relay it to a vasodilatory effect of testosterone on vascular and non-vascular smooth muscle by inhibiting the calcium-dependent elements of vascular contraction [21–23]. Low levels of testosterone are associated to insulin resistance [24, 25], supported by the observation that normalization of testosterone levels upregulating the expression of insulin signal genes (IRβ, IRS-1, Akt-2 and GLUT4) in adipose tissue [26].

Results from previous studies regarding the association between testosterone and prothrombotic factors are conflicting showing no significant association [27, 28], or a negative significant association [28–30]. We were not able to find a significant association between testosterone levels and prothrombotic factors in our study, the differences between these studies including our study may be due to differences in the number of study subjects, in the selection criteria of the subjects, and in the methods of statistical analyses. Several studies revealed an inverse relation between levels of testosterone and lipid metabolism [31, 32]. Moreover, lipid levels were reported to be higher in testosterone-deficient male mice [33, 34].

The above assumptions were confirmed by evidence from prospective longitudinal studies regarding the effect of testosterone replacement therapy on risk factors for atherosclerosis in men with hypogonadism. Thus, Yassin et al. [35] showed decreased BMI, blood pressure, fasting blood glucose, HbA1c, total cholesterol, low density lipoprotein cholesterol, triglycerides over a 5-year study period of 261 hypogonadal men with mean age 59.5 years, treated with testosterone. Also randomized, double-blind, placebo-controlled studies documented improvement of fasting glucose and reduction of visceral fat in 50 men aged between 45 and 65 years who treated with testosterone up to 2 years due to testosterone deficiency [7]. The authors also observed a significant improvement in markers of endothelial function and atherosclerosis progression such as high sensitive C-reactive protein and carotid intima-media thickness [7].
Our results and previous data have some clinical application. Thus, analysis of testosterone level should be part of the work up of men with increased risk for atherosclerosis.

The weaknesses of our study were mentioned elsewhere [8]. However, we would like to highlight that our study is based on a relatively small number of men as compared to other studies, however the statistical analysis revealed striking results in such a small population of men. Also, our results are based on a single analysis of testosterone. In fact, low testosterone values should be confirmed by repeated measurement. However, a single analysis of testosterone is considered sufficient to reflect the annual mean testosterone level in healthy middle-aged men as documented elsewhere [36].

In conclusion, we documented an inverse association between testosterone levels and risk factors for atherosclerosis, namely BMI, mean systolic blood pressure, fasting glucose levels, 2-hour glucose tolerance test, ApoB, and ApoB/ApoA-1 ratio. These results indicate that men with low testosterone levels associated with increased risk of atherosclerosis.

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