Clinical Research and Trials

Research Article ISSN: 2059-0377

Testosterone and coronary flow reserve: A study on Swedish men from the general population

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Abstract

Background: Coronary flow reserve (CFR) is an index of coronary microcirculation. No previous reports exist on the association between testosterone levels and CFR. Therefore, the aim of the present study is to explore the association between testosterone and CFR in a group of men from the general population.

Material and Methods: The study consisted of 66 men with no history of cardiovascular disease (CVD). Clinical data included age, body mass index (BMI), smoking, alcohol consumption, family history of CVD, and systolic blood pressure (SBP) were collected. Serum levels of testosterone and HbA1c were measured, and ApoB-to-ApoA-1 ratio was calculated. CFR was assessed using transthoracic colour Doppler echocardiography. CFR was calculated as the ratio between mean hyperemic coronary flow velocity (CFV) to mean baseline CFV. The men were classified into 3 groups based on CFR : G1 (CFR ≤3), G2 (CFR = 3.1-4.0), and G3 (CFR >4).

Results: Men in G1 had significantly lower testosterone levels compared to men in G3 (12nmol/L vs. 17nmol/L; p=0.021). On the other hand, no statistically significant differences were found between men in G1 and those in G2 (12nmol/L vs. 15nmol/L; p=0.299), as well as between men in G2 and G3 (15nmol/L vs. 17nmol/L; p=0.493). Age, BMI, smoking, alcohol consumption, family history of CVD, SBP, ApoB-to-ApoA-1 ratio, and HbA1c did not differ significantly between groups. In multivariate regression analysis adjusted for the age of subjects, BMI, smoking, alcohol consumption, family history of CVD, SBP, ApoB-to-ApoA-1 ratio, HbA1c; a significant positive association was found between testosterone and CFR (β=0.035; 95% CI=0.011, 0.069; p=0.04).

Conclusions: In men without symptomatic cardiovascular disease, there was a significant positive association between testosterone and CFR, which may indicate that testosterone levels could play a role for coronary microcirculation.

Introduction

In men, a low serum testosterone is associated with higher incidence of cardiovascular disease compared to men with normal testosterone [1,2]. Previous studies consistently found that testosterone replacement therapy lowers the risk of coronary heart disease in men with low testosterone levels at baseline [3,4]. Thus, testosterone is supposed to have a cardioprotective effect in men.

Early atherosclerotic coronary disease often manifests itself as impaired coronary microcirculation, without significant obstructive coronary stenoses. In response to vasodilators or physical activity, the microvasculature dilates and produces hyperaemia compared to the resting state, which can be quantified as coronary flow reserve (i.e., the ratio of flow during hyperaemia divided by flow at baseline). Transthoracic colour Doppler echocardiography allows us to estimate the coronary flow reserve by measuring the coronary flow reserve (CFR), which (in the absence of obstructive stenoses) is an index of coronary microcirculation in vivo [5,6]. A previous study has documented a positive effect of estrogen replacement therapy on CFR [7].

No published clinical reports have so far examined the association between serum testosterone levels and CFR. Therefore, the aim of the present study was to investigate the association between serum testosterone levels and CFR in middle-aged healthy Swedish men from the general population.

Material and Methods

Study subjects

The present study was based on data from 119 Swedish men from the general population, 45–60 years old, collected at the Department of Cardiology, Malmo University hospital between 2006 and 2011. The men were community-dwelling and recruited by random invitation, as previously described [8]. All subjects were asymptomatic, normotensive, non-diabetics. They had no history of physical or psychological diseases. No subject had taken any medications during the last 6 months before inclusion. The Institutional Review Board Committee at Lund University of Medical sciences reviewed and approved the design of the study. A written informed consent was obtained from each participant.

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Key words: *cardiovascular diseases, coronary flow reserve, microcirculation, testosterone*

Received: January 13, 2022; **Accepted:** February 01, 2022; **Published:** February 04, 2022

Collected data

We gathered information about the age of subjects; body mass index (BMI) was computed as the ratio of weight to the square of height (kg/m^2); smoking; alcohol consumption (defined as the number of alcoholic drinks consumed per week). Participants were asked; how many drinks do you have per week? and a dichotomous variable (yes or no) was created to identify drinkers from non-drinkers. The amount of pure alcohol (in mL) was calculated for each beverage using the following formula: the serving size of the drink (in mL) x the percentage alcohol by volume of the drink. The sum per week (mL/week) is then calculated and converted to cL/week; Family history of cardiovascular disease (CVD) was defined as a self-reported diagnosis of CVD in parents, siblings, or children that occurred at 60 years or younger.

Laboratory assay

Each man was asked to deliver a blood sample (5 mL) for analysis of testosterone. Blood samples were collected between 07:00 and 10:00 PM on day one. Blood sample was centrifuged at 1000 g for 10 minutes. After centrifugation, serum was kept at -80 °C until assay. Serum testosterone concentrations were measured using Two-step competitive method with Electro Chemi Luminiscence Immunoassay (ECLI) detection technique based on Ruthenium (Ru) derivatives, with an intra-and interassay coefficient of variation (CV) of 5%, and a reference range 5.0–30 nmol/L. ApoA-1 and ApoB were measured by Quest Diagnostics (San Juan Capistraon, CA), blinded to case-control status, using an immunonephelometric assay run on the Siemens BNII (Siemens, Newark, DE). The interassay variability was < 4.0% for both ApoA-1 and ApoB. Thereafter ApoB-to-ApoA-1 ratio was calculated. HbA1c is analyzed with Capillary's 3 TERA Hemoglobin A1c Kit program. The separation takes place on the capillary with subsequent calculation. The separation is optimized to eliminate interferences from hemoglobin variants, pre-HbA1c and carbamylated hemoglobin with an intra-and interassay coefficient of variation (CV) of CV: 3% at level ~ 36 mmol / mol and 3% at level ~ 68 mmol / mol (Department of Clinical Chemistry, Malmö University Hospital, Malmö, Sweden).

Measurement of systolic blood pressure: All study subjects underwent a 24-hour ambulatory blood pressure monitoring by means of a portable automatic device (SpaceLabs monitor 90207, Kontron) as previously described [9].

Coronary flow reserve: The day after the volunteers delivered the blood samples, each subject underwent an echocardiography exam at the department of Cardiology. They were then invited to perform an adenosine provocation for measurement of coronary flow velocity reserve. The coronary flow velocity was measured in the mid to distal left anterior descending coronary artery (LAD). Coronary flow velocity (CFV) was measured at baseline (baseline CFV) and after administrating the vasodilator using adenosine 140 μg/kg/min as an intravenous infusion for about 1 minute (hyperemic CFV) via peripheral vein using A Siemens Acuson platform equipped with a 4V1C transducer with 3.5 MHz spectral Doppler frequency (Siemens, Acuson Sequoia 521, Mountainview, California, USA). To ensure minimum variability, all measurements were performed by one operator. Cine-loops and Doppler images were stored for offline analysis using Tomtec image analysis software (Image Arena 2.9.1, Tomtec Imaging System, GmbH, Unterschleissheim, Germany). intraindividual variation for CFR readings in a similar population being 2.7 % (± 5.7%) [10]. CFR was calculated as the ratio between mean hyperemic CFV to mean baseline CFV. Of the 119 men, 87 (73 %) agreed to perform a CFR test. The remaining 32 (27 %) men declined receiving an intravenous vasodilator. The data were then checked by an expert where 21 out of 87 (24 %) cases were excluded due to inability to determine CFR or technical errors, resulting in 66 (76 %) valid CFR cases which were then used in analyses.

Statistical analyses

Statistical analyses were performed using the SPSS software, version 16 (SPSS, Inc; Chicago, IL).

CFR was < 2 in one (1 %), 2–3 in 25 (37 %), and > 3, ≤ 4 in 26 (38 %), and more than 4 in 14 (21 %) men. Therefore, the men were classified into three groups based on values of CFR ratio: G1 (CFR less than and equal to 3), G2 (CFR ratio between 3.1 and 4.0), and G3 (CFR more than 4). Age of subjects, BMI, smoking, alcohol consumption, family history of CVD, systolic blood pressure, ApoB-to-ApoA-1 ratio, HbA1c and testosterone levels were compared between groups using non-parametric Mann-Whitney test and Fisher exact test.

Thereafter, the association between testosterone levels (independent variable) and CFR groups (G1-G2-G3) (dependent variable) was tested in a multivariate regression analysis model adjusted for the age of subjects (contentious years), BMI (contentious kg/m^2), smoking (past/present, never), alcohol consumption (yes, no), family history of CVD (yes, no), systolic blood pressure (continuous mmHg), ApoB-to-ApoA-1 ratio, and HbA1c (continuous mmol/mol). P-values < 0.05 were considered statistically significant.

Results

Descriptive statistics of the study population is summarized in Table 1. Men in G1 (CFR ≤ 3) had significantly lower testosterone levels compared to men in G3 (CFR > 4) (12 nmol/L vs. 17 nmol/L; p = 0.021). On the other hand, no statistically significant differences were found between men in G1 and those in G2 (CFR ratio 3.1-4.0) (12 nmol/L vs. 15 nmol/L; p = 0.299), or between men in G2 and G3 (15 nmol/L vs. 17 nmol/L; p = 0.493). The age of subjects, BMI, smoking, alcohol consumption, family history of CVD, systolic blood pressure, ApoB-to-ApoA-1 ratio, and HBa1C did not differ significantly between groups (p >0.05) (Table 2).

In a multivariate regression analysis test adjusted for the age of subjects, BMI, smoking, alcohol consumption, family history of CVD, systolic blood pressure, ApoB-to-ApoA-1 ratio, and HbA1c; a significant positive association was found between testosterone and CFR (categorical) (β = 0.035; 95% CI = 0.011, 0.069; p = 0.04).

Discussion

In this study based on 66 healthy Swedish men from the general population, we found a significant positive association between testosterone levels and coronary flow reserve. These results suggest that testosterone may affect the coronary microcirculation. Thus, men with normal testosterone levels had a higher CFR compared to men with low testosterone levels. We were not able to find any significant association between CFR and the age of subjects, BMI, smoking, alcohol consumption, family history of CVD, systolic blood pressure, ApoB-to-ApoA-1 ratio, and HbA1c. Therefore, there is a possibility that the association between testosterone and CRF indicates a direct effect of testosterone on coronary microcirculation, and that testosterone may be an independent risk marker for early coronary heart disease.

We measured the CFR using transthoracic Doppler echocardiography, which is non-invasive, and provides measurement

Table 1. Descriptive statistics of the study population

Variables	Mean (± SD) or n (%)
Age (years):	56 (± 4.0)
BMI (kg/m^2):	27 (± 4.0)
Smoking:	
Never	13 (20 %)
Past/current	53 (80 %)
Alcohol consumption (Cl/week):	
Yes	55 (83 %)
No	11 (17 %)
Family history of CVD:	
Yes	17 (26 %)
No	49 (74 %)
Systolic blood pressure (mmHg)	125 (± 12)
ApoB-to-ApoA-1 ratio	0.79 (± 0.23)
HbA1c (mmol/mol)	4,7 (± 0.80)
Coronary flow reserve (CFR):	
Baseline CFV (cm/s)	23 (± 5.0)
Hyperemic CFV (cm/s)	74 (± 20)
CFR	3.3 (± 1.0)
Testosterone (nmol/L):	15 (± 7.0)

Number = 66; BMI = body mass index; CVD = cardiovascular diseases; CFV = Coronary flow velocity.

Table 2. Demographic data, systolic blood pressure, ApoB-to-ApoA-1 ratio, and testosterone concentration according to groups of CFR ratio from 66 healthy middle-aged Swedish men from the general population

Variables	G1 CRF ratio ≤ 3.0 n = 26	G2 CRF ratio 3.1–4.0 n = 26	G3 CRF ratio ≥ 4.0 n = 14
Age (years)	56 (± 4.0)	56 (± 3.0)	54 (± 4.0)
BMI (kg/m^2)	27 (± 4.0)	27 (± 3.0)	26 (± 4.0)
Smoking[a]	7 (27%)	5 (19%)	2 (13%)
Never	19 (73%)	21 (81%)	14 (87%)
Past/current	23 (88%)	22 (85%)	11 (69%)
Alcohol consumption[a]			
Yes	3 (12%)	4 (15%)	5 (31%)
No	7 (27%)	8 (31%)	2 (13%)
Family history of CVD[a]			
Yes	19 (73%)	18 (69%)	14 (87%)
No	124 (± 12)	127 (± 14)	124 (± 11)
Systolic blood pressure (mmHg)			
ApoB-to-ApoA-1 ratio	0.78 (± 0.22)	0.79 (± 0.26)	0.75 (± 0.23)
HbA1c (mmol/mol)	4.5 (± 0.48)	4.8 (± 1.10)	4.6 (± 0.40)
Testosterone (nmol/L)	12 (± 7.0)*	15 (± 6.0)	17 (± 6.0)*

Values are mean (± SD) or numbers (%); [a]Statistical analysis was done using fisher exact test; BMI = body mass index; CVD = cardiovascular diseases; *Values with same sign indicate significant differences.

of CFR with a relatively high success rate [5,6]. In general, CFR values are considered normal with values ≥ 2, and in previous studies most subjects with no coronary artery disease expressed values between 3 and 5 [11-13]. In our study, all but one subject had normal CFR, but there was a gradient within the normal range of CFR values, between the groups. Significant coronary artery stenoses or pronounced microvascular dysfunction are usually symptomatic, and it is therefore likely that our results indicate early microvascular dysfunction and reduction of CFR in relation to testosterone levels. The study recruited only asymptomatic men from the general population, and the effect on CFR was therefore free from confounding effects from comorbidities such as diabetes and hypertension.

Impaired or reduced coronary flow reserve has been previously reported to be reflective of coronary microcirculation abnormality in patients with hypercholesterolemia [14], diabetes [15] and hypertension [14,16]. The positive association between testosterone levels and CFR could be attributed to the cardioprotective effect of testosterone on men. Some studies have shown that lower risk of coronary heart disease is attributable to the favorable effects of testosterone on lipid and lipoprotein levels, glucose and insulin levels, and blood pressure [17]. Other mechanisms of potential benefit include antioxidant effects. For instance, Coenzyme Q-10 levels are significantly lower in men with isolated hypogonadism than in eugonadal men [18]. Testosterone treatment, performed in those patients with isolated hypogonadism, induced a significant enhancement both in Coenzyme Q-10 level and total antioxidant capacity [18]. Coenzyme Q-10 is suggested to improve the endothelial function, in this line intravenous injection of Coenzyme Q-10 was associated with rapid improvement of nitric oxide- dependent vasodilatation of the rat aorta [19].

Several studies have documented a role of low testosterone levels in microvascular dysfunction. Thus, testosterone has been shown to be negatively correlated with indices of aortic stiffness, and testosterone replacement therapy was associated with significant improvement of arterial stiffness [20,21]. Also, CFR was inversely related to indices of aortic stiffness [22]. One can assume that testosterone replacement therapy might also improve CFR.

We believe that our results are in line with previous observations regarding testosterone and its association to cardiovascular risk and taken together this may have clinical implications. Low levels of testosterone can be found by simple blood sample screening. If our results are replicated in larger materials, it may be warranted for clinicians to consider analysis of testosterone levels in middle-aged men who are suspected of coronary artery disease or have other cardiovascular risk factors. Abnormality of coronary microcirculation is an early manifestation of atherosclerosis, and if testosterone levels can add information in this respect, it should be taken to account in the clinical management of patients with increased risk for atherosclerosis. Whether or not testosterone replacement therapy can reverse coronary microvasculature impairment, remains to be studied. The next step in order to substantiate the clinical effect, could therefore be a randomized trial on men with low serum testosterone at baseline, comparing CFR pre- and post-testosterone substitution therapy.

The overall participation rate in this study was 16% of the invited persons. Since no information was available about the men who chose not to reply to the questionnaires, the characteristics of this group could not be compared to that of the included group of men, and one could question whether the included group of men represents the general population of middle-aged Swedish men. Our study is based on a relatively small sample size compared to other studies. In addition, 24% of the of the CFR measurements performed were excluded due to inability to determine CFR or technical errors, nevertheless the results were statistically significant even in this relatively small population. We believe, therefore, that the results are still valid and support the hypothesis that there is a relationship between serum levels of testosterone and coronary flow reserve in this group of men.

Conclusions

In conclusion, this the first study to find a significant positive association between testosterone levels and coronary flow reserve in men without known cardiovascular disease, indicating that testosterone may play a role in coronary microcirculation and early stages of atherosclerosis.

Declaration of interest

The authors report no conflict of interest.

References

1. Hu X, Rui L, Zhu T, Xia H, Yang X, et al. (2011) Low testosterone level in middle-aged male patients with coronary artery disease. Eur J Intern Med 22: e133-e136. [Crossref]
2. Pastuszak AW, Kohn TP, Estis J, Lipshultza LI (2017) Low Plasma Testosterone is Associated with Elevated Cardiovascular Disease Biomarkers. *J Sex Med* 14: 1095-1103. [Crossref]
3. Janjgava S, Zerekidze T, Uchava L, Giorgadze E, Asatiani K (2014) Influence of testosterone replacement therapy on metabolic disorders in male patients with type 2 diabetes mellitus and androgen deficiency. *Eur J Med Res* 19: 56.
4. Rouver WN, Delgado NTB, Menezes JB, Santos RL, Moyses MR (2015) Testosterone Replacement Therapy Prevents Alterations of Coronary Vascular Reactivity Caused by Hormone Deficiency Induced by Castration. *PLoS One* 10: e0137111. [Crossref]
5. Dimitrow PP (2003) Transthoracic Doppler echocardiography – noninvasive diagnostic window for coronary flow reserve assessment. *Cardiovasc Ultrasound* 1: 4.
6. Simova J (2015) Coronary Flow Velocity Reserve Assessment with Transthoracic Doppler Echocardiography. *Eur Cardiol* 10: 12-18. [Crossref]
7. Hirata K, Shimada K, Watanabe H, Muro T, Yoshiyama M, et al. (2001) Modulation of Coronary Flow Velocity Reserve by Gender, Menstrual Cycle and Hormone Replacement Therapy. *J Am Coll Cardiol* 38: 1879-1884. [Crossref]
8. Rezanezhad B, Borgquist R, Willenheimer R, Elzanaty S (2018) Association between serum levels of testosterone and biomarkers of subclinical atherosclerosis. *Aging Male* 21: 182-186. [Crossref]
9. Rezanezhad B, Borgquist R, Willenheimer R, Elzanaty S (2019) The Association between Serum Testosterone and Risk Factors for Atherosclerosis. *Curr Urol* 13: 101-106. [Crossref]
10. Borgquist R, Nilssonb PM, Gudmundssona P, Winterc R, Le´osdottı´r M, et al. (2007) Coronary flow velocity reserve reduction is comparable in patients with erectile dysfunction and in patients with impaired fasting glucose or well-regulated diabetes mellitus. *Eur J Cardiovasc Prev Rehabil* 14: 258-264. [Crossref]
11. Lowenstein JA, Caniggia C, Rousse G, Amor M, Sánchez ME, et al. (2014) Coronary flow velocity reserve during pharmacologic stress echocardiography with normal contractility adds important prognostic value in diabetic and nondiabetic patients. *Am Soc Echocardiogr* 27: 1113-1119. [Crossref]
12. Meimoun P, Sayah S, Tcheuffa JC, Benali T, Luycx-Bore A, et al. (2006) Transthoracic coronary flow velocity reserve assessment: comparison between adenosine and dobutamine. *J Am Soc Echocardiogr* 19: 1220-1228. [Crossref]
13. Forte EH, Rousse MG, Lowenstein JA (2011) Target heart rate to determine the normal value of coronary flow reserve during dobutamine stress echocardiography. *Cardiovasc Ultrasound* 9: 10. [Crossref]
14. Haraldsson I, Gan L-M, Svedlund S, Torngren K, Westergren HU, et al. (2019) PROspective evaluation of coronary FLOW reserve and molecular biomarkers in patients with established coronary artery disease the PROFLOW-trial: cross-sectional evaluation of coronary flow reserve. *Vasc Health Risk Manag* 28: 375-384. [Crossref]
15. Quiñones MJ, Hernandez-Pampaloni M, Schelbert H, Bulnes-Enriquez I, Jimenez X, et al. (2004) Coronary vasomotor abnormalities in insulin-resistant individuals. *Ann Intern Med* 140: 700-708. [Crossref]
16. Völz S, Svedlund S, Andersson B, Li-Ming G, Rundqvist B (2017) Coronary flow reserve in patients with resistant hypertension. *Clin Res Cardiol* 106: 151–157. [Crossref]
17. Tajar A, Huhtaniemi IT, O'Neill TW, Finn JD, Pye SR, et al. (2012) Characteristics of androgen deficiency in late-onset hypogonadism: results from the European Male Aging Study (EMAS). *J Clin Endocrinol Metab* 97:1508-1516. [Crossref]
18. Mancini A, Leone E, Festa R, Grande G, Silvestrini A, et al. (2008) Effects of testosterone on antioxidant systems in male secondary hypogonadism. *J Androl* 29: 622-629. [Crossref]
19. Kozaeva LP, Gorodetskaya EA, Ruuge EA, Kalenikova EI, Medvedev OS (2017) Beneficial effect of coenzyme Q 10 injection on nitric oxide -related dilation of the rat aorta. *Eur J Pharmacol* 5: 15-19.
20. Corrigan FE, Al Mheid I, Eapen DJ, Hayek SS, Sher S, et al. (2015) Low testosterone in men predicts impaired arterial elasticity and microvascular function. *Int J Cardiol* 194: 94-99. [Crossref]
21. Nichols WW, Denardo SJ, Davidson JB, Huo T,Merz CNB, Pepine CJ (2015) Association of Aortic Stiffness and Wave Reflections with Coronary Flow Reserve in Women without Obstructive Coronary Artery Disease: An Ancillary Study from the NHLBI-Sponsored Women's Ischemia Syndrome Evaluation (WISE). *Am Heart J* 170: 1243–1254. [Crossref]
22. Webb CM, Elkington AG, Kraidly MM, Keenan N, Pennell DJ, et al. (2008) Effects of oral testosterone treatment on myocardial perfusion and vascular function in men with low plasma testosterone and coronary heart disease. *Am J Cardiol* 101: 618-24. [Crossref]