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·*Original Article*·

Relationship between testosterone and indexes indicating endothelial function in male coronary heart disease patients

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Abstract

Aim: To investigate the relationship between androgen level and the indexes indicating endothelial function in male patients with coronary heart disease (CHD). **Methods:** We registered the following data for 106 50–70-year-old men: age, weight, blood lipid, including total cholesterol, low density lipoprotein cholesterol, high density lipoprotein cholesterol and triglyceride, whether a smoker, sugar levels, blood pressure, free testosterone (FT), vascular cell adhesion molecule-1 (VCAM-1) and the intima-media thickness (IMT) of common carotid artery, common carotid diameter, maximum velocity in systolic phase, minimum velocity in diastolic phase and resistent index. Among the 106 men, 51 were patients with CHD. The relationships between FT level, VCAM-1 concentration and IMT were examined, respectively, using a stepwise linear regression technique among all the 106 men. **Results:** There was no statistical difference in terms of age, blood pressure, whether a smoker, sugar levels, HDL-C, minimum velocity in diastolic phase, resistent index between male CHD patients and controls; whereas results for weight, total cholesterol, low density lipoprotein cholesterol, triglyceride, VCAM-1 and IMT of male CHD patients were higher than those of controls; FT level and maximum velocity in systolic phase were lower. It was found that among all the objects, FT level was inversely correlated with IMT and VCAM-1 concentration. **Conclusion:** FT level was inversely correlated with VCAM-1 concentration and IMT which are indicators of endothelial function. *(Asian J Androl 2008 Mar; 10: 214–218)*

Keywords: testosterone; coronary heart disease; endothelial function

1 Introduction

Coronary heart disease (CHD) is associated with several factors, including cigarette smoking, diabetes, hypertension and elevated serum lipids. Some data shows that men with proven coronary atherosclerosis have lower levels of endogenous androgens than healthy controls [1]. According to Channer and Jones [2] and Dobrzycki *et al.* [3], the decrease in genital function might play an important role during the progression of atherosclerosis in men. However, the mechanism is thus far unknown. Damage to endothelial function is acknowledged to be the initial step in the formation of atherosclerotic lesions, so we investigate the relationship between androgen level and some indexes indicating endothelial function.

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2 Materials and methods

2.1 Subjects

Fifty-one male patients aging from 56 to 76 years were recruited to cardiovascular department of the First Affiliated Hospital of Harbin Medical University. Among these patients, 11 were diagnosed with acute myocardial infarction (AMI), defined by the World Health Organization (WHO) criteria as a combination of typical symptoms, serial electrocardiogram (ECG) changes and elevation in cardiac enzymes; 12 patients were diagnosed with old myocardial infarction with certain history of AMI; 19 patients were diagnosed with angina pectoris with typical symptoms, ECG and Holter; and in nine patients coronary stenosis was discovered through coronary angiography, without any symptoms. Patients were excluded if they had any of the following diseases: diabetes mellitus, cerebral vascular disease, chronic diseases, dysfunction of liver and kidney and peripheral vascular disease. We enlisted 55 male controls aging from 55 to 74 years. Controls had no history of CHD attack or positive ECG changes, including 18 healthy volunteers from the community, 17 volunteers from the Center of Medical Examination of the First Affiliated Hospital of Harbin Medical University (Harbin, China), 20 people with normal coronary arteries as determined by coronary angiography. Men were excluded if they had any of the following diseases: diabetes mellitus, cerebral vascular disease, chronic diseases, dysfunction of liver and kidney, or peripheral vascular disease.

2.2 Reagents

A vascular cell adhesion molecule-1 (VCAM-1) enzyme-immunity association kit was obtained from Senxiong Science and Technology in Shanghai, China; a free testosterone (FT) enzyme-immunity association kit DSL-10-49100 was obtained from Diagnostic Systems Laboratories (Webster, TX, USA).

2.3 Apparatus

An echocardiograph (VIVID FIVE US GE, Fairfield, CA, USA) was used.

2.4 Procedures

2.4.1 Blood samples collection

The blood samples were collected in the fasting state on the next morning after admission and were separated and stored at –70ºC until assayed. Several blood samples of each patient were sent to clinical laboratories for assay of cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), triglyceride (TG) and sugar levels. The rest of the samples were tested for VCAM-1 and FT levels in the central lab at the same time. Each sample was separated into three holes in the platform and the average of the three values was taken as laboratory data.

2.4.2 Ultrasound studies

The studies were performed on fasting patients between 7: 30 am and 9: 00 am. To minimize external stimuli, all studies were carried out in a silent clinical research laboratory room. Blood pressure was measured twice during the ultrasound examination. All studies were performed following a predetermined, standardized scanning protocol for the right and left carotid arteries. Subjects were examined in a supine position, with head turned to the opposite side of the carotid artery under examination. The proximal part of the carotid bulb was identified, and the segment of the common carotid artery 1–2 cm proximal to the bulb was scanned. Two end-diastolic frames of the best image quality were selected and analyzed for maximum intima media thickness (IMT), and the average reading from these two frames was calculated for both the right and left carotid arteries. Common carotid diameter (D), maximum velocity in systolic phase (V_{smax}), minimum velocity in diastolic phase (V_{dmin}) and resistent index (RI) were also tested.

2.5 Statistical analysis

Results are expressed as mean ± SD. Comparisons between the groups were conducted by independent samples *t*-test and χ^2-test. Multivariate analyses were done through a stepwise linear regression analysis of all 106 men. The following explanatory variables were included in the analysis: age, weight, TC, LDL-C, HDL-C, TG, sugar, systolic blood pressure (SBP), diastolic blood pressure (DBP), whether a smoker and FT. All statistical analyses were performed using the SPSS version 13.0 statistical analysis system (SPSS, Chicago, IL, USA). $P < 0.05$ was taken as significant.

3 Results

Characteristics of the study groups are shown in Table 1. The association between VCAM-1 and risk factors of the 106 men are shown in Table 2. In the stepwise

Table 1. Characteristics of the study groups (mean ± SD). [a]$P > 0.05$, [b]$P < 0.05$, [c]$P < 0.01$ *vs.* controls. CHD, coronary heart disease; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglyceride; SBP, systolic blood pressure; DBP, diastolic blood pressure; FT, free testosterone; VCAM-1, vascular cell adhesion molecule-1; IMT, intima-media thickness; D, Common carotid diameter; V_{smax}, maximum velocity in systolic phase; V_{dmin}, minimum velocity in diastolic phase; RI, resistent index.

CHD	Patients	Controls
Age (year)	64.6 ± 10.7	63.3 ± 11.3[a]
Weight (kg)	78.1 ± 5.7	73.6 ± 4.8[c]
TC (mmol/L)	4.99 ± 0.88	4.52 ± 0.65[c]
LDL-C (mmol/L)	3.35 ± 0.63	2.94 ± 0.55[c]
HDL-C (mmol/L)	1.36 ± 0.22	1.43 ± 0.26[a]
TG (mmol/L)	2.35 ± 0.92	1.87 ± 0.61[c]
Hypertention (%)	49	49.1[a]
SBP (mmHg)	138.2 ± 22.2	136.9 ± 26.6[a]
DBP (mmHg)	84.0 ± 14.2	83.9 ± 17.5[a]
Smoking (%)	43.1	45.5[a]
Blood sugar (mmol/L)	4.77 ± 0.58	4.82 ± 0.50[a]
FT (10^{-9} g/L)	13.2 ± 3.6	16.6 ± 5.1[c]
VCAM-1 (10^{-6} g/L)	372.7 ± 111.8	202.8 ± 72.5[c]
IMT (10^{-3} m)	1.11 ± 0.24	0.96 ± 0.22[c]
D (10^{-3} m)	7.09 ± 0.62	6.82 ± 0.66[b]
V_{smax} (10^{-2} m/s)	65.5 ± 12.4	72.8 ± 11.3[c]
V_{dmin} (10^{-2} m/s)	17.9 ± 4.7	18.5 ± 4.6[a]
RI	0.71 ± 0.07	0.69 ± 0.07[a]

multivariate regression held across analyses of the 106 men, the independent explanatory variables for VCAM-1 include FT ($P < 0.01$), TG ($P < 0.01$), smoking ($P < 0.01$) and weight ($P < 0.05$). There is no correlation between VCAM-1 and age, TC, LDL-C, HDL-C, sugar, SBP and DBP.

Association between IMT and risk factors of the 106 men are shown in Table 3.

In the stepwise multivariate regression held across analyses of the 106 men, the independent explanatory variables for carotid IMT include FT ($P < 0.01$), smoking ($P < 0.01$), age ($P < 0.01$) and TC ($P < 0.05$). There is no correlation between IMT and weight, TG, HDL-C, LDL-C, sugar, SBP or DBP.

4 Discussion

The initial steps in the formation of atherosclerotic lesion involve the adherence of circulation monocytes to dysfunctional endothelium and transmigration into the arterial intima, so the expression of vascular cell adhesion molecule-1 (VCAM-1) might be a key regulatory point in controlling the atherosclerotic process. VCAM-1 is produced by endothelial cells, tumor cells, and so on, and its expression increases during inflammation and tumor. The

Table 2. Association between VCAM-1 and risk factors in the 106 men. VCAM-1, vascular cell adhesion molecule-1; FT, free testosterone; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglyceride; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Standardized	Coefficients	Partial correlation	*P*-value
FT	–0.463	–0.547	0.000
TG	0.227	0.297	0.002
Smoking	0.272	0.372	0.000
Weight	0.163	0.208	0.035
Age	–0.73	–0.95	0.341
TC	0.83	0.091	0.361
LDL-C	0.102	0.129	0.195
HDL-C	–0.051	–0.076	0.447
Sugar	0.022	0.033	0.744
SBP	0.034	0.051	0.611
DBP	0.058	0.087	0.386
Hypertention	0.057	0.086	0.392

Table 3. Association between IMT and risk factors in the 106 men. IMT, intima-media thickness; FT, free testosterone; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglyceride; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Standardized	Coefficients	Partial correlation	*P*-value
FT	–0.297	–0.358	0.000
Smoking	0.332	0.407	0.000
Age	0.294	0.377	0.000
TC	0.195	0.238	0.015
Weight	–0.026	–0.037	0.712
LDL-C	0.066	0.078	0.433
HDL-C	0.033	0.050	0.620
TG	0.082	0.116	0.244
Sugar	–0.070	–0.106	0.289
SBP	0.101	0.152	0.127
DBP	0.079	0.122	0.220
Hypertension	0.080	0.121	0.224

mechanism of atherosclerosis includes the theory of inflammation. When endothelial cells are activated by malignant stimulation, virus and inflammation factors, such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1) and interleukin-2 (IL-2), the expression of VCAM-1 increases. Had androgen inhibited the expression of endothelial adhesion molecules, it would have relieved atherosclerotic lesions. It was revealed that testosterone levels of male patients with CHD were lower than those of controls [1, 4]. It is well-known that CHD is associated with several factors, such as cigarette smoking, diabetes, hypertension and elevated serum lipids. Blood lipids, diabetes and hypertention are usually higher in CHD patients than in healthy people. During the experiment, the relationship between FT level and VCAM-1 concentration was analyzed, with the latter as the target. Multivariate analyses showed that FT was inversely correlated with VCAM-1 concentration. *In vitro* experiments revealed that androgen restrained endothelial cells from excreting VCAM-1 by preventing nuclear factor kappaB from activation [5]. Therefore, proper androgen levels might inhibit the elevation of VCAM-1 expression. Proper testosterone levels might improve endothelial function.

Carotid IMT is a widely accepted noninvasive measure of preclinical atherosclerosis and an independent predictor of future adverse cardiovascular and cerebrovascular events. A limited number of previous observations have suggested that carotid IMT is inversely associated with serum T levels in very elderly men [6], in men with type II diabetes [7], and in obese men [8]. Makinen *et al.* [9] reported that middle-aged men with symptoms of andropause, together with absolute or compensated testosterone deficiency, show increased carotid IMT. Therefore, hypotestosteronemia might accelerate the development of atherosclerosis and increase the risk of CHD. A recent study indicated the serum T concentration was inversely associated with the progression of carotid atherosclerosis in elderly men [10]. Our data showed the FT level of male patients with CHD was lower than that of controls, which corresponds with previous research [4]. The findings demonstrate an inverse association between serum FT and IMT; therefore, it is speculated that endogenous testosterone levels might play a protective role in the development of atherosclerosis. Hak *et al.* [11] demonstrate in a large population-based study that serum T levels inversely and independently correlate with the presence of aortic calcified plaques and the progression of aortic atherosclerosis. Experimental male animal studies show that androgens reduce diet-induced and injury-induced atherosclerosis [12, 13]. The current evidence suggests that normal androgen levels might protect aging men from the development of atherosclerosis. The possible underlying mechanisms might include the anti-inflammatory effects of normal physiological levels of sex hormones, regulation of apoptosis, and promotion of smooth muscle cell stability.

Endothelium is not only a barrier to blood stream, but also the biggest endocrine organ in the body. With its complex function, endothelium might be influenced by many factors, such as hypertention, diabetes, cigarette smoking, and so on. Several indexes are applied to reflect the endothelial function. The present experiment has shown that FT is inversely correlated with VCAM-1 and IMT, which are taken as some of the indicators of endothelial function. Further investigations are required to explore the relationship between androgen and endothelial function.

As men aging, their testosterone levels decline, and some changes in body and mind occur in accordance with decreases in androgen levels, such as increases in fat tissues, decreases in muscle, lipids changes, anaemia and fatigue. What was once called andropause is now referred to as partial androgen deficiency of the aging male. Low serum sex hormone binding globulin, low total testosterone and clinical androgen deficiency are associated with increased risk of developing metabolic syndrome over time. Therefore, they might provide early warning signs for cardiovascular risk and an opportunity for early intervention in men [14, 15]. Approved low-dose supplemental testosterone treatment in men with chronic stable angina reduces exercise-induced myocardial ischemia [16]. These issues bear on the potential use of testosterone replacement in aging men.

In fact, testosterone has been used as a compound for treatment of testosterone deficiency for almost 70 years. Researchers have been trying to develop new testosterone preparations or testosterone analogs because the traditional injective testosterone ester cannot maintain stable testosterone levels [17–19]. At present, there are several big volume multi-center clinical observations concerning testosterone supplement therapy being carried out worldwide.

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