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Original Research

Relationship between serum testosterone concentration and microvascular endothelial function in Japanese men

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Abstract

Background: Both endothelial dysfunction and low circulating androgen levels predict cardiovascular disease in men. Endothelial function evaluation is commonly performed by measuring flow-mediated vasodilatation of the brachial artery. However, studies have suggested that compared with evaluation of large arteries, microvascular function evaluation of peripheral arteries is a better predictor of increased cardiovascular disease risks. Although circulating levels of androgens, such as testosterone and dehydroepiandrosterone sulfate (DHEA-S), positively correlate with cardiovascular function, the association between circulating androgen levels and microvascular function is unknown. In this study, we investigated whether serum androgen levels correlate with microvascular endothelial function in men.

Methods: The study included 105 Japanese men (age 59 ± 1 years) in whom we measured serum testosterone and DHEA-S levels. The reactive hyperemia index (RHI) determined by the Endo-PAT system (finger plethysmography) was used to evaluate microvascular endothelial function.

Results: Serum testosterone levels were significantly correlated with the RHI ($r = 0.32$, $P < 0.01$). The association between serum testosterone levels and the RHI remained significant even after adjustment for confounders, including age and body mass index ($\beta = 0.31$, $P < 0.01$). Notably, serum DHEA-S levels were not associated with the RHI ($r = 0.01$, n.s.).

Conclusion: This study showed that serum testosterone levels were positively correlated with microvascular endothelial function in men. These results suggest that endogenous testosterone level is one of the determinants of microvascular endothelial function and may become a biomarker reflecting lifestyle modifications-induced improvement in cardiovascular function in men.

Keywords

Testosterone; Microvascular endothelial function; Dehydroepiandrosterone; Arterial stiffness; Endo-PAT system; Finger plethysmography

1. Introduction

Cardiovascular disease (CVD) accounts for 31% of all global deaths; an estimated 17.9 million individuals died of CVD in 2016 [1]. A low serum testosterone level is considered a risk factor for CVD events; men with low circulating testosterone levels (< 14.2 nmol/L) show a 4-fold higher risk of CVD [2], and long-term testosterone treatment was shown to lower the risk of CVD in men with hypogonadism [3], suggest-

ing that testosterone protects against CVD. The beneficial effects of testosterone are attributable to its endothelium-dependent action. Testosterone induces nitric oxide (NO) production via endothelial nitric oxide synthase (eNOS) activation in human aortic endothelial cells [4]. Circulating levels of testosterone and dehydroepiandrosterone sulfate (DHEA-S), a precursor of testosterone, positively correlate with vascular endothelial function, which is quantified by measuring flow-mediated dilatation (FMD) [5, 6]. We observed in our previous study that 12-week lifestyle modifications in overweight men and in those diagnosed with obesity led to a significant increase (7-10%) in serum testosterone levels, which correlated with decreased arterial stiffness and central blood pressure (BP) by 8-11% [7, 8]. Therefore, testosterone is essential for cardiovascular function regulation in men.

Age-induced endothelial dysfunction predicts future CVD events [9–11]. Endothelial function is most commonly measured by FMD, which is evaluated by brachial artery ultrasonography. However, FMD measurement requires specific training and results are operator-dependent. The Endo-PAT system measures microvascular endothelial function based on finger plethysmography and automatically calculates the reactive hyperemia index (RHI) [12]. In contrast to FMD measurement, this method does not require specific training and is not operator-dependent [13]. Additionally, the RHI positively correlates with FMD and coronary blood flow and predicts future CVD [12, 14, 15]. Studies have suggested that small early-stage atherosclerotic changes are more prominently detected in the peripheral arteries than in large arteries [16, 17], and the RHI is a reliable, non-invasive indicator of microvascular endothelial function [14, 18, 19].

Although testosterone administration improves microvascular endothelial function in men with hypogonadism [20], the association between endogenous androgen levels and microvascular endothelial function remains unclear. In this study, we investigated the association between circulating androgen levels and microvascular endothelial function in men. We hypothesized that higher circulating androgen levels correlate with higher microvascular endothelial function, quantified by the RHI.

2. Materials and methods

2.1 Participants

This study included 105 Japanese men (age 59 ± 1 years). We excluded men with a history of CVD and other chronic diseases and those who received hormone replacement therapy, to eliminate the effect of androgens on vascular function. This study was approved by the Ethics Committee of the Faculty of Health and Sport Sciences of the University of Tsukuba (#27-68) and conformed to the principles outlined in the Declaration of Helsinki. All participants provided written informed consent before inclusion in the study.

2.2 Experimental design

All measurements were performed after an overnight fast, including abstinence from caffeine and alcohol. Evaluation was performed after a rest period of at least 20 min with participants placed in the supine position in a quiet, temperature-controlled room (24-26 °C).

2.3 Blood biochemistry

Blood samples were obtained from each participant in the morning. Serum levels of triglycerides, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein cholesterol, and plasma levels of glucose and glycosylated hemoglobin were measured using standard enzymatic techniques. Serum total testosterone and DHEA-S levels were measured at a commercial laboratory (LSI Medience Corporation, Ibaraki, Japan) using chemiluminescent immunoassay and radioimmunoassay, respectively.

2.4 Microvascular endothelial function

Microvascular endothelial function was evaluated using finger plethysmography (Endo-PAT 2000, Itamar Medical Ltd., Caesarea, Israel). This device records the digital pulse wave amplitude using fingertip plethysmography and consists of two finger-mounted probes, which include a system of inflatable latex air cushions within a rigid external case. A BP pressure cuff was placed on the participant's upper arm (occluded arm), and the contralateral arm served as the control arm [14]. The pulse wave amplitude was recorded continuously during three measurement phases as follows: a quiet baseline phase (5 min), forearm occlusion phase (5 min) (with inflation of the arterial pressure cuff to 60 mmHg > the participant's systolic BP [SBP]), and a reactive hyperemia phase (5 min) following cuff deflation (Fig. 1). The RHI was calculated automatically by the system's software and represented the ratio between the post- to pre-occlusion mean signal size in both fingers, (corrected for systemic vascular changes) and the baseline level [12].

2.5 Heart rate, blood pressure and arterial stiffness

The heart rate, BP, and arterial stiffness were measured in a quiet temperature-controlled room (24-26 °C) after a rest period of at least 20 min. The heart rate, SBP, diastolic BP, the carotid-femoral pulse wave velocity (cfPWV), and brachial-ankle pulse wave velocity (baPWV) were measured using a noninvasive vascular profiling system (form PWV/ABI, Colin Medical Technology, Komaki, Japan), as previously described [21]. Of the direct carotid-femoral distances, 80% was applied to calculate the cfPWV [22]. Carotid and femoral artery pulse waves were simultaneously obtained using two applanation tonometers with 15 micro-piezoresistive transducers. Bilateral brachial and posterior tibial arterial pressure waveforms were recorded using extremity cuffs connected to air plethysmographic sensors wrapped on both arms and ankles of the participants. The distances traveled by the pulse
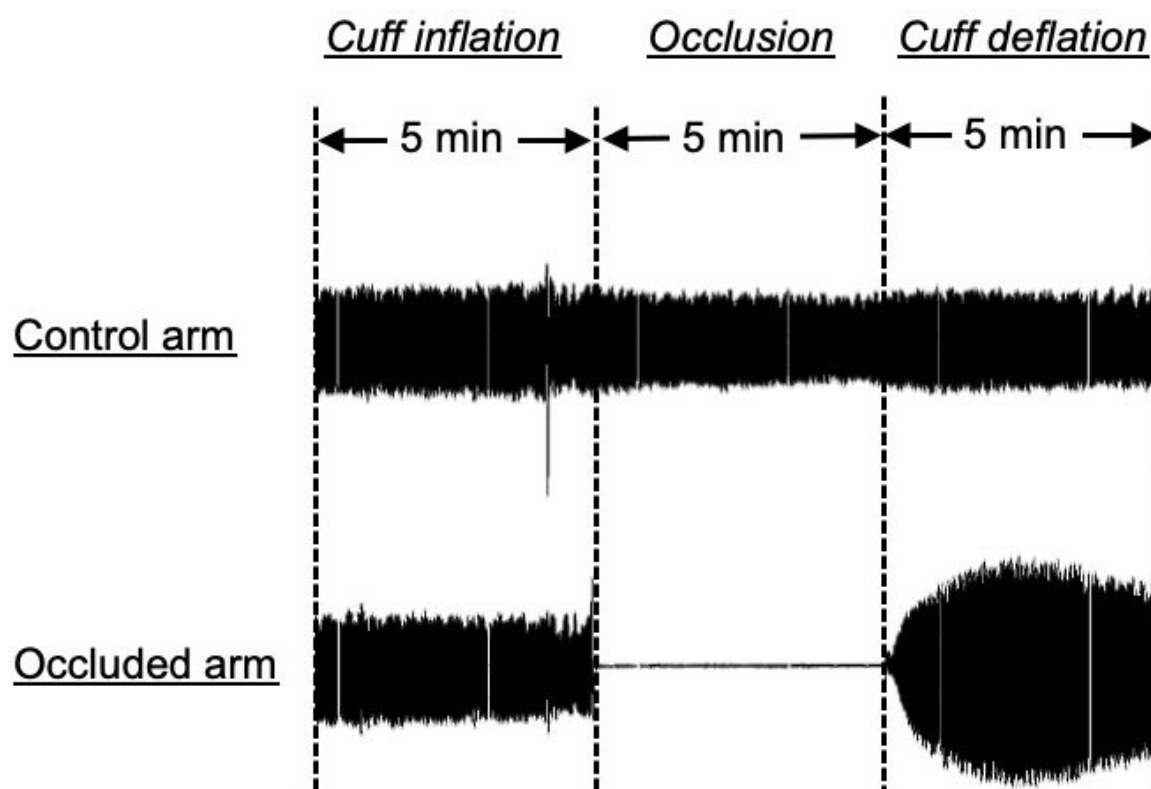
F I G . 1. The measurement protocol of RHI using Endo-pat 2000.

waves were recorded in triplicate with a random zero-length measurement over the body surface using a non-elastic tape measure. The pulse wave transit time was determined based on the time delay between the proximal and distal "foot" waveforms. The foot of the wave was automatically identified and detected as the commencement of a sharp systolic upstroke. PWV was assessed in duplicate and calculated as the distance divided by transit time.

2.6 Statistical analyses

Recorded values are expressed as mean ± standard error. Normality was tested using the Shapiro-Wilk test, and the correlations between vascular function and other variables were analyzed using Pearson's correlation coefficient or Spearman's rank correlation coefficient. Variables with skewed distributions were log-transformed to obtain normal distributions before multivariate linear regression analyses were performed. Independent correlates of log-transformed cfPWV and RHI were analyzed using multivariate linear regression analysis. Age, body mass index (BMI), smoking status, antihypertensive medication use, and serum testosterone or DHEA-S levels were subjected to multivariate linear regression analyses. A two-tailed P value < 0.05 was considered statistically significant. All statistical analyses were performed using the JMP Pro software, version 12 (SAS Institute).

T A B L E 1. Characteristics of the subjects

	Mean ± SE	Reference value
Age, years	59 ± 1	-
Height, cm	167.7 ± 0.6	-
Body mass, kg	65.5 ± 0.9	-
Body mass index, kg/m^2	23.3 ± 0.3	18.5-24.9 [a]
Waist circumference, cm	83.7 ± 0.8	-
Total cholesterol, mg/dL	210 ± 3	< 220 [b]
HDL cholesterol, mg/dL	63 ± 1	≥ 40 [b]
LDL cholesterol, mg/dL	126 ± 3	< 140 [b]
Triglycerides, mg/dL	102 ± 5	< 150 [b]
Glucose, mg/dL	102 ± 2	< 126 [b]
HbA1c, %	5.6 ± 0.1	< 6.5 [b]
DHEA-S, μg/dL	185.2 ± 9.8	-
Testosterone, nmol/L	21.6 ± 0.6	> 12.0 [c]
Anti-hypertensive medicine, n (%)	15 (14.3)	-
Anti-hypercholesterolemic medicine, n (%)	7 (6.7)	-
Current smoker, n (%)	8 (7.6)	-

HDL: high-density lipoprotein, LDL: low-density lipoprotein, HbA1c: glycosylated hemoglobin, DHEA-S: dehydroepiandrosterone-sulfate.

Values are expressed as mean ± SE.

[a]: Values are referenced from Japan Society for the Study of Obesity.

[b]: Values are referenced from Japan Atherosclerosis Society, total cholesterol value was removed from 2012.

[c]: Value is referenced from ISA, ISSAM and EAU recommendations.

TABLE 2. Hemodynamics of the subjects

	Mean ± SE
SBP, mmHg	128 ± 2
DBP, mmHg	80 ± 1
Heart rate, bpm	58 ± 1
cfPWV, cm/sec	818 ± 15
baPWV, cm/sec	1443 ± 23
RHI, %	2.1 ± 0.1

SBP: systolic blood pressure, DBP: diastolic blood pressure, cfPWV: carotid-femoral pulse wave velocity, baPWV: brachial-ankle pulse wave velocity, RHI: reactive hyperemia index.

Values are expressed as mean ± SE.

3. Results

Baseline and hemodynamic characteristics of the 105 men included in this study are summarized in Table 1 and 2, respectively. Significant correlations were observed between serum testosterone levels and the BMI ($r = -0.28$, $P < 0.01$), waist circumference ($r = -0.41$, $P < 0.001$), serum HDL cholesterol levels ($r = 0.23$, $P < 0.01$), serum triglyceride levels ($r = -0.26$, $P < 0.01$), and plasma glucose levels ($r = -0.20$, $P < 0.05$). Additionally, serum DHEA-S levels were significantly correlated with age ($r = -0.58$, $P < 0.001$). Fig. 2 shows the correlation between serum androgen levels and hemodynamic parameters. Serum DHEA-S levels were significantly correlated with the baPWV ($r = -0.42$, $P < 0.01$) and cfPWV ($r = -0.42$, $P < 0.01$), and serum testosterone levels were significantly correlated with the cfPWV ($r = -0.31$, $P < 0.01$) and RHI ($r = 0.32$, $P < 0.01$). We performed multivariate linear regression analyses to adjust for possible confounders such as age, BMI, smoking status, and antihypertensive medication use. We observed no significant correlation between serum DHEA-S levels and the baPWV ($\beta = -0.12$, n.s.) and cfPWV ($\beta = 0.003$, n.s.); however, serum testosterone levels were significantly correlated with the cfPWV ($\beta = -0.16$, $P < 0.05$) and RHI ($\beta = 0.31$, $P < 0.01$) even after adjustment for confounders.

4. Discussion

This study investigated the correlation between serum testosterone levels and microvascular endothelial function evaluated by finger plethysmography. We observed that serum testosterone levels were positively correlated with microvascular endothelial function in men. Furthermore, this association remained significant even after adjustment for confounders. These results suggest that high endogenous testosterone levels serve as a potent independent determinant of microvascular endothelial function in men.

A previous study has suggested beneficial effects of testosterone on cardiovascular function in men [2], which is partly attributable to NO-dependent vasodilatation [23]. Endothelium-derived NO is a potent endogenous vasodilator and regulates vascular tone, arterial compliance, and arterial stiffness [24–26]. Testosterone promotes NO production
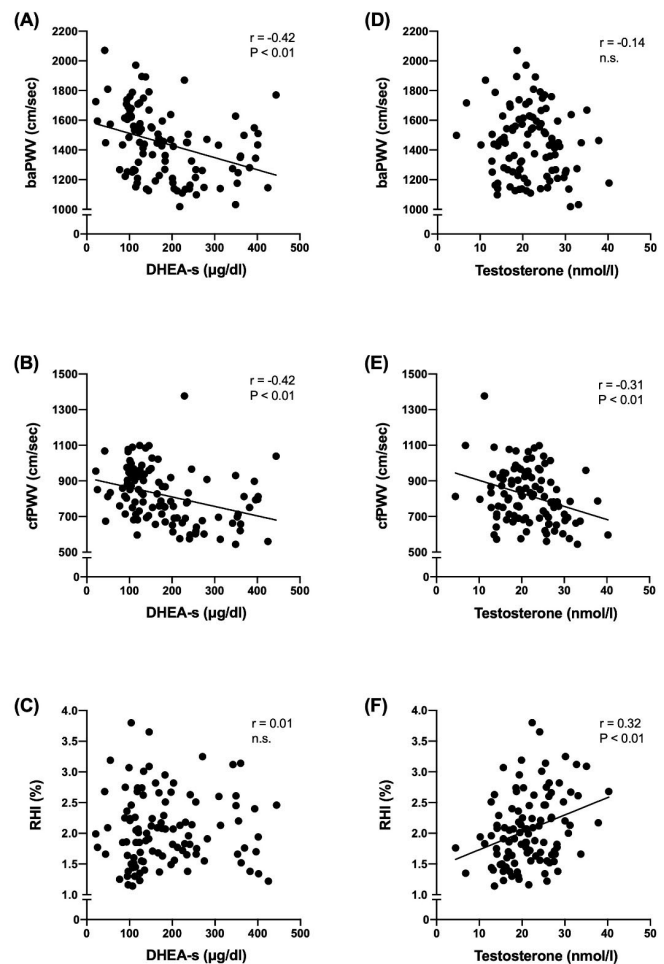

FIG. 2. The relationship between serum androgen levels and baPWV (A and D), cfPWV (B and C), and RHI (C and F). baPWV; brachial-ankle PWV, cfPWV; carotid-femoral pulse wave velocity, RHI; reactive hyperemia index.

via androgen receptor-mediated activation of eNOS in human aortic endothelial cells [4]. Akishita et al. reported that low circulating testosterone levels are associated with endothelial dysfunction at the brachial conduit artery in men [5]. Moreover, Francomano et al. observed that the administration of physiological levels of testosterone improved endothelial function in men with hypogonadism [20]. These findings prove that testosterone regulates endothelial function in men via NO production. Our present study showed that serum testosterone levels were significantly correlated with the RHI, which serves as an index of microvascular endothelial function in men. Multiple regression analysis revealed that serum testosterone levels were independently associated with the RHI. Therefore, it is reasonable to conclude that testosterone is a key regulator of microvascular endothelial function in men.

In this study, we observed that serum testosterone levels were significantly correlated with parameters of vascular function, such as the baPWV, cfPWV, and RHI in men. These results are consistent with those reported by previous studies, which have shown that circulating testosterone levels were significantly associated with arterial stiffness and

endothelial function [5, 27]. Many previous studies have reported that increased arterial stiffness and endothelial dysfunction serve as risk factors for CVD [28–30], and it has been suggested that low serum testosterone levels are associated with cardiovascular events in men [2, 3]. Tsai *et al.* reported that testosterone deprivation therapy in men with prostate cancer was associated with increased mortality to CVD [31]. Yaron *et al.* and Traish *et al.* reported that testosterone administration decreases arterial stiffness and BP in men with hypogonadism [32, 33]. Moreover, we observed in our previous study that lifestyle modifications led to a significant increase in circulating testosterone levels, which correlated with reduced cfPWV and central SBP in overweight men and in those diagnosed with obesity [7, 8]. These findings support the evidence that the serum testosterone level could be a useful biomarker for CVD risk factor assessment in men.

5. Conclusions

This study highlights that serum testosterone levels positively correlated with microvascular endothelial function in men, and the association remained significant even after adjustment for possible confounders, including age. These results suggest that the endogenous serum testosterone level is an important determinant of microvascular endothelial function and may serve as a useful biomarker that reflects lifestyle modification-induced improvements in cardiovascular function in men.

Author contributions

HK, A ZM, and SM designed this study. HK, A ZM, TY, KM, KT, and NA performed experiments and analyzed the data. HK and SM made the first draft of the manuscript and all authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Faculty of Health and Sport Sciences of the University of Tsukuba (#27-68) and conformed to the principles outlined in the Declaration of Helsinki. All participants provided written informed consent before inclusion in the study.

Conflict of interest

The authors have no conflicts of interest to disclose related to this manuscript.

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