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THE RELATIONSHIP BETWEEN SEX HORMONES, SEX HORMONE BINDING GLOBULIN AND PERIPHERAL ARTERY DISEASE IN OLDER PERSONS

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Abstract

Objective—The prevalence of peripheral artery disease (PAD) increases with aging and is higher in persons with metabolic syndrome and diabetes. PAD is associated with adverse outcomes, including frailty and disability. The protective effect of testosterone and sex hormone binding globulin (SHBG) for diabetes in men suggests that the biological activity of sex hormones may affect PAD, especially in older populations.

Methods—Nine hundred and twenty-one elderly subjects with data on SHBG, testosterone (T), estradiol (E2) were selected from InCHIANTI study. PAD was defined as an Ankle-Brachial Index (ABI) <0.90. Logistic regression models adjusted for age (Model 1), age, BMI, insulin, interleukin-6, physical activity, smoking, chronic diseases including metabolic syndrome (Model

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2), and a final model including also sex hormones (Model 3) were performed to test the relationship between SHBG, sex hormones and PAD.

Results—The mean age ($\pm$ SD) of the 419 men and 502 women was 75.0 ± 6.8 years (Sixty two participants (41 men, 21 women) had $ABI < 0.90$. Men with PAD had SHBG levels lower than men without PAD ($p=0.03$). SHBG was negatively and independently associated with PAD in men ($p=0.028$), but not in women. The relationship was however attenuated after adjusting for sex hormones ($p=0.07$). The E2 was not significantly associated with PAD in both men and women. In women, but not in men, T was positively associated with PAD, even after adjusting for multiple confounders, including E2 ($p=0.01$).

Conclusions—Low SHBG and high T levels are significantly and independently associated with the presence of PAD in older men and women, respectively.

Keywords

SHBG; sex hormones; PAD; older persons

Introduction

Lower extremities peripheral arterial disease (PAD) is a highly prevalent condition in the elderly^(1–3). Subjects with PAD have greater functional impairment and faster rates of functional decline than those without, independently from the presence of typical symptoms and signs⁽⁴⁾. The decline in physical performance, especially the slow gait speed, has been validated as a reliable measure of frailty, and more recently associated with a heightened risk of mortality^(5, 6).

The presence of PAD is also widely accepted as an indicator for generalized atherosclerosis, and a potential determinant of cardiovascular mortality and morbidity^(7–10). Patients with PAD frequently show signs of atherosclerosis in other vascular districts such as coronary⁽⁹⁾, cerebral⁽¹⁰⁾ and renal arteries⁽⁸⁾, and have an increased cardiovascular mortality⁽⁸⁾.

Subjects affected by metabolic syndrome (MS) and/or diabetes have a 2 to 3-fold higher risk of PAD than those without⁽¹¹⁾. Several lines of evidence suggest that sex hormones and Sex Hormone Binding Globulin (SHBG) levels may affect MS or its components^(12,13,14).

A recent meta-analysis performed on 52 observational studies suggests that total testosterone (T) levels are lower in men with MS than in those without, whereas in women higher T levels are associated with MS⁽¹²⁾.

Despite these promising epidemiological data, randomized controlled trials (RCT) confirming the protective role of T on cardiovascular risk are scant. If there is some evidence on the beneficial effects of T on insulin sensitivity⁽¹⁵⁾, data regarding the cardiovascular effects of T replacement in older men are conflicting⁽¹⁶⁾. A recent trial by Basaria et al assessed the effects of T supplementation on older men with mobility limitation. The revealed cardiovascular-related adverse events in T group, led to an early discontinuation of the study⁽¹⁷⁾. Similar results emerged in RCTs testing the effect of hormonal replacement therapy with estrogen plus progestin in postmenopausal women^(18,19). Therefore, it is still unclear as to whether the observed association between

male sex hormones and MS or atherosclerosis represents a causal effect or just the consequence of the aging and ill-health processes.

Moreover, there is growing evidence of an inverse relationship between SHBG levels and MS in both genders⁽¹²⁾. However, the effective presence of a causal relationship in this association is still debated and we cannot exclude that SHBG may represent a simple marker of insulin sensitivity rather than a determinant of atherosclerotic disease. The relationship between sex hormones, SHBG and MS may be supported by a link between gonadal status and atherosclerotic associated disease (e.g., PAD). The relationship between SHBG, sex hormones and lower extremity PAD has, however, received only little attention, with few and conflicting data in adult subjects^(20–22).

In a nested case-control study from the Edinburgh Artery Study, Price et al showed that in 83 cases with PAD (40 men and 43 women) and 88 age- and sex-matched controls, total and free T, estradiol (E2) and SHBG levels were not significantly different in cases as compared with controls⁽²⁰⁾. More recently, in a large population-based cohort of elderly men, the MrOS (Osteoporotic Fractures in Men), Tivesten et al found that low serum T and high serum estradiol levels were associated with a higher prevalence of lower extremity PAD in elderly men⁽²¹⁾. Finally, a recent analysis in 3034 participants of the Framingham Heart Study suggested that low SHBG levels, but not T or E2, are predictive of PAD in men but not in women⁽²²⁾.

Giving the controversial data in the current scientific literature, the aim of this study was to assess the association of sex hormones, SHBG levels and the presence of lower extremities PAD in elderly individuals of both genders. We also verified whether the hypothesized relationship was independent of confounders, including the MS and its components.

Methods

Study population

The data from The Aging in the CHIANTI Area, Invecchiare (InCHIANTI) study, an epidemiological study of a representative sample of the population living in Tuscany, Italy, were used for this investigation. The characteristics of the study population have been previously described elsewhere⁽²³⁾. Overall, 1,453 subjects were recruited. Of these, 634 men (65yrs: 492) and 802 women (65yrs: 648) with an age range of 21–103 years, were included in the final sample. The INRCA Ethical Committee ratified the entire study protocol. One thousand and two elderly community dwelling individuals of the InCHIANTI study had complete data on SHBG, total T, E2 and lower extremity PAD evaluation. From this initial number, 17 women who were taking hormone replacement therapy and 63 with a history of oophorectomy and 1 man who was on hormone replacement therapy were excluded. The final sample size included 921 men and women aged 65 yr and older (age range 65–102).

Hormone assays

Total testosterone (T) was assayed using a commercial immunoradiometric assay (Diagnostic Systems Laboratories, Webster, TX). The minimum detectable concentration

(MDC) was 0.05 ng/mL. The reference range for testosterone was 2.8–8.8 ng/mL. Intra- and inter-assay coefficient of variation (CV) for three different concentrations were 9.6, 8.1 and 7.8%, and 8.6, 9.1 and 8.4%, respectively. SHBG was measured using an immunoradiometric assay (Diagnostic Products, Los Angeles, CA) with a MDC of 3 nmol/L. The reference range was 9 – 111 nmol/L in men and 46 – 277 nmol/L in postmenopausal women. The inter- and intra-assay imprecision assessed for three different concentrations of analyte were 3.7, 1.1 and 3.4%, and 11.5, 10.3, and 8.7%, respectively. Total E2 was measured in the Laboratory of the University of Parma using an ultrasensitive radioimmunoassay (DSL-4800, Chematil, Angri, Italy), characterized by a MDC of 2.2 pg/mL and intra- and inter-assay CV of 8 and 10%, respectively. The reference range for E2 was 21.30–354.12 pg/mL.

Plasma insulin level was determined with a double-antibody, solid-phase radioimmunoassay (mean intra-assay imprecision 3.1%; Sorin Biomedica, Milan, Italy). Cross-reactivity with human proinsulin was lower than 0.3%. Serum levels of interleukin-6 (IL-6) were measured in duplicate by high-sensitivity ELISA (BioSource International, Camarillo, CA). The MDC was 0.1 pg/mL for IL-6. The total imprecision of the assay was below 7%. High-density lipoprotein cholesterol (HDL-C), total cholesterol and triglycerides were determined using commercial enzymatic tests (Roche Diagnostics, Mannheim, Germany).

PAD evaluation

The ABI is an objective diagnostic method to diagnose PAD, which shows high sensitivity (80–95%) and specificity (95–100%)^(24–25). ABI was measured during the clinical test session with a hand-held Doppler stethoscope (Parks model 41-A, Parks Medical Electronics, Inc., Aloha, Oregon). Systolic blood pressures were measured twice in the right brachial artery and twice in each posterior tibial artery. The highest pressure registered was used to calculate the ABI score by dividing the lower of the 2 systolic pressures for each leg by the brachial artery pressure. PAD was defined as ABI <0.90, whereas absence of PAD was defined as ABI from 0.90 to 1.50^(24–25). Patients were excluded if they had ABI >1.50, due to the inability to gauge arterial perfusion accurately.

Assessment of covariates

Weight and height were measured using standard techniques. BMI was calculated as weight (in kilograms) divided by the square of height (in meters). Physical activity in the year before the interview was coded as: 1) sedentary, completely inactive or light-intensity activity less than 1 h/wk; 2) light physical activity, light-intensity activity 2–4 h/wk; and 3) moderate to high physical activity, light activity at least 5 h/wk or more or moderate activity at least 1–2 h/wk. Smoking history was determined from self-report and dichotomized in the analysis as “current smoking” vs. “ever smoked” or “never smoked.”

Diseases were established by an experienced clinician according to defined criteria that combine information from self-reported physician diagnoses, current pharmacological treatment, medical records, clinical examinations, and blood tests⁽²³⁾. Diseases included in the current analysis were coronary heart disease (including angina and myocardial infarction), congestive heart failure (CHF), stroke, hypertension, diabetes and MS. Diabetes

mellitus was defined as a fasting plasma glucose ≥ 126 mg/dL (≥ 7.0 mmol/L) or treatment with a hypoglycemic agent. In agreement with the National Cholesterol Education Program Adult Treatment Panel III criteria (NCEP ATP-III), MS was defined as the presence of three or more of the following five features: 1) waist circumference ≥ 102 cm in men and ≥ 88 cm in women; 2) fasting serum triglycerides ≥ 150 mg/dL or lipid-lowering treatment; 3) fasting serum HDL-C < 40 mg/dL in men and < 50 mg/dL in women; 4) systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg or antihypertensive treatment; 5) fasting serum glucose levels ≥ 100 mg/dL or anti-diabetic treatment⁽²⁶⁾. Blood pressure was recorded using a standard mercury sphygmomanometer. All blood pressure measurements were performed three times with the participant in a supine position separated by intervals of two minutes; the average of the last two measures was used in the analysis.

Statistical analysis

Variables are reported as means (standard deviations, SDs) for normally distributed parameters or as numbers and percentages. Means were compared using Student's t-test and percentages were compared using chi-square tests. To approximate normal distributions, log-transformed values for insulin, testosterone, estradiol and SHBG levels were used in the analysis and back transformed for data presentation.

Factors statistically correlated with PAD were identified using age-adjusted partial correlation coefficient and Spearman partial rank-order correlation coefficients, as appropriate. Logistic regression models obtained by backward selection from initial fully adjusted models were used to identify independent risk factors of PAD. Model 1 was adjusted for age only. Then, covariates hypothesized as potential confounders of the association were progressively included in the initial model. Model 2 included, in addition to age, BMI, fasting insulin, IL-6, physical activity, smoking, MS, CHF. Finally, Model 3 including the covariates of Model 2 plus T, SHBG and total E2, was used to verify the association between SHBG, sex hormones and PAD. The SAS 8.2 statistical package (SAS Institute, Cary, NC) was used for all statistical analyses.

Results

The mean age of the whole sample population (921 subjects, 419 men and 502 women) was 75.0 ± 6.8 years (range 65–102 years). The mean age $\pm$ SD was 74.2 ± 6.5 and 75.6 ± 7.1 in men and women, respectively. Participants' characteristics according to the presence of PAD are shown in Table 1. Sixty two participants (41 men and 21 women) had ABI less than 0.90 and 859 participants (481 women and 378 men) had ABI > 0.90 . Older men with PAD (ABI < 0.90) had SHBG levels and physical activity significantly lower and IL-6 and insulin levels significantly higher than subjects with ABI > 0.90 . Men with PAD had also an higher prevalence of hypertension, CHF, MS and smoking, but no different prevalence of diabetes as compared with those with ABI > 0.90 (Table 1). Women with PAD (ABI < 0.90) were older, had significantly higher T levels and were significantly less physically active but had similar prevalence of diabetes compared to women with ABI > 0.90 (Table 1).

SHBG

Men with ABI <0.90 had mean SHBG levels of 96.9 ± 56.1 nmol/L, thus significantly lower than men with ABI >0.90 (109.3 ± 54.4 nmol/L; age adjusted $p=0.03$) (Figure 1). In men, SHBG was negatively associated with PAD, in both Model 1 ($p=0.0042$) and after adjustment for multiple confounders including MS (Model 2) ($p=0.03$). However, the association between SHBG and PAD was no longer significant after further adjusting for E2 and T, Model 3 ($p=0.07$) (Table 2). After excluding participants with diabetes, the results were substantially unchanged (data not shown).

Women with ABI < 0.90 had lower mean levels of SHBG (126.32 ± 49.9 nmol/L) than women with ABI >0.90 (136.8 ± 78.2 nmol/L), although the difference did not achieve statistical significance (age adjusted p value = 0.22) (Table 1). In women, SHBG was not associated with PAD both in Model 1 ($p=0.47$), after adjusting for additional confounders in Model 2 ($p=0.77$), and also after adjustment for T and E2 (Model 3) ($p=0.23$) (Table 2). We have already found that low SHBG are predictors of single components (HDL-cholesterol, waist circumference, hyperglycemia) and presence of metabolic syndrome in older men and women^(13–14). Only in women SHBG has been also shown inversely associated with inflammatory markers⁽²⁷⁾.

Testosterone

In men, T was not associated with PAD in Model 1 ($p=0.11$) and the results were substantially unchanged after adjusting for confounders and sex hormones. In women, T was positively associated with PAD, in both Model 1 and Model 2 ($p=0.03$, and $p=0.048$, respectively). The relationship was even stronger and still significant after further adjustment for E2 ($p=0.01$) (Table 3). After excluding participants with diabetes the relationship between testosterone and PAD was substantially unaffected (data not shown). We already reported that in older female population of the InCHIANTI Study, increased T levels are associated with higher inflammatory profile (C-reactive protein and interleukin-6)⁽²⁷⁾ but not with single components and the whole presence of MS⁽¹⁴⁾. Conversely, in men, total and free T have been inversely related to soluble interleukin receptor⁽²⁸⁾, waist circumference, triglycerides and positively associated with HDL-cholesterol⁽¹³⁾, all potential determinants of PAD.

Estradiol

Total E2 was not significantly associated with PAD in both men ($p=0.68$) and women ($p=0.21$) in Model 1 and the results were unchanged after adjusting for confounders (data not shown). This is somewhat unexpected, given the positive association previously reported in the older male population of the InCHIANTI Study between E2 and metabolic syndrome and inflammatory markers^(29–30).

Discussion

In this study, SHBG levels were found to be negatively associated with PAD in men only, whereas only total T levels were positively associated with PAD in women. PAD is highly prevalent and is associated with low gait speed, disability^(5, 7, 25) and mortality^(2, 5, 6, 8) in

the older population. Therefore, the identification of risk factors may help define new preventive strategies and effective therapies.

Previous studies found gender-related disparities in vascular disease. It is also known that gonadal status and SHBG can influence the course of MS and inflammation, suggesting a potential role of sex hormones and SHBG levels in the development and progression of PAD^(12–14, 27,31). Our findings are in agreement with the recent data by Haring et al, who studied 3,034 participants of the Framingham Heart Study and found that low SHBG levels, but not T or E2, were associated with PAD in men but not in women⁽²²⁾.

SHBG and PAD

The mechanism underlying the relationship between SHBG and PAD is not completely understood⁽³²⁾. SHBG is produced by liver and is down-regulated by insulin levels^(32–34). Along with the well-known role of transport protein SHBG is also involved in other independent functions^(32, 35–36). Emerging evidence attests that even sex hormones bound to SHBG may directly mediate cell-surface signaling, cellular delivery, and biologic action of sex hormones^(35–36). Moreover, some studies have associated low circulating levels of SHBG with impaired glucose control, diabetes and MS, suggesting its independent role in the maintenance of glucose homeostasis^(37–38). In particular, some polymorphisms in the *SHBG* gene, such as rs6257 and rs6259, are associated with insulin resistance, type 2 diabetes and MS^(32,37–39). Monosaccharide-induced lipogenesis reduces hepatic HNF-4 α levels, a transcription factor that plays a critical role in controlling the SHBG promoter, which in turn attenuates SHBG expression⁽³²⁾. These data are in agreement with the hypothesis that SHBG levels may positively influence insulin resistance and MS, and are potentially involved in other atherosclerotic processes such as PAD. It is also well known that participants affected by diabetes have an higher prevalence of this disease⁽⁴⁰⁾. However, due to the cross-sectional design of our study, we cannot exclude that SHBG represents only a marker of insulin resistance.

As expected, in our population, male participants with PAD had significantly higher levels of fasting insulin and higher prevalence of diabetes and MS than those without PAD. However, when we introduced insulin and MS in the multivariate models, the relationship between SHBG and PAD in men was weakened, but still statistically significant. The latter finding suggests that the higher prevalence of MS related to low SHBG levels explains only part of the association between SHBG and PAD in elderly men. Despite these suggestive data, the cross-sectional design of our study cannot provide a reliable answer to this question.

Another potential mechanism underlying the relationship between SHBG and PAD is the potential protective effect on endothelial function. Recent data suggest that altered transcriptional activity of SHBG gene during aging may be involved in the development of an earlier endothelial dysfunction and higher susceptibility to earlier cardiovascular disease^(41–42). We could not confirm this hypothesis because data on endothelial function was not collected in the InCHIANTI study. Interestingly, the relationship between SHBG and PAD was not further significant after adjustment for T and E2, suggesting that the

protective role of SHBG in PAD development may be partially mediated by the binding with these sex hormones.

Testosterone and PAD

During the past decade, laboratory and epidemiological studies have revealed a new link between low T levels, independent of the etiology, (Klinefelter's syndrome or androgen deprivation therapy in prostatic cancer) and cardiometabolic risk factors in men ^(43–44).

For many years the idea that androgens were a cardiovascular risk factor has been put forward. This hypothesis has been supported by studies showing a different prevalence of atherosclerotic disease in adult men and women ⁽⁴⁵⁾ and an increased number of cardiovascular events in athletes with androgens abuse ⁽⁴⁶⁾. More recently, evidence of a protective cardiometabolic effect of testosterone emerged from several observational ^(12, 43–44) and some intervention studies ^(16, 43, 47–48).

The decline of T during aging in men has been linked with all cause and cardiovascular mortality ^(44,49). Positive associations of T with a healthier cardiovascular system have been demonstrated in coronary artery disease (CAD), aorta and carotid atherosclerosis and other vascular districts ⁽⁴⁴⁾. The T deficiency is associated with higher levels of oxidative stress, greater endothelial dysfunction and a pro-inflammatory status, thus promoting the pathogenic process leading to atherosclerosis ⁽⁵⁰⁾. Empen et al found a positive relationship between T levels and endothelial function, suggesting that this hormone might modulate endothelium vasoreactivity ⁽⁵⁰⁾.

Data from Tivesten et seem to support the role of low T levels in the development of PAD in adult men ⁽²⁰⁾. Nevertheless, T levels were not associated with PAD in our study. The reason of this discrepancy is unclear, but it is noteworthy that there was a small sample of older subjects with PAD in our study population, although the prevalence observed is similar to that reported in previous studies ⁽²⁾.

In agreement with previous findings, our results support the potential and independent role of T in the pathogenesis of atherosclerosis and PAD in elderly women. The relationship between T and PAD in women is also suggested by the different trend of prevalence of PAD in men and women across ages. After menopause, the increased ratio between T and E2 is paralleled by an increased prevalence of PAD, which reaches a higher percentage in women compared with men ^(1,2). Giving the aromatization of T into E2, estradiol was entered into the multivariate models, but the association between T and PAD was substantially unchanged.

Estradiol and PAD

In men, estrogen levels may represent another possible risk factor in the development of MS ⁽⁵⁴⁾, although this topic is still debated. Similarly to the studies by Price et al and Haring et al, we found no relationship between E2 and PAD ^(20, 22). We acknowledge that our finding may be affected by the immunoassay used to measure estradiol levels, which is not sensitive enough to detect the very low levels often present in older men and even more in late postmenopausal women.

In women, estrogens play a kaleidoscope of functions in the regulation of vascular endothelium, proliferation, and the inflammatory response. Therefore, the decline in estrogen levels in postmenopausal women may lead to atherosclerosis^(51–52). Observational studies and clinical trials have demonstrated that E2 reduces the atherosclerotic progression at carotid level, whereas few studies have tested the relationship between E2 and lower extremity PAD^(53–54). Despite the well-known impact of estrogens on vascular disease, previous studies have failed to address their role in lower extremity PAD in postmenopausal women. Consistent with other studies, we did not find any association between estrogens and PAD in late postmenopausal women, suggesting that further investigations are required to clarify this issue.

Limitations

Our study has some limitations. These include the cross-sectional design, which does not allow to determine the temporal relationship between serum SHBG, sex hormones and PAD. Thus, our results may reflect hormonal changes induced by PAD disease rather than providing explanatory mechanisms by which SHBG or T may affect PAD. Further studies are needed to explore whether SHBG and T may be determinants of early causes of endothelial dysfunction. Moreover, we cannot exclude that both PAD and hormonal changes could be an adaptive response to the atherosclerotic process. Our survey is also limited by the relatively low number of the patients affected by PAD, although the observed prevalence of PAD is similar to that reported in previous studies. Therefore the sample size of our study offers limited power to detect potential relationship between sex hormones and PAD. We do also acknowledge that the immunoassays used to measure sex hormones may not be sensitive enough to detect small concentration of E2 and T in older men and late postmenopausal women. The mean SHBG levels for men and women appear rather high, in contrast with previous reports from large cohort studies. However the values are within the reference range for the SHBG assay.

Strengths

The limitations are offset by important strengths. This is the first study that tested the relationship between sex hormones and SHBG levels and PAD in elderly men and late postmenopausal women. The study included a relatively high number of subjects with a remarkable number of confounders which are not easily found in other epidemiological studies.

In conclusion, SHBG levels are negatively associated with PAD in older men, whereas in older women total T levels are positively associated with PAD.

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Highlights

Older Men with PAD have lower SHBG levels than men without PAD

Women with PAD had significantly higher Testosterone levels

Low SHBG and high Testosterone levels are independently associated with presence of PAD

Table 1

Characteristics of men (N=419) and women (N=502) according to the presence of peripheral artery disease.

Characteristics	Men (N=419)			Women (N=502)		
	ABI < 0.9	ABI ≥ 0.9	P ^o	ABI < 0.9	ABI ≥ 0.9	P ^o
Number	41	378		21	481	
Age, yr *	77.2 ± 6.5	73.89 ± 6.5	0.06	81.0 ± 7.5	75.4 ± 7.0	0.04
BMI, Kg/m ² *	26.8 ± 4.0	27.1 ± 3.2	0.86	26.1 ± 3.5	27.8 ± 4.6	0.22
TT (ng/dl) *	386 ± 115	433 ± 129	0.09	94 ± 85	62 ± 72	0.025
Bio-T (ng/dl) *	93.6 ± 39.1	94.2 ± 40.1	0.31	8.8 ± 7.0	12.0 ± 9.7	0.23
Estradiol (pg/ml) *	14.5 ± 8.3	13.3 ± 5.3	0.27	5.94 ± 3.29	6.5 ± 4.6	0.27
SHBG (nmol/L) *	97.0 ± 56.1	109.3 ± 54.4	0.03	126.3 ± 49.9	136.8 ± 78.2	0.22
Low HDL-C †	11 (26.8)	55 (14.6)	0.09	8 (38.1)	135 (28.1)	0.39
BP > 130/85 mmHg †	27 (65.9)	208 (55.0)	0.003	19 (90.5)	315 (65.5)	0.70
CHF †	20 (48.8)	85 (22.5)	0.002	7 (33.3)	118 (24.6)	0.72
Diabetes †	6 (14.6)	47 (12.4)	0.84	3 (14.3)	47 (9.8)	0.88
MS absence	6 (14.6)	95 (25.1)	0.003	0 (0)	57 (11.4)	0.008
Interleukin-6, pg/mL *	5.4 ± 13.9	2.4 ± 4.6	0.02	2.5 ± 1.6	1.8 ± 1.9	0.51
Insulin, mIU/L *	13.0 ± 7.5	11.0 ± 5.9	0.03	12.4 ± 8.4	11.49 ± 5.88	0.32
Physical activity †;			<0.0001			0.005
Sedentary	17 (41.5)	34 (9.0)		13 (62.0)	112 (23.3)	
Light-Moderate	22 (53.7)	308 (81.5)		8 (38.0)	73 (73.6)	
Moderate-High	2 (4.8)	36 (9.5)		0 (0.0)	15 (3.1)	
No smokers †	6 (14.6)	112 (29.6)	0.002	19 (90.5)	399 (82.9)	0.77

Legend: BMI body mass index

TT total testosterone

Bio-T Bioavailable testosterone

SHBG Sex Hormone Binding Globulin

HDL-C high density lipoprotein-Cholesterol

BP blood pressure

CHF chronic heart failure

*
mean $\pm$ Standard Deviation
† number (%)

The difference in the means and number and percentages of the parameters in men and women between participants with and without PAD were compared using t-test or chi-square tests in age adjusted analyses as appropriate

Table 2

Relationship between SHBG and PAD in men and women.

	Men			Women		
	OR	C.I.	P value	OR	C.I.	P value
Model 1 *	0.35	0.17–0.72	0.0042	0.71	0.29–1.76	0.47
Model 2 †	0.47	0.29–0.93	0.03	1.14	0.34–2.23	0.77
Model 3 ‡	0.49	0.21–1.09	0.07	0.87	0.16–1.56	0.23

* adjusted for age
† adjusted for age, BMI, interleukin-6, physical activity, fasting insulin, smoking, metabolic syndrome, chronic heart failure.
‡ adjusted for Model 2 and Testosterone, total estradiol.

Table 3

Relationship between testosterone and PAD in men and women.

	Men			Women		
	OR	C.I.	P value	OR	C.I.	P value
Model 1 *	1.23	0.95–1.58	0.11	5.43	1.13–26.06	0.03
Model 2 †	1.93	0.62–1.93	0.75	1.63	1.01–2.67	0.048
Model 3 ‡	1.01	0.52–1.99	0.96	2.00	1.15–3.47	0.01

* adjusted for age
† adjusted for age, BMI, interleukin-6, physical activity, fasting insulin, smoking, metabolic syndrome, chronic heart failure.
‡ adjusted for Model 2 and total estradiol and SHBG