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ORIGINAL DATA

Low Total Testosterone Levels are Associated With the Metabolic Syndrome in Elderly Men: The Role of Body Weight, Lipids, Insulin Resistance, and Inflammation; The Ikaria Study

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■ Abstract

BACKGROUND: The prevalence of the metabolic syndrome (MetS) increases with age. Among other changes, testosterone levels decline with age. The relationship between testosterone levels and MetS components in older subjects has not been clearly defined until today. **OBJECTIVES**: The aim of this work was to evaluate the relationship between total serum testosterone levels and MetS and its components. **METHODS**: The working sample consisted of 467 elderly individuals (mean age 75 ± 6 years old, n = 220 men) from Ikaria Island, Greece. MetS was defined according to the NCEP ATPIII criteria. **RESULTS**: MetS prevalence was 52% in men and 64% in women. Those with MetS had lower testosterone levels; a 10 ng/dl increase in testosterone was associated with a 3% reduction in odds of having MetS in men (95% CI: 0.95-0.99), but not in women. This remained the result after various adjustments had been made, including daily hours of sleep. Testosterone was inversely associated with abnormal waist circumference, high-sensitivity C-reactive protein (hs-CRP), insulin, and HDL cholesterol levels in men only. When lipid categories, hs-CRP, BMI, and insulin resistance levels were taken into account, testosterone lost its significance in predicting MetS ($p > 0.20$), suggesting that these markers possess a mediating effect. **CONCLUSIONS**: In elderly men, low serum testosterone was associated with MetS. Lipids, BMI, inflammation, and insulin resistance levels seem to explain this relationship, suggesting a potential mediating effect. This finding may support a research hypothesis relating serum testosterone to cardiovascular disease, which requires further research.

Keywords: metabolic syndrome · type 2 diabetes · elderly · testosterone · lipids · inflammation · body mass · C-reactive protein · insulin resistance · cardiovascular disease

Introduction

Aging is accompanied by a series of morphological and functional alterations which take place slowly over time. In humans, the aging process is altered or accelerated when metabolic and cardiovascular diseases (CVD) occur [1, 2]. Metabolic syndrome (MetS) shows increased prevalence in the elderly population and makes a further contribution to cardiovascular deterioration and total mortality [3, 4]. This syndrome has been defined as a cluster of conditions, including insulin resistance, hyperglycemia, hypertension, low high-density lipoprotein cholesterol (HDL-C), and increased very low density lipoprotein and triglyceride levels [5].

Abbreviations:
ANOVA - anylysis of variance
BMI - body mass index
CVD - cardiovascular disease
FFQ - food frequency questionnaire
HDL-C - high-density lipoprotein cholesterol
HOMA - homeostasis model assessment
IR - insulin resistance
hs-CRP - high sensitivity C-reactive protein
IPAQ - International Physical Activity Questionnaire
LDL-C - low-density lipoprotein cholesterol
MET - metabolic equivalent of task
MetS - metabolic syndrome
NCEP ATP - National Cholesterol Education Program Adult Treatment Panel
P-P plot - probability-probability plot
SHBG - sex hormone-binding globulin
SPSS - statistical package for social sciences

The prevalence of MetS varies from population to population, race to race, and between genders [6, 7]. This is attributed to many predisposing conditions which increase in prevalence during aging such as obesity, insulin resistance, inflammation, changes in the activity of the hypothalamus-hypophysis suprarenal axis, stress, and hypertension [8]. Among several endocrine mechanisms implicated in the pathogenesis of MetS, serum testosterone levels have been linked with lipolysis and reduced fatty acid production, while its deficiency seems to be associated with increased fat deposition resulting in insulin resistance [9, 10]. Recently, testosterone levels have been related to MetS among middle-aged men, in whom low testosterone levels have been linked with an increased risk of developing MetS and diabetes [11]. Although testosterone values decline with age, a decline that is attributed to hypogonadism and endocrine alterations [12], there is a lack of information regarding testosterone levels and MetS among the elderly.

The inhabitants of Ikaria Island, an isolated rural group with lifelong characteristics, have one of the highest longevity rates universally with a high percentage of healthy aging, despite the considerable prevalence of cardiovascular risk factors. While in Europe only the 0.1% of population lives to be over 90 years old, in Ikaria the percentage of persons over 90 years is almost 10 times higher [13]. This fact caused the "Blue Zones" investigators to include Ikaria Island as one of the places on Earth with increased longevity rates [14]. Several factors have been proposed to explain this ecological observation, including healthy eating, calm, relaxation, and physical activity as a part of daily living [13]. However, the pathophysiological role of various cardiometabolic characteristics has not been well studied and understood. Therefore, the aim of the present work was to evaluate the relationship between total serum testosterone levels with the prevalence of MetS and its components, in elderly individuals living on Ikaria Island.

Methods

Sample of the IKARIA Study

The IKARIA Study is a health survey that was carried out in the Province of Ikaria Island, from June 2009 to October 2009. 673 elderly people (i.e. above the age of 65 yrs, men n = 330, 75 ± 7 years, women n = 343, 75 ± 6 years), all permanent inhabitants of the island, were voluntarily enrolled (the participation rate was 84% of the elderly individuals invited to participate). After exclusion of those with known hypogonadism, hepatic cirrhosis and orchidectomy or those under estrogen treatment, as well as those without accurate biochemical or anthropometric measurements, 467 elderly people (n = 220 men, n = 247 women) were enrolled in this analysis (**Table 1**).

All participants were interviewed by trained personnel (cardiologists, general practitioners and nurses) who used a standard questionnaire developed for the purposes of the study. Details about the study have already been presented in the literature [15].

Bioethics

The study was approved by the Medical Research Ethics Committee of the 1st Cardiology Clinic of the University of Athens at Hippokration General Hospital and was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association. All participants were informed about the aims of the study, agreed to participate and signed an informed consent form.

Metabolic syndrome definition

MetS was defined according to the NCEP ATP III criteria [16]. In particular, a diagnosis can be established if 3 or more of the following risk factors are present: waist circumference >102 cm for men or >88 cm for women; triglyceride level ≥150 mg/dl; high density lipoprotein cholesterol (HDL-C) level <40 mg/dl for men or <50 mg/dl for women; blood pressure ≥130/85 mm Hg or fasting glucose >110 mg/dl.

Clinical and biochemical characteristics

Weight and height were measured following standard procedures and body mass index (BMI) in kg/m^2 was calculated. Obesity was defined as a BMI >29.9 kg/m^2. Waist and hip circumferences (to the nearest 0.5 cm) were also measured in all participants. Resting arterial blood pressure was measured three times in the right arm at the end of the physical examination with the subject in the sitting position. People who had blood pressure levels ≥140/90 mmHg or used antihypertensive medications were classified as hypertensive.

Fasting blood samples were collected from 08:00 to 10:00 h and after 12 hours' overnight fast. All serum samples for biochemical evaluations were immediately centrifuged at 3000 rpm for 10 minutes at +4°C. The biochemical evaluation was carried out in the same laboratory according to the criteria of the World Health Organization Reference Laboratories. Blood lipids (i.e. total serum cholesterol, HDL cholesterol and triglycerides) were measured using a chromatometric enzymatic method on an automatic chemistry analyzer.

Total serum testosterone was measured by a solid-phase, competitive chemiluminescent enzyme immunoassay system, IMMULITE 2000 [17]. High sensitivity C-reactive protein (hs-CRP) was assayed by immunoturbidimetry (AU 2700, Beckman Coulter, Germany). The method is linear within a range of 0.08-80 mg/l for hs-CRP. The intra-assay and inter-assay coefficient of variation was <5% for hs-CRP [18]. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula: (total cholesterol) - (HDL-C) - 1/5 (triglycerides). The intra- and inter-assay coefficients of variation did not exceed 3% for cholesterol or 4% for triglyceride. Hypercholesterolemia was defined as total serum cholesterol levels higher than 200 mg/dl or the use of lipid-lowering agents.

Diabetes mellitus type 2 was determined by fasting plasma glucose tests and was defined in accordance with the American Diabetes Association diagnostic criteria (fasting blood glucose levels greater than 125 mg/dl (7 mmol/l) or use of special medication indicated the presence of diabetes) [19]. The homeostasis model assessment (HOMA) for the description of glucose regulation was accomplished by the use of the following equation: HOMA-IR = (glucose x insulin)/405 glucose in mg/dl, insulin in μU/ml [20].

Socio-demographic and lifestyle measurements

Current smokers were defined as those who smoked at the time of interview; former smokers were defined as those who had stopped smoking for at least one year. Non-smokers were defined those who had never smoked. Physical activity was evaluated using the shortened version of the self-reported International Physical Activity Questionnaire (IPAQ) [21], which has been validated for the Greek population. Frequency (times per week), duration (minutes per time) and intensity of physical activity during sports, occupation and/or free-time activities were assessed. Participants who did not report any physical activities or reported very low levels (<600 MET/min/week) were defined as physically inactive, while the rest were defined as at least minimally active.

Dietary assessment was based on a validated and reliable food frequency questionnaire (FFQ) that has been developed especially for elderly people [22]. Consumption of all basic food groups and beverages (meat and meat products, fish and fish products, poultry, milk and other dairy products, fruits, vegetables, greens, legumes, refined and non-refined cereals, coffee, tea and soft-drinks) was measured in terms of daily, weekly or monthly consumption. Alcohol consumption was recorded in the number of 100 ml glasses drunk per day (1 glass was considered to be equivalent to 12 g ethanol). Furthermore, overall assessment of the quality of dietary habits was evaluated through a special diet score (the MedDietScore, theoretical range 0-55) [23]. Higher scores indicate greater adherence to this pattern and, consequently, healthier dietary habits.

Statistical analysis

Continuous variables are presented as mean values ± standard deviation (SD), while categorical variables are presented as relative frequencies. Normality was tested using the P-P plots. Comparisons between groups of participants in the case of normally distributed variables were performed using the Student's *t*-test, after testing for equality of variances. Associations between categorical variables were tested using the chi-square test. Correlations were evaluated by calculation of the Pearson *r* or the Spearman *rho* correlation coefficients for the normally distributed and skewed variables respectively.

Continuous variables were compared with testosterone tertile using ANOVA. Post-hoc analysis between groups was applied, after correcting the probability (p) value for multiple comparisons using the Bonferroni correction rule. For non-normally distributed triglyceride and insulin levels the non-parametric Mann-Whitney and the

Table 1. Distribution of the components of MetS and other clinical, lifestyle and biochemical characteristics, in male and female participants of the IKARIA study

Characteristic	Men (n = 220)	Women (n = 247)	p
Age (yr)	75.0 ± 7.0	75.0 ± 6.0	NS
MetS (%)	52	64	0.01
Components of MetS (%)			
Waist circumference[1]	58	87	0.001
Triglycerides ≥150 mg/dl	33	27	NS
HDL-C[2]	41	59	0.001
Arterial blood pressure[3]	75	70	NS
FBG ≥110 mg/dl	51	46	NS
Other clinical characteristics (% or mean ± SD)			
History of cardiovascular disease	22	19	NS
Diabetes	34	24	0.01
Obesity	31	30	NS
Arterial hypertension	64	73	0.03
Hypercholesterolemia	64	66	NS
Systolic BP (mmHg)	144.0 ± 18.0	143.0 ± 21.0	NS
Diastolic BP (mmHg)	80.0 ± 10.0	79.0 ± 12.0	NS
BMI (kg/m^2)	28.1 ± 4.0	28.5 ± 5.0	NS
Waist circumference (cm)	105.0 ± 11.0	102.0 ± 13.0	0.01
Lifestyle characteristics (% or mean ± SD)			
Currently smoking	23	12	0.001
Physical activity	90	82	0.01
MedDietScore (0-55)	37.9 ± 2.7	37.8 ± 3.8	NS
Hours night sleep per day	6.8 ± 1.3	6.3 ± 1.5	0.001
Biochemical measurements (mean ± SD)			
Testosterone (ng/dl)	354.0 ± 162	26.0 ± 53.0	0.001
Insulin (μIU/ml)	10.8 ± 18.0	10.0 ± 13.0	NS
Serum glucose levels (mg/dl)	110.0 ± 32.0	106.0 ± 35.0	NS
HOMA-IR	3.3 ± 6.4	2.9 ± 5.8	NS
Total cholesterol (mg/dl)	192.0 ± 43.0	201.0 ± 38.0	0.01
LDL-C (mg/dl)	120.0 ± 34.0	125.0 ± 33.0	0.08
HDL-C (mg/dl)	43.3 ± 10.5	49.1 ± 11.5	0.001
Triglycerides (mg/dl)	151.0 ± 90.0	132.0 ± 54.0	0.006
hs-CRP (mg/l)	2.6 ± 4.9	3.0 ± 3.7	NS

Legend: Data are mean ± SD, or percentages. P-values were derived using (*i*) chi-square test when comparing categorical variables, (*ii*) *t*-test when comparing normally distributed variables, and (*iii*) Mann-Whitney test when comparing skewed variables with gender. [1] >102 cm for men and >88 cm for women. [2] <40 mg/dl for men and <50 mg/dl for women. [3] ≥130 mmHG for systolic blood pressure and 85 mmHg for diastolic blood pressure. *Abbreviations*: BMI – body mass index, BP – blood pressure, FBG – fasting blood glucose, HDL-C – high-density lipoprotein cholesterol, HOMA-IR – homeostasis model assessment insulin resistance, hs-CRP – high sensitive C-reactive protein, LDL-C – low-density lipoprotein cholesterol, MetS – metabolic syndrome, NS – not significant.

Kruskal-Wallis tests were applied for between-group comparisons. Logistic regression analysis was used to estimate the odds of having metabolic syndrome, after controlling for various potential confounders. Results are presented as odds ratios and 95% confidence intervals. The Hosmer-Lemeshow test evaluated the models' goodness of fit.

All reported p-values were based on two-sided hypotheses. The Statistical Package for Social Sciences software, version 18.0 (SPSS Inc., Chicago, IL, USA) was used for all the statistical calculations.

Results

Demographic and clinical characteristics of the study participants

Women had lower triglycerides and higher HDL-C levels, while no gender differences were observed as regards arterial blood pressure, serum glucose, BMI, LDL cholesterol, and hs-CRP levels as compared with men (**Table 1**). Moreover, female participants were less physically active and reported lower prevalence of current smoking habits. No significant difference was observed between genders regarding the level of adherence to the Mediterranean diet (**Table 1**).

Demographic and clinical characteristics in relation to metabolic syndrome

The overall prevalence of MetS was 272 (58%) out of 467 participants (52% in men and 64% in women). Participants with MetS were more likely to be women and had a higher prevalence of CVD, diabetes mellitus, obesity, and hypertension. Also, they had a higher BMI, higher hs-CRP and insulin levels, and lower serum testosterone values compared to those without MetS (**Table 2**).

Demographic and clinical characteristics in relation to testosterone levels

Male participants in the highest tertile of testosterone levels (>407 ng/dl), were younger, had a lower prevalence of MetS and its components, had a lower prevalence of a history of CVD, diabetes, and obesity. They also had fewer hours of daily sleep and lower hs-CRP values compared with men in the lowest tertile (<289 ng/dl) of testosterone (**Table 3**).

Additionally, women in the highest tertile of testosterone (>27.4 ng/dl), showed no significant difference in the prevalence of MetS and its components as well as in the prevalence of a history of CVD, hypertension, diabetes, obesity, hypercholesterolemia, current smoking, physical activity, lipids, and hs-CRP levels compared with women in the lowest tertile (<1.71ng/dl, **Table 4**). A borderline significance was detected for MedDietScore only, with women in the highest tertile of testosterone levels having a lower score.

We also evaluated the distribution of testosterone levels in obese participants with and without pathological waist measurements. No differences were observed between obese and non-obese men and women with normal (p_{men} = 0.126, p_{women} = 0.589), as well as with abnormal waist circumference (p_{men} = 0.741, p_{women} = 0.463). Moreover, the correlation between testosterone and waist circumference levels was tested by obesity status. It was observed that testosterone levels were inversely correlated with waist circumference in non-obese men without MetS only (rho = -0.306, p = 0.003). No other significant correlations between testosterone and waist circumference levels were observed in men and women (all p-values >0.10).

Table 2. Distribution of socio-demographic, lifestyle, anthropometric, and biochemical markers in Ikarian study participants, according to the presence of MetS

Characteristic	Subjects with MetS (n = 272)	Subjects without MetS (n = 195)	p
Age (yr)	75.0 ± 6.0	75.0 ± 6.0	NS
Men (%)	42	54	0.01
Components of MetS (%)			
Waist circumference[1]	94	45	0.001
Triglycerides ≥150 mg/dl	46	8	0.001
HDL-C[2]	74	19	0.001
Arterial blood pressure[3]	85	54	0.001
FBG ≥110 mg/dl	70	19	0.001
Other clinical characteristics (% or mean ± SD)			
History of cardiovascular disease	24	15	0.01
Diabetes	40	13	0.001
Obesity	41	16	< 0.001
Arterial hypertension	78	56	< 0.001
Hypercholesterolemia	63	68	NS
BMI (kg/m^2)	30.0 ± 4.4	26.0 ± 3.7	0.001
Waist circumference, men (cm)	111.0 ± 11.0	99.0 ± 8.0	< 0.001
Waist circumfer., women (cm)	105.0 ± 11.0	95.0 ± 14.0	< 0.001
Lifestyle characteristics (% or mean ± SD)			
Currently smoking	11	19	NS
Physical activity	84	89	NS
MedDietScore (0-55)	38.0 ± 3.4	38.0 ± 3.0	NS
Hours night sleep per day	6.5 ± 1.5	6.6 ± 1.4	NS
Biochemical measurements (mean ± SD)			
Testosterone (ng/dl)	151.0 ± 180	221.0 ± 222	< 0.001
Insulin (μIU/ml)	12.9 ± 18.9	6.8 ± 10.0	< 0.001
Serum glucose levels (mg/dl)	118.0 ± 37.0	95.0 ± 22.0	< 0.001
HOMA-IR	4.0 ± 7.3	1.7 ± 3.5	< 0.001
Total cholesterol (mg/dl)	195.0 ± 41.0	199.0 ± 41.0	NS
LDL-C (mg/dl)	120.7 ± 34.0	125.0 ± 33.0	NS
HDL-C (mg/dl)	42.4 ± 9.0	52.2 ± 12.0	< 0.001
Triglycerides (mg/dl)	163.0 ± 81.0	110.0 ± 48.0	< 0.001
hs-CRP (mg/l)	3.1 ± 5.0	2.4 ± 3.1	0.07

Legend: Data are mean ± SD, or percentages. P-values were derived using (*i*) chi-square test when comparing categorical variables with the presence of MetS, (*ii*) *t*-test when comparing normally distributed variables with MetS, and (*iii*) Mann-Whitney test when comparing skewed variables with MetS. [1] >102 cm for men and >88 cm for women. [2] <40 mg/dl for men and <50 mg/dl for women. [3] ≥130 mmHG for systolic blood pressure and 85 mmHg for diastolic blood pressure. *Abbreviations*: BMI – body mass index, BP – blood pressure, FBG – fasting blood glucose, HDL-C – high-density lipoprotein cholesterol, HOMA-IR – homeostasis model assessment insulin resistance, hs-CRP – high sensitive C-reactive protein, LDL-C – low-density lipoprotein cholesterol, MetS – metabolic syndrome, NS – not significant.

Table 3. Distribution of socio-demographic, lifestyle, anthropometric, and biochemical markers in male Ikarian study participants, according to the presence of the distribution of testosterone levels

Characteristic	Testosterone levels in men (ng/dl)			p
	1st tertile (<289)	2nd tertile (289-407)	3rd tertile (>407)	
Age (yr)	78.0 ± 7.0	75.0 ± 6.0	73.0 ± 6.0*	0.001
MetS (%)	66	52	39	0.005
Components of MetS (%)				
Waist circumference >102 cm	69	63*	42*	0.002
Triglycerides >150 mg/dl	40	30	28	NS
HDL-C <40 mg/dl	49	44	32	NS
Arterial blood pressure[1]	84	73	67	0.06
FBG >110 mg/dl	60	50	43	NS
Other clinical characteristics (% or mean ± SD)				
History of CVD	30	20	15*	0.09
Diabetes	52	28	25*	0.001
Obesity	50	26	17*	< 0.001
Arterial hypertension	72	64	57*	NS
Hypercholesterolemia	58	67	67	NS
BMI (kg/m^2)	29.5 ± 3.7	27.8 ± 3.8	28.8 ± 3.3*	< 0.001
Systolic BP (mmHg)	147.0 ± 19.0	144.0 ± 20.0	140.0 ± 15.0	NS
Diastolic BP (mmHg)	81.0 ± 12.0	80.0 ± 10.0	79.0 ± 10.0	NS
Lifestyle characteristics (% or mean ± SD)				
Currently smoking	23	15	33	0.03
Physical activity	88	88	96*	NS
MedDietScore (0-55)	38.0 ± 2.6	37.8 ± 2.8	38.0 ± 3.8	NS
Hours night sleep per day	7.2 ± 1.4	6.8 ± 1.3	6.5 ± 1.4*	0.004
Biochemical measurements (mean ± SD)				
Insulin (μIU/ml)	15.1 ± 28.0	9.0 ± 7.7	8.2 ± 13.0*	0.05
Serum glucose levels (mg/dl)	108.0 ± 38.0	110.0 ± 29.0	103.0 ± 27.0*	0.01
HOMA-IR	4.6 ± 9.3	2.7 ± 2.9	2.5 ± 5.5	NS
Total cholesterol (mg/dl)	188.1 ± 38.0	194.2 ± 39.4	193.0 ± 51.0	NS
LDL-C (mg/dl)	116.0 ± 32.0	122.0 ± 31.0	121.0 ± 39.0	NS
HDL-C (mg/dl)	41.0 ± 11.0	42.0 ± 10.0	46.0 ± 11.0*	0.01
Triglycerides (mg/dl)	166.0 ± 61.0	144.0 ± 54.0	142.0 ± 46.0*	NS
hs-CRP (mg/l)	3.4 ± 7.7	2.4 ± 2.9	2.0 ± 2.4*	NS

Legend: Data are mean ± SD, or percentages. P-values were derived using (*i*) chi-square test when comparing categorical variables, (*ii*) ANOVA when comparing normally distributed variables, and (*iii*) Kruskall-Wallis test when comparing skewed variables with testosterone levels groups. The Bonferroni correction rule was used to adjust p-values for multiple comparisons. [1] ≥130 mmHG for systolic blood pressure and 85 mmHg for diastolic blood pressure. * $p < 0.05$ compared to first tertile. *Abbreviations*: BMI – body mass index, BP – blood pressure, CVD – cardiovascular disease, FBG – fasting blood glucose, HDL-C – high-density lipoprotein cholesterol, HOMA-IR – homeostasis model assessment insulin resistance, hs-CRP – high sensitive C-reactive protein, LDL-C – low-density lipoprotein cholesterol, MetS – metabolic syndrome, NS – not significant.

Multi-adjusted associations between MetS and serum testosterone levels

To assess the potential confounding effect of various participant characteristics, additive logistic regression models were customized (**Table 5**). In men, age-adjusted analysis showed that serum testosterone levels were inversely associated with the likelihood of having MetS, although no such association was observed in women (**Table 5**).

Furthermore, sex-specific tertiles of testosterone levels were calculated to test the linearity of the association between testosterone and the presence of MetS. A linear, dose-dependent association was revealed in men only (data not shown). Since testosterone levels seemed to be associated with smoking (**Table 3**), further analysis after adjusting for smoking habits confirmed that higher testosterone levels were associated with lower likelihood of having MetS in men but not in women (**Table 5**, model 2).

Testosterone levels remained a significant predictor of MetS in men, even upon adjustments for physical activity status, quality of dietary habits (as evaluated through the MedDietScore), and daily hours of sleep (**Table 5**, model 4). When BMI was entered in the model, there was a slight loss of significance of testosterone levels in predicting MetS in men (**Table 5**, model 5). Nevertheless, a 100 ng/dl increase

in testosterone levels in men was associated with a 10% lower likelihood (odds ratio = 0.90, p = 0.06) of having MetS, after all the aforementioned adjustments had been made (**Table 5**). As regards women, there was no association between testosterone levels and MetS in all the models presented in **Table 5**.

To evaluate further the potential mediating effect of lipids, insulin resistance, and hs-CRP levels, stratified analysis was carried out by quartile formation for HDL cholesterol, triglycerides, insulin, glucose, and hs-CRP in men and women separately. According to these subgroup analyses, testosterone levels were not associated with the presence of MetS in both men and women (all p-values >0.20).

Discussion

The metabolic syndrome shows an increased prevalence among elderly individuals, and has frequently been associated with pathophysiological conditions that involve increased inflammation and oxidation process, mitochondrial dysfunction and endothelial dysfunction. Testosterone levels have been linked with cardiovascular health, as low testosterone levels seem to accompany aging-related diseases, such as vascular dysfunction and atherosclerotic disease. Based on the present work, the prevalence of MetS was associated with serum tes-

Table 4. Distribution of socio-demographic, lifestyle, anthropometric, and biochemical markers in female Ikarian study participants, according to the presence of the distribution of testosterone levels

Characteristic	Testosterone levels in women (ng/dl)			p
	1st tertile (<1.71)	2nd tertile (1.71-27.4)	3rd tertile (>27.4)	
Age (yr)	75.0 ± 6.0	75.0 ± 5.0	76.0 ± 6.0	NS
MetS (%)	68	58	61	NS
Components of MetS (%)				
Waist circumference >102 cm	89	88	84	NS
Triglycerides >150 mg/dl	29	25	24	NS
HDL-C <40 mg/dl	62	65	51	NS
Arterial blood pressure[1]	66	70	74	NS
FBG >110 mg/dl	46	51	45	NS
Other clinical characteristics (% or mean ± SD)				
History of CVD	21	17	16	NS
Diabetes	20	30	25	NS
Obesity	27	27	37	NS
Arterial hypertension	73	70	75	NS
Hypercholesterolemia	66	65	67	NS
BMI (kg/m^2)	28.0 ± 4.5	28.6 ± 5.0	29.1 ± 5.4	NS
Systolic BP (mmHg)	141.0 ±18.0	143.0 ±24.0	146.0 ±22.0	NS
Diastolic BP (mmHg)	78.0 ± 9.0	78.0 ±14.0	79.0 ±13.0	NS
Lifestyle characteristics (% or mean ± SD)				
Currently smoking	12	12	12	NS
Physical activity	80	81	86	NS
MedDietScore (0-55)	38.5 ± 2.9	36.5 ± 5.7	37.2 ± 3.9	0.06
Hours night sleep per day	6.3 ± 1.6	6.3 ± 1.6	6.4 ± 1.6	NS
Biochemical measurements (mean ± SD)				
Insulin (μIU/ml)	9.9 ±11.3	8.9 ± 9.7	10.7 ±17.0	NS
Serum glucose levels (mg/dl)	104.0 ±36.0	112.0 ±43.0	105.0 ±29.0	NS
HOMA-IR	2.6 ± 3.7	2.5 ± 3.0	3.5 ± 8.8	NS
Total cholesterol (mg/dl)	203.0 ±39.0	196.0 ±45.0	200.0 ±34.0	NS
LDL-C (mg/dl)	127.0 ±33.0	121.0 ±40.0	124.0 ±29.0	NS
HDL-C (mg/dl)	49.0 ±12.0	49.0 ±10.0	50.0 ±11.0	NS
Triglycerides (mg/dl)	132.0 ±60.0	130.0 ±54.0	129.0 ±46.0	NS
hs-CRP (mg/l)	2.8 ± 2.8	5.0 ± 6.7	2.9 ± 2.6	NS

Legend: Data are mean ± SD, or percentages. P-values were derived using (*i*) chi-square test when comparing categorical variables, (*ii*) ANOVA when comparing normally distributed variables, and (*iii*) Kruskall-Wallis test when comparing skewed variables with testosterone levels groups. The Bonferroni correction rule was used to adjust p-values for multiple comparisons. [1] ≥130 mmHG for systolic blood pressure and 85 mmHg for diastolic blood pressure. * $p < 0.05$ compared to first tertile. *Abbreviations*: BMI – body mass index, BP – blood pressure, CVD – cardiovascular disease, FBG – fasting blood glucose, HDL-C – high-density lipoprotein cholesterol, HOMA-IR – homeostasis model assessment insulin resistance, hs-CRP – high sensitive C-reactive protein, LDL-C – low-density lipoprotein cholesterol, MetS – metabolic syndrome, NS – not significant.

Table 5. Gender-specific odds ratios (95% confidence intervals) derived from additive logistic regression models that evaluated the likelihood of having MetS according to testosterone levels

Men	**Model 1**	**Model 2**	**Model 3**	**Model 4**	**Model 5**
Age (per 1 yr)	0.985 (0.945-1.026)	0.989 (0.948-1.031)	0.989 (0.942-1.038)	0.988 (0.940-1.039)	1.025 (0.97-1.083)
Testosterone (per 1 ng/dl)	0.997 (0.995-0.999)	0.997 (0.995-0.999)	0.997 (0.995-0.999)	0.997 (0.994-0.999)	0.999 (0.996-1.001)
Currently smoking (yes vs. no)	-	1.299 (0.672-2.511)	1.689 (0.813-3.508)	1.743 (0.835-3.640)	2.657 (1.168-6.046)
Physical activity (moderate/high vs. low/sedentary)	-	0.959 (0.376-2.445)	0.823 (0.280-2.420)	0.802 (0.272-2.362)	0.736 (0.216-2.5)
MedDietScore (per 1/55 unit)	-	-	0.920 (0.816-1.037)	0.918 (0.813-1.038)	0.887 (0.776-1.013)
Hours of sleep per day (per 1 h)	-	-	-	0.897 (0.682-1.179)	0.902 (0.660-1.234)
Body mass index (per 1 kg/m^2)	-	-	-	-	1.342 (1.187-1.518)
Women					
Age (per 1 yr)	0.998 (0.945-1.033)	0.980 (0.935-1.026)	0.972 (0.912-1.035)	0.977 (0.916-1.041)	0.995 (0.928-1.068)
Testosterone (per 1 ng/dl)	0.999 (0.994-1.004)	0.999 (0.995-1.004)	1,00 (0.995-1.006)	1,00 (0.995-1.006)	0.999 (0.994-1.005)
Currently smoking (yes vs. no)	-	0.740 (0.325-1.688)	0.996 (0.344-2.883)	1.046 (0.356-3.077)	1.222 (0.371-4.023)
Physical activity (moderate/high vs. low/sedentary)	-	0.645 (0.306 -1.359)	1.18 (0.41-3.386)	1.187 (0.412-3.423)	1.854 (0.573-6)
MedDietScore (per 1/55 unit)	-	-	1.032 (0.94-1.132)	1.046 (0.948-1.154)	1.009 (0.909-1.121)
Hours of sleep per day (per 1 h)	-	-	-	1.087 (0.856-1.380)	1.072 (0.822-1.397)
Body mass index (per 1 kg/m^2)	-	-	-	-	1.220 (1.100-1.350)

Legend: *Model 1*: adjusted for age. *Model 2*: adjusted for age and smoking and physical activity. *Model 3*: adjusted for age and smoking and physical activity and level of adherence to the Mediterranean diet. *Model 4*: adjusted for age and smoking and physical activity and level of adherence to the Mediterranean diet and hours of sleep. *Model 5*: adjusted for age and smoking and physical activity and level of adherence to the Mediterranean diet and hours of sleep and body mass.

tosterone levels in men only, while no such relationship was observed in women, after various adjustments made. Furthermore, serum testosterone levels were inversely associated with components of MetS in both genders (**Table 3**).

When categories of lipids, hs-CRP, BMI and insulin resistance levels were taken into account, testosterone lost its significance in predicting MetS, suggesting a mediating effect of these markers on the relationship between testosterone and the syndrome. It requires further investigation to find out whether the associations demonstrated here describe the way in which testosterone levels affect CVD risk.

Metabolic syndrome and cardiovascular disease

Metabolic syndrome remains one of the leading threats for cardiovascular health in advanced age [3, 6]. The prevalence of MetS varies from country to country and region to region, around the world, but data regarding elderly populations are sparse in the literature. In a recent review work by Denys *et al*., the prevalence of MetS among the elderly varied from 23% to 55% (with a median prevalence equal to 31%), according to the NCEP ATP III definition. In this study, obesity and hypertension were the most prevalent components [24].

In the ATTICA study which included a representative sample of the Greek population, the prevalence of MetS among elderly men and women was 38% [24]. This is lower than the prevalence of the syndrome in the present study, which may be due to the different time of sampling. The sampling in the ATTICA study was carried out almost 10 years prior to the present study in individuals who were predominantly middle-aged in 2001-2002. It is probable that the distribution of the MetS in the reference population has now increased. In a Southern German study (the KORA Survey 2000), the prevalence of MetS according to the NCEP criteria was 28% in elderly men and 24% in elderly women [25]. In a recent Finnish study, based on data from 539 elderly inhabitants from northern Finland, the investigators observed that the prevalence of the syndrome was 25% in men and 21% in women (also according to the NCEP criteria) [26]. In those subjects, 90% of men and 90% of women had hypertension, and 53% and 33% respectively had hyperglycemia, while the proportions for elevated triglyceride and/or low HDL were 13% and 10%, respectively.

The prevalence of MetS was much higher in the IKARIA study compared with the results presented above. Whether the prevalence of MetS was higher in our elderly cohort, described as a long-living population, is a matter of further interest.

Metabolic syndrome and testosterone levels: the role of mediators

Metabolic syndrome has been described as a cluster of metabolic abnormalities, each of which contributes to an increase in cardiovascular risk. Therefore, previous studies have investigated the role of sex hormones on cardio-metabolic compounds. Among them total serum testosterone, which is associated with bone mineral density, body composition, mood aggression, cognitive function and sexual function, has also been linked with visceral obesity, atherosclerosis and type 2 diabetes mellitus in men. Testosterone is a sex hormone responsible for the development of secondary sex characteristics, which is synthesized in the interstitial Leydig cells of the testis and regulated by the interstitial cell-stimulating hormone of the anterior pituitary [10].

There is evidence that low testosterone levels (<8 nmol/l or 230 ng/dl) double the risk for all-cause mortality in men, independently of age, smoking habits, waist circumference and physical activity status [10, 27]. In accordance with this finding, elderly men in this study, with testosterone levels in the lowest tertile, had an increased prevalence of cardiovascular disease compared to those in the highest tertile. In contrast, this observation was not evident in women. This may reflect a difference between the genders in the association between sex hormone regulation and cardiovascular health. While many studies indicate that low testosterone levels represent a risk factor for CVD in men, the prevalence of CHD is higher in men than women, although this difference disappears for women after menopause [28].

In a recent meta-analysis [29], an evaluation of the relationship between androgens and CVD has revealed that the data are contradictory, and that it is not yet possible to state conclusively whether there is a direct association between levels of testosterone or other androgens and CVD, although low testosterone indicates poor general health. In addition, although testosterone levels in men are positively related to HDL-C and negatively to LDL-C, in women this ratio is negative, suggesting that men and women respond differently to androgens, and that some of the effects of testosterone that are beneficial in men may be deleterious in women. This may explain why no evident association was detected between CVD prevalence and serum testosterone levels in elderly women in this study. Furthermore, metabolic syndrome prevalence was unrelated to testosterone levels in elderly women, confirming a gender-dependent association between testosterone levels and MetS, which has been also revealed in a recent meta-analysis [11].

Elderly men with testosterone levels in the lowest tertile had increased prevalence of three out of the five components of MetS. Those components were waist circumference, which reflects central obesity, hyperinsulinemia and increased inflammatory activation in the adipose tissue. Hyperinsulinemia, as it suppresses sex hormone-binding globulin synthesis, may cause decreased serum testosterone, reflecting the association observed between serum testosterone and glucose levels in previous studies [30, 31]. The same applies for the present study, where total testosterone levels were associated with increased prevalence of diabetes mellitus in elderly men. When lipid categories, hs-CRP, BMI, and insulin resistance levels were taken into account, testosterone lost its significance in predicting MetS, implicating those factors as mediators. This finding is consistent with a study of 221 non-diabetic men, which was adjusted for regional adiposity by computed tomography

imaging. It showed that the inverse relationship of insulin resistance with testosterone was no longer significant when the analysis was adjusted for visceral fat [32].

Inflammation is one of the main mechanisms underlying endothelial dysfunction and therefore plays an important role in atherosclerosis and other CVD such as hypertension, insulin resistance, dyslipidemias and obesity [33]. Inflammation is a key factor in the progressive loss of lean tissue and impaired immune function observed in aging. IL-6 and CRP, known as "geriatric cytokines," are multifunctional cytokines produced in situations of trauma, stress and infection. During the aging process, levels of both IL-6 and CRP in plasma become elevated. The natural production of cytokines is likely to be beneficial during inflammation, but overproduction and the maintenance of an inflammatory state for long periods of time, as seen in elderly individuals, are detrimental for the maintenance of cardiovascular health [34, 35]. There is a debate as to whether these associations are independent of body fatness or, rather, an epiphenomenon of central obesity, which is an important source of inflammatory cytokines [36, 37].

It seems that the role of serum testosterone levels in the prediction of MetS among elderly men is masked by the presence of obesity. Previous studies have shown that changes in whole-body and regional fat mass are inversely correlated with testosterone levels [38]. A plausible explanation for this "hypogonadal obesity cycle" was first proposed by Cohen. According to this, increased deposition of visceral adipose in hypogonadal subjects causes a further decrease in testosterone concentrations through conversion to estradiol by the enzyme aromatase. This leads to even more abdominal fat deposition and a greater degree of testosterone deficiency [39].

Recent reports have refuted this explanation by showing that the suppression of the hypothalamo-hypophyseal-gonadal axis in patients with subnormal free testosterone concentrations and type 2 diabetes is not associated with increased estradiol concentrations [40]. In the generation 2 Framingham Heart Study, sex hormone-binding globulin (SHBG) levels and not testosterone levels were related to the prevalence of metabolic syndrome among men with a mean age of 60 years, but the causality link remains unclear [41]. Although circulating testosterone is partly bound to SHBG, factors like obesity and insulin resistance, which show high prevalence during aging, have been demonstrated to regulate both SHBG and testosterone levels. Additionally, aging causes a reduction in all serum testosterone concentrations, owing to testicular and endocrine alterations [43].

Thus, there is an age-dependent increase in SHBG levels by about 1.2% per year, causing a greater decrease in free serum testosterone levels than in total serum testosterone [42]. All the above suggests that the interpretation of SHBG levels becomes difficult as its values alter in a bidirectional way, especially in old age when metabolic abnormalities may begin to accumulate.

The study has limitations. Because of its cross-sectional design, the study could not establish causal relationships; it could only propose hypotheses that could be evaluated by prospective studies. The studied sample was relatively small for robust associations to be developed that can be transferred to other elderly populations. Furthermore, total testosterone levels, rather than free levels and SHBG, were measured. It is known that SHBG inhibits the function of testosterone, and therefore, the findings presented may be masked because of the lack of free testosterone levels.

Conclusions

In this cohort of elderly men, high serum testosterone levels were associated with low prevalence of MetS and some of its components, indicating a way in which testosterone may affect CVD risk. Lipids, inflammation and anthropometric markers, as well as insulin resistance levels, may suggest that the above relationship may have a potential mediating effect. Whether these associations may describe the route by which testosterone levels affect CVD risk, is something that needs further investigation.

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