Original Article

High normal thyroid-stimulating hormone is associated with arterial stiffness in healthy postmenopausal women

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Objective: Apart from the effects of a dysfunctional thyroid gland on the cardiovascular system, thyroid function within the reference range may have an impact on the vasculature. The present study aimed to evaluate the association between thyroid function and markers of arterial structure and function in euthyroid postmenopausal women.

Methods: The present cross-sectional study recruited 106 healthy postmenopausal women with a mean age of 55.0 years and thyroid-stimulating hormone (TSH) levels within the laboratory reference range (0.4–4.5 μIU/ml). Anthropometric and biochemical measures as well as blood pressure were determined in each individual. Vascular structure and function were assessed by intima–media thickness, pulse wave velocity (PWV), augmentation index and flow-mediated dilation, respectively. We evaluated the associations between arterial markers and serum TSH, free triiodothyronine, free thyroxin, as well as serum thyroid peroxidase and thyroglobulin autoantibodies.

Results: Mean levels of PWV increased linearly across increasing TSH quartiles (P value = 0.014). Individuals with serum TSH greater than 2.5 μIU/ml had significantly higher values of PWV when compared with individuals with TSH levels below 2.5 μIU/ml (9.68 ± 1.97 vs. 8.54 ± 1.83 m/s; P = 0.030). In multivariate analysis, age, insulin resistance and TSH above 2.5 μIU/ml were the only significant predictors of PWV (TSH, β-coefficient = 0.222; P = 0.014). No associations were found between the remaining markers and levels of thyroid hormones, whereas thyroid antibodies were not associated with any of the arterial markers.

Conclusion: Women with TSH levels in the upper reference range have increased arterial stiffness compared to women with lower TSH. The upper limit of normal TSH in postmenopausal women may need re-evaluation with respect to the effects on the vasculature.

Keywords: arterial stiffness, cardiovascular disease, menopause, thyroid-stimulating hormone

Abbreviations: anti-TPO, anti-thyroid peroxidase antibodies; BMI, body mass index; CVD, cardiovascular disease; FMD, flow-mediated dilation; FT3, triiodothyronine; FT4, thyroxine; HOMA-IR, homeostasis model assessment of insulin resistance; IMT, intima–media thickness; PWV, pulse wave velocity; TSH, thyroid-stimulating hormone; WHR, waist-to-hip ratio

INTRODUCTION

Thyroid dysfunction has a significant effect on the cardiovascular system [1]. Hypothyroidism is associated with abnormal lipid metabolism, increased C-reactive protein and homocysteine, impaired cardiac contractility, diastolic hypertension, impaired endothelial function as well as increased risk of atherosclerosis and ischemic heart disease [2,3]. On the contrary, hyperthyroidism leads to a hyperdynamic cardiovascular state with a faster heart rate and increased frequency of atrial fibrillation [1]. Even subclinical hyperthyroidism [1,4,5] and hypothyroidism [1,5,6] have been associated with higher frequency of cardiovascular events [1,4–8]. Autoimmune thyroid disorders, like Hashimoto's thyroiditis or Graves' disease, have also been linked to increased rates of hospitalizations for cardiovascular events [9]. Concerning postmenopausal women, the frequency of thyroid dysfunction appears to be surprisingly high: according to findings of the Study of Women's Health Across the Nation (SWAN), up to 9.4% of women aged 42–52 years have thyroid-stimulating hormone (TSH) values outside the euthyroid range of 0.5–5.0 μIU/ml. Furthermore, a statistically significant increase of 3.5% was observed in levels of TSH, for each 5-year increase in age ($P < 0.04$) [10].

Beyond clinical or subclinical thyroid dysfunction, fluctuation of thyroid hormones within the reference range

Journal of Hypertension 2012, 30:592–599

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Received 11 July 2011 **Revised** 12 October 2011 **Accepted** 16 November 2011

DOI:10.1097/HJH.0b013e32834f5076

has been associated with long-term health risks. Accumulating evidence suggests that the upper limit of the TSH reference range should be lowered and set around 2.5 μIU/ml [11], based on the distribution of TSH levels in a disease-free population [12]. Recent data demonstrate a significant association between circulating levels of TSH above 2.5 μIU/ml and the probability of developing metabolic syndrome, which was found as high as 1.7-fold in the general population [13] and 1.95-fold in postmenopausal women [14]. Furthermore, TSH levels above 2.5 μIU/ml have been associated with significantly elevated triglycerides and higher body mass index (BMI) compared with individuals having TSH levels below 2.5 μIU/ml [13]. Moreover, individuals with 'high-normal' TSH levels (2.0–4.0 μIU/ml) combined with positive antithyroid antibodies exhibit elevated levels of total cholesterol [15]. In addition, the guidelines of the Endocrine Society recommend a TSH upper range of 2.5 μIU/ml, before conception as well as during the pregnancy [16]. Finally, TSH levels above 2.5 μIU/ml are considered predictive of evolution into overt hypothyroidism according to longitudinal data [17].

The effect of thyroid hormone variation and in particular the effect of 'high-normal' TSH (>2.5 μIU/ml) on markers of early cardiovascular disease have not been adequately evaluated in the postmenopausal population. These markers have become an important tool in the cardiovascular risk stratification of an asymptomatic individual [18–20]. Intima–media thickness (IMT), a marker of arterial structure, is a strong predictor of future cardiovascular events [19,21,22]. Pulse wave velocity (PWV) is considered as the gold standard method to assess arterial stiffness. PWV is defined as the speed of systolic pulse transmission from the left ventricle to peripheral arteries, being higher in stiff arteries [23]. Augmentation index evaluates the stiffness of the systemic arterial tree, assessing the interaction between incident (from the heart to the periphery) and reflected (from the periphery to the central region) pulse wave [17], with a predictive role regarding cardiovascular outcomes and cardiovascular risk [20]. Endothelial function can be evaluated by flow-mediated dilation (FMD), an index of endothelial nitric oxide bioavailability associated with the risk of cardiovascular events in postmenopausal women [24,25]. These markers represent different mechanisms of vascular damage and they can be assessed during follow-up examinations in order to evaluate the extent of subclinical arterial disease [26]. The present study, therefore, aimed to assess the effect of thyroid hormones on surrogate markers of early cardiovascular disease (CVD) in a sample of healthy euthyroid postmenopausal women.

METHODS

Participants

The cross-sectional study included 106 postmenopausal women, recruited from the Menopause Clinic of Aretaieio Hospital, 2nd Department of Obstetrics and Gynecology, University of Athens. Before recruitment, all participants were subjected to a routine evaluation program which included plasma glucose measurement, thyroid, liver and renal function tests, gynecological evaluation, breast mammography, Papanicolaou smear and transvaginal sonography. Exclusion criteria were clinically overt or treated coronary artery disease, peripheral artery disease, thromboembolism, diabetes mellitus, familial hypercholesterolemia, inflammatory disease, thyroid dysfunction or any other endocrine disorder and treatment with lipid-lowering or antihypertensive medication. Furthermore, recruited women were in postmenopausal status for at least 1 year, had an endometrial thickness of 5 mm or less, absence of premature menopause, gynecological malignancy and were not current or past users of hormone therapy or raloxifene. In addition, we excluded patients with serum-free thyroxine (FT4) or TSH levels outside the reference range of our laboratory. Patients with adherence and retention concerns (e.g. alcoholism) were not included in the study.

Protocol study procedures

A detailed medical history was recorded for every participant, using questionnaires regarding demographic and lifestyle parameters, cardiovascular risk, obstetrical and gynecological history for women. Subsequently, blood pressure, waist and hip circumference, weight and height were recorded in the morning and in light clothing, and the waist-to-hip ratio (WHR) and BMI were calculated. Patients abstained from eating, smoking or drinking except water and all medications for 12 h before the study. Fasting venous blood samples were drawn at 0830–0930 h for the biochemical evaluation and the serum was stored at −80°C until assessment. Ultrasound examinations were performed immediately thereafter, in a fixed order, by an operator blinded to the medical history of the patients: IMT followed by PWV. An automated Omron 705IT device (Omron) was used to record the blood pressure of each patient by oscillometry (twice, 1 min apart), after resting in the sitting position for 5 min, and the average of these measures was used in data analysis. Hypertension was defined as systolic and/or diastolic blood pressure of more than 139 mmHg and/or 89 mmHg, respectively. Institutional Review Board approval was obtained by the Ethics Committee of Aretaieio Hospital.

Biochemical and hormone assays

Serum glucose, total cholesterol, triglycerides and high-density lipoprotein (HDL)-cholesterol were assessed enzymatically by an autoanalyzer (ARCHITECT-ci8200; Abbott Diagnostics Laboratories, Abbott Park, Illinois, 60064 USA; Abbott 65205, Wiesbaden, Germany). Insulin was measured on an Abbott Architect i1000 analyzer. The total CV% ranged from 1.9 to 5.2%, and the analytical sensitivity was 1 μU/ml. TSH 3d gen, free triiodothyronine (FT3), free thyroxin (FT4) were measured with the Abbott Architect i1000 analyzer. The Friedewald equation [low-density lipoprotein (LDL)-cholesterol = total cholesterol – triglycerides/5 – HDL-cholesterol] was used to estimate LDL-cholesterol. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as follows: fasting insulin (μU/ml) × fasting glucose (mmol/l)/22.5. The total coefficient of variation (CV%) and analytical sensitivity were as follows: TSH: CV%, 1.7–5.3% and analytical sensitivity 0.0025 μIU/ml; FT3: CV%, 2.3–5.0% and analytical

sensitivity 1.0 pg/ml; FT4: CV%, 3.6–7.8% and analytical sensitivity 0.4 ng/dl. Antithyroglobulin antibodies and anti-myeloperoxidase antibodies (anti-TPO) were measured with the Microparticle Enzyme Immunoassay kits: 'anti-TG Abbott Axsym' and 'anti-TPO Abbott Axsym', respectively, on Axsym analyzer (Abbott Laboratories). Reference ranges were: TSH, 0.40–4.5 μIU/ml; FT4, 0.71–1.85 ng/dl; FT3, 1.7–3.7 pg/ml; anti-TPO, below 5 IU/ml; antithyroglobulin below 5 IU/ml.

Ultrasound measurements

Intima–media thickness

Intima–media thickness was measured in three paired segments, of both right and left common carotid artery, carotid bulb, and internal carotid artery, from a fixed lateral transducer angle using B-mode ultrasound imaging (14.0-MHz multifrequency linear array probe; Vivid 7 Pro, GE). In each segment, three measurements of the maximal IMT in the far wall were averaged, and the average IMT was calculated for each of the two carotid arteries. The average value of right and left carotid IMT was defined as combined IMT. The femoral IMT was measured in the far wall of a 1-cm long arterial segment proximal to the femoral bifurcation [19]. All of the scans were digitally recorded for offline analysis. A single operator blinded to the cardiovascular risk profiles of the participants performed all scans and offline analyses (coefficient of variation for mean carotid IMT: 10.6 and 11.3% for femoral IMT). Atherosclerotic plaque was defined as a clearly identified area of focally increased IMT greater than 1.5 mm, a threshold of IMT which is also associated with increased risk of myocardial infarction and/or cerebrovascular disease [19].

Flow-mediated dilation

The ultrasound analysis was performed by two independent observers. FMD was assessed using a 7.0–14.0-Hz multifrequency linear array probe, attached to a high-resolution ultrasound machine (Vivid 7 Pro, GE). The right brachial artery of each woman was longitudinally imaged above the antecubital fossa in a supinated position of the forearm. A pneumatic cuff was placed around the forearm, and after the initial measurements at resting conditions, the cuff was rapidly inflated to 250 mmHg for 5 min and subsequently deflated. The increase of arterial flow (reactive hyperemia) was monitored for 90 s. FMD was calculated as the percentage of maximal change of lumen diameter between rest and reactive hyperemia. The inter-observer and intra-observer variability for brachial artery diameter measurements in our laboratory was 0.1 ± 0.12 and 0.08 ± 0.19 mm, respectively.

Arterial stiffness

Pulse wave velocity was calculated from measurements of pulse transit time and the distance travelled between two recording sites with a validated noninvasive device (Complior; Artech Medical) that allows online pulse-wave recording and automatic calculation of PWV [PWV equals distance (meters) divided by transit time (seconds)]. Using two transducers, we obtained two different pulse waves simultaneously at two different sites; the distance between of which was calculated by subtracting the carotid (sternal notch from the carotid) – femoral distance. PWV was assessed between the common carotid artery and the common femoral artery (coefficient of variation: 2.4% for two repeated measurements).

Central blood pressure assessment

Radial artery tonometry was used to acquire and analyze the pulse waveform of the aorta (SphygmoCor System-Atcor Medical). Central blood pressures add prognostic information over peripheral blood pressures and offer valuable information as an end point in the assessment of interventions targeting cardiovascular disease [27]. Peripheral pressure waveforms were recorded at the radial artery using a hand-held high-fidelity tonometer (Millar, Instruments) and calibrated by using arterial pressures measured at the brachial artery. Aortic pressure waveforms were then calculated by applying generalized transfer functions, as described previously [28]. Analysis of the derived aortic waveform allows the calculation of indices that correspond mainly with measures of arterial and particularly aortic stiffness and the intensity of reflected waves. The following parameters were measured from the central aortic waveform: augmentation index (percentage) normalized for the heart rate of 75 b.p.m. [29], which is the difference between the second and the first peaks of the central aortic waveform expressed as a percentage of the aortic pulse pressure; central systolic and diastolic pressures; time to the beginning of the reflected wave (in milliseconds); and blood pressure amplification calculated as the ratio of peripheral pulse pressure: central pulse pressure. Mean difference ± SD for two repeated measurements for augmentation index normalized for the heart rate of 75 b.p.m. was -0.2 ± 4.3 and for time to the beginning of the reflected wave was -5.2 ± 15.8.

Statistical analysis

Statistical analysis was performed by SPSS version 17.0 (SPSS, Chicago, Illinois, USA). Arterial structural and functional indices and their association with levels of thyroid hormones and thyroid antibodies were the main outcome measures, using appropriately analysis of variance (ANOVA) or chi-squared analysis (X^2). Levels of thyroid hormones were divided in quartiles; levels of thyroid antibodies were dichotomized as 'positive' and 'negative', if the value of the antibody was higher or lower than five-fold the upper normal limit, respectively [30]. Thyroid hormones that resulted in statistically significant outcomes were further evaluated using ANCOVA or multivariate linear regression analysis to examine the independence of the effect on markers of vascular function or structure. Data are expressed as percentage values or absolute numbers (mean ± SD). Statistical significance was set at the 0.05 level.

RESULTS

Table 1 presents the baseline hormonal, biochemical and demographic characteristics of the 106 women participating in the study. Table 2 presents the mean values of indices of vascular function and structure. Mean values of thyroid hormones did not differ significantly according to quartiles

TABLE 1. Baseline biochemical–hormonal and demographic characteristics of the study population

	Mean or frequency (%)	SD	Minimum	Maximum
Age (years)	55.0	5.4	41.0	68.0
YSM (years)	6.93	5.3	1.0	34
BMI (kg/m^2)	26.5	4.5	20.1	45.7
WHR	0.83	0.07	0.67	1.06
TSH (μIU/ml)	1.5	0.8	0.4	3.6
FT3 (pg/ml)	2.5	0.5	1.7	3.7
FT4 (ng/dl)	1.2	1.5	0.7	1.85
Anti-TPO (IU/ml)	12.8%			
Anti-TG (IU/ml)	26.9%			
Cholesterol (mg/dl)	236.11	37.0	145.0	322.0
TG (mg/dl)	90.7	40.9	37.0	278.0
HDL-C (mg/dl)	64.0	15.3	39.0	108.0
LDL-C (mg/dl)	147.8	35.5	67.0	240.0
HOMA-IR	1.6	1.1	0.4	4.8
Smoking	15.1%			

anti-TG, antithyroglobulin antibodies; anti-TPO, antimyeloperoxidase antibodies; BMI, body mass index; FT3, free triiodothyronine; FT4, free thyroxin; HDL-C, high-density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein-cholesterol; TG, triglycerides; TSH, thyroid-stimulating hormone; WHR, waist-to-hip ratio; YSM, years since menopause.

of TSH were as follows: mean levels of FT3, 2.47 ± 0.50 pg/ml, 2.39 ± 0.37 pg/ml, 2.42 ± 0.43 pg/ml, 2.50 ± 0.45 pg/ml for TSH quartiles Q1, Q2, Q3 and Q4, respectively; mean FT4 levels, 1.04 ± 0.19 ng/dl, 1.03 ± 0.13 ng/dl, 1.04 ± 0.16 ng/dl, 1.00 ± 0.17 ng/dl for TSH quartiles Q1, Q2, Q3 and Q4, respectively.

A significant association was observed between mean measures of PWV and levels of TSH in quartiles (8.23 ± 1.80 vs. 9.45 ± 2.18 m/s in quartiles Q1 and Q4, respectively, P value for linear trend 0.014 in the univariate analysis; Fig. 1). TSH levels correlated positively with PWV ($r = 0.2$, $P = 0.047$). No differences in central or peripheral blood pressures were observed across TSH quartiles (Table 3). Furthermore, no association was detected between central or peripheral blood pressures and TSH as a continuous variable. None of the remaining arterial indices, namely IMT, FMD or augmentation index, presented a significant association with any of the thyroid hormones. Likewise, none of the thyroid hormones exhibited significant differences regarding the presence of atherosclerotic plaques at any site in the univariate analysis. Among CVD risk factors, as expected, systolic blood pressure, peripheral mean arterial pressure and HOMA-IR were significantly associated with the presence of plaques ($P = 0.017$, $P = 0.026$ and $P = 0.014$, respectively).

Aiming to further examine the association of TSH with vascular indices, we dichotomized the group in women with higher TSH levels (TSH >2.5 μIU/ml) and in women with lower TSH levels (TSH <2.5 μIU/ml); the results of this association are presented in Table 4. Postmenopausal women with TSH greater than 2.5 μIU/ml had higher PWV (9.68 ± 1.97 vs. 8.54 ± 1.83 m/s, $P = 0.030$ in the univariate analysis) compared to women with lower TSH. Linear regression analysis revealed that the only significant predictors of PWV were age, insulin resistance and TSH values greater than 2.5 μIU/ml (Table 5). In contrast, TSH as

TABLE 2. Descriptive statistics of vascular function and structure

	Mean or frequency (%)	SD	Minimum	Maximum
FMD (%)	5.6	2.6	0.0	15.79
PWV (m/s)	8.7	1.9	5.5	15.10
AI (%)	31.8	7.6	9.0	50.0
Central SBP (mmHg)	111.7	18.2	71.0	169.0
Central DBP (mmHg)	75.1	9.7	55.0	94.0
Central PP (mmHg)	36.6	12.7	13.0	78.0
Central MAP (mmHg)	87.3	11.7	60.3	117.0
SBP (mmHg)	119.1	18.7	77.0	180.0
DBP (mmHg)	73.9	8.7	55.0	92.0
Peripheral PP (mmHg)	45.2	13.4	19.0	90.0
Peripheral MAP (mmHg)	88.9	11.2	62.3	120.0
Mean IMT (mm)				
CCA	0.703	0.294	0.400	3.300
CB	0.873	0.201	0.400	1.500
ICA	0.635	0.162	0.300	1.200
Combined	0.739	0.145	0.400	1.600
FA	0.600	0.250	0.300	1.600
Increased IMT (>0.9 mm)	8.5%			
Plaques in carotid arteries	22.0%			

AI, heart-rate adjusted augmentation index; CB, carotid bulb; CCA, common carotid artery; DBP, diastolic blood pressure; FA, femoral artery; FMD, flow-mediated dilation; ICA, internal carotid artery; IMT, intima–media thickness; MAP, mean arterial pressure; PWV, pulse wave velocity; SBP, systolic blood pressure.

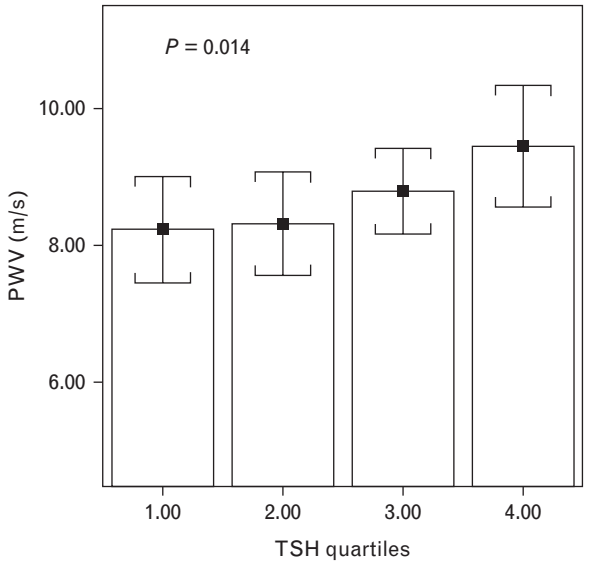

FIGURE 1 Mean values of pulse wave velocity (PWV) according to quartiles of thyrotropin (TSH) levels.

a continuous variable did not independently correlate with PWV, possibly indicating a nonlinear type of correlation between these two parameters with a 'step-up' biologic effect of TSH above the cut-off value of 2.5 μIU/ml in this population.

Concerning thyroid autoantibodies, women with levels of TSH above 2.5 μIU/ml showed increased prevalence of anti-TPO antibodies vs. those with lower TSH levels (30.0 vs. 12.5%, respectively). No significant association was found between the presence of atherosclerotic plaques at any site and positive anti-TPO antibodies (presence of plaque, positive antibodies 12.5% vs. negative antibodies 87.5%, P = NS) or antithyroglobulin antibodies (presence of plaques, positive antibodies 30.0%, negative antibodies 69.6%, P = NS). IMT, PWV and FMD were not associated with thyroid autoimmunity. The heart rate-adjusted augmentation index showed a significant association with the status of anti-TPO antibodies (32.8 ± 6.8 vs. 26.27 ± 8.33%, for negative vs. positive anti-TPO antibodies status, respectively, $P = 0.005$) in the univariate analysis. However, this association could not reach statistical significance in the multivariate approach, after adjusting for age, BMI, lipids, systolic blood pressure, HOMA-IR and smoking. Augmentation index was not associated with antithyroglobulin antibodies ($P = 0.321$).

DISCUSSION

The findings of the present study indicate that serum TSH associates positively with arterial stiffness in postmenopausal women with normal thyroid function. More specifically, TSH greater than 2.5 μU/ml was a significant predictor of PWV independently of traditional cardiovascular risk factors and particularly age and blood pressure. On the contrary, thyroid autoimmunity was not associated either with arterial structure or with arterial function.

Thyroid hormones and mainly circulating T3, derived from the conversion of T4, affect the cardiovascular system through both genomic and nongenomic pathways, which act in combination to regulate cardiac function and cardiovascular hemodynamics. Genomic actions of T3 are mediated by the nuclear thyroid hormone receptors. Two thyroid hormone receptor genes, alpha and beta, encode four isoforms TRα1, TRβ1, TRβ2 and TRβ3, the genes of which are expressed in the human heart, atria and ventricles [31], affecting thus cardiac contractile function and diastolic relaxation. Furthermore, the genomic effects of T3 maintain the endothelial integrity of the vasculature, while by decreasing resistance in peripheral arterioles, lead to enhanced cardiac output. The nongenomic actions of thyroid hormones consist of modulation of cellular metabolic activities, mitochondrial gene expression and function, influencing thus intracellular secondary messengers. In this way, thyroid hormones regulate the cardiac pace-maker gene expression, responses to β-adrenergic receptor stimulation, action potential duration, repolarization currents and ultimately heart rate [32]. In addition, thyroid hormones affect cardiovascular hemodynamics, with T3 influencing directly systolic function and contractility [2,32].

The presence of subclinical hypothyroidism, defined as increased serum TSH and normal circulating thyroid hormones, has been shown to affect structural and functional indices of CVD, as well as the occurrence of cardiovascular events. In the Rotterdam Study [33], which evaluated a random sample of 1149 elderly women, it was demonstrated that subclinical hypothyroidism, defined as TSH levels above 4 μIU/ml and normal free thyroxine levels, was associated with a higher age-adjusted prevalence of aortic atherosclerosis and myocardial infarction [odds ratio (OR) 1.7, 95% confidence interval (CI) 1.1–2.6; and 2.3, 95% CI 1.3–4.0, respectively]. Arterial stiffness has been repeatedly shown to be increased in patients with subclinical hypothyroidism [34–37]. According to the results of a recent meta-analysis [38], subclinical hypothyroidism is significantly

TABLE 3. Mean values of central and peripheral arterial pressure according to quartiles of thyroid hormones (numbers in brackets refer to standard deviation)

TSH quartiles (μIU/ml)	Central SBP	Central DBP	PP	Central PP	Peripheral SBP	Peripheral DBP	MAP
Q1 (0.40–0.85)	108.0 (17.8)	72.1 (9.9)	44.8 (12.4)	35.9 (10.7)	117.1 (12.3)	72.3 (9.3)	87.2 (11.6)
Q2 (0.86–1.35)	114.0 (13.1)	78.0 (8.1)	45.3 (10.8)	36.0 (7.7)	120.1 (15.1)	74.8 (7.4)	89.9 (9.3)
Q3 (1.36–2.10)	113.4 (15.3)	75.5 (9.5)	43.6 (12.5)	37.8 (13.1)	116.9 (15.2)	73.4 (7.8)	87.9 (9.1)
Q4 (2.11–3.70)	112.9 (24.6)	75.0 (10.8)	47.1 (17.8)	37.9 (16.9)	122.4 (25.0)	75.2 (10.1)	90.9 (14.3)
P value	0.429	0.448	0.658	0.569	0.447	0.341	0.362

DBP, diastolic blood pressure; FT3, free triiodothyronine; FT4, free thyroxin; MAP, mean arterial pressure; PP, pulse pressure; SBP, systolic blood pressure; TSH, thyroid stimulating hormone.

TABLE 4. Association between levels of TSH and vascular indices as well as CVD risk factors (numbers in brackets refer to standard deviation)

	TSH <2.50 ($n = 90$)	TSH >2.50 ($n = 15$)	*P* value
Age (years)	55.0 (5.3)	54.9 (6.7)	0.950
BMI (kg/m^2)	26.8 (4.6)	27.2 (4.8)	0.735
WHR	0.83 (0.07)	0.84 (0.06)	0.958
Cholesterol (mg/dl)	237.3 (37.6)	229.3 (33.6)	0.441
TG (mg/dl)	92.4 (42.1)	81.0 (32.4)	0.323
HDL-C (mg/dl)	63.5 (15.4)	67.2 (14.7)	0.392
LDL-C (mg/dl)	148.0 (36.4)	146.7 (30.6)	0.892
HOMA-IR	1.6 (0.9)	1.3 (0.4)	0.437
SBP (mmHg)	118.48 (18.5)	122.8 (19.9)	0.409
DBP (mmHg)	73.5 (8.6)	73.5 (8.6)	0.463
PP (mmHg)	44.9 (13.5)	46.5 (13.4)	0.674
MAP (mmHg)	88.5 (11.1)	91.8 (12.2)	0.299
FMD (%)	5.37 (2.68)	5.70 (2.36)	0.611
PWV (m/s)	8.54 (1.83)	9.68 (1.97)	0.030
AI (%)	31.34 (8.42)	30.00 (8.29)	0.523
Central SBP (mmHg)	111.0 (17.9)	116.7 (20.7)	0.343
Central DBP (mmHg)	74.4 (9.6)	77.8 (10.2)	0.283
Central PP (mmHg)	36.6 (12.6)	38.9 (13.3)	0.584
Central MAP (mmHg)	86.7 (11.4)	90.8 (13.1)	0.286
IMT combined (mm)	0.740 (0.149)	0.732 (0.126)	0.854

AI, heart-rate adjusted augmentation index; BMI, body mass index; DBP, diastolic blood pressure; FMD, flow-mediated dilation; IMT, intima–media thickness; MAP, mean arterial pressure; PP, pulse pressure; PWV, pulse wave velocity; SBP, systolic blood pressure; WHR, waist-to-hip ratio.

associated with increased risk of coronary heart disease (CHD) events and mortality, with the highest hazard ratio in patients with TSH levels above 10 μIU/ml (OR 1.89, 95% CI 1.28–2.80; and 1.58, 95% CI 1.1–2.27; $P < 0.001$ and 0.005 for trend, respectively). In an older meta-analysis subclinical hypothyroidism was shown to correlate with a higher risk of CHD cross-sectionally [relative risk (RR) 1.533, 95% CI 1.312–1.791] and prospectively (RR 1.188, 95% CI 1.024–1.379), with a follow-up period between 4 and 20 years. Moreover, the risk of death from cardiovascular causes at follow-up was significantly higher in patients with subclinical hypothyroidism (RR 1.278, 95% CI 1.023–1.597) [39].

Thyroid hormones within the reference range have also been associated with cardiovascular risk. Upper normal levels of TSH (2.48–4.00 μIU/ml) have been associated with higher prevalence of the metabolic syndrome, with an OR of 1.95 when compared to lower levels of TSH (0.3–1.44 μIU/ml) [14]. Similarly, patients with TSH in the range of 2.5–4.5 μU/ml had significantly higher levels of fasting triglycerides and BMI as well as a 1.7-fold higher risk for fulfilling the criteria of the metabolic syndrome [13].

According to the results of the present study, serum TSH in the upper normal range was associated with higher PWV in a population of healthy postmenopausal women, indicating increased arterial stiffness. In a previous study we demonstrated mild increases of arterial stiffness in a small group of younger men and women with high-normal levels of TSH (range 2.01–4.00 μIU/ml) when compared with those with lower TSH levels [35]. Interestingly, several studies support the association between serum TSH and blood pressure in euthyroid patients. The results of the Tromso Study [7] are in favor of a modest, but significant positive association between serum TSH and blood pressure within the normal serum TSH range. TSH was a significant predictor for systolic and diastolic blood pressure in women and for diastolic blood pressure in men. Furthermore, higher levels of TSH were found in hypertensive compared with normotensive euthyroid patients

TABLE 5. Linear regression analysis with pulse wave velocity as dependent variable and TSH high/low*, age, waist-to-hip ratio, smoking, mean arterial blood pressure and cholesterol as independent variables

	Pulse wave velocity (m/s)		
	Model $R^2 = 0.262$		
	β-coefficient	*P* value	Partial R^2
Age (years)	0.231	0.017	0.239
WHR	0.063	0.521	0.065
Smoking	−0.081	0.382	−0.089
MAP (mmHg)	0.140	0.176	0.137
Cholesterol (mg/dl)	0.159	0.291	0.107
LDL-cholesterol (mg/dl)	−0.192	0.211	−0.127
HOMA-IR	0.257	0.012	0.252
TSH high/low*	0.222	0.014	0.245

HOMA-IR, homeostasis model assessment of insulin resistance; MAP, mean arterial pressure; WHR, waist-to-hip ratio.
*TSHhigh/low = 0: levels of TSH <2.5 mIU/ml. TSHhigh/low = 1: levels of TSH >2.5 mIU/ml.

[40]. In a large cross-sectional, population-based study [26] women with serum TSH in the upper normal range (3.0–3.5 μIU/ml) had an OR 1.23 (95% CI 1.04–1.46) of having hypertension, whereas the respective figure for men was 1.98 (95% CI 1.56–2.53). In addition, the same study demonstrated a linear, positive association between systolic and diastolic blood pressure and levels of TSH within the reference range [26]. In a previous study conducted in the vascular laboratory of our hospital, based on 311 euthyroid men and women with mean age of 44 years, hypertensive individuals had higher TSH levels and belonged more frequently to the subgroup with TSH above 2 μIU/ml [30]. On the contrary, in a recent study evaluating 1319 Chinese men and women, although subclinical hypothyroidism was associated with hypertension in women, no association was found between serum TSH and blood pressure in euthyroid patients [41]. In our study TSH above 2.5 μIU/ml was a significant predictor of arterial stiffness, independently of arterial pressure, suggesting a possible direct effect on the vasculature. Accordingly, high-normal TSH has been independently associated with increased renal vascular resistance [40], whereas recently serum TSH in the upper normal range has been correlated with increased arterial stiffness in euthyroid hemodialysis patients [42]. Such a direct effect is supported by data indicating that the use of recombinant TSH increases circulating inflammatory molecules [43] and impairs the nitric oxide production in response to oxidative stress [44]. Both mechanisms are considered major mediators of arterial stiffening [45].

Cardiovascular mortality has also been associated with 'low' thyroid function within the reference range. The results of the Hunt Study [8], based on a large population of 17 311 women and 8002 men, indicated that serum TSH within the reference range was significantly associated with coronary artery disease mortality in women but not in men. Interestingly, women in the higher part of the TSH reference range (2.5–3.5 μIU/ml) had the highest hazard ratio for coronary death, namely 1.69 (95% CI 1.14–2.52), when compared with women in the lowest part of the TSH reference range, independently of age, BMI, smoking, hypertension or lipid profile.

Thyroid autoimmunity may have an impact on the cardiovascular system independently of thyroid function. In agreement with the present results, in a previous study we demonstrated that among euthyroid women, the presence of Hashimoto's thyroiditis was positively associated with arterial stiffness only in premenopausal women, whereas there was no association in postmenopausal women [46]. Thyroid autoimmunity, furthermore, has been associated with an increase in carotid IMT only in obese women, independently of thyroid function, BMI and cardiovascular risk factors [47]. This study, however, evaluated obese women attending an obesity clinic with a wide age range (18–66 years). On the contrary, the Rotterdam Study which evaluated elderly women reported that thyroid autoimmunity itself was not associated with CVD [33]. Taken together, the results of these studies, including the present one, may indicate that thyroid autoimmunity may affect cardiovascular indices only in younger women, whereas the effect may disappear in older women with stronger cardiovascular risk factors.

The present study bears certain limitations. Due to the cross-sectional design, causal associations cannot be demonstrated. Furthermore, sample size restrictions should also be noted, regarding the lack of association between vascular structure and thyroid function. Although, due to the large number of statistical tests performed, a statistical type I error cannot be excluded, the possibility of 'a by chance' finding should be rather low because our findings correlating PWV with TSH were consistent with our initial hypothesis; this association remained significant after adjusting for a large number of possible confounders; and the observed central blood pressure differences, albeit nonsignificant, also pointed to the same direction. The latter might be attributed to a high number of missing central blood pressure and augmentation index values possibly resulting in insufficient power to identify all possible associations. Finally, as we aimed to recruit healthy women and not patients with autoimmune thyroiditis, the prevalence of thyroid autoimmunity in our sample, reflecting that of the general population, may not have been high enough to enable us detect associations with vascular indices.

In conclusion, we demonstrated that serum TSH is an important predictor of arterial stiffness in euthyroid postmenopausal women. Serum TSH in the upper reference range is associated with significantly higher PWV, compared with lower TSH levels. These results are supportive of the need of redefining the upper normal TSH range in postmenopausal women, with respect to cardiovascular benefit. Larger prospective studies are needed to establish the significance of our findings.

ACKNOWLEDGEMENT

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector. This research did not receive any specific support in the form of equipment, drugs or any combination of these.

Conflicts of interest

There are no conflicts of interest.

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