

Normal flow-mediated vasodilatation of the brachial artery and carotid artery intima-media thickness in subclinical hypothyroidism

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Subclinical hypothyroidism (SHT) is a disease for which exact therapeutic approaches have not yet been established. Previous studies have suggested an association between SHT and coronary heart disease. Whether this association is related to SHT-induced changes in serum lipid levels or to endothelial dysfunction is unclear. The aim of this study was to determine endothelial function measured by the flow-mediated vasodilatation of the brachial artery and the carotid artery intima-media thickness (IMT) in a group of women with SHT compared with euthyroid subjects. Triglycerides, total cholesterol, HDL-C, LDL-C, apoprotein A (apo A), apo B, and lipoprotein(a) were also determined. Twenty-one patients with SHT (mean age: 42.4 ± 10.8 years and mean thyroid-stimulating hormone (TSH) levels: 8.2 ± 2.7 μ U/mL) and 21 euthyroid controls matched for body mass index, age and atherosclerotic risk factors (mean age: 44.2 ± 8.5 years and mean TSH levels: 1.4 ± 0.6 μ U/mL) participated in the study. Lipid parameters (except HDL-C and apo A, which were lower) and IMT values were higher in the common carotid and carotid bifurcation of SHT patients with positive serum thyroid peroxidase antibodies (TPO-Ab) (0.62 ± 0.2 and 0.62 ± 0.16 mm for the common carotid and carotid bifurcation, respectively) when compared with the negative TPO-Ab group (0.55 ± 0.24 and 0.58 ± 0.13 mm, for common carotid and carotid bifurcation, respectively). The difference was not statistically significant. We conclude that minimal thyroid dysfunction had no adverse effects on endothelial function in the population studied. Further investigation is warranted to assess whether subclinical hypothyroidism, with and without TPO-Ab-positive serology, has any effect on endothelial function.

Key words: Subclinical hypothyroidism; Lipid profile; Endothelial function; Carotid artery intima-media thickness

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Introduction

Subclinical hypothyroidism (SHT) is a disorder characterized by elevated serum thyroid-stimulating hormone (TSH) levels despite normal free-thyroid hormone values (1). The prevalence of SHT ranges from 5 to 10%, and the condition affects 6 to 10% of women (approximately 15% of women over 60 years of age) and 2.4 to 3% of the overall male population (2,3). There is much controversy regarding the morbidity and clinical significance of SHT (4-7). An unanswered question is whether individuals with SHT have only biochemical abnormalities that need to be closely monitored or if further laboratory investigation and thyroid hormone replacement are warranted.

Although not a consistent finding, some studies have found that subjects with SHT have higher total (TC) and low-density lipoprotein cholesterol (LDL-C) levels than euthyroid subjects (8-10). The reports of lipid and lipoprotein changes in response to thyroid hormone replacement in the setting of mild thyroid dysfunction have been somewhat conflicting (10-12). Although a consensus is still lacking, the benefit of levothyroxine replacement in patients with SHT seems to be linked to the reduction of cardiovascular risk (by lowering both TC and LDL-C levels) with restoration of euthyroid status (12-14). Previous studies have suggested an association between SHT and coronary heart disease (15,16). Whether this association is related to SHT-induced changes in serum lipid levels is unclear (11,14). In a population-based survey, SHT emerged as a significant risk factor for aortic atherosclerosis and myocardial infarction in elderly women, independent of serum cholesterol levels (16). Therefore, further mechanisms should be investigated to clarify the role of SHT in cardiovascular disease. Data from several studies of coronary heart disease in subjects with SHT are conflicting and the current available evidence for a causal relationship between SHT and mortality is still uncertain (17, 18).

The endothelium plays a major role in the maintenance of vascular function and integrity through the production of vasodilator and vasoconstrictor substances (19,20). Endothelial dysfunction, resulting in reduced availability of nitric oxide, has been identified as an early marker of atherosclerosis and can be used to predict coronary artery disease before the development of atherosclerotic changes (21). Recent studies have shown that hypothyroidism and SHT may have adverse effects on endothelial function independently of other well-known atherosclerotic risk factors (22-30).

The aim of the present study was to compare endothelial function measured by carotid artery intima-media thick-

ness (IMT) and the flow-mediated vasodilatation (FMD) of the brachial artery between women with SHT and normal controls.

Patients and Methods

All subjects (cases and controls) were women recruited from the Outpatient Clinic of the Clementino Fraga Filho University Hospital (HUCFF), Federal University of Rio de Janeiro (UFRJ).

Twenty-one women with SHT and 21 euthyroid women were included. The inclusion criterion was at least two documented laboratory determinations of SHT, at least 6 weeks apart, defined by both elevated TSH (>4.0 $\mu\text{IU/mL}$) and normal free-thyroxine (FT4) levels. The patients had no previous history of thyroid disease. The maximum TSH value accepted for the SHT group was 12.0 $\mu\text{IU/mL}$, while controls had TSH and serum anti-thyroid peroxidase antibodies (TPO-Ab) within the normal range and no history of thyroid disease. Patients were excluded from the study if they had a history of alcohol use, were suffering from concomitant non-thyroid illnesses (e.g., diabetes mellitus, arterial hypertension, liver, or renal diseases) or were using drugs that could interfere with thyroid, lipoprotein or endothelial function. Cases and controls were matched for body mass index (BMI), age and atherosclerotic risk factors. The study was approved by the UFRJ Institutional Ethics Committee and all subjects gave written informed consent to participate.

A general physical examination was performed including assessment of height (without shoes), weight and waist circumference (the minimum value between the iliac crest and the lateral costal margin). BMI was calculated as weight (kg) divided by height squared (m^2). Systolic and diastolic blood pressures were measured from the right brachial artery of the subjects in a supine position after 10 min of rest using a pneumatic sphygmomanometer.

Venous blood samples were drawn between 8:00 and 9:00 am after an overnight fast of 12 h. Serum was centrifuged and stored at -80°C until assayed. Serum TSH, FT4 and TPO-Ab, TC, high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), apoprotein B (apo B), apo A, and lipoprotein(a) were determined in both groups.

Serum TSH, FT4 and TPO-Ab were measured by immunochemiluminescence (Immulite 2000[®]; DPC, Diagnostic Products Corporation, USA). Reference ranges for TSH and FT4 were 0.4 - 4.0 $\mu\text{IU/mL}$ and 0.8 - 1.9 ng/dL , respectively. TPO-Ab levels of >35 IU/mL were considered positive. The intra-assay coefficients of variation were 3.8-12.5, 4.4-7.5, and 4.3-5.6% for TSH, FT4, and TPO-Ab, respectively. Inter-assay coefficients of variation were 4.6-

12.5, 4.8-9.0, and 7.8-10.5%. TC, TG (Vitros Chemistry Products assay, USA) and HDL-C (Boehringer Mannheim Systems, Germany) were determined enzymatically. LDL-C was calculated by the Friedewald equation: $LDL = (TC - HDL) - (TG/5)$. The reference ranges for TC, HDL-C, TG, and LDL-C were based on the III ATP of the National Cholesterol Education Program (NCEP) (31).

Lipoprotein(a) concentration was determined by an immunoradiometric assay (Diagnosis System International, Novatech, USA) and apo A and apo B were measured by rate nephelometric immunoassay (Beckman Coulter system, USA) and their reference ranges were lower than 30, 90-200, and 30-100 mg/dL, respectively.

The subjects were investigated by high-resolution color-Doppler ultrasound imaging (Toshiba Nemium, Japan; 14-mH linear probe) of the brachial artery in the dominant arm. The study was performed in a temperature-controlled room (25°C) with subjects resting in the supine position. Study participants fasted for 8 h prior to the exam. Blood pressure and heart rate were recorded on the opposite arm every 3 min using an automatic sphygmomanometer. The subjects' dominant arm was comfortably immobilized in the extended position to allow consistent access to the brachial artery. Doppler ultrasound measurements were performed before and 60 s after reactive hyperemia. To avoid interobserver variability, all measurements were performed by the same examiner, who was blind to the subjects' clinical status. Brachial artery vasodilation in response to reactive hyperemia was determined by a previously validated technique (32,33). The intraclass correlation coefficient of this technique has been reported previously by our laboratory and ranges from $R = 0.7001$ to $R = 0.8420$ ($P <$

0.05) (34,35). The scans were recorded on S-VHS videotape. The internal diameter of the brachial artery was assessed at the end of diastole, and arterial flow was measured using the pulse Doppler sample volume at an angle of 60° or less in the center of the artery. For each subject, optimal brachial artery images were obtained approximately 5 cm above the antecubital fossa. Arm pressure was caused by inflating a pneumatic arm cuff up to 30 mmHg higher than the subject's systolic arterial pressure for 5 min. The cuff was then deflated, the arterial flow was immediately recorded, and the diameter was measured 60 to 90 s after deflation. Figure 1 illustrates a typical example of an image obtained. For both diameters, one measurement was made and the value was recorded. FMD was calculated according to the formula: $FMD = (\text{post-occlusion diameter} - \text{baseline diameter}) \times 100 / \text{baseline diameter}$.

The IMT of the common carotid artery was calculated by the same examiner with high-resolution ultrasound imaging (Acuson Aspen Advanced model, 10 mH linear probe, USA), as described (36). Briefly, the common carotid arteries were scanned at the level of the bifurcation on both the right and left sides. Subsequently, the IMT was measured in the far wall of the arteries at sites of most advanced atherosclerotic lesions, identified as diffuse and continuous projections with the greatest distance between the lumen-intimal interface and the media-adventitial interface but without atherosclerotic plaques (Figure 2). Localized lesions of ≥ 2.0 mm thickness were considered to be atherosclerotic plaques. Three measurements were made for each subject and the mean value was used for analysis. The scans were recorded on S-VHS videotape. Reproduc-

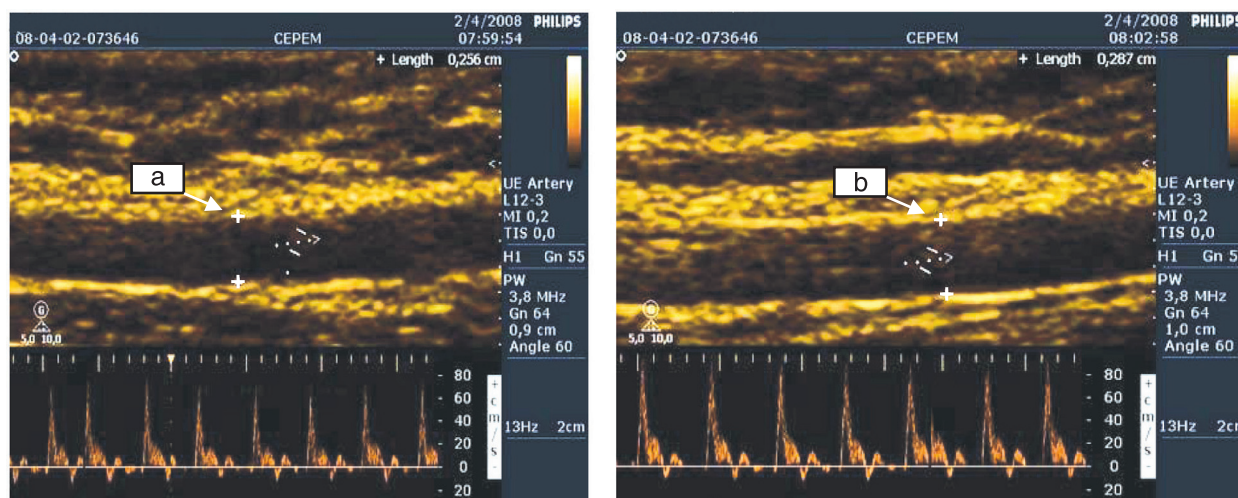


Figure 1. Brachial artery images obtained before arm pressure (a) and 60 s after deflation (b).

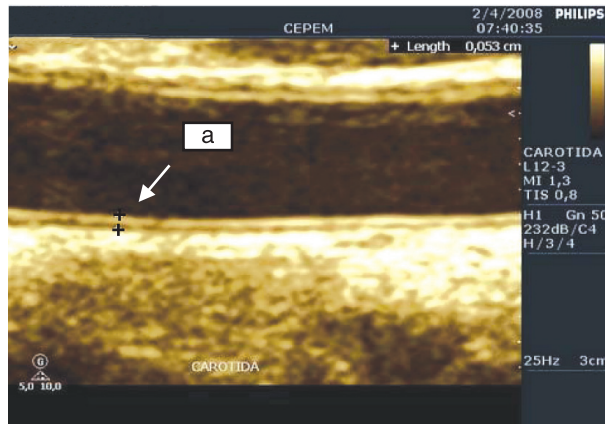


Figure 2. The greatest distance between the lumen-intima interface and the media-adventitia interface to calculate intima-media thickness of the carotid artery (a).

Table 1. Clinical and laboratory characteristics of the women with subclinical hypothyroidism.

	EU (N = 21)	SHT (N = 21)
Age (years)	44.2 ± 8.5	42.4 ± 10.8
Sedentarism (%)	88.2%	84.2%
Menopause (%)	61.9%	71.4%
Body mass index (kg/m ²)	24.6 ± 3.7	26.8 ± 4.8
Waist circumference (cm)	84.2 ± 13.6	85.1 ± 10.2
TSH (μIU/mL)	1.2 (0.7)	8.3 (4.3)*
FT4 (ng/dL)	1.2 (0.3)	1.0 (0.1)*
TG (mg/dL)	110 (61)	98 (47.5)
HDL-C (mg/dL)	54.4 ± 12.8	55.6 ± 10.8
TC (mg/dL)	201.1 ± 35.4	227 ± 49.4
LDL-C (mg/dL)	112.9 ± 33.3	148.7 ± 46.3*
apo A (mg/dL)	145.2 ± 26.2	156.9 ± 29.1
apo B (mg/dL)	104.4 ± 28.3	128.2 ± 37.7*
Lp(a) (mg/dL)	15.3 (28.6)	18.6 (71.05)

Data are reported as means ± SD or median (interquartile range). EU = euthyroid group; SHT = subclinical hypothyroid group; TSH = thyroid-stimulating hormone; FT4 = normal free thyroxine; TG = triglycerides; HDL-C = high-density lipoprotein cholesterol; TC = total cholesterol; LDL-C = low-density lipoprotein cholesterol; apo A = apoprotein A; apo B = apoprotein B; Lp(a) = lipoprotein(a). *P < 0.05 compared to EU group (*t*-test and Mann-Whitney test).

Table 2. Brachial and carotid artery parameters.

	EU (N = 21)	SHT (N = 21)
Flow-mediated dilatation (%)	16.1 (8.8%)	20.6 (11.2%)
Intima-media thickness of CCA (mm)	0.6 (0.1)	0.6 (0.1)
Intima-media thickness of bifurcation (mm)	0.6 (0.2)	0.6 (0.1)

Data are reported as median (interquartile range). EU = euthyroid group; SHT = subclinical hypothyroid group; CCA = common carotid artery. There were no statistically significant differences between the EU and SHT groups (Mann-Whitney test).

ibility of the IMT measurement was acceptable, as demonstrated by coefficients of variation of $7.7 \pm 4.3\%$ (36).

Statistical analysis

Statistical analysis was performed using SPSS for Windows program, version 10.0. The mean values of the numerical variables were compared between groups by the Mann-Whitney test; if not normally distributed by the Kolmogorov-Smirnov Lilliefors test. Parametric variables were compared by the *t*-test.

Data are reported as median values (interquartile range) of continuous non-parametric variables, as means ± SD values of parametric variables or frequencies of qualitative variables. Non-parametric variables are reported as median and range. A P value of <0.05 was considered to be significant.

Results

The mean age of the 21 SHT subjects was 42.4 ± 10.8 years and the mean age of the 21 subjects in the control group was 44.2 ± 8.5 years. The cause of SHT was spontaneous and 14 (70%) SHT subjects had abnormal TPO-Ab levels. No significant difference was observed with respect to age, BMI, waist circumference, activity level, or menopausal status. There were no statistically significant differences in lipid parameters between cases and controls, except for LDL-C (148.7 ± 46.3 vs 112.9 ± 33.3 mg/dL; *P* = 0.04) and apo B (128.2 ± 37.7 vs 104.4 ± 28.3 mg/dL; *P* = 0.02), which were significantly higher in the SHT group. The baseline clinical and laboratory characteristics of all participants are summarized in Table 1.

The median and range TSH values were 8.3 (4.3) μIU/mL for the SHT group and 1.2 (0.7) μIU/mL for the euthyroid group. TSH was 4 to 8 μIU/mL in eight patients (38%), and 8 to 12 μIU/mL in 13 (62%).

No significant differences were observed with respect to FMD or IMT between the SHT and control groups (Table 2). When the SHT group was subdivided into low (≥ 4 but > 8 μIU/mL) or high (≥ 8 but < 12 μIU/mL) TSH levels, no differences were observed in metabolic, or vascular parameters. Lipid parameters, FMD, and IMT values were higher in the common carotid artery and carotid bifurcation of SHT patients with positive serum TPO-Ab (0.62 ± 0.2 and 0.62 ± 0.16 mm, for common carotid artery and carotid bifurcation, respectively) compared to the negative TPO-Ab group (0.55 ± 0.24 and 0.58 ± 0.13 mm, for common carotid and carotid bifurcation, respectively) although the difference was not statistically significant.

Discussion

We did not find a significant association between thyroid function and vascular parameters in subjects who were similar with respect to age, BMI, smoking, menopausal status, and endothelial function modifiers, but who differed in thyroid function. The exclusion of participants with concomitant endocrinologic, renal, or hepatic diseases allowed us to control our analysis for confounding variables and yielded a homogenous population. In addition, by excluding co-morbid conditions, the mean age was reduced, allowing a smaller sample size for the study (21 subjects in each group). This may be the reason we did not detect a difference between SHT and control subjects in vasodilation parameters.

We examined endothelial function using a simple non-invasive, harmless method that enables accurate and reproducible assessment of the vascular response to flow increase. FMD is currently the main method used to assess endothelial function and is convenient for clinical practice (37).

Lekakis et al. (24) were the first to describe the negative association between borderline and mild hypothyroidism and FMD. In their study, cholesterol did not differ significantly among groups, but tended to be higher in the hypothyroidism and SHT groups and the authors concluded that higher cholesterol levels may be associated with endothelial dysfunction. LDL-C and apo B levels were significantly higher in our SHT group, and we did not find altered FMD compared to the euthyroid group.

Cikim et al. (26) compared 25 subclinical hypothyroid patients (mean age: 32.28 ± 9.67 years) and 23 euthyroid subjects (mean age: 35.87 ± 9.67 years) strictly matched for atherosclerotic risk factors and demonstrated that the subclinical hypothyroid group had significantly lower FMD values ($15.92 \pm 7.92\%$ for the euthyroid group and $10.68 \pm 3.71\%$ for the SHT group; $P < 0.05$). No significant differences were observed between groups with respect to other vascular parameters, including carotid artery IMT. Since the lipid profile was comparable between groups, the authors suggested that endothelial function could deteriorate prior to the emergence of hypothyroidism-induced metabolic changes. Cholesterol levels were lower in their

study than in the present one (181.04 ± 36.71 mg/dL for the euthyroid group and 179.56 ± 30.21 mg/dL for the SHT group). We found similar TSH levels (8.85 ± 6.86 mIU/L), but thyroid antibody levels were elevated in their SHT patients (mean: 496.71 ± 677.09 IU/mL), reflecting the autoimmune nature of hypothyroidism. Cikim et al. (26) evaluated the association of thyroid autoantibodies and the FMD/IMT ratio and found no significant correlation. We also could not find any significant association between anti-TPO concentrations and FMD or IMT.

The B-mode ultrasound measurement of IMT in the common carotid artery permits easy evaluation of atherosclerosis. Studies evaluating IMT values in hypothyroid and SHT patients showed conflicting results, which varied from low IMT values in subjects with elevated TSH levels (27) to high (22,23), or unchanged levels (38) compared to euthyroid subjects. The results are controversial but there are important differences between studies. Monzani et al. (22) excluded individuals over 55 years of age, and the mean IMT of their SHT patients was significantly higher in the subgroup older than 35 years. Levothyroxine replacement therapy reduced both LDL-C and mean IMT, suggesting that lipid infiltration of the arterial wall may represent the main mechanism underlying the increase in IMT in SHT. We previously reported no significant differences in mean carotid IMT in a group of subclinical hypothyroid patients compared to a euthyroid group, suggesting that mild SHT is not associated with an increase in cardiovascular risk when assessed by carotid IMT (38).

The causative agent of endothelial damage in thyroid dysfunction conditions is unclear, but seems to be associated with decreased endothelial nitric oxide synthesis due to low hormone levels or inflammation induced by thyroid autoantibodies (39).

Based on our data, minimal thyroid dysfunction had no adverse effects on endothelial function in the patients with SHT. Current evidence is insufficient to support the association between minimal thyroid disease and endothelial dysfunction. Larger studies are necessary to identify if there is any beneficial effect of levothyroxine treatment on endothelial function in patients with subclinical hypothyroidism.

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