Thyroid Function and High-Sensitivity C-Reactive Protein in Cross-Sectional Results from the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil): Effect of Adiposity and Insulin Resistance

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Key Words
Cardiovascular risk factors · C-reactive protein · Insulin resistance · Obesity · Subclinical hypothyroidism · Systemic inflammation · Thyroid dysfunction

Abstract

Background: Subclinical hypothyroidism (SCH) is associated with an increased cardiovascular risk, but little information is available about its association with high-sensitivity C-reactive protein (hs-CRP). Objectives: This study aims to analyze the association between SCH and hs-CRP using baseline data from the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil). Methods: The study has a cross-sectional design. We included subjects with normal thyroid function (thyroid-stimulating hormone, TSH, 0.4–4.0 μIU/ml and normal free thyroxine, fT 4 , 10.3–24.45 pmol/l) and SCH (TSH >4.0 μIU/ml and normal fT 4 ) who were evaluated for hs-CRP. We excluded individuals on medications that affect thyroid function and those who had prevalent cardiovascular disease. Multivariate linear regression evaluated hs-CRP and TSH as continuous variables, and logistic regression models assessed hs-CRP ≥19.05 nmol/l as the dependent variable and percent quintiles of TSH as the independent variables adjusted for demographic and cardiovascular risk factors. Results: We included 12,284 subjects with a median age of 50 years (interquartile range = 45–57); 6,408 (52.2%) were female, 11,589 (94.3%) were euthyroid, and 695 (5.7%) had SCH. Bivariate analyses of participants stratified into quintiles of TSH revealed differences according to hs-CRP but not the Framingham risk score. The fifth quintile of TSH was not associated with elevated hs-CRP, odds ratio = 1.11 (95% confidence interval = 0.98–1.26), p = 0.102, in a fully adjusted logistic model, also consistent with the linear model (β = 0.024, p = 0.145). Conclusions: TSH is not associated with hs-CRP. Obesity and insulin resistance are very important confounders in the study of the association between SCH and hs-CRP.

Introduction

The link between subclinical hypothyroidism (SCH), which is defined as high serum thyroid-stimulating hormone (TSH) concomitant with normal serum free thyroxine levels (fT 4 ) [1], and cardiovascular disease remains controversial. Some prospective cohort studies have
found an association [2, 3], while others have not [4, 5]. One large meta-analysis showed an association between SCH and an increased risk of coronary heart disease and cardiovascular mortality only in individuals with TSH values exceeding 7.0 mIU/l, and particularly above 10 mIU/l [6].

In spite of this controversy, some studies have linked SCH with hypertension [7], obesity [8], dyslipidemia [9], metabolic syndrome [10], insulin resistance [10], and subclinical atherosclerosis, especially aortic atherosclerosis [11], and carotid intima-media thickness [12] but not in a consistent manner.

High-sensitivity C-reactive protein (hs-CRP) is an acute-phase protein synthesized by the liver in response to infection and chronic inflammatory diseases [13]. Beyond that, hs-CRP is a predictor of future cardiovascular events and probably participates directly in the pathogenesis of atherosclerosis through activation of endothelial cells and coronary artery smooth muscle cells [13].

Large observational studies have established a relationship between hs-CRP, across a full range of values, and the morbidity and mortality associated with coronary heart disease [14, 15], and have improved the prediction of cardiovascular risk by adding hs-CRP to the Framingham risk score [16]. Other studies have found an association between hs-CRP and measures of subclinical atherosclerosis, such as coronary artery calcification and intima-media thickness [17]. The mechanism underlying this association is still unknown, but some clinical trials have shown an association between decreasing levels of atherogenic lipoproteins by statin therapy and concomitant decreases in systemic inflammation [18]. CRP also decreases as a result of interventions such as exercise, weight loss, smoking cessation, and the use of fibrates in a dose-response relationship [19].

The association between SCH and hs-CRP is still controversial [20, 21]; therefore, we aimed to cross-sectionally examine the association between SCH and hs-CRP as a surrogate marker for systemic inflammation using baseline data from the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil), an ongoing prospective cohort study in Brazil with information about thyroid function and hs-CRP levels.

**Methods**

**Study Population**

This study is a cross-sectional analysis of the baseline data from the ELSA-Brasil study.
two latter BP measurements was considered the value for defining hypertension. Hypertension was defined as the use of medication to treat hypertension, or systolic BP ≥140 mm Hg or diastolic BP ≥90 mm Hg. Body mass index (BMI) was calculated by dividing weight in kilograms by height in square meters. Obesity was defined as a BMI ≥30. Dyslipidemia was defined as an LDL cholesterol level ≥3.37 mmol/l or use of lowering cholesterol medications, and hypertriglyceridemia as serum triglyceride level ≥1.69 mmol/l. Diabetes was defined as previous medical history of diabetes, use of medication to treat diabetes, fasting plasma glucose ≥6.99 mmol/l, 2-hour plasma glucose ≥11.1 mmol/l, or HbA1c ≥6.5%. Chronic kidney disease (CKD) was defined as a glomerular filtration rate <60 ml/min/1.73 m² according to CKD-EPI [29, 30].

Insulin resistance was estimated using the homeostasis model assessment for insulin resistance (HOMA-IR) based on the following formula: fasting glucose (mg/dl) × insulin (μIU/ml)/405. HOMA-IR values above the 75th percentile were considered elevated. The metabolic syndrome was defined according to the National Cholesterol Education Program – Adult Treatment Panel III criteria as the presence of three of the following items: waist measurement ≥88 cm for women or ≥102 cm for men, HDL cholesterol <1.03 mmol/l in men and <1.29 mmol/l for women or <1.03 mmol/l in men, systolic BP ≥140 mm Hg or diastolic BP ≥90 mm Hg, serum triglyceride ≥1.69 mmol/l, and fasting plasma glucose ≥6.11 mmol/l [31]. The Framingham risk score for estimating the absolute 10-year cardiovascular risk (in %) was calculated according to criteria described elsewhere [31]. Briefly, it is a simplified coronary prediction model, building on age in years, gender, total cholesterol, HDL cholesterol, smoking, systolic BP, and use of medication to treat hypertension using data from the Framingham Heart Study after a 12-year follow-up [32].

**Laboratory Tests**

To measure fasting and post-load glucose levels, we used the hexokinase method (ADVIA 1200; Siemens): for fasting and post-load insulin, the immunoenzymatic assay; for glycated hemoglobin, high-performance liquid chromatography (Bio-Rad Laboratories, Hercules, Calif., USA), and for total and HDL cholesterol and triglycerides, the enzymatic colorimetric assay (ADVIA 1200). LDL cholesterol was calculated using the Friedewald equation, except in participants with elevated triglyceride levels (≥4.52 mmol/l), in whom the enzymatic colorimetric assay was used (ADVIA 1200).

**Statistical Analysis**

Continuous variables were expressed as means (SD) or medians (interquartile ranges, IQR), and compared using analysis of variance (ANOVA), the Mann-Whitney U test, or the Kruskal-Wallis test. Two-sample t-tests were used to compare means from normally distributed variables. Categorical variables were compared using the chi-square test or Fisher’s exact test, as appropriate.
Wallis test, as deemed appropriate after assessing normality assumptions. Categorical variables were expressed as proportions and compared using the χ² or Fisher’s exact test, as appropriate. HOMA-IR and hs-CRP were log transformed for comparison, but the values in the tables have been back transformed to their natural scales. TSH was divided by ascending quintiles: from first (lower values) to fifth (upper values). The first quintile was defined as the reference category.

Logistic regression models were built using hs-CRP categorized as <19.05 or ≥19.05 nmol/l as the dependent variable, and quintiles of TSH or SCH as the independent variable. Odds ratios (OR) and 95% confidence intervals (95% CI) are presented as crude and adjusted for sociodemographic characteristics, such as age, sex, and race/skin color in model 1. Model 2 includes all the variables in model 1 plus cardiovascular risk factors such as hypertension, diabetes, dyslipidemia, smoking, low HDL cholesterol, hypertriglyceridemia, and CKD; model 3 – model 2, plus BMI (continuous) and insulin resistance. The analyses were done using SPSS 20.0 software (IBM, Chicago, Ill., USA). p < 0.05 was regarded as significant, and all tests were two sided.

Results

A sample of 14,988 subjects retrieved from the 15,105 participants in the ELSA-Brasil study was evaluated for hs-CRP. After applying exclusion criteria, we included 12,284 subjects with a median age of 50 years (IQR = 45–57); 6,408 (52.2%) were females, 11,589 (94.3%) were eut...
thyroid, and 695 (5.7%) had SCH. Details on the study design can be found in the flowchart in online supplementary fig. 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000448683). In the euthyroid subjects, median TSH was 1.49 μIU/ml (IQR = 1.04–2.17; range 0.41–4.00); in the SCH group, TSH was 5.00 μIU/ml (IQR = 4.41–6.18; range 4.01–141), and in the total group, it was 1.55 μIU/ml (IQR = 1.07–2.34; range 0.41–141), p < 0.0001 (Mann-Whitney U test).

Table 1 shows the results by quintiles of TSH. Cutoffs for quintiles were as follows: 0.41–0.98 μIU/ml (first), 0.99–1.34 μIU/ml (second), 1.35–1.79 μIU/ml (third), 1.80–2.58 μIU/ml (fourth), and 2.59–141 μIU/ml (fifth). The fifth quintile included 1,768 euthyroid individuals and all 695 subjects with SCH. Age, BMI, and frequency of women, whites, never smokers, obesity, and CKD increased according to ascending quintiles.

Table 2 shows the logistic regression models. Analysis by quintiles in the total sample showed that the fifth quintile of TSH was associated with higher hs-CRP using the first as reference (OR = 1.20, 95% CI = 1.07–1.36, p = 0.003) in a model adjusted for sociodemographic characteristics and cardiovascular risk factors (model 2). However, the significance disappeared in the fifth quintile in comparison with the first after additional adjustment for insulin resistance and BMI: OR = 1.11 (95% CI = 0.98–1.26), p = 0.102 (model 3). Restricting the analysis to the euthyroid sample did not alter the results.

Table 3 shows the linear regression models, with results consistent with the logistic models. The model adjusted for sociodemographic characteristics and cardiovascular risk factors was significant (β = 0.057, p = 0.001), but further adjustment for insulin resistance and BMI (β = 0.024, p = 0.145) resulted in a lack of significance. Restricting the analysis to participants without diabetes, obesity, and insulin resistance did not change the results in logistic or linear models. We also analyzed the OR of a high hs-CRP level in participants with SCH as a categorical variable using euthyroid participants as the reference. Results were negative even in the crude logistic model (OR = 1.11, 95% CI = 0.95–1.29), p = 0.205 (data not shown).

**Discussion**

Although we found an association in the crude logistic models and models adjusted for age, sex, and race, this association lost significance after adjustment for insulin resistance and BMI. The association remained significant for the fourth quintile in the logistic models; in the linear regression models, these factors lost significance after further adjustment for insulin resistance and BMI.

Data about the association between hs-CRP and thyroid function are not very consistent. Two cross-sectional analyses with a design similar to this study found conflicting results [20, 21]. First, a cross-sectional analysis of a cohort of 2,494 participants recruited in Taiwan from 2006 to 2008 showed an association between SCH (TSH >5.6 μIU/ml) and elevated hs-CRP, defined as above the fourth quartile (>24.67 nmol/l), i.e. results consistent with ours [20]. In contrast with our population, they included a predominance of men (56%), with a lower mean age (38 years). However, the multivariate linear model was adjusted for triglycerides, HDL cholesterol, smoking, and systolic BP, but not for BMI, insulin resistance, or CKD.

Using data from the NHANES in the United States, a cross-sectional analysis of 1,551 euthyroid subjects and 57 with SCH did not find any association between ln(hs-CRP) and SCH (β = –0.017, p = 0.941). The sample in this study had a slight predominance of men (51%) and whites (52%), and was older (≥40 years of age). Multivariate linear adjustment was done for age, gender, race, use of cholesterol-lowering medication, and a composite of diabetes and prevalence of cardiovascular disease, hypertension, smoking, and obesity [21].

Similar to the NHANES data, other studies with different designs were not able to show a consistent association between SCH and increased hs-CRP. One Swiss double-blind placebo-controlled clinical trial showed that hs-CRP values increased progressively with thyroid failure in 63 subjects with SCH (p = 0.022), and particularly in 61 subjects with overt hypothyroidism (p = 0.016), in comparison to 40 euthyroid matched controls. However, levothyroxine therapy over 48 weeks did not result in decreased hs-CRP in 31 subjects with SCH compared to 32 subjects with SCH randomized to the placebo group [33]. Consistent with this clinical trial, one retrospective South Korean study that followed individuals with SCH who were treated or not with levothyroxine did not find decreased serum hs-CRP levels in either group [34].

Another large South Korean study found no association between SCH and hs-CRP. One cross-sectional study with 66,620 subjects, predominantly men (n = 43,588), did not find differences in mean hs-CRP levels among patients with SCH (defined as TSH >5.0 mIU/l and normal fT 4 ) in comparison to euthyroid subjects after adjustment for age and sex [35]. Similarly, another smaller
South Korean cross-sectional analysis examining data from outpatient checkups (4,410 euthyroid, 438 SCH, and 66 subjects with hyperthyroidism) [36] failed to demonstrate any association.

Our cross-sectional analysis did not detect an association between TSH and hs-CRP. Despite conflicting evidence about the relationship between thyroid function and CRP from cross-sectional analyses, some of them with large samples, other large observational studies have shown a strong relationship between overweight, obesity, abdominal obesity, metabolic syndrome, and increased hs-CRP in several populations [19, 37, 38]. Furthermore, other studies have proven that weight loss results in decreased hs-CRP levels in a dose-response relationship [19].

Similarly, Aronson et al. [38] found obesity to be the major determinant of elevated CRP in patients with the metabolic syndrome, while other large observational studies have also shown the association between insulin resistance and increased hs-CRP, suggesting cytokines originating from adipose tissue may play a role [39]. Instead of hs-CRP and thyroid function, the association between SCH, insulin resistance, and the metabolic syndrome has been clearly shown in other large observational studies [10, 40], including one using data from the ELSA-Brasil [10]. By contrast, little has been published about the direct inflammatory effect of SCH and its relation to the cardiovascular risk [20, 21]. Two different studies presented conflicting results. One cross-sectional study has reported that the cardiovascular risk in SCH patients is mediated by inflammatory pathways suggesting a dose-response effect [20]. However, cross-sectional data from the NHANES reported that SCH was not associated with cardiac inflammation [21]. Our findings suggest that high TSH levels are related to hs-CRP through its effects on fat depots and insulin resistance rather than a direct effect on this inflammatory marker.

Study Limitations
Although our results did not demonstrate a clear association between SCH and hs-CRP in an apparently healthy Brazilian adult population, it must be read within the context of the study design. This is a cross-sectional analysis which allows us to study associations but not causality. However, our results highlight the impact of adiposity and insulin resistance on hs-CRP as corroborated by evidence from large observational studies. Our study has also some strengths. Analysis by quintiles permitted us to draw conclusions about the impact of adiposity and insulin resistance on hs-CRP according to different levels of TSH in an apparently healthy population, especially in higher quintiles of TSH.

In conclusion, we did not find an association between increasing thyrotropin levels and higher hs-CRP levels when adjusted for cardiovascular risk factors, BMI, and insulin resistance, which was consistent with previous studies that suggested a potential role for cytokines originating from adipose tissue and systemic inflammation related to atherosclerosis and cardiovascular risks. We conclude that obesity and insulin resistance are potential mechanisms and very important confounders that mediate the effect of thyroid function on systemic inflammation in our population.

Acknowledgments
The ELSA–Brasil study was supported by the Brazilian Ministry of Health (Science and Technology Department) and the Brazilian Ministry of Science and Technology (Estudos e Projetos and CNPq National Research Council) (grants 01060010.00RS, 01060212.00BA, 01060300.00ES, 01060278.00MG, 01 060115.00 SP, 0106 0071.00RJ).

Disclosure Statement
No competing financial interests exist.

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