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Insulin resistance, lipid profile and low-grade inflammation in Hashimoto thyroiditis

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INSULIN RESISTANCE, LIPID PROFILE AND LOW-GRADE INFLAMMATION IN HASHIMOTO THYROIDITIS

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ASSINALE APENAS UMA DAS OPÇÕES:

É AUTORIZADA A REPRODUÇÃO INTEGRAL DESTE TRABALHO APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	☐
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Insulin resistance, lipid profile and low-grade inflammation in Hashimoto thyroiditis

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ABSTRACT

BACKGROUND: Hashimoto thyroiditis (HT) may present different levels of thyroid function impairment. It remains unclear how mild thyroid dysfunction, autoimmunity and chronic inflammation contribute to an increased cardiovascular risk in HT. Therefore, this study aims to assess insulin resistance, lipid panel and low-grade inflammation in HT patients.

METHODS: A total of 228 patients with HT were enrolled and divided into 3 groups, accordingly to TSH levels – TSH 0.35-2.49 μUI/ml, TSH 2.50-4.94 μUI/ml and TSH>4.94 μUI/ml. We assessed thyroid function tests and antibodies, lipid profile, insulin resistance indexes, high-sensitivity C-reactive protein, vitamin B12, folic acid and homocysteine. Statistical analysis was made using ANOVA, Student's t-test, Pearson's correlations and multiple linear regression.

RESULTS: 93.9% of our population were women and mean age was 47.1±15.4 years. No significant differences were found between groups, regarding age, sex and body mass index

(BMI). Homeostasis model assessment-insulin resistance (HOMA-IR) levels were significantly different in the three groups ($p<0.001$). In the total group, higher TSH values were associated to higher levels of triglycerides ($r=0.206$, $p=0.002$) and HOMA-IR ($r=0.209$, $p=0.002$), even after adjustment for age, sex and BMI. Thyroid peroxidase antibodies titers correlated positively with total cholesterol ($r=0.166$, $p=0.013$), LDL-cholesterol ($r=0.173$, $p=0.010$), ApoB ($r=0.190$, $p=0.006$) and HOMA-IR ($r=0.141$, $p=0.033$). Thyroglobulin antibodies correlated positively with triglycerides ($r=0.140$, $p=0.036$).

CONCLUSIONS: HT patients with mild thyroid dysfunction present a more atherogenic lipid profile and higher resistance to insulin action. Therefore, screening for cardiovascular comorbidities in these patients is essential to provide an early diagnosis and better treatment decisions.

KEY WORDS: Hashimoto disease, hypothyroidism, dyslipidemia, insulin resistance.

TEXT

Introduction

Hashimoto thyroiditis (HT), considered the most common autoimmune disease, is characterized by a chronic inflammation of the thyroid with a marked lymphocytic infiltration. It is a disorder that affects primarily women, with a female/male gender risk of 5:1 to 10:1, and the risk of developing it increases with age.[1] Clinically, HT patients may present with a goiter or, at a more advanced stage of the disease, an atrophic gland due to the gradual loss of thyroid function, estimated to be 5% per year. The presence of circulating antibodies to thyroid peroxidase (TPO-Ab) and thyroglobulin (Tg-Ab) is crucial for the diagnosis of this disease.[2,3] TPO antibodies, found in about 95% of HT patients, have a greater sensitivity and specificity when compared to Tg antibodies, which are positive in only 60-80% of these patients. The titer of TPO antibodies also presents a better correlation with the inflammatory activity in the gland.[2]

HT is considered the main cause of hypothyroidism, which can be classified as clinical or subclinical. At the initial stages of the disease, there may be a subclinical hypothyroidism (SCH) in which normal thyroid hormones levels are maintained by increased levels of thyroid-

stimulating hormone (TSH). As the disease progresses, free thyroxine (fT4) levels fall below the reference range and plasma TSH concentrations rise even further, which is referred to as overt hypothyroidism (OH).[2,4] SCH affects 3 to 15% of the population and the main risk factors are female sex, increasing age and suboptimal iodine intake. This condition has an annual rate of progression to overt disease of 2 to 6% and women with plasma TSH levels above 10mU/l and circulating anti-TPO antibodies have an increased risk of progression.[5] OH is associated to an increased risk of cardiovascular disease and atherosclerosis, partially due to the presence of risk factors, such as dyslipidemia, insulin resistance and obesity, common in these patients, but also through chronic inflammation observed in HT.[1,6] There is evidence that subclinical thyroid dysfunction also represents a risk factor for cardiovascular events and mortality, especially in younger patients with plasma TSH levels above 10mU/l.[7,8] The considered upper limit of the normal range of TSH levels is 4.94 μUI/ml. However, some authors defend that TSH levels above 2.50 μUI/ml are associated to mild thyroid dysfunction.[7]

Diabetes and dyslipidemia in hypothyroid patients may reflect the metabolic effects of hypothyroidism, which is associated to an energy conservation mode with reduced metabolism.[6,9] Hypothyroidism reduces clearance of low-density lipoprotein cholesterol (LDL-C) from the serum, affects cholesterol efflux, decreasing high-density cholesterol (HDL-C) plasma levels, and lowers lipoprotein lipase activity, elevating serum triglycerides (TG).[7,11] Patients with subclinical hypothyroidism show a similar lipid profile with higher plasma levels of total cholesterol and LDL-C.[5] Hypothyroidism also seems to be associated to insulin resistance, since thyroid hormones influence glucose metabolism by regulating insulin levels and the expression of glucose transporter GLUT4 in skeletal muscle, as well as hepatic genes expression.[9,10] Hyperhomocysteinemia, an additional risk factor for cardiovascular events, has also been associated to clinical hypothyroidism. Increased plasma levels of homocysteine in these patients may result from the diminished glomerular filtration rate and/or changes in homocysteine metabolism. Its values are also influenced by folic acid levels and serum vitamin B12 status.[11] Vitamin B12 deficiency is common in hypothyroid patients due to similar autoimmune process with circulating antibodies to

gastric parietal cells.[12] It has also been reported that patients with thyroid failure show a low-grade systemic inflammation with elevated high-sensitive C-reactive protein (hs-CPR) levels, which values correlate with the risk of future cardiovascular diseases.[13,14] However, these levels seem to be influenced by insulin resistance and increased body mass index (BMI) found frequently in these patients.[15]

The relationship between hypothyroidism and cardiovascular events is well established, however the pathways underlying this relation are not clear and may be independent of the traditional cardiovascular risk factors.[5] Aside from thyroid function, thyroid autoimmunity has been implicated as a risk factor for atherosclerosis, as some authors claim that the deregulated cytokines production, associated to the cell-mediated immunity defect, observed in HT contributes to obesity and hyperlipidemia.[16]

Autoimmune thyroiditis is a very common disease and it is essential to determine the impact of the different levels of thyroid dysfunction in the cardiovascular risk of these patients and explore its underlying mechanisms, in order to provide an early detection of comorbidities and better treatment decisions. Therefore, this study aims to assess cardiovascular risk, namely insulin resistance, lipid profile, low-grade inflammation and homocysteinemia, in subjects with autoimmune thyroiditis showing normal-high concentrations of TSH.

Materials and methods

Study population

The present study was conducted in the department of Endocrinology in University Hospital Center of São João, Porto, and enrolled 228 patients with HT followed in medical consultations from 2006 to 2017. This is a cross-sectional and retrospective study, in which patients' clinical and biochemical data were assessed through their respective medical records. The inclusion criteria were: (1) diagnosis of autoimmune thyroiditis with plasma anti-TPO antibodies levels higher than 5.61 UI/ml and/or anti-Tg antibodies levels superior to 4.11 UI/ml; and (2) TSH levels greater than 0.35 μUI/ml and normal thyroid hormones levels (FT3 levels between 1.71 and 3.71 pg/ml and FT4 levels between 0.7 and 1.48 ng/dl). Patients with previous cardiovascular events, other autoimmune diseases, diabetes mellitus, cancer or taking medication to dyslipidemia or thyroid disease or any other that could interfere with our results were excluded from this study.

Patients were divided into three groups, accordingly to serum TSH levels: group 1 comprised 166 subjects with TSH values between 0.35 and 2.49 μUI/ml; group 2 comprised 43 patients with TSH levels between 2.50 μUI/ml and 4.94 μUI/ml; group 3 comprised 19 patients with TSH levels higher than 4.94 μUI/ml. Both group 1 and 2 include euthyroid patients, but, as some authors claim that TSH levels above 2.50 μUI/ml are associated to an early form of thyroid dysfunction, TSH cut-off of 2.50 μUI/ml was defined. Group 3 includes patients with subclinical hypothyroidism, defined as TSH levels higher than 4.94 μUI/ml and normal FT3 and FT4 levels.

This study, protocol number 321-19, was approved by Ethical Committee of University Hospital Center of São João and its chairperson Prof. Dr. Filipe Almeida in October 24th of 2019.

Study protocol

From the medical records of the patients, thyroid function tests [TSH, free triiodothyronine (FT3), free thyroxine (FT4)] and TPO-Ab and Tg-Ab were registered. It was also assessed the lipid profile with total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), triglycerides (TG), apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB) and lipoprotein(a) [Lp(a)], as well as high sensitivity C-reactive protein (hs-CRP), homocysteine (Hcy), folic acid and vitamin B12 concentrations. The participants have had to perform oral glucose tolerance test (OGTT), which involves the assessment of blood glucose, insulin and C-peptide at 0, 30, 60, 90 and 120 minutes after the administration of 75 grams of glucose. The recorded values before and after the administration of this standardized dose of glucose were used to calculate insulin sensitivity and resistance indexes. The mathematical model HOMA (Homeostatic model assessment) abled the formulation of indexes that quantify insulin resistance (HOMA-IR), pancreatic β cells function (HOMA-β) and hepatic insulin sensitivity (HISI), using fasting plasma insulin and glucose values. QUICKI (Quantitative insulin sensitivity check index) is an indicator of insulin sensitivity, whereas IGI (Insulinogenic index) assesses β cells function making an estimate of insulin secretion. Matsuda index or WBISI (Whole-body insulin sensitivity index) represents hepatic and peripheral tissues sensitivity to insulin.[17,18]

Statistical analysis

Data analysis was performed using SPSS software version 26. The differences

between the three groups were evaluated by one-way analysis of variance (ANOVA). Two groups comparisons were performed using Student's t-test and equality of variances was evaluated by Levene's test. Pearson's correlation coefficient (r) was used to assess the relationships between variables in the total group and subgroups. Multiple linear regression models, adjusted for age, sex and body mass index (BMI), were made to assess the associations between thyroid function tests and evaluated metabolic and inflammatory parameters in the total group. All data are expressed as mean ± standard deviation in the text and tables. P values inferior to 0.05 were considered as statistically significant.

Results

The subjects enrolled in this study included 214 women (93.9%) and 14 men (6.1%). The mean age of this population is 47.1 ± 15.4 years and the mean body mass index (BMI) is 27.4 ± 5.1 kg/m2, which indicates that half of the population is overweight (BMI>25kg/m^2). The group with TSH between 0.35 and 2.49 µUI/mL included 166 subjects (72.8% of the total population) with a mean TSH of 1.41 ± 0.61 µUI/mL, the group with TSH between 2.50 and 4.94 µUI/mL included 43 subjects (18.9%) and had a mean TSH of 3.31 ± 0.72 µUI/mL and the group with TSH superior to 4.94 µUI/mL included 19 subjects (8.3%) with a mean TSH of 9.5 ± 7.29 µUI/mL. There are no significant statistical differences between the three groups regarding age (p=0.214), sex (p=0.880) and BMI (p=0.312). The demographic and clinical data of the population are presented in Table I.

Table I

In the comparison of the three groups, it was found a significant statistical difference in HOMA-IR values (p<0.001). The group with TSH higher than 4.94 µUI/ml showed significantly higher values of HOMA-IR in comparison to the group TSH 2.50-4.94 µUI/ml (3.77 ± 2.93 vs. 1.95 ± 1.25, p=0.006). The group TSH 2.50-4.94 µUI/ml, in comparison with the group TSH>4.94 µUI/ml, showed significantly superior values of QUICKI (0.70 ± 0.39 vs. 0.48 ± 0.14, p=0.049), as well as higher values of HISI (79.8 ± 63.7 vs. 41.7 ± 29.0, p=0.026). The group TSH 2.50-4.94 µUI/ml, in comparison to the group TSH 0.35-2.49 µUI/ml, presented significantly higher levels of Tg-Ab (152.5 ± 179.2 vs. 115.9 ± 131.7, p=0.037) and ApoB (102.1 ± 33.9 vs. 97.6 ± 21.0, p=0.036).

Considering the total group, positive correlations were found between TSH and both TG (r=0.206, p=0.002) and HOMA-IR (r=0.209, p=0.002) (Table II, Supplementary Figure 1). After adjustment for age, sex and BMI, TSH remained significantly associated to TG (β=0.203, p=0.034) and HOMA-IR (β=0.183, p=0.003) (Table III). FT3 was positively correlated with Tg-Ab levels (r=0.159, p=0.016). Tg-Ab titers also correlated positively with TG (r=0.140, p=0.036) and negatively with Lp(a) (r=-0.162, p=0.020) (Supplementary Figure 2). After adjustment for age, sex and BMI, the association between Tg-Ab titers and TG remained statistically significant (β=0.143, p=0.033), as well as the association between Tg-Ab and Lp(a) (β=-0.194, p=0.006). Positive correlations were found between TPO-Ab levels and TC (r=0.166, p=0.013), LDL-C (r=0.173, p=0.010), TG (r=0.148, p=0.027), ApoB (r=0.190, p=0.006), HOMA-IR (r=0.141, p=0.033) and vitamin B12 levels (r=0.138, p=0.047) (Supplementary Figure 3). After adjustment for age, sex and BMI, TPO-Ab titers remained significantly associated to TC (β=0.138, p=0.034), LDL-C (β=0.135, p=0.035) and ApoB (β=0.159, p=0.018). It was found a positive correlation between hs-CRP and IGI (r=0.156, p=0.024) and a negative correlation between hs-CRP and WBISI (r=-0.177, p=0.010) (Supplementary Table I).

Table II

Table III

Despite not being our main aim, we also assessed the interrelations between lipid profile and insulin resistance indexes. Positive correlations were found between HOMA-IR and LDL-C (r=0.205, p=0.002), TG (r=0.135, p=0.043) and ApoB (r=0.155, p=0.025) and negative correlations were found between HOMA-IR and both HDL-C (r=-0.195, p=0.003) and ApoA1 (r=-0.156, p=0.023) (Supplementary Table II). IGI correlated positively with TC (r=0.160, p=0.016), LDL-C (r=0.227, p=0.001), TG (r=0.174, p=0.009) and ApoB (r=0.23, p=0.001). IGI correlated negatively with HDL-C (r=-0.173, p=0.009). A positive correlation was found between HOMA-β and ApoA1 (r=0.142, p=0.039). WBISI correlated positively with HDL-C (r=0.161, p=0.015) and negatively with TG (r=-0.134, p=0.044). All these correlations may be confounded by BMI and, for that reason, we also assessed its correlations. In the total group, positive correlations were found involving BMI and TC (r=0.181, p=0.006), LDL-C (r=0.261, p<0.001), TG (r=0.154, p=0.021), hs-CRP (r=0.238,

p=0.001), ApoB (r=0.200, p=0.004), TPO-Ab (r=0.141, p=0.034), HOMA-IR (r=0.416, p<0.001), HOMA-β (r=0.175, p=0.008) and IGI (r=0.299, p<0.001). Negative correlations were found between BMI and HDL-C (r=-0.213, p=0.001), ApoA1 (r=-0.150, p=0.030), HISI (r=-0.155, p=0.02) and WBISI (r=-0.321, p<0.001).

In the group TSH 0.35-2.49 µUI/ml, FT4 levels correlated positively with ApoA1 (r=0.169, p=0.035) (Supplementary Table III). Tg-Ab correlated positively with LDL-C (r=0.168, p=0.031), ApoB (r=0.237, p=0.003), HOMA-IR (r=0.159, p=0.041) and IGI (r=0.205, p=0.008), and negatively with HDL-C (r=-0.176, p=0.024). It was also found a negative correlation between hs-CRP values and vitamin B12 (r=-0.199, p=0.017). In the group TSH 2.50-4.94 µUI/ml, it was found a negative correlation between FT4 and vitamin B12 (r=-0.380, p=0.027). As for the group with TSH higher than 4.94 µUI/ml, it was found a positive correlation between Tg-Ab and hs-CRP (r=0.647, p=0.004) and FT4 correlated negatively with both HDL-C (r=-0.552, p=0.018) and ApoA1 (r=-0.482, p=0.043). Hs-CRP also correlated positively with vitamin B12 concentration (r=0.852, p<0.001).

Discussion

It has been observed that HT patients present a higher risk of cardiovascular disease, even after adjustment for comorbidities common in these patients, such as hyperlipidemia and diabetes.[1] Hypothyroidism explains these associated comorbidities, however it has been suggested independent mechanisms underlying the higher incidence of hyperlipidemia and insulin resistance and also the increased cardiovascular risk, which would justify its presence in euthyroid patients. Subclinical hypothyroidism is also linked to an increased risk of cardiovascular events and mild thyroid dysfunction has been associated to metabolic syndrome. Therefore, TSH levels seem to be a good predictor for cardiovascular disease.[7] For this reason, we focused in the assessment of the variation of metabolic and inflammatory parameters accordingly to thyroid function and autoimmunity.

Lipid profile

No significant differences were found in lipid panel between the three groups, except in ApoB levels that were higher in the group TSH 2.50-4.94 µUI/ml in comparison to the group with lower TSH levels. ApoB is a major constituent of LDL-C, which suggests that these

particles increase in early thyroid dysfunction. This is in line with other previous findings, in which slight increases of TSH are associated to changes in lipid parameters, even for a normal range of TSH and thyroid hormones.[19-21]

Considering the total sample, our study revealed that increases in serum TG were proportional to increases of TSH levels. This correlation seems to be influenced by age and BMI of the population studied, but this correlation remained statistically significant after adjustment for those variables.[7,19,22] Xu et al. described that subjects with higher TSH levels, within the normal range, present higher levels of cholesterol and triglycerides, regardless of BMI, which is in agreement with the present study.[23]

Higher titers of TPO-Ab were associated to increased levels of TC, LDL-C, TG and ApoB and higher titers of Tg-Ab were associated to increased LDL-C, TG and ApoB and decreased HDL-C. This suggests that thyroid autoimmunity, represented by the presence of circulating TPO-Ab and Tg-Ab, is associated with a less favorable lipid panel, which contributes to an atherogenic profile in these patients. Tamer et al. obtained the same results, suggesting that autoimmunity may contribute independently for hyperlipidemia.[16] This autoimmune damage is possibly mediated through proinflammatory cytokines, predominantly IFN-γ that reduces cholesterol efflux to ApoA1.[24,25]

In the total group, it was found a negative correlation between Tg-Ab and Lp(a), which remained significant after adjustment for possible confounders. Lp(a) is an atherogenic lipoprotein and it is considered an independent risk factor for atherosclerotic cardiovascular disease. It has been reported that there is an association between thyroid autoimmunity and increased serum Lp(a) concentrations.[26] However, Bairaktari et al. found no significant differences in Lp(a) levels between subjects with and without thyroid antibodies.[27] Thus, the clinical relevance of this negative correlation found in our study is uncertain.

Insulin resistance

Our study revealed that patients with TSH levels superior to 4.94 μUI/ml have significantly higher values of HOMA-IR, in comparison to euthyroid patients, as well as lower values of QUICKI and HISI. This is supported by several studies that found that insulin resistance is present in subclinical hypothyroidism, justifying partially the increased cardiovascular risk reported in this group.[13,28-31] Maratou et al.

suggested that insulin resistance in these patients may be secondary to anomalous translocation of GLUT4 to cell surface, which results in a decreased glucose utilization by adipose and muscle tissues.[13]

An interesting finding is the positive correlation between TSH and HOMA-IR, showing that higher the TSH levels, higher the resistance of tissues to insulin, when considered a normal range of thyroid hormones. This association remained statistically significant after adjustment for possible confounders, such as age, sex and BMI. This correlation was also found in other studies in hypothyroid and euthyroid patients with HT.[29-33]

Positive correlations were found between TPO-Ab and HOMA-IR and between Tg-Ab and both HOMA-IR and IGI. These findings suggest that thyroid antibodies may also have a role in insulin resistance for euthyroid patients, possibly due to increased pro-inflammatory cytokines.[32]

Interrelations between lipid profile, insulin resistance and BMI

Our study showed that higher levels of HOMA-IR and IGI are accompanied with higher values of TC, LDL-C, TG, ApoB and lower levels of HDL-C and ApoA1, as well as that lower levels of WBISI and QUICKI are associated to higher levels of LDL-C and lower levels of HDL-C. The relationship between lipid profile and insulin resistance was not our primary goal, however it is interesting to observe that patients with HT and dyslipidemia also tend to present insulin resistance. It should be noted that small changes in thyroid hormones may cause variations in glucose-lipid metabolism, but these parameters also influence each other. It is suggested that the mechanisms associated to insulin resistance and hyperglycemia contribute to hypertriglyceridemia and other lipid alterations, but also that hypertriglyceridemia and low HDL-C induce hyperglycemia.[34,35] Therefore, glucose and lipid metabolism are intrinsically connected and affect each other. However, it should be noted that overweight and obesity may be confounders for these correlations, considering the great impact that they have on both variables.

Higher levels of BMI were found to be associated to higher levels of TPO-Ab, hs-CRP, TC, LDL-C, TG, ApoB, HOMA-IR, HOMA-β and IGI and to lower levels of HDL-C, ApoA1, HISI and WBISI. Mild thyroid dysfunction, represented by increased serum TSH levels, has been associated to higher risk of overweight and obesity, as TSH stimulates adipocytes proliferation. In contrast, increased BMI is

associated to higher TSH levels possibly due to larger serum leptin levels that affect hypothalamic-pituitary-thyroid axis.[23,29,36] Moreover, Marzullo et al. suggested that high leptin levels may have a relevant role in increasing susceptibility to thyroid autoimmunity in obesity, through changes in cell-mediated immunity.[37]

Low-grade inflammation and homocysteinemia

Hs-CRP is considered a low-grade inflammation marker and its values correlate with the risk of future cardiovascular diseases.[7,14] No significant differences were found in hs-CRP levels in the three groups. In the group with TSH higher than 4.94 µUI/ml, a positive correlation was found between Tg-Ab and hs-CRP, which suggests that higher antibodies titers are related to a systemic inflammation state. Higher levels of hs-CRP are associated to higher levels of IGI and lower levels of WBISI, in the total group, which implies that inflammation is related to increased insulin secretion and decreased insulin sensitivity. Insulin resistance has been associated to significantly higher CRP levels in various studies, but it is still controversial whether inflammation is secondary to insulin resistance or insulin resistance induces inflammation.[14,38,39]

Regarding vitamin B12, in the total group, it was found a positive correlation with TPO-Ab. However, after adjustment for age, sex and BMI, this correlation was not significant. Nevertheless, previous studies found an association between vitamin B12 deficiency and anti-thyroid antibodies, which is in accordance with the higher prevalence of pernicious anemia in HT patients, compared to general population.[1,40,41] In the group with lower TSH levels (TSH 0.35-2.49 µUI/ml), higher levels of hs-CRP were associated to lower vitamin B12 concentration, but, in the group with TSH superior to 4.94 µUI/mL, higher levels of hs-CRP were associated to higher levels of vitamin B12. Low-grade inflammation may be associated with micronutrients deficiency, but it does not seem to affect vitamin B12 absorption.[42] Therefore, the clinical relevance of these correlations is uncertain.

As for homocysteinemia, we found no significant differences between the groups nor significant correlations, which may suggest that these values only increase with a more severe thyroid function impairment.[11]

Our study has some limitations. It is a cross sectional study and the number of patients included in each group differ

considerably. As we performed multiple comparisons, the differences found as statistically significant should be interpreted with caution. The authors consider that this study contributes to a better description of the metabolic and inflammatory parameters and cardiovascular risk in patients with autoimmune thyroiditis and establishes the influence of the different states of thyroid function in those parameters. The multivariate analysis allowed elimination of possible confounders, such as age, sex and BMI, which is considered a strength of the study.

Conclusions

Our findings corroborate an association between thyroid function, autoimmunity and cardiovascular risk factors, namely insulin resistance, dyslipidemia and low-grade inflammation in patients with autoimmune thyroiditis. HT patients with mild thyroid dysfunction, showing normal-high TSH levels, present a more atherogenic lipid profile, as well as higher resistance to insulin action. Slight increases of TSH levels seem to be associated to significant increases in cholesterolemia and triglyceridemia, along with decreased insulin sensitivity. Thyroid autoimmunity also seems to be related to insulin resistance, to a less favorable lipid panel and low-grade inflammation.

These findings are relevant and demonstrate the importance of screening of cardiovascular comorbidities in these patients to provide an early diagnosis and better treatment decisions. More research is necessary to confirm if mild thyroid hormone deficiency, and the subsequent mild TSH increase, in HT patients is associated with a higher cardiovascular risk. If so, large controlled trials will be necessary to evaluate the benefits of levothyroxine replacement therapy in those associated comorbidities.

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NOTES

Conflicts of interest.— The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Authors' contributions.— Marta Afonso: manuscript design and conception, acquisition, analysis and interpretation of data, work draft, final approval of the manuscript; Celestino Neves: manuscript design and conception, work draft and critical review, final approval of the manuscript; João S. Neves: analysis and interpretation of data, critical review, final aproval of the manuscript; Miguel Pereira: acquisition and analysis of data, work draft, final approval of the version; Luís Delgado: interpretation of data, critical review, final approval of the manuscript; José L. Medina: interpretation of data, critical review, final approval of the manuscript; Davide Carvalho: interpretation of data, critical review, final approval of the manuscript.

TABLES

Table I. – Demographic and clinical data of the study population. Mean ± standard deviation.

	TSH 0.35-2.49 µUI/ml (n=166)	**TSH 2.5-4.94 µUI/ml** (n=43)	**TSH > 4.94 µUI/ml** (n=19)	***p* value**
Female sex (%)	93.4	95.3	94.7	0.880
Age (years)	48.1 ± 15.3	43.9 ± 14.7	44.6 ± 17.2	0.214
BMI (Kg/m²)	27.4 ± 5.0	26.6 ± 5.4	28.7 ± 5.2	0.312
TSH (µUI/ml)	1.41 ± 0.61	3.31 ± 0.72	9.50 ± 7.29	**<0.001**$^{+}$
FT4 (ng/dl)	1.03 ± 0.2	1.00 ± 0.23	0.93 ± 0.27	0.145
FT3 (pg/ml)	2.78 ± 0.56	3.09 ± 1.60	2.97 ± 0.76	0.092*
TPO-Ab (UI/ml)	604.4 ± 553.3	608.5 ± 580.4	725.1 ± 569.6	0.671
Tg-Ab (UI/ml)	115.9 ± 131.7	152.5 ± 179.2	151.2 ± 236.5	0.280*
TC (mg/dl)	200.8 ± 35.3	208.5 ± 48.1	218.1 ± 50.6	0.143
HDL-C (mg/dl)	57.7 ± 14.1	54.3 ± 14.9	57.4 ± 14.0	0.387
LDL-C (mg/dl)	126.3 ± 27.8	127.8 ± 40.7	136.3 ± 40.0	0.444
TG (mg/dl)	110.9 ± 70.7	130.9 ± 79.5	130.4 ± 78.7	0.203
ApoA1 (mg/dl)	143.3 ± 25.6	143.8 ± 27.2	146.3 ± 21.6	0.895
ApoB (mg/dl)	97.6 ± 21.0	102.1 ± 33.9	98.3 ± 30.6	0.612*
Lp(a) (mg/dl)	27.4 ± 28.8	26.7 ± 28.8	28.5 ± 43.0	0.981
HOMA-IR	1.90 ± 1.30	1.95 ± 1.25	3.77 ± 2.93	**<0.001**$^{+}$
HOMA-β	147.7 ± 179.8	182.4 ± 187.6	214.2 ± 113.9	0.198
QUICKI	0.67 ± 0.85	0.70 ± 0.39	0.48 ± 0.13	0.543^{+}
HISI	104.9 ± 238.5	79.8 ± 63.7	41.7 ± 29.0	0.392^{+}
WBISI	6.52 ± 5.65	6.10 ± 4.18	4.27 ± 3.53	0.207
IGI	0.74 ± 0.62	0.75 ± 0.55	1.10 ± 0.81	0.064
Hs-CRP (mg/dL)	0.39 ± 0.59	0.48 ± 0.58	0.54 ± 0.57	0.495
Homocysteine (µmmo/L)	8.66 ± 3.69	8.14 ± 2.39	8.11 ± 2.93	0.621
Folic acid	7.96 ± 7.58	6.91 ± 3.99	7.62 ± 3.37	0.715
Vitamin B12 (pg/mL)	432.7 ± 245.7	473.1 ± 251.6	571.9 ± 480.7	0.113

Table note: ApoA1 - apolipoprotein A1; ApoB – apolipoprotein B; BMI – body mass index; FT3 – free triiodothyronine; FT4 – free thyroxine; HDL-C – high density lipoprotein cholesterol; HISI – hepatic insulin sensitivity index; HOMA-IR – homeostasis model assessment-insulin resistance; Hs-CRP – high sensitivity C-reactive protein; IGI – insulinogenic index; LDL-C – low density lipoprotein cholesterol; Lp(a) – lipoprotein (a); QUICKI – quantitative insulin sensitivity check index; TC – total cholesterol; TG – triglycerides; Tg-Ab - thyroglobulin antibodies; TPO-Ab - thyroid peroxidase antibodies; TSH – thyroid stimulating hormone; WBISI – whole body insulin sensitivity index. P values inferior to 0.05 in bold. *p inferior to 0.05 when comparing first and second groups. $^{+}$ p inferior to 0.05 when comparing second and third groups.

Table II. – Pearson's correlations (r) between thryoid function, thryoid antibodies, lipid profile, insulin resistance, hs-CRP, homocysteine, folic acid and vitamin B12 in the total group.

		TSH	FT4	FT3	TPO-Ab	Tg-Ab
TC	r	0.072	-0.028	0.054	0.166	0.086
	p	0.282	0.672	0.423	**0.013**	0.198
HDL-C	r	-0.022	0.049	0.078	-0.058	-0.085
	p	0.737	0.468	0.241	0.384	0.204
LDL-C	r	0.028	0.001	-0.005	0.173	0.096
	p	0.676	0.989	0.944	**0.010**	0.152
TG	r	0.206	-0.103	-0.020	0.148	0.140
	p	**0.002**	0.125	0.762	**0.027**	**0.036**
ApoA1	r	0.082	0.095	0.075	-0.048	0.006
	p	0.238	0.170	0.280	0.490	0.932
ApoB	r	-0.015	-0.028	-0.007	0.190	0.103
	p	0.832	0.690	0.915	**0.006**	0.138
Lp(a)	r	0.076	0.128	0.033	0.004	-0.162
	p	0.278	0.068	0.642	0.949	**0.020**
HOMA-IR	r	0.209	0.027	0.023	0.141	0.028
	p	**0.002**	0.689	0.728	**0.033**	0.670
HOMA-β	r	0.080	0.050	0.025	0.062	-0.049
	p	0.231	0.451	0.706	0.353	0.461
QUICKI	r	-0.043	-0.073	-0.044	0.005	-0.036
	p	0.520	0.275	0.506	0.938	0.591
HISI	r	-0.049	-0.012	-0.032	-0.068	-0.062
	p	0.457	0.857	0.630	0.310	0.351
WBISI	r	-0.052	-0.041	-0.053	-0.084	-0.071
	p	0.438	0.536	0.429	0.205	0.285
IGI	r	0.056	0.063	0.003	0.109	0.062
	p	0.404	0.347	0.965	0.100	0.351
Hs-CRP	r	0.006	-0.056	-0.024	-0.098	0.105
	p	0.925	0.426	0.726	0.156	0.128
Hcy	r	-0.035	-0.056	0.071	-0.033	0.003
	p	0.617	0.429	0.313	0.643	0.961
Folic acid	r	-0.010	0.027	-0.040	0.006	0.064
	p	0.890	0.706	0.565	0.928	0.362
Vitamin B12	r	-0.001	-0.058	-0.053	0.138	0.121
	p	0.991	0.413	0.450	**0.047**	0.082

Table note: ApoA1 - apolipoprotein A1; ApoB – apolipoprotein B; FT3 – free triiodothyronine; FT4 – free thyroxine; HDL-C – high density lipoprotein cholesterol; HISI – hepatic insulin sensitivity index; HOMA-IR – homeostasis model assessment- insulin resistance; Hs-CRP – high sensitivity C-reactive protein; IGI – insulinogenic index; LDL-C – low density lipoprotein cholesterol; Lp(a) – lipoprotein (a); QUICKI – quantitative insulin sensitivity check index; TC – total cholesterol; TG – triglycerides; Tg-Ab - thyroglobulin antibodies; TPO-Ab - thyroid peroxidase antibodies; TSH – thyroid stimulating hormone; WBISI – whole body insulin sensitivity index. P values inferior to 0.05 in bold.

Table III. – Multiple linear regression models of the associations between thyroid function tests and metabolic parameters in the total group, after adjustment for age, sex and body mass index.

		TSH	FT4	FT3	TPO-Ab	Tg-Ab
TC	β	0.077	0.003	0.104	0.138	0.074
	p	0.237	0.968	0.113	**0.034**	0.258
HDL-C	β	-0.014	-0.056	0.063	-0.033	-0.083
	p	0.833	0.383	0.339	0.613	0.198
LDL-C	β	0.026	0.016	0.046	0.135	0.088
	p	0.682	0.797	0.478	**0.035**	0.170
TG	β	0.203	0.016	0.007	0.127	0.143
	p	**0.002**	0.817	0.923	0.058	**0.033**
ApoA1	β	0.074	-0.040	0.053	-0.034	0.024
	p	0.284	0.554	0.445	0.618	0.726
ApoB	β	0.004	0.002	0.048	0.159	0.073
	p	0.954	0.970	0.481	**0.018**	0.280
Lp(a)	β	0.090	0.045	0.550	0.002	-0.194
	p	0.203	0.526	0.441	0.981	**0.006**
HOMA-IR	β	0.183	-0.056	0.079	0.079	0.045
	p	**0.003**	0.363	0.204	0.202	0.465
HOMA-β	β	0.052	-0.060	0.031	0.038	-0.027
	p	0.430	0.361	0.637	0.563	0.677
QUICKI	β	-0.025	0.081	-0.050	0.025	-0.052
	p	0.714	0.221	0.457	0.711	0.438
HISI	β	-0.035	0.017	-0.045	-0.047	-0.072
	p	0.601	0.798	0.502	0.484	0.277
WBISI	β	-0.023	0.030	-0.085	-0.038	-0.090
	p	0.721	0.637	0.186	0.557	0.157
IGI	β	0.038	-0.018	0.041	0.063	0.073
	p	0.552	0.777	0.527	0.332	0.259
Hs-CRP	β	-0.017	-0.087	0.001	-0.142	0.126
	p	0.803	0.200	0.989	**0.038**	0.065
Homocysteine	β	-0.023	-0.084	0.103	-0.044	-0.018
	p	0.752	0.236	0.152	0.535	0.805
Folic acid	β	-0.008	0.002	-0.029	-0.005	0.060
	p	0.913	0.975	0.690	0.941	0.406
Vitamin B12	β	0.026	-0.059	-0.018	0.128	0.089
	p	0.706	0.398	0.798	0.064	0.201

Table note: ApoA1 - apolipoprotein A1; ApoB – apolipoprotein B; FT3 – free triiodothyronine; FT4 – free thyroxine; HDL-C – high density lipoprotein cholesterol; HISI – hepatic insulin sensitivity index; HOMA-IR – homeostasis model assessment- insulin resistance; Hs-CRP – high sensitivity C-reactive protein; IGI – insulinogenic index; LDL-C – low density lipoprotein cholesterol; Lp(a) – lipoprotein (a); QUICKI – quantitative insulin sensitivity check index; TC – total cholesterol; TG – triglycerides; Tg-Ab - thyroglobulin antibodies; TPO-Ab - thyroid peroxidase antibodies; TSH – thyroid stimulating hormone; WBISI – whole body insulin sensitivity index. P values inferior to 0.05 in bold.

SUPPLEMENTARY MATERIAL

TABLES

Supplementary Table Ia. – Pearson's correlations (r) between hs-CRP, homocysteine, folic acid, vitamin B12, BMI and evaluated metabolic parameters in the total group and in the group TSH 0.35-2.49 µUI/ml.

		Total group					TSH 0.35-2.49 µUI/ml			
		Hs-CRP	Hcy	Folic acid	Vitamin B12	BMI	Hs-CRP	Hcy	Folic acid	Vitamin B12
TC	r	0.010	-0.011	-0.013	0.038	0.181	0.004	-0.044	-0.022	-0.006
	p	0.883	0.882	0.852	0.592	**0.006**	0.959	0.591	0.787	0.938
HDL-C	r	-0.097	-0.087	0.018	-0.084	-0.213	-0.116	-0.099	0.012	-0.099
	p	0.163	0.216	0.802	0.233	**0.001**	0.152	0.230	0.881	0.222
LDL-C	r	0.044	0.056	-0.014	0.088	0.261	0.014	0.019	-0.022	0.060
	p	0.532	0.433	0.845	0.212	**<0.001**	0.867	0.814	0.786	0.458
TG	r	0.115	0.070	-0.063	-0.031	0.154	0.103	0.068	-0.054	-0.050
	p	0.097	0.322	0.373	0.654	**0.021**	0.204	0.406	0.503	0.541
ApoA1	r	-0.011	-0.037	0.021	-0.101	-0.150	-0.047	-0.025	0.001	-0.132
	p	0.880	0.603	0.767	0.150	**0.030**	0.577	0.762	0.993	0.104
ApoB	r	0.032	0.017	-0.046	0.008	0.200	0.049	-0.009	-0.056	-0.033
	p	0.659	0.812	0.516	0.914	**0.004**	0.556	0.915	0.493	0.687
Lp(a)	r	0.028	-0.068	0.007	0.010	0.056	-0.006	-0.076	-0.007	0.039
	p	0.699	0.342	0.920	0.883	0.423	0.948	0.357	0.929	0.633
HOMA-IR	r	0.110	0.023	0.009	0.052	0.416	0.102	0.067	0.053	0.119
	p	0.114	0.748	0.895	0.455	**<0.001**	0.210	0.414	0.517	0.143
HOMA-β	r	0.083	-0.007	-0.034	0.029	0.175	0.088	0.026	-0.028	0.034
	p	0.233	0.924	0.626	0.681	**0.008**	0.278	0.750	0.732	0.671
QUICKI	r	-0.053	0.026	-0.022	-0.035	-0.119	-0.036	0.046	-0.025	0.009
	p	0.443	0.712	0.752	0.620	0.073	0.656	0.578	0.762	0.907
HISI	r	-0.092	-0.034	-0.013	-0.054	-0.155	-0.083	-0.041	-0.018	-0.062
	p	0.185	0.634	0.855	0.443	**0.020**	0.307	0.622	0.822	0.447
WBISI	r	-0.177	-0.076	0.003	-0.027	-0.321	-0.171	-0.080	-0.022	-0.026
	p	**0.010**	0.279	0.967	0.694	**<0.001**	**0.034**	0.330	0.790	0.753
IGI	r	0.156	0.021	-0.054	-0.016	0.299	-0.168	0.062	-0.030	-0.035
	p	**0.024**	0.766	0.444	0.820	**<0.001**	**0.037**	0.452	0.714	0.669
Hs-CRP	r		0.040	-0.063	-0.018	0.238		0.052	-0.083	-0.199
	p		0.582	0.393	0.810	**0.001**		0.539	0.327	**0.017**
Hcy	r			-0.111	-0.004	0.085			-0.094	0.002
	p			0.119	0.954	0.230			0.255	0.985
Folic acid	r				0.113	0.028				0.094
	p				0.108	0.688				0.246
Vitamin B12	r					0.031				
	p					0.660				

Table note: ApoA1 - apolipoprotein A1; ApoB – apolipoprotein B; Hcy – homocysteine; HDL-C – high density lipoprotein cholesterol; HISI – hepatic insulin sensitivity index; HOMA-IR – homeostasis model assessment-insulin resistance; Hs-CRP – high sensitivity C-reactive protein; IGI – insulinogenic index; LDL-C – low density lipoprotein cholesterol; Lp(a) – lipoprotein (a); QUICKI – quantitative insulin sensitivity check index; TC – total cholesterol; TG – triglycerides; WBISI – whole body insulin sensitivity index. P values inferior to 0.05 in bold.

Supplementary Table Ib. - Pearson's correlations (r) between hs-CRP, Hcy, folic acid, vitamin B12 and evaluated metabolic parameters in the group TSH 2.5-4.94 µUI/ml and in the group TSH >4.94 µUI/ml.

		TSH 2.5-4.94 µUI/ml				**TSH > 4.94 µUI/ml**			
		Hs-CRP	**Hcy**	**Folic acid**	**Vitamin B12**	**Hs-CRP**	**Hcy**	**Folic acid**	**Vitamin B12**
TC	r	-0.053	0.064	-0.019	0.107	0.062	0.220	0.227	0.025
	p	0.754	0.714	0.915	0.540	0.813	0.396	0.398	0.924
HDL-C	r	-0.069	-0.229	<0.001	-0.223	0.046	0.186	0.064	0.141
	p	0.686	0.185	0.999	0.197	0.862	0.474	0.815	0.590
LDL-C	r	0.066	0.146	-0.031	0.177	0.124	0.271	0.196	0.043
	p	0.698	0.401	0.858	0.310	0.634	0.293	0.468	0.871
TG	r	0.188	0.269	-0.206	0.039	-0.041	-0.143	0.170	-0.118
	p	0.272	0.118	0.235	0.825	0.876	0.585	0.529	0.652
ApoA1	r	0.042	-0.236	0.120	-0.242	0.185	0.246	0.270	0.173
	p	0.823	0.173	0.493	0.162	0.476	0.325	0.294	0.493
ApoB	r	-0.005	0.172	-0.053	0.247	-0.035	-0.001	0.119	-0.164
	p	0.980	0.323	0.763	0.153	0.893	0.997	0.649	0.516
Lp(a)	r	0.149	0.047	-0.079	-0.083	0.127	-0.143	0.336	-0.016
	p	0.432	0.792	0.657	0.642	0.626	0.572	0.187	0.948
HOMA-IR	r	0.088	0.084	-0.017	-0.136	0.100	-0.101	-0.275	-0.155
	p	0.598	0.631	0.923	0.437	0.694	0.690	0.286	0.539
HOMA-β	r	0.038	-0.015	0.056	0.188	0.002	-0.131	0.329	0.020
	p	0.822	0.933	0.748	0.280	0.994	0.603	0.197	0.937
QUICKI	r	-0.164	-0.222	-0.051	-0.315	-0.175	-0.047	-0.444	-0.088
	p	0.324	0.199	0.773	0.065	0.488	0.854	0.074	0.729
HISI	r	-0.222	-0.041	-0.027	0.171	-0.200	-0.076	0.291	-0.008
	p	0.181	0.817	0.879	0.326	0.425	0.765	0.257	0.975
WBISI	r	-0.164	-0.114	0.184	0.106	-0.207	-0.104	0.226	-0.021
	p	0.325	0.516	0.289	0.544	0.409	0.681	0.383	0.935
IGI	r	0.062	-0.020	-0.202	-0.096	0.150	-0.188	-0.192	-0.008
	p	0.710	0.908	0.245	0.583	0.552	0.454	0.461	0.975
Hs-CRP	r		-0.078	-0.113	-0.144		0.184	0.496	0.852
	p		0.681	0.552	0.449		0.478	0.050	**<0.001**
Homocysteine	r			-0.322	-0.136			-0.266	0.139
	p			0.059	0.435			0.303	0.582
Folic acid	r				0.175				0.469
	p				0.314				0.058
Vitamin B12	r								
	p								

Table note: ApoA1 - apolipoprotein A1; ApoB – apolipoprotein B; Hcy – homocysteine; HDL-C – high density lipoprotein cholesterol; HISI – hepatic insulin sensitivity index; HOMA-IR – homeostasis model assessment-insulin resistance; Hs-CRP – high sensitivity C-reactive protein; IGI – insulinogenic index; LDL-C – low density lipoprotein cholesterol; Lp(a) – lipoprotein (a); QUICKI – quantitative insulin sensitivity check index; TC – total cholesterol; TG – triglycerides; WBISI – whole body insulin sensitivity index. P values inferior to 0.05 in bold.

Supplementary Table II. – Pearson's correlations (r) between insulin resistance and lipid profile in the total group.

		TC	HDL-C	LDL-C	TG	ApoA1	ApoB	Lp(a)
HOMA-IR	r	0.107	-0.195	0.205	0.135	-0.156	0.155	-0.041
	p	0.109	**0.003**	**0.002**	**0.043**	**0.023**	**0.025**	0.559
HOMA-β	r	0.013	0.119	0.011	0.009	0.142	-0.036	-0.035
	p	0.845	0.073	0.873	0.889	**0.039**	0.605	0.621
QUICKI	r	-0.013	-0.033	-0.039	0.043	0.022	0.032	0.005
	p	0.844	0.626	0.563	0.517	0.754	0.650	0.943
HISI	r	0.044	0.063	0.019	-0.045	0.017	0.027	0.047
	p	0.508	0.342	0.773	0.501	0.801	0.696	0.503
WBISI	r	-0.028	0.161	-0.100	-0.134	0.118	-0.076	0.080
	p	0.677	**0.015**	0.137	**0.044**	0.086	0.274	0.254
IGI	r	0.160	-0.173	0.227	0.174	-0.111	0.230	-0.091
	p	**0.016**	**0.009**	**0.001**	**0.009**	0.107	**0.001**	0.192

Table note: ApoA1 - apolipoprotein A1; ApoB – apolipoprotein B; HDL-C – high density lipoprotein cholesterol; HISI – hepatic insulin sensitivity index; HOMA-IR – homeostasis model assessment-insulin resistance; IGI – insulinogenic index; LDL-C – low density lipoprotein cholesterol; Lp(a) – lipoprotein (a); QUICKI – quantitative insulin sensitivity check index; TC – total cholesterol; TG – triglycerides; WBISI – whole body insulin sensitivity index. P values inferior to 0.05 in bold.

Table IIIIa. – Pearson's correlations (r) between thryoid function, thryoid antibodies, lipid profile, insulin resistance, hs-CRP, homocysteine, folic acid and vitamin B12 in the groups TSH 0.35-2.49 µUI/ml and TSH 2.5-4.94 µUI/ml.

		TSH 0.35-2.49 µUI/ml					TSH 2.5-4.94 µUI/ml				
		TSH	**FT4**	**FT3**	**TPO-Ab**	**Tg-Ab**	**TSH**	**FT4**	**FT3**	**TPO-Ab**	**Tg-Ab**
TC	r	0.001	0.021	0.033	0.235	0.152	0.148	-0.030	0.018	0.006	-0.270
	p	0.993	0.786	0.674	**0.002**	0.050	0.351	0.851	0.912	0.972	0.083
HDL-C	r	-0.008	0.099	0.117	-0.019	-0.176	-0.035	0.159	0.077	-0.276	0.023
	p	0.914	0.204	0.132	0.806	**0.024**	0.825	0.319	0.630	0.077	0.884
LDL-C	r	0.026	0.047	-0.001	0.213	0.168	0.088	-0.037	-0.069	0.098	-0.230
	p	0.739	0.551	0.987	**0.006**	**0.031**	0.578	0.817	0.660	0.535	0.142
TG	r	0.004	-0.091	0.020	0.223	0.340	0.032	0.015	-0.082	0.062	-0.268
	p	0.963	0.246	0.795	**0.004**	**<0.001**	0.840	0.927	0.610	0.698	0.090
ApoA1	r	0.033	0.169	0.145	-0.033	-0.036	0.227	0.129	0.014	-0.097	-0.114
	p	0.679	**0.035**	0.071	0.686	0.656	0.183	0.459	0.936	0.576	0.509
ApoB	r	0.014	-0.050	-0.006	0.272	0.237	0.103	-0.045	-0.027	-0.009	-0.274
	p	0.859	0.537	0.937	**0.001**	**0.003**	0.550	0.796	0.874	0.959	0.106
Lp(a)	r	-0.062	0.153	0.021	0.050	-0.124	0.245	0.082	0.030	-0.173	-0.360
	p	0.444	0.060	0.794	0.536	0.126	0.156	0.645	0.865	0.322	**0.034**
HOMA-IR	r	0.182	0.054	0.008	0.145	0.159	-0.211	0.146	0.019	0.032	-0.115
	p	**0.019**	0.492	0.923	0.063	**0.041**	0.175	0.355	0.906	0.838	0.463
HOMA-β	r	-0.002	0.015	0.033	0.060	-0.050	0.097	0.162	0.018	0.035	-0.058
	p	0.979	0.852	0.675	0.445	0.521	0.538	0.305	0.911	0.823	0.709
QUICKI	r	-0.005	-0.081	-0.055	0.009	-0.032	0.018	-0.136	-0.064	0.024	-0.113
	p	0.951	0.299	0.478	0.907	0.678	0.911	0.391	0.682	0.879	0.469
HISI	r	0.066	-0.015	-0.022	-0.078	-0.069	0.051	-0.136	-0.081	0.058	-0.083
	p	0.397	0.844	0.782	0.316	0.380	0.744	0.392	0.607	0.713	0.597
WBISI	r	0.046	-0.050	-0.023	-0.102	-0.104	-0.012	-0.022	-0.071	0.032	0.012
	p	0.553	0.521	0.771	0.190	0.181	0.941	0.890	0.649	0.840	0.941
IGI	r	0.150	0.093	-0.034	0.128	0.205	-0.066	-0.042	-0.032	-0.058	-0.199
	p	0.054	0.235	0.659	0.101	**0.008**	0.674	0.791	0.839	0.711	0.200
Hs-CRP	r	0.038	-0.106	-0.082	-0.119	0.030	0.003	0.055	-0.015	-0.182	-0.009
	p	0.639	0.191	0.314	0.142	0.710	0.988	0.746	0.930	0.273	0.955
Hcy	r	0.027	-0.034	0.051	-0.061	-0.111	0.077	-0.060	0.253	0.063	0.329
	p	0.739	0.681	0.536	0.456	0.178	0.661	0.738	0.143	0.720	0.054
Folic acid	r	0.042	0.012	-0.035	0.013	0.045	-0.273	0.117	-0.063	-0.031	0.127
	p	0.606	0.878	0.668	0.872	0.582	0.112	0.509	0.718	0.858	0.466
Vitamin B12	r	-0.090	0.041	0.025	0.119	0.021	-0.153	-0.380	-0.284	-0.036	-0.329
	p	0.268	0.619	0.759	0.141	0.795	0.380	**0.027**	0.098	0.838	0.054

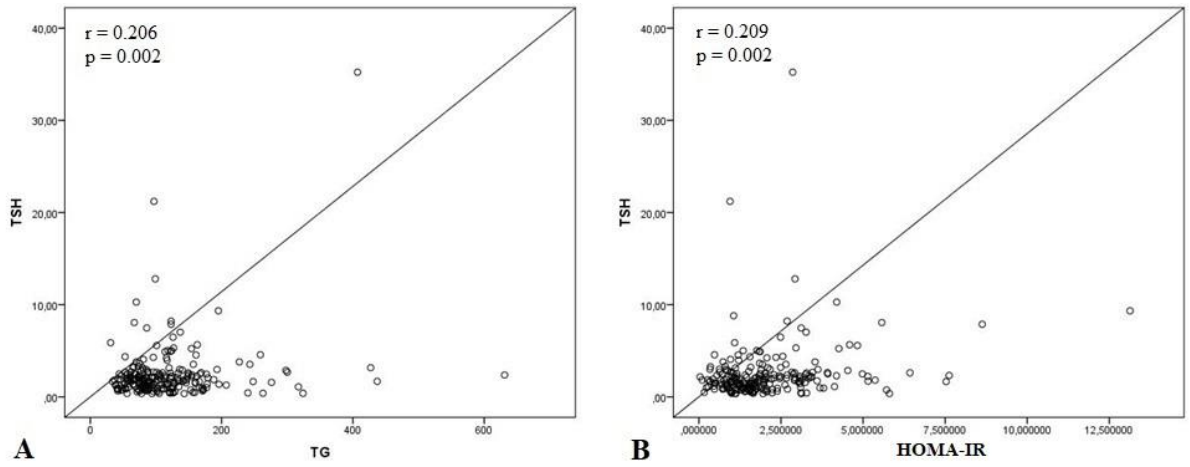
Table note: ApoA1 - apolipoprotein A1; ApoB – apolipoprotein B; FT3 – free triiodothyronine; FT4 – free thyroxine; HDL-C – high density lipoprotein cholesterol; HISI – hepatic insulin sensitivity index; HOMA-IR – homeostasis model assessment- insulin resistance; Hs-CRP – high sensitivity C-reactive protein; IGI – insulinogenic index; LDL-C – low density lipoprotein cholesterol; Lp(a) – lipoprotein (a); QUICKI – quantitative insulin sensitivity check index; TC – total cholesterol; TG – triglycerides; Tg-Ab - thyroglobulin antibodies; TPO-Ab - thyroid peroxidase antibodies; TSH – thyroid stimulating hormone; WBISI – whole body insulin sensitivity index. P values inferior to 0.05 in bold.

Table IIIIb. – Pearson's correlations (r) between thryoid function, thryoid antibodies, lipid profile, insulin resistance, hs-CRP, homocysteine, folic acid and vitamin B12 in the group TSH > 4.94 μUI/ml.

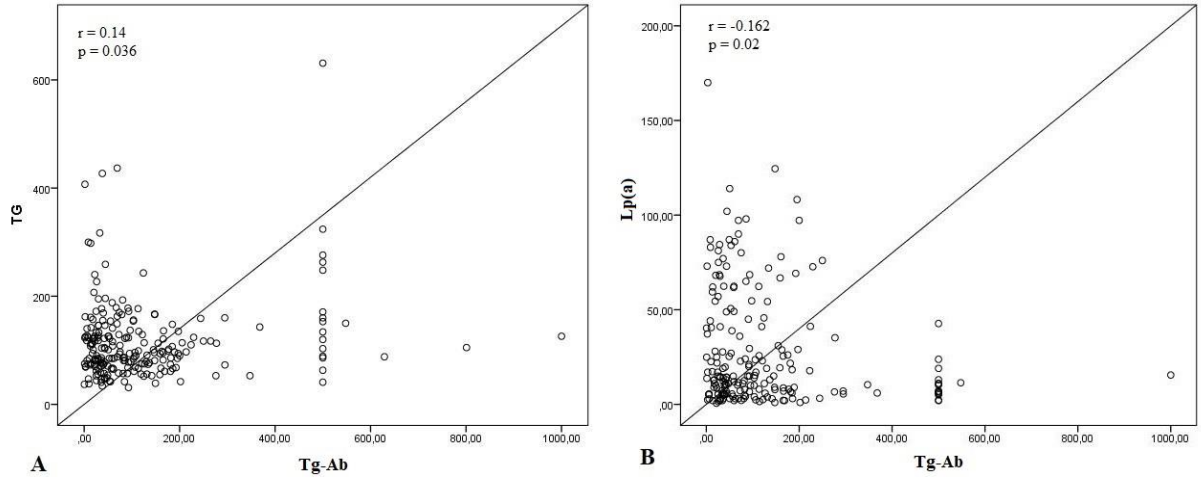
		TSH	FT4	FT3	TPO-Ab	Tg-Ab
TC	r	-0.114	-0.133	0.200	0.035	0.382
	p	0.652	0.599	0.427	0.890	0.118
HDL-C	r	-0.021	-0.552	0.146	0.141	0.231
	p	0.934	**0.018**	0.563	0.576	0.355
LDL-C	r	-0.177	-0.047	0.226	0.069	0.395
	p	0.483	0.852	0.367	0.787	0.105
TG	r	0.709	-0.321	-0.235	-0.331	-0.151
	p	**0.001**	0.194	0.348	0.180	0.550
ApoA1	r	0.281	-0.482	-0.027	-0.123	0.431
	p	0.258	**0.043**	0.915	0.626	0.074
ApoB	r	-0.144	0.126	-0.052	0.173	0.171
	p	0.570	0.618	0.838	0.491	0.497
Lp(a)	r	0.255	0.093	0.130	-0.020	-0.160
	p	0.306	0.713	0.608	0.936	0.526
HOMA-IR	r	-0.098	0.076	-0.004	0.199	-0.228
	p	0.691	0.759	0.987	0.415	0.348
HOMA-β	r	-0.050	0.310	-0.253	0.148	-0.196
	p	0.840	0.196	0.297	0.546	0.422
QUICKI	r	0.133	-0.237	-0.160	-0.109	0.126
	p	0.588	0.328	0.514	0.656	0.606
HISI	r	0.136	-0.226	-0.211	-0.085	0.095
	p	0.578	0.351	0.385	0.728	0.698
WBISI	r	0.218	-0.247	-0.283	-0.059	0.071
	p	0.371	0.307	0.240	0.811	0.774
IGI	r	-0.296	0.212	0.234	0.182	-0.225
	p	0.219	0.383	0.334	0.457	0.355
Hs-CRP	r	-0.282	0.159	0.129	0.234	0.647
	p	0.257	0.529	0.609	0.350	**0.004**
Homocysteine	r	-0.017	-0.324	-0.109	0.184	0.407
	p	0.946	0.190	0.667	0.465	0.094
Folic acid	r	0.058	0.017	-0.040	0.092	0.332
	p	0.826	0.948	0.878	0.727	0.193
Vitamin B12	r	-0.269	0.013	0.180	0.361	0.632
	p	0.280	0.960	0.475	0.141	**0.005**

Table note: ApoA1 - apolipoprotein A1; ApoB – apolipoprotein B; FT3 – free triiodothyronine; FT4 – free thyroxine; HDL-C – high density lipoprotein cholesterol; HISI – hepatic insulin sensitivity index; HOMA-IR – homeostasis model assessment- insulin resistance; Hs-CRP – high sensitivity C-reactive protein; IGI – insulinogenic index; LDL-C – low density lipoprotein cholesterol; Lp(a) – lipoprotein (a); QUICKI – quantitative insulin sensitivity check index; TC – total cholesterol; TG – triglycerides; Tg-Ab - thyroglobulin antibodies; TPO-Ab - thyroid peroxidase antibodies; TSH – thyroid stimulating hormone; WBISI – whole body insulin sensitivity index. P values inferior to 0.05 in bold.

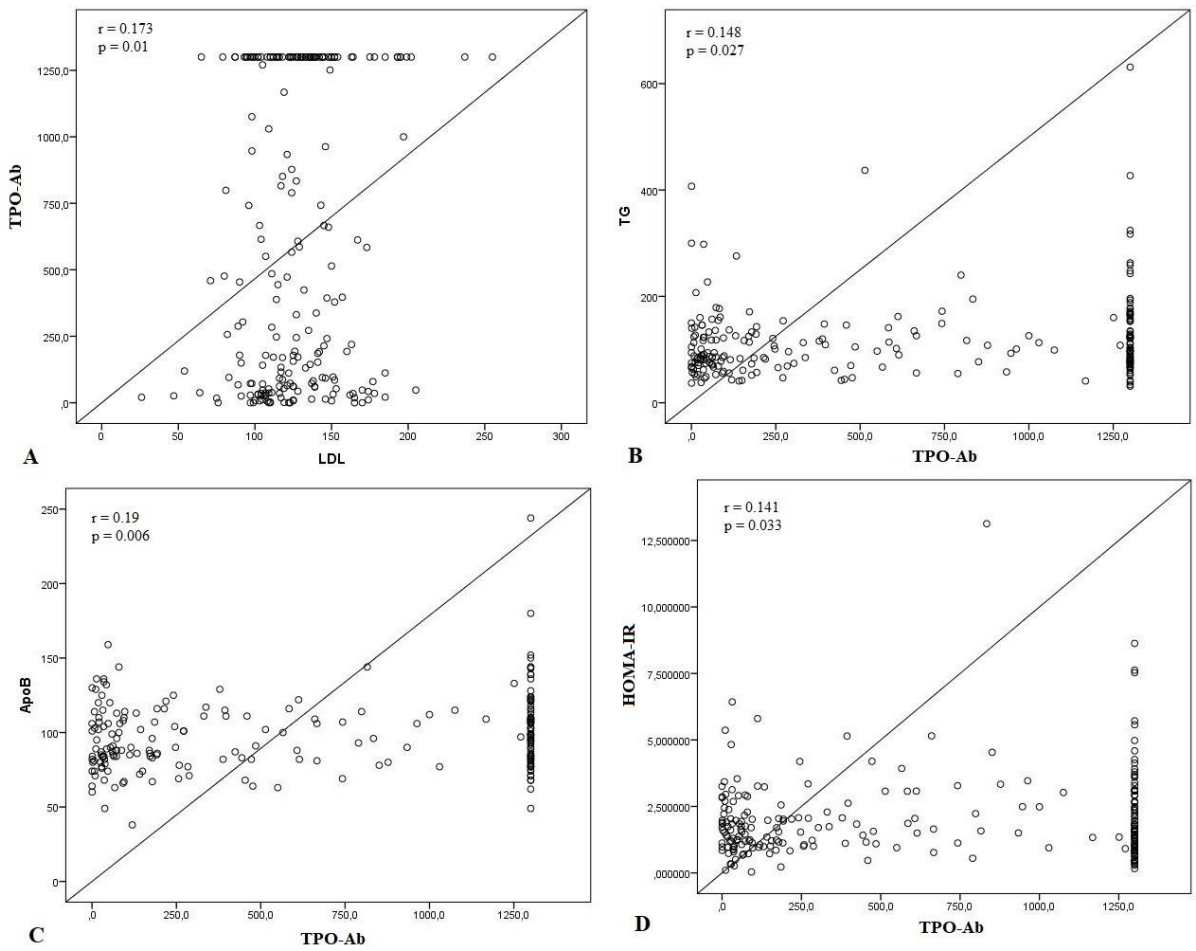
FIGURES



Supplementary Figure 1 — Scatterplot of the Pearson's correlations (r) between TSH and both triglycerides (A) and HOMA-IR (B) in the total group.



Supplementary Figure 2 — Scatterplot of the Pearson's correlations (r) between thyroglobulin antibodies (Tg-Ab) and both triglycerides (TG) (A) and lipoprotein (a) [Lp(a)] (B) in the total group.



Supplementary Figure 3 — Scatterplot of the Pearson's correlations (r) between thyroid peroxidase antibodies (TPO-Ab) and LDL-C (A), triglycerides (TG) (B), ApoB (C) and HOMA-IR (D) in the total group.

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ANEXOS

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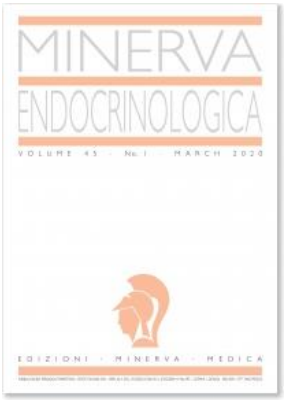

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Instructions for the most frequent types of articles submitted to the journal. **Editorials**. Commissioned by the Editor in Chief or the Managing Editor, editorials deal with a subject of topical interest about which the author expresses his/her personal opinion. The text must not be subdivided. No more than 1000 words (3 typed, double-spaced pages) and up to 15 references will be accepted.

Original articles. These should be original contributions to the subject. The text should be 3000-5500 words (8 to 16 typed, double-spaced pages) not including references, tables, figures. No more than 50 references will be accepted. The article must be subdivided into the following sections: introduction, materials (patients) and methods, results, discussion, conclusions. The introduction should describe the theoretical background, the aim of the study and the hypothesis to be tested. The materials and methods section should describe in a logical sequence how the study was designed and carried out, how the data were analyzed (what hypothesis was tested, what type of study was carried out, how randomization was done, how the subjects were recruited and chosen, provide accurate details of the main features of treatment, of the materials used, of drug dosages, of unusual equipments, of the statistical method ...). In the results section the answers to the questions posed in the introduction should be given. The results should be reported fully, clearly and concisely supported, if necessary, by figures, graphs and tables. The discussion section should sum up the main results, critically analyze the methods used, compare the results obtained with other published data and discuss the implications of the results. The conclusions should briefly sum up the significance of the study and its future implications. For randomised controlled trials it is suggested to the authors to conform the structure of their paper to the checklist requirements of the following guidelines reported by the CONSORT statement: http://www.consort-statement.org.

Review articles. These articles are commissioned by the Editor in Chief or the Managing Editor. They should discuss a topic of current interest, outline current knowledge of the subject, analyze different opinions regarding the problem discussed, be up-to-date on the latest data in the literature. Systematic reviews and meta-analyses must be subdivided into the following sections: introduction, evidence acquisition, evidence synthesis, conclusions. For systematic reviews and meta-analyses it is suggested to the authors to conform the structure of their paper to the checklist requirements of the following guidelines reported by the PRISMA statement: http://www.prisma-statement.org. The text should be 6000-12000 words (17 to 34 typed, double-spaced pages) not including references, tables, figures. No more than 100 references will be accepted.

Special articles. These are articles on the history of medicine, health care delivery, ethics, economic policy and law concerning endocrinology. The text should be 3000-7000 words (8 to 20 typed, double-spaced pages) not including references, tables, figures. No more than 50 references will be accepted.

Letters to the Editor. These may refer to articles already published in the journal or to particularly interesting observations or scientific data that the authors wish to present to readers in a concise form. The text must not be subdivided and should be 500-1000 words (1 to 3 typed, double-spaced pages) not including references, tables, figures. No more than 5 references will be accepted.

Guidelines. These are documents drawn up by special committees or authoritative sources. The number of figures and tables should be appropriate for the type and length of the paper.

PREPARATION OF MANUSCRIPTS

Text file

Manuscripts must be drafted according to the template for each type of paper (editorial, original article, review, special article, letter to the Editor, guidelines).

The formats accepted are Word (.DOC and .DOCX) and RTF. The text file must contain title, authors' details, abstract, key words, text, references, notes, tables and titles of tables and figures. Figures should be submitted as separate files. The file should not contain active hyperlinks.

Title and authors' details

Short title, with no abbreviations. First name in full, middle name's initial, surname of the authors. Collective name, if any, as last author. Corresponding author marked with an asterisk. Affiliation (section, department and institution) of each author. Name, address, e-mail of the corresponding author.

Abstract and key words

Articles should include an abstract of between 200 and 250 words. For original articles, the abstract should be structured as follows: background (what is already known about the subject and what the study intends to examine), methods (experimental design, patients and interventions), results (what was found), conclusions (meaning of the study). For systematic reviews and meta-analyses, the abstract should be structured as follows: introduction, evidence acquisition, evidence synthesis, conclusions. Key words should refer to the terms from Medical Subject Headings (MeSH) of MEDLINE/PubMed. No abstracts are required for editorials or letters to the Editor.

Text

Identify methodologies, equipment (give name and address of manufacturer in brackets) and procedures in sufficient detail to allow other researchers to reproduce results. Specify well-known methods including statistical procedures; mention and provide a brief description of published methods which are not yet well known; describe new or modified methods at length; justify their use and evaluate their limits. For each drug generic name, dosage and administration routes should be given. Brand names for drugs should be given in brackets. Units of measurement, symbols and abbreviations must conform to international standards. Measurements of length, height, weight and volume should be given in metric units (meter, kilogram, liter) or their decimal multiples. Temperatures must be expressed in degrees Celsius. Blood pressure must be expressed in millimeters of mercury. All clinical chemistry measurements should be expressed in metric units using the International System of Units (SI). The use of unusual symbols or abbreviations is strongly discouraged. The first time an abbreviation appears in the text, it should be preceded by the words for which it stands.

References

It is expected that all cited references will have been read by the authors. The references must contain only the authors cited in the text, be numbered in Arabic numerals and consecutively as they are cited. Bibliographical entries in the text should be quoted using superscripted Arabic numerals. References must be set out in the standard format approved by the International Committee of Medical Journal Editors (http://www.icmje.org).

Journals

Each entry must specify the author's surname and initials (list all authors when there are six or fewer; when there are seven or more, list only the first six and then "*et al*."), the article's original title, the name of the Journal (according to the abbreviations used by MEDLINE/PubMed), the year of publication, the volume number and the number of the first and last pages. When citing references, please follow the rules for international standard punctuation carefully.

Examples:

- Standard article.

Sutherland DE, Simmons RL, Howard RJ. Intracapsular technique of transplant nephrectomy. Surg Gynecol Obstet 1978; 146:951-2.

- Organization as author

International Committee of Medical Journal Editors. Uniform requirements for manuscripts submitted to biomedical journals. Ann Int Med 1988;108:258-65.

- Issue with supplement

Payne DK, Sullivan MD, Massie MJ. Women's psychological reactions to breast cancer. Semin Oncol 1996;23(1 Suppl 2):89-97.

Books and monographs

For occasional publications, the names of authors, title, edition, place, publisher and year of publication must be given. Examples:

- Books by one or more authors

Rossi G. Manual of Otorhinolaryngology. Turin: Edizioni Minerva Medica; 1987.

- Chapter from book

De Meester TR. Gastroesophageal reflux disease. In: Moody FG, Carey LC, Scott Jones R, Ketly KA, Nahrwold DL, Skinner DB, editors. Surgical treatment of digestive diseases. Chicago: Year Book Medical Publishers; 1986. p. 132-58.

- Congress proceedings

Kimura J, Shibasaki H, editors. Recent advances in clinical neurophysiology. Proceedings of the 10th International Congress of EMG and Clinical Neurophysiology; 1995 Oct 15-19; Kyoto, Japan. Amsterdam: Elsevier; 1996.

Electronic material

- Standard journal article on the Internet

Kaul S, Diamond GA. Good enough: a primer on the analysis and interpretation of noninferiority trials. Ann Intern Med [Internet]. 2006 Jul 4 [cited 2007 Jan 4];145(1):62-9. Available from: http://www.annals.org/cgi/reprint/145/1/62.pdf

- Standard citation to a book on CD-ROM or DVD

Kacmarek RM. Advanced respiratory care [CD-ROM]. Version 3.0. Philadelphia: Lippincott Williams & Wilkins; ©2000. 1 CD-ROM: sound, color, 4 3/4 in.

- Standard citation to a homepage

AMA: helping doctors help patients [Internet]. Chicago: American Medical Association; ©1995-2007 [cited 2007 Feb 22]. Available from: http://www.ama-assn.org/.

Footnotes and endnotes of Word must not be used in the preparation of references. References first cited in a table or figure legend should be numbered so that they will be in sequence with references cited in the text taking into consideration the point where the table or figure is first mentioned. Therefore, those references should not be listed at the end of the reference section but consecutively as they are cited.

Notes

Conflicts of interest; mention of any funding, research contracts; authors' contribution statement; list of the members of the collective name (author's name in full, middle name's initial in capital letters and surname, with relevant affiliation); contributors' names; dates of any congress where the paper has already been presented; acknowledgements.

Tables

Tables should be submitted in the text file. Each table should be created with the Table menu of Microsoft Word table editor, by selecting the number of rows and columns needed. Tabulations are not allowed. Each table must be numbered in Roman numerals and accompanied by the relevant title. Each table must include heading, body and notes, if needed, at the foot of the table. Tables should be referenced in the text sequentially.

Figures

Each figure should be submitted as a separate file. Formats accepted: JPEG set at 300 dpi resolution preferred; other formats accepted are TIFF and PDF (high quality). Figures should be numbered in Arabic numerals and accompanied by the relevant title. Titles of figures should be repeated also in the text file. Figure should be referenced in the text sequentially. Reproductions should be limited to the part that is essential to the paper. Histological photographs should always be accompanied by the magnification ratio and the staining method.

If figures are in color, it should always be specified whether color or black and white reproduction is required.

Supplementary Digital Material

Authors may submit supplementary material to support and enhance their article's text to be published in the online edition only. Supplementary material should be submitted online during the submission process and may include the following types of content: text files, tables, figures, audios and videos. Authors are requested to submit as supplementary material tables that are too long to fit on a single printed page of the journal and any appendices.

One or more files of supplementary material may be attached to the article. Such files must be submitted separately and cited in consecutive order in the text. There are no restrictions on the content of a file (it may include a text and a table, a single table, a figure and a table, two figures, a video, etc..).

Each in-text citation of supplementary material should be clearly labeled as “Supplementary Digital Material” followed by the relevant number and the description of the material submitted (Supplementary Digital Material 1: Supplementary Text File, Supplementary Figure 1, Supplementary Table I and Supplementary Table II online content only). Audio and video citations should also include the length and size of the file (Supplementary Digital Material 2: Supplementary Video 1, online content only, 5 minutes, 10MB). Text files, figures and tables of supplementary materials should be accompanied by the relevant title.

Formats accepted for text files and tables: Word (.DOC and .DOCX) and RTF; formats accepted for figures: JPEG set at 300 dpi resolution preferred; other formats accepted are TIFF and PDF (high quality); formats accepted for audio files: MP3, WAV; formats accepted for video files: MP4, AVI, WMV. To ensure a quality experience, it is suggested that authors submit supplementary audios and videos no larger than 10 MB each.

If accepted, supplementary material will be published as submitted and will not be checked or corrected.