The effect of obesity and dietary habits on oxidative stress in Hashimoto’s thyroiditis

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Abstract

Objective: Increased oxidative stress has been described in patients with Hashimoto’s thyroiditis (HT). The aim of the present study was to investigate whether high oxidative stress is further influenced by obesity and dietary habits in euthyroid women with HT.

Methods: Two hundred eighteen consecutive euthyroid women with HT were studied and separated in two groups; 102 with thyroxine replacement and 114 without. For the evaluation of oxidative stress, total lipid peroxide levels in serum (TOS) were measured and recorded as ‘high TOS’ vs ‘medium/low TOS’. The type of food and consumption frequency were recorded. Two binary variables were considered; normal vs low fruit consumption and daily vs sporadic vegetable consumption.

Results: ‘High TOS’ was more frequent in women under thyroxine replacement (31.4% vs 14.7%, OR = 2.7, 95% CI: 1.4–5.2). The prevalence of ‘high TOS’ was higher among overweight/obese women compared to women with normal BMI (30.4% vs 12.5%, OR = 3.1, 95% CI: 1.5–6.4). Low fruit consumption was associated with increased ‘high TOS’ prevalence (30.6% vs 12.9%, OR = 3.0, 95% CI: 1.4–6.2). Sporadic vegetable consumption was associated with increased ‘high TOS’ prevalence compared to daily consumption (29.9% vs 13.5%, OR = 2.7, 95% CI: 1.3–5.7). The examined risk factors were independent and additive in their effect on TOS. At least three risk factors had to be concomitantly present for the likelihood of ‘high TOS’ to be significantly elevated.

Conclusions: Oxidative stress is increased in women with HT under thyroxine replacement. Nevertheless, normal BMI, daily fruit and vegetable consumption, all contribute in maintaining oxidative stress at low levels.

Introduction

Reduction-oxidation (redox) processes are a fundamental part of human metabolism (1, 2). Oxidants are a large group of reactive species including both free-radicals and nonradicals (i.e. reactive oxygen species, reactive nitrogen species, reactive chlorine/bromine species, reactive sulfur species) (1, 2). At low/moderate concentrations oxidants are necessary for normal cell functions such as signaling mechanisms, defense against infectious agents and the process of mitosis (1, 2, 3, 4). On the other hand, at high concentrations, oxidants react with biomolecules and alter their functions (3, 4). These reactions can lead to DNA modification, lipid peroxidation, disturbance of the lipid membrane arrangement, fragmentation of the peptide chain and alteration of the electrical charge of proteins.
The harmful effect of oxidants is counterbalanced by a variety of antioxidants, both enzymatic (i.e. catalases, superoxide dismutases, glutathione peroxidases, thioredoxin system) and nonenzymatic (i.e. β-carotene, glutathione vitamin E, vitamin C, vitamin A) (1, 2, 3, 4). disturbed the balance between oxidants and antioxidants in favor of the oxidants results in oxidative stress (1, 2, 3, 4).

Environmental factors and lifestyle can affect the balance between oxidants and antioxidants and oxidative stress accordingly (4, 5, 6, 7, 8, 9, 10, 11). Exposure to exogenous sources of oxidants such as cigarette smoke, ozone, ionizing radiation and heavy metal ions promotes oxidative stress production (4, 5). Diet and physical activity both have an impact on oxidative stress levels (5, 6, 7, 8, 9, 10, 11). Frequent consumption of food rich in antioxidants such as fruit and vegetables reinforce antioxidant defense mechanisms (6, 7, 8), whereas alcohol abuse leads to overproduction of oxidants (5). Concerning physical activity, regular moderate-to-high-intensity exercise contributes to antioxidant defense system (9). Nevertheless, both exhausting exercise and sedentary life lead to oxidative stress (10, 11).

The balance between oxidants and antioxidants is critical for the proper function of the thyroid gland (12, 13, 14, 15). Reactive oxygen species are present in thyroid tissue and are necessary for the physiological process of thyroid hormone synthesis (12, 13, 14, 15). To control the damage from reactive oxygen species, thyroid cells are equipped with antioxidant enzymes (superoxide dismutases, catalase and glutathione peroxidases) (12, 13, 14, 15). The role of oxidative stress in thyroid diseases has been the object of many studies (16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26). High oxidative stress is involved in the pathogenesis of thyroid cancer (16, 17). Moreover, increased oxidative stress levels have been described in patients with Grave’s disease (18, 19). In the same direction, studies among patients with HT have shown increased oxidants and decreased antioxidants’ levels in these patients (20, 21, 22, 23, 24, 25, 26).

Hashimoto’s thyroiditis (HT) is the most common autoimmune thyroid disease and it affects people of any age with an increased incidence in middle-aged women (27, 28). Both genetic and environmental factors are implicated in the pathogenesis and the development of this disease (29, 30, 31). Concerning oxidative stress in patients with HT, it is interesting to note that, despite the fact that the oxidative stress indices used varied, all studies reported increased oxidative stress levels (20, 21, 22, 23, 24, 25, 26). Moreover, high oxidative stress was present irrespective of the thyroid functional status – euthyroidism (20, 21, 22), subclinical hypothyroidism (23, 24, 26) or overt hypothyroidism (23, 24, 25, 26).

Studies evaluating the effect of thyroxine replacement therapy in newly diagnosed hypothyroid HT patients showed an improvement in the oxidative status of these patients when they became euthyroid (32, 33). Recent studies showed that high oxidative stress is associated with the severity and the progression of HT from euthyroidism to overt hypothyroidism (23, 34, 35). The aim of the present study was to investigate whether high oxidative stress is further influenced by obesity and dietary habits in euthyroid women with Hashimoto thyroiditis.

Materials and methods
This cross-sectional study was conducted in Greece; it involved 218 consecutive euthyroid women with mean age 46.0±12.7 years (range 19–69 years). All women were examined as outpatients at the Department of Endocrinology and Metabolism of Athens University School of Medicine from November 2010 to May 2014. The Institutional Ethics Committee of Athens University School of Medicine (Alexandra’s Hospital Scientific Committee, 13/7/2012, protocol number 9468) approved this investigation and all patients were informed and gave consent to participate in the study. All women had Hashimoto’s thyroiditis and had been diagnosed at least 6 months before entering the study. All participants were euthyroid (FT4: 9–20.5 pmol/L, TSH: 0.4–4.0 μIU/mL, T3: 0.7–1.8 ng/mL), had hypoechogenic pattern in the ultrasound and at least one of thyroid autoantibodies was positive (anti-Tg ≥30 U/L, anti-TPO ≥40 U/L). None of them was pregnant (within the past 6 months) or breast-feeding at the time of the study and none of them was consuming vitamin supplements. First- and second-degree relatives of those already enrolled in the study, who had HT, were excluded. Information about height, weight, waist and hip measurements were collected and waist-to-hip ratio and BMI were calculated for all participants. Clinical and demographic data were recorded and a detailed medical and family history was taken for all women. Thyroid volume and the presence of clinical goiter were evaluated through ultrasound and clinical examination. Based on World Health Organization’s classification, women with BMI from 18.5 to 25 kg/m² were defined as normal weight, women with BMI from 25 to 30 kg/m² as overweight and women with BMI >30 kg/m² as obese (36). Depending on thyroxine replacement...
therapy, women were classified into two groups. The first group consisted of 102 women on thyroxine replacement (treatment group), while the remaining 114 women did not receive thyroxine treatment and belonged to the no treatment group.

Thyroid function tests were performed for all patients. Blood samples were obtained by venipuncture between 08:00 and 09:00 h fasting. The specimens were centrifuged, and the serum was kept frozen at −20°C until analyses. Serum TSH, free thyroxine (FT4), triiodothyronine (T3) and anti-thyroid antibodies (anti-TPO, anti-TG) were estimated by using chemiluminescence immunometric assays with the DPC Immulite 2000 (Siemens). Reference range was TSH: 0.4–4.0 µIU/mL, FT4: 9–20.5 pmol/L, T3: 0.7–1.8 ng/mL, anti-TPO <30 U/L, anti-TG <40 U/L.

For evaluation of the oxidative stress, total lipid peroxide levels in serum (TOS) were measured by using the PerOx-(TOS/TOC) kit (photometric test system for the determination of the total oxidative status/capacity – TOS/TOC – by Immundiagnostics) as previously reported (37, 38). The PerOx-(TOS/TOC) kit defines TOS values <180 µmol/L as low oxidative stress, TOS values 180–310 µmol/L as moderate oxidative stress and TOS values >310 µmol/L as high oxidative stress. In a previous study using the PerOx-(TOS/TOC) kit in the same population, it was shown that TOS had significant ability to discriminate between the two HT groups (35). Further analysis showed that the cutoff value that offered the best tradeoff between sensitivity and specificity was 590 µmol/L (35). Based on these results, TOS values for the present study were recoded as ‘high TOS’ (≥590 µmol/L) vs ‘medium/low TOS’ (<590 µmol/L).

A semi-quantitative food frequency questionnaire was developed for this study based on the study of Tyrovolas et al. (39) with adjustments. The questionnaire was designed to measure the frequency of consumption of various food groups and beverage categories that are usually consumed in Greece (i.e. red meat, poultry, fish and seafood, milk, yogurt, eggs, cheese, fruits, dried fruit, nuts, vegetables, greens, salads, legumes, pasta, potatoes, cereals, olive oil, sweets, junk food, soft drinks, spirits, tea and coffee) during the previous month. The answers were given in six predefined options (never, rare, 1–3 times per month, 1–2 times per week, 3–5 times per week, daily and ‘how many times per day’). As portion size, we used the reference portion size described in the study of Tyrovolas et al. (39); this was explained in detail to all participants before the filling of the questionnaire. Using open-ended questions, we collected more information about the type of fruit, vegetables, bread, cheese, coffee and tea that were usually consumed during the past month. Based on the antioxidant capacity of food (40, 41, 42), all answers were evaluated for possible associations with oxidative stress. Two binary variables were considered – fruit consumption and vegetable consumption. The cut-off for fruit consumption was at least 14 portions weekly which equals to at least two portions daily and the groups were named accordingly, normal (more than 14 portions weekly/more than two portions daily) vs low fruit consumption (less than 14 portions weekly/less than two portions daily). The cut-off for vegetable consumption was at least seven portions weekly which equals to at least one portion daily and the groups were named accordingly, daily (more than seven portions weekly/more than one portion daily) vs sporadic vegetable consumption (less than seven portions weekly/less than one portion daily).

**Statistical analysis**

The primary outcome was the presence of ‘high TOS’ (≥590 µmol/L). There were four binary independent predictors (risk factors): thyroxine treatment (Yes vs No), BMI (overweight/obese vs normal), fruit consumption (less than two portions daily vs at least two portions daily) and vegetable consumption (less than one portion daily vs at least one portion daily). Concerning the fruit and vegetable consumption, the original six-level ordinal variables regarding the quantity and frequency of fruit and vegetable consumption, that were recorded in the questionnaire, were checked for the existence of a linear-by-linear association with the presence of high TOS using the chi-square for trends statistic. Subsequently, they were transformed into binary, dichotomous variables based on ROC analysis of the variables on TOS levels that produced the cutoffs providing the best tradeoff between sensitivity and specificity. The association of TOS levels with thyroxine treatment, BMI and dietary habits was checked with 2×2 contingency tables reporting the OR with its associated 95% confidence intervals, followed by stepwise logistic regression models. The level of significance was set at 0.05.

**Results**

Table 1 shows median values (plus interquartile ranges) for demographic, clinical and biochemical characteristics of the study population. All women were euthyroid at the time of the study. TOS levels were significantly higher in the treatment group in comparison to the no treatment group. Further analysis showed that the cutoff value that offered the best tradeoff between sensitivity and specificity was 590 µmol/L (35). Based on these results, TOS values for the present study were recoded as ‘high TOS’ (≥590 µmol/L) vs ‘medium/low TOS’ (<590 µmol/L).

A semi-quantitative food frequency questionnaire was developed for this study based on the study of Tyrovolas et al. (39) with adjustments. The questionnaire was designed to measure the frequency of consumption of various food groups and beverage categories that are usually consumed in Greece (i.e. red meat, poultry, fish and seafood, milk, yogurt, eggs, cheese, fruits, dried fruit, nuts, vegetables, greens, salads, legumes, pasta, potatoes, cereals, olive oil, sweets, junk food, soft drinks, spirits, tea and coffee) during the previous month. The answers were given in six predefined options (never, rare, 1–3 times per month, 1–2 times per week, 3–5 times per week, daily and ‘how many times per day’). As portion size, we used the reference portion size described in the study of Tyrovolas et al. (39); this was explained in detail to all participants before the filling of the questionnaire. Using open-ended questions, we collected more information about the type of fruit, vegetables, bread, cheese, coffee and tea that were usually consumed during the past month. Based on the antioxidant capacity of food (40, 41, 42), all answers were evaluated for possible associations with oxidative stress. Two binary variables were considered – fruit consumption and vegetable consumption. The cut-off for fruit consumption was at least 14 portions weekly which equals to at least two portions daily and the groups were named accordingly, normal (more than 14 portions weekly/more than two portions daily) vs low fruit consumption (less than 14 portions weekly/less than two portions daily). The cut-off for vegetable consumption was at least seven portions weekly which equals to at least one portion daily and the groups were named accordingly, daily (more than seven portions weekly/more than one portion daily) vs sporadic vegetable consumption (less than seven portions weekly/less than one portion daily).
Fig. 1 shows the percentage of women with ‘high TOS’ (≥590 μmol/L) depending on the weekly consumption of fruit (A) and vegetables (B). The significance of the chi-square for trends proves that there is a linear-by-linear association between fruit and vegetable consumption and TOS levels. The reduction of the portions of fruit and vegetables consumed is associated with higher probability of the appearance of ‘high TOS’. Visual inspection of Fig. 1 reveals the best cutoffs for fruit (more than 14 portions weekly equals to more than two portions daily named as ‘normal’ vs less than 14 portions weekly equals to less than two portions daily named as ‘low’) and vegetable consumption (more than seven portions weekly equals to more than one portions daily named as ‘daily’ vs less than seven portions weekly equals to less than one portion daily named as ‘sporadic’). These cutoffs were confirmed by ROC analysis. The cutoff of normal vs low fruit consumption for the prediction of ‘high TOS’ has a sensitivity of 73.9% and a specificity of 51.3%. The cutoff of daily vs sporadic vegetable consumption for the prediction of ‘high TOS’ has a sensitivity of 74.6% and a specificity of 48.4%.

The chi-square for trends did not achieve statistical significance regarding the association with TOS levels and the weekly consumption of meat (P = 0.833), seafood (P = 0.402), dairy products (P = 0.523), alcohol (P = 0.059), coffee (P = 0.495) and tea (P = 0.057).

Fig. 2 shows the percentage of women with ‘high TOS’ (≥590 μmol/L) depending on thyroxine treatment (a), BMI (b), fruit consumption (c) and vegetable consumption (d). ‘High TOS’ was more frequent in the treatment group compared to the no treatment group (31.4% vs 14.7%, OR = 2.7, 95% CI: 1.4–5.2, Fig. 2A). The prevalence of ‘high TOS’ was higher among overweight and obese women compared to women with normal BMI (30.4% vs 12.5%, OR = 3.1, 95% CI: 1.5–6.4, Fig. 2B). Regarding dietary habits, low fruit consumption was associated with increased ‘high TOS’ prevalence compared to normal fruit consumption (30.6% vs 12.9%, OR = 3.0, 95% CI: 1.3–5.7, Fig. 2C). Sporadic vegetable consumption was associated with increased ‘high TOS’ prevalence compared to daily consumption (29.9% vs 13.5%, OR = 2.7, 95% CI: 1.3–5.7, Fig. 2D). Overall, Fig. 2 shows that all four risk factors have roughly comparable effect on TOS. The OR values around three mean that the ratio of ‘high TOS’ to ‘medium/low TOS’ among women with the risk factor is about three.

Table 1 Demographic, clinical and biochemical characteristics of the study population.

<table>
<thead>
<tr>
<th></th>
<th>No treatment group</th>
<th>Treatment group</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>116</td>
<td>102</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45.1 (13.7)</td>
<td>47.0 (11.4)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.0 (5.0)</td>
<td>27.1 (5.9)</td>
</tr>
<tr>
<td>W/H (ratio)</td>
<td>0.78 (0.07)</td>
<td>0.79 (0.08)</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>71 (9)</td>
<td>69 (9)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>114 (13)</td>
<td>114 (15)</td>
</tr>
<tr>
<td>TSH (μIU/mL)</td>
<td>2.4 (1.6)</td>
<td>2.0 (1.6)</td>
</tr>
<tr>
<td>FT4 (pmol/L)</td>
<td>14.0 (2.6)</td>
<td>15.5 (2.4)</td>
</tr>
<tr>
<td>T3 (ng/mL)</td>
<td>1.1 (0.2)</td>
<td>1.0 (0.2)</td>
</tr>
<tr>
<td>Anti-TPO (U/L)</td>
<td>343 (341)</td>
<td>433 (1014)</td>
</tr>
<tr>
<td>Anti-TG (U/L)</td>
<td>110 (250)</td>
<td>392 (780)</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>96.5 (29.3)</td>
<td>96.6 (29.2)</td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td>1.8 (3.2)</td>
<td>1.9 (3.5)</td>
</tr>
<tr>
<td>TOS (μmol/L)</td>
<td>421 (238)</td>
<td>509 (308)</td>
</tr>
</tbody>
</table>

Median value and interquartile range for the two groups. BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; TOS, total oxidative status; W/H, waist to hip ratio.
times than the same ratio among women without the risk factor.

Stepwise logistic regression showed that all four factors (thyroxine treatment, BMI, fruit and vegetable consumption) were significant independent predictors of ‘high TOS’ (Table 2). In each case, the related OR signifies how many times the odds of ‘high TOS’ to ‘medium/ low TOS’ are multiplied in the presence of the risk factor against the baseline of the absence of the risk factor.

Figure 3 exemplifies the additive effect of the presence of the examined risk factors on the development of ‘high TOS’. It takes at least the conjoint effect of any three risk factors to increase significantly the probability of high TOS levels. Nonetheless, even the presence of all four risk factors cannot completely qualify the appearance of high TOS levels.

Discussion

The aim of the present study was to investigate the influence of thyroxine treatment, obesity and dietary habits on the development of high oxidative stress in euthyroid women with HT. The current findings revealed that ‘high TOS’ was more prevalent in women with HT who were on thyroxine replacement therapy compared to those without. ‘High TOS’ was also more frequent in overweight and obese women compared to those with normal BMI. In addition, limited consumption of fruit and vegetables was also associated with increased risk of ‘high TOS’. The examined risk factors were independent and additive in their effect on TOS. It takes the coincident presence of at least three risk factors for the likelihood of ‘high TOS’ to be significantly elevated.

‘High TOS’ was more frequent in euthyroid women with HT who were on thyroxine replacement therapy. Studies in euthyroid patient with HT showed that the oxidative balance in these patients is shifted toward the oxidant side (20, 21, 22). Also, there are few studies that showed an association between high oxidative stress and the progression of HT (23, 34, 35). In the same direction,

Table 2 Results of the stepwise logistic regression of the effect of the four independent predictors (thyroxine treatment, BMI, fruit and vegetable consumption) on ‘high TOS’.

<table>
<thead>
<tr>
<th>Independent predictor</th>
<th>B</th>
<th>Wald</th>
<th>df</th>
<th>P</th>
<th>OR (e^B)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1.3</td>
<td>9.64</td>
<td>1</td>
<td>0.002</td>
<td>3.6</td>
<td>1.6–8.0</td>
</tr>
<tr>
<td>BMI</td>
<td>0.9</td>
<td>5.33</td>
<td>1</td>
<td>0.021</td>
<td>2.5</td>
<td>1.2–5.6</td>
</tr>
<tr>
<td>Fruit consumption</td>
<td>1.2</td>
<td>7.36</td>
<td>1</td>
<td>0.007</td>
<td>3.2</td>
<td>1.4–7.3</td>
</tr>
<tr>
<td>Vegetable consumption</td>
<td>0.8</td>
<td>3.89</td>
<td>1</td>
<td>0.049</td>
<td>2.3</td>
<td>1.1–5.3</td>
</tr>
</tbody>
</table>

BMI, body mass index; TOS, total oxidative status.
the present finding showed that women with HT who were in need of thyroxine replacement therapy to be euthyroid had higher oxidative stress levels compared to those who were euthyroid without therapy. The need of thyroxine replacement therapy to achieve euthyroidism shows that HT was more severe and thyroid function was more impaired in these women. Moreover, none of the women in the present study was newly diagnosed and all of them were euthyroid at the time of the study; this fact may explain the diversity in the findings from those in studies concerning the effect of thyroxine replacement therapy on oxidative status of HT patients (32, 33). We should note that studying only one parameter of oxidative stress is an important limitation of this study.

The prevalence of ‘high TOS’ was found to be higher among overweight and obese women compared to women with normal BMI. This finding is in accordance with studies about the oxidative status in obese population. These studies have shown that both obesity and obesity-derived complications promote the generation of oxidative stress (43, 44, 45, 46, 47). Some of the conditions that contribute to the increased oxidative stress in obesity are chronic low-grade inflammation, insulin resistance, hyperglycemia, elevated lipid levels, vitamin and mineral deficiencies, hyperleptinemia increased muscle activity, endothelial dysfunction and impaired mitochondrial function (44, 45, 46, 47). In addition, health problems caused by obesity such as type 2 diabetes, cardiovascular disease, systemic arterial hypertension, liver and renal dysfunction, carcinogenesis and respiratory disorders are associated with elevated oxidative stress levels (45, 46, 47).

Another finding of the present study was that limited daily consumption of fruit and vegetables was associated with increased risk of high oxidative stress levels. The benefits of regular consumption of fruits and vegetables on human health are well established (48). Fruit and vegetables have high antioxidant capacity (40, 41, 42); their addition to the daily diet reinforces the antioxidant defense system (49). Furthermore, the finding that all four factors examined in this study (thyroxine treatment, BMI, fruit and vegetable consumption) were significant independent predictors of ‘high TOS’, implies that following a healthy way of life (normal BMI, daily consumption of fruit and vegetables) contributes to maintaining low oxidative stress levels in this population as well.

There are, however, several important limitations in our study. The sample size of the study was relatively small. Moreover, we studied only one of the many parameters of oxidative stress. Unfortunately, we had no access to measure other oxidative stress indices. One further limitation of the present study was that the food frequency questionnaire that was developed for this study was not cross-validated, which prevents generalization of the present findings. In addition, the use of non-validated FFQ didn’t allow estimation of total energy intake and therefore energy-adjusted consumption of foods and beverages has not been used. Finally, self-reported dietary intake has many limitations like recall and memory bias. Also, the FFQ is subjected to a number of limitations compared to other self-reported tools like 24 h recalls and food diaries. Some of these limitations include, but are not limited to, absence of details regarding dietary intake and reported inaccuracies originated from the use of a predefined list of food items, as well as memory bias and respondent bias according to social desirability (50). Further studies are warranted to validate the present findings.

In conclusion, oxidative stress was found increased in euthyroid women with HT under thyroxine replacement therapy. Nevertheless, a normal BMI and daily consumption of fruit and vegetables, all contribute in maintaining low oxidative stress levels. The findings of the present study suggest that patients with HT should be encouraged to modify their diet and achieve a healthy BMI.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.
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Received in final form 13 July 2018
Accepted 6 August 2018
Accepted Preprint published online 8 August 2018

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