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Hypothyroid women have persistently higher oxidative stress compared to healthy controls

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Abstract

Objective: Some studies suggest that hypothyroidism is associated with increased oxidative stress. Urinary excretion of 8-oxo-7,8-dihydroguanosine (8-oxoGuo) and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) represents whole-body RNA and DNA oxidation, respectively. These biomarkers have only been explored sparsely in patients with thyroid disorders.

Methods: In 45 Danish women with newly diagnosed hypothyroidism, we compared 8-oxoGuo and 8-oxodG before or shortly after initiating levothyroxine with the excretion rates at euthyroidism. We also compared the excretion of 8-oxoGuo and 8-oxodG in the patients after restored euthyroidism with 18 healthy control subjects.

Results: Compared with baseline, none of the biomarkers changed significantly in the patients after becoming euthyroid. The geometric mean of 8-oxoGuo was 1.63 (95% CI: 1.49–1.78) nmol/mmol creatinine at baseline and 1.67 nmol/mmol at euthyroidism (95% CI: 1.53–1.83) ($P = 0.39$), while that of 8-oxodG was 1.28 nmol/mmol creatinine at baseline (95% CI: 1.14–1.44) and 1.32 nmol/mmol at euthyroidism (95% CI: 1.18–1.48), respectively ($P = 0.47$). The relative mean differences were 0.97 (95% CI: 0.91–1.04) for 8-oxoGuo and 0.97 (95% CI: 0.88–1.06) for 8-oxodG. At baseline, multiple linear regression revealed a positive association between free thyroxine and both biomarkers (8-oxoGuo, $P < 0.001$; 8-oxodG, $P = 0.04$). Furthermore, 8-oxoGuo was positively associated with age ($P = 0.04$) and negatively associated with thyrotropin ($P = 0.02$). In the control group, the geometric mean of 8-oxoGuo was 1.23 nmol/mmol creatinine (95% CI: 1.07–1.42), while that of 8-oxodG was 1.04 nmol/mmol creatinine (95% CI: 0.88–1.23). Thus, compared with control subjects, euthyroid patients showed a significantly higher level of both 8-oxoGuo ($P < 0.001$) and 8-oxodG ($P = 0.03$).

Keywords

- ▶ oxidative stress
- ▶ 8-oxo-7,8-dihydroguanosine
- ▶ 8-oxoGuo
- ▶ 8-oxo-7,8-dihydro-2'-deoxyguanosine
- ▶ 8-oxodG
- ▶ hypothyroidism

Conclusion: In hypothyroid women, no significant effect of levothyroxine treatment on the oxidative stress biomarkers 8-oxoGuo and 8-oxodG could be demonstrated. However, the excretion of these biomarkers was significantly higher than in healthy controls.

Introduction

Hypothyroidism, most often caused by autoimmune thyroiditis, is a common disease with a prevalence of approximately 3% in the European population (1). Epidemiological studies have shown that hypothyroidism is associated with an increased risk of comorbidity (2, 3, 4), which impacts ability to work (5) and quality of life (6) and leads to a shorter life span, at least in younger individuals (7, 8). The pathogenesis behind this impact on health is unresolved.

An imbalance between oxidative and antioxidative processes, in favor of the oxidative processes, leads to a disruption of redox signaling and/or molecular damage, and is defined as oxidative stress (9). Prooxidants include reactive oxygen species (ROS) that can be generated from oxidative phosphorylation in the mitochondria. Cells also harbor antioxidative countermeasures. Oxidation of the genome and other macromolecules is either part of the cells' regulatory functions and adaptation, or it can be pathogenic. Some chemical modifications of DNA and RNA might trigger mutagenesis, which can result in accelerated aging or malignant transformation (10). If cellular structures other than DNA and RNA are degenerated, it might result in cell dysfunction and apoptosis (11). Regarding thyroid function, the impact of oxidative stress is of particular interest, since thyroid hormones are crucial for oxidative metabolism and energy expenditure (12).

There are numerous ways of measuring oxidative stress. The most reliable methods do not only focus on the amounts of prooxidants or antioxidants but measure the integrated effect of the oxidative stress burden. The stability of the genome is crucial for the normal function of the cell. The guanine nucleoside derivatives 8-oxo-7,8-dihydroguanosine (8-oxoGuo) and 8-oxo-dihydro-2'-deoxyguanosine (8-oxodG) result from oxidative modifications to RNA and DNA, respectively (13). These biomarkers reflect the aggregated oxidative stress load in the organism (14). 8-oxoGuo and 8-oxodG are excreted into the urine and can therefore be measured noninvasively (13). Increased excretion rates of 8-oxoGuo and 8-oxodG have been identified in cardiovascular diseases and various conditions like hypertension and cancer (11). The excretion of

these biomarkers also increases with smoking and aging (11) and is predictive of mortality in patients with type 2 diabetes (15, 16) and cancer development (17, 18).

Oxidized guanine nucleosides have only been explored sparsely in thyroid disorders. We recently demonstrated that 8-oxoGuo and 8-oxodG decrease by 10–25% after treatment of hyperthyroidism (19). Whether the same applies to the hypothyroid state is unknown. Thus, in the present study, we aimed to investigate the effect of levothyroxine (LT4) treatment on the oxidative stress load in hypothyroid women. To this end, we determined the urinary excretions of 8-oxoGuo and 8-oxodG and compared the excretion rates of these biomarkers with those in healthy individuals.

Methods

Study population and design

Hypothyroid patients from two different cohorts were included. Cohort A was part of a longitudinal prospective cohort study of the impact of hypothyroidism on bone microarchitecture, as described elsewhere (20). In brief, newly diagnosed hypothyroid women were recruited from the outpatient clinic at the Department of Endocrinology, Odense University Hospital, Denmark, between May 2011 and June 2016. The patients had to be 20–85 years of age and diagnosed with autoimmune thyroiditis to meet the inclusion criteria. Autoimmune thyroiditis was defined by positive antibodies against thyroid peroxidase (TPOAb) or thyroglobulin in serum. Hypothyroidism was defined as overt (plasma thyrotropin (TSH) above the reference range of 0.3–4.0 mIU/L and total plasma thyroxine (TT4) below the reference range of 60–130 nmol/L) or mild (plasma TSH above the reference range, and plasma TT4 within the reference range). Exclusion criteria were pregnancy or planned pregnancy during the study period, treatment with glucocorticoids, or reduced kidney function (serum creatinine: >100 µmol/L). Patients with known osteoporosis and treatment with antiosteoporotic agents were excluded.

The study was approved by the Regional Research Ethics Committee of Southern Denmark (S-2011-0018), and is registered on www.clinicaltrials.gov (NCT02005250).

Cohort B was part of a study aiming to evaluate changes in appetite and food intake in newly diagnosed hypothyroid patients during the first 6 months after starting LT4, as described elsewhere (21). Participants were included from the Copenhagen General Population Study (22) or recruited from the outpatient clinic at the Department of Endocrinology, Herlev-Gentofte Hospital, Denmark, between August 2015 and February 2018. The participants had to be 20–75 years of age and had a TSH >10.0 mIU/L at diagnosis as well as TPOAb positivity. Exclusion criteria were severe comorbidity, pregnancy, previous thyroidectomy, amiodarone treatment, and inability to understand and speak Danish. The study was approved by the Regional Research Ethics Committee of the Capital Region of Denmark (H-15001954) and is registered on www.clinicaltrials.gov (NCT02993562).

In both cohorts, the urinary excretion of the oxidized guanine nucleosides 8-oxoGuo and 8-oxodG was monitored. Thus, all patients were examined twice, shortly after diagnosis and when euthyroidism had been restored by LT4 treatment for at least 6 months. The dose of LT4 was adjusted according to routine clinical practice and aimed at a plasma TSH level within the reference range.

Healthy female volunteers were recruited from a Danish website (<https://www.forsoeegsperson.dk>) used to advertise medical research. The control group was matched by age and body mass index (BMI) with the patients enrolled in cohort B. Informed written consent was obtained from all participants.

Oxidative stress markers

Spot urine samples from both study visits were stored at -80°C until analysis using a validated method of ultraperformance liquid chromatography–tandem mass spectrometry (23). The concentrations of the biomarkers 8-oxoGuo and 8-oxodG were normalized against the urinary creatinine concentration. Chromatographic separation was performed using PerkinElmer Series 200 HPLC with two pumps. The HPLC columns were a Phenomenex Prodigy ODS column (100 × 2 mm, 3 μm) and a C18 ODS guard column (4 × 2 mm), both from Phenomenex (Torrance, CA, USA). The mass spectrometry detection was performed on an API 3000 triple quadrupole mass spectrometer (Sciex, Toronto,

Ontario, Canada) equipped with an electrospray ionization ion source (TurboSpray) operated in the positive mode (24). Urinary creatinine was measured using an in-house Jaffe's method (25).

Blood samples

In cohort A, fasting blood samples from all study visits were drawn and stored at -20°C until analysis. After study completion, plasma was analyzed for TSH and free T4 (FT4) by a two-site chemiluminescent immunometric assay on Cobas 8000 (Roche Diagnostics). The coefficients of variation (CVs) for TSH were 8.3% and 4.2% at levels of 0.084 and 11.3 mIU/L, respectively; for FT4, the CVs were 7.1% and 4.4% at levels of 17 and 33 pmol/L, respectively (20).

In cohort B, blood samples were analyzed during each visit. TSH and FT4 were analyzed by a chemiluminescent immunoassay on ADVIA Centaur XP or Centaur CP (Siemens). The CVs for TSH were 8.17% and 6.95% at levels of 0.287 and 6.94 mIU/L, respectively; for FT4, the CVs were 8.0% and 3.1% at levels of 11.7 and 25.5 pmol/L, respectively.

TPOAb were analyzed using different commercial methods employed routinely at the local hospital laboratories. In cohort A, TPOAb were measured using AutoDELFA (PerkinElmer). In cohort B, TPOAb were determined by a chemiluminescent immunoassay on ADVIA Centaur XP (Siemens), and the CV was 5.7%. The cutoff value defining a positive value was 60 kIU/L.

Statistical analysis

Numerical variables are presented as mean \pm s.d. or median with full range where appropriate. Categorical variables are shown as numbers and ratios. Differences in excretion rates of the oxidative stress markers between baseline and follow-up were evaluated using a paired *t*-test. To compare the oxidative stress markers between hypothyroid women and controls, an independent *t*-test was used. Categorical and numerical baseline characteristics were compared with Fisher's exact test and independent *t*-test, respectively. The normal distribution of data was evaluated with the Shapiro–Wilk test. If data were not normally distributed, logarithmic transformation was applied. Accordingly, the geometric mean with a 95% CI was calculated for the oxidative stress biomarkers, as these were not normally distributed.

The relationship between baseline characteristics and the baseline excretion of the oxidative stress

biomarkers was evaluated with a forward stepwise multiple linear regression analysis; variables included in the full model were age, BMI, TPOAb, and TSH or FT4. Because of the correlation between TSH and FT4, separate models were fitted, including only one of these variables to avoid multicollinearity. As BMI, TSH, and TPOAb were not normally distributed, a logarithmic transformation was applied before being included in the regression analysis.

To account for the fact that smoking increases the generation of the oxidized guanine nucleosides (26), we made a sensitivity analysis of the independent *t*-test (comparing the oxidative stress markers between hypothyroid women and controls), excluding smokers and those with unknown smoking status.

Statistical tests were two-sided, and statistical significance was defined as $P < 0.05$. Statistical analyses were performed using the IBM Statistical Package for the Social Sciences Statistics (SPSS) for Macintosh, version 28.0.1.0 (released 2021; IBM Corp, Armonk, NY, USA).

Results

Characteristics of participants

Thirty-two women newly diagnosed with autoimmune thyroiditis were included in cohort A. Three women were excluded during the follow-up period due to pregnancy ($n=1$), diagnosis of an eating disorder ($n=1$), and donation of a kidney ($n=1$). One participant was lost to follow-up, leaving 28 patients from cohort A for the final analysis. In cohort B, 19 women with newly diagnosed autoimmune thyroiditis were included. Two

women were excluded due to missing oxidative stress markers at baseline ($n=1$) and self-exclusion ($n=1$), leaving 17 patients from cohort B for the final analysis. Thus, 45 patients in total completed the study. In addition, 18 control subjects were recruited and included in the analysis.

Relevant demographic, clinical, and laboratory characteristics of the study participants are presented in Table 1. Twenty-two participants were diagnosed with mild (subclinical) hypothyroidism, and 23 with overt hypothyroidism. The median time between baseline (at diagnosis) and follow-up (at euthyroidism) was 442 days, ranging from 183 to 1354 days. There were no significant differences in age, BMI, and smoking status between the patients and the control subjects (Table 1).

Table 2 shows the thyroid hormone status at baseline and follow-up. The median LT4 dose for achieving euthyroidism was 114 µg/day. One participant, initially hypothyroid, regained euthyroidism spontaneously, and the LT4 treatment could be discontinued.

Urinary excretion of 8-oxoGuo and 8-oxodG

The geometric mean of the excretion of 8-oxoGuo was 1.63 nmol/mmol creatinine at baseline and 1.67 nmol/mmol at euthyroidism ($P=0.39$), while that of 8-oxodG was 1.28 nmol/mmol at baseline and 1.32 nmol/mmol at euthyroidism, respectively ($P=0.47$) (Table 2 and Fig. 1). Thus, no significant change in either 8-oxoGuo or 8-oxodG was seen after restored euthyroidism, i.e. the relative mean differences were 0.97 (95% CI: 0.91–1.04) and 0.97 (95% CI: 0.88–1.06), respectively.

The individual changes in 8-oxoGuo and 8-oxodG are shown in Fig. 2. Twenty-five patients exhibited

Table 1 Characteristics of the hypothyroid women and healthy control subjects. Data are presented as ratios, n (%), mean \pm s.d., or median (full range).

	Patients	Controls	P^a
n	45	18	
Mild:overt hypothyroidism	22:23		
Age (years)	47.4 \pm 11.4	45.2 \pm 13.1	0.51
Body mass index (kg/m ²)	28.4 \pm 6.0	29.4 \pm 5.5	0.58
Smoking, n (%)			
Yes	6 (13.3)	0	0.23
No	37 (82.2)	18 (100)	0.23
Unknown	2 (4.5)	0	0.23
TPOAb at diagnosis (kIU/L)	590 (2–13,000) ^b		
Time between baseline and follow-up (days)	442 (183–1354)		
LT4 dose at follow-up (µg/day)	114 (0–200) ^c		

^aDifference between hypothyroid participants and control subjects; ^bThree patients were negative for TPOAb but positive for anti-thyroglobulin antibodies (>60 mIU/L); ^cOne participant, initially hypothyroid, regained euthyroidism spontaneously, and the LT4 treatment could be discontinued. LT4, levothyroxine; TPOAb, thyroid peroxidase antibodies.

Table 2 Thyroid function parameters and urinary excretion of oxidative stress markers in hypothyroid women and healthy control subjects. Thyroid function parameters are given as mean \pm s.d., and the oxidative stress markers are presented as geometric mean with a 95% CI. The mean difference (with 95% CI) between baseline and follow-up is a relative measure as the oxidative stress markers are logarithmically transformed.

	Patients (<i>n</i> = 45)				Controls (<i>n</i> = 18)	<i>p</i> ^b
	Baseline	Follow-up	Mean difference	<i>p</i> ^a		
TSH (mIU/L)	21.89 \pm 35.04	2.11 \pm 1.84		<0.001	1.74 \pm 0.98	
FT4 (pmol/L)	14.00 \pm 5.01	18.10 \pm 3.25		<0.001	14.47 \pm 1.68	
8-oxoGuo (nmol/mmol Cr)	1.63 (1.49–1.78)	1.67 (1.53–1.83)	0.97 (0.91–1.04)	0.39	1.23 (1.07–1.42)	<0.001
8-oxodG (nmol/mmol Cr)	1.28 (1.14–1.44)	1.32 (1.18–1.48)	0.97 (0.88–1.06)	0.47	1.04 (0.88–1.23)	0.03

^aPaired *t*-test comparing hypothyroid participants at baseline and follow-up; ^bIndependent *t*-test comparing oxidative stress markers in hypothyroid participants at follow-up with control subjects.

8-oxoGuo, 8-oxo-7,8-dihydroguanosine; 8-oxodG, 8-oxo-7,8-dihydro-2'-deoxyguanosine; Cr, creatinine; FT4, free thyroxine; TSH, thyrotropin.

an increase, while 20 patients showed a decrease in 8-oxoGuo. The corresponding numbers for 8-oxodG were 26 and 19, respectively. Patients demonstrating a reduction in the biomarkers did not differ in baseline characteristics from those who showed an increase (data not shown).

The geometric mean of the urinary excretion of 8-oxoGuo and 8-oxodG in the control subjects was 1.23 (95% CI: 1.07–1.42) and 1.04 (95% CI: 0.88–1.23) nmol/mmol creatinine, respectively. Significantly higher excretions of 8-oxoGuo ($P < 0.001$) and 8-oxodG ($P = 0.03$) were found in the patients at euthyroidism compared to the control subjects (Table 2 and Fig. 1). In our study, six patients were smokers, and none were in the control group. We carried out a sensitivity

analysis, excluding smokers and those with unknown smoking status, whereby there were no significant changes in our results (data not shown).

Both the full and the stepwise multiple regression analyses (Table 3) demonstrated a negative correlation between 8-oxoGuo and baseline TSH ($P = 0.015$) and positive correlations between 8-oxoGuo and baseline FT4 ($P < 0.001$) and age ($P = 0.035$) (Fig. 3). There was no correlation between 8-oxoGuo and BMI or TPOAb. The excretion of 8-oxodG did not correlate significantly with TSH at baseline ($P = 0.119$), nor were there any significant associations with age, BMI, or TPOAb. However, a positive correlation between baseline FT4 and 8-oxodG ($P = 0.038$) was demonstrated (Table 3 and Fig. 3).

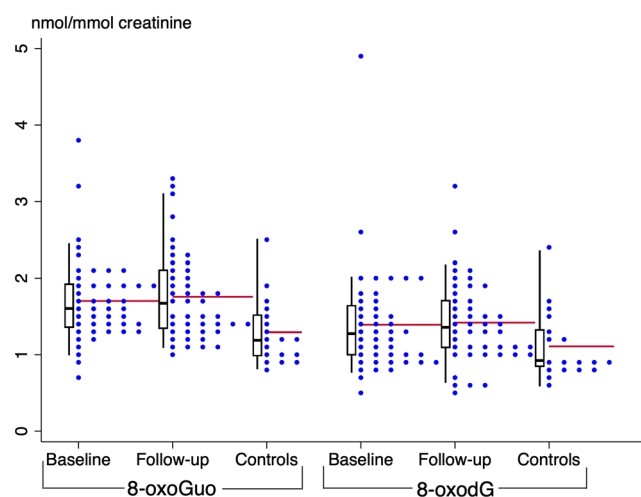


Figure 1 Individual urinary excretion rates of the biomarkers 8-oxo-7,8-dihydroguanosine (8-oxoGuo) and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) in hypothyroid women at diagnosis and at euthyroidism when treated with levothyroxine, and in the healthy control subjects. Box-and-whisker plots illustrate the 5th, 25th, 50th (median), 75th, and 95th percentile. The red lines represent mean values.

Discussion

The present study is the first to evaluate, using a validated method, the effect of LT4 treatment in hypothyroid patients on the urinary excretion of the oxidized guanine nucleosides 8-oxoGuo and 8-oxodG (27, 28) and to compare the results with a healthy control group. No

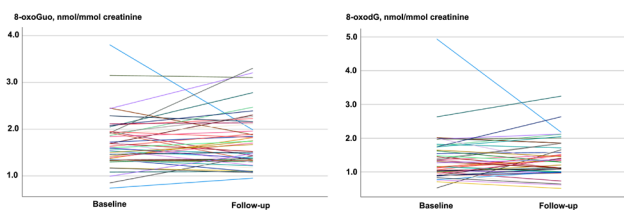


Figure 2 The urinary excretion of 8-oxoGuo and 8-oxodG in 45 hypothyroid women at diagnosis and at euthyroidism when treated with levothyroxine. Each line represents one individual. Urinary samples were taken before or shortly after initiating levothyroxine (baseline) and again after euthyroidism was achieved (follow-up). 8-oxoGuo, 8-oxo-7,8-dihydroguanosine; 8-oxodG, 8-oxo-7,8-dihydro-2'-deoxyguanosine.

Table 3 Multiple regression associations of baseline excretion of 8-oxoGuo and 8-oxodG with demographic characteristics and baseline thyroid function test in 45 hypothyroid women. TPOAb, BMI, and TSH were not normally distributed, and a logarithmic transformation was applied.

Variables included	8-oxoGuo (n = 45)			8-oxodG (n = 45)		
	β coefficient ^a	P	R ²	β coefficient ^a	P	R ²
Variables included			0.230			0.057
Age	0.309	0.049		0.169	0.321	
BMI	0.107	0.459		0.042	0.791	
TPOAb	0.021	0.899		0.202	0.276	
TSH	-0.382	0.019		-0.217	0.215	
Variables included			0.331			0.164
Age	0.277	0.055		0.155	0.328	
BMI	0.157	0.252		0.101	0.505	
TPOAb	0.063	0.680		0.276	0.112	
FT4	0.525	<0.001		0.429	0.012	
By stepwise multiple regression			0.219			
Age	0.297	0.035				
TSH	-0.348	0.015				
By stepwise multiple regression			0.241			0.096
FT4	0.491	<0.001		0.310	0.038	

^aStandardized coefficient.

8-oxoGuo, 8-oxo-7,8-dihydroguanosine; 8-oxodG, 8-oxo-7,8-dihydro-2'-deoxyguanosine; BMI, body mass index; TSH, thyrotropin; FT4, free thyroxine; TPOAb, thyroid peroxidase antibodies.

significant effect of LT4 treatment on the excretion of either 8-oxoGuo or 8-oxodG was demonstrated. However, a significantly higher oxidative stress level in hypothyroid patients than in healthy individuals persisted after restoration of euthyroidism. In accordance with previous studies, we found that

age was positively correlated to the excretion of 8-oxoGuo (19, 29).

Increased oxidative stress has been associated with aging, various diseases, and mortality (11) and might also be a contributor to the increased morbidity (2, 3, 4) and mortality documented in hypothyroid patients (7, 8). However, several interacting factors seem to be involved. The lower energy expenditure resulting from hypothyroidism should, in theory, lead to a reduced generation of ROS but may be counterbalanced by a diminished antioxidant capacity. Complexity is underlined by the observation that untreated hypothyroidism, unless severe, may benefit elderly people in terms of longevity (30), although controversies exist (8). The underlying mechanism is obscure, but less oxidative stress burden in the hypothyroid condition may play an important and protective role. Such a theory is supported by our present study, considering the inverse correlation between the severity of hypothyroidism and the excretion of 8-oxoGuo.

Numerous studies using different methods have investigated oxidative stress in hypothyroidism, with conflicting results. Some methods assessed the oxidative impact on cell membranes, lipids, or proteins, while others measured the antioxidant capacity or amount of prooxidants. The hyperlipidemia resulting from hypothyroidism increases the risk of lipid peroxidation (31), which in turn leads to the formation of malondialdehyde and thiobarbituric acid reactive

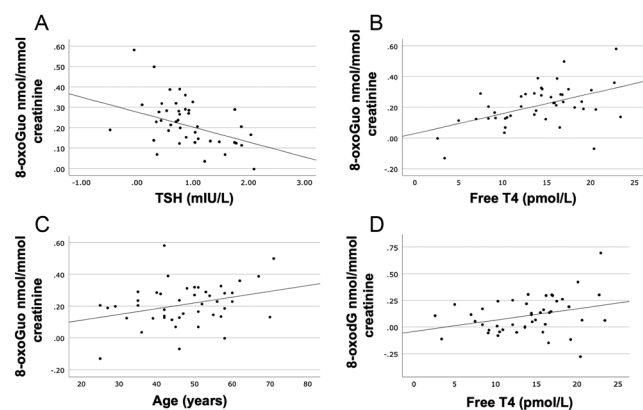


Figure 3

Correlations between the oxidative stress markers 8-oxoGuo and 8-oxodG and the predictor variables age, thyrotropin (TSH), and free thyroxine (T4) in hypothyroid women. A: Correlation between baseline 8-oxoGuo and baseline TSH ($R^2 = 0.130$, $P = 0.015$). B: Correlation between baseline 8-oxoGuo and baseline free T4 ($R^2 = 0.241$, $P < 0.001$). C: Correlation between baseline 8-oxoGuo and age ($R^2 = 0.098$, $P = 0.015$). D: Correlation between baseline 8-oxodG and baseline free T4 ($R^2 = 0.0096$, $P = 0.038$). Logarithmic transformation was applied for 8-oxoGuo, 8-oxodG, and TSH. 8-oxoGuo, 8-oxo-7,8-dihydroguanosine; 8-oxodG, 8-oxo-7,8-dihydro-2'-deoxyguanosine.

substances. Compared to healthy control subjects, elevated concentrations of thiobarbituric acid reactive substances (32, 33) and malondialdehyde (34, 35, 36, 37, 38) have been reported in both overt and mild hypothyroidism. In a study by Baskol *et al.* (39), the malondialdehyde level decreased after LT4 treatment for 6 months. Still, it remained elevated compared to healthy individuals (39), in accordance with the findings of the present study.

Regarding the antioxidant capacity in hypothyroidism, results are ambiguous (40). One study demonstrated a decrease in superoxide dismutase (41), an enzyme that inactivates ROS, in hypothyroid patients compared to healthy controls. At variance, other studies found opposite results (32, 42) or could not demonstrate any difference in superoxide dismutase activity between hypothyroid patients and healthy individuals (35, 39, 42). Other antioxidant enzymes include catalase and glutathione peroxidase, which act by preventing the production of ROS or inactivating these species. Elevated concentrations of catalase (32, 36) and glutathione peroxidase (33, 41, 42) in hypothyroid patients, compared to healthy individuals, have been reported, while other studies found no difference (41, 43), or lower concentrations of catalase (42). Studies of other markers, including vitamin E (32, 35), total antioxidant status (44, 45), paraoxonase 1, and arylesterase, also showed ambiguous results (39, 44, 46, 47).

The conflicting results in previous studies underline that measuring a single component in the oxidative or antioxidative pathways does not reliably assess the oxidative burden due to counteracting mechanisms. In contrast, the urinary excretion of the oxidized guanine nucleosides, as measured in the present study, reflects the overall damage to DNA and RNA, being pivotal in the processes of cell aging, degeneration, and apoptosis. Thus, measurement of 8-oxodG and 8-oxoGuo can be considered the biologically most relevant method for assessing whole-body oxidative stress (14).

Recently, we investigated with the same laboratory method the 8-oxoGuo and 8-oxodG urinary excretions in hyperthyroid patients before and after treatment (19). Both biomarkers decreased by 10–25% after the patients were rendered euthyroid by treatment. The oxidative stress markers were higher in patients with Graves' disease than in those with toxic nodular goiter (19), implying that the degree of hyperthyroidism is most pronounced in the former disease. Still, the presence of autoimmunity *per se* may also be an important factor.

It is unclear why hyperthyroid patients benefit from treatment with respect to oxidative stress while hypothyroid patients do not. A possible explanation might be that the hyperthyroid state is characterized by markedly elevated energy expenditure and oxygen turnover, exceeding the antioxidant capacity. Treatment will reverse these processes, leading to measurable changes in oxidative stress biomarkers. In the hypothyroid state, changes in the energy expenditure may be more subtle and below the threshold at which changes in the biomarkers are detectable. This is challenged by the fact that we found a positive correlation between plasma FT4 and the oxidative stress biomarkers at baseline. However, in this context, FT4 may be considered merely a surrogate marker for the severity of thyroid autoimmunity, leaving the oxidative stress level unchanged after treatment. Thus, the underlying pathophysiological condition, i.e. thyroid autoimmunity, might also explain why the level of oxidative stress biomarkers in our patients remained higher than that in the control subjects, despite biochemical euthyroidism obtained by LT4 substitution. Such a hypothesis is supported by previous observations (43). We found no influence of the TPOAb level *per se* on the level of oxidative stress, but this may rely on the fact that TPOAb shows huge variations among patients with autoimmune thyroiditis. Finally, it cannot be excluded that LT4 is unable to restore complete euthyroidism within the cell, in line with the ongoing debate about the ideal treatment of hypothyroid patients (48). If true, hypothyroid individuals will not improve their oxidative stress level following LT4 treatment, in contrast to the effects seen when treating hyperthyroid patients (19). Whether LT4 combined with liothyronine proves better for the oxidative stress burden remains to be shown, ideally in the setting of a randomized controlled trial.

The presence of comorbidity may potentially contribute to increased oxidative stress (11). Most of our patients did not suffer from disorders other than hypothyroidism. However, in cohort A, four participants had coexisting chronic diseases such as type 2 diabetes mellitus, asthma, fibromyalgia, and previous ovarian cancer without evidence of residual disease, respectively. Drugs may also affect whole-body oxidative stress, although data are scarce. Previous reports support that drugs of different kinds have either a favorable or a neutral effect on the oxidative stress level (49, 50, 51, 52, 53, 54). Thus, any effect of coadministered drugs would probably tend to ameliorate the oxidative burden in our patients. If such a drug-induced effect was excluded, the

difference in the oxidative stress biomarkers would be even more pronounced between patients and control subjects. No patient received statins, while five patients were treated with proton pump inhibitors or antihypertensive drugs. When excluding these individuals and those with comorbidity, our results remained significant (data not shown).

Our study has some limitations. The population comprised selected patients from two cohorts, and the study was not designed specifically for the present purpose. Further, the duration between the two study visits varied between patients, and we cannot exclude the fact that the burden of oxidative stress may depend on the length of the euthyroid state. We did not consistently monitor the use of antioxidant supplements, e.g. selenium, which potentially may have affected the oxidative stress level (55) and a number of other variables (56). However, as the use of supplements most likely remained unchanged during the study period, both in patients and in controls, any impact on our results is probably negligible. Finally, the urine and blood samples from the two study cohorts were not analyzed simultaneously, which is probably of little or no importance, as the oxidative stress biomarkers show high chemical stability and no diurnal variation (26, 57).

In conclusion, the whole-body oxidative stress biomarkers 8-oxoGuo and 8oxodG were inversely correlated with the severity of hypothyroidism in patients with newly diagnosed autoimmune thyroiditis. No significant change in these biomarkers could be demonstrated after restoring euthyroidism. Importantly, despite treatment, we demonstrated a persistently higher level of oxidative stress in our patients than in healthy control subjects. The mechanism behind the latter observation remains to be clarified. Finally, whether a causal link exists between these biomarkers of oxidative stress and the increased morbidity observed in patients with hypothyroidism remains to be elucidated.

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.

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Author contribution statement

SJB, THB, LH, and BN contributed to the study conception and design. SJB, THB, CBL, BM, FKK, CZJ, and BN conducted the data collection. HEP were involved in the development of methodology (biomarkers). KRR was responsible for data analysis. KRR and SJB interpreted the data. KRR wrote the first draft of the manuscript. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability

Some or all datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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