Treatment of hyperthyroidism reduces systemic oxidative stress, as measured by markers of RNA and DNA damages

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Words for indexing: Oxidative stress, hyperthyroidism, 8-oxo-7,8-dihydroguanosine, 8-oxo-7,8-dihydro-2'-deoxyguanosine, Graves' disease, toxic nodular goiter

Clinical trials registration number: NCT02005250

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Abstract

Background: Whole-body oxidative stress can be estimated by the urine excretion of oxidized guanosine species, 8-oxo-7,8-dihydroguanosine (8-oxoGuo) and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), derived from RNA and DNA, respectively. These oxidative stress markers are not well explored in thyroid disorders.

Methods: Urinary excretion of 8-oxoGuo and 8-oxodG was measured in 51 hyperthyroid patients (toxic nodular goiter (TNG), n=30; Graves' disease (GD), n=21) before or shortly after initiation of therapy and when stable euthyroidism had been achieved for at least 12 months.

Results: Adjusting for age, the baseline urinary excretion of oxidative stress markers correlated positively with plasma thyroxine (8-oxoGuo: p=0.002; 8-oxodG: p=0.021) and was significantly higher in GD than in TNG patients (p=0.001 for both oxidative stress markers). Restoration of euthyroidism significantly affected the excretion of the oxidative stress markers. In TNG, 8-oxoGuo decreased from geometric mean 2.11 nmol/mmol creatinine (95%CI:1.85-2.39) to 1.91 nmol/mmol (95%CI:1.67-2.19), p=0.001, while 8-oxodG decreased from 1.65 nmol/mmol (95%CI:1.41-1.93) to 1.48 nmol/mmol (95%CI:1.27-1.74), p=0.026. In GD, 8-oxoGuo decreased from 2.25 nmol/mmol (95%CI:1.95-2.59) to 1.79 nmol/mmol (95%CI:1.63-1.97), p=0.0003, while 8-oxodG decreased from 2.02 nmol/mmol (95%CI:1.73-2.38) to 1.54 nmol/mmol (95%CI:1.31-1.81), p=0.001. In the euthyroid state, there were no differences between groups.

Conclusion: Restoration of euthyroidism in patients with hyperthyroidism significantly decreased the systemic oxidative stress load by 10-25%. Our findings may help to explain the higher morbidity and mortality linked to hyperthyroid diseases, as shown in observational studies.

Introduction

Hyperthyroidism, often caused by Graves' disease (GD) and toxic nodular goiter (TNG), is a common condition with a lifetime risk of 2-5% (1, 2). Hyperthyroidism is associated with increased morbidity and mortality (1, 3, 4), but the pathophysiological mechanisms behind the detrimental impact on health remain obscure. One explanation might be the influence of oxidative stress (5-7).

Oxidative stress is defined as an imbalance between the production of oxidants and antioxidants, leading to oxidative damage to macromolecules, including nucleic acids, or disrupt redox signaling. Pro-oxidants are categorized based on the chemical composition, with reactive oxygen species as an important part of redox processes. Reactive oxygen species and other pro-oxidants may result from external factors, e.g. ionizing radiation, but under homeostatic conditions most of these species are generated as a by-product of the mitochondrial oxidative respiration and enzymes. Increased oxidative stress load potentially causes mutations, premature cellular aging, or cell death (8, 9).

The guanine nucleosides 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) and 8-oxo-7,8-dihydroguanosine (8-oxoGuo) can be measured in the urine and represent the cumulated impact of oxidative stress on the nucleic acids in the whole organism (10, 11). Increased urinary excretion of 8-oxodG and 8-oxoGuo has been demonstrated in a range of conditions like aging, smoking, cardiovascular diseases, type 2 diabetes mellitus, hypertension, neurodegenerative diseases, and certain cancers (10, 12-16). Moreover, these oxidative stress markers are predictive for the development of diabetic complications (14, 17).

Although the association between hyperthyroidism and oxidative stress has been investigated in previous studies using different methodologies, (5, 6, 18-23), the impact on the cellular nucleus and the transcriptive apparatus, in terms of the oxidative damage to deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), is unknown. Thus, this study aimed to investigate whether treatment of patients suffering from GD or TNG affects the urinary excretion of the oxidative stress markers 8-oxoGuo and 8-oxodG.

4

Methods

Study design and participants

This prospective study was originally designed to examine the impact of hyperthyroidism on bone microarchitecture, as previously reported (24). In brief, the study was conducted at the Endocrine Outpatient Clinic, Odense University Hospital, Denmark, in the period May 2011 through January 2016. Women between 20 and 85 years of age with newly diagnosed overt or mild hyperthyroidism due to either Graves' disease (GD) or toxic nodular goiter (TNG) were invited to participate. Exclusion criteria were pregnancy or a wish to become pregnant during the study period, glucocorticoid treatment, decreased kidney function as defined by a plasma creatinine >100 µmol/L (reference interval 45-90 µmol/L) and/or eGFR<90 ml/min/1.73m^2. Overt hyperthyroidism was defined as subnormal plasma thyrotropin (TSH), and thyroxine (T4) and/or triiodothyronine (T3) above the normal reference ranges, while mild hyperthyroidism was defined as subnormal or plasma TSH and normal levels of plasma T4 and T3. Patients harboring thyrotropin receptor antibodies (TRAb) were defined as having GD, while patients being TRAb negative and showing a thyroid scintigraphy compatible with thyroid nodularity were defined as having TNG.

The impact of hyperthyroidism as well as the effect of treatment on the whole-body oxidative stress load were measured by the urinary excretion of the oxidized guanine nucleosides 8-oxodG and 8-oxoGuo. Thus, the patients were examined twice: at diagnosis or shortly thereafter, and when euthyroidism had been restored for at least twelve months. The treatment of the patients followed standard clinical guidelines, and included anti-thyroid drugs, thyroidectomy, and radioiodine. The thyroid function was monitored by routine laboratory tests every 4-8 weeks. The follow-up examination was performed when the patient had been euthyroid for a minimum of twelve months, defined by a plasma TSH level within reference range.

5

Blood samples

All patients were diagnosed with hyperthyroidism by their general health practitioner, employing total T4 (TT4) and total T3 (TT3) assessments according to the routine methods at the laboratory. Many patients, primarily those with GD, started antithyroid drug treatment at this point. TT4 and TT3, reflecting the thyroid function in the treatment naïve patient, were analyzed by an immunoassay (Architect, Abbott, Chicago, Il).

Blood samples from both study visits were drawn in a non-fasting condition and stored at -20°C until analysis. After study completion, these samples were analyzed for TSH (reference level: 0.3-4.0 mIU/L), free T3 (4.0-6.8 pmol/L) and free T4 (10.0-22.0 pmol/L) by a two-site chemiluminescent immunometric assay using Cobas® 8000 (Roche Diagnostics, U.S). The coefficient of variation (CV) was 8.3% and 4.2% at TSH levels of 0.084 and 11.3 mIU/L, respectively; CVs were 2.1% and 2.0% at free T3 levels of 5.75 and 14.0 pmol/L, respectively; CVs were 7.1% and 4.4% at free T4 levels of 17 and 33 pmol/L, respectively.

Thyroid peroxidase antibody was measured using AutoDelfia (Perkin Elmer, Waltham, MA), and TRAb was measured using a radioimmunoassay from Thermo Fisher Scientific (BRAHMS, Hennigsdorf, Germany).

Oxidative stress markers

Spot urine samples from both study visits were stored at −80°C until analysis for 8-oxodG and 8-oxoGuo using ultra-performance liquid chromatography tandem mass spectrometry. 8-oxodG and 8-oxoGuo results were normalized against the urinary creatinine concentration measured by an in-house Jaffe's method (25). Chromatographic separation was performed using Perkin Elmer 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, Calif., USA). The mass spectrometry detection was performed on an API 3000 triple quadrupole mass spectrometer (Sciex, Toronto, Canada) equipped with an ESI ion source (Turbospray) operated in the positive mode (25).

6

Statistical analysis

Due to deviation from the normal distribution, values of 8-oxodG and 8-oxoGuo were log-transformed before calculation. Data are presented as means (±SD or 95% confidence interval (CI)) or medians (range). Parametric paired/unpaired t-tests, one-way ANOVA and ANCOVA with adjustment for co-variate (age=55.6 years) were used to compare differences within individuals (hyperthyroidism vs. euthyroidism) and between groups (GD vs. TNG). A backward stepwise regression analysis was used for testing correlations between variables. The analyses were performed using SPSS Statistics, version 25 (IBM, Armonk, NY), and statistical significance was defined as p<0.05. All statistical tests were two-sided.

Results

Patient characteristics

Characteristics of the patients, stratified for type of disease, are shown in Table 1. Ten patients had arterial hypertension, all being well treated on anti-hypertensive medication. The remaining patients had no comorbidity and were not receiving any medication unless it was for the thyroid disease. Table 2 shows the baseline values obtained at the first study visit and follow-up values at the second study visit, when euthyroidism had been achieved for at least 12 months. TNG patients were older than those with GD (59.3±12.1 years vs. 50.4±8.1 years, p=0.005), and were less hyperthyroid (Total T4 before initiation of treatment: 106.9±27.2 nmol/L in TNG vs. 182.4±66.2 nmol/L in GD; p<0.001). GD patients were treated with methimazole at diagnosis, and the median time to the first study visit was 33 days. Two GD patients received radioiodine after the first study visit, resulting in permanent hypothyroidism which was treated with levothyroxine. Seven GD patients continued the antithyroid drug treatment throughout the study period, while 12 patients achieved remission and remained euthyroid at follow-up. All patients with TNG were treated with radioiodine, except one who underwent thyroidectomy. Radioiodine was given before

7

study entry in eight TNG patients. Considering all TNG patients, the median time from the first study visit to treatment was six days. Radioiodine resulted in permanent hypothyroidism in three TNG patients, who were then treated with levothyroxine.

Excretion of 8-oxoGuo and 8-oxodG at hyperthyroidism

The values of the oxidative stress markers at baseline are shown in Table 2. The baseline urinary excretion of both 8-oxoGuo and 8-oxodG was not significantly higher in GD than in TNG. For both 8-oxoGuo and 8-oxodG, the excretion increased with age, as shown in Figure 1. Consequently, age adjustment of the data was performed as patients with TNG were nine years older on average than those with GD. After such an adjustment the between-group difference in the baseline urinary excretion of 8-oxoGuo and 8-oxodG became significantly different, as shown in Figure 2. Thus, the excretion of 8-oxoGuo was 2.52 nmol/mmol creatinine (95%CI: 2.26-2.80) in GD and 1.95 nmol/mmol (95%CI: 1.78-2.12) in TNG (p=0.001). For 8-oxodG, the corresponding values were 2.24 nmol/mmol (95%CI: 1.92-2.62) in GD and 1.53 nmol/mmol (95%CI: 1.34-1.74) in TNG (p=0.001). No between group differences were found at follow-up (data shown in supplementary materials (26)). No difference was found either between smokers and never/former smokers, or between patients with and without arterial hypertension (data not shown).

A multiple regression analysis explored the extent to which potential variables, shown in Table 3, were predictive for the excretion of the oxidative stress markers. A positive correlation was found with age (8-oxoGuo: p<0.001; 8-oxodG: p=0.002) and the severity of the hyperthyroidism, reflected by the TT4 plasma level at diagnosis (8-oxoGuo: p<0.001; 8-oxodG: p=0.008, Table 3 and Figure 1).

8

Excretion of 8-oxoGuo and 8-oxodG at euthyroidism

Treatment of hyperthyroidism significantly reduced the oxidative stress markers, as illustrated in Table 2 and in Figure 3. In patients with TNG we found a 10% reduction in both oxidative stress markers, as 8-oxoGuo decreased from geometric mean (GM) 2.11 nmol/mmol creatinine (95%CI:1.85-2.39) to 1.91 nmol/mmol (95%CI:1.67-2.19), p=0.001, while 8-oxodG decreased from 1.65 nmol/mmol (95%CI:1.41-1.93) to 1.48 nmol/mmol (95%CI:1.27-1.74), p=0.026. An even more pronounced reduction was seen among patients with GD, in whom 8-oxoGuo decreased 20% from 2.25 nmol/mmol (95%CI:1.95-2.59) to 1.79 nmol/mmol (95%CI:1.63-1.97), p=0.0003, while 8-oxodG decreased 25% from 2.02 nmol/mmol (95%CI:1.73-2.38) to 1.54 nmol/mmol (95%CI:1.31-1.81), p=0.001.

A multiple regression analysis (supplementary material (26)) was performed to demonstrate how a number of relevant variables correlated with the dynamics of the oxidative stress markers during follow-up (termed delta: value at first visit minus value at second visit). Delta 8-oxoGuo and delta 8-oxodG correlated significantly with the baseline values of 8-oxoGuo and 8-oxodG, respectively (supplementary material (26)). Further, delta 8-oxoGuo correlated positively with baseline s-FT3 and the presence of TRAb (Graves' disease), while delta 8-oxodG correlated negatively with age.

Discussion

Thyroid hormones are crucial for regulating the activity of a wide range of metabolic pathways. Hyperthyroidism leads to an increased mitochondrial oxygen consumption, increased energy expenditure, and a higher basal metabolic rate. Therefore, it is a logical assumption that more reactive oxygen species are produced in the hyperthyroid state. However, the net effect may be neutral to the body, if the antioxidative capacity is increased in parallel; otherwise, oxidative stress will occur.

Several studies aimed to estimate the effects of oxidative stress in humans with thyroid diseases, using different methods and with focus on either oxidation products or the antioxidative capacity, or both

9

aspects (5, 6, 18-23). In a study by Aslan et al. (5), a measure of the total antioxidant capacity (TAC) was decreased, while the total oxidant status (TOS) as well as the oxidative stress index (TOS/TAC) were increased in hyperthyroid patients compared to healthy control subjects. TAC and TOS were estimated by the amounts of free hydroxyl radicals and hydrogen peroxide in serum, respectively (5). In another study (23), using the same automated measurement methods as by Aslan et al. (5), the oxidative stress index was significantly higher in patients suffering from either Graves' disease or Hashimoto's thyroiditis, compared to a healthy control group. However, previous estimates of oxidative stress focused on components in serum, the cell membrane, or the cytosol, while the net effect on the core cellular machinery, i.e., gene translation and protein transcription, has been less explored. The guanine nucleosides, 8-oxoGuo and 8-oxodG, are derived from oxidative damage to DNA and RNA, respectively. Therefore, these metabolites represent a biologically more relevant estimate of the consequences from inappropriately high levels of reactive oxygen species and pro-oxidants.

Our study is the first to investigate concentrations of 8-oxoGuo and 8-oxodG in patients with hyperthyroidism, and to investigate the effect of treatment. The age-adjusted baseline levels of both oxidative stress markers were significantly higher in patients with GD as compared to TNG. This may be explained by the fact that these patients were significantly more hyperthyroid at diagnosis than those with TNG. Another explanation may be that autoimmunity *per se* is of importance, perhaps in a synergetic action with the hyperthyroid state. This is supported by previous studies indicating that presence of TRAb is linked to oxidative stress by generation of reactive oxygen species and/or induction of lipid peroxidation (27-29). If autoimmunity is a major determinant in disorders where increased oxidative stress plays a pathophysiological role, supplementation with antioxidants, e.g. selenium, might be of benefit. Indeed, selenium supplementation seems to reduce the thyroid peroxidase antibody titer in patients with Hashimoto's thyroiditis, which might be mediated by less oxidative stress (30-32). However, it remains to be demonstrated whether exogenous antioxidants can reduce the excretion of oxidized nucleosides, in hyperthyroidism as well as in other diseases.

An important finding in our study was that restoration of euthyroidism decreased the oxidative stress load. Patients with TNG showed a decrease by 10% in both 8-oxoGuo and 8-oxodG, whereas patients with GD showed a 20% decrease in 8-oxoGuo and a 25% decrease in 8-oxodG, probably reflecting the hyperthyroidism being more severe in the latter condition. The changes observed at follow-up in both oxidative stress markers (delta) correlated positively with the baseline levels. Further, at follow-up, patients with GD showed higher excretion of both 8-oxoGuo and 8-oxodG than seen among patients with TNG. Although the difference did not reach statistical significance, this observation supports the hypothesis that autoimmunity is of importance in this context.

Alteration of the nucleic acids and changes of their intramolecular bonds have profound consequences in terms of mutations of genes and misfolding of protein during transcription. Importantly, the correlation between the generation of oxidized guanine nucleosides and mutations may not be simple and linear. Some of the DNA and RNA damages may be restored by intracellular reparatory enzymes, which to some extent counterbalance the detrimental impact imposed by oxidative stress (33, 34). Such a post-impact repair of the genetic apparatus cannot be detected by the method used in our study. Nevertheless, we believe that measurement of the urinary excretion of oxidized guanine nucleosides for the detecting of whole-body oxidative stress is a more valid method than those used previously in studies of thyroid disorders.

The urinary excretion of 8-oxoGuo and 8-oxodG may have prognostic value, rather than merely representing a co-existing phenomenon. Thus, we previously reported that individuals with diabetes mellitus type 2 showing high levels of oxidized guanine nucleosides at the time of diagnosis had increased risk of diabetic complications, as well as mortality (10, 12-15, 35). Until now, oxidized guanine nucleosides have only sparsely been explored in relation to thyroid diseases. We have previously shown that levels of 8-oxoGuo and 8-oxodG were unaltered following radioiodine therapy in patients with benign nodular goiter (36). In patients with Graves' orbitopathy, Tsai et al. (37) reported that urinary excretion of the DNA metabolite 8-hydroxy-2'-deoxyguanosine, another marker of oxidative DNA damage, was increased and

11

correlated with disease activity and smoking status. To our knowledge, no studies have investigated markers of RNA oxidation in thyroid disorders.

A few limitations of our study exist. Only women were included, and the study was not designed specifically to investigate whole-body oxidative stress in hyperthyroidism. Further, treatment was in some patients initiated prior to the first study visit. Patients with GD were started on an antithyroid drug regimen at diagnosis, while most patients with TNG received radioiodine after the first study visit. However, the period from initiation of treatment to the baseline measurement of 8-oxoGuo and 8-oxodG was short. Thus, we believe that the influence of this time gap was minimal, considering the fact that euthyroidism ensues several weeks to months following commencing antithyroid drug treatment. In fact, had all patients with GD been examined in the untreated state at the first study visit, this might have accentuated the difference in biomarker excretion between TNG and GD patients. Seven patients still received antithyroid drug treatment at follow-up, as the GD had not remitted. In theory, the oxidative stress burden may be affected either by ongoing autoimmune inflammation or by the antithyroid drug *per se*. However, we did not find any significant difference between individuals with and without need for continuous antithyroid drug treatment (data not shown).

Our study focused on the dynamics of 8-oxoGuo and 8-oxodG during treatment of hyperthyroidism, and it did not include a healthy control group. As the generation of 8-oxoGuo and 8-oxodG are influenced by a range of internal and external factors, a control group must be carefully matched. This should be addressed in future studies to clarify whether restoration of euthyroidism results in similar levels of 8-oxoGuo and 8-oxodG, as those found in healthy individuals without any history of thyroid disease.

In conclusion, our study shows that among newly diagnosed hyperthyroid patients, the systemic oxidative stress load, measured as nucleic acids derivates, correlated with age and the severity of hyperthyroidism. Further, treatment of hyperthyroidism decreased the oxidative stress markers by as much as 25%. Accepting that oxidative stress has serious consequences to health, if not counterbalanced by adequate regulatory mechanisms, our results may help explain the increased morbidity and mortality

12

associated with hyperthyroidism, as demonstrated in large cohort studies (3, 4). If this holds true, it paves the way for new treatment strategies, including use of antioxidant supplementation. Furthermore, it remains to be evaluated whether 8-oxoGuo and 8-oxodG have any prognostic value in thyroid diseases, as shown for diabetes mellitus (14).

Acknowledgements

Statement of Ethics

All procedures performed in this study were in accordance with the ethical standards of the Regional Research Ethics Committee of Southern Denmark (protocol: S-2011-0018) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The patients gave written and informed consent.

Author disclosure statement

The authors have nothing to declare. No competing financial interests exist.

Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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15

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16

Table 1: Patient characteristics.

	GD n=21	TNG n=30	All patients n= 51	P^a
Age (years)	50.4 ± 8.11	59.3 ± 12.12	55.6 ± 11.4	0.005
BMI (kg/m²)	22.9 ± 3.17	24.7 ± 4.7	24.0 ± 4.2	0.133
Smoking status, number (%)				
Never	8 (38)	16 (53)	24 (47)	
Former	7 (33)	6 (20)	13 (25.5)	
Smoker	6 (29)	8 (27)	14 (27.5)	
Plasma TSH at diagnosis (mIU/L)	0.011 ± 0.0048	0.057 ± 0.071	0.038 ± 0.06	< 0.001
Plasma TT3 at diagnosis (nmol/L)	3.92 ± 2.78	2.07 ± 0.47	2.83 ± 2.01	< 0.001
Plasma TT4 at diagnosis (nmol/L)	182.4 ± 66.2	106.9 ± 27.2	138.0 ± 59.9	< 0.001
Thyrotropin receptor antibody (IU/L)	10.4 (2.2 - 40)			
Thyroid peroxidase antibody (IU/mL)	350 (3-2615)	7.8 (1-36)	149 (1-2615)	< 0.001
Time from initiation of treatment to first study visit (days)	33 (10 - 241)	-6 (-83 - 105)	22 (-83-241)	< 0.001
Length of follow-up (months from first study)	22.9 ± 8.9	17.2 ± 4.6	19.6 ± 7.2	0.004

Data are presented as mean ± SD or median and range.

[a] Difference between Graves' disease (GD) and toxic nodular goiter (TNG).

BMI: Body mass index; TSH: Thyrotropin; TT3: Total triiodothyronine; TT4: Total thyroxine.

Table 2: Thyroid function and oxidative stress markers at first (baseline) and second (follow-up) study visit.

	GD (n=21)			TNG (n=30)			All patients (n=51)		
	Baseline	Follow-up	*P*	Baseline	Follow-up	*P*	Baseline	Follow-up	*P*
8-oxoGuo nmol/mmol creatinine	2.25 (1.95-2.59)	1.79 (1.63-1.97)	0.0003	2.11 (1.85-2.39)	1.91 (1.67-2.19)	0.001	2.16 (1.97-2.37)	1.86 (1.71-2.03)	< 0.001
8-oxodG nmol/mmol creatinine	2.02 (1.73-2.38)	1.54 (1.31-1.81)	0,001	1.65 (1.41-1.93)	1.48 (1.27-1.74)	0.026	1.79 (1.60-2.01)	1.51 (1.35-1.68)	< 0.001
Plasma TSH (mIU/L)	0.09 ± 0.3	2.0 ± 1.9	0.0003	0.2 ± 0.2	1.5 ± 1.1	< 0.001	0.1 ± 0.3	1.7 ± 1.5	< 0.001
Plasma FT3 (pmol/L)	6.8 ± 3.6	4.8 ± 0.5	0.021	5.9 ± 1.6	4.7 ± 0.5	0.001	6.3 ± 2.6	4.8 ± 0.5	< 0.001
Plasma FT4 (pmol/L)	16.9 ± 8.0	15.9 ± 2.8	0.615	17.2 ± 5.1	15.4 ± 2.3	0.099	17.1 ± 6.4	15.6 ± 2.5	0.151
[b]Delta 8-oxoGuo nmol/ mmol creatinine	-0.52 ± 0.56			-0.19 ± 0.32		0.01[a]	-0.32 ± 0.46		
[b]Delta 8-oxodG nmol/ mmol creatinine	-0.52 ± 0.59			-0.17 ± 0.39		0.05[a]	-0.31 ± 0.51		
[b]Delta plasma TSH (mIU/L)	1.90 ± 2.00			1.35 ± 1.18		0.22[a]	1.58 ± 1.58		
[b]Delta plasma FT3 (pmol/L)	-2.02 ± 3.68			-1.16 ± 1.74		0.27[a]	-1.5 ± 2.71		
[b]Delta plasma FT4 (pmol/L)	-0.99 ± 8.85			-1.76 ± 5.69		0.70[a]	-0.45 ± 7.09		

Data are presented as mean ± SD or 95% confidence interval.

8-oxoGuo: 8-oxo-7,8-dihydroguanosine; 8-oxodG: 8-oxo-7,8-dihydro-2'-deoxyguanosine

TSH: Thyrotropin; FT3: Free triiodothyronine; FT4: Free thyroxine.

[a] Difference between Graves' disease (GD) and toxic nodular goiter (TNG).

[b] Delta represents the difference between the first and second study visit.

Table 3: Key variables included in the regression analysis for prediction of baseline levels of 8-oxoGuo and 8-oxodG in all patients.

	8-oxoGuo		8-oxodG	
All variables included in the equation	**Beta coefficient**	*P*	**Beta coefficient**	*P*
Plasma TT3 at diagnosis	0.512	0.117	0.809	0.042
Plasma TT4 at diagnosis	0.195	0.272	0.233	0.277
Plasma FT3 at first study visit	0.259	0.079	1.497	0.03
Plasma FT4 at first study visit	-0.825	0.143	-1.731	0.013
Plasma FT3/FT4 ratio	-0.844	0.095	-1.544	0.013
Age	0.729	< 0.001	0.600	0.0002
Smoking	-0.218	0.049	0.021	0.87
BMI	-0.052	0.637	-0.109	0.413
Time from treatment initiation to first study visit	-0.133	0.288	-0.78	0.606
Thyroid peroxidase antibody titer at diagnosis	0.003	0.977	0.022	0.879
Presence of TRAb at diagnosis (GD)	0.123	0.469	0.065	0.748
By backward stepwise regression				
Age	0.674	< 0.001	0.420	0.002
Plasma TT4 at diagnosis	0.405	< 0.001	0.354	0.008

Dependent variables in the regression analyses are baseline 8-oxoGuo and 8-oxodG, respectively.

GD: Graves' disease; 8-oxoGuo: 8-oxo-7,8-dihydroguanosine; 8-oxodG: 8-oxo-7,8-dihydro-2'-deoxyguanosine; T3: triiodothyronine; T4: thyroxine; FT3: Free T3; FT4: Free T4; TT3: Total T3; TT4: Total T4; BMI: Body mass index; TSH: Thyrotropin. TRAb: Thyrotropin receptor antibody

Fig 1: A: correlation between baseline 8-oxo-7,8-dihydroguanosine (8-oxoGuo) and age (r=0.591, p<0.001); B: correlation between baseline 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) and age (r=0.348, p=0.006); C: correlation between baseline 8-oxoGuo and total plasma T4 at diagnosis (r=0.268, p=0.029); D: Correlation between baseline 8-oxodG and total plasma T4 at diagnosis (r=0.268, p=0.029).
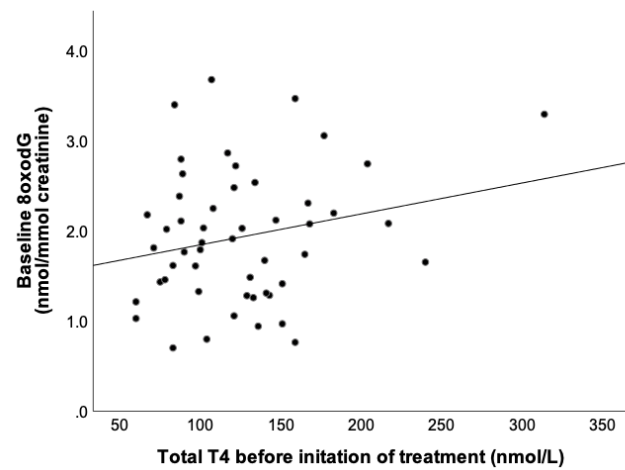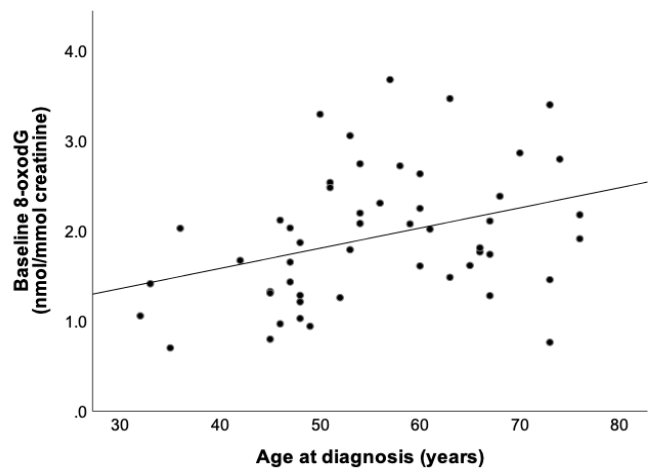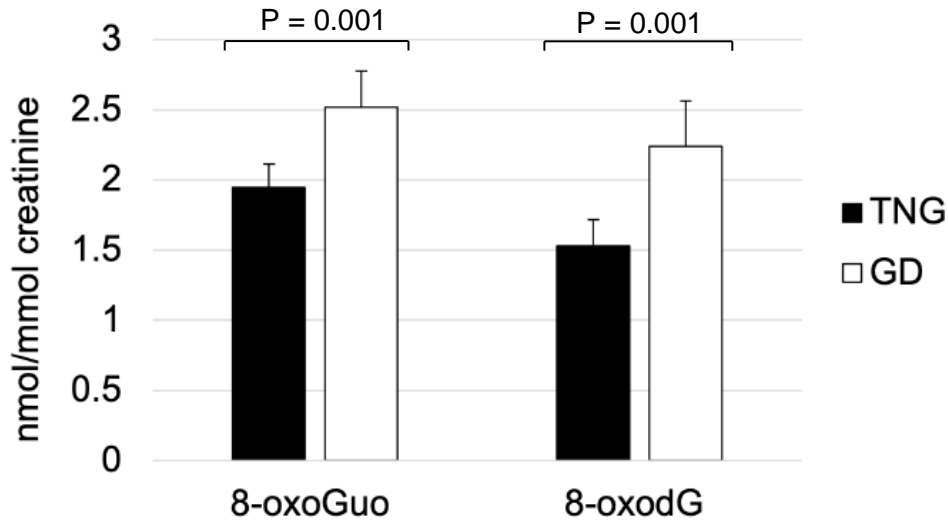
A)

B

C)

D)

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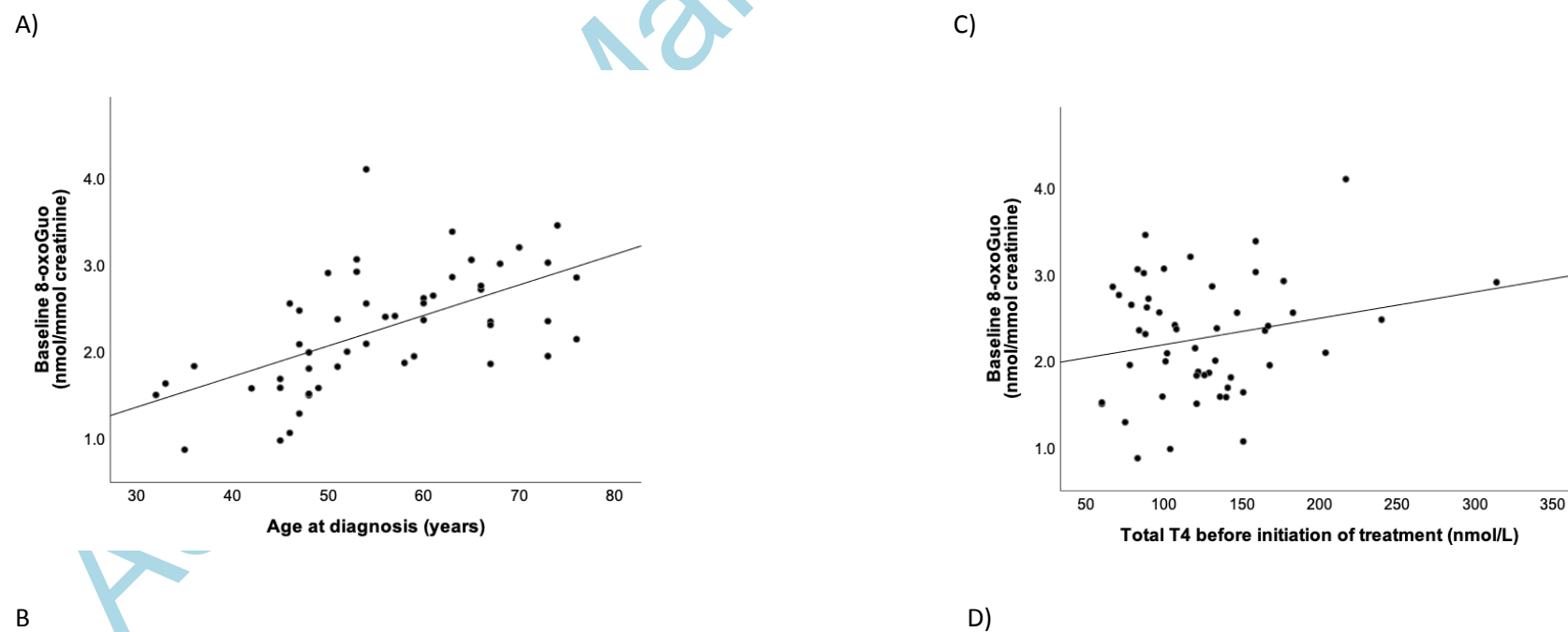
Fig. 2: Age adjusted levels of oxidative stress markers in Graves' disease (GD) and toxic nodular goiter (TNG) at first study visit.
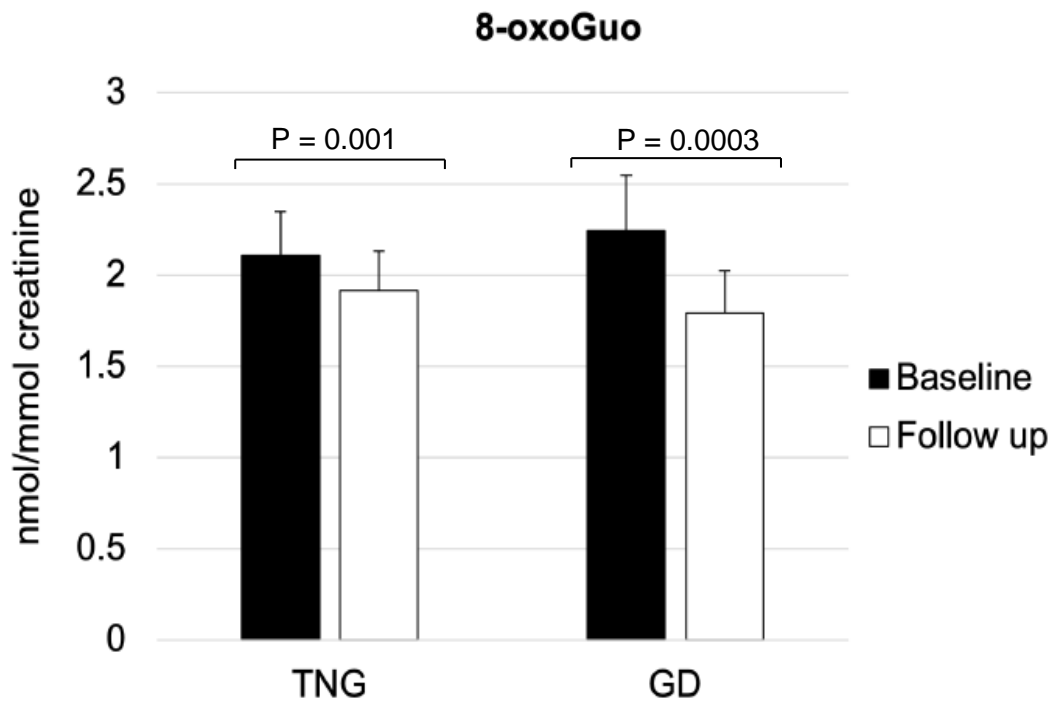

Age as co-variate is included in the model at the following value: age = 55.6 years.

8-oxoGuo: 8-oxo-7,8-dihydroguanosine nmol/mmol creatinine; 8-oxodG: 8-oxo-7,8-dihydro-2'-deoxyguanosine nmol/mmol creatinine.
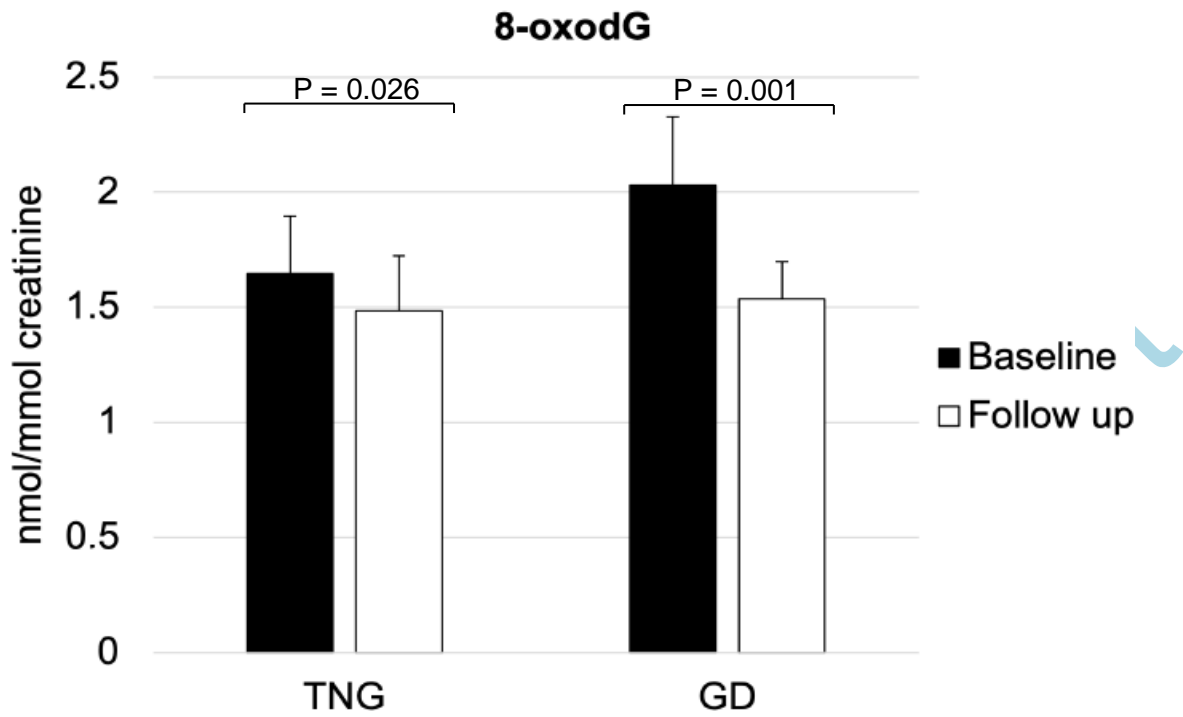
Fig 3: Effect of treatment of hyperthyroidism on urinary excretion of oxidative stress markers.

A)

8-oxoGuo

B)

8-oxodG

Mean levels of 8-oxo-7,8-dihydroguanosine nmol/mmol creatinine (8-oxoGuo) (A) and 8-oxo-7,8-dihydro-2'-deoxyguanosine nmol/mmol creatinine (8-oxodG) (B) in patients with toxic nodular goiter (TNG) and Graves' disease (GD), respectively.