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ORIGINAL ARTICLE

Markers of oxidative stress in obese men with and without hypertension

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ABSTRACT

Objectives: The aim of our study was to investigate if the 24-hour excretion of the urinary markers for oxidative stress to DNA and RNA, measured as 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) and 8-oxo-7,8-dihydro-guanosine (8-oxoGuo), respectively, were increased in obese individuals with or without hypertension compared to lean controls.

Methods: A total of 63 obese hypertensive men (obeseHT), 40 obese normotensive men (obeseNT) and 27 lean normotensive men (leanNT) were included in the study. Body mass index (BMI) was between 20.0 and 24.9 kg/m^2 in leanNT participants and ≥30 kg/m^2 in obese participants. Hypertension was defined as a mean 24-hour systolic ambulatory blood pressure (AMBP) ≥ 130 mmHg or a mean 24-hour diastolic AMBP ≥80 mmHg and normotension as mean 24-hour AMBP <130/80 mmHg. Twenty-four hour urinary 8-oxoGuo and 8-oxodG excretion (nmol/24 h) were measured by a validated liquid chromatography-tandem mass spectrometry method (UPLC-MS/MS).

Results: Urinary 8-oxoGuo excretion was (median and [interquartile range]) 30.8 [27.8–32.2] nmol/24 h in leanNT, 36.8 [31.3–40.2] nmol/24 h in obeseNT and 40.6 [31.7–48.5] nmol/24 h in obeseHT. The difference was statistically significant ($p = .002$) and *post hoc* tests showed a significant difference between leanNT and obeseHT ($p = .001$) as well as obeseNT ($p = .002$), whereas the two obese groups did not differ ($p = .6$). No statistically significant differences in 8-oxodG concentrations were observed between the three groups ($p = .3$).

Conclusion: The measurement of urinary excretion of 8-oxoGuo suggests that obesity in men, but not hypertension, is associated with increased oxidative damage to RNA.

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Introduction

Oxidative stress to DNA and RNA can be measured as the urinary excretion of the oxidized guanine nucleosides 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) and 8-oxo-7,8-dihydro-guanosine (8-oxoGuo), respectively [1]. Oxidative stress is characterized by the production of reactive oxidative species (ROS) which exceeds the anti-oxidative capacity of the cell, leading to increased cellular damage to e.g. DNA and RNA [2]. We have previously shown that increased urinary excretion of the RNA oxidation marker 8-oxoGuo, measured both in patients with newly diagnosed type 2 diabetes (T2D) and patients with established and treated T2D, was associated with poor long-term survival independently of known risk factors [3,4].

It has been known for many years that obesity, especially visceral obesity, is the main risk factor for developing T2D [5,6]. Indeed, in the 1970s Sims et al. suggested the term 'diabesity' [7]. Adipose tissue releases a number of hormones and cytokines involved in the development of insulin resistance, a key factor in developing T2D [8]. Furthermore, obesity has been associated with chronic low-grade systemic inflammation and oxidative stress [9]. Additionally, patients with T2D frequently suffer from hypertension (HT), the risk being higher with increasing body weight [5,6]. Increased oxidative stress has been implicated both in the basic pathogenesis of HT and in the end organ damage caused by HT [10].

We hypothesized that obese individuals have increased oxidative stress and that obese individuals with HT have even further increased oxidative stress. Therefore, we aimed to investigate if the excretion of the urinary markers for oxidative stress, 8-oxoGuo and 8-oxodG, were increased in obese individuals with or without HT compared to lean controls.

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Subjects and methods

Subject characteristics, including inclusion and exclusion criteria, as well as experimental procedures have previously been published [11] and are briefly summarized below.

Study population

The study population consisted of 63 obese hypertensive men (obeseHT), 40 obese normotensive men (obeseNT) and 27 lean normotensive men (leanNT). The lean normal weight participants had a body mass index (BMI) between 20.0 and 24.9 kg/m^2 and the obese ≥30 kg/m^2. HT was defined as a mean 24-hour systolic ambulatory blood pressure (AMBP) ≥130 mmHg or a mean 24-hour diastolic AMBP ≥80 mmHg and normotension as mean 24-hour AMBP <130/80 mmHg. The study was conducted over three visits. None of the participants were taking any medication in the 3 weeks prior to the study. Further, none of the participants were treated for hypertension, diagnosed with cardiovascular disease, diabetes or other chronic diseases, and they all had a screening fasting plasma glucose <7.0 mmol/L.

Participants gave informed written consent before inclusion and the study was performed in agreement with the Helsinki declaration. The study was approved by the Local Ethical Committee (HB-2007-040).

Measurements

The participants collected urine for 24 hours and the urine samples were stored at −20 °C until analysis. The 8-oxoGuo and 8-oxodG concentrations were measured by a validated liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method [12]. The 8-oxoGuo and 8-oxodG urinary concentrations in nmol/L were multiplied by the diuresis volume, resulting in a measure of the daily 8-oxoGuo and 8-oxodG excretion in nmol/24 h.

In previous publications we have in detail described the measurements of glucose and insulin concentrations in the fasting state and during an oral glucose tolerance test (OGTT), calculations of insulin resistance (IR) [11,13], measurements of the renin-angiotensin system components and urinary catecholamines [11], quantification of high sensitive C-reactive protein (hsCRP), white blood cell (WBC) count [14], and finally, the measurements of adiponectin [15] and leptin concentrations [16].

Statistical analyses

The statistical analyses were performed using R version 3.1.2 [17]. Differences in 8-oxoGuo/8-oxodG urinary excretion between leanNT, obeseNT and obeseHT were compared using the nonparametric Kruskal–Wallis rank sum test. When the Kruskal–Wallis rank sum test was statistically significant, post hoc testing with the Wilcoxon rank sum test was performed. A p-value of <.05 was considered statistically significant. Non-parametric statistics were performed due to non-normal distribution of the dependent variables 8-oxoGuo and 8-oxodG. Accordingly, data are given as median and interquartile range (IQR). We performed a number of *post hoc* regression analyses focusing on the two groups with obese participants ($n = 103$). Multiple linear regression analyses were performed to test if there were any associations between 8-oxoGuo/8-oxodG and waist:hip ratio adjusted for age, smoking habit (yes/no) and hypertension status (yes/no). Multiple linear regression analyses, adjusted for age, smoking status and BMI, were used to test for associations between 8-oxoGuo/8-oxodG and systolic mean 24-hour AMBP, diastolic mean 24-hour AMBP and plasma levels of the renin-angiotensin system components (renin, angiotensin II and aldosterone). Furthermore, using multiple linear regression models adjusted for age, smoking status, hypertension status and BMI, we tested if the urinary markers were associated with leptin, adiponectin, glucose metabolism (the glucose concentration at 2 hours during the OGTT), insulin resistance (HOMA-IR) and peripheral insulin sensitivity (whole body insulin sensitivity index; WBISI), inflammation (hsCRP and white blood cell (WBC) count) and the stress hormones noradrenaline and adrenaline. Correlation analyses were performed estimating the Pearson correlation coefficient (r) for waist:hip ratio and 8-oxoGuo and 8-oxodG, respectively.

Results

8-oxoGuo and 8-oxodG in obese men with or without HT

Table 1 shows baseline characteristics of the subjects and Figure 1 shows the 24-hour urinary excretion of 8-oxoGuo and 8-oxodG in leanNT, obeseNT and obeseHT participants. For 8-oxoGuo the daily excretion was (median and [IQR]) 30.8 [27.8–32.2] nmol/24 h in the leanNT group, increasing to 36.8 [31.3–40.2] nmol/24 h in the obeseNT group, and reaching 40.6 [31.7–48.5] nmol/24 h in the obeseHT group. When comparing the three groups using the Kruskal–Wallis rank sum test, the difference in 8-oxoGuo concentrations was statistically significant ($p = .002$). A *post hoc* analysis using Wilcoxon rank sum tests demonstrated a significant difference between levels in obeseHT and leanNT ($p = .001$) as well as between obeseNT and leanNT ($p = .002$), whereas the difference between obeseNT and obeseHT was insignificant ($p = .6$).

For 8-oxodG the daily excretion was (median and [IQR]) 22.1 [19.6–33.0] nmol/24 h among leanNT, 29.1 [23.3–33.9] nmol/24 h among obeseNT and 25.7 [22.6–33.5] nmol/24 h among obeseHT men. The Kruskal–Wallis rank sum test did not show any differences when comparing the three groups ($p = .3$).

Additional analyses in the obese participants

Table 2 shows all the p-values from the adjusted multiple linear regression analyses. There was an association between waist:hip ratio and 8-oxoGuo ($p = .009$), but no association between waist:hip ratio and 8-oxodG ($p = .7$). Similarly, the

Table 1. Baseline characteristics in lean normotensive (leanNT), obese normotensive (obeseNT) and obese hypertensive (obeseHT) men. Data is shown as median and interquartile range (IQR). *p*-values are from the Kruskal-Wallis rank sum test.

Median (IQR)	leanNT	obeseNT	obeseHT	*p*-value
Age (years)	54 (46–58)	48 (39.5–54.25)	50 (42.4–58.5)	.24
BMI (kg/m^2)	22.6 (22.0–24.1)	32.4 (31.5–33.4)	33.3 (31.3–35.2)	<.0001
Mean 24-hour systolic AMBP (mmHg)	110 (108–116)	118 (113.8–121)	136 (130.5–144.5)	<.0001
Mean 24-hour diastolic AMBP (mmHg)	72 (69.5–74)	74.5 (71.8–76)	82 (79.5–87)	<.0001
8-oxoGuo (nmol/24h)	30.8 (27.8–32.2)	36.8 (31.3–40.2)	40.6 (31.7–48.5)	.002
8-oxodG (nmol/24h)	22.1 (19.6–33.0)	29.1 (23.3–33.9)	25.7 (22.6–33.5)	.3

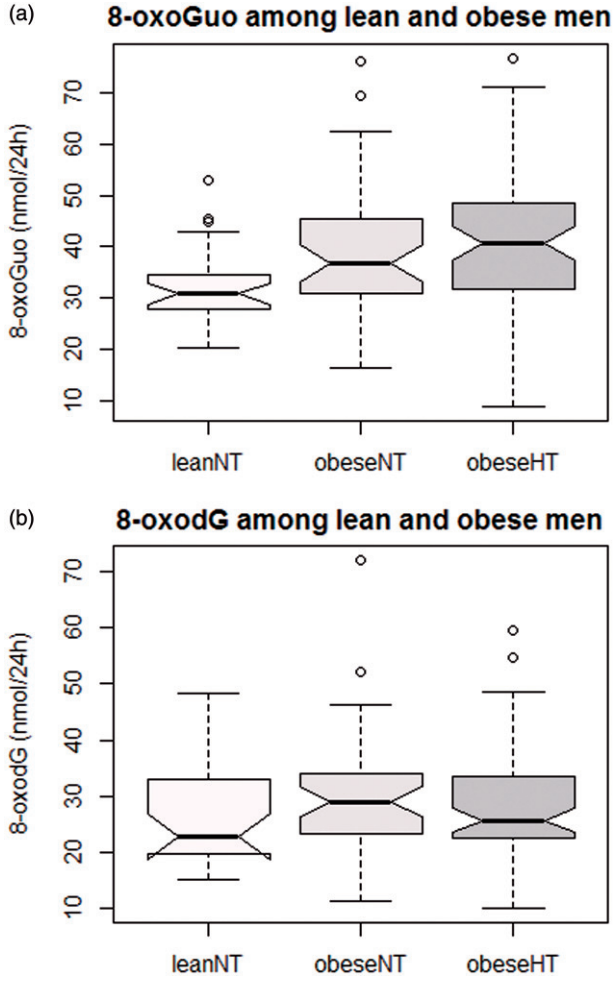

Figure 1. Notched boxplots showing 8-oxoGuo (a) and 8-oxodG (b) excretion (nmol/24 h) in lean normotensive (leanNT), obese normotensive (obeseNT) and obese hypertensive (obeseHT) men. The boxplots are shown with the median (bold line) and the interquartile range IQR (the box). The whiskers stretch outward from the 1st and 3rd quartiles and are 1.5 times the IQR. Outliers beyond that are marked separately with small dots. The notch displays the confidence interval around the median. There is a statistically significant difference in 8-oxoGuo concentrations when comparing the 3 groups ($p = .002$). We could not show any differences for 8-oxodG ($p = .3$).

correlation analysis showed a correlation between waist:hip ratio and 8-oxoGuo (Pearson's r = .27, $p = .007$) and no correlation between waist:hip ratio and 8-oxodG (Pearson's r = .02, $p = .8$). Mean 24-hour systolic and 24-hour diastolic AMBP were associated with 8-oxodG ($p = .02$ and $p = .03$, respectively), but they were not associated with 8-oxoGuo ($p = .3$ and $p = .4$, respectively). Plasma aldosterone associated with 8-oxoGuo ($p = .04$) and borderline-significantly with 8-oxodG ($p = .06$). Urinary adrenaline was not associated with 8-oxoGuo ($p = .8$) but with 8-oxodG ($p = .04$). Urinary noradrenaline was not associated with either of the urinary markers (8-oxoGuo: $p = .2$, 8-oxodG: $p = .1$). There were no associations between the urinary markers and adipocytokines (leptin, adiponectin), renin, angiotensin II, glucose metabolism (2-hour glucose in obese/obese with impaired glucose tolerance), insulin resistance (HOMA-IR), insulin sensitivity (WBISI) nor inflammation markers (hsCRP and WBC).

Discussion

Oxidative stress in obesity and hypertension

In this study, we tested the hypothesis that obese men have increased oxidative stress, measured as oxidative stress to RNA and DNA. Our findings show that oxidative stress to RNA is increased about 1.3-fold in obese compared to lean men. Further, we found a similar increase in oxidative stress to RNA in obese men regardless of whether they had hypertension or not. In contrast to the increased oxidative stress to RNA, the oxidative stress to DNA was not different in obese and lean men. Additionally, we demonstrated a significant association in obese men between 24-hour urinary 8-oxoGuo excretion and waist:hip ratio, the latter a better marker of visceral obesity than BMI. Obesity has previously been linked to oxidative stress, probably induced by hormones/cytokines ('adipocytokines') secreted from the adipose tissue [9], although in this study we did not find any associations between the urinary oxidative stress markers and leptin or adiponectin. Previous studies investigating the relation between obesity and 8-oxodG report conflicting findings; while some studies show increased urinary excretion of DNA oxidation products among obese subjects [18,19], others do not confirm such an association or they report a negative correlation between DNA oxidation and body weight [20–22]. However, previous studies were performed using ELISA techniques with inferior performance due to high variability and insufficient specificity as compared to the UPLC-MS/MS method used in the present study [23]. The previously reported results may therefore be the result of inaccurate quantifications. To our knowledge, there are no previous studies on the relation between obesity and the RNA oxidation marker 8-oxoGuo.

We expected to find higher oxidative stress among the obese subjects with hypertension, since ROS generation and subsequent damage of vascular tissue has been suggested to play a role in the pathophysiology of hypertension [10].

Table 2. The *p*-values from multiple regression analyses testing associations between 8-oxoGuo/8-oxodG and a number of variables.

	8-oxoGuo (nmol/24h)	8-oxodG (nmol/24h)
Waist:hip ratio[a]	.009*	.7
Adipocytokines[c]		
Leptin	.2	.8
Adiponectin	.1	.6
Blood pressure[b]		
24-hour systolic AMBP	.3	.02*
24-hour diastolic AMBP	.4	.03*
Renin-angiotensin system[b]		
Plasma renin	.2	.4
Plasma angiotensin II	.6	.5
Plasma aldosterone	.04*	.06*
Glucose metabolism and insulin resistance/sensitivity[c]		
2-hour glucose during OGTT	.8	.7
2-hour glucose during OGTT in individuals with impaired glucose tolerance[d]	.6	.9
HOMA-IR	.5	.4
WBISI	.8	.7
Inflammation[c]		
hsCRP	.2	.09
WBC	.9	.5
Urinary cathecolamines[c]		
Adrenaline	.8	.04*
Noradrenalin	.2	.1

[a]Adjusted for age, smoking habit (yes/no) and hypertension status (yes/no).
[b]Adjusted for age, smoking status and BMI.
[c]Adjusted for age, smoking status, hypertension status (yes/no) and BMI.
[d]Impaired glucose tolerance was defined as 2-hour glucose during OGTT $\geq$7.8 mmol/L.
*Statistically significant (*p*-values $<$.05).

However, we could not identify any statistically significant difference in the urinary markers between the obese subjects with normotension vs. hypertension, despite relatively large differences in blood pressure between the two groups. The mean difference between obeseHT and obeseNT was 18 mmHg for the average 24-hour systolic AMBP and 7.5 mmHg for average 24-hour diastolic AMBP. We did, on the other hand, find a statistically significant association between mean 24-hour systolic and diastolic AMBP and 8-oxodG among obese men. A previous study also found increased 8-oxodG among elderly individuals with hypertension compared to controls, but this was by means of an ELISA method [24]. From the present study we conclude that oxidative stress to DNA or RNA is not associated with hypertension since there is no clear relation. The association between systolic and diastolic blood pressure and the DNA oxidation marker in obese men is likely to be a chance finding. The cross-sectional study design does not enable an analysis of causality and we speculate that a more specific marker for vascular oxidative stress might be better for measurement of oxidative damage in hypertension.

Supplementary analyses (obese participants)

For hypothesis generation purposes, we performed several regression analyses. However, only plasma aldosterone was associated with the urinary excretion of 8-oxoGuo, while the urinary excretion of adrenalin and 8-oxodG were associated. It has been reported that elevated levels of cortisol, e.g. due to Cushing's syndrome or high-dose treatment with steroids, can induce a phenotype similar to the metabolic syndrome and even cause overt diabetes [25]. Cortisol is, along with adrenal catecholamines (adrenalin and noradrenalin), known to be involved in the stress response [26] and we have previously found an association between cortisol and both DNA and RNA oxidation [27]. We therefore hypothesized that the urinary markers were associated with the urinary concentration of catecholamines, but we only found the urinary excretion of adrenalin to be associated with 8-oxodG. This association needs further investigation as a primary endpoint in future studies. We found no clear association between hypertension and the urinary markers, and we suspect the association between aldosterone and 8-oxoGuo to be a chance finding.

Urinary 8-oxoGuo & 8-oxodG as biomarkers

We measured oxidative stress to RNA and DNA by measuring the excretion of the oxidized nucleosides 8-oxoGuo and 8-oxodG in urine. The urinary excretion of the markers is a measurement of the whole-body oxidative stress and is increased in diseases where all cell types are affected, such as diabetes and hemochromatosis [28]. Therefore, conditions with oxidative stress located to a specific organ or tissue type will not increase urinary excretion of 8-oxoGuo or 8-oxodG because of its relatively small contribution of the total-body urinary excretion of the markers.

Both the oxidized base and the oxidized nucleosides are found in urine. There are two reasons for measuring the nucleoside. Firstly, when measuring the nucleoside, i.e. the nucleobase attached to the pentose sugar by a N-glycosidic bond, it is possible to determine whether the oxidation occurred in RNA or in DNA, since RNA and DNA differs by type of attached pentose. This is not the case when measuring the oxidized base that can originate from any of the nucleic acids or their nucleotide pools. Secondly, the diet, particularly meat, contains oxidized nucleic acids in particular after high temperature cooking. Studies with labelled nucleic acids have demonstrated that the N-glycosidic bond is cleaved in the intestine before absorption [29]. Therefore, the oxidized base found in urine can originate from both the diet and from internal sources. The oxidized nucleoside, however, can only originate from the body's own cells. The intracellular origin of the oxidized nucleosides is not established, but theoretically it can originate from either DNA/RNA or from the corresponding intracellular nucleotide pools. There are indications that oxidation does occur in the nucleotide pool [30], however, the quantitative contribution is not established. It is therefore possible that the oxidized nucleosides found in urine originate from the oxidized nucleic acid either from oxidation of the nucleic acids DNA/RNA and subsequent repair/breakdown, or from incorporation of the oxidized nucleotide into the nucleic acid and subsequent removal or breakdown, or from the nucleotide pool. DNA and RNA are intracellular, and therefore the urinary excretion reflects the intracellular oxidative stress [28].

Smoking and sex hormones are associated with increased urinary excretion of the oxidation product of DNA,

8-oxodG, [31,32]. For the RNA oxidation product only a few studies have investigated factors associated with increased urinary excretion, but now we have established that obesity is such a factor, whereas it is not associated with increased excretion of the DNA oxidation product. The difference between DNA and RNA oxidation indicates the presence of different types of intracellular oxidative stress that may determine the disease development. This is corroborated by the fact that only DNA oxidation has been associated with cancer [32,33], whereas only RNA oxidation has been associated with hemochromatosis genotype and mortality among patients with T2D [3,4,34]. The differentiated intracellular oxidative stress can be explained by the differences in compartmentalization and structure of DNA and RNA in the cell; DNA is located in the nucleus while RNA is located in the cytosol near the mitochondria which is the main producer of ROS. DNA is double-stranded, while RNA mostly is single-stranded, and, furthermore, DNA is protected by histone proteins [35]. However, these histone molecules can be modulated by histone deacetylases and demethylase [36] which altogether could explain the versatile pattern of associations between DNA and RNA oxidation and conditions/diseases.

The present study supports previous findings that only RNA oxidation is associated with mortality among patients with T2D [3,4]. Here we report that also the RNA oxidation is increased in obese men, and it is well established that obesity is a major risk factor in the development of diabetes. Our study design does not investigate the causal link between RNA oxidation and obesity/obesity related co-morbidities. Regardless, the urinary excretion of the RNA oxidation product 8-oxoGuo seems to have prognostic value and has the potential to be utilized as a biomarker not only in T2D, but also the pre-diabetic condition obesity.

Conclusions

We conclude that obesity in men is associated with oxidative damage to RNA. The finding of a 30% increased RNA oxidation, but not DNA oxidation, in obese men is in line with our previous finding that only the RNA oxidation predicts mortality in T2D. Whether the increased oxidative damage to RNA among obese individuals leads to increased morbidity and mortality still needs to be clarified. We did not find differences in oxidative stress to DNA or RNA in obese men with or without hypertension. Perhaps a more specific marker of vascular oxidative damage is required to investigate the hypothesis of oxidative stress-mediated hypertension.

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Disclosure statement

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