



Original article

Urinary markers of nucleic acid oxidation increase with age, obesity and insulin resistance in Danish children and adolescents



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ARTICLE INFO

Keywords:

8-oxoGuo

8-oxodG

Cardiometabolic risk factors

Childhood obesity

Insulin resistance

Oxidative stress

ABSTRACT

Purpose: Oxidative stress may play an important role in childhood obesity and increased cardiometabolic risk. 8-oxo-7,8-dihydroguanosine (8-oxoGuo) from oxidation of RNA and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) from oxidation of DNA are excreted into urine and function as biomarkers for oxidative stress reflecting the modification rate of nucleic acids by oxidation. This study investigates the associations between urinary markers of nucleic acid oxidation and Body Mass Index (BMI), age, sex and cardiometabolic risk factors in children and adolescents with and without obesity.

Methods: We studied 543 children and adolescents from an obesity clinic cohort (n = 418) and a population-based cohort (n = 125), all aged 6–18 years. Anthropometrics, urine and blood samples were collected. A validated liquid chromatography-tandem mass spectrometry method was used to measure the nucleic acid oxidation markers.

Results: Compared with the population-based cohort, children and adolescents in the obesity clinic cohort had higher calculated 24-h excretion of 8-oxoGuo (p = 0.045) and 8-oxodG (p = 0.014) adjusted for basal metabolic rate. Both oxidation markers were positively associated with age and female sex (all p < 0.002). In the obesity clinic cohort the RNA oxidation marker 8-oxoGuo correlated with serum insulin (rho = 0.18, p = < .001) and insulin resistance (rho = 0.19, p = < .001).

Conclusions: Childhood obesity associate with higher urinary excretion of nucleic acid oxidation biomarkers, and increase with age throughout childhood, mirroring the obesity- and age-related increase shown in adults. Finally, children with obesity and insulin resistance had higher RNA oxidation markers than children with obesity and no insulin resistance, supporting a possible link between oxidative stress and the pathogenesis of cardiometabolic risk including type 2 diabetes.

1. Introduction

Childhood obesity remains an important health problem, and the worldwide prevalence of overweight or obesity in children has now reached 18% [1,2]. WHO estimates that 60% of children with

overweight before puberty will exhibit overweight or obesity in adulthood [2–4]. High levels of oxidative stress have been proposed as a common aetiology for the increased risk of cardiovascular disease and type 2 diabetes observed in adult obesity [5,6]. Whether markers of oxidative stress associate with obesity and cardiometabolic risk already

Abbreviations: 8-oxodG, 8-oxo-7,8-dihydro-2'-deoxyguanosine; 8-oxoGuo, 8-oxo-7,8-dihydroguanosine; BMI, Body mass index; BMR, Basal metabolic rate; DXA, Dual-energy X-ray absorptiometry; GFR, Glomerular filtration rate; HOMA-IR, Homeostasis model of assessment - insulin resistance; ROS, Reactive oxygen species; SDS, Standard deviation score

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<https://doi.org/10.1016/j.freeradbiomed.2020.05.009>

Received 20 March 2020; Received in revised form 5 May 2020; Accepted 12 May 2020

Available online 25 May 2020

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in childhood remains largely unknown.

The classic definition of oxidative stress is a situation that occurs when generation of reactive oxygen species (ROS) overwhelm the antioxidant defence mechanisms of the cell [7]. The complexity of oxidative stress is nuanced by the concepts of eustress and distress. Eustress is beneficial physiological regulation of oxidative processes, whereas distress is damaging oxidative modifications [8]. When oxidation modifies or damages nucleic acids the degradation products from RNA and DNA are detectable in urine as 8-oxo-7,8-dihydroguanosine (8-oxoGuo) and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), respectively [9].

Urinary concentrations of these oxidation markers reflect an average rate of oxidatively generated modifications on RNA and DNA in the organism. Both the ROS formation and the antioxidant defense mechanisms affect this rate; accordingly a rise in urinary concentrations of these oxidation markers can reflect a change in various cellular mechanisms [10]. ROS are highly reactive but have short half-lives and therefore predominantly react with molecules close to their site of generation. The compartmentalization of both RNA and DNA plays a crucial role in the oxidation-process by ROS. RNA is single stranded and dominant in the cytosol close to the mitochondria. DNA is located in the nucleus, is double-stranded and protected by proteins. This is assumedly one reason why RNA oxidation exceeds DNA oxidation [11].

Recent studies have reported associations between urinary concentrations of 8-oxoGuo and 8-oxodG and the pathogenesis of chronic disorders, such as obesity, type 2 diabetes and cardiovascular disease [5,12–14]. The non-invasive nature of these urinary biomarkers suggests a large clinical potential, but further investigations are necessary to use and understand these markers, especially in children [15].

While oxidative stress is recognized as an inherent part of aging biology from adulthood to senescence, far less focus has been given to the early aging process from childhood to early adulthood [16]. A pilot study from our group investigated urinary markers of nucleic acid oxidation in a small pediatric sample and found indications that children with obesity and impaired glucose tolerance had increased oxidative stress, but no significant associations with the degree of obesity [17].

To test the hypothesis that children with obesity have increased levels of oxidative stress, we examine how concentrations of the urinary markers 8-oxoGuo and 8-oxodG vary with body mass index (BMI) standard deviation scores (SDS), age and sex in a large sample of children and adolescents with normal weight and with overweight/obesity. Finally, we investigate associations between 8-oxoGuo and 8-oxodG and cardiometabolic risk factors.

2. Materials and methods

2.1. Design and study population

This cross-sectional study included children and adolescents from an obesity clinic cohort and a population-based cohort included in the Danish Childhood Obesity Data- and Biobank [18,19], who had delivered a urine sample. The obesity clinic cohort (n = 445) was recruited from January 2009 to August 2016, as they were enrolled in a multidisciplinary treatment program at Copenhagen University Hospital Holbæk [18]. The population-based cohort (n = 130) was recruited at local schools, public dentistry and health care nurses from 11 municipalities in the region of Zealand and the Capital region in Denmark from March 2012 to February 2015 to reflect the contemporary Danish pediatric population.

The same exclusion criteria was applied for both cohorts: 1) age younger than 6 or older than 18.9 years (n = 12); 2) insufficient urine sample volume (n = 5); 3) more than 60 days between urine samples and anthropometrics (n = 11); 4) known chromosome disorder (n = 0); 5) diagnosed type 1 diabetes (n = 2); 6) diagnosed type 2 diabetes (n = 0); 7) intake of insulin, Liraglutide, or metformin

(n = 2).

2.2. Ethics

All participants and their parents gave informed consent. Written consent was obtained from children's parents if younger than 18 years. The study was approved by the Scientific Ethics committee of Region Zealand, Denmark (protocol no. SJ-104) and is a part of The Danish Childhood Obesity Biobank; [ClinicalTrials.gov](https://clinicaltrials.gov) ID-no: NCT00928473, registered June 25, 2009.

2.3. Anthropometrics and blood pressure measurements

Medical professionals measured anthropometrics with the children wearing light indoor clothes and without shoes. A stadiometer was used to measure the height to the nearest 1 mm and weight was measured to the nearest 0.1 kg. The weight scale used on the obesity clinic cohort was a Tanita Digital Medical Scale, WB-110 MA (Tanita Corp., Tokyo, Japan) and the population-based cohort used a Tanita BC-418 Scale (Tanita Corp., Tokyo, Japan).

We used the oscillometric device Omron 705IT (Omron Healthcare Co., Ltd., Kyoto, Japan) and performed three blood pressure measurements in supine position, after a 5 min rest and with 5 min between each measurement. The last two measurements were used to calculate average SDS according to American National Institutes of Health reference values [20].

BMI was calculated from the measured height and weight, and BMI-SDS values were derived according to a Danish reference using the Lambda-Mu-Sigma method [21].

2.4. Biochemical analysis

Blood samples were drawn from a peripheral venous catheter between 7-9 am after an overnight fast. Samples of fresh voided urine was obtained from the participants and immediately stored at -80 °C until analysis. Blood biomarkers included plasma glucose, serum insulin, and whole blood HbA1c, as well as plasma total-, low-density (LDL)- and high-density (HDL)-cholesterol, triglycerides and alanine aminotransferase (ALAT). Analyses were conducted at Department of Clinical Biochemistry, University Hospital Holbæk and followed standard optimization and quality control as per hospital policy [19,22,23].

The urinary concentrations of 8-oxoGuo, 8-oxodG and creatinine were measured by ultra-performance liquid chromatography coupled with tandem mass spectrometry as previously described [24]. Analyses were conducted at Laboratory of Clinical Pharmacology at Rigshospitalet, Department of Clinical Pharmacology, Bispebjerg Frederiksberg Hospital, University Hospital Copenhagen. The laboratory is ISO accredited by the Danish Laboratory Accreditation Fund DANAK.

Based on a recent publication presenting a physiological model that estimates 24-h urinary excretion for both 8-oxoGuo and 8-oxodG [25] we calculated 24-h urinary excretion for all individuals based on urinary concentrations of the oxo-guanine values and creatinine, plasma concentrations of creatinine, age, sex, height, weight and glomerular filtration rate (GFR) [26,27]. The calculated 24-h excretion is a more reliable estimate for repeated measurements with changing physiology or for comparing populations with different physiological characteristics, as is the case in pediatric cohorts, than simply adjusting for urinary creatinine concentration [25]. Furthermore, 24-h excretion was adjusted for basal metabolic rate (BMR) [28] to adjust for the difference in cell activity and enable comparison across the pediatric age range. The BMR-adjusted values will represent a measure of modifications to nucleic acids related to oxygen consumption, i.e. for 8-oxoGuo it can be interpreted as mitochondrial efficiency.

2.5. Definition of insulin resistance

Insulin resistance was defined as a homeostasis model of assessment insulin resistance (HOMA-IR), value above the 90th percentile of published age- and sex specific population-based reference values from our group [22]. HOMA-IR was calculated as serum insulin (mU/L) x plasma glucose (mmol/L) /22.5.

2.6. Statistical analysis

All analysis were performed using R v. 3.5.2 [29]. Normality of data was assessed with histograms and qq-plots. As data was non-normally distributed, non-parametric analyses were performed. Differences between the cohorts were tested using Wilcoxon rank-sum or chi-squared test. For each cohort percentile curves for 8-oxoGuo and 8-oxodG were calculated using the Generalized Additive Models for Locations Scale and Shape (GAMLSS) package [30], with the Box-Cox distribution family; determining best fit by the Akaike Information Criterion. Associations between 8-oxoGuo and 8-oxodG and age and sex were examined with linear regression models, while associations with cardiometabolic risk factors were tested with Spearman partial correlation coefficients adjusted for age and sex. Multiple comparisons were controlled for using Bonferroni correction and robustness of significant associations was further tested in a sensitivity analysis without the 5% high and low oxidation marker values.

3. Results

3.1. Baseline characteristics and effect of obesity

A total of 543 children and adolescents were included in the study (n = 418 from the obesity clinic cohort; n = 125 from the population-based cohort). Baseline characteristics are shown in Table 1. Children in the obesity clinic cohort were on average 1 year older, 9 cm taller and had significantly higher BMI-SDS (2.86 vs. 0.38, p > 0.001) than the population-based cohort.

Calculated 24-h excretion of 8-oxoGuo (RNA oxidation marker) and 8-oxodG (DNA oxidation marker) were higher in the obesity clinic cohort compared with the population-based cohort, both unadjusted and adjusted for BMR (8-oxoGuo: 9.4 vs. 8.4 nmol/24h/kcal, p = 0.045; 8-oxodG: 6.6 vs. 5.4 nmol/24h/kcal, p = 0.014) (Table 1). No differences were found between the concentrations of 8-oxoGuo and 8-oxodG in the two cohorts when simply adjusting for urinary creatinine concentration

Table 1

Descriptive information and biomarkers of the 543 study participants.

	Obesity clinic cohort	Population-based cohort	P-value
N	418	125	
Age (years)	11.4 [9.4, 13.3]	10.4 [8.4, 12.7]	0.021
Sex (girl/boy, %)	51/49	58/42	0.146
Height (cm)	152 [142, 163]	146 [134, 160]	< .001
Weight (kg)	59.7 [48.7, 78.7]	38.0 [28.5, 48.4]	< .001
Waist (cm)	89 [80, 99]	65 [60, 72]	< .001
BMI SDS	2.86 [2.48, 3.30]	0.38 [-0.13, 1.32]	< .001
Pubertal status (prepubertal/pubertal, %)	43/57	36/64	0.300
<i>24-h values, unadjusted</i>			
8-oxoGuo (RNA) (nmol/24h)	14.8 [12.2, 19.1]	11.5 [7.7, 16.8]	< .001
8-oxodG (DNA) (nmol/24h)	10.7 [8.4, 13.8]	7.2 [4.8, 12.9]	< .001
<i>24-h values, BMR-adjusted</i>			
8-oxoGuo (RNA) (nmol/24h/kcal)	9.4 [7.8, 11.0]	8.4 [5.5, 12.1]	< .001
8-oxodG (DNA) (nmol/24h/kcal)	6.6 [5.3, 8.2]	5.4 [3.6, 9.4]	< .001
<i>Spot urine values, creatinine-corrected</i>			
8-oxoGuo (RNA) (nmol/mmol creatinine)	2.0 [1.7, 2.4]	2.0 [1.4, 2.9]	0.762
8-oxodG (DNA) (nmol/mmol creatinine)	1.4 [1.2, 1.8]	1.4 [0.9, 2.2]	0.485

Data are medians and IQR, or prevalence in %. Urinary biomarkers are calculated 24-h values based on glomerular filtration rate, plasma creatinine, urinary 8-oxoGuo/8-oxodG and urinary creatinine from morning spot urine; values are further presented relative to basal metabolic rate (BMR). Creatinine corrected spot urine values are included for comparison. BMI SDS: Body mass index standard deviation score. P values are calculated by Wilcoxon rank sum test or chi-squared test.

(Table 1). Repeating the analyses including only the normal weight participants in the population-based cohort did not change the results.

3.2. Correlations with age and sex

Fig. 1 presents percentile curves for BMR-adjusted 24-h 8-oxoGuo and 8-oxodG values from the two cohorts. In a linear regression model, BMR-adjusted 24-h 8-oxoGuo and 8-oxodG were found increase with age in the obesity clinic cohort (p = 0.001 and p < 0.001, respectively). The same trend was visually observed in the population-based cohort (Fig. 1, right panels), but only reached statistical significance for 8-oxodG (p = 0.203 and p = 0.033, respectively). Adjusted for age, female sex was associated with 1.0 nmol/24h/kcal higher BMR-adjusted 8-oxoGuo and 1.3 nmol/24h/kcal higher BMR-adjusted 8-oxodG values in the obesity clinic cohort (both p < 0.001). Similar associations were found in the population-based cohort, without reaching statistical significance (p = 0.479 and 0.251, respectively).

3.3. Cardiometabolic risk factors

Table 2 shows age- and sex-adjusted correlations between BMR-adjusted 24-h 8-oxoGuo and 8-oxodG and cardiometabolic risk factors in the obesity clinic cohort. After Bonferroni correction for multiple comparisons the RNA oxidation marker 8-oxoGuo was significantly correlated with insulin (rho = 0.18, p < 0.001) and HOMA-IR (rho = 0.19, p < 0.001), and the DNA oxidation marker 8-oxodG was negatively correlated with total cholesterol (rho = -0.15, p = 0.003). Only the association between 8-oxoGuo and insulin and HOMA-IR persisted following a further sensitivity analysis without the 5% high and low oxidation marker values (p = 0.002, p = 0.003 and P = 0.081, respectively). Lastly, 44% of children and adolescents in the obesity clinic cohort exhibited insulin resistance. These patients had a larger BMI SDS (3.06 [interquartile range (IQR) 2.72, 3.47] vs. 2.73 [IQR 2.37, 3.08], p < 0.001) and higher BMR-adjusted 24-h 8-oxoGuo concentrations (9.8 [8.1, 11.4] vs. 9.0 nmol/24h/kcal [7.5, 10.7], p = 0.009) than patients without insulin resistance. No such differences were found for BMR-adjusted 24-h 8-oxodG concentrations.

4. Discussion

In the current study, we demonstrated correlations between the urinary nucleic oxidation markers 8-oxoGuo and 8-oxodG and the degree of obesity, age and female sex in a cohort of children and

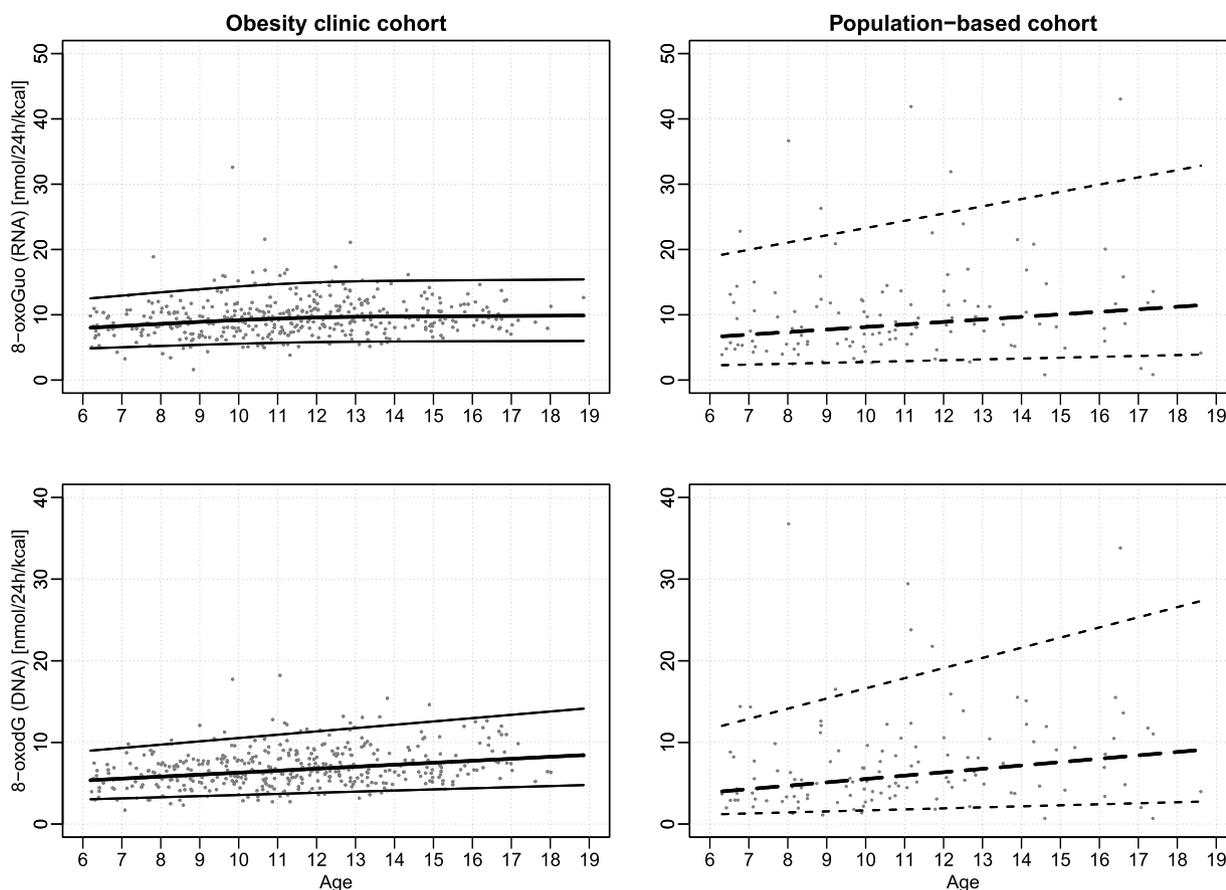


Fig. 1. Smoothed fifth, 50th and 95th percentile curves for urinary markers of RNA and DNA oxidation for the obesity clinic cohort (left panels, full lines) and the population-based cohort (right panels, dotted lines).

Table 2

Spearman partial correlation coefficients between urinary RNA and DNA oxidation markers and cardiometabolic risk factors in children and adolescents enrolled in obesity treatment.

	8-oxoGuo (RNA)	8-oxodG (DNA)
	<i>rho</i> (<i>P</i> -value)	<i>rho</i> (<i>P</i> -value)
Anthropometrics		
BMI SDS	0.01 (0.894)	-0.02 (0.694)
Waist (cm)	0.12 (0.019)	0.06 (0.249)
Total fat mass% (DXA)	0.14 (0.006)	0.10 (0.049)
Glucose Metabolism		
Glucose (mmol/L)	0.11 (0.027)	0.09 (0.070)
HbA1c (mmol/mol)	0.04 (0.368)	-0.07 (0.161)
Insulin (pmol/L)	0.18 (< .001)	0.11 (0.019)
HOMA-IR	0.19 (< .001)	0.12 (0.017)
Lipid profile		
Cholesterol (mmol/L)	0.02 (0.732)	-0.15 (0.003)
LDL-C (mmol/L)	0.02 (0.649)	-0.14 (0.005)
HDL-C (mmol/L)	-0.09 (0.081)	-0.07 (0.179)
Triglycerides (mmol/L)	0.14 (0.006)	0.06 (0.263)
Liver marker		
ALAT (U/L)	-0.00 (0.971)	-0.09 (0.070)
Blood pressure		
Systolic BP z-score	0.04 (0.496)	0.08 (0.126)
Diastolic BP z-score	0.03 (0.520)	0.06 (0.240)

Values are age- and sex-adjusted Spearman partial correlation coefficients and *P* values for basal metabolic rate (BMR)-adjusted 24-h urinary 8-oxoGuo (RNA) /8-oxodG (DNA) concentrations and cardiometabolic risk factors. Abbreviations: BMI SDS = Body mass index standard deviation score. HbA1c = Hemoglobin A1c. HOMA-IR = Homeostasis Model of Assessment Insulin Resistance. ALAT = Alanine aminotransferase. Significant results are bold following Bonferroni correction.

adolescents with and without overweight/obesity. These findings extend the findings of both correlations with obesity and an age-related increase found in studies in adults [5,16]. Interestingly, children and adolescents with obesity and insulin resistance had higher RNA oxidation markers than children with obesity and no insulin resistance.

4.1. Physiology and existing knowledge

RNA and DNA oxidation reflect different consequences of redox imbalance. The mitochondria are well-known contributors of ROS and assumedly the predominant source of ROS oxidizing RNA. The leak of ROS from the mitochondria can vary especially under pathophysiological circumstances and can result in increased ROS exposure.

RNA oxidation and the excretion of 8-oxoGuo may thus be understood as a marker of mitochondrial dysfunction. In newer studies RNA oxidation, measured by urinary excretion of 8-oxoGuo, is associated with all-cause mortality [12,31] and cardiovascular mortality [13] in patients with type 2 diabetes. Historically, DNA oxidation measured as 8-oxodG excretion have mainly shown associations with carcinogenesis, such as colorectal [32], breast [33–35], and lung cancer [33]. Underlying molecular events are pre-mutagenic transversion mutations of G-C to A-T from oxidation of the guanine base in DNA [36].

Empirically, RNA oxidation is associated with complications to metabolic disease and DNA is associated with development of cancer. A recent study also associated high RNA and DNA oxidation with development of type 2 diabetes, however, the underlying molecular events are not clearly defined. The applied technique with urinary marker OxoGua cannot determine if RNA or DNA oxidation is dominant or both equally involved [37].

4.2. Obesity and resilience

Obesity in adults is known to cause both increased oxidative stress levels and inflammation [38]. A large number of epidemiological studies have demonstrated the effect of high BMI-SDS and an associated high risk of developing chronic diseases. In this study we tested the hypothesis that children with obesity would have increased levels of oxidative stress. Our findings showed higher values of RNA and DNA oxidation markers in the obesity clinic cohort than in the population-based cohort, suggesting that obesity already early in life can affect oxidative stress levels. The physiological link between obesity and oxidative stress is not clarified yet, but might be related to induced production of obesity-related hormones/cytokines as part of a general (metabolic) stress.

Furthermore, aging has been linked to both increased oxidative stress and low grade inflammation in adults [39,40]. In our study we demonstrated an increase in oxidative stress with age in children with and without obesity for the BMR-adjusted 24-h nucleic acid excretion. This supports our hypothesis based on epidemiological studies in adults, that states that free oxygen radicals over time will lead to cumulative damage within the cell, suggesting a correlation between ROS-production and life longevity [16].

4.3. Nucleic acid oxidation and cardiometabolic risk

We showed an association between 8-oxoGuo concentrations and insulin resistance. Additionally, we found that the 44% of the children in the obesity clinic exhibited insulin resistance and that these children had higher 8-oxoGuo concentrations. This finding indicates a link between glucose metabolism and RNA oxidation, that has not previously been established in children [41].

Adults with type 2 diabetes have increased urinary excretion of 8-oxoGuo [39]. Generally the increase in patients with type 2 diabetes is about 30%, similar to what is observed in patients with obesity [5], in contrast the individual variation is more than 5–10 fold [42]. The first and fourth quartile in patients with type 2 diabetes differ with a factor of about 2–3 and the corresponding HR for death is 1 vs 1.82 [12]. This would indicate that an increase of 10% in 8-oxoGuo corresponds to an increase in HR for death of about 5–10%, which is of importance for the individual patient and from a public health point of view.

Large cohort studies comparing 8-oxoGuo excretion in patients with and without type 2 diabetes and their mortality have not been published. It is further unclear whether increased RNA oxidation, as detected by 8-oxoGuo, plays a role of the pathogenesis of insulin resistance or is a cellular response to insulin resistance. To strengthen these findings further studies will be necessary to unravel RNA-oxidations' role in insulin resistance and aging for both children and adults, as they might have an important predictive and clinically beneficial value for patients with type 2 diabetes.

4.4. Strength and limitations

Strengths of this study include the homogenous paediatric cohorts from a well-defined geographic location, making the two cohorts easily comparable. Additionally, all samples were collected in a narrow timeframe early morning after an overnight fast, and analysis done in the same batch using standardized laboratory procedures for all participants.

A limitation to this study is the cohort selection. The children included in the present study represent a subgroup from a larger total patient cohort. All participants in the total patient cohort were asked to deliver urine samples, but only 1/3 delivered urine samples, why our results from the population-based cohort might differ from the population as a whole. Another limitation is the use of spot urine samples in this study. The golden standard would be a 24-h collection of urine, which would represent all guanines oxidized in RNA and DNA in the

body in the period. A 24-h collection was not feasible in this study, but a new physiological model with equations that take body composition and kidney function into account, has made it possible to estimate a more precise, reliable and individual 24-h excretion for each spot urine sample. The model does not solve all problems related to measuring and comparing urinary values across the pediatric age range, but it is a considerable improvement from the former approach for calculating oxidative stress levels, especially when investigating patient groups with considerable variations in body physiology.

5. Conclusion

We have shown a positive association between the degree of obesity and urinary oxidation markers of RNA and DNA in children and adolescents. We further demonstrated an age-dependent increase in these nucleic acid oxidation biomarkers, mirroring the obesity- and age-related increase found in adults. Lastly, the RNA oxidation marker 8-oxoGuo was increased in children with obesity and insulin resistance compared with children with obesity and no insulin resistance. In adults with type 2 diabetes increased concentrations 8-oxoGuo are known to associate with mortality, but the role of 8-oxoGuo as a biomarker for progression of insulin resistance already in childhood is unknown. Therefore, further investigations are necessary to clarify the role of oxidative stress in childhood obesity and the potential of these biomarkers for prediction of cardiometabolic morbidity and type 2 diabetes.

Acknowledgements

This study is part of the research activities in TARGET (The Impact of our Genomes on Individual Treatment Response in Obese Children), BIOCHILD (Genetics and Systems Biology of Childhood Obesity in India and Denmark), Tripartite Immunometabolism Consortium (TriC) and MicroLiver. The study is part of The Danish Childhood Obesity Data- and Biobank; [ClinicalTrials.gov](https://doi.org/10.1186/1745-2974-1-1) ID-no.: NCT00928473, registered June 25th, 2009. The Novo Nordisk Foundation Center for Basic Metabolic Research is an independent Research Center at the University of Copenhagen partially funded by an unrestricted donation from the Novo Nordisk Foundation. This research has been conducted using the Danish National Biobank resource, supported by the Novo Nordisk Foundation.

The authors wish to thank Mrs. Oda Troest, Mrs. Birgitte Holløse and Mrs. Trine Henriksen for their invaluable assistance with biological samples and database. The authors also wish to thank the Department of Clinical Pharmacology at Copenhagen University Hospital, Rigshospitalet.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.freeradbiomed.2020.05.009>.

Funding

This work was supported by the Innovation Fund Denmark [grant numbers 0603-00484B, 0603-00457B]; the Novo Nordisk Foundation [grant number NNF15OC0016544, NNF15CC0018486, NNF15OC0016692]; and The Danish Heart Foundation [grant number 18-R125-A8447].

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