Free Radical Biology and Medicine 129 (2018) 532–536

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original article

RNA oxidation and iron levels in patients with diabetes

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ARTICLE INFO

Keywords:
Oxidative stress
Oxidative modification
8-oxo-7,8-dihydro-guanosine
8-oxo-7,8-dihydro-2′-deoxyguanosine
Iron
Diabetes

ABSTRACT

Aim: The urinary biomarker for oxidative stress to RNA, 8-oxo-7,8-dihydro-guanosine (8-oxoGuo) is associated with mortality in patients with type 2 diabetes. Iron has also been linked to diabetes. In individuals with untreated hereditary iron overload it has been observed that 8-oxoGuo was higher compared to controls. In the current study, we hypothesized that 8-oxoGuo was associated with diagnosis of diabetes, and that iron confounded this association.

Methods: Participants from a general Danish population were included in the study (n = 3567). UPLC-MS/MS method was used for 8-oxoGuo (nmol/mmol creatinine) measurement in spot urine. Iron biomarkers included total plasma iron, ferritin, transferrin saturation (TS) and transferrin.

Results: 8-oxoGuo was 17% higher in diabetes patients (n = 208) compared to non-diabetes controls. Unadjusted logistic regression model showed an odds ratio of diabetes of 1.38 (95%CI:1.21–1.57, P < 0.0001) per unit increase of 8-oxoGuo. When the model was adjusted for possible confounders the odds ratio was 1.09 (95%CI:0.94–1.26, P = 0.24). When additional adjustment was performed including ferritin, TS, or transferrin, respectively, the OR were 1.14 (95%CI:0.97–1.33, P = 0.09), 1.10 (95%CI: 0.95–1.28, P = 0.18), and 1.17 (95%CI:1.01–1.38, P = 0.04).

Conclusions: Our study indicates that 8-oxoGuo is higher in diabetes patients. The lack of association between 8-oxoGuo and diabetes in the adjusted model may be due to the cross-sectional design including post-treatment bias. Our data did not show consistent effect of all iron biomarkers in relation to diabetes. Most likely, the iron biomarkers were affected by inflammation thus not reflecting true iron levels.

1. Introduction

Presence of oxidative stress has been proposed to be an important component in the pathogenesis of any type of diabetes [1]. A contributing biological mechanism that increases oxidative stress is based on the Fenton reaction, where iron shifts from ferrous to ferric state accompanied by generation of the hydroxyl radical. A biomarker of oxidative stress is urinary excretion of the oxidative modification to RNA 8-oxo-7,8-dihydro-guanosine (8-oxoGuo). The hydroxyl radical, belonging to the group of Reactive Oxygen Species (ROS) may induce multiple modifications by oxidation to cellular structures such as RNA [2]. This is supported by previous findings of a higher 8-oxoGuo in untreated genetic iron overload (hereditary hemochromatosis) compared to controls, and a decrease in 8-oxoGuo after iron removal by phlebotomy [3]. In a previous Asian study of hospital-ascertained patients with type 2 diabetes (T2D) of which one third had severe complications, levels of 8-oxoGuo were higher in T2D patients compared to controls [4]. But no previous study in Caucasians or from the general population comparing levels of 8-oxoGuo in patients with T2D and controls has been performed. In Danish patients with T2D followed up for up to 18 years, we have previously shown that 8-oxoGuo is associated with mortality [5,6] Moreover, several studies have linked iron biomarkers to risk of T2D, diabetic complications, and metabolic disturbances [7].

Despite control of blood glucose, blood pressure and dyslipidemia, T2D patients have considerable excess co-morbidity and mortality

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https://doi.org/10.1016/j.freeradbiomed.2018.10.420
Received 18 April 2018; Received in revised form 10 September 2018; Accepted 9 October 2018
Available online 16 October 2018

indicating non-glucose pathogenic factors and a need for their identification and exploration of their importance. We hypothesise that oxidative stress to RNA and iron dysregulation could be a relevant contributing pathogenic mechanism in T2D patients. Therefore, we aimed to investigate 1) if 8-oxoGuo urinary excretion was associated with diabetes and 2) if iron biomarkers confounded this association.

2. Material and methods

2.1. Study population

The study population consisted of participants from the Danish General Suburban Population Study (GESUS) and details are described elsewhere [8]. In brief, information on participants originates from a paper-questionnaire, health examination, biochemical measurements and genotyping. All participants were Danish citizens and > 99% of Danish descent. Eligibility criteria for participation were: living in the Naestved municipality, being above 20 + years, and having a Danish citizenship. We included all participants with measured levels of 8-oxoGuo, the corresponding oxidative modification of DNA 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), and creatinine concentration (n = 3608).

The study was approved by the Regional Ethics committee for Region Zealand (SJ-113, SJ-114) and was reported to the Danish Data Protection Agency (REG-27–2014). The study was performed in agreement with the Helsinki declaration and all participants gave informed written consent.

2.2. Primary endpoint

We classified participants as having known diabetes if they reported in the questionnaire a diagnosis of diabetes or treatment with insulin or other types of antidiabetic medication. Any type of diabetes was included since the self-administered questionnaire did not include distinction between different types of diabetes. We classified participants as having undiagnosed diabetes if they did not report diagnosis of diabetes/use of antidiabetic medication but had a non-fasting plasma glucose ≥ 11.1 mmol/l and/or HbA_{1c} ≥ 6.5% (48 mmol/mol) measured at the health examination. Participants without self-reported diabetes and with non-fasting plasma glucose/HbA_{1c} levels below the abovementioned cut-off levels were regarded as not having diabetes and used as controls.

2.3. Biochemical measurements

Spot urine specimens were collected at the day of the health examination (May 2011 to October 2013) and were stored in −20 °C freezers until analysis (June 2013–June 2015). We simultaneously measured 8-oxoGuo and 8-oxodG by UPLC-MS/MS analysis, which is considered the "gold standard" for measurement of oxidized nucleic acids, and described in detail elsewhere [9]. The urinary 8-oxoGuo and 8-oxodG concentrations were adjusted for urinary creatinine concentration to reduce variability [10].

Iron biomarker measurements consisted of plasma ferritin (μg/L), plasma transferrin (μmol/L) and total plasma iron (μmol/L), and were all measured with Cobas-6000 (Roche). Transferrin saturation (TS, in %) was calculated as 100 × [iron]/(2 × [transferrin]) since each molecule of transferrin can bind two iron atoms.

2.4. Other co-variates

Alanine amino transferase (ALAT, U/L), total cholesterol (mmol/L), high-sensitivity C-reactive-protein (hsCRP, mg/L) and non-fasting plasma glucose (mmol/L) were analysed with Cobas-6000 (Roche). HbA_{1c} (mmol/mol) was measured with Tosoh G7 and G8 (TOSOH). We present HbA_{1c} in both mmol/mol and percent based on the equation HbA_{1c} (%) = [0.0914* HbA_{1c} (mmol/mol)] + 2.152 [11]. Blood pressure (mmHg) was measured on the left upper arm after 5 min of rest (instrument type A&D UA-787, A&D Medical, Tokyo, Japan); measurement was performed twice and the second measurement registered. Waist circumference was measured using a tape measure and measured at the lowest rib (cm). Participants completed a self-administered questionnaire from where information was retrieved on smoking habit (never, former, current) and alcohol habits (units per week; 1unit = 12 g pure alcohol).

2.5. Statistical analyses

Statistical analyses were performed using R version 3.2.2 [12]. We observed one participant with extreme ferritin value above 6000 μg/L and concurrently ALAT above 1500 U/L. Two participants had extremely high concentration of 8-oxoGuo > 25nmol/mmol creatinine (~ 10x standard deviation above mean). These 3 participants were considered as outliers and excluded from analyses (see Supplementary Fig. S1). Thirty-seven participants had unknown status of diabetes (due to either missing HbA_{1c} measurements or missing questionnaire information), and additionally one participant did not have iron biomarker measurements; they were therefore excluded from the analyses. In total, we included n = 3567 in the analyses.

We explored the basic characteristics of the participants according to diabetes status. For normally distributed numerical variables mean, standard deviation (SD) and P values from *t*-test are presented. For non-normally distributed numerical variables median, interquartile range (IQR), and Mann-Whitney *U* test are presented. Number, frequencies, and chi-squared test are presented for categorical variables.

We examined the association between diabetes (no/yes) and the predictor 8-oxoGuo using logistic regression analyses of individuals with no missing values (n = 3557). The diabetes patients included both known diabetes and undiagnosed diabetes. Analyses were performed unadjusted as well as multivariable adjusted based on a priori knowledge of confounding. The co-variates consisted of age (years), sex (female/male), systolic blood pressure, waist circumference, smoking status, total cholesterol, hsCRP, alcohol intake, and ALAT. The co-variates ALAT and hsCRP were log-transformed and alcohol intake divided into quartiles (< 1.71, 1.71–12.0, 12.1–17.0 and > 17.0 units/week) because of non-normal distribution. In order to investigate the relationship between 8-oxoGuo and iron biomarkers, odds ratios (OR) from the adjusted logistic regression model with 8-oxoGuo as predictor was compared to an adjusted logistic regression of 8-oxoGuo controlled for each iron biomarker separately (i.e. additional adjustment with each iron biomarker).

Additionally, we investigated the association between diabetes (no/yes) and iron biomarkers (ferritin, transferrin saturation, and transferrin) using multivariable adjusted logistic regression. We adjusted for age (years), sex (female/male), systolic blood pressure, waist circumference, smoking status, total cholesterol, hsCRP, alcohol intake, and ALAT. The iron biomarkers were log2-transformed in all regression analyses and the exponentiated coefficient can thus be interpreted as the odds ratio of diabetes associated with a doubling of the corresponding iron biomarker.

Pearson correlation coefficient (Pearson's *r*) was used to investigate the correlation between 8-oxoGuo and HbA_{1c}. Linear regression for HbA_{1c} (log-transformed) as outcome regressed on 8-oxoGuo was performed unadjusted and adjusted for age and gender. We compared mean 8-oxoGuo between prevalent known diabetes patients with HbA_{1c} ≥ 6.5% (48 mmol/mol), known diabetes patients with HbA_{1c} < 6.5% (48 mmol/mol)), and undiagnosed diabetes using ANOVA.

3. Results

Basic characteristics of the participants according to diabetes status (no/yes) are presented in Table 1. The diabetes patients had a higher

Table 1
Basic characteristics of participants.

	No diabetes (controls) n = 3359	Diabetes n = 208	P value
Age, years			
Mean (SD)	57.1 (13.1)	64.9 (10.5)	< 0.0001
Sex, n (%)			< 0.001
Female	2007 (59.8)	99 (47.6)	
Male	1352 (40.2)	109 (52.4)	
8-oxoGuo, nmol/mmol creatinine			< 0.0001
Mean (SD)	2.4 (0.9)	2.8 (0.8)	
8-oxodG, nmol/mmol creatinine			0.99
Mean (SD)	1.9 (0.8)	1.9 (0.7)	
Ferritin, µg/L			0.08
Median (IQR)	120.0 (62.0–203.0)	137.5 (77.0–221.0)	
Transferrin, µmol/L			< 0.01
Mean (SD)	33.0 (4.9)	34.1 (5.4)	
Transferrin saturation, %			0.03
Mean (SD)	21.5 (7.8)	20.3 (7.4)	
Iron, µmol/L			0.18
Mean (SD)	13.9 (4.9)	13.5 (4.5)	
HbA_{1c}, mmol/mol			< 0.0001
Median	37.0 (35.0–39.0)	50.0 (45.0–57.0)	
Non-fasting glucose, mmol/L			< 0.0001
Median (IQR)	5.5 (5.1–5.9)	7.3 (6.2–9.5)	
WC, cm			< 0.0001
Mean (SD)	91.0 (12.9)	102.8 (12.5)	
Smoking status, n (%)			0.01
Never	1504 (44.8)	71 (34.1)	
Former	1283 (38.2)	92 (44.2)	
Current	572 (17.0)	45 (21.6)	
Alcohol intake, units/week,			< 0.01
Median (IQR)	6.9 (1.7–17.1)	3.4 (0–17.1)	
Systolic BP, mmHg			0.02
Mean (SD)	140.5 (21.1)	144.0 (20.8)	
Total cholesterol, mmol/L			< 0.0001
Mean (SD)	5.5 (1.0)	4.9 (1.2)	
ALAT, U/L			< 0.0001
Median (IQR)	22.0 (17.0–29.0)	26.0 (19.0–34.0)	
hsCRP, mg/L			< 0.0001
Median (IQR)	1.3 (0.6–2.8)	1.8 (1.0–3.8)	

Basic characteristics (n = 3567) according to diabetes status: The group 'no diabetes' include participants who report having no diagnosis of diabetes, no treatment with insulin or other antidiabetic medication, and whose level of non-fasting glucose and HbA_{1c} are < 11.1 mol/l and < 48 mmol/mol, respectively. The groups 'diabetes' include known diabetes patients (i.e. participants who reported having a diagnosis of diabetes, being treated with insulin or other antidiabetic medication) as well as participants with undiagnosed diabetes (i.e. those who did not report having a diagnosis of diabetes /treatment with antidiabetic medication but whose non-fasting glucose level was ≥11.1 mol/L and/or HbA_{1c} ≥48 mmol/mol when measured at the heath examination). T-test and Man-Whitney U were used to test differences in means for continuous normally distributed variables or medians for continuous non-normally distributed variables, respectively. For categorical variables, chi-squared test were used. BP: blood pressure, hsCRP: high sensitive C-reactive protein, WC: waist circumference.

mean age with a predominance of men compared to the group without diabetes. Mean 8-oxoGuo urinary excretion differed between diabetes patients and non-diabetes controls (P < 0.0001) being 17% higher in those with diabetes. Mean of the corresponding DNA modification 8-oxodG did not differ between the groups (P = 0.99) (Table 1).

Total plasma iron and ferritin did not differ between diabetes patients and controls (Table 1). Mean TS was 5.6% lower in diabetes patients (P = 0.03) while mean transferrin was 3.3% higher in diabetes patients (P < 0.01). *Post hoc* analyses revealed that ferritin and TS were higher in undiagnosed compared to prevalent known diabetes, however only median ferritin difference was significant (Supplementary Table S1).

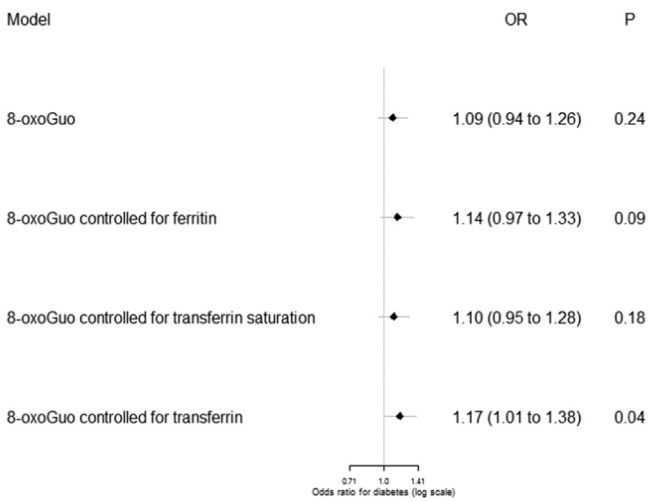

Fig. 1. Odds ratios for diabetes per unit change in 8-oxoGuo. All models were adjusted for age, sex, smoking, alcohol, systolic blood pressure, waist circumference, ALAT, cholesterol, and hsCRP. Iron biomarkers were log2-transformed. Ten observations were deleted due to missing values among these covariates; i.e. the adjusted regression models included n = 3557. There were 207 cases of diabetes (known diabetes: 169, undiagnosed diabetes: 38).

3.1. 8-oxoGuo, iron biomarkers and diabetes

Logistic regression revealed an OR of diabetes of 1.38 (95%CI: 1.21–1.57, P < 0.0001) per unit increase in 8-oxoGuo and OR of 1.23 (95%CI:1.06–1.41, P = 0.007) after adjustment for age and sex. Multivariable adjustments resulted in OR of 1.09 (95%CI:0.94–1.26, P = 0.24) (Fig. 1). Analyses for 8-oxoGuo and diabetes were also performed controlled for ferritin, TS, and transferrin, respectively (Fig. 1); resulting in OR of 1.14 (95%CI: 0.97–1.33, P = 0.09), 1.10 (95%CI:0.95–1.28, P = 0.18), and 1.17 (95%CI:1.01–1.38, P = 0.04). Logistic regression analyses for each of the iron biomarkers as predictor showed that only transferrin was associated with diabetes (OR = 4.86, 95%CI:2.25–10.53, P < 0.0001)(Supplementary Fig. S2).

3.2. 8-oxoGuo in relation to HbA_{1c}

Mean 8-oxoGuo concentration did not differ between known diabetes with HbA_{1c} below 48 mmol/mmol (n = 71, mean 8-oxoGuo = 2.76 nmol/mmol creatinine), known diabetes with HbA_{1c} above 48 mmol/mmol (n = 98, mean 8-oxoGuo = 2.78 nmol/mmol creatinine), and undiagnosed diabetes patients (n = 38, mean 8-oxoGuo = 2.69 nmol/mmol creatinine) (ANOVA: P = 0.88). Analysis of correlation between 8-oxoGuo and HbA_{1c} showed a Pearson's *r* of 0.11 (95%CI: 0.08–0.14, P < 0.0001)). Linear regression with HbA_{1c} (log-transformed) as outcome regressed on 8-oxoGuo showed an association (beta coefficient of 1.02 (95%CI: 1.01–1.02), P < 0.0001), however the association was not present when the model was adjusted for age and sex (beta coefficient of 0.99 (95%CI: 0.99–1.00), P = 0.36).

4. Discussion

4.1. 8-oxoGuo and diabetes

As the urinary biomarker 8-oxoGuo is associated with mortality in T2D patients, investigation of the association between 8-oxoGuo and diagnosis of diabetes is merited [5,6]. In the current study we find a higher urinary excretion of 8-oxoGuo in diabetes patients, observed

both in diagnosed and undiagnosed, compared to controls. Our study indicates that high RNA oxidation is associated with higher odds for having diabetes; however due to observational study design we cannot deduce the causal pathway. It is not known if increased oxidative damage to RNA may induce diabetes (i.e. 8-oxoGuo is a disease risk biomarker) or vice versa that the diabetic increases oxidative damage to RNA that in turn promotes diabetic complications (i.e. 8-oxoGuo is a complication risk biomarker). Examples of predisposed individuals for developing T2D (young men born with low birthweight, and obese children) do not seem to have higher RNA oxidation compared to controls [13,14]. However, we have previously observed that 8-oxoGuo was higher in adult obese men compared to lean controls [15]. We hypothesise that 8-oxoGuo may particularly increase after onset of diabetes and take part in the pathogenesis of diabetic complications, which is supported by the aforementioned study where 8-oxoGuo was especially high in patients with macrovascular complications, besides being higher in T2D patients compared to age-matched controls [4]. They also observed that the corresponding DNA modification by oxidation, 8-oxodG, was only moderately higher, which is in accordance with the finding that 8-oxodG is not prognostic in T2D mortality nor higher in obesity [5,6,15]. Likewise, the current study do not observe differences in mean 8-oxodG between diabetes patients and controls.

We observed that there was a correlation between 8-oxoGuo and HbA_{1c} but the correlation coefficient was small. Furthermore, 8-oxoGuo did not differ between undiagnosed diabetes patients, prevalent known diabetes patients with HbA_{1c} within treatment goal (according to current guidelines) and prevalent known diabetes patients not within HbA_{1c} treatment goal. This indicates that the current glycaemic control management does not lower 8-oxoGuo, although it may be a desirable treatment target due to its association with mortality.

4.2. Iron's links to 8-oxoGuo and diabetes

There are presumably several risk factors for increased 8-oxoGuo, such as age and obesity [10,15]. Recently, we published a study indicating a causal relationship between high iron levels (measured as ferritin and TS) and high 8-oxoGuo [16]. 8-oxoGuo has also been found to be considerably elevated in untreated hereditary hemochromatosis, however, levels decrease in response to phlebotomy [3]. Hereditary hemochromatosis is an iron accumulating condition which can manifest as diabetes if untreated, and iron overload in the general population has also been proposed to be involved in the pathology of diabetes [17]. A strong indication of a link between iron and diabetes is the improved metabolic profile observed after phlebotomy in diabetes patients where iron is removed through bloodletting [18]. A meta-analysis on the association between iron biomarkers and T2D from year 2014 reported that high levels of ferritin was associated with an elevated relative risk of T2D compared to low levels [19]. The association between TS and T2D was more unclear, and they found no evidence of association between transferrin and T2D. The present study cannot confirm associations between all iron biomarkers and diabetes. Iron biomarkers are unfortunately not specific for iron because they are influenced by other factors such as inflammation. Hence, an association between ferritin/TS and diabetes may be masked by presence of inflammation in diabetes patients; ferritin is an acute-phase protein that increases in inflammation, while TS decreases in inflammation [20]. This influence makes it difficult to interpret if iron confounds the association between 8-oxoGuo and diabetes. Our results did show an association between transferrin and diabetes and indicated that transferrin confounded the association between 8-oxoGuo and diabetes. The association between increased transferrin and diabetes has also been described in a prospective case-control study design [21]. Transferrin is a protein functioning as an iron transporter, and whose concentration is incorporated in the equation for calculation of TS. Transferrin has been proposed to be an anti-oxidant as it binds and prevents free iron from participating in the Fenton reaction [20]. Normally, transferrin is low in individuals with iron overload and is elevated in individuals with iron deficiency [19]. The higher level of transferrin observed among diabetes patients compared to controls in our study is therefore likely a reaction to inflammation characterized by functional iron deficiency [22].

4.3. Strengths and limitations

RNA is located in the cytosol, in contrast to the conventionally used extracellular biomarkers albumin and HbA_{1c} used in assessment of diabetes. Among strengths of the study is therefore the intracellular and hence potentially additional information 8-oxoGuo contributes with in evaluation of diabetes risk and/or disease progression. The cross-sectional study design is prone to limitations. The study covers a heterogeneous group of diabetes patients with heterogeneous disease duration, macro-and microvascular complications, and therapy; factors we did not assess in the current study design. We therefore speculate if the adjusted model may have included co-variates that were affected by antidiabetic treatment (non-pharmacological and pharmacological) thereby resulting in post-treatment bias, hence weakening the association to diabetes.

Our study included multiple iron biomarkers, thereby evaluating both iron storage (ferritin) and iron availability (TS). The iron biomarkers are to some extent exposed to reverse causation; i.e. the presence of inflammation in diabetes (or antidiabetic treatment) alters the iron biomarker concentrations. Nevertheless, by including several iron biomarkers our study elucidates that a snapshot based on a single blood sample in evaluation of iron overload in diabetes patients should be interpreted with caution. Even though analyses of the iron biomarkers did not confirm our hypothesis, we cannot rule out that true iron overload does not induce a causal pathway. We examined iron levels in a monotonically increased fashioned way; however the role of iron in diabetes may be especially relevant for a subgroup of the population with extremely high levels of iron, e.g. in hereditary or acquired hemochromatosis.

Our data includes both type 1 diabetes and T2D which may induce some variation. It is however presumed that T2D constitutes the main type (~90–95%) [23]. Finally, as previously shown, individuals participating in GESUS were healthier than non-participants [8], introducing selection bias with fewer diabetes cases compared to the background population and consequently leading to lower power.

4.4. Conclusions and perspective

This study indicates that 8-oxoGuo is higher in diabetes patients. Despite longstanding developments in antidiabetic treatment and improvement of micro-vascular complications, the improvement of the diabetes-associated cardiovascular mortality is questioned [24]. In the long term, identifying an intervention that reduces RNA oxidation, assessed by urinary 8-oxoGuo, may be beneficial for diabetes patients. The newer antidiabetic drug empagliflozin (SGLT-2 inhibitor) seems to reduce the cardiovascular mortality in diabetes patients [25]. In addition, it has been proposed that the drug may have an antioxidant effect [26]. Hypothetically, this effect may be one of the underlying biological mechanisms that decreases the diabetes related mortality.

Our data is inconclusive regarding iron's effects on the association between 8-oxoGuo and diabetes, since there was not a consistency in all iron biomarkers on the association between 8-oxoGuo and diabetes. Most likely, the iron biomarkers were influenced by other factors than iron per se (e.g. inflammation) thus not reflecting true iron levels. In order to evaluate if iron reduction may be beneficial in diabetes management, an intervention study is needed with inclusion of diabetes patients undergoing iron removal (by phlebotomy, iron chelator therapy or even dietary control) and measurement of all iron biomarkers with 8-oxoGuo as a surrogate endpoint. Follow-up via nationwide registers of good quality, as existent in Denmark, of co-morbidity and mortality could help identify if iron management and lowering of

8-oxoGuo has a clinically relevant role.

Acknowledgments

Funding: This study was supported by the Research Committee of the Capital Region of Denmark and the Toyota Foundation Denmark. The Danish General Suburban Population Study was founded by the Region Zealand Foundation; Naestved Hospital Foundation; Naestved Municipality; Johan and Lise Boserup Foundation; TrygFonden; Johannes Fog's Foundation; Region Zealand; Naestved Hospital; The National Board of Health; and the Local Government Denmark Foundation. V. Cejvanovic has received a scholarship from Faculty of Health and Medical Sciences, University of Copenhagen.

None of the funding sources were involved in the design, conduct of study, collection, management, analysis, or interpretation of data, preparation or review of manuscript, and had no right to approve or disapprove of the submitted manuscript.

We thank laboratory technician Katja Røhsle Luntang Christensen from the Laboratory of Clinical Pharmacology Q7642 for assisting UPLC-MS/MS analyses of the urine samples from GESUS.

Contribution statement

CE and HEP initiated the study; GESUS was initiated by CE and urinary measurements by HEP. All authors contributed to the concept of the study and interpretation of results. Urinary measurements were performed by TH. VC performed the statistical analyses guided by HEP, CE, and HKMB. VC drafted the manuscript and all authors contributed to the revisions.

Conflict of interest

The authors declare that they have no conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.freeradbiomed.2018.10.420.

References

[1] M.S. Shah, M. Brownlee, Molecular and cellular mechanisms of cardiovascular disorders in diabetes, Circ. Res. 118 (2016) 1808–1829, https://doi.org/10.1161/CIRCRESAHA.116.306923.
[2] F. Oliveira, S. Rocha, R. Fernandes, Iron metabolism: from health to disease, J. Clin. Lab Anal. 28 (2014) 210–218, https://doi.org/10.1002/jcla.21668.
[3] K. Broedbaek, H.E. Poulsen, A. Weimann, et al., Urinary excretion of biomarkers of oxidatively damaged DNA and RNA in hereditary hemochromatosis, Free Radic. Biol. Med. 47 (2009) 1230–1233, https://doi.org/10.1016/j.freeradbiomed.2009.08.004.
[4] X. Liu, W. Gan, Y. Zou, et al., Elevated levels of urinary markers of oxidative DNA and RNA damage in type 2 diabetes with complications, Oxid. Med. Cell Longev. 2016 (2016) 1–7, https://doi.org/10.1155/2016/4323198.
[5] K. Broedbaek, V. Siersma, T. Henriksen, et al., Urinary markers of nucleic acid oxidation and long-term mortality of newly diagnosed type 2 diabetic patients, Diabetes Care 34 (2011) 2594–2596, https://doi.org/10.2337/dc11-1620.
[6] K. Broedbaek, V. Siersma, T. Henriksen, et al., Association between urinary markers of nucleic acid oxidation and mortality in type 2 diabetes: a population-based cohort study, Diabetes Care 36 (2013) 669–676, https://doi.org/10.2337/dc12-0998.
[7] J.M. Fernández-Real, M. Manco, Effects of iron overload on chronic metabolic diseases, Lancet Diabetes Endocrinol. 2 (2014) 513–526, https://doi.org/10.1016/S2213-8587(13)70174-8.
[8] H.K.M. Bergholdt, L. Bathum, J. Kvetny, et al., Study design, participation and characteristics of the Danish general suburban population study, Dan. Med. J. 60 (2013) A4693.
[9] S.T. Rasmussen, J.T. Andersen, T.K. Nielsen, et al., Simvastatin and oxidative stress in humans: a randomized, double-blinded, placebo-controlled clinical trial, Redox Biol. 9 (2016) 32–38, https://doi.org/10.1016/j.redox.2016.05.007.
[10] R. Andreoli, A. Mutti, M. Goldoni, et al., Reference ranges of urinary biomarkers of oxidized guanine in (2′-deoxy)ribonucleotides and nucleic acids, Free Radic. Biol. Med. 50 (2011) 254–261, https://doi.org/10.1016/j.freeradbiomed.2010.11.009.
[11] ⟨http://www.ngsp.org/convert1.asp⟩.
[12] R Core TeamR: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL ⟨http://www.R-project.org/⟩.
[13] P.R. Hillestrøm, A. Weimann, C.B. Jensen, et al., Consequences of low birthweight on urinary excretion of DNA markers of oxidative stress in young men, Scand. J. Clin. Lab. Investig. 66 (2006) 363–370, https://doi.org/10.1080/00365510600696402.
[14] J.T. Kloppenborg, C.E. Fonvig, J. Johannesen, et al., Urinary markers of nucleic acid oxidation in Danish overweight/obese children and youths, Free Radic. Res. 50 (2016) 691–697, https://doi.org/10.3109/10715762.2016.1164310.
[15] V. Cejvanovic, C. Asferg, L.K. Kjær, et al., Markers of oxidative stress in obese men with and without hypertension, Scand. J. Clin. Lab. Invest. (2016) 1–6, https://doi.org/10.1080/00365513.2016.1230776.
[16] V. Cejvanovic, L.K. Kjær, H.K.M. Bergholdt, et al., Iron induced RNA-oxidation in the general population and in mouse tissue, Free Radic. Biol. Med. 115 (2018) 127–135, https://doi.org/10.1016/j.freeradbiomed.2017.11.013.
[17] C. Ellervik, T. Mandrup-Poulsen, H.U. Andersen, et al., Elevated transferrin saturation and risk of diabetes: three population-based studies, Diabetes Care 34 (2011) 2256–2258, https://doi.org/10.2337/dc11-0416.
[18] J.M. Fernández-Real, G. Peñarroja, A. Castro, et al., Blood letting in high-ferritin type 2 diabetes: effects on insulin sensitivity and beta-cell function, Diabetes 51 (2002) 1000–1004.
[19] E. Orban, S. Schwab, B. Thorand, C. Huth, Association of iron indices and type 2 diabetes: a meta-analysis of observational studies, Diabetes Metab. Res. Rev. 30 (2014) 372–394, https://doi.org/10.1002/dmrr.2506.
[20] M.E. Elsayed, M.U. Sharif, A.G. Stack, Transferrin saturation: a body iron biomarker, Adv. Clin. Chem. 75 (2016) 71–97, https://doi.org/10.1016/bs.acc.2016.03.002.
[21] C. Podmore, K. Meidtner, M.B. Schulze, et al., Association of multiple biomarkers of iron metabolism and type 2 diabetes: the EPIC-interact study, Diabetes Care 39 (2016) 572–581, https://doi.org/10.2337/dc15-0257.
[22] J.B. Wish, Assessing iron status: beyond serum ferritin and transferrin saturation, Clin. J. Am. Soc. Nephrol. CJASN 1 (Suppl 1) (2006) S4–S8, https://doi.org/10.2215/CJN.01490506.
[23] American Diabetes Association, Diagnosis and classification of diabetes mellitus, Diabetes Care 37 (Suppl 1) (2014) S81–S90, https://doi.org/10.2337/dc14-S081.
[24] Control Group, F.M. Turnbull, C. Abraira, et al., Intensive glucose control and macrovascular outcomes in type 2 diabetes, Diabetologia 52 (2009) 2288–2298, https://doi.org/10.1007/s00125-009-1470-0.
[25] B. Zinman, C. Wanner, J.M. Lachin, et al., Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes, N. Engl. J. Med. 373 (2015) 2117–2128, https://doi.org/10.1056/NEJMoa1504720.
[26] M. Oelze, S. Kröller-Schön, P. Welschof, et al., The sodium-glucose co-transporter 2 inhibitor empagliflozin improves diabetes-induced vascular dysfunction in the streptozotocin diabetes rat model by interfering with oxidative stress and glucotoxicity, PloS One 9 (2014) e112394, https://doi.org/10.1371/journal.pone.0112394.