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ORIGINAL ARTICLE

Consequences of low birthweight on urinary excretion of DNA markers of oxidative stress in young men

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Abstract

Objective. Low birthweight (LBW) has been associated with an increased risk of development of type 2 diabetes in adult life. Both type 1 and type 2 diabetes mellitus are characterized by increased oxidative stress. The purpose of this study was to investigate whether young healthy adults born with LBW showed differences in oxidative stress under normal conditions and during the added challenge of a physiological Intralipid infusion. ***Material and methods.*** Urinary excretion of DNA markers of oxidative stress were analyzed by LC-MS/MS in 19 men (aged 19 years) with LBW and in 19 age matched, normal birthweight (NBW) controls pre- and post a 3-fold increase of plasma free fatty acids. ***Results.*** Mean excretion rates of 8-oxo-guanine (8oxoGua), 8-oxo-guanosine (8oxoGuo), 8-oxo-2′deoxyguanosine (8oxodG), and 1,N^6-ethenodeoxyadenosine (εdA) did not statistically differ between subjects with LBW and NBW (66.9 versus 73.9 nmol/15 h, 17.8 versus 18.5 nmol/15 h, 11.9 versus 14.4 nmol/15 h and 44.0 versus 43.2 pmol/15 h, respectively). Furthermore, Intralipid infusion did not affect excretion of DNA adducts in LBW or NBW subjects. Statistically significant correlations were found between body mass index and urinary excretion of 8oxoGua (r=0.64, p=0.003) and 8oxoGuo (r=0.64, p=0.003) in the LBW group only. ***Conclusions.*** These findings suggest that oxidative stress may be a consequence of diabetes and is not, or at least only partly, involved in the early pathogenesis of type 2 diabetes.

Key Words: *8oxoGua, 8oxoGuo, 8oxoG, εdA, fatty acids, type 2 diabetes, urinary excretion*

Introduction

There is a growing body of evidence documenting a significant association between low birthweight (LBW) and an increased risk of developing type 2 diabetes in adult life [1–3]. The Fetal Origins, or ''Barker'', hypothesis has been proposed as an adaptive response to intrauterine malnutrition, which results in programming of organ function with lifelong consequences for disease risk. However, the exact mechanism(s) are as yet unknown.

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Experimental and clinical studies suggest that oxidative stress plays a major role in the development of insulin resistance, β-cell dysfunction and in the late complications of diabetes [4,5]. Evidence of oxidative stress has been indicated by high levels of lipid peroxidation products in diabetic patients [6]. 8-oxo-2′deoxyguanosine (8oxodG) is a specific biomarker of oxidative stress [7]. Elevated levels of 8oxodG in the DNA of mononuclear cells [8], along with increased urinary excretion of 8oxodG [9], have been reported in diabetic patients. However, the question remains whether oxidative stress is a causal factor or rather an injurious secondary response to metabolic disorders.

Several studies have documented that LBW is a risk factor for various diabetes-associated conditions, including obesity, hypertension, renal disease, atherosclerosis, and cardiovascular disease in adult life [10,11]. In addition, some studies have shown that even healthy young men with LBW display subtle changes in glucose metabolism, such as increased whole-body [12] and tissue-specific [13,14] insulin resistance and impaired insulin secretion [15]. The fact that these metabolic abnormalities occur so early, many years before clinical onset of diabetes, indicates that they may indeed be primary and result from programming *in utero*. Furthermore, the above-mentioned conditions are characterized by oxidative stress. Thus, oxidative DNA damage may be an underlying mechanism mediating development of insulin resistance, as well as a consequence of clinical disease.

The aim of the present study was to investigate whether low birthweight is associated with increased oxidative DNA damage and potential correlations with metabolic and anthropometrical measurements. Urinary excretion of 8-oxo-guanine (8oxoGua), 8-oxo-guanosine (8oxoGuo) and 8oxodG were quantified to describe primary DNA damage. An additional approach to assess oxidative DNA damage was made by measuring εdA excretion, a product used as a marker of lipid peroxidation generated by free radical attack on polyunsaturated fatty acids.

Elevated plasma free fatty acids (FFA) may be involved in causing insulin resistance in obese subjects as well as in patients with type 2 diabetes [16]. High plasma FFA concentration is associated with increased oxidative stress [17]. Therefore, in addition we investigated whether a prolonged physiological increase in FFA achieved by Intralipid infusion could increase oxidative DNA damage and potentially unmask differences between the two groups.

Material and methods

Participants

Forty 19-year-old men born at term (39–41 weeks) in 1980 in Copenhagen County were identified and recruited from the Danish Medical Birth Registry according to birthweight, as previously described [15]. In summary, 20 men had birthweights below the 10th percentile for gestational age (LBW), and 20 men had birthweights in the upper normal range (50–75th percentile) (NBW). This corresponded to <2800 g (week 39), <2960 g (week 40), and <3010 g (week 41) for the LWB group and 3390–3700 g (week 39), 3500–3800 g (week 40), and 3660–4000 g (week 41) for the NBW group. None of the participants had a family history of diabetes (parents, grandparents), hypertension, or ischemic heart disease. In addition, none of them had received medication known to affect glucose or lipid homeostasis. All participants had normal glucose tolerance after a standard 75-g oral glucose tolerance test (OGTT), according to the World Health Organization criteria. The participants provided written informed consent before participation. The

protocol was approved by the regional ethics committee, and procedures were performed according to the principles of The Helsinki Declaration.

Experimental design

Each subject was studied in randomized order on two occasions (3–5 weeks apart), during Intralipid or saline infusion [18]. A polyethylene catheter was placed in the antecubital vein for test infusions (Intralipid: Intralipid (20 %), 0.4 mL kg^{-1} h^{-1}; Heparin (200U) (bolus), 0.2 U kg^{-1} h^{-1}; or saline: NaCl 9 g/L, 0.4 mL kg^{-1} h^{-1}). The study subjects reported to the laboratory at 0800 h, after a 10-h overnight fast. Urine was collected from 0700 h to 2200 h (t=15 h) during the first day (day 1) of the experiment. The urine samples were thoroughly mixed and aliquots were stored at −20°C until further analysis.

Measurements of Rd (glucose disposal), EGP (endogen glucose production), GF (glycolytic flux), GOX (glucose oxidation), GS (glucose storage), EE (energy expenditure), RQ (respiratory quotient), and insulin secretion disposition indices were obtained on study day 2 of the experiment [18]. Maximal aerobic capacity $V_{O_{2\max}}$ was determined by a submaximal exercise test, as reported previously [15]. Body composition and fat tissue distribution had been determined by dual-energy X-ray absorptiometry, as reported previously [19].

Quantification of 8oxo-Gua as the nucleobase, ribonucleoside and deoxynucleoside forms and εdA in human urine by LC-MS/MS

Quantification of 8oxoGua, 8oxoGuo, and 8oxodG in the urine samples were measured using a published LC/ESI(+)-MS/MS method [20]. The 1,N^6-ethenodeoxyadenosine (εdA) concentrations were measured in the urine samples by a column-switching LC/APCI(+)-MS/MS assay as previously described [21]. Urinary excretion rates were based on 15-h collection during both saline and Intralipid infusion on study day 1.

Statistical analysis

All results are reported as the means ± standard deviation (SD). Statistical analysis of two groups was performed by the Mann-Whitney U-test or paired *t*-test, and a *p*-value of less than 0.05 was considered significant. The Spearman rank test was used for correlation analysis. STATISTICA version 6.0 (StatSoft, Inc., Tulsa, Okla., USA) was used for these analyses.

Results

Subject characteristics

Selected baseline characteristics are shown in Table I. These have been published separately by Jensen et al. [15].

FFA concentrations

Fifteen hours of Intralipid infusion increased plasma FFA by approximately 3-fold in both groups (t=15 h; LBW: 0.13 ± 0.02 versus 0.44 ± 0.04 mmol/L, NBW: 0.15 ± 0.03 versus 0.44 ± 0.05 mmol/L).

Table I. Subject characteristics.

	LBW		Control		*p*-value
Birthweight (g)	2702	±202	3801	±99	>0.001
Height (cm)	178.5	±4.0	181.7	±4.8	0.03
Weight (kg)	73.6	±8.5	74.7	±13.1	NS
BMI (kg/m^2)	23.1	±2.7	22.6	±3.6	NS
Waist-to-hip ratio	0.82	±0.04	0.797	±0.04	NS
Total fat mass (%)	20.9	±7.1	20.1	±5.3	NS
Abdominal fat mass (%)	22.3	±8.6	19.5	±7.7	NS
$Vo_{2\ max}$ (l/min)	3.4	±0.4	3.5	±0.7	NS

Abbreviations: LBW=low birthweight; BMI=body mass index; NS=not significant.

Urinary excretion rates of DNA adducts

Comparison of urinary excretion per 15 h in LBW and NBW subjects is shown in Table II. There were no differences between the two groups for any of the four adducts, regardless of prior infusion.

Urinary excretion rates of 8oxoGua and 8oxoGuo correlated positively with body mass index (BMI) in the LBW group only, as shown in Figure 1 (8oxoGua: r=0.64, p=0.003; 8oxoGuo: r=0.64, p=0.003; 8oxodG: r=0.02, p=NS; εdA: r=0.21, p=NS). This correlation was not present during Intralipid infusion. Maximal aerobic capacity $V_{O_{2\max}}$ correlated positively with excretion of εdA in the LBW group during both saline (r=0.52, p=0.023) and Intralipid (r=0.65, p=0.002) infusion; these relationships are depicted in Figure 2. The oxidative markers did not correlate with $V_{O_{2\max}}$.

There were no associations between excreted adducts and any of the metabolic parameters assessed during clamp or dual-energy X-ray absorptiometry. Furthermore, there was no relationship between DNA markers of oxidative stress and the previously reported differences in insulin secretion in early adulthood of subjects with LBW [15] or the reported differences in fat tissue distribution [19].

Discussion

Our working hypothesis was that LBW and/or increased plasma FFA would be associated with an increase in oxidative stress, assessed by increased urinary excretion of DNA

Table II. Comparison of excretion rates among control and LBW groups on two occasions[a].

	Saline infusion[b]					Intralipid infusion[b]				
	LBW		Control		*p*-value	LBW		Control		*p*-value
	(n=19)		(n=19)			(n=20)		(n=19)		
8oxoGua[c]	66.9	±24.1	73.9	±47.8	0.92	60.7	±25.1	65.0	±15.3	0.50
8oxoGuo [c]	17.8	±4.7	18.5	±5.0	0.85	18.2	±6.1	18.4	±3.3	0.69
8oxodG[c]	11.9	±3.7	14.4	±6.1	0.17	13.3	±6.4	16.1	±4.6	0.06
εdA[d]	44.0	±16.0	43.2	±17.9	0.83	44.1	±17.7	44.1	±15.4	0.87

Abbreviations: LBW=low birthweight; 8oxoGua=8-oxo-guanine; 8oxoGuo=8-oxo-guanosine; 8oxodG=8-oxo-2′-deoxyguanosine; εdA=1,N^6-ethenodeoxyadenosine. [a]Urinary excretion rates during infusion (t=15 h) of either saline or intralipid; [b]the *p*-values were obtained by comparing adduct levels among LBW versus control using the non-parametric Mann-Whitney U-test; [c]nmol/15 h; [d]pmol/15 h.

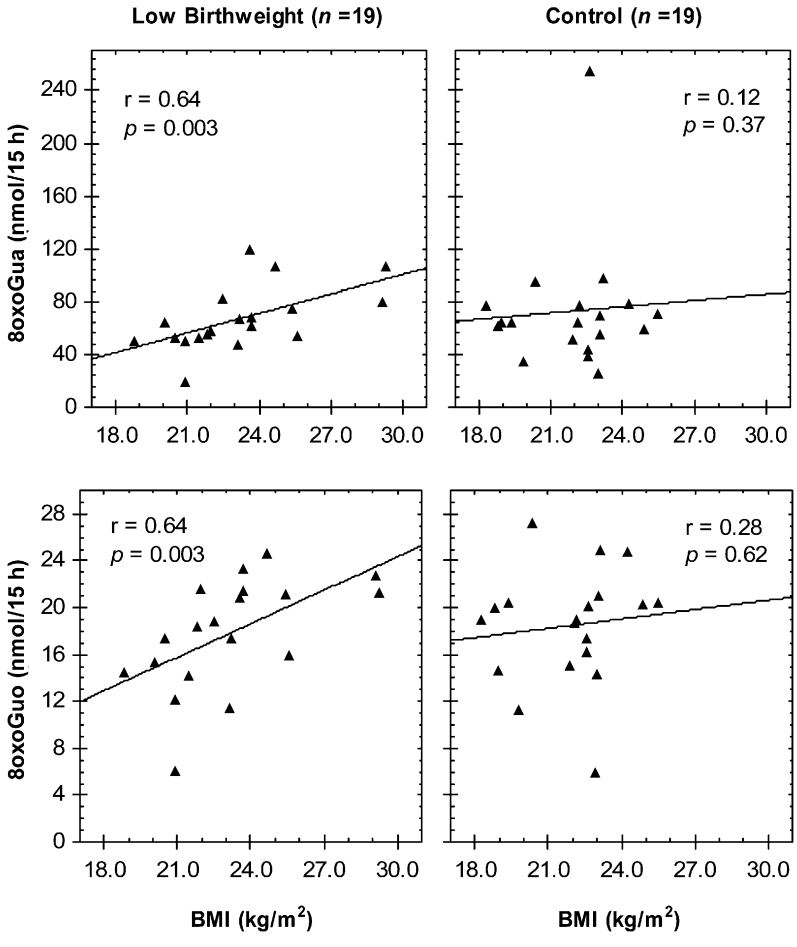

Figure 1. Correlation between body mass index (BMI) (kg/m^2) and urinary excretion (nmol/15 h) of 8-oxo-guanine (8oxoGua) or 8-oxo-guanosine (8oxoGuo) in the low birthweight (LBW) and control groups during the saline infusion study.

markers of oxidative stress. This hypothesis was founded on several observations: 1) LBW is associated with an increased risk of type 2 diabetes [10,11], 2) increased oxidative stress has been reported in patients with type 1 and type 2 diabetes [6,8], e.g. by increased excretion of 8oxodG [22], 3) prediction of diabetic nephropathy by urinary excretion of 8oxodG [9], and 4) improvement in microalbuminuria in patients with type 2 diabetes treated with high doses of antioxidants [23].

We measured four different markers of oxidative stress to nucleic acids: oxidation of the guanine moiety, i.e. 8-hydroxylation of guanine and deoxyguanosine representing DNA oxidation, 8-hydroxylation of guanosine representing oxidation of RNA, and εdA representing lipid peroxidation modification of DNA, at the adenine base.

It is quite clear from the data in Table II that there were no differences in DNA markers of oxidative stress between men with low and normal birthweight at age 19 years, either in the control situation or during a lipid challenge. If anything, the controls had slightly higher excretion rates. Hence, we were not able to support our hypothesis about oxidative stress being higher in men with LBW.

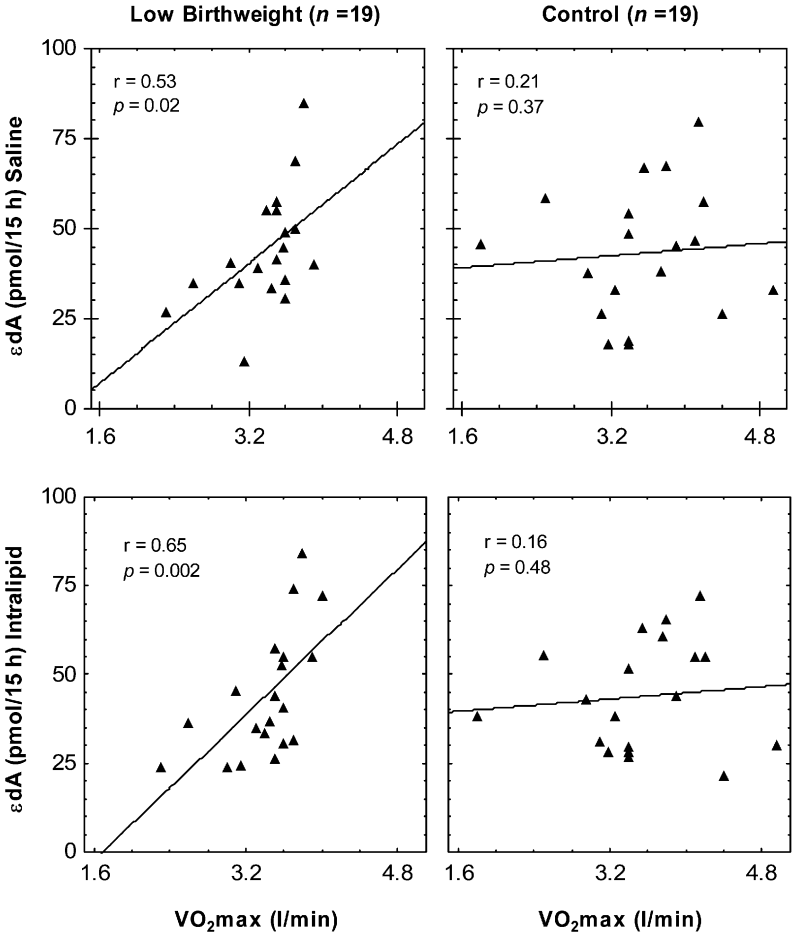

Figure 2. Relationship between 1,N^6-ethenodeoxyadenosine (εdA) excretion (pmol/15 h) and $V_{O_{2\max}}$ (l/min) in the low birthweight (LBW) and control groups during the saline (top) and Intralipid (bottom) infusion study.

Interestingly though, there did seemed to be subtle differences between the LBW and NBW men, as shown in Figure 1. We found significant correlations between the oxidative stress markers and BMI in the LBW group only. Over the past decade several studies have reported higher risk of abdominal obesity (BMI, W/H ratio) in middle-aged LBW adults [24–27] and we recently reported increased abdominal fat accumulation in young healthy adults with LBW [19]. Hypothetically, increasing abdominal obesity in LBW, leading in turn to hyperlipidemia, hyperinsulinemia, and hyperglycemia, could promote oxidative stress, which is further aggravated by our present sedentary lifestyle pattern. A study by Collins et al. showing a positive correlation between BMI and DNA strand breaks in patients with insulin-dependent diabetes mellitus (IDDM) characterized by high glucose, but not in controls, supports of this notion [28].

High levels of FFAs in plasma are also linked to insulin resistance, and a high plasma FFA concentration is associated with increased oxidative stress. Paolisso et al. found that a 3-fold increase in FFA caused an increase in lipid peroxidation measured by plasma thiobarbituric acid-reactive substances (TBARS) and LPO (lipid peroxidation), and a

decrease in plasma reduced/oxidized glutathione ratio, indicative of increased oxidative stress [17]. In contrast, with a similar increase in FFA, we did not detect any changes in oxidative DNA damage during Intralipid infusion, compared with saline infusion. Whereas plasma TBARS and LPO increase immediately with FFA infusion, oxidative stress induced by high metabolism, e.g. long-distance running, peaks days after the insult proper [29]. This may be due to the localization of DNA deep within the cell, whereas TBARS and LPO presumably are due to oxidation of cell membrane lipids in close contact with plasma. Our observation of a positive correlation between $V_{O_{2\max}}$ and εdA in the LBW group supports this view. Another potential limitation of this study is the small sample size, which is further exacerbated when comparing subgroups. However, based on the previously reported levels we should be able to detect a relevant difference [8,9].

In conclusion, we found no evidence of increased oxidative stress in 19-year-old healthy men with LBW. Furthermore, a 3-fold increase in plasma FFA for 15 h had no effect of oxidized DNA, and did not uncover any defects in the LBW group. Therefore, generalized oxidative stress as assessed by excretion of oxidized DNA does not appear to be an early or primary pathophysiological primary event in the development of diabetes associated with LBW. However, in young LBW men there is an adverse interaction between increasing BMI and urinary excretion of oxidized DNA that may in time contribute to the continued impairment of glucose metabolism and cardiovascular disease.

Contributors

The paper was jointly written by all the authors. CBJ and AAV produced the original study design, and CBJ and HS collected the data, carried out the baseline survey, and collected the urine samples. AW was responsible for the various urinary guanine measurements, PRH for the urinary εdA measurements, and PRH and HEP were responsible for the statistical data analysis and interpretation.

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