

CLINICAL STUDY

Oxidative DNA damage and obesity in type 2 diabetes mellitus

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Abstract

Objective: This study illustrates the relationship between oxidative DNA damage and obesity in patients with prediabetes and type 2 diabetes compared with controls.

Design and methods: Participants attended the School of Community Health, Diabetes Screening Clinic, Charles Sturt University, Australia, between February 2006 and June 2008. A total of 162 participants (35 type 2 diabetic patients; eight prediabetic subjects; and 119 age-, gender-, and weight-matched controls) were investigated. All patients were selected on clinical grounds.

Results: Serum 8-hydroxy 2'-deoxy-guanosine (8-OHdG) level was significantly greater in the prediabetic subjects (671.3 ± 140 pg/ml) compared with controls (210.1 ± 166 pg/ml; $P < 0.01$). The diabetic group (1979.6 ± 1209 pg/ml) had the highest level of 8-OHdG. There was a significant increase in serum 8-OHdG in obese subjects (848.5 ± 103 pg/ml; $P < 0.001$) and overweight subjects (724 ± 102 pg/ml; $P = 0.005$) compared with the lean subjects (196.5 ± 327 pg/ml).

Conclusion: Our results indicate that serum 8-OHdG is increased already in prediabetes suggesting oxidative DNA damage to be present with minor elevation of blood glucose levels (BGLs). The statistically significant positive correlation between serum 8-OHdG and body mass index in the diabetic group indicates that obesity has an additive effect to increased BGL contributing to oxidative DNA damage.

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Introduction

Obesity is a major risk factor for increased cardiovascular mortality in type 2 diabetes mellitus, with a strong positive correlation between obesity and ischemic heart disease, hypertension, and stroke (1), via its role in the development of insulin resistance and its associated complications (2, 3). This high mortality rate can be reversed with 15–20% weight loss in the first year after diagnosis of type 2 diabetes mellitus. A deliberate weight loss of 0.5–9.0 kg, depending on the initial weight, is associated with a 30–40% reduction in diabetes-related mortality (4).

Increases in blood glucose levels (BGLs) above 5.5 mmol/l but below the diabetes cutoff of 7 mmol/l have been shown to increase free radicals and lead to diabetes-associated complications (5). Oxidative stress involves reactive molecules that are composed of only oxygen or oxygen and hydrogen (e.g. hydrogen peroxide) as well as reactive nitrogen species (nitrosyl stress) that are reactive molecules composed of nitrogen and reactive oxygen (6). These molecules can damage

nucleic acids, lipids, and proteins and figures prominently in the etiology and progression of diabetes (7, 8), numerous cancers, and coronary and carotid atherosclerosis (9, 10).

An increased body mass index (BMI), among overweight individuals, has been shown to be associated with increased risk of DNA damage due to oxidative stress (11) and of cardiovascular disease (12, 13). Type 2 diabetic patients significantly exhibit elevated oxidative DNA damage, as measured by 8-hydroxy 2'-deoxy-guanosine (8-OHdG) such that its use as a biomarker in the diagnosis of diabetes has been considered (14). 8-OHdG, a result of DNA base modification produced by the oxidation of deoxyguanosine, is considered as the most sensitive and useful marker of oxidative DNA damage (15). The importance of this lesion stems from the fact that it is both abundant in DNA and it is mutagenic (16). Current evidence suggests that 8-OHdG lesions present in DNA during cellular replication results in somatic mutation, which may contribute to smooth muscle proliferation in the pathogenesis of atherosclerotic plaques (17). It is essential that cellular free

Table 1 Characteristics of the subjects included in this study.

	Control subjects	Prediabetic subjects	Type 2 diabetic patients
Number	119	8	35
Age (years)	66.4 ± 11.1	57.5 ± 9	69.9 ± 8.6
Sex (man/women)	49/70	5/3	20/15
Fasting BGL (mmol/l)	4.9 ± 0.5	6.4 ± 0.22 [†]	7.2 ± 0.3*
HbA1c %	5.6 ± 0.26	5.8 ± 0.2 [†]	6.7 ± 0.1*
SBP (mmHg)	131.5 ± 15.2	130.1 ± 12.4	135.3 ± 15
DBP (mmHg)	75.7 ± 8.4	72.9 ± 5.7	74.6 ± 10.1
Nephropathy	1	0	0
Statin	19 (15.9%)	1 (12.5%)	20 (57.1%)
Antihypertensive medication	37 (31%)	2 (25%)	27 (77.1%)
Single medication	30	3	4
Two medications	7	2	11
Three medications	1	1	1
> three medications	0	0	1

*Significant difference between control and type 2 diabetes ($P < 0.001$).

[†]Significant difference between control and prediabetes ($P < 0.001$). BGL, blood glucose; SBP, systolic BP; DBP, diastolic BP.

radical-induced damage as well as mitochondrial and nuclear DNA damage is repaired. However, the antioxidant system is overwhelmed with increases in BGL and obesity, which leads to increased free radical activity and further tissue damage (18).

Materials and methods

The study protocol was reviewed and approved by the ethics in human research committee of Charles Sturt University. Informed consent was obtained from each participant. Participants of the Diabetes Screening Clinic were drawn from the community through announcements in the local newspaper, radio, and television between February 2006 and June 2008. Only participants in whom complete data were available as required for this study were included in the analysis. The exclusion was not to include any people with co-morbidities such as kidney disease and cardiovascular disease.

Measurement of BMI

Weight was measured using standardized beam weight scales without footwear and with only light clothes. Height was measured with the subjects barefoot and standing with the feet together. BMI is defined as weight in kilogram per height in meters squared and is independent of gender and age. Overweight is defined as a BMI of 25.0–29.9 kg/m² and obese is a BMI ≥ 30.0 kg/m² (19).

Prediabetes was defined as a person with impaired fasting BGL (BGL > 6 mmol/l but < 7 mmol/l) according to the World Health Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications

(20). Diabetic participants were selected on the basis of having been diagnosed previously using an oral glucose tolerance test and/or being on antihyperglycemic medication. Attendees at the diabetes screening clinic were recruited in the controls if they had no background of diabetes, cardiac, respiratory, or renal disease. Participants need to have normal fasting plasma glucose before being considered as control.

Participants were comparable for age, gender, smoking habit, diet, and physical activity.

Measurements of BGL and HbA1c

BGL and HbA1c were measured at South West Pathology; BGL was assessed using fasting blood samples obtained through venipuncture and HbA1c was measured using HPLC.

Measurements of oxidative DNA damage

Serum 8-OHdG was measured using an ELIZA Kit, Cayman Chemical, MI, USA (21). The test utilizes an anti-mouse IgG-coated plate and a tracer consisting of an 8-OHdG-enzyme conjugate. This format has the advantage of providing low variability and increased sensitivity compared with assays that utilize an antigen-coated plate. The results of 8-OHdG will be compared with BMI and total serum cholesterol levels for the three groups. Serum cholesterol was measured colorimetrically at South West Pathology.

Statistical analysis

Descriptive analysis was performed. Categorical data are presented as a frequency table, and quantitative data were analyzed using the Statistical Package for Social Sciences (SPSS version 17, Sydney, NSW, Australia) and Microsoft Excel (Office2007, Microsoft). All values are expressed as mean \pm s.d. Statistical analysis was performed using ANOVA followed by the least squared difference (LSD) *post hoc* test to compare serum 8-OHdG with BMI and serum total cholesterol. Pearson's

Table 2 Tests of between-subjects effects.

Source	Dependent variable	df	F	Sig.
^a 8-OHdG (pg/ml)	Age of the participants	1	0.896	0.346
	Body mass index	1	9.523	0.002
	HbA1c	1	8.978	0.003
^b Gender	Age of the participants	1	1.179	0.279
	Body mass index	1	0.122	0.727
	HbA1c	1	0.157	0.692
^c Group classification	Age of the participants	2	2.553	0.081
	Body mass index	2	0.135	0.874
	HbA1c	2	6.808	0.002
Gender \times group classification	Age of the participants	2	0.997	0.372
	Body mass index	2	0.102	0.903
	HbA1c	2	0.096	0.908

^a $r^2 = 0.074$ (adjusted $r^2 = 0.035$). ^b $r^2 = 0.152$ (adjusted $r^2 = 0.116$). ^c $r^2 = 0.406$ (adjusted $r^2 = 0.381$).

Table 3 Multivariate tests of the following markers with age, body mass index, and HbA1c.

Effect	Value	F	df	Sig.
8-OHdG (pg/ml)				
Pillai's trace	0.124	6.539 ^a	3	0.000
Wilks' lambda	0.876	6.539 ^a	3	0.000
Hotelling's trace	0.141	6.539 ^a	3	0.000
Roy's largest root	0.141	6.539 ^a	3	0.000
Gender				
Pillai's trace	0.010	0.490 ^a	3	0.690
Wilks' lambda	0.990	0.490 ^a	3	0.690
Hotelling's trace	0.011	0.490 ^a	3	0.690
Roy's largest root	0.011	0.490 ^a	3	0.690
Group classification				
Pillai's trace	0.120	2.977	6	0.008
Wilks' lambda	0.881	3.031 ^a	6	0.007
Hotelling's trace	0.134	3.084	6	0.006
Roy's largest root	0.126	5.882 ^b	3	0.001
Gender × group classification				
Pillai's trace	0.017	0.404	6	0.876
Wilks' lambda	0.983	0.402 ^a	6	0.877
Hotelling's trace	0.017	0.401	6	0.878
Roy's largest root	0.017	0.811 ^b	3	0.490

^aExact statistic.

^bThe statistic is an upper bound on F that yields a lower bound on the significance level.

correlation was performed to illustrate the correlation between 8-OHdG, HDL-C, BMI, and HbA1c. Power analysis using a high-power coefficient and effect size indicated that the number of samples included in the analysis was sufficient.

Results

Table 1 illustrates the baseline values of the 162 participants. Disease status, fasting BGL, age, sex, and HbA1c are shown.

BMI, blood pressure, BGL, and HbA1c increased from the control to the prediabetic and diabetic groups. BMI was significantly increased in the diabetic group ($30.7 \pm 5 \text{ kg/m}^2$) compared with the controls ($27.3 \pm 4.4 \text{ kg/m}^2$; $P < 0.001$). Increases in BGL and HbA1c were found to be statistically significant in the prediabetic and diabetic groups compared with the control group. There were also statistically significant elevations of these parameters in the diabetic group when compared with the prediabetic group ($P < 0.001$; Tables 1 and 2).

Multivariate analysis was performed to evaluate the effect of study variables (age, sex, HbA1c, and BMI; Tables 2 and 3). No significant association between serum 8-OHdG and age or gender was found.

Post hoc Fischer's LSD test for between-group differences demonstrated statistically significant differences between the control and diabetic, or prediabetic group, or both for serum lipid profile, except for triglycerides that showed a non-significant difference between the control and prediabetic group. The level of

total cholesterol was found to be lower in the type 2 diabetic group ($4.4 \pm 1.2 \text{ mmol/l}$) compared with the controls ($5.1 \pm 1 \text{ mmol/l}$; $P < 0.001$). There was also a significant reduction in the level of low-density lipoprotein cholesterol (LDL-C) in the type 2 diabetic group ($2.4 \pm 1 \text{ mmol/l}$) compared with the controls ($3.2 \pm 0.8 \text{ mmol/l}$; $P < 0.001$; Table 4). A statistically significant reduction in the level of high-density lipoprotein cholesterol (HDL-C) in type 2 diabetes group ($1.1 \pm 0.2 \text{ mmol/l}$; $P < 0.001$) compared with the control subjects (1.35 ± 0.3) was also noted. (Table 4).

8-OHdG showed a significant elevation in the diabetic ($1979.6 \pm 1209 \text{ pg/ml}$; $P < 0.001$) and the prediabetic groups ($671.3 \pm 140 \text{ pg/ml}$; $P = 0.03$) compared with the controls ($210.4 \pm 166 \text{ pg/ml}$; Table 4) using a one-way ANOVA followed by Fischer's LSD *post hoc* test. In addition, there was also a significantly higher 8-OHdG level for the diabetic compared with the prediabetic group ($P < 0.001$; Table 4).

Pearson's correlation showed a significant positive correlation between 8-OHdG with BMI and HbA1c (Table 5 and Fig. 1) at $P < 0.01$.

However, there was a significant negative correlation between 8-OHdG and HDL-C (Table 5 and Fig. 2) at $P < 0.01$.

Discussion

Due to the global epidemic of obesity, there is also a large increase in the prevalence of type 2 diabetes mellitus (22). It has been estimated that every 1 kg increase in weight is associated with a 9% relative increase in prevalence of diabetes (23), whereas weight loss appears to be a more dominant predictor of reduced diabetes incidence than changes in diet or activity level (24). A multivariate analysis was carried out to evaluate the effects of age, gender, BMI, and the HbA1c on 8-OHdG

Table 4 Biomarkers of oxidative stress (mean ± s.d.) in control, prediabetic, and diabetic groups.

Oxidative stress biomarkers	Control	Prediabetic group	Type 2 diabetic group
Total cholesterol (mmol/l)	5.1 ± 1	4.89 ± 1.03	4.4 ± 1.2 ^f
Triglycerides (mmol/l)	1.3 ± 0.6	1.7 ± 1.3	1.8 ± 0.9 ^f
HDL-C (mmol/l)	1.35 ± 0.3	1.3 ± 0.3*	1.1 ± 0.2 ^f
LDL-C (mmol/l)	3.2 ± 0.8	3 ± 1.3	2.4 ± 1 ^f
BMI (kg/m ²)	27.3 ± 4.4	27.9 ± 2.5	30.7 ± 5 ^f
8-OHdG (pg/ml)	210.4 ± 166	671.3 ± 140* ⁺	1979.6 ± 1209 ^f

*Significant difference between diabetic and prediabetic group with control ($P < 0.05$). ^fSignificant difference between diabetic and prediabetic group with the control ($P < 0.001$). ⁺Significant difference between diabetic and prediabetic group ($P < 0.05$). HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; BMI, body mass index; 8-OHdG, 8-hydroxy 2'-deoxy-guanosine.

Table 5 Pearson's (*r*) correlation between 8-OHdG, HDL-C, HbA1c, and BMI.

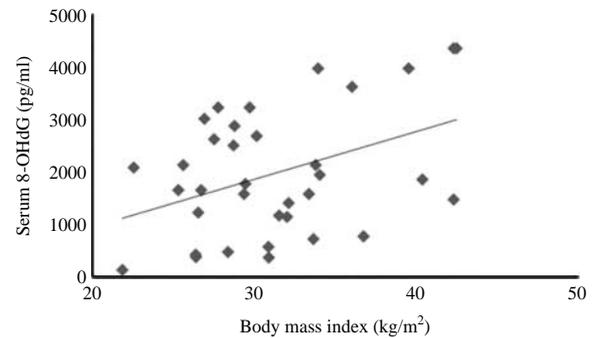
	8-OHdG (pg/ml)	HDL-C (mmol/l)	HbA1c (%)	BMI (kg/m ²)
8-OHdG (pg/ml)				
<i>r</i>	1	-0.313 ^a	0.584 ^a	0.274 ^a
<i>P</i> (two-tailed)		0.000	0.003	0.002
<i>n</i>	162	161	157	152
HDL-C (mmol/l)				
<i>r</i>	-0.313 ^a	1	-0.234 ^a	-0.263 ^a
<i>P</i> (two-tailed)	0.000		0.003	0.001
<i>n</i>	161	161	157	151
HbA1c %				
<i>r</i>	0.584 ^a	-0.234 ^a	1	0.218 ^a
<i>P</i> (two-tailed)	0.003	0.003		0.008
<i>n</i>	157	157	157	147
BMI (kg/m ²)				
<i>r</i>	0.274 ^a	-0.263 ^a	0.218 ^a	1
<i>P</i> (two-tailed)	0.002	0.001	0.008	
<i>n</i>	152	151	147	152

^aCorrelation is significant at the 0.01 level (two-tailed).

(Tables 2 and 3). The results indicated a significant relation between serum 8-OHdG with BMI and HbA1c, also there was a significant relationship between group classification (control, prediabetes, and type 2 diabetes) and HbA1c. This finding may partially explain the strong correlation between hyperglycemia and the significant rise in oxidative DNA damage and the role of obesity in increasing the risk of diabetes.

Our study showed a significant elevation in BMI in the diabetic group compared with the controls (Table 4), explaining the strong association of body weight with insulin resistance, as higher BMI is associated with hyperinsulinemia and insulin resistance.

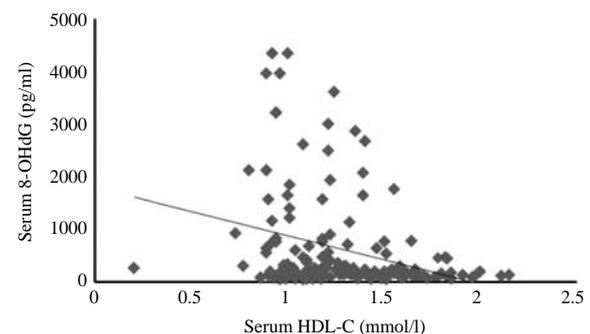
A significant rise in the level of 8-OHdG was recorded in prediabetic and diabetic group compared with the control group (Table 4). 8-OHdG is known to be a sensitive marker of oxidative DNA damage and of total systemic oxidative stress *in vivo*. Interestingly, 8-OHdG appears to play a role in tissue cell injury via the induction of apoptotic cell death (25). Previous studies have shown that 8-OHdG is one of the commonly used markers for evaluation of oxidative DNA damage (26). It is also considered to be a measurable risk factor for co-morbid illnesses like cancer, atherosclerosis, and diabetes (27). This may be explained by the high level of H₂O₂ generated from free radicals emerged from oxidative stress conditions (28). In previous studies, an increased number of 8-OHdG-positive islet cells were found in the human pancreas from type 2 diabetic subjects (29). Insulin treatment rapidly normalizes these elevated 8-OHdG levels, suggesting the involvement of hyperglycemia in oxidative DNA damage (30). Importantly in our study, the sensitivity of 8-OHdG is such that we observed a significant elevation in the prediabetic group compared with the controls despite good HbA1c readings in all groups. An elevation of 8-OHdG indicates an increase in the degree of oxidative

**Figure 1** Significant positive correlation between serum 8-OHdG and BMI was seen in type 2 diabetic subjects ($r=0.04$, $P<0.01$).

stress affecting tissue function and integrity and therefore provides useful information on oxidative stress and disease progression (31). Our current findings agree with the earlier work (32); however, the prediabetic group in this study was defined according to the WHO criteria with blood glucose ranging between 6 and 7 mmol/l (20), whereas we used the ADA classification of prediabetes in the previous study with blood glucose ranging between 5.5 and 7 mmol/l.

As diverse pathologies affect 8-OHdG level, we excluded all participants with other pathology from this study. Therefore, the level of 8-OHdG in the first instance indicates oxidative stress and DNA damage due to the glycaemic state of the participants. As the prediabetic group had higher glucose levels compared with the control group, the resulting reactive oxygen species formed will lead to more oxidative DNA damage.

There are many controversies in explaining the relationship between 8-OHdG and BMI, with some studies showing a negative correlation existing between them (33), others showed no such correlation exist (34). In our study, we did find a positive correlation between 8-OHdG and BMI in people with type 2 diabetes mellitus (Tables 2 and 5 and Fig. 1), but such correlation was not found in the prediabetic nor the control group. This explains the importance of the changes in the relationship between hyperglycemia and

**Figure 2** Significant negative correlation between serum 8-OHdG and serum HDL-C was seen in all the participants ($r=-0.31$, $P<0.01$).

hyperlipidemia in the development of diabetes with increased BGL being more important initially, as is the case in the prediabetic state. Body weight takes on an increased influence with increased BGLs and in the diabetic state.

Lower HDL-C was more frequent in the prediabetic and in type 2 diabetic group compared with the controls (Table 2), with a significant negative correlation with 8-OHdG (Table 5 and Fig. 2). This observation is consistent with the finding of Taskinen in 2003 (35). Reduced HDL-C in diabetic subjects result from higher catabolic rate of HDL with normal activity of cholesteryl ester transfer protein and hepatic lipase in insulin resistance conditions (36). The low level of HDL-C will eventually increase the risk of coronary artery disease in diabetic patients (37). However, cholesterol levels may also have been affected due to statin medication taken by the majority of the diabetic group (see Table 1), which was not stopped for this research. Statins impact negatively on antioxidant coenzyme-Q as well as HDL, of which the former exacerbates oxidative stress. It is noteworthy that lipid peroxidation acts principally on LDL, and therefore hypothetically, LDL level would be positively correlated with 8-OHdG. In our study, we found neither a statistically significant difference in LDL between groups nor a correlation with 8-OHdG. Therefore, it is probable that the observed negative correlation between serum 8-OHdG and serum HDL-C is due to the statins' indirect increase in oxidative stress and direct depletion of HDL.

In conclusion, serum 8-OHdG is a useful indicator to assess disease severity or extent of oxidative stress in the presence of high serum lipid levels in prediabetes and in type 2 diabetes. Increased weight takes on more of a role in disease progression once glucose levels have reached diabetic status. This might be utilized for treatment and management of high BMI cases associated with disturbed BGLs.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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