Oxidative DNA damage: antioxidant response in postprandial hyperglycaemia in type 2 diabetes mellitus

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Abstract

The mechanism by which postprandial glucose load and sudden cardiac death are linked is not fully understood. This study compares the postprandial response of 8-hydroxy-deoxy-guanosine (8-OHdG) and erythrocyte glutathione (GSH) in control and type 2 diabetes groups. 8-OHdG was significantly elevated in type 2 diabetic patients (824.1±331.2 and 1087±273.1 pg/ml at the first and second hours respectively, p<0.05, versus 600.4±214.4 pg/ml at baseline) following a glucose load. This was associated with a significant reduction in the level of erythrocyte GSH after the first hour (59.1±9 mg/100ml; p<0.001) compared with the basal level (72.1±9 mg/100ml), followed by a significant elevation in the second hour (71.5±11.1 mg/100ml; p<0.001) compared with the first hour, bringing the GSH level only back to base level. The increase in 8-OHdG in people with type 2 diabetes during the postprandial period further supports previous evidence of a defective antioxidant response and greater risk of heart attack due to blood vessel endothelial cell damage and smooth muscle proliferation.


Key words: type 2 diabetes mellitus, oral glucose tolerance test, 8-hydroxy-deoxy-guanosine, erythrocyte reduced glutathione

Introduction

Postprandial hyperglycaemia is an independent risk factor for cardiovascular disease in patients with type 2 diabetes mellitus or with the prediabetic state characterised by IGT. ROS are increased in the diabetic state and are important contributing factors to the development of atherosclerosis involving structural and functional changes of endothelial cells.1-9 8-OHdG is a product of oxidative DNA damage following specific enzymatic cleavage after ROS induced 8-hydroxylation of the guanine base in mitochondrial and nuclear DNA.10 8-OHdG is known to be a sensitive marker of oxidative DNA damage and of the total systemic oxidative stress in vivo as well as contributing to smooth muscle proliferation in the pathogenesis of atherosclerotic plaques.11,12

The antioxidant reserve is related to the activity of GSH in the body. GSH constitutes the principal antioxidant defence system,13 acting as a substrate for GSH peroxidase-1. GSH peroxidase-1 deficiency has been shown to be associated with increased risk of coronary artery disease and cardiovascular events.14 Previous studies have shown changes in erythrocyte antioxidant levels in diabetes-related cardiovascular disease resulting in enhancement of procoagulant tissue factors and proinflammatory mediators that lead to endothelial dysfunction, smooth muscle proliferation and cardiovascular disease.15,16 However, acute changes associated with the postprandial period and a possible role and involvement of GSH and 8-OHdG have not been thoroughly investigated.

Cardiovascular disease is more prevalent in patients with type 2 diabetes and is epidemiologically correlated to postprandial stress as demonstrated by numerous observational studies such as in the Honolulu Heart Programme, the Helsinki Policeman...
Study and the Hoorn Study. Contrary findings that lowering postprandial glucose lowers cardiovascular risk, as shown by the NAVIGATOR trial, have been inconclusive. Therefore further mechanistic studies in this area are still required.

The primary purpose of this article is to illustrate the difference in the acute response of 8-OHdG and GSH in people with type 2 diabetes and control participants following an oral glucose load. We were interested in answering several questions:

1. Do patients with type 2 diabetes have different levels of 8-OHdG and GSH to healthy control subjects?
2. Are there acute changes in the levels of 8-OHdG and GSH after a glucose load?
3. With regard to 8-OHdG and GSH, how does the response to an oral glucose load differ in patients with type 2 diabetes mellitus and the control group?
4. Is there a specific correlation between the level of 8-OHdG and GSH? If yes, does this differ in the diabetic group compared with the control group?

**Research design and methods**

**Protocol**

The study protocol was reviewed and approved by the Ethics in Human Research Committee of Charles Sturt University. Informed consent was obtained from each subject. Participants for the research carried out at the diabetes screening clinic at Charles Sturt University were drawn from the community through announcements in the local newspaper, radio and television, during the period of the study between February 2006 and June 2008. Only participants for whom complete data were available as required for this study were included in the analysis. There were no exclusion criteria set. Participants with type 2 diabetes had been diagnosed previously using an OGTT or were on glucose-lowering medication. Control subjects were also recruited from the diabetes screening clinic if they had no background of diabetes or cardiac, respiratory or renal disease. Participants were comparable for age, sex, smoking habit, diet and physical activity.

**OGTT measurement**

All participants were asked to fast for 8 hours before the test with the exception of water and any drugs normally taken. Basal blood and urine samples were taken before starting the test, and repeated every half an hour for 2 hours after administration of a 75 g glucose load.

**Measurements of oxidative stress**

Erythrocyte malondialdehyde was measured using thiobarbituric acid reactive substances. Levels of methaemoglobin were assessed using spectrophotometry of haemoglobin absorption before and after addition of cyanide. Oxidative DNA damage was measured using the 5,5’-dithiobis-2-nitrobenzoic acid (DTNB) reaction. The level of erythrocyte GSH was determined using the 5,5’-dithiobis-2-nitrobenzoic acid (DTNB) reaction.

**Statistical analysis**

The data were analysed using SPSS (Version 14) and Microsoft Excel (Office 2007, Microsoft). All values were expressed as mean ± standard deviation. Statistical analysis was performed using a one-way ANOVA followed by Scheffe post-hoc test for between group comparisons. Independent Student's t-tests for between group comparisons were performed for blood glucose, systemic blood pressure and HbA1c. Correlation regression analysis, taking p<0.05 as the significance limit, was used to determine whether there was a significant correlation between 8-OHdG and the other parameters associated with oxidative stress measured in this study.

**Results**

Table 1 illustrates the baseline values of 37 participants. Disease status, fasting blood glucose level, age, sex, HbA1c, blood pressure, statin therapy and BMI are shown.

Type 2 diabetes participants were diagnosed according to the American Diabetes Association’s diagnostic criteria of diabetes mellitus. Of the patients with diabetes, 9% had hypertension and 33.3% were on statin therapy for treatment of hyperlipidaemia. Participants in the control and diabetes groups were comparable for age, sex, smoking habit, diet and physical activity. Age, BMI, blood pressure, blood glucose and HbA1c were greater in the diabetes group than the control group (table 1). Using an independent samples t-test, blood glucose was significantly different between the control and diabetes groups (p<0.001), and systolic blood pressure and HbA1c also showed significant differences between the control and diabetes groups (p<0.05) (table 1).

Following the glucose load, blood glucose levels increased for both the control and diabetes groups, with blood glucose remaining significantly elevated in the diabetes group after 2 hours (5.8±0.9 and 14.2±4.2 mmol/L respectively, p<0.001). In the control group, blood glucose returned to baseline values with no significant difference between baseline and the two-hour level following the glucose load (table 2, figure 1).

At baseline level, there was already an approximately three-fold greater amount of 8-OHdG in the serum of subjects with diabetes than controls (600.4±214.4 and 240.9±103.4 pg/ml respectively).

Following the OGTT, the already elevated 8-OHdG in the diabetes group rose significantly after the first and second hours (824.1±331.2 pg/ml, p<0.05 and 1087±273.1 pg/ml, p<0.001) (table 2, figure 2), whereas only a slight increase in 8-OHdG was found in the control group in line with the smaller increase in blood glucose level after the first hour and a return to baseline after the second hour over the post-glucose load period.

The ANOVA test showed significant differences between all groups for blood glucose (F=27.27, p<0.001), erythrocyte GSH (F=7.6, p<0.001) and serum 8-OHdG (F=29.59, p<0.001).

In the diabetes group, using the Scheffe post-hoc test, a significant reduction in the level of erythrocyte GSH (p<0.001)
was observed after the first hour (59.1±9 mg/100 ml) followed by a significant elevation (p<0.001) after the second hour (71.5 ± 11.1 mg/100 ml) back to levels close to baseline (72.1±9 mg/100 ml). In the control group, there was no significant change in the level of GSH over the two hours after glucose load, (table 2, figure 3).

**Discussion**
Postprandial hyperglycaemia represents an increased risk of heart attack especially in people with diabetes due to a large extent to changes in the oxidative stress biochemistry. However, a link between oxidative stress induced damage to blood vessels and the antioxidant response during this period has not been thoroughly investigated.

Changes in oxidative balance, or in antioxidant defences, during acute hyperglycaemia have been demonstrated in subjects with and without type 2 diabetes. The DNA base-modified product 8-OHdG is one of the most commonly used markers for evaluating oxidative DNA damage. In this study, free radical induced tissue damage was already present in the diabetes group as indicated by the higher 8-OHdG levels at baseline compared with the control group. Despite the low

**Table 1.** Characteristics of study participants

<table>
<thead>
<tr>
<th>Participants</th>
<th>Control subjects</th>
<th>Type 2 diabetic patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>8</td>
<td>29</td>
</tr>
<tr>
<td>Age (years)</td>
<td>62.7±14</td>
<td>67.8±10</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>4/4</td>
<td>13/16</td>
</tr>
<tr>
<td>Average blood glucose level (fasting plasma glucose, mmol/L)</td>
<td>5.1±0.2</td>
<td>7.6±2**</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.3±1.4</td>
<td>30±5.8*</td>
</tr>
<tr>
<td>Glycated haemoglobin A1c %</td>
<td>5.3±0.6</td>
<td>6.57±1**</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>126.5±14.8</td>
<td>147.5±20.6*</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>75.8±7</td>
<td>79.7±8.2</td>
</tr>
<tr>
<td>Subjects with cardiovascular disease</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Subjects with statin use</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

*significant difference between control and type 2 diabetes (p<0.05)
**significant difference between control and type 2 diabetes (p<0.001)

**Table 2.** Biomarkers of oxidative stress (mean ± SD) in control and diabetic groups

<table>
<thead>
<tr>
<th>Biochemical markers</th>
<th>Participants</th>
<th>Basal measurements</th>
<th>Measurements after 1 hour</th>
<th>Measurements after 2 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>BGL (mmol/L)</td>
<td>Control group</td>
<td>5.1±0.2</td>
<td>7.7±0.5</td>
<td>5.8±0.9</td>
</tr>
<tr>
<td></td>
<td>Type 2 diabetes</td>
<td>7.6±2</td>
<td>13.9±4.1</td>
<td>14.2±4.2</td>
</tr>
<tr>
<td>8-OHdG (pg/ml)</td>
<td>Control group</td>
<td>240.9±103.4</td>
<td>261.8±112.5</td>
<td>289.8±162.5</td>
</tr>
<tr>
<td></td>
<td>Type 2 diabetes</td>
<td>600.4±214.4</td>
<td>824.1±331.2</td>
<td>1087±273.1</td>
</tr>
<tr>
<td>GSH (mg/100 ml)</td>
<td>Control Group</td>
<td>73.0±9</td>
<td>73.1±8</td>
<td>75.9±7</td>
</tr>
<tr>
<td></td>
<td>Type 2 diabetes</td>
<td>72.1±9</td>
<td>59.1±9</td>
<td>71.5±11.1</td>
</tr>
</tbody>
</table>

*Key:* 8-OHdG = 8-hydroxy-deoxy-guanosine; BGL = blood glucose level; GSH = erythrocyte reduced glutathione
number of participants in the control group, the large significant difference in the rise of serum 8-OHdG in the diabetes group compared with the controls (approximately three-fold higher) with lower control variance provides a sound basis for our conclusions. Our results demonstrate greater oxidative DNA damage in the diabetes group with a significant elevation in serum 8-OHdG in the first and second hours following glucose load. There was no such effect of this parameter in control subjects: the rate of increase was much less in the second hour, reducing the oxidative damage to DNA in line with the reduction in blood glucose (table 2, figure 2). The extent of the increase in 8-OHdG following the glucose load in the diabetes group suggests a sensitisation to free radical induced biochemical changes that can explain in part the increase in cardiovascular risk in people with hyperglycaemia. These results came in accordance with the results obtained by Ceriello and Andreassi and Botto.

In addition it has been suggested that much of the toxicity of ROS in living organisms is due to the iron-dependent generation of hydroxyl radicals, or other powerful oxidants through the Fenton chemical reaction that results in a marked increase in 8-OHdG. Kyong et al. and Huang et al. found that hydrogen peroxide, a source of hydroxyl radicals, is detoxified primarily through GSH activity. Therefore, in our study we investigated the level of GSH as an indicator of antioxidant state of the body. Significant changes in the antioxidant system expressed by the level of erythrocyte GSH in type 2 diabetes mellitus may be part of the pathology leading to sudden cardiac arrest in people with diabetes by increased free radical activity and endothelial damage or a reduction in free radical regulation as shown by the increase in 8-OHdG. In this study a statistically significant reduction in GSH levels in the diabetes group following the first hour after a glucose load was observed. This reduction in the function of the antioxidant system can be linked to a decrease in activities of the enzymes involved in GSH synthesis (such as γ-glutamycysteine synthetase) or in the transport rate of GSSG from erythrocytes and an enhanced sorbitol pathway, as shown by Ciuchi and co-workers. In the control group no significant changes in erythrocyte GSH concentration was observed, indicating that the global antioxidant response is compromised in the diabetes group to the extent that erythrocyte GSH levels are drastically reduced to counteract the free radical activity (table 2, figure 3). Conversely in the control group the global antioxidant system is able to deal with the increase in free radicals following glucose load and thus not require erythrocyte GSH stores initially; the erythrocytes synthesise more GSH during the second hour, through biochemical messaging, which can be released if required. Our results suggest that the efficiency of this defence mechanism is altered in diabetes and, therefore, the ineffective scavenging of free radicals may play a crucial role in determining tissue damage and increasing vulnerability to vascular disease.

The reduction in the level of GSH observed in type 2 diabetic patients reflects the significant rise in the level of 8-OHdG
Key messages

In type 2 diabetes postprandial:
- erythrocyte GSH is initially reduced, returning to baseline by 2h
- 8-OHdG is elevated, suggesting defective antioxidant response

due to the high amount of hydroxyl radicals generated as a response to an oral glucose load. The increased level of 8-OHdG in the diabetes group indicates endothelial and smooth muscle damage associated with atherosclerosis and a possible increased risk of cardiovascular disease in people with diabetes.

References