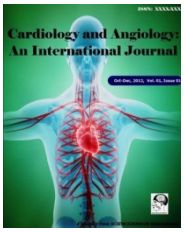

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Glutathione: Glutathione Sulfide Redox Imbalance in Early Impaired Fasting Glucose

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Authors' contributions

This work was carried out in collaboration between all authors. Authors HFJ and HAAA designed the study, wrote the protocol. Authors LM and SM wrote the first draft of the manuscript. Authors EB and DAJ managed the literature searches and analyses of the study. All authors agreed on the final edition of the paper.

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ABSTRACT

Aim: The current study aims to examine the balance between glutathione and glutathione sulfide and how this was disturbed in patients with impaired fasting glucose (IFG) level. The study also included 8-hydroxy-2'-deoxyguanosine to provide a more comprehensive picture of the overall redox state.
Methodology: A cross-sectional analysis of ninety medication free participants without reported history of cardiovascular disease and/or diabetes mellitus was undertaken with data collected from the Diabetes Complications Research Initiative database at Charles Sturt University. Fasting blood glucose, HbA1c and cholesterol as standard markers for diabetes mellitus and associated complications were measured in addition to the emerging biomarkers glutathione (GSH), glutathione disulfide (GSSG), and urinary 8-hydroxy-2'-deoxyguanosine (8OHdG).

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Results: The IFG group had a mean blood glucose level above 6.1mmol/L being significantly higher compared to control (P<0.001). Traditional clinical markers were all within the normal range for both groups. However the GSH/GSSG ratio (8.53±5.4 vs 6.62±2.2, P=.04) was significantly lower in the IFG group. GSH and 8OHdG, being markers for oxidative stress, were not significantly different between the two groups.
Conclusion: The free radical related changes in metabolic redox pathways are linked to oxidative stress and related pathologies but may not be associated with disease progression, providing an explanation why conflicting results are presented in the literature concerning any individual biomarkers and risk of diabetes. Our study included individuals with no medication use and mild hyperglycemia (impaired fasting glucose) and indicates a pro-oxidant response to mild-moderate hyperglycemia with a moderate rise in oxidative DNA damage.

Keywords: Impaired fasting glucose; oxidative stress; antioxidants; glutathione; GSH/GSSG ratio.

1. INTRODUCTION

Oxidative stress is implicated in diabetes mellitus, obesity, atherosclerosis, coronary artery disease, heart failure, and renal disease [1]. Metabolic syndrome may form a common link between these disorders due to hyperglycemia induced oxidative stress mechanisms. Glutathione is one of the main cellular antioxidants which has been found to increase in prediabetes and diabetes mellitus [2,3]. However the diversity of redox associated changes in redox signaling pathways associated with diabetes progression requires the identification of suitable reaction products that correlate with the pathophysiological processes associated with oxidative stress from normoglycemia to chronic hyperglycemia [4]. The oxidative stress can generate free radicals which are able to cause damage to the DNA, proteins and lipids [2–4]. These radicals react with guanine bases in DNA to form 8-hydroxy-2'-deoxyguanosine (8OHdG) [5]. Previous studies have shown that 8OHdG is associated with increased risks of diabetes mellitus [6,7] and atherosclerosis [8]. In our previous studies, we observed that serum 8OHdG was significantly elevated among the impaired fasting glucose (IFG) group compared to the control group [9].

2. MATERIALS AND METHODS

Data for this study was obtained from patients attending the diabetes complications clinic at Charles Sturt University, Australia. Participants were recruited via public media announcements. 428 participants (female: male, 247:181) were screened and after excluding those with diabetes mellitus, cardiovascular (CVD) or renal disease as well as those taking any medication, data from 62 control and 28 impaired fasting glucose participants were analyzed. Impaired fasting glucose was set at 5.6mmol/L in accordance with the American Diabetes Association [10]. Anthropometric data was obtained (Table 1) in addition to blood glucose levels (BGL), and cholesterol profile. Differences in the level of biochemical markers of oxidative stress (erythrocyte reduced glutathione (GSH)/glutathione disulfide (GSSG)), endothelial dysfunction (urinary 8OHdG) and lipid were determined for IFG and control subjects.

After an overnight fast, whole blood specimens were collected into heparin and EDTA tubes for analysis. Plasma was separated within 1 hour by centrifugation at 1000xg for 10min.

Plasma from heparin-containing tubes was immediately used for lipid analysis. Plasma from EDTA-containing tubes was kept at -80℃ for serum 8OHdG and GSH analysis. Fasting plasma total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) were measured by standard techniques. TC and TG were determined with a commercial enzymatic kit. HDL-C was determined by immunoinhibition assay. Low-density lipoprotein cholesterol (LDL-C) was calculated according to the Friedewald formula [11]. Serum 8OHdG was measured using an EIA Kit, Cayman Chemical, MI, USA [12]. The test utilizes an anti-mouse IgG-coated plate and a tracer consisting of an 8OHdG-enzyme conjugate which detects all three oxidized guanine species; 8-hydroxy-2'-deoxyguanosine from DNA, 8-hydroxyguanosine from RNA, and 8-hydroxyguanine from either DNA or RNA. This format has the advantage of providing low variability and increased sensitivity compared with assays that utilize an antigen coated plate and only detect 8OHdG. Fresh blood was kept on ice for not more than 1 hour to measure GSH. The level of erythrocyte GSH was determined using the 5, 5′-dithiobis-2-nitrobenzoic acid (DTNB) reaction [13]. The GSH: GSSG ratio was determined using the formula (total GSH-2GSSG)/GSSG.

3. RESULTS

Participants were divided into two groups: (i) a control group with normal blood glucose levels and (ii) an IFG group with impaired fasting blood glucose according to the recommendations of the American Diabetic Association [4]. (Table 1) shows the main demographic characteristics of the two groups. The control and impaired fasting glucose subjects were comparable for the body mass index, and for systolic and diastolic blood pressure but the IFG group was significantly older. No participants were taking either antiarrhythmic, antihypertensive medication or statins.

Table 1. Anthropometric results of the participants involved in this study

	Control (n=62)	IFG (n=28)	*P* value
Age (years)	62±11.6*	66.4±11	ns**
BMI (kg/m^2) §	26±4	25.8±4	ns
SBP (mmHg)	120±14	124±13	ns
DBP (mmHg)	74±7	74±7	ns

**mean±s.d.; ** nonsignificant; § BMI = body mass index, SBP–systolic blood pressure, DBP–diastolic blood pressure*

Table 2. Biomarker results

	Control (n= 62)	IFG (n=28)	*P* value
BGL(mmol/L)§	4.8±0.3*	6.1±0.4	*P*<0.001**
HbA1c(%)	5.6±0.5	5.8±0.5	ns
TC (mmol/L)	5.2±0.9	5.5±1	ns
Triglycerides(mmol/L	1.1±0.6	1.1±0.4	ns
HDL-C(mmol/L)	1.6±0.5	1.7±0.5	ns
LDL-C(mmol/L)	3.1±0.8	3.2±1	ns
GSH(mg/100ml)	68.62±19.6	70.2±20.8	ns
GSSG(mg/100ml)	21.9±13.6	22.7±9.1	ns
GSH:GSSG	8.53±5.4	6.62±2.2	*P*=.04
8OHdG(ng/ml)	204.5±157.4	232±161.7	ns

**mean±s.d.; **non significant; § BGL-blood glucose level, HbA1c – glycated hemoglobin, TC–total cholesterol, HDL-C–high density lipoprotein-cholesterol, LDL-C – low density lipoprotein-cholesterol, GSH–glutathione, GSSG–glutathione disulfide, 8OHdG - 8-hydroxy-2'-deoxyguanosine*

(Table 2 above) shows the biomarker results for the study. There were no significant differences in blood lipid levels between the two groups ($p>0.05$). Reduced GSH and glutathione disulfide were not significantly different between the two groups, nor was 8OHdG. The GSH/GSS Gratio was significantly ($p<0.05$) decreased in the IFG group.

4. DISCUSSION

IFG is the preclinical stage of diabetes mellitus and is a useful target for early intervention therapies to prevent the development of this wide-spread disease and its associated complications. We focused on investigating changes in biomarkers associated with moderate increases in BGL as observed for IFG in individuals with no CVD, hypertension or kidney dysfunction and free of medication. Based on the definition of IFG, we expected to find adverse effects of mild hyperglycaemia in this group. However the HbA1c, blood lipid levels and blood pressure did not differ between the groups.

Increased blood sugar levels are associated with oxidative stress leading to a myriad of free radical formation and antioxidant response [14-16]. Free radicals, non radical oxidants and non radicalthiol-reactive chemicals all play a role in the multiple metabolic pathways associated with oxidative stress and diabetes mellitus [17].

A very important finding of this study is the significant decrease in GSH/GSSG ratio in the IFG group compared to control. The significant difference in GSH:GSSG between the two groups may be indicating an early thiol related oxidative stress response and impaired redox status with erythrocyte GSH levels slightly up but close to the control group. This is consistent with our earlier findings and those reported in the literature, which showed that changes in the antioxidant status, especially the erythrocyte glutathione system, characterize the initial phase of oxidative stress in diabetes mellitus progression and commences prior to the establishment of the disease when BGL are above 7mmol/L [18-20]. Reduced glutathione is an ubiquitous antioxidant, which has been shown to be lower in IGT subjects with similar levels to that reported in frank diabetes mellitus [20,21]. Glutathione protects against peroxides and toxic aldehydes by undergoing oxidation to GSSG. However lower GSH and higher GSSG levels are not only associated with disease processes but also with aging, where the GSH pool is more oxidized [22,23]. From our data it can be seen that the GSH pool size and reduced GSH remained relatively constant between controls and IFG participants. This suggests that age-related changes do not play a major role in the current cohort and the glutathione redox system is reacting to the increases in blood glucose levels associated with IFG, keeping oxidative stress to a minimum.

Previous work of ours has shown that although a decrease in reduced GSH is seen in IGT, the change was not significant [9] and may be associated with lipid peroxidation and endothelial damage in the vascular walls, which increases homocysteine and therefore reduces cysteine availability to GSH. Dincer et al. reported that oxidative stress inhibits the activity of glutathione reductase, the enzyme that regenerates GSH from GSSG [24], an effect, which could have contributed to the increased GSSG levels in our study. In the current study we demonstrate a significant reduction in GSH:GSSG redox balance indicating oxidative stress. GSH:GSSG may be a robust early marker of oxidative stress associated with impaired fasting glucose in the presence of a normal cholesterol profile and has been associated with type 2diabetes mellitus.

We have also previously shown that 8OHdG is increased significantly in IFG but requires an increase in triglycerides. However this association between cholesterol and 8OHdG is

controversial with some authors showing positive and others negative associations to cholesterol levels [25-28]. In our work a drop in HDL or increase in LDL does not seem to affect 8OHdG when BGL is below 7mmol/L and HbA1c below 6.4% [29]. The current study still indicates this increase in 8OHdG, although not to a significant extent. These findings highlight the complex cellular interactions associated with diabetes mellitus and diabetes progression.

The pathophysiology of diabetes mellitus progression and associated changes in multiple biomarkers do not constitute a separate linear model for each biomarker such as HbA1c, lipids, oxidative stress and inflammatory markers but are a part of a complex interactive model, which changes depending on additional factors such as age and comorbidity [30]. Our model of mild IFG, within this framework of redox balance, suggests that redox balance can be impaired due to minor increases in blood glucose levels, which are not enough to decrease erythrocyte GSH or increase GSSG levels but do effect the GSH:GSSG balance. Changes in the GSH/GSSG redox state have been proposed to occur also in the absence of oxidative stress and depending on the level gluconeogenesis [31-33].

5. CONCLUSION

The free radical related changes in metabolic redox pathways are linked to oxidative stress and related pathologies but may not be associated with disease progression, providing an explanation why conflicting results are presented in the literature concerning any individual biomarkers and risk of diabetes mellitus. Our study included individuals with no medication use and mild hyperglycemia (IFG) and indicates a pro-oxidant response to mild-moderate hyperglycemia with a moderate rise in oxidative DNA damage.

CONSENT

All authors declare that ‘written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the Human Research Ethics Committee, Charles Sturt university and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Montuschi PLJ, Barnes P. Fau-Roberts 2nd, and LJ. Roberts 2nd. Insights into oxidative stress: The isoprostanes. FASEB J. 2004;18(0929-8673):1791-1800.
2. Al-Aubaidy HA, Jelinek HF. Oxidative DNA damage: Antioxidant response in postprandial hyperglycaemia in type 2 diabetes mellitus. BJDVD. 2011;11(2):87-91.
3. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature. 2001;414(6865):813-820.

4. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res. 2010;107(9):1058-1070.
5. Erhola M, et al. Biomarker evidence of DNA oxidation in lung cancer patients: association of urinary 8-hydroxy-2'-deoxyguanosine excretion with radiotherapy, chemotherapy and response to treatment. FEBS Lett. 1997;409(2):287-91.
6. Endo K, et al. Probucol and atorvastatin decrease urinary 8-hydroxy-2'-deoxyguanosine in patients with diabetes and hypercholesterolemia. J Atheroscler Thromb. 2006;13(1):68-75.
7. Nakanishi S, et al. Increasing of oxidative stress from mitochondria in type 2 diabetic patients. Diabetes Metab Res Rev. 2004;20(5):399-404.
8. Mahmoudi M, Mercer J, Bennett M. DNA damage and repair in atherosclerosis. Cardiovasc Res. 2006;71(2):259-68.
9. Al-Aubaidy HA, Jelinek HF. Preclinical DNA damage assessed by 8 Hydroxy-2-deoxy-Guanosine in a rural diabetes screening clinic. Redox Rep. 2010;15(3):1-7.
10. Endocrinology ACo. Prediabetes Consensus Statement. Diagnosis and management of prediabetes in the continuum of hyperglycemia—When do the risks of diabetes begins? Endocr Pract. 2008;14(7):934-946.
11. Friedewald W, Levy R, Frederickson D. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifugation. Clin Chem. 1972;18:499-502.
12. Noiri E, et al. Oxidative and nitrosative stress in acute renal ischemia. Am J Physiol-Renal. 2001;281(5):948-57.
13. Lowry O, et al. Effects of ischemia on known substrates and cofactors of the glycolytic pathway in the brain. J Biol Chem. 1964;239:18.
14. Bandeira SDM, et al. Oxidative stress as an underlying contributor in the development of chronic complications in diabetes mellitus. Int J Mol Sci. 2013;14(2):3265–3284.
15. Menon V, et al. Oxidative stress and glucose levels in a population-based sample. Diabet Med. 2004;21(12):1346-52.
16. Whiting PH, et al. The relationship between chronic glycaemic control and oxidative stress in type 2 diabetes mellitus. Br J Biomed Sci. 2008;65(2):71-4.
17. Jones DP. Radical-free biology of oxidative stress. Am J Physiol Cell Physiol. 2008;295(4):C849-C868.
18. Hayden MR, Tyagi SC. Intimal redox stress: Accelerated atherosclerosis in metabolic syndrome and type 2 diabetes mellitus. Atheroscleropathy. Cardiovascular Diabetology. 2002;1:3.
19. Nwose EU, et al. Atherothrombosis and oxidative stress: The connection and correlation in diabetes. Redox Rep. 2009;14(2):55-60.
20. Vijayalingam S, et al. Abnormal antioxidant status in impaired glucose tolerance and non-insulin-dependent diabetes mellitus. Diab Med. 1996;13(8):715-719.
21. Sekhar RV, et al. Glutathione synthesis Is diminished in patients with uncontrolled diabetes and restored by dietary supplementation with cysteine and glycine. Diabetes Care. 2011;34(1):162-167.
22. Samiec PS, et al. Glutathione in human plasma: Decline in association with aging, age-related macular degeneration, and diabetes. Free Radic Biol Med. 1998;24(5):699-704.
23. Rebrin I, Sohal RS. Pro-oxidant shift in glutathione redox state during aging. Adv Drug Deliv Rev. 2008;60(13-14):1545-52.
24. Dincer Y, et al. Susceptibility of glutatione and glutathione-related antioxidant activity to hydrogen peroxide in patients with type 2 diabetes: effects of glycemic control. Clin Biochem. 2002;35:297-301.

25. Subash P, et al. Urinary 8OHdG: A marker of oxidative stress to DNA and total anioxidant status in essential hypertension with South Indian population. Indian Journal of Clin Biochm. 2010;25(2):127-132.
26. Miyamoto M, et al. The relationship between urinary 8-hydroxydeoxyguanosine and metabolic risk factors in asymptomatic subjects. Med Princ Pract. 2011;20(2):187-90.
27. Sakano N, et al. Oxidative stress biomarkers and lifestyles in Japanese healthy people. J Clin Biochem Nutr. 2009;44(2):185-95.
28. Kikuchi H, et al. Lower serum levels of total cholesterol are associated with higher urinary levels of 8-hydroxydeoxyguanosine. Nut and Metab. 2013;10(1):59.
29. Al-Aubaidy H, Jelinek HF. Oxidative stress and triglycerides as predictors of subclinical atherosclerosis in prediabetes. Redox Rep. 2014;19(2):87-91.
30. Mazurak N, et al. Heart rate variability as a measure of cardiac autonomic function in anorexia nervosa: A review of the literature. Eur Eat Disord Rev; 2010.
31. Miller LT, et al. Oxidation of the glutathione/glutathione disulfide redox state Is Induced by cysteine deficiency in human colon carcinoma HT29 Cells. Nutrition; 2002;132(8):2303-2306.
32. Winiarska K, et al. Relationship between gluconeogenesis and glutathione redox state in rabbit kidney-cortex tubules. Metabolism. 2003;52(6):739-46.
33. Winiarska K, et al. Diabetes-induced changes in glucose synthesis, intracellular glutathione status and hydroxyl free radical generation in rabbit kidney-cortex tubules. Mol Cell Biochem. 2004;261(1-2):91-8.

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