Research article

Oxidative stress and triglycerides as predictors of subclinical atherosclerosis in prediabetes

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Background: The role of triglycerides in early preclinical atherosclerosis is controversial. Antioxidant markers may be associated with triglyceride levels in early preclinical atherosclerosis especially when fasting plasma glucose is raised.

Methods: This cross-sectional study included 127 participants attending the Diabetes Screening Clinic, Charles Sturt University, Australia.

Results: Serum 8-hydroxy-2-deoxy-guanosine (8-OHdG) was significantly greater in the impaired fasting glucose (IFG) group compared with the control group (536.7 pg/ml ± 249.8 versus 171.4 pg/ml ± 96.9, respectively). The increase in 8-OHdG was associated with a mildly non-significant elevation in low-density lipoprotein level (3.2 ± 1.1 mmol/l) and a poor level of high-density lipoprotein (1.31 ± 0.3 mmol/l) in the IFG group. However, a significant increase in triglycerides (1.6 ± 0.97 mmol/l; $P < 0.05$) in the IFG group was observed. Erythrocyte reduced glutathione (GSH) levels in the IFG group, although increased, were also not significantly different to control.

Conclusion: A significant increase in 8-OHdG is associated with increased levels of triglycerides in the absence of significant changes in reduced GSH and normal levels of cholesterol in the IFG cohort, suggesting that oxidative stress may be present and indicative of subclinical atherosclerosis.

Keywords: Triglycerides, Impaired fasting glucose, Glutathione, 8-OHdG, Atherosclerosis

Introduction

Previous studies have shown that endothelial dysfunction in the vasculature is traditionally associated with elevated low-density lipoprotein cholesterol (LDL-C), which is involved in the progression of atherosclerosis through mechanisms including lipid peroxidation.[1] However, recent work in animal models highlighted that these complex pathophysiological mechanisms associated with arterial wall cholesterol level, lipid peroxidation, and endothelial dysfunction are not correlated with plasma total cholesterol (TC) level.[2] The mechanism by which oxidative stress and serum cholesterol causes cardiovascular disease (CVD) therefore remains to be clarified.[3]

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An association between 8-hydroxy-2-deoxy-guanosine (8-OHdG) and endothelial dysfunction have been proposed.[4,5] Our previous studies confirmed these results and showed significant differences between the degree of oxidative stress and lipid peroxidation measured by 8-OHdG, erythrocyte reduced glutathione (GSH), and erythrocyte malondialdehyde (MDA) between prediabetes/impaired fasting glucose (IFG) and type 2 diabetes mellitus.[6,7] However, possible biochemical or structural mechanisms that can further explain the pathophysiology at the IFG stage have not been clarified specifically, in disease progression, when only minor elevations, in blood glucose levels (BGLs) with normal levels of cholesterol but elevated triglycerides (TGs), have not been investigated.

Oxidative stress and DNA strand breakage can be caused by hyperglycaemic events that lead to the

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formation of reactive oxygen species that attack DNA strands and cause destruction of endothelial function and atherosclerosis.[8] Oxidative DNA damage and repair due to free-radical activity is present in arterial endothelial cells of patients with atherosclerosis as well as in diabetes, CVD.[9–11] 8-OHdG is considered to be the most sensitive biomarker of oxidative DNA damage and repair and is an early marker of endothelial dysfunction,[7,12] indicating not only DNA and RNA strand damage and repair but also 8-OHdGMP levels in the serum.[13]

Erythrocyte reduced GSH is the main cellular antioxidant found in red blood cells, acting as a scavenger for free radicals such as hydrogen peroxide entering the red blood cell.[14–16] GSH levels have been shown to be dependent on diurnal rhythm, stress, exercise, and diabetes progression associated with glucose and insulin levels.[17,18] Thus, emerging biomarkers, such as 8-OhdG or GSH, may play an important role in understanding the association between atherosclerosis and IFG and the associated biological processes.

The primary purpose of the current work was to investigate whether the 8-OHdG and the GSH levels are different in IFG compared with the normal levels of BGL and the role of the serum lipids, and/or the glycated haemoglobin (HbA1c) in disease progression.

Materials and methods

Protocol

The study protocol was reviewed and approved by the ethics in human research committee of Charles Sturt University in accordance with the provisions set out in the Declaration of Helsinki. Informed consent was obtained from each participant after a full explanation of the purpose, nature, and risk of all the procedures used was provided by the principal investigator. IFG and control participants at the CSU Diabetes Screening Clinic were drawn from the community through announcements in the local newspaper, radio, and television between February 2006 and June 2012. Only participants for whom complete data were available as required for this study were included in the analysis. The exclusion criteria included any person with comorbidities such as kidney and/or cardiovascular/cerebrovascular and hypertension disease.

Population sample

According to the American Diabetes Association, prediabetes is defined as an impaired fasting BGL (5.6–6.9 mmol/l).[19] The control participants had normal BGL and no history of diabetes, cardiac, respiratory, or renal disease.

After an overnight fast, whole blood specimens were collected into heparin and ethylenediaminetetraacetic acid (EDTA) tubes for analysis. Plasma was separated within 1 hour by centrifugation at 1000 *g* for 10 minutes. Plasma from heparin-containing tubes was immediately used for MDA analysis. MDA analysis was based on the protocol of thiobarbituric acid reactive substances.[20]

Plasma from EDTA-containing tubes was kept at −80 °C for serum 8-OHdG and GSH analysis.

BGL and HbA1c were measured according to Australian Laboratory standards by the local pathology laboratory. Fasting plasma TC and TGs were determined with a commercial enzymatic kit. High-density lipoprotein cholesterol (HDL-C) was determined by immunoinhibition assay. LDL-C was calculated according to the Friedewald formula.[21]

The atherogenic index of plasma (AIP) was defined as the logarithm to the base 10 of the ratio of fasting plasma TGs to HDL-C measured in mmol/l.[22] Dobiasova and Frohlich classified the risk of atherogenicity depending on the level of AIP into: AIP < 0.11 – low risk; AIP between 0.11–0.21 intermediate risk; and AIP > 0.21 high risk.[23] The logarithm of the ratio log (TG/HDL-C) corrects for the lack of a normal distribution of this parameter in a population sample and correlates with the presence of smaller LDL-C.[23]

Serum 8-OHdG was measured using an ELISA Kit, Cayman Chemical, MI, USA.[24] The test utilizes an anti-mouse IgG-coated plate and a tracer consisting of an 8-OHdG-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 8-hydroxy-2′-deoxy-guanosine.

Fresh blood was kept on ice for not more than 1 hour to measure GSH. The level of erythrocyte reduced GSH was determined using the 5,5′-dithiobis-2-nitrobenzoic acid reaction.[25]

Statistical analysis

The results of 8-OHdG, GSH, HbA1c, MDA, cholesterol, and AIP levels were compared between the control and the IFG groups. Categorical data are presented as a frequency table, and quantitative data were analysed using the Statistical Package for Social Sciences (SPSS Version 20, Sydney, NSW, Australia) and Microsoft Excel (Office2007, Microsoft). All values are expressed as mean ± SD. Statistical analysis was performed using Student's *t*-test. Pearson's correlation was performed to illustrate the correlation between 8-OHdG, HDL-C, AIP, and GSH where appropriate non-parametric statistics based on median and interquartile range were used. Power analysis using a high-power coefficient (0.8) and

Table 1 Characteristics of the subjects included in this study

	Control	IFG	*P* value
Number	103	24	
Age (years)	66.2 ± 11.2	64.3 ± 11.3	NS[a]
Sex (man/women)	42/61	13/11	NS[a]
Fasting plasma glucose (mmol/l)	4.8 ± 0.5	5.95 ± 0.3	<0.001
HbA1c (%)	5.6 ± 0.26	5.7 ± 0.2	NS[a]
BMI (kg/m^2)	27.6 ± 4.4	26.9 ± 3.4	NS[a]
Systolic blood pressure (mmHg)	130.1 ± 19.7	129.5 ± 14.4	NS[a]
Diastolic blood pressure (mmHg)	76.6 ± 8.6	75.9 ± 7.7	NS[a]
Statin	20 (19.4%)	9 (37.5%)	
Antihypertensive medication	46 (44.7%)	10 (41.6%)	

[a]NS, non-significant.

Table 2 Biomarkers of oxidative stress and atherogenicity (mean ± SD) in the control and the IFG groups

Biomarkers	Control	IFG	*P* value
Total cholesterol (mmol/l)	5.1 ± 1	5.1 ± 1.2	NS[a]
Triglycerides (mmol/l)	1.26 ± 0.6	1.6 ± 0.97	<0.05
HDL-C (mmol/l)	1.35 ± 0.3	1.31 ± 0.3	NS[a]
LDL-C (mmol/l)	3.3 ± 0.85	3.2 ± 1.1	NS
AIP	−0.06 ± 0.02	0.056 ± 0.03	NS
MDA (nmol/l)	11.63 ± 6.7	12.8 ± 5.9	NS
GSH (mg/100 ml)	70.3 ± 12	71.7 ± 18	NS
8-OHdG (pg/ml)	171.4 ± 96.9	536.7 ± 249.8	<0.001

[a]NS, non-significant.
HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; AIP, atherogenic index of plasma; 8-OHdG, 8-hydroxy-deoxy-guanosine; GSH, reduced erythrocyte glutathione.

medium effect size (0.6) indicated that the number of samples included in the analysis was sufficient to obtain a significant difference at $P < 0.05$. Missing data constituted no more than 20% of data.

Results

Disease status, fasting BGL, age, sex, body mass index (BMI), blood pressure, and HbA1c values are illustrated in Table 1 for the 127 participants. There were no significant differences between the control and the IFG groups for sex, age, blood pressure, HbA1c, and BMI (Table 1). BGL was significantly increased in the IFG group compared with the control groups ($P < 0.001$).

There was no significant difference in the use of antihypertensive medications between the two groups. On the other hand, 19.4% of the control group used statins compared with 37.5% of the IFG group.

TGs were significantly elevated in the IFG group ($P < 0.05$). This profile corresponds with a slight but non-significant increase in MDA (Table 2), but no significant differences were noted for TC, LDL-C and HDL-C profile. The AIP was in the low-risk range for both the groups although it was higher in the IFG group.

Serum 8-OHdG showed a significant elevation in the IFG group (536.7 ± 249.8 pg/ml; $P < 0.001$) compared with the control group (171.4 ± 96.9 pg/ml; Table 2). While erythrocyte reduced GSH did not significantly change in the IFG group (71.7 ± 18 mg/100 ml) compared with the control group (70.3 ± 12 mg/100 ml) shown in Table 2.

Cholesterol levels are indicative of atherosclerosis risk. A significant positive correlation was observed between serum 8-OHdG and the AIP and a negative correlation between 8-OHdG and HDL-C suggesting a relationship between atherosclerosis risk and oxidative stress. No significant correlations were observed with GSH or LDL-C. In addition, 8-OHdG and GSH levels were not correlated in this study (Table 3).

Discussion

Our study suggests that early, preclinical atherosclerosis changes in the vasculature may be associated with increased 8-OHdG if the TGs are elevated in the presence of increased BGL but below 6.1 mmol/l and normal levels of cholesterol, lipid peroxidation, and

Table 3 Pearson correlation between 8-OHdG, GSH, AIP, and HDL-C

		AIP	8-OHdG (pg/ml)	HDL (mmol/l)	GSH in mg/100 ml
AIP	Correlation	1	0.239	−0.714[a]	−0.015
	Sig. (two-tailed)		0.007	0.001	0.898
8-OHdG (pg/ml)	Correlation		1	−0.228[a]	−0.081
	Sig. (two-tailed)			0.01	0.4
HDL (mmol/l)	Correlation			1	0.013
	Sig. (two-tailed)				0.909

[a]Correlation is significant at $P \leq 0.05$ (two-tailed).

HbA1c. This milieu suggests a low-level oxidative stress environment with GSH not yet elevated.

Studies investigating early or subclinical CVD risk in IFG have shown no greater adverse CVD risk.[26] Similarly, insulin resistance and metabolic syndrome was not associated with subclinical atherosclerosis in the Multi-Ethnic Study of Atherosclerosis (MESA).[27] The Atherosclerosis Risk in Communities Study (ARIC) showed no correlation between HbA1c < 6% and coronary heart disease or death from any cause, but a 21% risk of diabetes regardless of the level of BGL.[28,29] However, subclinical atherosclerosis was not investigated.

The current study suggests a possible model for preclinical atherosclerosis and oxidative stress associated with increased TGs by including 8-OHdG and GSH. Although the association between TGs and the risk of CVD is controversial, our findings extend previous reports that TGs are more likely to be associated with atherosclerosis [30,31] and suggests that small increases in BGL combined with an increase in TGs may be a stimulus for oxidative DNA damage measured by 8-OHdG prior to an antioxidant GSH response.[16,32,33] MDA was slightly but not significantly increased in the IFG group, confirming that lipid peroxidation is not a strong contributor to the observed biomarker levels in the IFG unless stimulated by acute increases in the BGL as is the case when an oral glucose tolerance test is applied.[34]

Medication use may have contributed to some of the results noted in our study. However, there was no significant difference in the use of antihypertensive medication between the two groups. Statin use was higher in the IFG group and may have contributed to the borderline normal levels of LDL in both the groups but despite their reported antioxidant effects, the IFG group had significantly higher 8-OHdG. The number of subjects may have also influenced the results. However, our study was adequately powered and the significance of the results is high. Larger cohort studies are required to determine the influence of duration of IFG that can lead to oxidative DNA damage by determining the activity of additional oxidative stress and inflammatory markers including catalase enzyme activity and protein oxidation as well as NADPH.[34,35]

Conclusion

We propose that preclinical atherosclerosis is associated with raised BGL (below 6.1 mmol/l), normal cholesterol with no significant lipid peroxidation but an increased TG level, which results in increased 8-OHdG but normal GSH. Medications such as antihypertensive medication or statins do not protect endothelial cells from free-radical damage in our model. Therefore, serum TGs remain a strong indicator for the risk of atherosclerosis and coronary artery disease, but may need to be combined with measures of 8-OHdG and GSH if the clinical aim is to reduce the likelihood of atherosclerosis development, especially in individuals with IFG.

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