

Original investigation

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Plasma matrix metalloproteinases, low density lipoprotein oxidisability and soluble adhesion molecules after a glucose load in Type 2 diabetes

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Published: 18 June 2004

Received: 06 February 2004

Cardiovascular Diabetology 2004, **3**:7

Accepted: 18 June 2004

This article is available from: <http://www.cardiab.com/content/3/1/7>

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Abstract

Background: Acute hyperglycaemia is an independent cardiovascular risk factor in Type 2 diabetes which may be mediated through increased oxidative damage to plasma low density lipoprotein, and in vitro, high glucose concentrations promote proatherogenic adhesion molecule expression and matrix metalloproteinase expression.

Methods: We examined these atherogenic risk markers in 21 subjects with Type 2 diabetes and 20 controls during an oral 75 g glucose tolerance test. Plasma soluble adhesion molecule concentrations [E-selectin, VCAM-1 and ICAM-1], plasma matrix metalloproteinases [MMP-3 and 9] and plasma LDL oxidisability were measured at 30 minute intervals.

Results: In the diabetes group, the concentrations of all plasma soluble adhesion molecules fell promptly [all $p < 0.0001$] related principally to glycaemic excursions, but such changes also occurred in the control group. Plasma MMP-3 and -9 concentrations were lower [$p < 0.05$], and LDL oxidisability greater [$p < 0.01$] in the diabetes group but did not change in either group. There was a direct relationship between plasma MMP-9 and s ICAM-1 in the controls [$r = 0.62$; $p = 0.006$] perhaps suggesting a functional relationship between s ICAM-1 shedding and MMP-9.

Conclusions: A glucose load leads to a rapid fall in plasma soluble adhesion molecule concentrations in Type 2 diabetes and controls, perhaps reflecting reduced generation of soluble from membrane forms during enhanced leukocyte – endothelial adhesion or increased hepatic clearance, without changes in plasma matrix metalloproteinase concentrations or low density lipoprotein oxidisability. These in vivo findings are in contrast with in vitro data.

Introduction

Epidemiological and prospective studies have shown a consistent direct relationship between blood glucose lev-

els and cardiovascular morbidity and mortality [1] in Type 2 diabetes, but some data suggest acute or post-prandial hyperglycaemia may be an independent predictor of

cardiovascular morbidity in these patients and in people without diabetes [2,3]. Surrogates for macro vascular disease such as carotid intimal – medial thickness are more closely related to acute rather chronic hyperglycaemia [4] and much of the controversy over the recent diagnostic classification of diabetes was based on the predictive power of post glucose load hyperglycaemia as a marker for increased vascular risk [5], which could be mediated by acute oxidative damage to lipoproteins, proteins and DNA [6]

Oxidatively modified plasma low density lipoproteins and elevated glucose concentrations promote the expression of adhesion molecules on vascular endothelial cells in vitro [7,8], through a mechanism sensitive to oxidative balance [8], and soluble forms of some of these adhesion molecules are a marker of increased macrovascular risk in people with and without diabetes [9] although there is uncertainty if they contribute directly to this risk. A further unexamined link between acute hyperglycaemia and macrovascular disease in Type 2 diabetes is the effect of acute hyperglycaemia on matrix metalloproteinase [MMP] expression by cells involved in the atherogenic process. The MMP system has a role in maintaining the stability of coronary atheromatous plaque [10], and in vitro some cellular MMP message and product are actively promoted by high glucose concentrations and oxidatively damaged lipoproteins [11], and elevated plasma MMP concentrations may be a feature of coronary atherosclerosis, carotid plaque instability and a range of inflammatory and malignant processes [12-14]. Finally, acute hyperglycaemia may

be associated with proatherogenic modifications to plasma lipoproteins [15,16]

We hypothesised that acute glycaemic excursions in subjects with or without Type 2 diabetes would promote LDL oxidative susceptibility, increase the cell membrane shedding and plasma concentrations of soluble forms of key adhesion molecules, and promote increased plasma concentrations of MMP.

Materials and Methods

Patient selection

After local Ethical Committee approval and written informed consent, we studied 21 patients with Type 2 diabetes and 20 controls. All were non-smokers between 40 and 65 years old, and patients were considered to have Type 2 diabetes if they had been diagnosed after the age of 40 years, with no history of ketosis and with stable glycaemic control on diet or oral hypoglycaemics. Patients with treated hypertension, with clinical evidence or history of coronary artery disease, or receiving hormone replacement therapy or insulin were excluded. Patients with microalbuminuria [defined as an elevated urine albumin: creatinine ratio] or macroproteinuria [defined as albuminuria positive proteinuria] were also excluded. Seven of the 21 patients had background retinopathy, by definition none had microalbuminuria and 6 were diet controlled alone. All controls had a fasting plasma glucose below 6.1 mmol/l and had no significant past medical history of hypertension or cardiovascular disease. Baseline data are shown in Table 1.

Table 1: Baseline data on 21 subjects with Type 2 diabetes and 20 controls without diabetes.

	Type 2 diabetes	Controls	p
Number	21	20	
Age [yrs]	57.1 [6.3]	59.0 [9.9]	ns
Known Diabetes duration [yrs]	5.7 [7.1]	-	
M:F	10:11	11:9	ns
Body Mass Index [kg/m ²]	30.1 [4.8]	26.7 [3.8]	ns
Waist – hip ratio	0.91 [0.09]	0.86 [0.08]	ns
Diabetes treatment		-	
Diet	6		
Sulphonylurea	6		
Metformin	5		
Combination	4		
HbA1c [%]	7.3 [1.3]	5.1 [0.3]	0.0001
Total cholesterol [mmol/l]	5.8 [0.7]	5.8 [0.9]	ns
LDL cholesterol [mmol/l]	3.6 [0.5]	3.7 [0.9]	ns
Triglycerides [mmol/l]	2.5 [0.9]	1.6 [0.7]	0.03
HDL cholesterol [mmol/l]	1.04 [0.2]	1.36 [0.3]	0.03

Data are shown as a mean and one [standard deviation] NS = not significant; p > 0.05

Glucose tolerance tests [GTT] and glycaemic control

Oral glucose tolerance tests [75 g anhydrous glucose orally] were undertaken at 0800 hours with peripheral venous blood samples taken at 0, 30, 60, 90 and 120 minutes from an indwelling venous cannula. Patients omitted all oral hypoglycaemics on the morning of testing and had been fasting for 15 hours. Patients remained seated from time-15 minutes to 120 minutes.

Plasma soluble adhesion molecule concentrations

Plasma soluble intercellular adhesion molecule [s-ICAM-1], soluble vascular cell adhesion molecule [s-VCAM-1] and soluble E-selectin were measured during each glucose tolerance test at 0 minutes and at 60, 90 and 120 minutes after glucose loading. Concentrations of each soluble adhesion molecule were quantified using commercially available ELISA kits [R&D Systems plc, Abingdon, UK].

Plasma matrix metalloproteinase concentrations

Plasma MMP-3 and MMP-9 were measured at 0 and 90 minutes during the glucose tolerance test using BIOTRAK human ELISA systems [Amersham Pharmacia Biotech; Amersham, UK]. The MMP-3 assay detects total MMP-3 levels [pro, active, complexed] whereas the MMP-9 assay detects pro and complexed forms of the enzyme.

Plasma low density lipoprotein oxidisability

Plasma low density lipoprotein [LDL] oxidisability was measured at 0 and 90 minutes during each glucose tolerance test. The measurement of LDL susceptibility to oxidation was determined by the delay [lag] in the production of conjugated diene production at 234 nm by copper stimulated oxidation based on the method described by Kleinvelde et al. [17].

Measurement of lipid fractions

Plasma lipid profiles were measured at time 0 and 90 minutes during each glucose tolerance test using commercially available kits [Roche, Herts, UK] on an automated biochemistry analyser [COBAS MIRA, Roche, Welwyn, UK] with estimation of low density lipoprotein cholesterol [18] from total cholesterol, HDL cholesterol and triglycerides.

Measurement of glycaemic control

HbA1c was assessed using a commercially available kit [Roche Diagnostic systems, Welwyn, Herts, UK] on an automated biochemistry analyser [COBAS MIRA, Roche Diagnostic systems, Welwyn, UK] with the normal range quoted as 4.5 – 5.7%.

Statistical analysis

Data are shown as a mean and [one standard deviation] and all variables were normally distributed other than MMP-3 and MMP-9 concentrations [median and range].

Differences in individual variables during glucose tolerance tests were analysed by repeated measure one-way ANOVA [significance expressed as an F value for differences across the 4 time points] with paired t tests where a significant difference [$p < 0.05$] was found. Unpaired t tests or Mann Whitney U tests were used as appropriate for between group comparisons. Relationships between variables were analysed by simple linear regression, or stepped multiple regression analysis with entry at $p < 0.1$. Data were analysed on Apple Macintosh Statview software [1996].

Results**Clinical features and baseline data [Tables 1, 2]**

Clinical and baseline data are shown in the Tables. Groups did not differ in mean basal s ICAM-1 [$p = 0.45$] or in s E selectin [$p = 0.14$], but sVCAM-1 was significantly higher in the diabetes group compared to controls [$p = 0.001$].

Associations with baseline plasma soluble adhesion molecule concentrations

In the diabetes group, the only variables independently directly associated with s ICAM-1 at baseline [0 mins] were HbA1c [$R^2 = 0.29$, $F_{1,20} = 7.45$; $p = 0.01$] and fasting blood glucose [$R^2 = 0.16$; $F_{1,20} = 3.7$; $p = 0.06$]. The only variable independently associated with s E-selectin at baseline [0 mins] was fasting blood glucose [$R^2 = 0.18$; $F_{1,20} = 4.3$; $p = 0.05$]. However, s VCAM-1 concentrations at baseline during were and unrelated solely to any measure of glycaemic control. In the control group, there were no significant independent relationships between s ICAM-1, s E-selectin, or s VCAM-1 concentrations and fasting glucose or HbA1c measurements.

Plasma soluble adhesion molecules during GTT [Table 2]

In the diabetes group during GTT there was a significant fall in mean plasma soluble ICAM-1 [$F_{1,3} = 17.8$; $p < 0.0001$], VCAM-1 [$F_{1,3} = 14.1$; $p < 0.0001$] and E-selectin concentrations [$F_{1,3} = 16.2$; $p < 0.0001$]. The change was significant by 60 minutes, and maximum decrements were 9.7% [s ICAM-1], 12.9% [s VCAM-1] and 8.3% [s E-selectin], reached at either 90 minutes [E-selectin] or 120 minutes [s ICAM-1, s VCAM-1]. In the control group there were significant [$p < 0.001$] and equally prompt falls in plasma s-ICAM-1 and s E-Selectin, but not in s VCAM-1 [Table 2] during GTT. At all time points during GTT, s VCAM-1 concentrations were significantly higher in the Type 2 diabetes group compared to controls [$p < 0.05$], with no significant differences in s ICAM-1 or s E-selectin concentrations between groups during GTT.

Table 2: Changes in plasma soluble adhesion molecule, matrix metalloproteinase concentrations and LDL oxidisability during a glucose tolerance test in 21 patients with Type 2 diabetes and 20 controls.

	Time [mins]				p
	0	60	90	120	
Type 2 diabetes					
Blood glucose [mmol/l]	8.6 [2.7]	16.1 [3.8] *	17.8 [3.5] *	17.0 [3.6] *	0.0001
s ICAM-1 [ng/ml]	276 [85]	249 [76] *	249 [79] *	247 [78] *	0.0001
s VCAM-1 [ng/ml]	528 [222]	486 [216] *	472 [208] *	459 [194] *	0.0001
s E-Selectin [ng/ml]	82 [33]	76 [31] *	74 [32] *	75 [32] *	0.0001
LDL lag time [mins]	54.8 [9.8]	-	52.2 [9.3]	-	ns
Plasma triglycerides [mmol/l]	2.5 [0.9]	-	2.4 [0.7]	-	ns
MMP-3 [ng/ml]	13.7 [0 – 71]	-	18.0 [0 – 85]	-	ns
MMP-9 [ng/ml]	0.0 [0 – 129]	-	0.0 [0 – 75]	-	ns
Controls					
Time [mins]	0	60	90	120	p
Blood glucose [mmol/l]	4.6 [0.3]	7.0 [1.2] *	6.0 [1.1] *	5.4 [1.1] *	0.0001
s ICAM-1 [ng/ml]	280 [82]	219 [57] *	257 [70] *	230 [71] *	0.0001
s VCAM-1 [ng/ml]	321 [198]	349 [197]	321 [174]	338.7 [182]	0.23
s E-Selectin [ng/ml]	64 [24]	60 [21]	58 [22] **	59 [21]	0.003
LDL lag time [mins]	71.3 [8.1]	-	74.3 [10.2]	-	ns
Plasma triglycerides [mmol/l]	1.6 [0.7]	-	1.7 [0.8]	-	ns
MMP-3 [ng/ml]	30.8 [12.8 – 39]	-	26.4 [4.3 – 37.4]	-	ns
MMP-9 [ng/ml]	4.7 [1.2 – 41]	-	11.1 [2.5 – 41.9] *	-	0.03

Abbreviations as in text. All data shown as mean [SD] or as a median and range [MMP-3 and 9] and p values are for one way repeated measure ANOVA for each variable with * = $p < 0.001$, ** = $p < 0.01$ compared to time 0.

Associations with change in plasma adhesion molecule concentrations during GTT

In the diabetes group, the major determinant of the maximum change in adhesion molecule concentrations during GTT was the maximum blood glucose increment [mmol/l] for s ICAM-1 [$F_{1,20} = 4.41$; $p = 0.048$; $R^2 = 0.18$] and s E-selectin [$F_{1,20} = 7.4$; $p = 0.013$; $R^2 = 0.28$], but not s VCAM-c 1 [$F_{1,20} = 0.09$; $p = 0.75$; $R^2 = 0.04$]. This relationship was stronger when percentage changes in plasma s ICAM-1 [$F_{1,20} = 4.9$; $p = 0.028$; $R^2 = 0.23$] and s E-selectin [$F_{1,20} = 7.8$; $p = 0.01$; $R^2 = 0.29$] were used. In the controls, the maximum decrement in plasma s ICAM-1 and s-selectin concentrations were unrelated to blood glucose increment [$p = 0.84$ and 0.47 respectively]

Plasma MMP-3 and MMP-9 concentrations [Table 2]

Median plasma MMP-3 [$p = 0.02$] and MMP-9 [$p = 0.04$] concentrations were significantly higher in the controls. Median plasma MMP-3 and MMP-9 concentrations did not change significantly during acute hyperglycaemia in either group.

Associations with plasma MMP-3 & MMP-9 concentrations

The only variable independently inversely related to plasma MMP-3 levels at baseline was s-ICAM-1 [$R^2 = 0.30$; $F_{1,20} = 6.6$; $p = 0.03$] in the diabetes group. The relationship between MMP-3 and s-ICAM-1 was independent of all other variables, and there were no significant relationships between plasma MMP-3 levels and any measure of glycaemic control, other adhesion molecules or plasma LDL oxidisability. MMP-9 was undetectable in the diabetes group. In the controls there was a strong direct independent relationship only between plasma MMP-9 levels and s ICAM-1 [$R^2 = 0.36$; $F = 9.73$; $p = 0.006$].

LDL oxidisability [Table 2]

There was no significant difference between LDL lag times at baseline and at 90 minutes in either diabetic or controls groups, although lag times were significantly longer in the control group [$p = 0.01$]

Discussion

The prompt highly significant fall in all plasma soluble adhesion molecule concentrations following an oral glucose load in Type 2 diabetes is a surprising observation as

in vitro, upregulation of membrane adhesion molecule expression and message in endothelial and other cell lines is associated with both high glucose concentrations and increased intracellular oxidative stress [6-8], and other smaller studies have described increased plasma concentrations of soluble adhesion molecules [particularly s ICAM-1] in Type 2 diabetes and controls after a glucose load [19-21]. In common with other studies in Type 2 diabetes, we also demonstrated a direct relationship between measures of long term glycaemic control and plasma concentration of some soluble adhesion molecules

It is unlikely that the rapid fall in all plasma soluble adhesion molecules concentrations represented decreased synthesis and expression of all these proteins, as message for the membrane bound forms of these adhesion molecules takes several hours to regulate in vitro [22]. Also, the mechanisms underlying these changes following a glucose load must account for the rapid and highly significant fall in all three measured plasma soluble adhesion molecules and must be independent of acute substantial hyperglycaemia as they also occurred in the control group, where the changes were unrelated to glycaemic excursions. Plasma soluble adhesion molecules are derived by the proteolytic cleavage of the membrane bound adhesion molecule at the point of membrane insertion [23]. The mechanisms controlling the shedding of these soluble forms from the cell surface remain unclear, but in some cell lines the membrane bound proteases that release the soluble forms are zinc containing non-matrix metalloproteinases [24]. Although there is no data on the regulation of these membrane bound proteases by acute glycaemic excursions, it is possible that any change within minutes as occurred in the present study reflects reduced proteolytic release of the adhesion molecule from the cell surface, but this would imply that the release mechanisms for all these adhesion molecules was similarly regulated by acute hyperglycaemia, and of course the control group [who did not experience acute hyperglycaemia] also showed marked reductions in plasma soluble adhesion molecule concentrations. We have shown recently in separate studies [25,26] that an oral glucose load leads to the increased cell surface expression of peripheral blood monocyte adhesion molecules CD11b [Mac-1] and LFA-1 in controls and in Type 2 diabetes, and increased expression of the monocyte LDL scavenger receptor CD36 in controls without diabetes. Leukocyte CD11b and LFA-1 are central to leukocyte-endothelium adhesion, and we also recorded a similar significant fall in plasma soluble adhesion molecules in parallel with increased monocyte expression of these adhesion molecules [25]. One possible interpretation of the data in the present study is that increased leukocyte adhesion molecule expression following a glucose load in Type 2 diabetes and controls leads to enhanced leukocyte - endothelial interactions and a tran-

sient decrease in endothelial and leukocyte shedding of the soluble forms into the plasma.

Another possibility is that the rapid, consistent fall in a plasma soluble adhesion molecules in both groups after a glucose load reflects increased clearance of these plasma proteins. An oral or intravenous glucose load increases splanchnic and hepatic blood flow by up to 40% within minutes in man and animal models and in both diabetes and control subjects [27,28] and is in part a function of changes in portal vein osmolarity which might increase hepatic clearance of these proteins. Finally, acute hyperinsulinaemia is unlikely to have produced this effect as an acute 30-fold increase in plasma insulin concentrations has no effect on plasma soluble adhesion molecule concentrations [29,30]. Our findings differ from those of Ceriello et al [19], Marfella et al [20] and Nappo et al [21] who performed a single oral [19] or intravenous [20] glucose tolerance test in 9 [19] and 10 [20] patients respectively with Type 2 diabetes and reported a significant increase in plasma s ICAM-1 concentrations at 60 - 90 minutes, or increased plasma soluble adhesion molecules concentrations in 20 Type 2 patients following a carbohydrate meal [21]. The patients in these studies were similar in baseline glycaemic control, glycaemic excursions and basal s ICAM-1 levels to the present study, the major difference being the larger sample size in the present study.

There was no significant change in LDL oxidisability during hyperglycaemia although increased oxidative stress and antioxidant consumption post-prandially has been suggested as a possible mechanism for increased lipoprotein oxidisability in diabetes [15]. Others have described increased LDL oxidisability in Type 2 diabetes after a fat containing meal, using a methodology similar to ours [16]. A meal rich in oxidised lipids may lead to an acute rise in lipoprotein conjugated dienes in Type 2 diabetes [31] and this could be a confounding mechanism for apparent post-prandial changes in LDL oxidisability after a fat meal, which do not occur when a glucose load is used. The present study had 89% power at the 5% level to detect one standard deviation difference in LDL lag times between time points in the diabetes group. This difference [9.8 mins] is biologically relevant as it is similar to or less than the difference seen between groups with or without atherosclerosis [32].

Despite the interest in MMP as mediators of the development of cardiovascular disease there is surprisingly little data on plasma MMP levels in Type 2 diabetes. Other groups have been able to detect plasma MMP-9 in Type 1 and 2 diabetes [33,34], and we have shown that peripheral venous monocytes derived from patients with Type 2 diabetes do not differ in the expression of MMP-3, MMP-9 or TIMP-1 compared to controls [35], although fibrob-

lasts derived from the healthy skin of diabetic patients show increased expression of MMP-2 and MMP-3 compared to controls [36]. The significant inverse relationship between sICAM-1 and plasma MMP-3 levels in the diabetes group alone and the strong direct relationship between sICAM-1 and MMP-9 in the controls is intriguing. Others have shown that endothelial cell expression of MMP-9 is promoted by LFA-1/ICAM-1 mediated cell adhesion to the endothelium [37], and that ICAM-1 release from some cell lines is mediated by MMP-9 [38], and which may have been detected in this study.

Although the changes in plasma soluble adhesion molecule concentrations in Type 2 diabetes and controls following an oral glucose load were highly significant, and have been reproduced in our other studies [25], this study has limitations. Firstly, as the mechanisms underlying these observations are unclear, it would be interesting to examine the effects of an oral fat load, intravenous glucose load or calorie free oral load on these variables. Secondly, other key adhesion molecules such as p-selectin were not studied. P-selectin storage and release from intracellular compartments [39,40] would make it an attractive candidate for further study as it may be more sensitive to physiological changes following a glucose load. Finally it would have been informative to study leukocyte counts, as polymorphonuclear leukocytes are a rich source of proteases and potentially of shed sICAM-1, and to examine MMP levels with gelatin or casein zymography rather than ELISA assays to better distinguish active from inactive forms of the enzymes.

In summary we have shown that a glucose load in Type 2 diabetes and controls leads to a rapid decrement in plasma soluble adhesion molecule concentrations and this may be a function of impaired shedding of these proteins or increased hepatic clearance. These findings in vivo are in contrast to that those expected from in vitro data on glucose and adhesion molecule expression. Glycaemic excursions following a glucose load do not influence plasma MMP concentrations or LDL oxidisability in Type 2 diabetes.

Abbreviations

ICAM-1 Intercellular adhesion molecule-1

VCAM-1 Vascular cell adhesion molecule-1

LDL Plasma low density lipoprotein

Competing interests

None declared.

Authors contributions

MS initiated the study, helped write the paper and analysed the data

ID helped write the paper and undertook analyses

JG directed work on MMP analysis and helped write the paper

BS helped write the paper and undertook analysis

JB helped write the paper and undertook analysis

SA helped write the paper and advised on LDL analysis

DH directed work on adhesion molecule analysis and helped write the paper

Acknowledgements

We are grateful to Dawn Wright, Colin Ward and Tracey Williams and for their assistance in this project. We are grateful to the Norwich and Norfolk Diabetes Trust for supporting some of the costs of this project. JB, SA and DAH are supported by the Biotechnology and Biological Sciences Research Council, UK.

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