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ORIGINAL CONTRIBUTION

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A study of the effect of oral glucose loading on plasma oxidant:antioxidant balance in normal subjects

Summary *Background* Antioxidant defence has been reported to decrease, and oxidative stress to increase, after oral glucose loading in both normal and diabetic subjects. If confirmed in normal subjects, glucose-induced antioxidant depletion has important implications for health in relation to the modern, sugar-rich diet. *Aims of the study* To investigate changes in plasma biomarkers of oxidant:antioxidant balance in non-diabetic subjects following oral glucose loading. Baseline inter-relationships between biomarkers of glycaemic control, oxidant:antioxidant balance and inflammation were also explored. *Methods* A single-blinded, placebo-controlled, cross-over intervention trial involving 10 healthy, consenting subjects. Venous blood was collected after ingestion of 75 g glucose in 300 mL water, or of water alone. Blood was collected at 0 time (fasting) and 30, 60, 90, 120 min post-ingestion. Within 2 weeks the procedure was repeated with volunteers crossed-over onto the other treatment. Plasma total antioxidant capacity (as the FRAP value), ascorbic acid, α-tocopherol, uric acid, malondialdehyde (MDA), allantoin and high sensitivity C-reactive protein (hsCRP), glucose and insulin, were measured in all samples. Paired results post-glucose and post-water at each time interval were compared using the Wilcoxon matched-pairs signed-ranks test. *Results* Normal glucose tolerance was observed in all subjects, although, as expected, plasma glucose and insulin increased significantly ($p < 0.05$, $n = 10$) after glucose loading. Post-glucose responses in plasma FRAP and the individual antioxidants tested were not significantly different to the responses seen post-water, although both FRAP and α-tocopherol decreased slightly. Neither were post-glucose changes in plasma MDA and allantoin, putative biomarkers of oxidative stress, significantly different to those after intake of water alone. Plasma FRAP and α-tocopherol also decreased slightly, but not significantly, after intake of water. A significant direct correlation ($r = 0.867$, $p < 0.001$, $n = 10$) was found between fasting allantoin and (log transformed) hsCRP concentrations. *Conclusions* These new data from a controlled intervention trial indicate that acute, transient increases in plasma glucose following oral intake of a large glucose load do not, as previously reported, cause a significant decrease in plasma antioxidants or increase oxidative stress in non-diabetic subjects. This is reassuring given the large quantities of sugar ingested by children and adolescents. However, a small decrease in plasma antioxidant capacity was seen after ingestion of water and of glucose, and it is possible that intake of glucose without concomitant intake of antioxidants in susceptible individuals may cause oxidative stress. Further work is needed in relation to diabetic subjects and a possible glucose threshold for this. The finding of a direct relationship between allantoin, a biomarker of oxidative stress, and hsCRP, a marker of inflammation and CHD predictor, in healthy subjects is interesting and indicates a link between sub-clinical inflammation and oxidative stress.

Key words OGTT – plasma glucose – oxidant:antioxidant balance – antioxidants – oxidative stress – FRAP – ascorbic acid

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Introduction

Hyperglycaemia, the characteristic of diabetes, is reported to deplete antioxidants and to increase oxidative stress, and this may be central to the development of vascular complications in diabetes [1, 2]. Increased oxidative stress, caused by a relative or absolute decrease in antioxidant defence, is suggested as a major contributor to insulin resistance and associated accelerated atherosclerosis [2]. Oxidative stress is also implicated as a causal factor in ageing and in many chronic diseases, including coronary heart disease (CHD) and cancer [3–5].

A transient, acute increase in plasma glucose induced by a standard oral glucose tolerance test (OGTT) was reported to deplete plasma antioxidants and to increase oxidative stress in both normal subjects and in Type 2 diabetic subjects [6]. This reported pro-oxidant effect of glucose loading, if confirmed for normal subjects, is worrying. Intake of glucose rich foods is common and frequent in the modern diet, particularly by children and adolescents, and could have important implications beyond 'empty calorie' concerns because of the link between increased oxidative stress and chronic diseases [3–5]. The primary aim of this study, therefore, was to determine the effect of OGTT on plasma biomarkers of oxidant:antioxidant balance in non-diabetic subjects. Owing to the reported links between inflammation, ascorbic acid and CHD [7–11], high sensitivity C-reactive protein (hsCRP) was also measured, and its relationships with biomarkers of oxidant:antioxidant balance and responses of plasma glucose and insulin to OGTT were explored.

Subjects and methods

This was a single blinded, placebo-controlled, cross-over intervention trial. Ten apparently healthy, non-smoking volunteers (4 males, 6 females), aged 20–55 years (mean ± SD, 37.1 ± 12.3) were recruited into the study with their informed consent. Fasting venous blood samples were collected into commercial heparinised and fluoride-oxalate blood collection tubes. Subjects were then given, on a non-selective basis, either 75 g glucose in 300 mL water (n = 5) or water alone (n = 5). Blood samples were taken 30, 60, 90, 120 min later. Subjects returned within 2 weeks, were crossed over onto the other treatment, and the procedure described above was repeated.

Blood samples were stored at 4 °C for no longer than 2 h after collection before centrifugation and plasma separation. Immediately after separation, ascorbic acid and total antioxidant capacity (as the FRAP value) were measured, using heparinised plasma, by a modified version of the Ferric Reducing/Antioxidant Power (FRAP) assay (US patented), known as the FRASC assay [12], and using a Cobas Fara centrifugal analyser (Roche Diagnostics, Basle, Switzerland). Plasma glucose (in fluoride oxalate plasma) was measured by an enzymatic kit method (Biosystems, Barcelona) within 2 h of separation. Aliquots of fresh heparinised plasma were stored at –70 °C for < 2 months before batch analysis of uric acid, using a commercial enzymatic kit method (Biosystems, Barcelona) on the Cobas Fara, hsCRP by latex bound particle enhanced immunoturbidimetry (Roche/Hitachi Modular Analytics system, Roche Diagnostics, Mannheim), and insulin by an enzyme linked immunosorbant assay kit method (DAKO Cytomation Ltd., Cambridgeshire, UK). Within-run and between-run CVs of hsCRP tests and all tests performed on the Cobas Fara were < 2 % and < 4.5 % respectively (n = 20); between-run CV of insulin measurement was < 12 %. Measurements of α-tocopherol and malondialdehyde (MDA) were performed by reverse phase high performance liquid chromatography (HPLC) in batches [13–15], using heparinised plasma aliquots stored at –70 °C for < 6 months, and with no intermediate freeze/thaw. Within-run and between-run CVs of α-tocopherol were < 6 % and < 9 % respectively (n = 8). Within-run and between-run CVs of MDA were < 8 % (n = 8). Plasma allantoin was measured by reverse phase HPLC in batches [16]; within-run and between-run CVs of allantoin were < 10 % (n = 8).

Results post-glucose were compared to those post-water at each time point using Wilcoxon matched-pairs signed-ranks test. Pearson's correlation was performed on fasting data to explore inter-relationships between the biomarkers of interest, with log transformation of data where appropriate. Software was InStat version 3.00 (GraphPad Software Inc., San Diego); $p < 0.05$ (two-tailed) was regarded as statistically significant.

This study was approved by the Ethics Sub-Committee of The Hong Kong Polytechnic University, and all procedures involving human subjects complied with the Declaration of Helsinki (2000).

Results

After glucose ingestion, plasma glucose and insulin levels increased significantly ($p < 0.05$) as expected (Table 1). All subjects showed normal glucose tolerance by both WHO and locally established criteria [17, 18]. Plasma FRAP and α-tocopherol decreased slightly with time after both treatments, but decreases after glucose were not significantly different to those after water. Plasma ascorbic acid was unaffected by either treatment. In terms of relationships between fasting levels of biomarkers measured, significant correlation was seen between glucose and log transformed insulin ($r = 0.758$, $p < 0.01$), and between allantoin and log transformed hsCRP ($r = 0.867$, $p < 0.001$). No other significant correlations of interest were seen.

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Table 1 Plasma concentrations (mean (SD); n = 10 except # = 9) of biomarkers of interest in non-diabetic subjects following OGTT

Biomarker	0 min (fasting)		+ 30 min		+ 60 min		+ 90 min		+ 120 min	
	+ glucose	+ water	+ glucose	+ water	+ glucose	+ water	+ glucose	+ water	+ glucose	+ water
Glucose (mmol/L)	5.4 (0.2)	5.5 (0.3)	8.8* (1.6)	5.4 (0.4)	7.0* (1.8)	5.4 (0.4)	6.2 (1.2)	5.5 (0.3)	5.8 (0.9)	5.5 (0.5)
Insulin (pmol/L)	32 (24)	21 (11)	323*(109)	17 (8)	259* (84)	24 (8)	231* (161)	22 (8)	171* (120)	20 (15)
FRAP# (µmol/L)	1206 (135)	1203 (122)	1209 (125)	1191 (110)	1176 (115)	1197 (124)	1174 (113)	1196 (119)	1159 (112)	1201 (135)
Ascorbic acid# (µmol/L)	64 (16)	66 (12)	66 (15)	65 (12)	63 (15)	71 (15)	65 (12)	69 (18)	67 (15)	74 (17)
Uric acid (µmol/L)	319 (61)	311 (53)	310 (54)	305 (53)	321 (66)	301 (50)	315 (51)	294 (49)	299 (60)	297 (50)
α-tocopherol (µmol/L)	29.3 (14.1)	29.2 (11.7)	26.7 (13.1)	27.4 (11.2)	26.6 (12.5)	27.8 (12.4)	27.6 (14.0)	27.7 (12.6)	26.1 (12.1)	27.7 (11.4)
MDA (µmol/L)	1.1 (0.4)	0.9 (0.2)	0.9 (0.2)	0.9 (0.2)	0.9 (0.3)	0.9 (0.2)	0.9 (0.2)	0.9 (0.2)	0.8 (0.3)	0.9 (0.2)
Allantoin (µmol/L)	7.7 (3.0)	7.6 (3.6)	8.5 (3.4)	7.8 (3.4)	7.7 (2.9)	7.0 (2.9)	7.2 (2.5)	7.3 (3.3)	6.6 (1.9)	7.1 (3.3)
hsCRP# (mg/L)	0.7 (0.5)	0.6 (0.4)	0.7 (0.5)	0.6 (0.4)	0.7 (0.5)	0.6 (0.4)	0.7 (0.5)	0.6 (0.5)	0.6 (0.4)	0.6 (0.4)

* $p < 0.05$ compared to matching time with water

Discussion

This study was performed because of concerns raised by published data that reported a decrease in plasma total antioxidant capacity, ascorbic acid [vitamin C], α-tocopherol [vitamin E]) and protein sulphydryl groups in normal subjects following OGTT, changes which were interpreted as indicating induction of oxidative stress [6]. Results of this current, controlled, cross-over trial, however, indicate that transient, acute increases in plasma glucose *per se* do not cause a significant decrease in plasma antioxidants or a significant increase in biomarkers of oxidative stress in non-diabetic subjects. Furthermore, increased plasma glucose does not induce a pro-oxidative inflammatory response in non-diabetic subjects, as no significant change in hsCRP levels was seen.

It is possible that some of the differences between this and the previous study [6] may have been due to different analytical techniques, and to different ages of subjects (mean age 37 years in this current study, versus 56 years in that of Ceriello et al. [6]). However, the published data are puzzling in that statistically higher ascorbic acid concentrations were found in the diabetic subjects, compared to the non-diabetic subjects studied [6]. This is puzzling when the thrust of the paper was that increased glucose levels deplete antioxidants. Indeed, it is generally accepted that diabetic subjects have low plasma ascorbic acid [1, 5, 10, 19]. The published data [6] are problematical also in that the ascorbic acid and vitamin E levels reported appear unrealistically high. Mean plasma ascorbic acid is presented as between 160–260 µmol/L, and that of vitamin E is between 240–320 µmol/L [6]. This compares to data reported by us and others of around 30–70 and 20–45 µmol/L, respectively, for ascorbic acid and vitamin E in fasting plasma. Even after a large oral dose of vitamin C (1 g or more), plasma ascorbic acid levels do not generally exceed 200 µmol/L [5, 7–10, 12, 19–22].

Differences in design of the two studies are likely to be important also. The study of Ceriello et al. [6] was not controlled, in that no group ingested only water. We suggest that the significant decreases seen [6] in plasma antioxidants may have been owing, at least in part, to an additive effect of prolonged fasting and ingestion of glucose. In a previous study [23] we noticed a small decline in plasma FRAP values with prolonged fasting. In this current study, small decreases in FRAP and α-tocopherol were seen after glucose ingestion, and also after ingestion of water. In a post-hoc analysis, the changes after glucose were slightly, but not significantly greater, than those after water. Interestingly, no difference was seen in ascorbic acid concentration after either treatment in this study.

While the published data [6] are problematical, the issue raised by Ceriello et al. is, nonetheless, an impor-

tant one. Hyperglycaemia has been suggested to be one of the inducers of oxidative stress [10, 24, 25], and may occur via various routes, such as autoxidation of glucose, concomitant production of superoxide, nonenzymatic glycation of proteins, and increased glucose flux through the polyol pathway leading to depletion of NADPH [5, 10]. Furthermore, concomitant elevation of NADH favours prostaglandin synthesis, which increases production of superoxide [5, 25].

The new data presented here show no evidence of significantly increased oxidative stress or decreased antioxidants occurring as a result of glucose ingestion *per se* in non-diabetic subjects. It is possible, however, that the previously published data [6] highlight a phenomenon that may be of considerable importance. Intake of glucose-rich food by otherwise fasting, non-diabetic subjects may cause a small but significant decrease in plasma antioxidants owing to the combination of the effects of increased plasma glucose and concurrent lack of dietary replenishment of plasma antioxidants. Furthermore, the effect may be exaggerated in subjects with impaired glucose tolerance or diabetes. In this regard, it is of interest that oxidative stress (as measured by plasma nitrotyrosine, an indirect marker of peroxynitrite formed) increased in healthy subjects during a hyperglycaemic glucose clamp test in which plasma glucose was maintained (by infusion) at ~15 mmol/L for 120 min [26]. It is possible to speculate, therefore, that there is a threshold of plasma glucose above which there is a significant increase in oxidative stress, and that this effect may be exaggerated in those with inadequate intake of antioxidants. Further study in this area is warranted. The novel finding of a direct correlation between allantoin (a biomarker of oxidative stress) and hsCRP (a biomarker of inflammation and CHD predictor) in non-diabetic subjects also deserves further study, as it suggests that sub-clinical inflammation may exert oxidative stress, or vice versa.

In conclusion, results of this controlled study indicate that healthy subjects who undergo a standard OGTT, with a resultant acute increase in plasma glucose, do not suffer a significant change in plasma oxidant: antioxidant balance. This is reassuring, given the high intake of glucose in the modern diet. However, data also indicate that high intake of glucose without a concomitant intake of antioxidants is a combination to avoid. These new data show for the first time also that sub-clinical inflammation is linked to oxidative stress. Further study is needed in insulin resistant states and in overt diabetes.

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