Lack of effect of oral glucose loading on conduit vessel endothelial function in healthy subjects

Aris SIAFARIKAS*, Katie WATTS†, Petra BEYE*, Timothy W. JONES*‡, Elizabeth A. DAVIS*‡ and Daniel J. GREEN†§

*Department of Endocrinology and Diabetes, Princess Margaret Hospital, Subiaco, WA 6007, Australia; †School of Human Movement and Exercise Science, University of Western Australia, Nedlands, WA 6009, Australia; ‡Centre for Child Health Research, University of Western Australia, Telethon Institute of Child Health Research, Subiaco, WA 6008, Australia, and §West Australian Institute for Medical Research, Perth, WA 6000, Australia

ABSTRACT

The aim of the present study was to investigate the impact of an oral glucose load on circulating insulin and glucose levels and arterial function in healthy non-diabetic subjects. Thirty-nine non-obese, healthy subjects (24 female, 15 male), aged 21.0 ± 1.8 years of age, were randomly assigned to undergo either an OGTT (oral glucose tolerance test; 75 g of glucose) or administration of a placebo. Analyses of lipids, liver function and HbA1c (glycated haemoglobin) at baseline revealed results which were within the standard reference range. Insulin and glucose levels as well as vascular function [FMD (flow-mediated dilation)] were measured at 0, 60 and 120 min. Compared with baseline, the control subjects did not exhibit any significant changes in glucose or insulin levels, whereas, in the OGTT group, blood glucose levels at both 60 (5.4 ± 1.7 mmol/l) and 120 (5.0 ± 1.1 mmol/l) min increased significantly relative to baseline (4.1 ± 0.4 mmol/l; both \( P < 0.001 \)) and, similarly, insulin levels were higher at both 60 (30.1 ± 21.3 m-units/l) and 120 (34.9 ± 23.6 m-units/l) min compared with baseline (4.7 ± 4.3 m-units/l; both \( P < 0.001 \)). Although blood glucose and insulin levels changed, FMD did not significantly differ between time-points or between groups. In summary, despite significantly elevated glucose and insulin concentrations in these subjects, we observed no change in vascular function, suggesting that acute elevations of glucose and insulin within the clinically normal range are not associated with impaired vascular function in vivo.

INTRODUCTION

The importance of the endothelium in maintaining a healthy vasculature has been increasingly recognized, particularly with respect to endothelial release of NO (nitric oxide) and its various functions [1,2]. NO causes vasodilation and possesses a number of anti-atherogenic properties, including inhibition of platelet and monocyte adhesion to the endothelium of vessel walls and inhibition of cellular transmigration, vascular smooth muscle proliferation and LDL (low-density lipoprotein) oxidation [3]. Several lines of evidence indicate that endothelial dysfunction is an early manifestation of atherosclerotic disease and that interventions which decrease cardiovascular mortality and morbidity are associated with improved endothelial function [4]. In addition, recent studies indicate that coronary and brachial artery endothelial dysfunction predict cardiovascular mortality and morbidity [1]. Some previous studies suggest that hyperglycaemia suppresses endothelium-dependent vasodilation, possibly via production of oxygen-derived free radicals,
which decrease the bioavailability of endothelial-derived NO [5,6]. It has also been reported that patients with Type II diabetes and insulin resistance, who typically exhibit prolonged hyperinsulinaemia, possess impaired NO-mediated endothelial function [7,8]. In contrast, acute intra-arterial infusion of insulin is associated with endothelium-dependent vasodilation [9]. Some uncertainty therefore exists regarding the effects of oral glucose administration and elevated insulin levels on vascular function, particularly in young healthy subjects.

The aim of the present study was to investigate the impact of an oral glucose load with associated increases in circulating insulin and glucose levels on vascular arterial function in healthy non-diabetic subjects.

METHODS

Subjects
Non-obese healthy subjects (n=39; 24 female, 15 male), aged 21.0 ± 1.8 years of age, were recruited and assigned randomly to undergo either an OGTT (oral glucose tolerance test) or administration of a placebo (Table 1). None of the subjects was diabetic, had impaired glucose tolerance test) or administration of a placebo (Table 1). None of the subjects was diabetic, had impaired glucose tolerance, any history of cardiovascular disease or was receiving any medications. Written consent was obtained from all subjects, and the study was approved by the Princess Margaret Hospital Ethics Committee.

Study design
Subjects were seen at 09.00 hours after a 12 h fasting period. Height and weight were recorded during a physical examination. An indwelling catheter was inserted in the cubital vein of the right arm for repeated venous samples. Vascular function assessment using FMD (flow-mediated dilation) was measured approximately 20 min later. Following baseline blood sampling, subjects in the OGTT group received a glucose load (75 g of glucose; Glucaid®; Histo-Labs, Riverstone, Australia), whereas control subjects were administered a glucose-free placebo drink. Blood sampling and FMD analysis were repeated at 60 and 120 min in each subject.

Analyses
Using routine methods and standard assays, biochemical analyses of cholesterol, triacylglycerols (triglycerides), high-density lipoprotein (HDL), LDL, AST (aspartate aminotransferase), ALT (alanine aminotransferase) and HbA1c (glycated haemoglobin) were obtained at 0 min. Insulin levels and blood glucose levels were analysed at 0, 60 and 120 min. Insulin was measured in heparinized plasma using a solid-phase two site chemiluminescent immunoassay (Inmulite 2000; Diagnostic Products Corporation, Los Angeles, CA, U.S.A.).

Vascular function assessment
Each assessment of vascular function was undertaken using a 12 MHz multi-frequency, linear array probe attached to a high-resolution ultrasound machine (Aspen; Acuson, Mountain Vie, CA, U.S.A.). HR (heart rate) was monitored continuously by three-lead ECG. SBP (systolic blood pressure), DBP (diastolic blood pressure) and MAP (mean arterial pressure) were measured using an automated sphygmomanometer on the contralateral arm (Dinamap 8100; Critikon, Tampa, FL, U.S.A.). The monitored non-dominant arm was positioned at an arm-to-torso angle of approx. 80° with the distal forearm supinated and immobilized by foam supports encompassing the limb. The brachial artery was visualized in the distal third of the upper arm. The probe was held in a constant stable position for the duration of the study by a stereotactic clamp and its precise location recorded and standardized. Ultrasonic parameters were set to optimize longitudinal B-mode imaging of the lumen–arterial wall interface. The focus zone was positioned to optimize visualization of the near wall. Once set, operating parameters remained constant throughout each study.

A pneumatic cuff, placed around the forearm immediately distal to the humeral epicondyles, was rapidly inflated to 200 mmHg for 5 min. B-mode recording commenced 30 s before cuff deflation and continued for 2.5 min. FMD of the brachial artery, resulting from ischaemia-induced reactive hyperaemia following release of this cuff, provided a measure of endothelium-dependent-mediated vasodilation [10,11]. FMD measures were repeated at 60 and 120 min after the initial baseline measure.

Analysis of brachial artery diameter and FMD responses was performed using custom-designed edge-detection and wall-tracking software, which minimizes
investigator bias and has the power to detect an absolute change in FMD of 2.5% in a parallel design study with only eight subjects [12]. Briefly, an edge-detection algorithm averages >300 diameter measurements/frame, with 20–30 frames assessed/s. Those average diameter measures, which coincide with the ECG R-wave (also auto-detected), were subsequently analysed using a third-order polynomial curve [13]. FMD responses were then calculated from the peak value derived from this polynomial curve, related to the average of all R-wave-gated diameters collected during the baseline period preceding the FMD manipulation. The mean intra-observer coefficient of variation of repeated measures of FMD using this software is 6.7%, which is significantly lower than that for traditional manual methods [12].

Analysis
Differences in subject characteristics between those administered glucose or placebo were analysed using unpaired Student’s t-tests. In the case of vascular function, blood glucose and insulin levels, where repeated measures were taken, two-way ANOVA with post-hoc Student’s t-tests was used and P < 0.05 considered statistically significant (SPSS statistical software package).

RESULTS

Subject characteristics
All subjects completed all time-points of the analysis and ultrasound scans of sufficient quality were achieved in all cases. There was no significant difference between the OGTT and control groups in terms of height, weight or BMI (body mass index). Similarly, no difference existed in cholesterol, triacylglycerols, HDL, LDL, HBA1c, AST and ALT between groups (Table 1), and all subject data were within the standard reference range [14].

Analyses of blood glucose and insulin levels
Across the three time-points measured, a significant difference existed in the blood glucose responses between the OGTT and control groups (Figure 1A, P < 0.05, two-way ANOVA). Although the control subjects did not exhibit any significant changes in glucose compared with baseline, blood glucose levels increased significantly relative to baseline at both 60 (5.4 ± 1.7 compared with 4.1 ± 0.4 mmol/l; P < 0.001) and 120 (5.0 ± 1.1 compared with 4.1 ± 0.4 mmol/l; P < 0.001) min in the OGTT group. No difference existed between the 60 to 120 min time-points.

Similarly, plasma insulin levels were significantly different between the groups (Figure 1B; P < 0.001, two-way ANOVA). In response to the glucose load, insulin levels were significantly higher than baseline at both 60 (30.1 ± 21.3 compared with 4.7 ± 4.3 m-units/l; P < 0.001) and 120 (34.9 ± 23.6 compared with 4.7 ± 4.3 m-units/l; P < 0.001) min, with no difference evident between 60 and 120 min. No changes were observed in the control subjects administered placebo. No individual subject exhibited insulin levels which would indicate the presence of insulin resistance.

Forearm conduit vessel function
Baseline brachial artery diameters immediately preceding FMD did not significantly differ between time-points or between groups (Table 2). Similarly, no differences were evident over time or between groups for HR, SBP, DBP or MAP.

FMD of the brachial artery, predominantly a measure of endothelium-dependent vasodilation in conduit vessels [10,11], did not differ significantly between time-points after placebo or glucose administration (Table 2), and no significant difference between groups in response to these interventions could be detected (Figure 1C).
DISCUSSION

The present study is the first randomized placebo-controlled study to examine the effects of an oral glucose load in young non-diabetic healthy subjects. The results indicate that, in this particular group, a glucose load of 75 g induced a significant elevation in blood glucose within the non-diabetic range and a consequent significant increase in insulin levels within the non-hyperinsulinaemic range. However, neither metabolic change had any impact on conduit vessel NO-dependent function, as FMD responses did not alter across the course of the study.

The rationale for investigating conduit vessel function in response to acute changes in blood glucose and insulin levels was based on disparity between previous studies, which have measured the impact of these factors on vascular function and CVD (cardiovascular disease). A meta-analysis of 20 studies by Coutinho et al. [15] concluded that elevated glucose levels, even below the diabetic cut-off, increase the risk of cardiovascular disease as a function of time. This finding may be confounded by the presence and development of additional risk factors, such as obesity, hypertension or hyperlipidaemia, but suggests an epidemiological link between elevated blood glucose and CVD. Mechanistically, several studies have supported such a link. It is well established that hyperglycaemia induces superoxide production by the mitochondrial electron transport chain, which, in turn, increases oxidant stress and atherosclerosis [5,6]. In addition, activation of PKC (protein kinase C) isoforms via DAG (diacylglycerol) increases formation of AGE (advanced glycation end products) and activation of the proinflammatory transcription factor NF-κB, which may also contribute to an atherogenic milieu [5,6,16]. These molecular mechanisms ultimately lead to a decreased bioavailability of endothelium-derived NO and impaired vasodilation.

Similarly, changes in insulin levels have been associated with modulation of vascular function [17,18]. Various effects of insulin are mediated after binding to specific insulin receptors on endothelial cells [19]. At physiological concentrations, insulin can protect against atherosclerosis by locally stimulating NO production, which results in relaxation of vascular smooth muscle cells [19]. In addition, insulin reverses the effects of hyperglycaemia on PKC and DAG [19,20]. Conversely, the prolonged hyperinsulinaemia evident in insulin resistance may impair endothelial function by activating local vascular growth factors [VEGF (vascular endothelial growth factor)], by increasing local concentrations of the vasoconstrictor ET-1 (endothelin-1) and by elevating sympathetic neural outflow [17,19].

The evidence described above has encouraged research investigating the acute clinical impact of elevated blood glucose and insulin levels on vascular endothelial function. Akbari et al. [21] reported impaired endothelium-dependent vasodilation in healthy subjects 60 min after glucose administration during OGTT. However, values at 120 min were not reported and a placebo group was not included in that study. A further major limitation of that study [21] related to the method used to determine endothelium-dependent vasodilation; FMD was analysed in response to ischaemia induced by occlusion with a cuff placed above the scanned brachial artery. A study by Doshi et al. [11] indicated that this response is not, in fact, endothelium-dependent and that cuff placement below the artery is required to stimulate flow-mediated endothelium-dependent vasodilation in the brachial artery. Title et al. [22] also demonstrated impaired endothelial function after an oral glucose load. That study [22] included only 10 subjects and did not have a placebo control group. In another study investigating the FMD response to OGTT in healthy subjects, Kawano et al. [23] showed a tendency towards suppressed endothelium-dependent vasodilation from 60 min. Interestingly, the FMD response had returned to normal levels at 120 min, despite continued elevation in blood glucose levels. Similar findings were evident in a study of 20 normal subjects by Ceriello et al. [24], which indicated that, although the combination of a high-fat meal and oral glucose load impaired FMD from 60–180 min, endothelial function returned to normal 120 min after glucose administration alone. One reason for the slight impairment in FMD in these studies, relative to our present results at 60 min, may relate to the fact that both Kawano et al. [23] and Ceriello et al. [24] studied much older subjects, who may have possessed more CVD risk factors than our young cohort. In contrast with these studies, Houben et al. [25] showed that 24 h of local hyperglycaemia did not affect endothelium-dependent vasoreactivity.

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<th>Table 2 Brachial artery diameter response to FMD following an oral glucose or placebo administration</th>
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<td>Values are means ± S.D. No significant changes in FMD (% change in diameter from preceding baseline) were evident following oral glucose or placebo administration.</td>
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Recently, Reed et al. [26] published results of a carefully performed glucose clamp study. In agreement with our present findings, they did not show any changes in FMD during moderate hyperglycaemia (peak glucose, 7.7 mmol/l) after suppression of insulin effects by somatostatin infusions. In contrast, Williams et al. [27] performed a clamp study in which subjects exhibited impaired vascular function after hyperglycaemia of 16.1 mmol/l. The results of these studies and the present data therefore suggest that the effect of glucose loading on vascular endothelial function in healthy subjects may be dependent upon the level of hyperglycaemia or, alternatively, that raised blood glucose and insulin may possess countervailing effects, which mask gross changes in FMD. In support of the latter possibility, it has been reported that acute arterial infusion of insulin causes vasodilation, which is endothelium- and NO-dependent [17,28], whereas acute infusion of glucose impairs endothelium-dependent vascular function.

There are several important limitations of the present study. We studied young healthy subjects in whom glucose administration induced changes in blood glucose and insulin concentration within the clinically normal range. We therefore cannot exclude the possibility that higher glucose or insulin levels may have been associated with altered vascular function or that impaired function may have been evident in a cohort of older, insulin resistant or Type II diabetic subjects. Similarly, the duration of elevated glucose and insulin may be germane and it is possible that different results may be evident if the stimulus is repeated or prolonged. We did not clamp insulin levels in the present study and cannot be sure that the countervailing effects of glucose and insulin may have masked a change in FMD. This seems unlikely, however, given the previous study of Reed et al. [26]. Finally, we did not undertake repeated assessments of smooth muscle sensitivity with, for example, glyceryl trinitrate. This was due to concerns regarding the possibility that repeated administration of this agent may have influenced the FMD results. We therefore cannot specifically make conclusions regarding the endothelium dependency of the FMD responses in the present study, rather that NO-mediated vascular function did not appear impaired by oral glucose loading.

In summary, the purpose of the present study was to determine the impact of an oral glucose load, which mimics a postprandial state, on circulating insulin and glucose levels and vascular arterial function in healthy non-diabetic subjects. Despite significantly elevated glucose and insulin concentrations in these subjects, we observed no change in vascular function, suggesting that acute elevations of glucose and insulin within the clinically normal range are not associated with impaired vascular function in vivo.

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