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IMPAIRED FASTING BLOOD GLUCOSE IS ASSOCIATED WITH INCREASED ENDOTHELIN-1 VASOCONSTRICTOR TONE

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Abstract

Aim/Hypothesis—The experimental aim of this study was to determine whether ET-1-mediated vasoconstrictor tone is elevated in adult humans with impaired fasting blood glucose concentrations, independent of other cardiovascular risk factors.

Methods—Forearm blood flow (FBF: plethysmography) responses to intra-arterial infusion of selective ET_A receptor blockade (BQ-123: 100 nmol/min for 60 min) and non-selective $ET_{A/B}$ blockade (BQ-123 + BQ-788: 50 nmol/min for 60 min) were determined in 28 middle-aged, sedentary adults (17 M/11 F): 14 with normal fasting blood glucose (age: 57±2 yr; 6F/8M; BMI: 29.2±0.9 kg/m2; glucose: 4.9±0.1 mmol/L) and 14 impaired fasting blood glucose (58±1 yr; 5F/9M; 29.6±1.1 kg/m2; 5.8±0.1 mmol/L) concentrations.

Results—Selective ET_A receptor blockade elicited a significantly greater (~20%) increase in FBF in the impaired fasting glucose adults compared with the normoglycemia controls. $ET_{A/B}$ blockade resulted in a further 2-fold increase ($P<0.05$) in FBF above that elicited by ET_A receptor antagonism in the impaired fasting glucose but not normal fasting glucose adults. There was a positive correlation between fasting blood glucose levels and the peak vascular responses to ET_A ($r=0.44$; $P<0.05$) and $ET_{A/B}$ ($r=0.62$; $P<0.05$) blockade. No other anthropometric, hemodynamic or metabolic variable was correlated with the blood flow responses to ET-1 receptor blockade.

Conclusions/Interpretation—ET-1-mediated vasoconstrictor tone is elevated in adults with impaired fasting blood glucose concentrations, independent of other cardiometabolic risk factors. Enhanced ET-1 system activity may underlie endothelial vasomotor dysfunction and increased cardiovascular risk in adults with impaired fasting blood glucose concentrations.

Keywords

endothelin-1; glucose; vasoconstriction

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Introduction

Approximately 80 million adults in the United States have impaired fasting blood glucose concentrations[1], defined as fasting plasma glucose between 5.6–6.9 mmol/L[2]. It has recently been reported that middle-aged adults without diabetes, but with elevated fasting plasma glucose, are at an increased risk for coronary heart disease (4). For example, Alexander et al[3] demonstrated that adults with impaired fasting glucose are at a 50% higher risk of developing cardiovascular disease compared with adults with normal fasting glucose. The mechanisms responsible for this apparent increase in vascular risk are not fully understood. Glucose has been shown to adversely affect endothelial cell function, which may propagate the atherosclerotic process[4]. Several clinical studies have shown that impaired fasting glucose is associated with endothelial dependent vasodilator dysfunction[5, 6], a central feature of atherogenesis[7].

Endothelin-1 (ET-1) is a potent vasoconstrictor peptide released by the endothelium that contributes to the regulation of vascular tone and has been implicated in the etiology of atherosclerotic vascular disease[8]. Interestingly, *in vitro*, a high glucose environment results in an elevation in endothelin-1 converting enzyme, suggesting a link between glucose and the ET-1 system[9]. Currently, it is unknown whether ET-1 system activity is altered in adult humans with impaired fasting plasma glucose. If so, this may contribute mechanistically to impaired endothelial vasomotor function and increased cardiovascular risk in this population. Thus, the aim of this study was to determine whether ET-1-mediated vasoconstrictor tone is elevated in adult humans with impaired fasting blood glucose concentrations, independent of other cardiovascular risk factors.

Methods

Subjects

Twenty-eight sedentary adults participated in this study: 14 (6F/8M) with normal plasma glucose (<5.6 mmol/L); and 14 (5F/9M) with impaired fasting plasma glucose (5.6–6.9 mmol/L) concentrations. Groups were stratified according to American Diabetes Association criteria[2]. Subjects were non-smokers and free of overt cardiovascular disease. Fasting plasma lipid, lipoprotein, glucose and insulin concentrations were determined using standard techniques. HOMA-IR was calculated as previously described[10]. All women were at least 1 year postmenopausal and not taking hormone replacement therapy. Written informed consent was obtained according to the guidelines of the University of Colorado at Boulder.

Intra-arterial Infusion Protocol

All studies were performed between 7 AM and 10 AM after a 12-hour overnight fast as previously described by our laboratory[11]. Briefly, following arterial catheterization, forearm blood flow (FBF: venous occlusion plethysmography) responses to BQ-123 (Clinalfa, AG), a selective ET_A receptor antagonist, infused for 60 minutes with FBF measured every 10 minutes. Thereafter, FBF was assessed every 10 minutes for an additional 60 minutes with the coadministration of BQ-123 and BQ-788 (Clinalfa, AG), a specific antagonist of ET_B receptors. Due to product availability, BQ-788 was infused in 7 of the 14 subjects in each group.

Statistical Analysis

Differences in subject characteristics were determined by between-group analysis of variance (ANOVA). Group differences in FBF responses to BQ-123 and BQ-123 + BQ-788 were determined by repeated-measures ANOVA. Relation between variables of interest was

assessed by linear regression analysis. There were no significant main effects of gender on FBF responses to endothelin blockade of FBF × gender interactions, therefore the data were pooled and presented together. Data are expressed as means±SEM. Statistical significance was set at $P<0.05$.

Results

Subject characteristics are presented in the Table. There were no significant differences in baseline FBF between the normal (4.4±0.3 mL/100 mL of tissue/min) and impaired (4.0±0.3 mL/100 mL of tissue/min) fasting blood glucose groups. FBF responses to ET-receptor blockade are shown in the Figure. BQ-123 elicited a significantly greater (~20%) increase in FBF in the impaired fasting glucose than normal fasting glucose groups. Moreover, the addition of BQ-788 to BQ-123 resulted in a further 2-fold increase ($P<0.05$) in FBF in the impaired fasting glucose but not normal fasting glucose adults. In the overall study population, there was a strong and positive correlation between fasting blood glucose levels and the peak vascular responses to BQ-123 ($r=0.44$; $P<0.05$) and BQ-123 + BQ-788 ($r=0.62$; $P<0.05$). No other anthropometric, hemodynamic or metabolic variable was significantly correlated with the vascular responses to ET-1 receptor blockade.

Discussion

The key finding of the present study is that ET-1 mediated vasoconstrictor tone is elevated in adults with impaired fasting blood glucose concentrations independent of other cardiometabolic risk factors. Moreover, the enhancement in ET-1 vasoconstriction is facilitated by both the ET_A and ET_B receptors. To our knowledge, this is the first study to assess the influence of impaired fasting blood glucose concentrations on ET-1 system activity.

In a recent study, DeVan and colleagues[6] reported that endothelial vasodilator function is impaired in middle-aged adults with impaired fasting blood glucose. Indeed, brachial artery flow-mediated dilation was ~30% lower in the adults with impaired fasting glucose compared with healthy controls of similar age. The results of the present study compliment and extend these findings by demonstrating that endothelial vasomotor dysfunction with impaired fasting glucose is not limited to vasodilation. Indeed, the seminal findings presented herein demonstrate that ET-1-mediated vasoconstrictor tone is markedly higher in middle-aged adults with impaired fasting glucose. FBF responses to both selective and non-selective ET-1 receptor blockade were markedly higher (20% and 40%, respectively) in the adults with impaired fasting glucose compared with their normal fasting plasma glucose counterparts. Of note, $ET_{A/B}$ receptor blockade resulted in a further increase in FBF above that observed with ET_A blockade alone in the impaired fasting glucose adults only, demonstrating that the ET_B receptor also contributes to the elevation in ET-1 vasoconstrictor tone with impaired fasting blood glucose. It should be noted that we assessed ET-1 system activity by pharmacologically blocking both ET-1 receptors (located on vascular smooth muscle cells and endothelium) instead of relying on circulating plasma concentrations of ET-1. The physiological relevance of plasma ET-1 levels is questionable as ET-1 is predominantly (>80%) released abluminally toward the vascular smooth muscle[12]. Thus, circulating levels provide little information on the vascular effects of the peptide at the level of the vessel wall.

The mechanisms underlying greater ET-1 vasoconstrictor tone with impaired fasting glucose are not well understood. It is important to emphasize that there were no differences between our groups with respect to body composition, blood pressure or plasma lipid and lipoproteins, all factors that are independently associated with increased ET-1 system

activity and often coexist with the impaired fasting glucose condition. For example, overweight and obesity, a common co-morbidity with impaired fasting glucose, has been shown to adversely influence endothelial vasomotor regulation via increased ET-1 system activity[11]. The subjects in the present study, however, were remarkable similar anthropometrically; discounting the influence of body composition on our findings. Notably, the only variable in the present study that correlated with the vascular responses to ET-1 receptor blockade was fasting plasma glucose. Peak blood flow responses to both selective ET_A receptor (r=0.44) and non-selective $ET_{A/B}$ receptor (r=0.62) blockade were significantly and positively associated with plasma glucose concentrations. At the very least, these correlative data provide directional support that the observed group differences were indeed glucose-related. While not measured in the present study, it is possible that oxidative stress and inflammatory burden may underlie the impaired fasting glucose-related increase in ET-1-mediated vasoconstrictor tone. Both oxidative stress and inflammation have been shown to exacerbate ET-1 system activity[13] and are prevalent with the impaired fasting glucose condition[14]. Future studies are needed to determine whether the increase in ET-1 vasoconstriction with impaired fasting glucose is due, at least in part, to oxidative and inflammatory processes.

There are two experimental considerations regarding the present study that deserve mention. Firstly, given our cross-sectional study design, we cannot discount the possibility that genetic and/or lifestyle behaviors may have influenced our results. To minimize the influence of lifestyle behaviors, we studied sedentary adults who were non-smokers and not currently taking any medication that could influence endothelial vasomotor function. Moreover, to isolate the primary influence of impaired fasting blood glucose concentrations, we studied adults of similar age who were free of other cardiometabolic abnormalities that are known to influence endothelial function, such as hypertension[15] and dyslipidemia[16]. Secondly, the present study has a modest sample size and all of the subjects were Caucasian. Thus, any generalizations to larger, more diverse populations require further study.

In summary, the results of this study demonstrate that ET-1-mediated vasoconstrictor tone is elevated in adults with impaired fasting blood glucose concentrations, independent of other cardiometabolic risk factors. From a public health perspective, endothelial vasomotor dysfunction, characterized by ET-1 system hyperactivity, is a well-established vascular abnormality with type II diabetes that contributes to cardiovascular risk[17]. Our findings indicate that augmented ET-1 vasoconstrictor tone is already apparent in the impaired fasting glucose prediabetic state. This provides further support for early intervention in adults with impaired fasting blood glucose concentrations not only to reduce their risk of developing diabetes but cardiovascular abnormalities as well.

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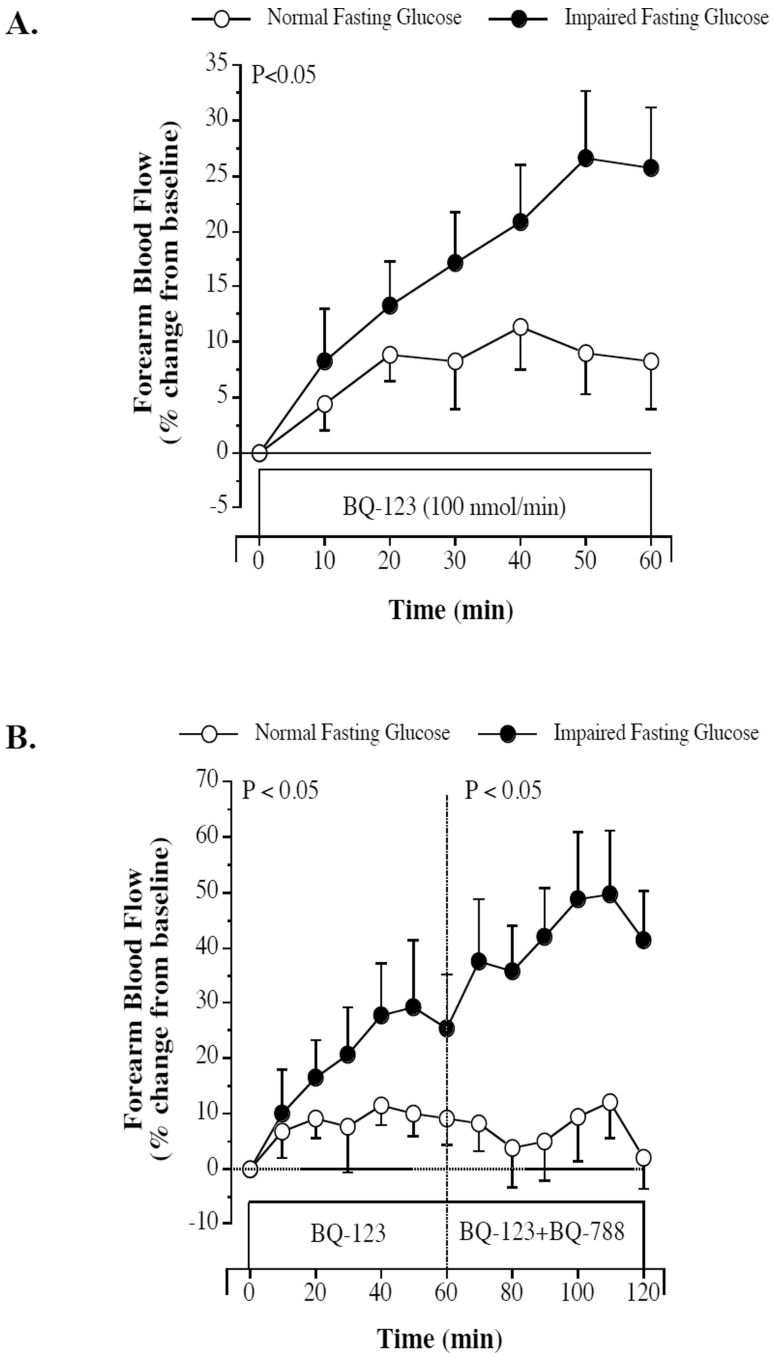

Figure.

Forearm blood flow responses to BQ-123 (100 nmol/min), a selective ET_A receptor antagonist (panel A) and BQ-788 (50 nmol/min), a selective ET_B receptor antagonist (panel B), in normal fasting glucose and impaired fasting glucose adults. Values are mean±SEM. The P value refers to the difference in the FBF response to ET_A and $ET_{A/B}$ blockade in the normal vs. impaired fasting glucose groups.

Table

Selected subject characteristics

Variable	Normal Fasting Glucose (n=14)	Impaired Fasting Glucose (n=14)
Age (yrs)	57 ± 2	58 ± 1
Gender, M/F	8/6	9/5
Body Mass (kg)	84.9 ± 3.9	87.5 ± 3.9
Body Mass Index (kg/m^2)	29.1 ± 0.9	29.7 ± 1.0
Body Fat (%)	36.1 ± 1.5	35.4 ± 1.6
Waist Circumference (cm)	94.3 ± 3.4	99.1 ± 2.8
Systolic BP (mmHg)	124 ± 2	127 ± 3
Diastolic BP (mmHg)	79 ± 2	79 ± 2
Total Cholesterol (mmol/L)	5.0 ± 0.2	5.3 ± 0.3
LDL-Cholesterol (mmol/L)	3.0 ± 0.2	3.4 ± 0.2
HDL-Cholesterol (mmol/L)	1.3 ± 0.1	1.2 ± 0.1
Triglycerides (mmol/L)	1.5 ± 0.2	1.5 ± 0.2
Glucose (mmol/L)	4.9 ± 0.1	5.8 ± 0.1 *
Insulin (pm/L)	49.8 ± 6.6	51.0 ± 6.6
HOMA-IR	1.9 ± 0.2	2.4 ± 0.32

BP = blood pressure; HDL=high-density lipoprotein; LDL=low-density lipoprotein; HOMA-IR=homeostasis model assessment of insulin resistance; values are mean ± SEM

* P < 0.05 vs. normal fasting glucose