Non-invasive assessment of vascular function

Paradoxical vascular response to intravenous glucose in coronary heart disease

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Background In healthy individuals, insulin administration causes an increase in forearm blood flow which is dependent on the effects of insulin on the vascular endothelium. Glucose, administered as an intravenous bolus, produces a transient hyperinsulinaemic response. We hypothesized that the insulin response to an intravenous glucose challenge during the intravenous glucose tolerance test might lead to increases in forearm blood flow in healthy individuals, and that such a response might be altered in patients with coronary heart disease.

Methods and Results Healthy individuals (n=10, aged 41.6±3.0 years, mean ± SEM) and patients with angiographically proven coronary heart disease (n=13, aged 65.5±2.4 years) underwent an intravenous glucose tolerance test with simultaneous measurement of right forearm blood flow at 28 time points, using mercury-in-silastic venous occlusion plethysmography. In controls, forearm blood flow increased to a mean of 31.7% above baseline values at 7 min and remained above baseline up to 180 min after intravenous glucose. In contrast, patients with coronary heart disease exhibited an opposite response, with forearm blood flow decreasing to a mean of −16.2% below baseline values at 7 min and −25.8% at 180 min. Marked group differences emerged in net changes from baseline in forearm blood flow throughout the intravenous glucose tolerance test, expressed as incremental areas under the forearm blood flow profiles (controls: +351.3±121.7; coronary heart disease patients: −244.3±72.4 min ml\(^{-1}\). 100 ml\(^{-1}\), \(P=0.001\)).

Conclusions We have demonstrated for the first time that in healthy individuals forearm blood flow increases after an intravenous bolus of glucose, and that paradoxically, this response is reduced below baseline forearm blood flow in patients with coronary heart disease. Further studies are needed to determine whether plethysmographic measurement of forearm blood flow after an intravenous bolus of glucose could provide a clinically useful non-invasive test for the diagnosis of occult coronary heart disease.


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Key Words: Endothelium, insulin, atherosclerosis.

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Introduction

Endothelial dysfunction occurs early in atherogenesis\textsuperscript{[1]} and is present in patients with coronary heart disease \textsuperscript{[2–4]}. In vivo, stimuli used for the assessment of endothelial dysfunction include intra-arterial administration of acetylcholine\textsuperscript{[5]}. Less invasive procedures entail the assessment of the vascular response to changing blood flow\textsuperscript{[6,7]}, which relies on the fact that increased flow causes dilatation of the vessel\textsuperscript{[8]} via the release of nitric oxide\textsuperscript{[9]}. In the study of endothelium-dependent vascular function it would be desirable to identify agents whose vasoactive actions are endothelium-dependent, which do not require intra-arterial administration, and which are free of side effects.

It is well established that insulin has vascular actions\textsuperscript{[10]}. These are not due to insulin’s metabolic
effects but rather, are a distinct phenomenon attributable to insulin per se\cite{11-13}. Although insulin affects the sympathetic system, tissue capillarization and vascular conductivity, it also acts via the vascular endothelium. In this regard, endothelial denudation has been shown to abolish its insulin’s vascular effects in aortic rings\cite{14}. In healthy individuals, insulin-stimulated blood flow correlates with nitric oxide-dependent blood flow\cite{15} and accordingly, insulin-induced blood flow responses are abolished by $N^\omega$-monomethyl-L-arginine (L-NMMA)\cite{16-18}. This has also been seen in studies of the forearm blood flow response to hyperinsulinaemia in healthy individuals\cite{19}. These data indicate that insulin is an endothelium-dependent vasoactive agent, acting through endothelial nitric oxide synthesis-dependent pathways.

With the exception of studies employing oral glucose challenges\cite{20}, studies of the vascular effects of insulin have invariably used exogenous insulin, usually in the context of clamp studies. The intravenous glucose tolerance test, unlike clamp studies, gives rise to a physiological insulin response and has been used extensively for the assessment of glucose and insulin metabolism, but never for the assessment of vascular function. The original aim of this study was to determine whether in healthy individuals forearm blood flow changes during the intravenous glucose tolerance test. The incidental finding of a reduction in forearm blood flow during the intravenous glucose tolerance test in one patient with previously undiagnosed coronary heart disease led us to postulate that the forearm blood flow response during the intravenous glucose tolerance test might be altered in this condition. The study of 13 patients with coronary heart disease are included in this preliminary report.

**Subjects and methods**

All patients in the study group had angiographically-proven coronary heart disease. Healthy controls were selected from participants in an ongoing study of metabolic predictors of coronary heart disease and diabetes mellitus — the Heart Disease and Diabetes Risk Indicators in a Screened Cohort Study (HDDRISC). Controls were selected whose total cholesterol, triglycerides, high density lipoprotein cholesterol and fasting insulin levels were similar to previously-assessed patients with coronary heart disease. This was to minimize possible confounding interactions between basal metabolic status and endothelial function. Concurrent medications in the study group included: digoxin (n=2), beta-blockers (n=1), calcium antagonists (n=3), angiotensin-converting enzyme inhibitors (n=4), loop diuretics (n=4), nitrates (n=3), aspirin (n=4), statins (n=1), either alone or in combination. None of the medications was discontinued prior to the study and all subjects were non-smokers. All patients gave written informed consent and the study was approved by the local Ethics Committee.

**Intravenous glucose tolerance test**

Participants were asked to fast for 12 h prior to the study session on the metabolic ward, which commenced at 0900 h. The study was conducted with subjects in a semi-recumbent position in an air-conditioned room with a room temperature of approx. 25°C, having allowed 15 min for the subjects to familiarize themselves with the study conditions. Following sampling for baseline measurements, dextrose, at a concentration of 50% (dose of $0.5 \text{ g kg}^{-1}$) was administered intravenously into the left brachial vein over a period of 2 min 40 s. Prior to venous sampling, the cannula and three-way tap were flushed with saline, the three-way tap replaced with another, and this flushed again. Venous blood was extracted from the left brachial vein at multiple time points, according to a previously published protocol\cite{21} for measurement of plasma insulin and glucose. Plasma insulin concentration was determined by a microplate chemilumimometric assay specific for insulin (supplied by Molecular Light Technology Research Limited, Cardiff, U.K.). The assay was seen to exhibit negligible molar cross-reactivity with intact pro-insulin, split 32-33 proinsulin and des 31,32 proinsulin (<2%) and no detectable reactivity with C-peptide at 18 ng ml$^{-1}$. Between-assay coefficient of variation was <7% over the range 13–110 μU ml$^{-1}$. Plasma glucose was measured using a glucose oxidase method.

**Forearm blood flow**

This was measured in the right arm throughout the course of the intravenous glucose tolerance test, at $-10$, $-3$, 0, 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 75, 90, 107, 120, 125, 130, 135, 140, 145, 150, 155, 160, 175 and 180 following glucose administration. Blood flow measurements were obtained using the venous occlusion technique and a mercury-in-silastic strain-gauge plethysmograph (Hokanson EC4, PMS Instruments, Maidenhead, U.K.), with the silastic gauges placed at approx 5 cm below the antecubital creases. Forearm blood flow (ml min$^{-1}$ 100 ml forearm) was calculated from the rate of increase in forearm volume over four pulses, while venous return was interrupted by inflation of the cuff to 50 mmHg. The average of three measurements was taken as the blood flow at each time point.

**Statistical analyses**

Areas under the curve were calculated for glucose, insulin and forearm blood flow using the trapezoidal rule. Incremental areas, calculated by subtracting the mean fasting value, multiplied by 180 from the area under the curve, were used as a measure of the net change in these parameters from baseline values. The latter areas were used for analysis to minimize confounding by inter-subject variation in fasting values.
Group differences were assessed by the Mann–Whitney U test. ANOVA with repeated measures was used for analysis of changes in forearm blood flow from baseline. All statistical analyses were performed using the SYSTAT statistical package (Evanston, Illinois, USA). A $P$ value of $<0.05$ was considered statistically significant.

**Results**

The characteristics of the study and control groups are shown in Table 1. Patients with coronary heart disease (aged 65.5 ± 2.4 years, mean ± SEM) and controls (aged 41.6 ± 3.0 years) had similar plasma lipid levels, fasting glucose and insulin, and post-glucose challenge glucose and insulin levels. There were highly significant group differences in the net changes in forearm blood flow following intravenous glucose administration, expressed as incremental areas under the forearm blood flow profile. Group differences in incremental areas under the forearm blood flow profile were significant when age ($P=0.651$) or body mass index ($P=0.975$) were entered as covariates in ANOVA.

Figure 1 shows the % change from baseline in forearm blood flow following intravenous administration of glucose in both groups. Negative incremental forearm blood flow areas were observed in 11 out of 13 patients with coronary heart disease, but only in two out of 10 healthy individuals ($P=0.015$).

**Discussion**

This study provides the first demonstration that in healthy individuals forearm blood flow increases after an intravenous bolus injection of glucose. Paradoxically, intravenously administered glucose led to a reduction in forearm blood flow in patients with coronary heart disease, who had similar plasma lipid, glucose and insulin levels to healthy controls. Inclusion of age or body mass index as covariates in ANOVA did not affect the significance of such differences. These preliminary findings suggest that differences in the forearm blood flow response to glucose relate to the presence of coronary heart disease.

There is evidence to suggest that insulin’s vascular effects[16,22,23] are due to insulin per se rather than to its effect on glucose uptake[23]. We have found that increases in forearm blood flow during the intravenous glucose tolerance test occur as early as 7 min after the glucose injection, before insulin has any significant effect on glucose uptake. Baron’s group have elegantly shown that the vasodilatory effect of insulin in skeletal muscle is NO-dependent[24], which is consistent with the finding that endothelial denudation abolishes insulin’s vascular effects on aortic rings[25]. Other workers have demonstrated a correlation in healthy individuals between insulin-stimulated blood flow and NO-dependent blood flow[23] and that insulin-induced blood flow responses are abolished by Nω-monomethyl-L-arginine (L-

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**Table 1 Characteristics, metabolic variables and forearm blood flow responses during the intravenous glucose tolerance test**

<table>
<thead>
<tr>
<th></th>
<th>Healthy (n=10)</th>
<th>CHD (n=13)</th>
<th>$P$ value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>41.6 (3.0)</td>
<td>65.5 (2.4)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Body mass index (kg.m$^{-2}$)</td>
<td>30.1 (3.1)</td>
<td>24.8 (1.1)</td>
<td>0.011</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>132.0 (4.5)</td>
<td>113.0 (4.4)</td>
<td>0.01</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>83.5 (2.2)</td>
<td>73.4 (2.8)</td>
<td>0.043</td>
</tr>
<tr>
<td>Cholesterol (mmol.1$^{-1}$)</td>
<td>5.4 (0.4)</td>
<td>5.5 (0.2)</td>
<td>ns</td>
</tr>
<tr>
<td>Triglycerides (mmol.1$^{-1}$)</td>
<td>1.8 (0.4)</td>
<td>1.7 (0.2)</td>
<td>ns</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol.1$^{-1}$)</td>
<td>1.4 (0.1)</td>
<td>1.3 (0.1)</td>
<td>ns</td>
</tr>
<tr>
<td>Fasting glucose (mmol.1$^{-1}$)</td>
<td>5.1 (0.12)</td>
<td>5.7 (0.3)</td>
<td>ns</td>
</tr>
<tr>
<td>Fasting insulin (μU.m$^{-1}$)</td>
<td>5.9 (1.2)</td>
<td>8.3 (1.5)</td>
<td>ns</td>
</tr>
<tr>
<td>Incremental glucose area</td>
<td>541.8 (46.7)</td>
<td>516.5 (38.9)</td>
<td>ns</td>
</tr>
<tr>
<td>Incremental insulin area [mmol.1$^{-1}$ min]</td>
<td>2567.1 (549.0)</td>
<td>2391.3 (437.6)</td>
<td>ns</td>
</tr>
<tr>
<td>Forearm blood flow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (ml min$^{-1}$ . 100 ml$^{-1}$)</td>
<td>4.85 (0.65)</td>
<td>6.2 (0.8)</td>
<td>ns</td>
</tr>
<tr>
<td>7 min (ml min$^{-1}$ . 100 ml$^{-1}$)</td>
<td>5.84 (0.55)</td>
<td>5.0 (0.9)</td>
<td>ns</td>
</tr>
<tr>
<td>180 min (ml min$^{-1}$ . 100 ml$^{-1}$)</td>
<td>10.06 (1.66)</td>
<td>4.9 (1.1)</td>
<td>0.005</td>
</tr>
<tr>
<td>Incremental blood flow area (min ml . 100 ml$^{-1}$)</td>
<td>351.3 (121.7)</td>
<td>-244.3 (72.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>Change from baseline (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 min</td>
<td>31.7 (14.7)</td>
<td>-16.2 (11.5)</td>
<td>0.013</td>
</tr>
<tr>
<td>180 min</td>
<td>112.8 (24.9)</td>
<td>-25.8 (7.3)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as mean (± SEM).† $P$ values refer to differences from Mann–Whitney U tests, except for those relating to group differences in forearm blood flow from baseline, which are derived from ANOVA with repeated measures. CHD=coronary heart disease, HDL=high density lipoprotein.
NMMA)\cite{16-18}. Using plethysmography, Cardillo et al. have recently shown that in healthy individuals, the forearm blood flow response in response to insulin administration, measured by venous occlusion plethysmography, is NO-dependent\cite{19}.

The involvement of NO in forearm blood flow responses after intravenous glucose could offer an explanation for the impaired responses observed in patients with coronary heart disease. In this regard, in conditions which involve endothelial injury, such as atherosclerosis\cite{25,25}, hypertension\cite{26} and diabetes mellitus\cite{27,28}, there is a paradoxical vascular response to endothelium-dependent vasoactive agents, such as acetylcholine, which causes vasoconstriction in patients with these conditions and vasodilation in healthy subjects. Our finding that intravenous glucose causes an increase in forearm blood flow in healthy individuals and a reduction in patients with coronary heart disease suggests a parallelism between the effects of glucose and those of acetylcholine. The observed differential response to intravenous glucose may be related to the fact that insulin also affects inducible NO synthase (iNOS) in vascular smooth muscle cells\cite{29}. Accordingly, the net in vivo effects of insulin on the vasculature may depend on the balance between stimulation of endothelial constitutive NO synthase (cNOS) and vascular smooth muscle iNOS. A failure of the NO system to respond to insulin could explain an absence of forearm blood flow response to insulin in coronary heart disease, but not an actual reduction, as we have observed. Thus, it is possible that, upon removal of the normal vasodilatory effects of insulin-stimulated NO release, the vasculature becomes susceptible to vasoconstrictive factors, be they endothelium-dependent or -independent.

Another mechanism for the paradoxical response to intravenous glucose observed in patients with coronary heart disease may relate to the vascular actions of glucose per se. In agreement with in vitro\cite{30} and in vivo\cite{31} animal studies, Williams et al.\cite{32} have recently shown that hyperglycaemia attenuates the forearm blood flow response to the endothelium-dependent agent methacoline. This could offer an explanation for our findings, insofar as the paradoxical forearm blood flow response in patients with coronary heart disease could result from an impairment of forearm blood flow by hyperglycaemia which is unopposed by insulin-mediated vasodilation.

In conclusion, we have shown for the first time that glucose, administered as an intravenous bolus injection, leads to an increase in forearm blood flow in healthy individuals. A further novel finding from this study is that, paradoxically, a reduction in forearm blood flow during the intravenous glucose tolerance test occurs in patients with coronary heart disease. On the basis of our findings, measuring forearm blood flow in response to intravenous glucose has the potential for use as a non-invasive procedure for the diagnosis of clinically occult coronary heart disease. The diagnostic specificity of this procedure will depend on whether other conditions which are associated with endothelial damage

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Mean percent change ($\%$, SEM) from baseline in forearm blood flow (FBF) during the intravenous glucose tolerance test in healthy individuals (C) and in patients with coronary heart disease (●). Each point is the mean of three readings at each time point.}
\end{figure}
such as hypertension, diabetes, dyslipidaemias, peripheral and cerebrovascular disease are associated with similar alterations in forearm blood flow during the intravenous glucose tolerance test. Further studies are needed to explore these issues.

Limitations of the study

Our findings on a small number of individuals should be considered as preliminary. Amongst the limitations of this study, it should be noted that patients with coronary heart disease were significantly older than controls. Although the differences in forearm blood flow responses could not be accounted for by age when this was entered as a covariate in ANOVA, further evaluation of the effects of age on forearm blood flow responses during the intravenous glucose tolerance test are clearly needed. The reason for selecting younger individuals as controls was to minimize the possibility of occult coronary heart disease and vascular endothelial damage.

It should also be noted that patients with other conditions which involve endothelial damage, such as hypertension, diabetes, dyslipidaemias, and cerebrovascular disease may exhibit similar intravenous glucose tolerance test forearm blood flow responses to patients with coronary heart disease. This would clearly affect the specificity of this technique in the diagnosis of coronary heart disease. Furthermore, we cannot exclude the fact that inter-individual variations in the forearm blood flow response in patients with coronary heart disease may be attributable to concomitant therapies which are known to improve endothelial dysfunction, such as statins and angiotensin-converting enzyme inhibitors. These issues require further evaluation.

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References


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