Carbohydrate ingestion, with transient endogenous insulinaemia, produces both sympathetic activation and vasodilatation in normal humans

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ABSTRACT

It has been shown that sustained insulin infusion causes an increase in sympathetic vasoconstrictor discharge but, despite this, also causes peripheral vasodilatation. The present study was designed to determine in healthy subjects the effect of ingestion of a carbohydrate meal, with its attendant physiological insulinaemia, on vascular resistance in and sympathetic vasoconstrictor discharge to the same vascular bed, and the relationship between these parameters. Fifteen healthy subjects were studied for 2 h following ingestion of a carbohydrate meal. Calf vascular resistance was measured by venous occlusion plethysmography, and muscle sympathetic nerve activity was assessed by peroneal microneurography. Five of the subjects also ingested water on a separate occasion, as a control. Following the carbohydrate meal, the serum insulin concentration increased to 588 ± 72 pmol/l. This was associated with a 47% increase in skeletal muscle blood flow (P < 0.001), a 39% fall in vascular resistance (P < 0.001) and a 57% increase in sympathetic activity (P < 0.001). There was a significant correlation between the increase in insulin and the changes in blood flow, vascular resistance and sympathetic activity. In conclusion, we have shown that ingestion of a carbohydrate meal, with its attendant physiological insulinaemia, was associated with overriding skeletal muscle vasodilatation, despite an increase in sympathetic vasoconstrictor discharge to the same vascular bed. These mechanisms may be important in ensuring optimal glucose uptake and maintenance of blood pressure postprandially.

INTRODUCTION

Insulin causes sympathetic vasoconstrictor activation in the nerves destined to supply the skeletal muscle vasculature [1–5]. However, it also causes vasodilatation in the same vascular bed, and this overrides the sympathetic pressor influence [1,3,4]. These effects have been demonstrated in the controlled environment of a euglycaemic hyperinsulinaemic clamp, and as such may not occur in the normal physiological situation of insulinaemia, that of eating a meal. As insulin-induced skeletal muscle vasodilatation and sympathetic neural activation are...
potentially important events in determining insulin resistance [6,7] and blood pressure (BP) [8,9], the magnitude of, and balance between, these two effects of insulin could be important in the everyday context of meal ingestion. Studies that have assessed these effects in response to the transient insulinemia of meal ingestion have shown that there is sympathetic vasoconstrictor activation [10–13], but most studies have shown no effect on vascular resistance [14–17], with variable effects on BP [10–17]. These studies have not examined the effects simultaneously in the same skeletal muscle vascular bed, so it is possible that, following a meal, vasodilatation is prevented by the increased sympathetic vasoconstrictor activity.

The present study was designed to determine, in healthy subjects, whether ingestion of a carbohydrate meal, with its attendant physiological increase in endogenous insulin, was associated with changes in skeletal muscle blood flow and sympathetic vasoconstrictor discharge to the same region, and to examine the relationship between these changes.

METHODS

Subjects
The study group comprised 15 healthy Caucasian subjects: four women and eleven men, age range 34–75 years. All were screened by medical history and physical and laboratory examination, and were excluded if there was evidence of chronic disease influencing the autonomic nervous system. The subjects were not on any medication. The study was approved by Local Ethics Committee, and conformed to the principles outlined in the Declaration of Helsinki (1989) of the World Medical Association. Each subject gave informed, written consent prior to taking part.

Experimental protocol
Studies were performed between 08.00 and 12.00 hours. All subjects had fasted for 12 h, and had avoided nicotine and caffeine for 12 h and alcohol and strenuous exercise for 24 h, prior to investigation.

After emptying their bladder, all subjects were studied semi-supine. They relaxed for 10 min to reach steady state before starting the study. Measurements were made in a darkened, temperature-constant (22–24 °C) laboratory. Heart rate (HR) and arterial BP were recorded using a standard ECG and a Finapres device (Model 2300; Ohmeda). Respiration was monitored using a pneumograph. Arterialized venous blood samples were obtained to measure blood glucose and serum insulin. A measure of insulin resistance was calculated from fasting insulin and glucose values using the HOMA (Homeostasis Model Assessment) method [18].

After baseline measurements had been obtained, subjects remained semi-supine and ingested a 500 ml liquid carbohydrate meal, at room temperature, within 5 min. The energy content of the meal was 2.5 MJ, with 85% as carbohydrate (80 g of Maxijul and 20 g of Duocal). Data were then obtained every 10 min for 120 min. During each data acquisition period, muscle sympathetic nerve activity (MSNA), calf blood flow (CBF), BP and HR were recorded over a minimum period of 2 min. Blood samples were taken before the meal and every 20 min afterwards. Before and after the meal, all subjects performed a standard Valsalva manoeuvre [19] in order to calculate baroreflex sensitivity.

Separate control studies were performed in five of the subjects. The protocol was identical, except that 500 ml of water, served at room temperature, replaced the carbohydrate meal. Repeat studies were performed in a random order.

Microneurography
Post-ganglionic MSNA was recorded from the right peroneal nerve using the technique of microneurography, as described in detail previously [20–22]. MSNA was differentiated from skin sympathetic activity and afferent activity by previously accepted criteria [21,22]. Only vasoconstrictor discharge was accepted and examined, the criteria of acceptance being appropriate responses to spontaneous changes in BP, the Valsalva manoeuvre [23] and isometric hand-grip exercise [24].

Vascular resistance
CBF was measured simultaneously in the left leg using venous occlusion plethysmography (D. E. Hokansen, Bellevue, WA, U.S.A.). Calf vascular resistance (CVR), expressed in arbitrary units, was obtained from mean BP divided by mean CBF (ml·min⁻¹·100 ml⁻¹ tissue), obtained as the average value of at least six measurements for each 2-min period.

Analytical methods
Blood glucose was measured using a glucose analyser (Yellow Springs Instruments, Yellow Springs, OH, U.S.A.). Serum insulin was measured using the 1235 Auto DELFIA automatic immunoassay system (Wallac). The intra- and inter-assay variation of this assay, expressed as the coefficient of variation, was 2.1–3.7% and 3.3–3.8% respectively.

Data analysis and statistics
CVR, CBF, MSNA and results of the blood assays were analysed independently, by operators blinded to the intervention received. ECG, BP and the mean voltage neurogram were analysed off-line by a single experienced
operator using signal-processing software (FASTDAQ; Lectromed UK). The multi-unit bursts of MSNA on the mean voltage neurogram were identified by inspection when the signal/noise ratio was greater than 3, and were quantified as bursts/min and bursts/100 cardiac beats. Baroreflex sensitivity was determined from the slope of the best linear relationship between the increase in systolic BP and the pulse interval during phase IV of the Valsalva manoeuvre [19].

The changes in measured variables following meal ingestion were examined using repeated-measures ANOVA with Newman–Keuls post test analysis. Differences between the group of subjects who had meal ingestion and the control (water ingestion) subjects were examined by the paired Student t test. The relationships between changes in measured variables were examined using the Pearson correlation coefficient (r). The insulin values were logarithmically transformed prior to performing the correlations. Values of P < 0.05 were considered statistically significant, and all data are presented as means ± S.E.M.

**RESULTS**

**Subjects**

Subject characteristics are shown in Table 1. All subjects completed the carbohydrate protocol, and five of the subjects also completed the water control protocol.

<table>
<thead>
<tr>
<th>Subjects Carbohydrate only Carbohydrate and water</th>
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<tbody>
<tr>
<td>Number 15 (11 male/4 female) 5 (2 male/3 female)</td>
</tr>
<tr>
<td>Age (years) 53 ± 3.3 (34–75) 42 ± 3.0 (36–53)</td>
</tr>
<tr>
<td>Weight (kg) 81 ± 3.2 (61–101) 76 ± 2.9 (66–83)</td>
</tr>
<tr>
<td>Body mass index (kg/m²) 26 ± 0.7 (21–30) 27 ± 1.2 (24–30)</td>
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</table>

**Insulin and glucose**

The meal was followed by increases in blood glucose and serum insulin concentrations in every subject. The increases began in both cases at 20 min, and reached a peak at 40 and 80 min respectively (Figure 1). They remained significantly elevated at 120 min. The mean HOMA insulin resistance value of the subjects was 1.10 ± 0.16.

**Sympathetic activity**

Following the peak serum insulin concentration, there was an increase in the mean frequency of MSNA bursts in each subject. Following the meal (Figure 2), group increases in MSNA (bursts/min) attained statistical significance at 100 min and continued to increase for the remainder of the experiment. The magnitude of these increases following the peak serum insulin concentration amounted to 57%. The increase in MSNA (bursts/min) was correlated significantly with that in serum insulin concentration (r = 0.34, P < 0.003). When analysed as bursts/100 cardiac beats, MSNA increased throughout the study, reaching statistical significance at 120 min. The magnitude of this increase was 33%, and it was also correlated with the serum insulin concentration (r = 0.20, P < 0.03).

**CBF and vascular resistance**

In each subject there was an increase in CBF and a decrease in CVR following the peak serum insulin concentration. The group data (Figure 2) showed a significant increase in CBF at 60 min and a significant decrease in CVR at 40 min. The increase in CBF and the decrease in CVR following the peak serum insulin concentration amounted to 47% and 39%, respectively. The increase in CBF (r = 0.21, P < 0.02) and the decrease in CVR (r = −0.25, P < 0.006) were correlated significantly with the increase in serum insulin concentration. There was a significant increase in CVR in the first 10 min after meal ingestion (P < 0.05).
Figure 2  Changes in MSNA, CBF and CVR following ingestion of a carbohydrate meal in 15 healthy subjects
Data are expressed as means ± S.E.M. Significance of changes relative to baseline (ANOVA): * P < 0.05, † P < 0.01, ‡ P < 0.001.

Figure 3  Changes in HR and BP following ingestion of a carbohydrate meal in 15 healthy subjects
Data are expressed as means ± S.E.M. Significance of changes relative to baseline (ANOVA): * P < 0.05, † P < 0.01, ‡ P < 0.001.

BP and HR
Following peak serum insulin, the mean BP decreased in nine, did not change in three and increased in three subjects. Taking the group as a whole (Figure 3), BP decreased by 7% to reach a nadir at 30 min, but increased thereafter. There was no change in systolic BP (P > 0.7), but a significant fall in diastolic BP corresponded to the change in mean BP. The decrease in mean BP did not correlate with serum insulin concentration (r = 0.05, P > 0.30). The age of the subjects did not correlate with change in BP (r = 0.14, P > 0.30).

HR increased in the group as a whole by 17%. The increases in HR were correlated with those in insulin concentration (r = 0.41, P < 0.0001), but not with changes in BP (r = −0.15, P > 0.06).

After 120 min, when serum insulin was still raised at 483 ± 69.7 pmol/l, baroreflex sensitivity was not significantly different from that during the baseline period. Before the meal baroreflex sensitivity was 7.4 ± 1.2 ms/mmHg, and postprandially it was 6.3 ± 1.3 ms/mmHg (P > 0.5).

Controls
The changes that occurred in the five subjects who ingested the carbohydrate meal and also water are given in Table 2. Following water ingestion, there were no significant change in blood glucose or serum insulin concentrations. Water ingestion did not reproduce any of the observed carbohydrate-induced responses in terms of HR, BP, MSNA, CBF or CVR. As we have described previously [20], there was an early increase in MSNA associated with peripheral vasoconstriction in the subjects who ingested water. These changes had all resolved within 50 min, while the changes observed with carbohydrate did not occur until after this period, reaching maximum at 120 min. Therefore the second hour fol-
following water ingestion was used a control experiment for the carbohydrate meal.

**DISCUSSION**

This is the first study to have looked at the relationship between ingestion of a carbohydrate meal, with its attendant physiological insulinaemia, and sympathetic vasoconstrictor discharge and blood flow in the same skeletal muscle vascular bed simultaneously. We found that a carbohydrate meal was associated with overriding vasodilatation of the skeletal muscle vasculature, despite an increase in sympathetic vasoconstrictor activity. The vasodilatation preceded the increase in sympathetic activity, and the time of the increase in MSNA corresponded to the return of BP towards baseline values.

A high-carbohydrate meal was chosen, in contrast with some other studies [14–17], as this elicited an increase in insulin to the physiological concentrations obtained using an insulin clamp [1–5]. It also prevented the confounding vasoconstrictive effects seen with fat ingestion [25] that may be observed in the context of a mixed meal. A liquid form of the meal was chosen to enable it to be consumed easily while semi-supine.

Care was taken to study healthy subjects without insulin resistance, and to control for all the confounding factors that might interfere with the measurement of autonomic function [26–30]. Water was used a control substance to ensure that the changes observed were not related to the procedure. We have shown previously that water ingestion leads to early sympathoactivation and vasoconstriction, thought to be secondary to the reflex effect of stomach distension [20]. It was clear that, despite the possible effects of gastric distension, there was overriding vasodilatation following carbohydrate ingestion.

In the context of a meal, sympathoactivation could occur via a variety of mechanisms. Insulin has been shown to increase sympathetic activity through a central neural action, crossing the blood–brain barrier [32]. Studies have also shown reflex increases in sympathetic activity due to stomach distension [30], and baroreceptor reflexes lead to a rapid, transient increase in sympathetic activity in response to a fall in arterial BP. However, the timing of sympathetic activation following carbohydrate ingestion did not correspond to that expected if gastric distension were playing a large part [31], but occurred much later in the study, coinciding instead with the timing of the insulin peak. While the magnitude of the increase in MSNA was similar to that observed in other postprandial studies, those studies have consistently shown an increase in MSNA in the first 30 min after eating a meal [10–13]. It is widely acknowledged that the vasoactive effects of insulin are time- and dose-dependent [1–5,32] and it seems likely that the earlier increase in MSNA was due to the earlier insulin peak seen in these previous studies, as well as possible additional effects of gastric distension.

In the present investigation, there was no evidence linking sympathetic activation to baroreceptor reflex effects. While there was an overall fall in BP within the group in the first 20 min postprandially, this did not correspond to the increase in MSNA, which occurred only in the final period of the study. In addition, all subjects showed activation of MSNA, despite a heterogeneous response of BP. The increase in serum insulin concentration was not associated with significant changes in baroreceptor reflex sensitivity, and any individual changes in the latter did not correlate with sympathetic activation.

Only one study had previously demonstrated vasodilatation in response to oral carbohydrate ingestion [33]. The time to vasodilation was similar to that of the present study, and would fit with the proposed mechanism of insulin-induced vasodilatation by a local endothelial mechanism. It is possible that other studies failed to demonstrate vasodilatation as a result of using

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**Table 2 Data from five subjects following separate carbohydrate and water meals**

Details are presented as means ± S.E.M. Also shown is the significance of differences between values obtained following ingestion of carbohydrate and water at 120 min postprandially.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Postprandial</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>Water</td>
<td>Carbohydrate</td>
<td>Water</td>
</tr>
<tr>
<td>Blood glucose (mmol/l)</td>
<td>4.4 ± 0.1</td>
<td>4.6 ± 0.1</td>
<td>10.3 ± 0.95</td>
</tr>
<tr>
<td>Serum insulin (pmol/l)</td>
<td>37 ± 12.0</td>
<td>44 ± 15.2</td>
<td>696 ± 233</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>50 ± 4.3</td>
<td>55 ± 4.4</td>
<td>69 ± 6.2</td>
</tr>
<tr>
<td>Mean BP (mmHg)</td>
<td>83 ± 4.4</td>
<td>80 ± 5.6</td>
<td>78 ± 2.6</td>
</tr>
<tr>
<td>MSNA (bursts/min)</td>
<td>21 ± 2.7</td>
<td>21 ± 2.7</td>
<td>39 ± 2.9</td>
</tr>
<tr>
<td>MSNA (bursts/100 beats)</td>
<td>34 ± 3.8</td>
<td>38 ± 3.6</td>
<td>59 ± 8.0</td>
</tr>
<tr>
<td>CVR (units)</td>
<td>51 ± 4.7</td>
<td>56 ± 6.2</td>
<td>35 ± 4.5</td>
</tr>
<tr>
<td>CBF (ml·min⁻¹·100 ml⁻¹)</td>
<td>1.7 ± 0.12</td>
<td>1.3 ± 0.13</td>
<td>2.4 ± 0.27</td>
</tr>
</tbody>
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meals with a lower carbohydrate content and/or mixed meals containing a higher proportion of fat [14–17].

There has been a suggestion that the effect of insulin in causing vasodilatation is pharmacological and may not be applicable under ordinary physiological conditions [34]. The present study demonstrates that ingesting a carbohydrate meal reproduces the effects seen with intravenous insulin [1,3–5]. The effects are unlikely to have been a direct action of the carbohydrate, as glucose infusion fails to lead to vasodilatation when the attendant rise in insulin is prevented by simultaneous octreotide infusion [35]. In addition, the present study showed significant correlations between the rise in insulin and both sympathetic activation and vasodilatation. The weakness of the correlation is perhaps not surprising, in view of the other variables occurring following a meal, with gastric distension and its reflex effects particularly pertinent. The vascular effects of insulin should therefore be considered as an ordinary part of daily physiological function.

Although the present study did not look at insulin-mediated glucose uptake by skeletal muscle, work by Baron and co-workers [6,33] has established a potential role of insulin to affect insulin sensitivity through its ability to cause vasodilatation. The present study showed that the vasodilatation occurs early compared with the sympathoactivation. In a physiological context, this would seem to direct nutrients to skeletal muscle so as to facilitate glucose uptake and storage. The sympathoactivation may then occur to pre-empt and prevent an excessive fall in BP, preventing syncope. This would fit with results from studies in subjects with autonomic dysfunction, where there is vasodilatation without sympathoactivation at the expense of a fall in BP [8,9].

It remains to be discovered whether an imbalance between the pressor and depressor effects of insulin has a role to play in the development or worsening of insulin resistance, but this is an exciting possibility. Certainly one might hypothesize that overwhelming sympathetic vasoconstrictor influence may prevent subsequent insulin-induced vasodilatation and glucose uptake, or that failure of vasodilatation as a result of another mechanism could prevent glucose uptake in the context of a meal, leading to insulin resistance. If there was then an increase in sympathetic vasoconstrictor discharge, this could serve to increase peripheral vascular resistance and have detrimental effects on the cardiovascular system, including perhaps the development of hypertension.

In conclusion, ingestion of a carbohydrate meal leading to physiological insulinaemia is associated with overriding skeletal muscle vasodilatation, despite the simultaneous increase in sympathetic vasoconstrictor discharge to the same vascular bed. It is possible that these haemodynamic mechanisms in skeletal muscle are important to ensure optimal glucose uptake and storage postprandially, while maintaining BP. Alteration of the balance between them, preventing the predominance of vasodilatation, may contribute to impaired glucose tolerance and insulin resistance on a daily basis.

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REFERENCES


Postprandial insulinaemia and vasoregulation