

# Differential Effects of Hyperinsulinemia and Carbohydrate Metabolism on Sympathetic Nerve Activity and Muscle Blood Flow in Humans

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## Abstract

Euglycemic hyperinsulinemia evokes both sympathetic activation and vasodilation in skeletal muscle, but the mechanism remains unknown. To determine whether insulin per se or insulin-induced stimulation of carbohydrate metabolism is the main excitatory stimulus, we performed, in six healthy lean subjects, simultaneous microneurographic recordings of muscle sympathetic nerve activity, plethysmographic measurements of calf blood flow, and calorimetric determinations of carbohydrate oxidation rate. Measurements were made during 2 h of: (a) insulin/glucose infusion (hyperinsulinemic [6 pmol/kg per min] euglycemic clamp), (b) exogenous glucose infusion at a rate matched to that attained during protocol a, and (c) exogenous fructose infusion at the same rate as for glucose infusion in protocol b. For a comparable rise in carbohydrate oxidation, insulin/glucose infusion that resulted in twofold greater increases in plasma insulin concentrations than did glucose infusion alone, evoked twofold greater increases in both muscle sympathetic nerve activity and calf blood flow. Fructose infusion, which increased carbohydrate oxidation comparably, but had only a minor effect on insulinemia, did not stimulate either muscle sympathetic nerve activity or calf blood flow. These observations suggest that in humans hyperinsulinemia per se, rather than insulin-induced stimulation of carbohydrate metabolism, is the main mechanism that triggers both sympathetic activation and vasodilation in skeletal muscle. (*J. Clin. Invest.* 1993. 92:147–154.) Key words: energy expenditure • fructose infusion • glucose infusion • microneurography • hyperinsulinemic euglycemic clamp

## Introduction

Insulin, apart from its effects on intermediary metabolism, also has effects on the heart and the peripheral vasculature (1–4). For example, in humans acute euglycemic hyperinsulinemia stimulates both sympathetic efferent activity (5–9), and blood

flow in skeletal muscle (6, 9, 10). More recently, evidence has accumulated in humans, indicating that insulin's cardiovascular effects may be involved in metabolic regulation (10, 11). Insulin-induced stimulation of blood flow to skeletal muscle, a major insulin-sensitive tissue, has been found to be closely correlated with insulin-mediated muscle glucose uptake. However, the mechanism by which insulin stimulates sympathetic activity and blood flow in skeletal muscle remains unknown. More specifically, it is not known whether such stimulatory effects are caused by hyperinsulinemia per se or hyperinsulinemia-induced stimulation of carbohydrate metabolism.

Accordingly, the purpose of this study was to determine whether hyperinsulinemia or carbohydrate metabolism is the primary stimulus that triggers sympathetic activation and vasodilation in humans. To accomplish this aim, we performed simultaneous microelectrode recordings of sympathetic nerve discharge to skeletal muscle, plethysmographic measurements of calf blood flow, and calorimetric determinations of carbohydrate oxidation in lean, healthy volunteers during euglycemic hyperinsulinemic glucose clamp, and exogenous glucose or fructose infusion. These interventions evoke comparable increases in carbohydrate oxidation but different increases in plasma insulin concentrations.

## Methods

### Subjects

Six lean, healthy subjects (wt 70.8±4.3 kg, height 180.2±5.3 cm, body mass index 21.8±0.8 kg/m<sup>2</sup>, age 28±4 yr, mean±SE) participated in this study after providing informed written consent. All subjects were normotensive, had normal glucose tolerance, were taking no medications, and had no evidence of metabolic or cardiovascular disease at the time of the study. Tests were performed within an interval of 5–8 wk, and were all conducted in the morning after an overnight fast. Subjects had been on a weight maintaining diet containing at least 40% carbohydrates for 3 d before the tests. The experimental protocol was approved by the Institutional Review Board on Human Investigation.

### General procedures

Subjects were studied in the supine position. Heart rate (electrocardiogram), respiratory excursions (pneumobelt), blood pressure (Finapres blood pressure monitor; Ohmeda, Englewood, CO [12]), calf blood flow (venous occlusion plethysmography [13]), and efferent muscle sympathetic nerve activity (MSNA),<sup>1</sup> were recorded continuously on an electrostatic recorder and on a tape recorder (R71; TEAC Corp., Tokyo, Japan). Respiratory excursions were monitored to detect inadvertent performance of a Valsalva maneuver or prolonged expiration; these respiratory maneuvers can markedly stimulate MSNA (14). Intravenous catheters were inserted in a right and a left antecubital vein,

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1. Abbreviation used in this paper: MSNA, muscle sympathetic nerve activity.

one for substrate infusion, the other for blood sampling. Urine was collected before and at the end of the study for nitrogen determination.

### Experimental protocols

**Protocol 1: hyperinsulinemic euglycemic clamp.** After instrumentation and 1 h of baseline measurements, all six subjects received a primed continuous infusion of crystalline insulin (Actrapid HM; Novo Industri S/A, Bagsvaerd, Denmark) at a rate of 6 pmol/kg per min for 2 h. Euglycemia was maintained by determining plasma glucose concentration every 5 min and periodically adjusting a variable infusion of 20% dextrose (15). Hypokalemia was prevented by administration of KCl infused at a rate of 10 meq/h. Hemodynamic measurements and sympathetic nerve activity were recorded for 5 out of every 15 min throughout the study. Blood samples were collected in the basal state and at timed intervals throughout the study for analysis of substrate and hormone concentrations.

**Protocol 2: glucose infusion.** All six subjects repeated the same protocol as above, except that instead of insulin, they received a continuous infusion of glucose for 2 h, at a rate matched to that observed during the last 30 min of the hyperinsulinemic euglycemic clamp.

**Protocol 3: fructose infusion.** Five of the six subjects participated in this protocol, during which they received a continuous infusion of fructose for 2 h at a rate matched to that of glucose observed during the last 30 min of the hyperinsulinemic euglycemic clamp. No KCl was infused during this protocol.

**Protocol 4: determination of glucose oxidation during fructose infusion.** The rationale of these experiments, was to examine whether during fructose infusion, glucose oxidation contributes to the observed stimulation of carbohydrate oxidation. To address this issue, in six male lean healthy subjects (age  $30 \pm 4$  yr, body mass index  $22.9 \pm 0.6$  kg/m<sup>2</sup>) after a bolus injection of NaH<sup>13</sup>CO<sub>3</sub> (0.3 mmol), a prime (120 μg/kg)-continuous (1.5 μg/kg per min) infusion of [U-<sup>13</sup>C]glucose (90% enrichment; Cambridge Isotope Laboratories, Woburn, MA) was administered from time -150 to +120 min. From time 0 to +120 min, a 2-h continuous infusion of fructose at a rate identical to the one used in protocol 3 was superposed upon the [U-<sup>13</sup>C]glucose infusion. Respiratory gas exchanges (indirect calorimetry) were monitored throughout the experiment. Breath and plasma samples were collected (at 5-min intervals for expired air, and at 10-min intervals for blood samples) at basal (-180 to -150 min), between -30 and 0 min, and between +90 and +120 min for determination of <sup>13</sup>CO<sub>2</sub> and <sup>13</sup>C plasma glucose enrichments.

### Recording of sympathetic nerve activity

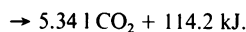
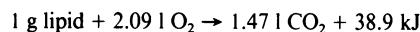
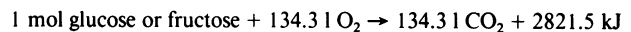
Multiunit recordings of sympathetic nerve activity were obtained with unipolar tungsten microelectrodes inserted selectively into muscle nerve fascicles of the peroneal nerve posterior to the fibular head by the microneurographic technique of Vallbo, Hagbarth, et al. (16). The neural signals were amplified (by  $20\text{--}50 \times 10^3$ ), filtered (bandwidth 700–2,000 Hz), rectified, and integrated (time constant 0.1 s) to obtain a mean voltage display of sympathetic activity. A recording of MSNA was considered acceptable when it revealed spontaneous, pulse-synchronous bursts of neural activity that increased during the Valsalva maneuver, but not during arousal stimuli such as loud noise. Sympathetic bursts were identified by inspection of the filtered and mean voltage neurograms. To determine intra- and interobserver variability in identifying bursts, two of us (U. Scherrer, P. Vollenweider), blinded to patient condition, systematically analyzed 21 consecutive recordings obtained during this and a related project (9). We found that the intraobserver coefficients of variation of the mean averaged 2.9% (with a range of 0–10%), and 6.0% (with a range of 0–17%), respectively; the coefficient of variation exceeded 10% in only one record. The interobserver coefficient of variation of the mean in identifying bursts in these 21 recordings was 8.7%, with a range of 0–21%; the coefficient of variation exceeded 15% in only two recordings. Nerve traffic was expressed both as bursts per minute, an index of the frequency of the activity, and as bursts per minute times mean burst amplitude, an index of integrated (total) activity.

### Calf blood flow

Blood flow in the calf was measured with venous occlusion plethysmography, using mercury-in-silastic strain gauges (13). The calf was elevated 10–15 cm above the level of the right atrium to collapse the veins. The circulation to the foot was arrested by inflating a cuff around the ankles during blood flow determinations, which were performed at 15-s intervals for 5 min.

### Indirect calorimetry

Energy expenditure and substrate utilization were calculated from respiratory gas exchanges (determined by a computerized, flowthrough canopy gas analyzer system [Deltatrac; Datex, Helsinki, Finland], and urinary nitrogen excretion, after correction for changes in the body urea nitrogen pool (17, 18). The following stoichiometric equations were used:



This procedure does not allow differentiation of glucose from fructose oxidation. During the euglycemic clamp and during glucose infusion, total glucose uptake was assumed to be equal to exogenous glucose infusion. The rate of nonoxidative glucose disposal was calculated by subtracting the rate of glucose oxidation from the rate of steady state glucose uptake. These calculations were not performed during fructose infusion, during which endogenous glucose production (not measured) is not suppressed (19).

### Measurement of glucose oxidation during fructose infusion

Breath <sup>13</sup>CO<sub>2</sub> isotopic enrichment was measured by continuous-flow isotope ratio mass spectrometry (20) on a Roboprep G-Tracermass (Europa Scientific Ltd., Crewe, UK). Plasma samples (2 ml) were deproteinized with perchloric acid (3% final concentration), neutralized with 3.2 M K<sub>2</sub>CO<sub>3</sub>, and partially purified over sequential anion-exchange resins (AG 1-X8 and AG 50W-X8; Bio-Rad Laboratories, Richmond, CA). The neutral fraction was evaporated to dryness, resuspended in 140 μl H<sub>2</sub>O, and plasma glucose was purified by HPLC on a column (HPX-87-C; Bio-Rad Laboratories) eluted with H<sub>2</sub>O at a temperature of 80°C and at a flow rate of 0.6 ml/min. This procedure allows complete separation of plasma glucose from glycerol. Purified glucose was again evaporated to dryness, resuspended in 15 μl H<sub>2</sub>O, and its <sup>13</sup>C enrichment was measured by combustion and continuous flow isotope ratio mass spectrometry on a Roboprep CN-Tracermass (Europa Scientific Ltd). Breath <sup>13</sup>CO<sub>2</sub> and plasma [<sup>13</sup>C]glucose were expressed as atom percent excess. Glucose oxidation was calculated as

glucose oxidation (μmol/min)

$$= \{ \dot{V}\text{CO}_2 \cdot {}^{13}\text{CO}_2 / [{}^{13}\text{C}]\text{glucose} \cdot 0.8 \} / 0.134$$

where  $\dot{V}\text{CO}_2$  is ml/min, and <sup>13</sup>CO<sub>2</sub> and [<sup>13</sup>C]glucose are isotopic enrichments in atom percent excess. 0.134 ml CO<sub>2</sub> is produced from oxidation of 1 μmol glucose. 0.8 is <sup>13</sup>CO<sub>2</sub> recovery as determined previously in our laboratory (21).

### Analytical methods

Plasma glucose was determined in duplicate by the glucose oxidase method on a glucose analyzer (Beckman Instruments, Fullerton, CA), and plasma fructose by an enzymatic method (22). Plasma insulin was measured by radioimmunoassay (23), catecholamines by HPLC (24), blood urea nitrogen using a urea analyzer (Beckman Instruments), plasma free fatty acid concentrations by a colorimetric method using a kit from Wako (Freiburg, Germany), and urinary nitrogen by the Kjeldahl method (25).

Table 1. Responses to 2-h Infusion of Insulin (Euglycemic Clamp), Glucose, or Fructose

	Insulin infusion			Glucose infusion			Fructose infusion		
	Basal	60 min	120 min	Basal	60 min	120 min	Basal	60 min	120 min
Heart rate (beats/min)	58±3	58±3	62±4*	60±4	58±4	64±4*	57±4	63±3*	65±4*
Mean arterial pressure (mmHg)	96±3	97±4	98±3	97±4	96±3	99±3	98±3	102±3	99±2
Systolic pressure (mmHg)	127±8	127±8	132±7	133±4	135±4	137±5	134±2	135±5	132±7
Diastolic pressure (mmHg)	81±3	81±3	82±4	78±4	77±3	80±3	81±3	85±2	83±3
MSNA (bursts/min)	13±2	22±4*	26±4*	13±3	15±3‡	20±3**	15±2	14±1§	15±1§
(Units) <sup>  </sup>	151±27	263±36*	367±60*	129±27	166±31	211±34**	159±15	138±13‡	156±14‡
Calf blood flow (ml/100 ml per min)	2.3±0.1	2.9±0.2*	3.1±0.2*	2.1±0.1	2.3±0.2‡	2.6±0.2‡	2.0±0.2	2.2±0.3‡	2.2±0.3‡
Calf vascular resistance (Units)	43.3±1.3	34.3±1.6*	32.8±2.0*	45.6±2.2	42.9±2.7‡	40.5±3.2‡	49.7±5.3	49.0±5.7‡	45.7±5.2‡
Insulin (pmol/liter)	45.6±5.4	402.6±22.8*	376.2±30.0*	38.4±3.3	140.4±12.0**	282.6±19.8**	37.2±1.9	53.4±2.9**	57.0±2.7**
Norepinephrine (nmol/liter)	1.53±0.13	1.84±0.25	1.66±0.30	1.37±0.19	1.53±0.28	1.44±0.26	1.20±0.20	1.25±0.08	1.19±0.13
Epinephrine (nmol/liter)	454±67	507±49	521±84	439±74	436±79	378±64	324±66	296±59	287±63
Glucose (mmol/liter)	5.5±0.1	5.0±0.2	5.0±0.2	5.5±0.1	11.5±0.4*§	10.5±0.6*§	5.3±0.2	5.4±0.2	5.3±0.2
Fructose (mmol/liter)	—	—	—	—	—	—	0.0±0.0	2.1±0.2*	2.0±0.2*

Entries are mean±SE for six (insulin, glucose) and five (fructose) subjects, respectively. Hemodynamic and MSNA measurements represent the average value of three 5-min periods of basal and the average of the last 5 min of the first and second hour of infusion. <sup>||</sup> MSNA given in units (bursts/min · mean burst amplitude).

\*  $P < 0.05$  vs. corresponding baseline period.

‡  $P < 0.05$  vs. insulin infusion.

§  $P < 0.01$  vs. insulin infusion.

||  $P < 0.001$  vs. insulin infusion.

### Data analysis

Mean arterial pressure was calculated as diastolic pressure plus  $\frac{1}{3}$  pulse pressure. Vascular resistance in the calf was calculated as mean arterial pressure in millimeters of mercury divided by blood flow in milliliters per minute per 100 ml tissue, and expressed in units. The 5 min of data from intraneural recordings of MSNA, calf blood flow, blood pressure, and heart rate collected every 15 min were averaged to a single value. Whole body glucose uptake, energy expenditure, and substrate oxidation were averaged for 30-min periods. Statistical analysis was performed using analysis of variance for repeated measures, and paired  $t$  tests with the Bonferroni adjustment for multiple comparisons. Correlation coefficients were calculated according to the method of least squares. A  $P$  value  $< 0.05$  was considered statistically significant. Data are given as mean±SE.

### Results

**Plasma glucose, fructose, and insulin.** Plasma glucose and insulin concentrations were comparable during the basal periods of all three protocols (Table I). During the hyperinsulinemic euglycemic clamp the coefficient of variation in plasma glucose concentration was 4.3%. During glucose infusion, glucose concentrations nearly doubled and reached a plateau by the end of the first hour of infusion. During fructose infusion, plasma glucose concentrations remained stable, whereas plasma fructose concentrations rose from undetectable levels to  $2.0 \pm 0.2$  mmol/liter by the end of the first hour of infusion, and remained unchanged thereafter (Table I).

As expected, the effects of the three experimental protocols on plasma insulin concentrations differed markedly (Table I, Fig. 1). During the euglycemic hyperinsulinemic clamp, plasma insulin concentrations rose more rapidly and to significantly ( $P < 0.05$ ) higher levels than during glucose infusion ( $376.2 \pm 30.0$  vs  $282.6 \pm 19.8$  pmol/liter). During fructose infusion, we observed only a minor, albeit statistically significant ( $P < 0.05$ ) rise in plasma insulin concentrations from  $37.2 \pm 1.9$  to  $57.0 \pm 2.7$  pmol/liter.

**MSNA, calf blood flow, and calf vascular resistance.** The 2-h euglycemic hyperinsulinemic clamp, which increased plasma insulin concentrations roughly tenfold above baseline, increased MSNA burst frequency by  $93 \pm 18\%$  and calf blood flow by  $47 \pm 14\%$ , whereas it decreased calf vascular resistance by  $19 \pm 4\%$  (Table I, Figs. 1 and 2). The 2-h infusion of exogenous glucose alone, which stimulated carbohydrate metabolism similarly as did insulin infusion, but increased plasma insulin concentrations only about half as much, also evoked twofold lesser ( $P < 0.05$ ) increases of both MSNA burst frequency ( $54 \pm 10\%$ ), and calf blood flow ( $20 \pm 6\%$ ) (Table I, Figs. 1 and 2). The 2-h infusion of fructose, which stimulated carbohydrate oxidation rate even more than did insulin/glucose or glucose infusion, but had only a minor effect on plasma insulin concentrations, had no effect on MSNA, calf blood flow, or calf vascular resistance (Table I, Figs. 1 and 2). The latter finding is not caused by a nonspecific impairment of sympathetic outflow by fructose infusion, since sympathetic responses to a Valsalva maneuver were not altered by fructose infusion; peak sympathetic responses were  $69 \pm 8$  bursts/min during insulin/glucose infusion, and  $72 \pm 5$  bursts/min during fructose infusion ( $P > 0.1$ ).

During insulin/glucose infusion, not only the magnitude of the MSNA and calf blood flow responses was markedly greater, but also the latency in the onset of these responses was markedly shorter than during glucose infusion alone. MSNA and calf blood flow had increased significantly ( $P < 0.05$ ) above baseline 45 min after the start of insulin/glucose infusion, but only 90 min after the start of glucose infusion (Fig. 1).

During both insulin/glucose infusion and glucose infusion alone, there was a positive correlation between plasma insulin concentration and MSNA, calf blood flow, and carbohydrate oxidation. During insulin/glucose infusion, the  $r$  values for the correlation between insulin and MSNA, insulin and calf blood flow, and insulin and carbohydrate oxidation were 0.57 ( $P = 0.003$ ), 0.42 ( $P = 0.02$ ), and 0.57 ( $P = 0.001$ ), respectively;

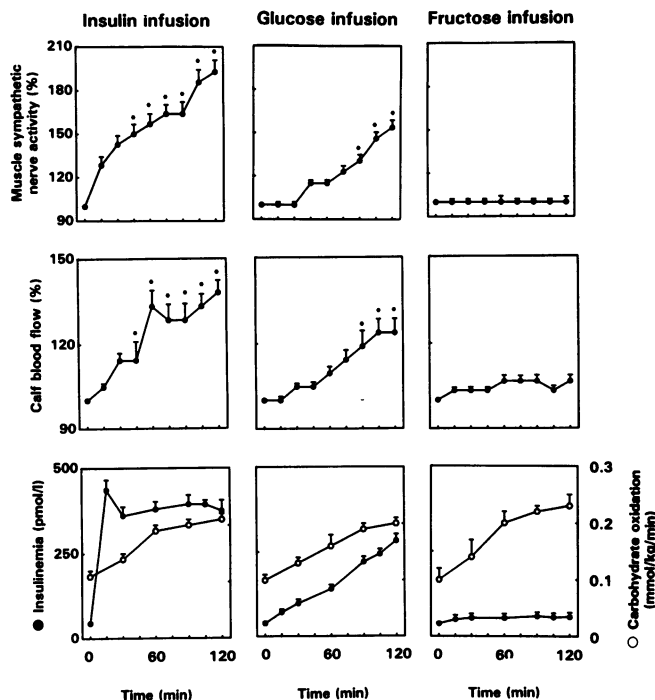


Figure 1. Line graphs showing the effects on plasma insulin concentration, carbohydrate oxidation rate, calf blood flow, and MSNA, of 2-h infusions of insulin/glucose (euglycemic hyperinsulinemic clamp), glucose, or fructose. Data represent mean  $\pm$  SE for six (insulin/glucose, glucose), and five (fructose) subjects, respectively. \*  $P < 0.05$  vs. corresponding basal value. For comparable stimulation of carbohydrate oxidation, insulin/glucose infusion, that resulted in a twofold greater increase in plasma insulin concentrations than did glucose infusion alone, also evoked twofold greater increases in MSNA and calf blood flow. Fructose infusion that increased carbohydrate oxidation similarly, but had only minor effects on insulinemia, did not stimulate either MSNA or calf blood flow.

during glucose infusion alone, the corresponding  $r$  values were 0.68 ( $P = 0.0002$ ), 0.56 ( $P = 0.001$ ), and 0.73 ( $P = 0.0001$ ), respectively.

**Plasma catecholamines and potassium.** During none of the three experimental protocols did plasma norepinephrine or epinephrine levels change significantly (Table I).

Serum potassium remained within the normal range during all three protocols. Plasma potassium concentrations at the end of the 2-h infusions of insulin/glucose, glucose, and fructose were  $3.6 \pm 0.1$ ,  $3.9 \pm 0.1$ , and  $3.9 \pm 0.1$  mmol/liter, respectively.

**Blood pressure and heart rate.** In all three studies, systolic, diastolic, and mean arterial blood pressure remained unchanged throughout the protocol. In contrast, there was a small but significant ( $P < 0.05$ ) increase in heart rate during the second hour in all three protocols (Table I).

**Carbohydrate metabolism and energy expenditure.** In all three studies, carbohydrate oxidation increased similarly (Table II, Fig. 1) to attain rates twofold above baseline during the last 30 min of infusion. To examine in more detail the kinetics of carbohydrate oxidation during the first 30 min of infusion, carbohydrate oxidation rates for this period were calculated using 15-min (rather than 30-min) time intervals. The increases in carbohydrate oxidation during the first 15 min of insulin/glucose and glucose infusion were  $0.2 \pm 0.7$ , and

$0.5 \pm 1.4$   $\mu\text{mol/kg}$  per min, respectively; during the second 15 min of infusion, increases were  $2.7 \pm 0.7$ , and  $1.7 \pm 0.8$   $\mu\text{mol/kg}$  per min, respectively ( $P > 0.1$ , insulin/glucose vs. glucose infusion alone). These data indicate that during insulin/glucose infusion, carbohydrate oxidation did not increase more rapidly than during glucose infusion alone.

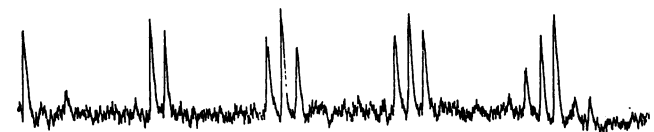
During fructose infusion, glucose oxidation as calculated from isotopic enrichments of breath  $^{13}\text{CO}_2$  and plasma [ $^{13}\text{C}$ ]-glucose (protocol 4) increased by  $22 \pm 7\%$  (Table III). This finding indicates that during fructose infusion, part of the increase in carbohydrate oxidation measured by indirect calorimetry is caused by stimulation of glucose oxidation.

Energy expenditure also increased comparably by  $\sim 10\%$  above baseline during the last 30 min of either the hyperinsulinemic clamp or glucose or fructose infusions. However, the time course of the increase in energy expenditure was different: during the hyperinsulinemic clamp, the major part of the increase occurred already during the first hour of infusion,

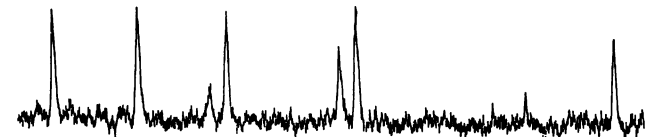
#### Insulin infusion



#### Glucose infusion



#### Fructose infusion



— 15 sec —

Figure 2. Segments of illustrative recordings of MSNA from the same subject obtained during the last 5 min of 2-h infusions of each, insulin (euglycemic clamp), glucose, and fructose. On these mean voltage displays of MSNA, each peak represents a spontaneous burst of sympathetic discharge. For comparable rates of carbohydrate oxidation, insulin/glucose infusion, which in this subject increased plasma insulin concentration by 373 pmol/liter above baseline, evoked a much greater increase in MSNA than did glucose infusion alone, which increased plasma insulin concentration only by 253 pmol/liter. The recording obtained during the last 5 min of fructose infusion, which had only a minor effect on plasma insulin concentration ( $+18$  pmol above baseline), was superposable to the recording obtained during the basal period (not shown).

Table II. Responses to 2-h Infusion of Insulin (Euglycemic Clamp), Glucose, or Fructose

	Insulin infusion			Glucose infusion			Fructose infusion		
	Basal	30-60 min	90-120 min	Basal	30-60 min	90-120 min	Basal	30-60 min	90-120 min
Energy expenditure (kJ/min)	4.46±0.12	4.88±0.15*	5.03±0.18*	4.46±0.08	4.57±0.21	4.94±0.17*	4.57±0.16	4.86±0.20*	5.09±0.31*
[kcal/min]	[1.06±0.03]	[1.17±0.04*]	[1.20±0.04*]	[1.07±0.02]	[1.09±0.05]	[1.18±0.04*]	[1.09±0.03]	[1.16±0.05*]	[1.22±0.07*]
Carbohydrate oxidation (μmol/kg per min)	11±1	19±1*	21±1*	10±1	16±2*	20±1*	10±2	20±2*	23±2*
[mg/kg per min]	[1.9±0.2]	[3.4±0.2*]	[3.9±0.2*]	[1.7±0.2]	[2.8±0.3*]	[3.7±0.2*]	[1.8±0.4]	[3.5±0.4*]	[4.1±0.3*]
Total glucose uptake (μmol/kg per min)	—	40±3	44±3	—	28±1	49±10	—	—	—
[mg/kg per min]	—	[7.3±0.5]	[7.7±0.4]	—	[5.0±0.6‡]	[8.8±1.1]	—	—	—
Nonoxidative glucose disposal (μmol/kg per min)	—	21±4	22±8	—	6±1‡	29±5	—	—	—
[mg/kg per min]	—	[3.9±0.6]	[4.0±0.6]	—	[1.0±0.1‡]	[5.2±1.0]	—	—	—

Entries are mean±SE for six (insulin, glucose) and five (fructose) subjects, respectively. Substrate oxidation measurements represent the average value of the last 30 min of control, and the average of the last 30 min of the first and second hour of infusion.

\*  $P < 0.05$  vs. corresponding basal period. ‡  $P < 0.05$  vs. insulin infusion.

whereas during glucose infusion, energy expenditure increased mostly during the second hour of infusion (Table II).

## Discussion

The respective roles played by hyperinsulinemia per se, and hyperinsulinemia-induced stimulation of carbohydrate metabolism, in the regulation of sympathetic outflow and blood flow in skeletal muscle have been difficult to elucidate using incremental insulin infusions (6), and indirect indices of sympathetic nerve activity (26). The ability to combine direct measurements of sympathetic nerve discharge and plethysmographic measurements of blood flow in skeletal muscle with calorimetric determinations of carbohydrate oxidation, during experimental interventions in which insulinemia was altered independently of carbohydrate metabolism, provided the opportunity to develop a straightforward approach to this problem. The major new finding is that both stimulation of sympathetic discharge and vasodilation in skeletal muscle are related to hyperinsulinemia rather than to carbohydrate metabolism. For comparable rates of carbohydrate metabolism and oxidation, insulin/glucose infusion, that resulted in a twofold greater increase in plasma insulin concentrations than did glu-

cose infusion alone, also evoked twofold greater increases in MSNA and in calf blood flow. Furthermore, fructose infusion that increased carbohydrate oxidation to a rate similar to that observed during insulin/glucose infusion, but had only minor effects on insulinemia, did not stimulate either MSNA or calf blood flow. These findings provide evidence in humans that hyperinsulinemia per se is the main mechanism which triggers both sympathetic activation and vasodilation in a major insulin-sensitive tissue, skeletal muscle.

This interpretation is predicated upon the assumption that the stimulation of carbohydrate metabolism indeed was comparable in the present experimental protocols. Plasma insulin concentrations such as those observed during the present insulin/glucose or glucose infusion studies, are known to completely suppress hepatic glucose production (27); thus, under these conditions, total glucose uptake is equal to exogenous glucose infusion. During fructose infusion at the rates used in the present experiments, endogenous glucose production is not suppressed (19) because fructose uptake and subsequent stimulation of carbohydrate metabolism occurs in the absence of hyperinsulinemia (28). Therefore, total carbohydrate uptake may have been slightly higher during fructose infusion than during insulin/glucose or glucose infusion. Thus, in the present experiments, similar stimulation of carbohydrate metabo-

Table III. Glucose Oxidation during Fructose Infusion

	Breath <sup>13</sup> CO <sub>2</sub> atom % excess	Plasma [ <sup>13</sup> C]glucose atom % excess	VCO <sub>2</sub> ml/min	Glucose oxidation μmol/kg per min	Carbohydrate oxidation μmol/kg per min
Basal	0.0133±0.0004	0.0442±0.0022	198±5	7.55±0.65	7.13±1.63
Fructose infusion	0.0129±0.0006	0.0434±0.0024	244±8*	9.20±0.96*	16.42±2.45*

Entries are mean±SE for six subjects. For both measurement periods breath <sup>13</sup>CO<sub>2</sub> and plasma [<sup>13</sup>C]glucose values represent the average of three duplicate determinations. VCO<sub>2</sub> values represent the average of 30 min by min determinations. Glucose oxidation was calculated as  $\{(\dot{V}CO_2 \times ^{13}CO_2)/([^{13}C]glucose \times 0.8)\}/\{0.134 \times wt(kg)\}$ .

Carbohydrate oxidation measurements were determined using indirect calorimetry, and represent the average value of the last 30 min of control, and the last 30 min of fructose infusion, respectively.

\*  $P < 0.01$  vs. corresponding basal period.

lism was associated with strikingly different degrees of sympathetic activation and vasodilation in skeletal muscle.

There is abundant evidence that estimates of carbohydrate oxidation obtained by indirect calorimetry, and adjusted for changes in urea pool size, are highly reliable and reproducible (17, 18). In the present studies, carbohydrate oxidation increased similarly in all three protocols to attain rates twofold above basal during the last 30 min of infusion. Kinetics of the increases in carbohydrate oxidation during the initial phase (i.e., the first 30 min) of insulin/glucose infusion and glucose infusion alone also were similar, as shown by analysis of calorimetric determinations of carbohydrate oxidation using 15-min time intervals, whereas kinetics of the increases in MSNA and blood flow differed markedly during the initial phase of these two experimental protocols.

Finally, several lines of evidence indicate that, even though during fructose infusion the initial step of fructose metabolism is mainly hepatic, skeletal muscle accounts for an important proportion of fructose disposal (29–31). During fructose infusion in humans, muscle glycogen content in skeletal muscle biopsies increases similarly as during glucose infusion (29, 30). This increase has been attributed in part to direct fructose uptake in skeletal muscle, as demonstrated both by determination of arteriovenous fructose concentrations in human forearm tissue (32), and by direct measurements of splanchnic and renal substrate exchange during fructose infusion in humans, showing that 30–40% of fructose disposal could not be accounted for by splanchnic or renal tissue and presumably was largely taking place in skeletal muscle (31). In addition, uptake of lactate and glucose, resulting from fructose-induced stimulation of endogenous glucose production (31) and splanchnic and renal lactate release (31), may also contribute to stimulation of muscle metabolism. In this regard, our data provide direct evidence that fructose infusion in the amounts used in the present experiments is accompanied by a 22% increase in glucose oxidation. Thus, even though some of the precise steps involved in fructose metabolism are still unknown, there is agreement that a substantial part of this metabolism is taking place in skeletal muscle (33). In the present studies, such stimulation of muscle carbohydrate metabolism during fructose infusion was not associated with sympathetic activation and vasodilation in skeletal muscle.

By comparing sympathetic and vasodilatory responses at the same metabolic endpoints in the same subjects, we were able to dissociate the effects of carbohydrate uptake, oxidation, and storage, from the effects of hyperinsulinemia on the regulation of sympathetic outflow and blood flow in skeletal muscle. We found that during insulin/glucose infusion, MSNA and calf blood flow increased markedly already during the first hour of infusion. In contrast, during glucose infusion, nerve traffic and calf blood flow did not increase during the first hour of infusion but only during the second hour of infusion, when plasma insulin concentrations had more than doubled as compared to the first hour of infusion. The present findings suggest that this delayed pattern of sympathetic activation and stimulation of blood flow during glucose infusion is related to the delayed increase in plasma insulin concentrations observed under these conditions. The studies using fructose infusion strengthen this interpretation by demonstrating that the stimulation of MSNA and muscle blood flow during insulin/glucose and glucose infusion is not an artifact caused, for example, by

increasing discomfort of the subjects resulting in nonspecific sympathetic activation.

The present data represent the first demonstration in humans that not only insulin/glucose infusion, but also glucose infusion alone, stimulates sympathetic outflow to skeletal muscle. This conclusion differs from that of two previous studies in which intravenous administration of glucose had no detectable effect on sympathetic activity (5, 26). However, the conclusions of one study were based upon the absence of a detectable effect of glucose infusion on plasma norepinephrine levels (5, 26), a relatively insensitive index of sympathetic discharge (34–36). In the present study, infusion of exogenous glucose in amounts comparable to those used in this earlier study (26) also did not increase plasma norepinephrine levels significantly, but evoked highly significant increases in sympathetic discharge targeted at skeletal muscle. The other study used intravenous bolus injections of glucose, which caused only very short lasting increases in plasma insulin concentrations (5) that may have been insufficient to increase sympathetic outflow. Furthermore, these bolus injections resulted in acute volume expansion, which, in turn, could have reflexly decreased sympathetic outflow by stimulating inhibitory cardiopulmonary afferents (37), thereby masking a potential sympathoexcitatory effect of such short lasting glucose-induced hyperinsulinemia.

Our interpretation, that during glucose infusion hyperinsulinemia is the primary stimulus not only for sympathetic activation, but also for vasodilation in skeletal muscle, is strengthened by the recent observation in humans that somatostatin administration during glucose infusion suppresses not only glucose-induced stimulation of endogenous insulin secretion, but vasodilation in skeletal muscle as well (38).

This insulin-induced vasodilation may be of physiological importance. First, there is increasing evidence that stimulation of blood flow to insulin-sensitive tissues during insulin/glucose and glucose infusion may be an important determinant of the rate of *in vivo* glucose uptake (10, 11, 38). Second, a balance between insulin's opposing sympathoexcitatory pressor and vasodilatory depressor effects may offer a potential explanation for the present, and earlier (6, 7, 26) observations, that in lean healthy subjects acute hyperinsulinemia at high physiologic concentrations does not raise arterial pressure.

The present experiments in humans do not elucidate the underlying mechanisms of insulin-induced sympathetic activation and vasodilation. The present observation during insulin/glucose infusion, that MSNA and calf blood flow did not show a peak increase during the initial peak increase of plasma insulin concentration, and showed a further increase when plasma insulin levels were at steady state, could suggest that insulin may have to reach the interstitial space to exert its excitatory effects. In this regard, findings during insulin infusion in dogs, showed a time lag between the steep initial increase in plasma insulin concentration and the appearance of insulin in the lymph, an indicator of interstitial insulin concentration, and a further increase in lymph insulin concentrations when plasma insulin levels were decreasing or at steady state (39). One potential mechanism by which insulin may exert its sympathoexcitatory effects, is that insulin-induced vasodilation in skeletal muscle may lead to slight decreases in arterial pressure and baroreflex mediated increases in MSNA. In this regard, an earlier study found a small but significant decrease in diastolic

blood pressure during insulin/glucose infusion in humans (6). Alternatively, a central neural action of insulin may also contribute to stimulation of sympathetic outflow in this setting (8, 40–43).

Insulin/glucose or glucose infusion in humans not only stimulates sympathetic activity and blood flow in skeletal muscle, but also increases energy expenditure by stimulating obligatory and facultative thermogenesis (44). The latter has been thought to be sympathetically mediated because it can be suppressed by propranolol (44, 45). Here, we provide direct evidence that insulin/glucose and glucose infusion indeed stimulate MSNA that, in turn, may contribute to the thermogenesis observed under these conditions. However, fructose infusion that increased thermogenesis even more than did insulin/glucose or glucose infusion (45), did not stimulate MSNA, and did not have any detectable effect on plasma norepinephrine levels. This is an unexpected finding, since it has been thought that fructose-induced thermogenesis also is sympathetically mediated, because it can be suppressed by beta-blocker administration (19, 46). However, regional sympathetic responses can be highly differentiated (47), and the present observations based upon direct measurements of sympathetic nerve action potentials targeted specifically at skeletal muscle, do not exclude the possibility of a fructose-induced stimulation of sympathetic outflow targeted at other tissues such as the liver which, in turn, may have contributed to thermogenesis.

In conclusion, these experimental findings in healthy humans suggest that hyperinsulinemia per se, rather than hyperinsulinemia-induced stimulation of carbohydrate metabolism, is the primary stimulus for both sympathetic activation and vasodilation in a major insulin-sensitive tissue, skeletal muscle. This vascular effect of insulin may be of physiological importance, as recent evidence indicates that insulin resistance is associated with attenuated hyperinsulinemia-induced vasodilation in skeletal muscle (10, 11).

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