Blunted sympathetic neural response to oral glucose in obese subjects with the insulin-resistant metabolic syndrome

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ABSTRACT
Background: Glucose ingestion stimulates sympathetic nervous system (SNS) activity in lean subjects, whereas blunted responses have been reported in the obese.

Objective: The objective was to investigate the impact of insulin resistance on the SNS response to oral glucose.

Design: Nineteen insulin-resistant (IR) and 12 insulin-sensitive (IS) obese subjects with the metabolic syndrome and matched for age, sex, and blood pressure participated. Simultaneous measurements of muscle sympathetic nerve activity (MSNA) by microneurography, whole-body norepinephrine spillover rate, cardiac baroreflex sensitivity (BRS), calf blood flow, and arterial blood pressure were made at baseline and 30, 60, 90, and 120 min after a 75-g glucose load.

Results: IR subjects had a higher insulin area under the curve from 0 to 120 min (AUC0–120: 13,468 ± 677 compared with 6399 ± 612 μU/L · min; P < 0.001), glucose AUC0–120 (P < 0.05), and resting MSNA (41 ± 3 compared with 31 ± 3 bursts/min; P = 0.03) than did IS subjects. MSNA and the norepinephrine spillover rate increased from baseline (29 ± 7% and 40 ± 13%, respectively; P ≤ 0.001 for both) in IS subjects after the glucose load. In contrast, there was a blunted and delayed sympathetic response in IR subjects. Cardiac BRS and diastolic blood pressure decreased, whereas calf blood flow increased after the glucose load and by a similar magnitude in both groups (P < 0.01). Body mass index, abdominal fat, and insulin AUC0–120 were independent (inverse) predictors of the SNS response.

Conclusions: IR subjects with the metabolic syndrome have a blunted SNS response to oral glucose compared with IS subjects and the metabolic syndrome, which is related to central adiposity and the insulin response but not to differences in skeletal muscle vasodilation or BRS.


INTRODUCTION

It has been known for >25 y that food intake, particularly carbohydrate, increases the activity of the sympathetic nervous system (SNS) in healthy humans (1–6). A strong and sustained increase in muscle sympathetic nerve activity (MSNA), recorded by microneurography, and a parallel rise in plasma norepinephrine concentrations has been shown after oral glucose loading by several authors (1, 3, 4). The increased sympathetic outflow after glucose ingestion has been interpreted as a combined effect of insulin and the entrance of a nutrient into the gastrointestinal tract (3, 6). Fructose, protein, and fat—which do not stimulate endogenous insulin secretion—have either no effect or a blunted effect on MSNA (3, 6, 7). The presumed physiologic role of the postprandial increase in MSNA is to induce compensatory peripheral vasoconstriction for maintenance of blood pressure after splanchnic vasodilatation (6). In addition, norepinephrine stimulates the metabolic rate, so the increase in SNS activity after meals contributes to the postprandial rise in energy expenditure (2). Data suggest that obesity and the insulin-resistant state are accompanied by impaired sympathetic neural responsiveness to physiologic hyperinsulinemia, glucose ingestion, and changing energy states. Vollenweider et al (8) showed, in lean young subjects, that MSNA increased by 94% in response to euglycemic hyperinsulinemia, whereas in age-matched obese subjects the increase was only 9%. Moreover, the MSNA responses to oral glucose are diminished in elderly insulin-resistant subjects (4) and in insulin-resistant Pima Indians (9), and short-term under- and overfeeding is accompanied by a blunted sympathetic response in obese subjects (10).

The metabolic syndrome (MetS) is a multifaceted condition associated with excess cardiovascular disease risk (11). Several indexes of SNS activity, such as urinary norepinephrine excretion (12), norepinephrine spillover from adrenergic nerve terminals (13), and MSNA at rest are known to be elevated in subjects with the MetS, even in the absence of hypertension (14, 15) and may potentially contribute to both the pathogenesis (16) and complications of this condition (17). Insulin resistance has been proposed as the key underlying pathophysiologic mechanism in the MetS, and several lines of evidence suggest that insulin has sympathostimulating effects (18). This effect of insulin is mediated through the central nervous system (CNS), either as

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a reflex response to vasodilation or as a direct effect of insulin on forebrain areas regulating sympathetic outflow (18). It is of note, however, that the role of hyperinsulinemia and elevated SNS activity as mediators of the relation between obesity and blood pressure can differ between different ethnic groups. Pima Indians, for example, have a high prevalence of obesity and hyperinsulinemia, but a low resting MSNA compared with weight-matched whites (19).

To date, no study has examined the sympathetic response to glucose loading in MetS subjects and whether insulin resistance modifies this response. Accordingly, the purpose of this study was to compare the sympathetic neural response to a standard oral-glucose-tolerance test (OGTT) in a group of obese subjects with the MetS, stratified on the basis of their insulin sensitivity. To accomplish this aim, we simultaneously measured arterial glucose, insulin, MSNA, whole-body norepinephrine spillover, spontaneous cardiac baroreflex sensitivity, blood pressure, heart rate during a 2-h OGTT. We hypothesized that insulin-resistant subjects would have increased insulin and blunted SNS responses to a glucose load.

SUBJECTS AND METHODS

Subjects

The study group comprised 31 white middle-aged subjects (17 men and 14 postmenopausal women) who were selected to participate in a weight-loss and exercise-intervention trial. To be eligible, they had to have central obesity (waist circumference ≥ 102 cm in men and ≥ 88 cm in women) and ≥2 MetS criteria as per the updated National Cholesterol Education Adult Treatment Panel III criteria for the MetS (20). Specifically, these criteria include elevated triglycerides (≥1.7 mmol/L), low HDL cholesterol (<1.03 mmol/L in men and <1.3 mmol/L in women), elevated fasting glucose (≥5.6 mmol/L), and elevated blood pressure (≥130 mm Hg systolic or ≥85 mm Hg diastolic). None of the subjects smoked. Exclusion criteria included type 2 diabetes (fasting glucose ≥7 mmol/L or drug use to treat elevated glucose concentrations), use of drugs known to affect the measured variables, and a history of secondary hypertension or cardiovascular, cerebrovascular, renal, liver, or thyroid disease. Participants currently treated for hypertension (n = 2) or hypercholesterolemia (n = 1) were studied after antihypertensive or cholesterol-lowering medications had been discontinued for ≥4 wk. At screening, all subjects underwent a fitness assessment, during which maximal oxygen consumption (VO₂max) was measured with an incremental cycle ergometry protocol (Quark b® Cosmed, Rome, Italy). Body composition was determined by conducting a dual-energy X-ray absorptiometry (DXA) scan (GE-Lunar Prodigy Advance PA + 130510; GE Medical Systems Lunar, Madison, WI). Dietary intake was evaluated by 4-d prospective diet records with the use of the Australian food-composition tables (Xyris Software; FoodWorks, Highgate Hill, Australia). Adiposity and fat mass distribution were calculated on the basis of DXA measurements of total body, trunk, and abdominal fat (measured in the abdominal cut at the L1-L4 level) and by waist circumference and body mass index (BMI; in kg/m²). Whole-body insulin sensitivity was calculated from OGTT variables according to the formula of Matsuda and DeFronzo (21):

Whole-body insulin sensitivity = \(10,000 / \sqrt{\text{FPI} \times \text{FPG}} \times \text{mean PG} \times \text{mean PI} \)

where FPI is fasting plasma insulin (in mU/L), FPG is fasting plasma glucose (in mg/dL), and mean values are the average of concentrations at times 0, 30, 60, 90, and 120 min. This index represents a composite of hepatic and peripheral tissue insulin sensitivity and considers insulin sensitivity in the basal state (FPG × FPI) and after the ingestion of a glucose load (mean plasma insulin × mean plasma glucose). The index was previously validated against the euglycemic insulin clamp and is highly correlated with the rate of whole-body glucose disposal during the clamp (r = 0.73 and r = 0.78; P < 0.0001) (21, 22). Subjects were stratified into insulin-sensitive and insulin-resistant groups on the basis of cutoffs of >2.1 and ≤2.1, respectively. These cutoffs were chosen because they correspond to an insulin area under the curve (AUCₐ₋₁₂₀) of <10,000 and of ≥10,000 mU/L · min, respectively, and because they represent values in populations with normal and impaired glucose tolerance (22). All studies were carried out in the Heart Centre of the Alfred Hospital. The study protocol was approved by the institutional ethics committee and written informed consent was obtained from each participant.

Experimental protocol

Subjects attended the clinic at 0800, after having fasted for 12 h, abstained from caffeine for 18 h, and abstained from alcohol and strenuous exercise for 36 h. The experiments were performed in a quiet room with an ambient temperature of 22 °C while the subjects were lying in a supine position. The subjects voided before the study began. A 20G venous cannula was inserted into an antecubital vein for infusion of tritiated norepinephrine, and an arterial cannula was placed into the brachial artery for blood sampling. Thereafter, microneurography was initiated.

Sympathetic nervous system activity

Recordings of multunit postganglionic MSNA were made from a muscle fascicle of the right peroneal nerve at the fibular head, as previously described (23). The tungsten microelectrode (FHC, Bowdoinham, ME) was adjusted until satisfactory spontaneous MSNA was observed. The nerve signal was amplified (×50,000), filtered (bandpass: 700–2000 Hz), and integrated. Intravascular blood pressure, echocardiogram, respiration, and MSNA were digitized with a sampling frequency of 1000 Hz (PowerLab recording system, model ML 785/8SP; ADI Instruments). Resting measurements were recorded over a 15-min period. The subjects then ingested 75 g glucose (Glucaid; Fronine Pty, Ltd, Australia) and, for the next 2 h, MSNA was recorded for 5 min at 30, 60, 90 and 120 min. Sympathetic bursts were counted manually and expressed as burst frequency (bursts/min) and burst incidence (bursts/100 heart beats). The amplitude of the largest burst during the analyzed period of the recording was defined as 100, and all other bursts were expressed as a percentage of the largest one. Total MSNA was calculated by multiplying the mean burst amplitude per minute by burst rate, expressed as units per minute and units per 100 heart beats.
Whole-body sympathetic activity was assessed by measuring the apparent rate of appearance of endogenous norepinephrine in plasma (norepinephrine spillover rate) with the isotope dilution technique (24). Plasma concentrations of norepinephrine depend on 2 concurrent processes: spillover of norepinephrine into plasma after release from sympathetic nerves and subsequent removal from the circulation. The plasma concentrations of $[^3\text{H}]$norepinephrine during its constant infusion to steady state were used to estimate the rate of clearance of norepinephrine from arterial blood according to the following equation:

\[
\text{Clearance of norepinephrine} = \frac{[\text{H}]\text{norepinephrine infusion rate (dpm/min)}}{\text{plasma } [\text{H}]\text{norepinephrine concentration (dpm/mL)}}
\]

(2)

The unit of clearance measurement (L/min) signifies the given volume of plasma that is cleared of norepinephrine per minute. The product of the plasma clearance of norepinephrine and arterial concentrations of endogenous norepinephrine yields a value for the plasma spillover of norepinephrine (expressed as ng/min). After a priming bolus of 6 µCi of 1-[ring-2,5,6-[3H]norepinephrine (specific activity: 10–30 µCi/mmol; Perkin-Elmer, Norwalk, CT), an infusion was started at 0.07 µCi · m² · min⁻¹. To ensure that steady state concentrations of infusate were attained, baseline arterial blood samples (time 0) were taken ≥30 min after the infusion began. Arterial samples were obtained 30, 60, 90, and 120 min after the glucose load.

**Spontaneous cardiac baroreflex function**

Baroreflex sensitivity was assessed with the sequence method by using BaroCor software (AtCor Medical, West Ryde, Australia) as previously described (25). The slope between cardiac interval and systolic blood pressure was calculated for each validated sequence, and an average slope was calculated for each recording. Resting measurements were recorded over 15 min and averaged. Measurements were made 30, 60, 90, and 120 min after the glucose load and were calculated as the average recording over 20 min.

**Calf vascular measurements**

Calf arterial blood flow was measured in the left leg with automated venous occlusion plethysmography equipment (DE Hokansen, Bellevue, WA). During the measurements, an arterial occlusion cuff was attached around the thickest part of the calf. Ankle cuffs were inflated ≥60 s before measurements of flow to allow calf blood flow to stabilize. Strain gauges were attached around the thickness part of the calf. Throughout the experiment, the measured leg was supported 10 cm above heart level to empty the venous system. Calf vascular resistance (mL · 100 mL⁻¹ · min⁻¹ · mm Hg⁻¹) was calculated from mean arterial pressure divided by mean calf blood flow (mL · min⁻¹ · 100 mL⁻¹ tissue), obtained as the average of 12 measurements at times 0, 30, 60, 90, and 120 min.

**Laboratory measurements**

Plasma concentrations of neurochemicals were measured by HPLC with electrochemical detection. Intraassay CVs in our laboratory are 1.3% for norepinephrine and 2.3% for $[^3\text{H}]$norepinephrine; interassay CVs are 3.8% and 4.5%, respectively. Arterial plasma glucose was quantified by enzymatic methods with an Architect C18000 analyzer (Abbott Laboratories), and insulin and leptin were measured by radioimmunoassay (Linco Research Inc, MO). Plasma total cholesterol, HDL cholesterol, and triglycerides were measured with automated enzymatic methods, and plasma high sensitivity C-reactive protein (hsCRP) was measured by immunoturbidimetric assay.

**Statistical methods**

Data are expressed as means ± SEMs. Statistical analysis was performed by using SigmaStat software (version 2.03; SPSS Inc, Chicago, IL) for Windows. Insulin-sensitive and insulin-resistant subject demographics were compared with Student’s unpaired t tests. The courses of MSNA, norepinephrine kinetics, cardiac baroreflex sensitivity, calf blood flow, arterial blood pressure, and plasma glucose and insulin during the OGTT were analyzed by 2-factor repeated-measures analysis of variance; multiple pairwise comparisons were made with the Holm-Sidak test. Non-parametric data were log-transformed before analyses. The areas under the plasma concentration-time curve (AUC₀₋₁₂₀) were calculated by using the trapezoidal rule. Associations between variables were evaluated by Pearson’s and Spearman’s rank correlations, as appropriate. Forward stepwise regressions were carried out for those univariate correlations with a P value < 0.05. Statistical significance was set at a 2-sided P value < 0.05.

**RESULTS**

**Subject characteristics**

Clinical and metabolic characteristics of the participants are presented in Table 1. Compared with insulin-sensitive subjects, insulin-resistant subjects had significantly higher fasting plasma insulin, homeostasis model assessment of insulin resistance (HOMA-IR), and glucose AUC₀₋₁₂₀ and double the insulin AUC₀₋₁₂₀ value during the OGTT (Figure 1). Fasting glucose, fitness level, and plasma leptin did not differ significantly between the 2 groups. Dietary analysis showed that insulin-resistant subjects consumed significantly more total fat, saturated fat, and protein than did insulin-sensitive subjects (Table 1). BMI correlated significantly with insulin AUC₀₋₁₂₀ ($r = 0.37, P = 0.04$) and inversely with log insulin sensitivity index ($r = -0.36, P = 0.05$), whereas abdominal fat mass correlated with fasting insulin concentration ($r = 0.40, P = 0.03$) and HOMA-IR ($r = 0.44, P = 0.01$). Saturated fat consumption (% of energy) was associated with insulin AUC₀₋₁₂₀ ($r = 0.35, P = 0.05$) and inversely related to log insulin sensitivity index ($r = -0.38, P = 0.04$).

**Sympathetic nervous system activity**

Complete microneurographic recordings were available for all 31 subjects (19 insulin-resistant and 12 insulin-sensitive). Baseline resting MSNA was higher in insulin-resistant than in
TABLE 1
Characteristics of insulin-resistant and insulin-sensitive metabolic syndrome subjects

<table>
<thead>
<tr>
<th></th>
<th>Insulin resistant (n = 19)</th>
<th>Insulin sensitive (n = 12)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>55 ± 1</td>
<td>56 ± 1</td>
<td>—</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>10/9</td>
<td>7/5</td>
<td>—</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>34.2 ± 1.1</td>
<td>31.7 ± 0.9</td>
<td>—</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>108.6 ± 2.9</td>
<td>107.2 ± 2.7</td>
<td>—</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>2.1 ± 0.3</td>
<td>1.8 ± 0.4</td>
<td>—</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.24 ± 0.06</td>
<td>1.32 ± 0.07</td>
<td>—</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>5.9 ± 0.1</td>
<td>5.6 ± 0.2</td>
<td>—</td>
</tr>
<tr>
<td>Fasting insulin (mU/L)</td>
<td>21.1 ± 1.1</td>
<td>13.8 ± 0.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>5.5 ± 0.3</td>
<td>3.5 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2-h Glucose (mmol/L)</td>
<td>10.2 ± 0.4</td>
<td>9.5 ± 0.4</td>
<td>—</td>
</tr>
<tr>
<td>Glucose AUC0–120 (mmol/L · min)</td>
<td>1215 ± 34</td>
<td>1110 ± 33</td>
<td>0.047</td>
</tr>
<tr>
<td>Insulin AUC0–120 (mU/L · min)</td>
<td>13,468 ± 677</td>
<td>6,399 ± 612</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SI</td>
<td>1.62 ± 0.07</td>
<td>3.32 ± 0.41</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>20.3 ± 3.4</td>
<td>17.3 ± 4.6</td>
<td>—</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)*</td>
<td>135 ± 3</td>
<td>129 ± 5</td>
<td>—</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)*</td>
<td>77 ± 2</td>
<td>74 ± 3</td>
<td>—</td>
</tr>
<tr>
<td>VO₂max/FFM (mL · min⁻¹ · kg⁻¹)</td>
<td>28.3 ± 1.0</td>
<td>30.5 ± 1.6</td>
<td>—</td>
</tr>
<tr>
<td>Family history of type 2 diabetes (n)</td>
<td>4</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>Energy (kcal/d)</td>
<td>2090 ± 141</td>
<td>1902 ± 113</td>
<td>—</td>
</tr>
<tr>
<td>Fat (% of energy)</td>
<td>35.4 ± 1.1</td>
<td>30.9 ± 1.3</td>
<td>0.01</td>
</tr>
<tr>
<td>Saturated (% of energy)</td>
<td>15.5 ± 0.6</td>
<td>12.8 ± 0.8</td>
<td>0.009</td>
</tr>
<tr>
<td>Polyunsaturated (% of energy)</td>
<td>5.5 ± 0.31</td>
<td>5.3 ± 0.4</td>
<td>—</td>
</tr>
<tr>
<td>Monounsaturated (% of energy)</td>
<td>14.3 ± 0.6</td>
<td>12.8 ± 0.7</td>
<td>—</td>
</tr>
<tr>
<td>Carbohydrate (% of energy)</td>
<td>45.1 ± 1.4</td>
<td>44.5 ± 1.9</td>
<td>—</td>
</tr>
<tr>
<td>Protein (% of energy)</td>
<td>17.2 ± 0.8</td>
<td>20.1 ± 0.8</td>
<td>0.02</td>
</tr>
<tr>
<td>Alcohol (% of energy)</td>
<td>4.4 ± 1.3</td>
<td>4.5 ± 1.5</td>
<td>—</td>
</tr>
</tbody>
</table>

* AUC0–120 area under the curve at 0–120 min during the oral-glucose-tolerance test determined by using the trapezoidal rule; FFM, fat-free mass; HOMA-IR, homeostasis model assessment of insulin resistance; SI, whole-body insulin sensitivity index derived by using the method of Matsuda and DeFronzo (21); VO₂max, oxygen consumption at maximal effort during an incremental cycle ergometry protocol.

** Groups were compared by Student’s unpaired t test.

† Mean ± SEM (all such values).

‡ Blood pressure was measured while the subjects were supine with a Dinamap monitor (average of 5 readings; model 1846SX; Critikon Inc, Tampa, FL).

§ Family history refers to a parent or sibling with type 2 diabetes.

The sympathetic response was blunted and delayed in insulin-resistant subjects, which reached statistical significance only for total MSNA (units/min) at 90 min. The percentage increase in burst frequency from baseline was significantly greater in insulin-sensitive than in insulin-resistant subjects at 60 min (29.1 ± 6.8% compared with 6.0 ± 5.2%; P = 0.01) and at 120 min (29.5 ± 6.1% compared with 8.8 ± 5.6%; P = 0.02 respectively). Corresponding values for burst incidence were 18.1 ± 6.0% compared with 30.9 ± 6.8% for the time effect and P = 0.12 for the group effect. *P < 0.05 and **P < 0.01 compared with IS subjects.

FIGURE 1. Mean (± SEM) arterial plasma glucose (A) and insulin (B) concentrations in insulin-sensitive (IS; n = 12) and insulin-resistant (IR; n = 19) subjects after oral intake of 75 g glucose. Data were analyzed by 2-factor repeated-measures ANOVA using the Holm-Sidak test for pairwise comparisons. Glucose: P < 0.001 for the time effect and P = 0.04 for the group effect; *P < 0.05 and **P < 0.01 compared with IS subjects. Insulin: P < 0.001 for the time effect, P < 0.001 for the group effect, and P = 0.02 for the time-by-group interaction; ***P < 0.001 compared with IS subjects.
compared with 1.1 ± 5.8% at 60 min (P = 0.06) and 16.3 ± 7.0% compared with −0.9 ± 5.6% at 120 min (P = 0.07), respectively. Median burst amplitude did not change significantly during the OGTT in either group.

There were significant inverse correlations between anthropometric measures (BMI, trunk fat, and abdominal fat mass) and the increase in MSNA to glucose (Table 2), which indicated that central adiposity was associated with a blunted MSNA response. In concordance with this finding, higher insulin responses to glucose (change in insulin and insulin AUC_{0–120}) were also associated with a blunted MSNA response, whereas the whole-body insulin sensitivity index correlated positively with the MSNA response (Table 2). Saturated fat intake was also inversely associated with the MSNA response.

Whole-body norepinephrine kinetic data were available for only 30 subjects, because we had difficulty placing an arterial line in 1 participant (Figure 3). At baseline, endogenous norepinephrine concentrations were similar in insulin-resistant and insulin-sensitive subjects (223 ± 32 and 224 ± 36 pg/mL, respectively). However, norepinephrine plasma clearance was significantly higher in insulin-sensitive subjects (2.4 ± 0.3 compared with 1.6 ± 0.1 L/min; P < 0.01), but there was no significant difference in the calculated norepinephrine spillover rate (543 ± 92 compared with 320 ± 41 ng/min; P = 0.11). After the glucose load, the rate of norepinephrine spillover to plasma increased significantly at 30 min by 40 ± 13% and at 120 min by 32 ± 8% (P < 0.01 compared with baseline for both) in insulin-sensitive subjects. In contrast, the response was blunted and delayed, reaching statistical significance only at 120 min in insulin-resistant subjects. The plasma clearance rate of norepinephrine did not change in response to the glucose load; however, the clearance was significantly greater in insulin-sensitive subjects at all time points (Figure 3C).

The increase in norepinephrine spillover at 30 min correlated positively with fitness level and inversely with baseline triglyceride concentration (P < 0.01 for both; Table 3). There was also an inverse relation with insulin AUC_{0–120}; however, it was not statistically significant (r = −0.30, NS). Baseline hsCRP concentrations were inversely correlated with the norepinephrine spillover response at 90 min.

Stepwise regression analysis showed that abdominal fat mass, BMI, insulin AUC_{0–120}, and dietary saturated fat consumption were the strongest independent predictors (inverse) of the MSNA response, accounting for between 15% and 40% of the variance (Table 4). Fitness (V_{O2max}/fat-free mass) and plasma hsCRP (inverse) were the strongest independent predictors of the norepinephrine spillover response, accounting for 22% of the variance (Table 4).

**Blood pressure and heart rate**

Systolic blood pressure did not change significantly from baseline in response to the glucose load in insulin-sensitive or insulin-resistant subjects (Figure 4A). In contrast, diastolic blood pressure decreased significantly at 30 (by 4.8 ± 1.6 and 3.0 ± 1.2 mm Hg) and 60 (by 4.8 ± 1.6 and 3.8 ± 1.1 mm Hg) min in insulation-sensitive subjects. In contrast, the heart rate increased significantly 15 min after the glucose load in both insulin-resistant and insulin-sensitive subjects, reaching a peak at 60 min (by 12 ± 1.9 and 12 ± 1.7 bpm, respectively).

**FIGURE 2.** Mean (± SEM) muscle sympathetic nerve activity (MSNA) in insulin-sensitive (IS; n = 12) and insulin-resistant (IR; n = 19) subjects in response to 75 g oral glucose expressed as burst frequency (A), burst incidence (B), total MSNA burst frequency (C), and total MSNA burst incidence (D). Data (log-transformed when appropriate) were analyzed by 2-factor repeated-measures ANOVA using the Holm-Sidak test for pairwise comparisons. MSNA burst frequency: *P < 0.001 for the time effect and P = 0.03 for the time-by-group interaction; **P < 0.05 compared with IS group and ***P < 0.001 compared with time 0. MSNA burst incidence: *P = 0.06 for the time effect; *P < 0.05 compared with IS subjects. Total MSNA burst frequency: *P < 0.001 for the time effect and P = 0.05 for the time-by-group interaction; *P < 0.05 compared with IS subjects and **P < 0.01 and ***P < 0.001 compared with time 0. Total MSNA burst incidence: *P = 0.04 for the time effect. hb, heart beats.
the 2 groups, respectively (P < 0.01 compared with baseline for all) and at 90 min by 3.2 ± 1.3 mm Hg in insulin-resistant subjects only (P = 0.002). Heart rate increased significantly from 30 min onward in both groups (P < 0.001 compared with baseline for all; Figure 4B). The change in MSNA and whole-body norepinephrine spillover did not correlate significantly with the change in diastolic blood pressure at any of the time points in the insulin-sensitive, insulin-resistant, or pooled subject groups.

Calf vascular measurements

Baseline arterial calf blood flow was greater in insulin-resistant than in insulin-sensitive subjects (2.85 ± 0.25 compared with 2.16 ± 0.21 mL · min⁻¹ · 100 mL⁻¹ tissue; P = 0.004 by unpaired t test). Calf blood flow increased, whereas calf vascular resistance decreased from 60 min onward in both insulin-sensitive and insulin-resistant subjects (P < 0.01 compared with baseline for all; Figure 4C). The magnitude of change did not differ between the groups. There were no significant associations
Whole-body NE spillover, n
MSNA burst frequency, n
MSNA burst incidence, n
sensitive subjects and from time 90 min onward (P served at any of the time points (Figure 4D).
measurements. No significant between-group differences were ob-
in insulin-resistant subjects when compared with resting mea-
by using the method of Matsuda and DeFronzo (21); TG, triglycerides; hsCRP, high-sensitivity C-reactive protein; SI, whole-body insulin sensitivity index derived

Dependent variable Step Predictor variable Std. coeff \( r^2 \) \( P \)

**TABLE 3**

Univariate correlates of the whole-body norepinephrine (NE) spillover response to oral glucose (n = 30)\(^1\)

<table>
<thead>
<tr>
<th>Variable</th>
<th>( \Delta V_O_{2\text{max}}/FFM \text{ (mL \cdot min}^{-1} \cdot kg^{-1}) )</th>
<th>Log TG (mmol/L)</th>
<th>Log hsCRP (mg/L)</th>
<th>Insulin AUC(_{0-120} \text{ (mU/L \cdot min)} )</th>
<th>Log SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Delta \text{NE spillover, 30 min} )</td>
<td>0.48(^2)</td>
<td>-0.44(^2)</td>
<td>—</td>
<td>-0.30</td>
<td>0.25</td>
</tr>
<tr>
<td>( \Delta \text{NE spillover, 90 min} )</td>
<td>—</td>
<td>—</td>
<td>-0.42(^2)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>( \Delta \text{NE spillover, 120 min} )</td>
<td>0.44(^3)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

\(^1\) AUC\(_{0-120}\) area under the curve at 0–120 min during the oral-glucose-tolerance test determined by using the trapezoidal rule; FFM, fat-free mass; hsCRP, high-sensitivity C-reactive protein; SI, whole-body insulin sensitivity index derived by using the method of Matsuda and DeFronzo (21); TG, triglycerides; \( \Delta V_O_{2\text{max}} \), maximal oxygen consumption during an incremental cycle ergometry protocol; \( \Delta \), change. Normal variables were correlated by using Pearson’s product moment correlation and nonparametric variables by using Spearman’s rank-order correlation.

\(^2\) \( P < 0.01. \)

\(^3\) \( P \leq 0.05. \)

between resting calf blood flow and sympathetic activity or between change in calf blood flow and change in sympathetic activity for the pooled data and within subgroups.

**Cardiac baroreflex sensitivity**

Spontaneous cardiac baroreflex sensitivity decreased significantly from time 60 min onward (\( P \leq 0.001 \) for all) in insulin-sensitive subjects and from time 90 min onward (\( P \text{ all} \leq 0.001 \)) in insulin-resistant subjects when compared with resting measurements. No significant between-group differences were observed at any of the time points (Figure 4D).

**DISCUSSION**

This was the first study to date to examine the stimulation of the SNS after an oral glucose load in middle-aged subjects who met MetS criteria. The main finding is that insulin-resistant subjects display a blunted sympathetic neural response to glucose ingestion compared with age- and blood pressure–matched insulin-sensitive subjects, despite a 2-fold greater increase in plasma insulin concentrations (because insulin putatively stimulates SNS activity). Two methods were used to quantify sympathetic activity: microneurography, which directly quantifies sympathetic neural outflow to the smooth muscle of blood vessels within skeletal muscle, and the isotope dilution technique, which estimates the rate at which norepinephrine released from sympathetic nerve endings enters and is cleared from the plasma compartment and which represents an estimate of whole-body sympathetic activity. The fact that the main finding was consistent across the 2 techniques strengthens these observations. Additional findings were as follows: 1) resting MSNA, but not whole-body norepinephrine spillover, was higher in insulin-resistant than in insulin-sensitive obese subjects; 2) the blunted sympathetic response to glucose in insulin-resistant subjects, as measured by MSNA, was related to BMI, abdominal fat mass, and the insulin response but appeared not to be mediated by differences in the vasodilatory responses to insulin or in cardiac baroreflex sensitivity; and 3) fitness is positively associated, whereas hsCRP is negatively associated, with the whole-body norepinephrine spillover response to glucose.

Our findings concur with those of Fagius et al (4), who reported higher resting MSNA and blunted responses to oral glucose in elderly insulin-resistant subjects. A similar flat response of MSNA was shown in obese subjects during a hyperinsulinemic euglycemic clamp (8) and also after oral glucose intake, despite the hypersecretion of insulin (9). One interpretation of these results is that the higher rate of resting MSNA in insulin-resistant subjects may be similar to the maximal insulin-induced sympathetic

**TABLE 4**

Stepwise regression analyses of the sympathetic neural response to oral glucose\(^1\)

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Step</th>
<th>Predictor variable</th>
<th>Std. coeff</th>
<th>( r^2 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Delta \text{MSNA burst frequency, n = 31 (bursts/min)} )</td>
<td>1</td>
<td>Insulin AUC(_{0-120} \text{ (mU/L \cdot min)} )</td>
<td>-0.41</td>
<td>0.17</td>
<td>0.02</td>
</tr>
<tr>
<td>( \Delta \text{MSNA burst incidence, n = 31 (bursts/100 heart beats)} )</td>
<td>1</td>
<td>Saturated fat intake (% of energy)</td>
<td>-0.41</td>
<td>0.23</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>BMI (kg/m(^2))</td>
<td>-0.32</td>
<td>0.33</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Log SI</td>
<td>0.39</td>
<td>0.15</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Saturated fat intake (% of energy)</td>
<td>-0.43</td>
<td>0.22</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Abdominal fat mass (kg)</td>
<td>-0.38</td>
<td>0.36</td>
<td>0.02</td>
</tr>
<tr>
<td>( \Delta \text{Whole-body NE spillover, n = 30 (ng/min)} )</td>
<td>1</td>
<td>( V_O_{2\text{max}}/FFM \text{ (mL \cdot min}^{-1} \cdot kg^{-1}) )</td>
<td>0.48</td>
<td>0.23</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Log hsCRP (mg/L)</td>
<td>-0.47</td>
<td>0.22</td>
<td>0.01</td>
</tr>
</tbody>
</table>

\(^1\) AUC\(_{0-120}\) area under the curve at 0–120 min during the oral-glucose-tolerance test determined by using the trapezoidal rule; FFM, fat-free mass; hsCRP, high-sensitivity C-reactive protein; NE, norepinephrine; MSNA, muscle sympathetic nerve activity; SI, whole-body insulin sensitivity index derived by using the method of Matsuda and DeFronzo (21); \( V_O_{2\text{max}} \), maximal oxygen consumption during an incremental cycle ergometry protocol; \( \Delta \), change. Statistical analysis was performed by using forward stepwise linear regression.
activation in skeletal muscle and may preclude the demonstration of any further increase during acute physiologic elevation of plasma insulin (8). However, an inverse relation between resting sympathetic activity and sympathetic response to glucose is not supported by the norepinephrine spillover data in the present study or by data in Pima Indians, who, as a population are insulin-resistant and have a low resting MSNA, but who also display a blunted MSNA response to glucose (9).

The sympathoexcitatory effects of insulin are thought to be mediated by ≥2 mechanisms: 1) a direct increase in central sympathetic outflow and 2) baroreceptor reflex–mediated sympathetic activation in response to insulin’s peripheral vasodilatory actions. Insulin enters the CNS through the blood-brain barrier by receptor-mediated transport, and insulin receptors have been shown in several distinct regions, such as the median hypothalamus (26). In rats, intracerebroventricular insulin infusion (at a dose that did not have systemic action) acutely increased the activity of multiple sympathetic nerves, an effect that was abolished by anterovernal third ventricle lesions (27). In healthy humans, euglycemic hyperinsulinemia in the postprandial range increases MSNA, consistent with the concept that insulin stimulates central sympathetic outflow (28). Alterations in the efficiency with which insulin enters the CNS could theoretically modify the sympathetic response to glucose. Reduced insulin delivery into the brain has been shown in canine models of insulin resistance, induced by high fat feeding or dexamethasone administration (29). Moreover, Kern et al (30) found a low ratio of cerebrospinal fluid to plasma insulin in obese humans, which was independently associated with the degree of insulin resistance. Low insulin concentrations in the brain may favor weight gain and increase peripheral insulin resistance, which, in turn, might further hamper brain uptake of insulin and eventually lead to a vicious cycle (30).

Insulin-stimulated nitric oxide production mediates capillary recruitment, vasodilation, increased arterial blood flow, and subsequent glucose disposal in skeletal muscle. Several studies have shown blunted insulin-induced vasodilation in skeletal muscle of obese compared with lean subjects (8, 31). In the present study, calf blood flow and heart rate both increased and diastolic blood pressure decreased similarly after the glucose load in obese insulin-sensitive and obese insulin-resistant subjects. Therefore, the blunted sympathetic responses in insulin-resistant subjects was not explained by differences in vasodilation-mediated (baroreflex) mechanisms. In concordance with this finding, experimental insulin resistance induced by infusion of fat emulsion in lean subjects did not alter vasodilatory responses during a euglycemic hyperinsulinemic clamp (32).

The cardiac baroreflex is a reflex loop with cardiac, vascular, and cerebral components involved in short-term blood pressure regulation. Euglycemic hyperinsulinemia produces cardiac vagal withdrawal, as assessed by power spectral analysis in healthy subjects (33). Laitinen et al (34) showed greater cardiac vagal withdrawal in response to acute hyperinsulinemia in overweight insulin-resistant than in lean insulin-sensitive subjects. In the present study, however, both subject groups experienced a significant reduction in spontaneous cardiac baroreflex sensitivity, which did not differ in magnitude. To examine the specific role of insulin resistance on cardiac sympathovagal balance, Frontoni

**FIGURE 4.** Mean (± SEM) intraarterial systolic (top) and diastolic (bottom) blood pressure (A), heart rate (B), calf blood flow (C), and spontaneous cardiac baroreflex sensitivity (D) in insulin-sensitive (IS; n = 12) and insulin-resistant (IR; n = 19) subjects during the oral-glucose-tolerance test. Data were analyzed by 2-factor repeated-measures ANOVA using the Holm-Sidak test for pairwise comparisons. Systolic blood pressure: no significant time, group, or interaction effects. Diastolic blood pressure: P < 0.001 for the time effect; significant decrease from time 30 min onward in both groups. Heart rate: P < 0.001 for the time effect; significant increase from 30 min onward in both groups. Calf blood flow: P < 0.001 for the time effect and P = 0.07 for the group effect; significant increase from 60 min onward in both groups. Spontaneous cardiac baroreflex sensitivity: P < 0.001 for the time effect and P = 0.03 for the time-by-group interaction; ***P < 0.001 compared with time 0.
et al (35) compared low- with high-frequency (LF:HF) ratio responses during an intravenous glucose tolerance test in normal-weight, insulin-resistant and insulin-sensitive offspring of type 2 diabetic subjects. Endogenous hyperinsulinemia was associated with an increase in the LF:HF ratio in insulin-resistant but not in insulin-sensitive subjects. Insulin resistance may therefore be responsible for the early derangements in autonomic nervous tone control, independent of the obese state (35).

A consistent body of data indicate that abdominal visceral, rather than subcutaneous, fat is associated with sympathetic neural activation in humans (36, 37). This appears to be related to metabolic factors, such as the greater insulin resistance characterizing central obesity (36). In agreement with this, we observed a positive association at baseline between BMI, abdominal fat mass, and measures of insulin resistance. It is also likely that the greater consumption of saturated fat contributed to the insulin resistance in the insulin-resistant group. Saturated fats may also directly influence sympathetic neuronal function. Reductions in field stimulation evoked norepinephrine release and blunted thermogenic responses have been shown after high saturated fat feeding in animals (38, 39), which is consistent with the blunted sympathetic response to glucose observed in our study. We further observed that measures of central adiposity (BMI, trunk, and abdominal fat) and attendant metabolic abnormalities (insulin AUC0–120, plasma triglycerides, and hsCRP concentrations) were all inversely related to the SNS response to glucose, whereas fitness was a positive predictor. This finding concurs with data in Pima Indians and matched whites in whom the increase in MSNA during the OGTT correlated negatively with percentage body fat independently of race (9). Exercise training lowers resting sympathetic nerve traffic, potentiates baroreflex sensitivity (40, 41), and improves insulin sensitivity (42). Therefore, fitness may influence the sympathetic response to glucose by improving insulin resistance.

Diet-induced changes in sympathetic activity are physiologically important in metabolic heat production. Mixed-meal studies have shown that β-blockade or clonidine administration reduces the thermic effect of food by 23% (2). The clinical significance of a blunted sympathetic response is that it may promote weight gain and further aggravate insulin resistance and hyperinsulinemia, thus acting as a self-perpetuating mechanism. Additional studies are required to examine whether improvements in insulin sensitivity with weight loss and/or physical activity reverse the blunted SNS response to glucose.

We thank Dianne Payne for performing and analyzing the DXA scans and Donna Vizi and Jenny Starr for nursing assistance.

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REFERENCES


