Effects of mental stress on insulin-mediated glucose metabolism and energy expenditure in lean and obese women

G. SEEMATTER, E. GUENAT, P. SCHNEITER, C. CAYEUX, E. JÉQUIER, AND L. TAPPY
Institute of Physiology, University of Lausanne Medical School, 1005 Lausanne, Switzerland

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THE SYMPATHETIC NERVOUS SYSTEM (SNS) exerts complex effects on insulin sensitivity and energy metabolism. This complexity is illustrated by the observation that various conditions known to elicit SNS activation have markedly different metabolic effects. A lower body negative pressure (LBNP) decreases insulin-mediated glucose disposal (17) but does not significantly alter resting energy expenditure (26). In contrast, mental stress acutely enhances insulin-induced glucose utilization (14, 20, 27). Whether it affects energy expenditure has, to our knowledge, not been documented. These divergent metabolic effects of LBNP and mental stress may correspond to a compartmentalization of the SNS, with activation of distinct sympathetic fibers according to the mode of SNS activation. Thus it has been observed that LBNP increases muscle, but not skin, sympathetic nerve activity (29) while mental stress stimulates SNS in both muscle and skin (28).

The hemodynamic effects of LBNP and mental stress differ in several respects. LBNP results in a decrease in leg blood flow, related with α2-adrenergic receptor-mediated vasoconstriction (12). In contrast, mental stress acutely stimulates limbs blood flow through mechanisms incompletely elucidated but which involve endothelial NO release (7, 15) and β-adrenoceptor activation (10, 18). Since alterations of muscle blood flow may modulate insulin sensitivity by altering the rate of delivery of both glucose and insulin itself to skeletal muscle (1), these hemodynamic effects of LBNP and mental stress may be involved in the regulation of insulin-mediated glucose disposal.

Several reports indicate the presence of alterations of SNS activity in obese patients. In obese Pima Indians, reduced norepinephrine turnover has been observed, suggesting an overall decrease in SNS activity. No such alteration was observed in obese Caucasians (5). In contrast, obese Pima Indians and Caucasians have an increased muscle sympathetic nerve activity related to their increased body fat (21, 22).

To further evaluate the role of the SNS in metabolic control and the possible relationship between metabolic and hemodynamic actions of SNS, we evaluated the effects of mental stress in lean and obese subjects. The specific aims were to further delineate the effects of mental stress on insulin sensitivity and energy expenditure in healthy lean females and to assess whether the metabolic effects of mental stress were altered in obesity.

SUBJECTS AND METHODS

Subjects

Two groups of subjects were selected to take part in this study. Group 1 consisted of eight obese women with a mean age of 25.5 ± (SD) 0.7 yr (r = 24–47), body mass index (BMI) 33.9 ± 2.2 kg/m² (r = 29–47), percentage of body fat (deter-
mined from skinfold thickness measurement; Ref. 8) 33.4 ± 1.3% (r = 28–40), and fat-free mass (FFM) 60.9 ± 1.9 kg (r = 56–70). Group 2 consisted of 11 lean women with a mean age of 24.6 ± 0.8 yr (r = 20–28), BMI 21.8 ± 0.4 kg/m² (r = 20–24), percentage of body fat 17.6 ± 0.8% (r = 14–23), and FFM 50.5 ± 1.1 kg (r = 45–56). All subjects were in good physical condition and were nonsmokers. They were not currently taking any medication and had no family history of diabetes or hypertension. The experimental protocol was approved by the Ethical Committee of the Lausanne University Medical School, and every subject provided informed written consent.

**General Procedure**

Experiments began in the morning after an overnight fast. Subjects were requested not to consume caffeine- or alcohol-containing drinks for at least 24 h before the study; furthermore, they were asked not to get involved in any strenuous physical activity during the 3 days preceding the study. All the women were studied during the follicular phase of the menstrual cycle.

Each subject took part in one or two protocols aimed at assessing the metabolic and hemodynamic effects of mental stress during euglycemic hyperinsulinemic clamp. On the arrival of the subjects in the metabolic laboratory, one indwelling venous cannula was inserted into a vein of their right wrist. The right hand was subsequently placed into a thermostabilized box heated at 56°C to achieve partial arterIALIZATION of venous blood. Blood samples were periodically collected through this catheter. A second indwelling cannula was inserted into an antecubital vein of the contralateral arm for infusion of glucose tracer, insulin, glucose, and propranolol.

In protocol 1, aimed at assessing the metabolic effects of mental stress during hyperinsulinemia, a primed continuous infusion of [6,6-2H₂]glucose (MassTrace, Woburn, MA; 11.1 μmol/kg bolus, 111 nmol-kg⁻¹-min⁻¹ continuous) was started at 7:30 AM. Sixty minutes after the beginning of the [6,6-2H₂]glucose infusion, a hyperinsulinemic-euglycemic clamp (0.4 μU·kg⁻¹·min⁻¹; Ref. 6) was started (time 0). The exogenous glucose was labeled with 1.25% [6,6-2H₂]glucose to avoid dilution of the tracer by glucose infusion. After 150 min of euglycemic hyperinsulinemia, a mental stress was applied for 30 min while the clamp was continued. Mental stress consisted of a succession of 5-min periods of mental stress during hyperinsulinemia, a primed continuous 

**Analysis**

Plasma glucose concentration was determined with a Beckman glucose analyzer 2 (Beckman Instruments, Fullerton, CA). Plasma lactate concentration was measured with a lactate analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma epinephrine and norepinephrine concentrations were determined by high-performance liquid chromatography (11). Plasma insulin concentration was determined by RIA (kit from Biodata, Guidoni, Montecello, Italy). Isotopic enrichment of glucose was determined by gas chromatography-mass spectrometry (GC 5890-MS 5971, Hewlett-Packard, Palo Alto, CA) after preparation of pentaoctanol derivates.

**Calculations**

Glucose appearance and utilization were calculated from [6,6-2H₂]glucose dilution analysis using “hot infusion” equations (9). Endogenous glucose production was calculated as glucose appearance minus glucose perfusion. Systemic vascular resistance was calculated as the ratio between mean arterial pressure and cardiac index and was expressed in terms of arbitrary units (U).

**Statistics**

All results are given as means ± SE. The effect of mental stress on all parameters mentioned was assessed with analysis of variance for repeated measurements. Between-group comparisons were done by a two-way analysis of variance and Fisher’s protected least significant difference (PLSD) tests.

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**Table 1. Hemodynamic parameters**

<table>
<thead>
<tr>
<th></th>
<th>Lean (n = 11)</th>
<th>Obese (n = 8)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Mental stress</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>58 ± 3</td>
<td>86 ± 3</td>
</tr>
<tr>
<td>Blood pressure, mmHg</td>
<td>77 ± 3</td>
<td>85 ± 3</td>
</tr>
<tr>
<td>Cardiac index, 1-min⁻¹·m⁻²</td>
<td>3.6 ± 0.3</td>
<td>5.2 ± 0.5</td>
</tr>
<tr>
<td>Peripheral resistance, U</td>
<td>21 ± 2</td>
<td>16 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 between baseline and mental stress; †P < 0.05 between lean and obese subjects. bpm, Beats/min.
RESULTS

Cardiovascular Parameters

Protocol 1. As shown in Table 1, Baseline data were similar in lean and obese subjects. Mental stress caused a significant increase in heart rate, mean blood pressure, and cardiac index in both groups. The increase in cardiac index was, however, 25% smaller ($P < 0.05$), and the increase in mean blood pressure was 18% higher ($P < 0.05$) in the obese group compared with the lean group (Table 1). Systemic vascular resistance decreased by 24% in the lean group ($P < 0.05$) but not in the obese group.

Protocol 2. As shown in Table 2, propranolol decreased baseline heart rate ($P < 0.05$) and cardiac index ($P < 0.05$), but systolic and diastolic blood pressure did not change. Mental stress thereafter increased mean blood pressure ($P < 0.05$), but heart rate, cardiac index, and systemic vascular resistance remained unchanged (Table 2).

Hormones and Substrates

Protocol 1. Basal plasma insulin concentration was $59 \pm 9$ pmol/l in the lean group and $132 \pm 27$ pmol/l ($P < 0.02$) in the obese group. During the hyperinsulinemic clamp, plasma insulin concentration was $250 \pm 16$ pmol/l in the control group and $366 \pm 66$ pmol/l ($P < 0.05$) in the obese group and remained stable throughout the experiment (Fig. 1). Plasma glucose concentrations were $4.94 \pm 0.03$ mmol/l (coefficient of variation 2%) and $4.96 \pm 0.02$ mmol/l (coefficient of variation 2%) in lean and obese individuals, respectively. Plasma potassium concentrations decreased slightly, from $3.7 \pm 0.1$ and $3.8 \pm 0.1$ meq/l in the basal state to $3.5 \pm 0.1$ and $3.6 \pm 0.1$ meq/l during hyperinsulinemia in lean and obese subjects, respectively. Mental stress caused a significant increase in plasma epinephrine concentration in both lean and obese groups and a nonsignificant increase in norepinephrine 10 min after the beginning of the mental stress (min 160; Fig. 2). Plasma insulin, glucose, and potassium concentrations remained unchanged, but blood lactate increased from $1.08 \pm 0.05$ to $1.43 \pm 0.13$ mmol/l, ($P < 0.01$) in lean subjects and from $1.05 \pm 0.07$ to $1.45 \pm 0.07$ mmol/l, ($P < 0.01$) in obese patients.

Protocol 2. Propranolol infusion did not alter hormones and substrates concentrations (Fig. 3). The peak increase in epinephrine (from $0.14 \pm 0.01$ to $0.24 \pm 0.02$ nmol/l) and norepinephrine (from $1.51 \pm 0.17$ to $1.85 \pm 0.19$ nmol/l) observed was also comparable with those recorded in the same subjects under control conditions. Propranolol abolished the rise in lactate (from $1.08 \pm 0.05$ to $1.43 \pm 0.13$ mmol/l).
Glucose Utilization

In lean subjects, mental stress increased the rate of glucose infusion required to maintain euglycemia (from $24.0 \pm 2.3\ \mu\text{mol} \cdot \text{kg FFM}^{-1} \cdot \text{min}^{-1}$ to $30.7 \pm 2.6\ \mu\text{mol} \cdot \text{kg FFM}^{-1} \cdot \text{min}^{-1}$; $P < 0.05$). Endogenous glucose production was not different from zero during hyperinsulinemia ($0.8 \pm 0.5\ \mu\text{mol} \cdot \text{kg FFM}^{-1} \cdot \text{min}^{-1}$) and did not increase during mental stress. Glucose utilization assessed from $[6,6^{2}\text{H}_2]\text{glucose}$ dilution increased from $24.5 \pm 2.6\ \mu\text{mol} \cdot \text{kg FFM}^{-1} \cdot \text{min}^{-1}$ during hyperinsulinemia to an average of $32.5 \pm 3.1\ \mu\text{mol} \cdot \text{kg FFM}^{-1} \cdot \text{min}^{-1}$ after mental stress (Fig. 1).

In obese patients, both the rate of glucose infusion ($20.9 \pm 2.6\ \mu\text{mol} \cdot \text{kg FFM}^{-1} \cdot \text{min}^{-1}$) and glucose utilization ($22.7 \pm 2.4\ \mu\text{mol} \cdot \text{kg FFM}^{-1} \cdot \text{min}^{-1}$) were similar to those observed in lean subjects. Endogenous glucose production was nearly completely suppressed by hyperinsulinemia ($0.9 \pm 0.5\ \mu\text{mol} \cdot \text{kg FFM}^{-1} \cdot \text{min}^{-1}$). After mental stress, the increases in both glucose infusion and glucose utilization were markedly blunted (Fig. 1). As in lean subjects, mental stress did not increase glucose production.

Protocol 2. In the study without propranolol, the stimulation of glucose utilization during mental stress was not completely compensated by the increase in glucose infusion, resulting in a slight, transient drop in glycemia (from $4.91 \pm 0.9\ \text{mmol/l}$ to $4.78 \pm 0.04$, $P < 0.05$). Baseline clamp parameters were not affected by propranolol, but the stimulation of glucose uptake elicited by mental stress was abolished (from $25.0 \pm 3.1$ to $26.8 \pm 3.2\ \mu\text{mol} \cdot \text{kg lean mass}^{-1} \cdot \text{min}^{-1}$, NS) compared with the data obtained in the same subjects without propranolol (from $25.3 \pm 2.7$ to $33.6 \pm 2.6\ \mu\text{mol} \cdot \text{kg lean mass}^{-1} \cdot \text{min}^{-1}$, $P < 0.05$; Fig. 3). Endogenous glucose production remained suppressed throughout the tests.

Oxygen Consumption

Protocol 1. Mental stress increased $\dot{V}O_2$ by 20% (from $192 \pm 5$ to $229 \pm 6\ \text{ml/min}$, $P < 0.0001$) in the control.
group and by 21% in the obese group (from 248 ± 15 to 299 ± 12 ml/min, P < 0.0001). There was no difference in the amplitude of the V\(_2\) increase between obese and lean patients (Fig. 4).

Protocol 2. Basal V\(_2\) was not significantly altered by propranolol, but mental stress increased V\(_2\) by only 6% (from 189 ± 8 to 201 ± 8 ml/min, NS), which was significantly less (P < 0.05) than the increase observed without propranolol in the same subjects (from 191 ± 8 to 227 ± 11 ml/min, P < 0.001) (Fig. 5).

DISCUSSION

Mental stress acutely increases insulin-stimulated glucose utilization in healthy lean humans. The major new observation of this study is that this effect is abolished in obese nondiabetic patients.

In lean subjects, mental stress elicited an increase in glucose uptake in the forearm, as already reported by Jern (14), and at the whole body level, as reported by Moan et al. (20) and Touma et al. (27). This effect of mental stress differs from that observed during other sympathetic activation procedures. It has indeed been observed that a LBNP decreases glucose uptake (17). This difference may be explained by the hemodynamic effects of mental stress. In our study, as in previous reports that used either mental arithmetic (20) or the color word conflict test (10), mental stress increased cardiac index and decreased peripheral vascular resistance, presumably in skeletal muscle.

Several studies have documented an increase in limb blood flow during mental stress in humans. This effect was completely inhibited by \(\beta\)-adrenergic antagonists (10, 18) and by inhibitors of NO synthase, indicating that endothelial NO release was involved (4, 7, 15). There is no definitive explanation for this inhibition of vasodilation by both \(\beta\)-adrenergic antagonists and NO synthase inhibitors. The vascular responses to mental stress may be complex, with simultaneous vasodilation through activation of \(\beta\)-adrenergic receptors and endothelial NO release. Alternatively, it is possible that stimulation of \(\beta\)-adrenergic receptors is involved in endothelial NO release in this condition. The recent observation that NO release contributes to \(\beta\)-adrenergic-mediated vasodilation would be consistent with this explanation (19).

It has been proposed that stimulation of muscle blood flow enhances insulin actions in humans during mental stress (20). The inhibition of both vasodilation and stimulation of insulin sensitivity by \(\beta\)-adrenergic antagonists further supports this hypothesis. Furthermore, mental stress failed to stimulate insulin sensitivity in obese patients. This was concomitant with an absent vasodilatory response already reported by other investigators (25). It was not related to a lower sympathetic stimulation, because the increase in plasma epinephrine and norepinephrine, the stimulation of heart rate, and the increase in V\(_2\) were all similar to what was recorded in lean subjects. These observations indicate a specific impairment of the vascular response to mental stress in obesity. Obese patients also have impaired vasodilation in response to other NO-dependent vasodilatory stimuli such as hyperinsulinemia and intra-arterial cholinergic agonists (24). It is, therefore, likely that these impaired responses to mental stress were related to endothelial dysfunction associated with obesity. Because insulin was infused according to body weight rather than lean body mass, plasma insulin concentrations during the clamp were 46% higher in obese patients than in lean subjects. This is likely to account for the similar glucose utilization observed in the two groups of subjects. The higher insulin concentrations attained in obese subjects is, however, unlikely to affect our conclusions. Although insulin has potent vasodilatory properties, it is unlikely that a vasodilation induced by the higher insulin concentrations in obese patients marked the effects of a subsequent mental stress, because insulin vasodilatory actions have been shown to be markedly blunted in obesity (16, 30).
The second new observation of this study is that mental stress elicits a significant 20% increase in energy expenditure that was sustained during 30 min. This stimulation was secondary to an endogenous activation of the SNS, because it was associated with increases in plasma norepinephrine and epinephrine concentrations. Moreover, this stimulation was abolished by propranolol. Interestingly, mental stress elicited a comparable increase in energy expenditure in obese and lean subjects. This observation suggests that the stimulation of the SNS by mental stress and the metabolic effectors responsible for the increased V̇O₂ activated by the SNS are not altered in obesity. This contrasts with the observation that thermogenesis induced by norepinephrine infusion is blunted in obese patients (2, 3). The reason for these differing effects of exogenously administered and endogenously released catecholamines on energy expenditure in obese patients remains unclear.

In summary, our data indicate that a mental stress performed during moderate hyperinsulinemia in healthy volunteers stimulates insulin-mediated glucose disposal, possibly in relation to a decreased systemic vascular resistance; in addition, it increases energy expenditure. These two effects are abolished by β-adrenergic receptor antagonists. In obese patients, the stimulation of insulin-mediated glucose disposal and the decrease in vascular resistance by mental stress are both abolished, possibly in relationship with endothelial cell dysfunction. In contrast, the thermogenic effect of mental stress is retained in obesity.

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REFERENCES


