Subcutaneous obesity is not associated with sympathetic neural activation

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Alvarez, Guy E., Tasha P. Ballard, Stacy D. Beske, and Kevin P. Davy. Subcutaneous obesity is not associated with sympathetic neural activation. Am J Physiol Heart Circ Physiol 287: H414–H418, 2004. First published February 26, 2004; 10.1152/ajpheart.01046.2003.—We tested the hypothesis that muscle sympathetic nerve activity (MSNA) would not differ in subcutaneously obese (SUBOB) and nonobese (NO) men with similar levels of abdominal visceral fat despite higher plasma leptin concentrations in the former. We further hypothesized that abdominal visceral fat would be the strongest body composition–or regional fat distribution-related correlate of MSNA among these individuals. To accomplish this, we measured MSNA (via microelectrode technique), body composition (via dual-energy X-ray absorptiometry), and abdominal fat distribution (via computed tomography) in 15 NO (body mass index 35 kg/m²; 22.4 ± 1.4 yr) and 9 SUBOB (25 ± body mass index 35 kg/m²; 23.4 ± 2.1 yr) sedentary men. As expected, body mass (94 ± 4 vs. 71 ± 2 kg), total fat mass (25 ± 2 vs. 12 ± 1 kg), and abdominal subcutaneous fat (307 ± 36 vs. 132 ± 12 cm²) were significantly higher in the SUBOB group compared with NO peers. However, the level of abdominal visceral fat did not differ significantly in the two groups (69 ± 7 vs. 55 ± 5 cm²). MSNA was not different between SUBOB and NO men (23 ± 3 vs. 24 ± 2 bursts/min; P > 0.05, respectively) despite a 2.6-fold higher (P < 0.05) plasma leptin concentration in the SUBOB men. Furthermore, abdominal visceral fat was the only body composition– or regional fat distribution-related correlate (r = 0.45; P < 0.05) of MSNA in the pooled sample. In addition, abdominal visceral fat was related to MSNA in NO (r = 0.58; P = 0.0239) but not SUBOB (r = 0.39; P = 0.3027) men. Taken together with our previous observations, our findings suggest that the relation between obesity and MSNA is phenotype dependent. The relation between abdominal visceral fat and MSNA was evident in NO but not in SUBOB men and at levels of abdominal visceral fat below the level typically associated with elevated cardiovascular and metabolic disease risk. Our observations do not support an obvious role for leptin in contributing to sympathetic neural activation in human obesity and, in turn, are inconsistent with the concept of selective leptin resistance.

Leptin, the product of the ob gene, is secreted from adipocytes in proportion to fat mass, circulates in the blood, and crosses the blood-brain barrier to act on hypothalamic neuronal targets to alter energy intake and expenditure (1). Leptin also exerts an important influence on cardiovascular and renal function in experimental animals that is sympathetically mediated (15, 17). However, some (26, 33) but not all (27) studies in humans support such a link. As such, the concept that the sympathoexcitatory action of leptin is preserved despite resistance to its anorectic effects (i.e., selective leptin resistance) remains unsubstantiated in humans. Importantly, leptin expression and secretion is higher in subcutaneous compared with visceral adipocytes (30, 37), and circulating concentrations of the protein are higher in subcutaneous compared with visceral obesity (22). Thus we reasoned that similar levels of MSNA in SUBOB compared with NO men despite higher leptin concentrations in the former would provide evidence against the notion of selective leptin resistance.

We have previously reported reduced vagal but not sympathetic baroreflex gain in men with higher levels of abdominal visceral fat. As expected, the relation was independent of total fat mass. However, whether obese individuals with a subcutaneously obese phenotype (SUBOB) demonstrate higher levels of MSNA compared with NO individuals with similar levels of abdominal visceral fat is unknown.

Accordingly, we tested the hypothesis that MSNA would not differ in SUBOB and NO men with similar levels of abdominal visceral fat. We further hypothesized that abdominal visceral fat would be the strongest body composition–or regional fat distribution-related correlate of MSNA in these individuals with levels of abdominal visceral fat below the threshold typically associated with elevated cardiovascular and metabolic disease risk factors.

The sympathetic nervous system (SNS) plays a critical role in the regulation of cardiovascular and metabolic homeostasis. Sustained activation of the SNS has been implicated in a number of pathophysiological states associated with obesity including hypertension and congestive heart failure (10). Muscle sympathetic nerve activity (MSNA) is elevated in even healthy normotensive obese compared with nonobese (NO) humans (12, 14, 31, 34). However, there is considerable interindividual variability in the level of MSNA among obese individuals. We previously demonstrated that MSNA is higher in men with elevated abdominal visceral fat compared with their age- and total fat mass-matched peers with lower levels (2). MSNA was more closely associated with abdominal visceral fat than subcutaneous fat, and the relation was independent of total fat mass. However, whether obese individuals with a subcutaneously obese phenotype (SUBOB) demonstrate higher levels of MSNA compared with NO individuals with similar levels of abdominal visceral fat is unknown.

EXCESS WEIGHT IS A MAJOR public health problem in the United States (9). In the past decade, the prevalence of overweight and obesity has increased dramatically (11, 25). Currently, 65% of U.S. adults are considered overweight and 31% are obese (11). Overweight and obesity are associated with an increased risk of cardiovascular and metabolic diseases (9). However, not all obesity phenotypes are similar in their association with cardiovascular and metabolic disease risk. Abdominal visceral fat is more closely associated with elevated cardiovascular and metabolic disease risk than subcutaneous fat (6, 7).

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visceral fat compared with their age- and total fat mass-matched peers with lower levels (2, 4). Therefore, we sought to determine whether vagal baroreflex gain was also reduced in SUBOB men compared with NO men.

METHODS

Subjects. Fifteen NO (body mass index \(\leq 25\) kg/m\(^2\)) and 9 SUBOB (25 \(\leq\) body mass index \(\leq 35\) kg/m\(^2\)) men with an abdominal visceral fat level < 110 cm\(^2\) were included in the present investigation. This level was selected because it is below the level typically associated with elevated cardiovascular and metabolic disease risk factors (39). These individuals were selected from a larger group of 66 men who volunteered to participate in our ongoing studies on obesity and autonomic nervous system control of cardiovascular function (2, 4). All subjects were normotensive and free from overt chronic disease (other than overweight/obesity) as determined from individual health histories. All subjects were free of overt cardiopulmonary disease, were non-diabetic, and were not taking any medications including those that could affect autonomic circulatory function. All subjects were sedentary and did not participate in regular physical activity (defined as \(>20\) min on \(>2\) days/wk). All men meeting these criteria were included. The nature, purpose, risks, and benefits were explained to each subject before informed consent was obtained. The experimental protocols were approved by our University Human Research Committee.

Experimental procedures. Body mass and height were measured using a physician’s balance scale and a stadiometer, respectively. Body mass index was calculated as weight (in kg) divided by height (in m\(^2\)). The waist-to-hip ratio was calculated from the measured respective circumferences. Body composition was measured using dual-energy X-ray absorptiometry (DPX-IQ, Lunar Radiation version 4.5c). Computed tomography scans were obtained as described previously (2, 4). Maximal oxygen consumption (\(\dot{V}O_{2\text{max}}\)) was measured during graded treadmill exercise to exhaustion using open-circuit spirometry (TrueMax 2400, ParvoMedics). Heart rate was determined from lead II of the electrocardiogram, and beat-by-beat arterial pressure was measured in the finger using photoplethysmography (Finapres Medical Systems) and “adjusted” to brachial arterial pressures with an automated device (Dinamap, Critikon) before the injection of vasoactive drugs (see Experimental protocol). Respiration was monitored continuously using a pneumobelt placed around the upper abdomen. Recordings of multunit MSNA values were obtained from the right peroneal nerve using microneurography and were considered acceptable according to published criteria (40). Sympathetic and vagal baroreflex responses were measured using the modified Oxford technique (8) as described previously (2–4).

Experimental protocol. All subjects reported to the laboratory between 7:00 and 11:00 AM following a 12-h overnight fast. Subjects were instructed to refrain from caffeine and alcohol consumption and to avoid participation in any vigorous activity 24 h before testing. After steady-state levels of all variables were achieved, a 10-min recording of basal MSNA was obtained. Subsequently, a bolus injection of sodium nitroprusside (100 \(\mu\)g iv) was given; 60 s later, a bolus injection of phenylephrine HCl (150 \(\mu\)g) was administered. These pharmacological perturbations decreased and increased arterial blood pressure \(\sim 15\) mmHg from baseline levels during a 3-min period. Three trials were completed, and each was separated by a minimum of 15 min of quiet rest.

Data analysis. Commercially available medical imaging software (SliceOmatic, version 4.2, Tomovision) was used to quantify abdominal visceral and subcutaneous fat regions as described previously (2, 4). MSNA, heart rate, arterial blood pressure, and respiration were recorded continuously and digitized at 500 Hz to a laboratory computer for later analysis. Basal MSNA was quantified as burst frequency (bursts/min). MSNA recordings for each subject were also normalized by assigning the largest sympathetic burst under resting conditions to an amplitude of 1,000. All other bursts from that recording were calibrated against that value. Zero nerve activity level was determined from the mean voltage neurogram during a period of silence between sympathetic bursts. Sympathetic baroreflex responses were determined from the relation between MSNA and diastolic blood pressure during vasoactive drug injections. To perform a linear regression between neural activity and diastolic blood pressure, MSNA was binned over 3-mmHg diastolic blood pressure ranges using a segregated signal averaging approach (16). Vagal baroreflex responses were determined from the relation between R-R interval and systolic blood pressure during vasoactive drug infusions. A four-parameter sigmoid was fit to the data to determine vagal baroreflex gain. The gain was calculated after systematic removal of bin values in the threshold and saturation regions as described in detail previously (3). The average of at least two of the three trials performed with each subject was used to determine each individual’s average sympathetic and vagal baroreflex gain. Plasma leptin concentrations were measured by radioimmunoassay (LINCO Research). There is a strong positive relation between plasma leptin concentration and cerebrospinal fluid leptin concentration (\(r = 0.92; P = 0.0001;\) Ref. 32). Thus plasma leptin concentrations are considered a reasonable reflection of brain leptin concentrations.

Statistical analysis. Differences in characteristics of the NO and SUBOB subjects and in the dependent variables were analyzed by independent Student’s t-test. Bivariate correlation analysis was used to assess relationships among variables in the pooled sample. All data are expressed as means \(\pm\) SD. The significance level was set a priori at \(P < 0.05\) for all comparisons for which we hypothesized (MSNA, vagal, and sympathetic baroreflex gain) or expected by design (age, height, systolic and diastolic blood pressure, heart rate, and all expressions of \(\dot{V}O_{2\text{max}}\)) no difference between NO and SUBOB. However, we used a Bonferroni correction to adjust the \(P\) value (\(P < 0.0045\)) for number of \(t\)-tests performed (total = 11) on the body composition- and body fat distribution-related variables (except abdominal visceral fat) and plasma leptin concentration, which we expected to be higher in SUBOB compared with NO men.

RESULTS

Characteristics for SUBOB and NO men. Subject characteristics of NO and SUBOB men are presented in Table 1. Body mass, body mass index, waist circumference measurement, hip circumference measurement, waist-to-hip ratio, body fat percent, total fat mass, lean body mass, subcutaneous abdominal fat measurement, and total abdominal fat mass were greater in SUBOB compared with NO men (all, \(< 0.0045\) except waist-to-hip ratio). \(\dot{V}O_{2\text{max}}\) expressed relative to body weight and in absolute terms tended to be lower (\(P = 0.0278\) and 0.0648, respectively) in SUBOB compared with NO men but not when expressed relative to fat-free mass (\(P = 0.5025\)). Age, height, blood pressure (systolic and diastolic), and heart rate did not differ between the two groups. Abdominal visceral fat was not significantly different in SUBOB and NO men.

Basal MSNA, baroreflex responsiveness, and plasma leptin concentrations in SUBOB and NO men. There were no differences in MSNA burst frequency (23 \(\pm\) 3 vs. 24 \(\pm\) 2 bursts/min; \(P = 0.7007;\) Fig. 1A) in SUBOB and NO men. Vagal baroreflex gain (17.9 \(\pm\) 2.8 vs. 14.9 \(\pm\) 2.0 mmHg/L; \(P = 0.3808\)) and sympathetic baroreflex gain (\(-8.1 \pm 1.0\) vs. \(-8.5 \pm 0.4\) arbitrary integrative units mmHg\(^{-1}\)beat\(^{-1}\); \(P > 0.6447\)) also were not different in SUBOB and NO men. Plasma leptin concentration was significantly higher in SUBOB compared with NO men (7.38 \(\pm\) 5.41 vs. 2.86 \(\pm\) 0.97 ng/ml; \(P = 0.0043;\) Fig. 2B).
Table 1. Subject characteristics of nonobese and subcutaneously obese men

<table>
<thead>
<tr>
<th>Variable</th>
<th>NO</th>
<th>SUBOB</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>22.4±5.3</td>
<td>23.4±6.4</td>
<td>0.6692</td>
</tr>
<tr>
<td>Height, cm</td>
<td>180.6±10.4</td>
<td>181.0±8.9</td>
<td>0.9189</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>70.6±6.8</td>
<td>94.2±12.8</td>
<td>0.0000</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>21.7±1.8</td>
<td>28.7±2.4</td>
<td>0.0000</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>79.8±5.1</td>
<td>97.1±8.5</td>
<td>0.0000</td>
</tr>
<tr>
<td>Hip circumference, cm</td>
<td>97.3±4.6</td>
<td>110.5±7.7</td>
<td>0.0000</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.82±0.04</td>
<td>0.88±0.05</td>
<td>0.0047</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>16.0±4.9</td>
<td>26.2±6.4</td>
<td>0.0002</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>11.5±4.2</td>
<td>24.8±7.2</td>
<td>0.0000</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>55.9±4.3</td>
<td>66.3±9.0</td>
<td>0.0009</td>
</tr>
<tr>
<td>Abdominal subcutaneous fat, cm²</td>
<td>131.7±47.2</td>
<td>306.9±107.9</td>
<td>0.0000</td>
</tr>
<tr>
<td>Abdominal visceral fat, cm²</td>
<td>55.1±18.3</td>
<td>68.5±20.2</td>
<td>0.1075</td>
</tr>
<tr>
<td>Total abdominal fat, cm²</td>
<td>186.8±61.5</td>
<td>375.4±112.2</td>
<td>0.0000</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>114.7±7.9</td>
<td>119.2±7.9</td>
<td>0.1820</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>66.1±6.5</td>
<td>62.7±7.7</td>
<td>0.2577</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>63.3±8.1</td>
<td>61.0±9.7</td>
<td>0.5137</td>
</tr>
<tr>
<td>VO₂max, ml·kg⁻¹·min⁻¹</td>
<td>52.4±6.6</td>
<td>45.0±8.9</td>
<td>0.0278</td>
</tr>
<tr>
<td>VO₂max, ml·kg·fat-free mass⁻¹·min⁻¹</td>
<td>65.9±7.3</td>
<td>63.5±10.0</td>
<td>0.5025</td>
</tr>
<tr>
<td>VO₂max, l/min</td>
<td>3.69±0.5</td>
<td>4.18±0.7</td>
<td>0.0648</td>
</tr>
</tbody>
</table>

All values are means ± SE; n = 15 nonobese (NO) men and 9 subcutaneously obese (SUBOB) men. VO₂max, maximal oxygen consumption.

Body composition and anthropometric values, and abdominal fat distribution-related correlates. Abdominal visceral fat was the only significant body composition- or abdominal fat distribution-related correlate of MSNA (r = 0.45; P < 0.0285; Fig. 2A) in the pooled sample (n = 24). In addition, abdominal visceral fat was related to MSNA in NO (r = 0.58; P = 0.0239; Fig. 2B) but not SUBOB (r = 0.39; P = 0.3027) men. Abdominal visceral fat was also correlated with vagal baroreflex gain (r = 0.49; P = 0.0309), but the relation was biased by one SUBOB individual with a particularly high vagal baroreflex gain (~35 ms/mmHg). The relation was no longer significant (P = 0.0820) after this individual’s data was removed from the analysis. This individual’s data had no impact on the relation between abdominal visceral fat and MSNA. There were no significant correlates of sympathetic gain in these men.

Plasma leptin concentration was correlated with all indexes of total adiposity (r = 0.70–0.73; all, P ≤ 0.0001). In addition, plasma leptin concentration was correlated with total abdominal fat (r = 0.86; P < 0.0001) and subcutaneous abdominal fat (r = 0.87; P < 0.0000). However, plasma leptin concentration was not significantly related to abdominal visceral fat (r = 0.40; P = 0.0530) or MSNA (r = 0.16; P = 0.4567).

**DISCUSSION**

There are two important new findings from the present study. First, MSNA was similar in SUBOB and NO men with comparable levels of abdominal visceral fat despite ~2.6-fold higher plasma leptin concentrations in the SUBOB men. Second, abdominal visceral fat was the only significant body composition- or abdominal fat distribution-related correlate of MSNA in these men. Importantly, the relation between abdominal visceral fat and MSNA was evident in NO men but not in
SUBOB men and at levels of abdominal visceral fat below the level typically associated with elevated cardiovascular and metabolic disease risk. Taken together with our previous observations (2), the results of the present study suggest that the association between obesity and MSNA is phenotype dependent.

The mechanism(s) linking specific adipose tissue depots with sympathetic neural activity in humans remains unclear. However, there are several possibilities. First, leptin, the product of the ob gene, is secreted from adipocytes in proportion to fat mass, circulates in the blood, crosses the blood-brain barrier, and acts on hypothalamic neuronal targets to alter energy intake and expenditure (1). Leptin also exerts an important influence on cardiovascular and renal function in experimental animals that is sympathetically mediated (15, 17). Thus hyperleptinemia may be a potential “adiposity signal” contributing to elevated MSNA in obese humans. However, the results of human studies are conflicting; some (26, 33) but not all (27) support a relation between plasma leptin concentration and MSNA. Importantly, leptin expression and secretion is higher in subcutaneous compared with visceral adipocytes (30, 37) and circulating concentrations of the protein are higher in subcutaneous compared with visceral obesity (22). In the present study, we observed a closer association of plasma leptin concentrations with abdominal subcutaneous fat than with abdominal visceral fat. In addition, MSNA was not elevated in SUBOB compared with NO men despite an ~2.6-fold higher plasma leptin concentration in the former. Consistent with this observation, Lambert et al. (21) have reported similar levels of subcortical brain noradrenergic activity in obese compared with NO humans. Taken together, these observations do not support an obvious role for leptin in contributing to sympathetic neural activation and, in turn, are inconsistent with the concept of selective leptin resistance in human obesity (23). Future studies are necessary to confirm or refute our findings.

Second, angiotensinogen expression and secretion is greater in visceral compared with subcutaneous adipocytes (36), and angiotensinogen is an important factor for the formation of angiotensin II. Furthermore, plasma angiotensinogen concentrations are elevated in obesity (35), and angiotensin II stimulates central sympathetic outflow in humans (24). Thus it is possible that angiotensin II could increase MSNA in individuals with visceral obesity. There is presently no information available that directly addresses this issue. However, the observation that angiotensin II receptor blockade reduces MSNA in obese hypertensive humans (13) is consistent with this concept. The similar levels of MSNA in SUBOB and NO men in the present study may be related to their comparable renin-angiotensin system activity.

Third, Bjorntorp et al. (5) hypothesized that hypertension and the metabolic syndrome have a closely related central origin resulting from parallel activation of the hypothalamic-pituitary axis and the SNS (i.e., a hypothalamic arousal syndrome). In addition, visceral obesity is more closely associated with hypothalamic-pituitary axis dysfunction than subcutaneous obesity (29). Therefore, it is possible that hypothalamic-pituitary axis function may be important in understanding the influence of obesity phenotype on MSNA. Interestingly, Grassi et al. (14) reported greater reductions in MSNA after 1 wk of dexamethasone treatment in obese compared with NO normotensive individuals. Unfortunately, regional fat distribution was not assessed in this study. Thus, although intriguing, future studies are necessary to define the role of the hypothalamic-pituitary axis in obesity.

Finally, it is possible that other factors or a combination of factors are acting collectively or synergistically to influence sympathetic neural outflow in different obesity phenotypes. In addition, it is conceivable that a third factor, e.g., hypothalamic-pituitary axis (dys)function, could result in visceral obesity and sympathetic neural activation (5). However, visceral obesity and sympathetic neural activity may be only indirectly related. Future studies are necessary to determine the mechanisms that link specific adipose tissue depots and sympathetic neural activity in humans.

There are some limitations of the present study that should be considered. First, the present study was cross sectional in design. Thus it is possible that genetic or other factors could contribute to our observations. Future interventional studies are necessary to confirm or refute our observations.

Second, the sample sizes in the present study were small. Therefore, the inclusion of a larger number of subjects in each group may yield a different outcome.

Third, we studied only healthy young men who were free of overt cardiovascular and metabolic diseases. Thus these findings should not be generalized beyond the population studied. Additional studies are necessary to determine whether sympathetic neural activation is obesity phenotype dependent in women, older adults, or obese hypertensive individuals. Age- and gender-related differences in MSNA do appear to depend at least in part on age- and gender-related differences in abdominal adiposity (i.e., waist-to-hip ratio and waist circumference, respectively; Refs. 18–20).

Finally, obesity is an important risk factor for obstructive sleep apnea, and its prevalence appears to be more closely associated with abdominal visceral fat than with total adiposity (38, 41). In addition, Narkiewicz et al. (28) reported that sympathetic neural activation is present only in obese individuals with evidence of obstructive sleep apnea. Therefore, it is possible that a lower prevalence of sleep-disordered breathing in the SUBOB men in the present study could account for their relatively “normal” levels of MSNA. However, we believe it is unlikely that the presence of obstructive sleep apnea could simultaneously explain the relation between abdominal visceral fat and MSNA particularly among the NO men in the present study. Future studies are necessary to address this issue.

In summary, the results of the present study suggest that MSNA is not elevated in SUBOB compared with NO men despite an ~2.6-fold higher plasma leptin concentration in the former. Furthermore, the relation between abdominal visceral fat and MSNA is evident at levels of abdominal visceral fat below that typically associated with elevated cardiovascular and metabolic disease risk, but such a relation is not obvious in SUBOB men. Taken together with our previous observations (2), our findings suggest that the association between obesity and MSNA is phenotype dependent. Our observations do not support an obvious role for leptin in contributing to sympathetic neural activation in human obesity and, in turn, are inconsistent with the concept of selective leptin resistance.
SYMPATHETIC NEURAL ACTIVITY IN SUBCUTANEOUS OBESITY

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