Modest weight gain is associated with sympathetic neural activation in nonobese humans

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Gentile CL, Orr JS, Davy BM, Davy KP. Modest weight gain is associated with sympathetic neural activation in nonobese humans. Am J Physiol Regul Integr Comp Physiol 292: R1834–R1838, 2007. First published January 11, 2007; doi:10.1152/ajpregu.00876.2006.—We tested the hypothesis that modest, overfeeding-induced weight gain would increase sympathetic neural activity in nonobese humans. Twelve healthy males (23 ± 2 years; body mass index, 23.8 ± 0.7 kg/m²) were overfed -1,000 kcal/day until a 5-kg weight gain was achieved. Muscle sympathetic nerve activity (MSNA, microneurography), blood pressure, body composition (dual energy X-ray absorptiometry), and abdominal fat distribution (computed tomography) were measured at baseline and following 4 wk of weight stability at each individual’s elevated body weight. Overfeeding increased body weight (73.5 ± 3.1 vs. 78.4 ± 3.2 kg, P < 0.001) and body fat (14.9 ± 1.2 vs. 18 ± 1.1 kg, P < 0.001) in 42 ± 8 days. Total abdominal fat increased (220 ± 22 vs. 266 ± 22 cm², P < 0.001) with weight gain, due to increases in both subcutaneous (158 ± 15 vs. 187 ± 12 cm², P < 0.001) and visceral fat (63 ± 8 vs. 79 ± 12 cm², P = 0.004). As hypothesized, weight gain elicited increases in MSNA burst frequency (32 ± 2 vs. 38 ± 2 burst/min, P = 0.002) and burst incidence (52 ± 4 vs. 59 ± 3 bursts/100 heart beats, P = 0.026). Systolic, but not diastolic blood pressure increased significantly with weight gain. The change in MSNA burst frequency was correlated with the percent increase in body weight (r = 0.59, P = 0.022), change in body fat (r = 0.52, P = 0.043) and percent change in body fat (r = 0.51, P = 0.045). The results of the current study indicate that modest diet-induced weight gain elicits sympathetic neural activation in nonobese males. These findings may have important implications for understanding the link between obesity and hypertension.

adiposity; autonomic nervous system; overfeeding

THE SYMPATHETIC NERVOUS SYSTEM (SNS) plays a pivotal role in cardiovascular and metabolic homeostasis. Sympathetic neural activation is characteristic of numerous cardiovascular disorders and is associated with adverse clinical outcomes in individuals with chronic heart failure and essential hypertension (4, 15, 26). In addition, pharmacological inhibition of SNS activity is a common therapeutic approach for reducing risk in these populations (23, 24).

There has been considerable controversy in the past regarding the effects of obesity on SNS behavior (37). The results of more recent studies (2, 10, 11, 27, 28, 36) consistently reveal higher muscle sympathetic nerve activity (MSNA) in obese compared with nonobese individuals. However, Huggett et al. (14) reported that MSNA was nearly identical in normal weight and overweight men, whereas obese diabetic men displayed 50% higher levels. Therefore, whether modest weight gain increases sympathetic neural activity in humans is unclear.

Masu et al. (19, 20) have reported that plasma norepinephrine concentrations increase following weight gain in Japanese men. While these findings from an observation study suggest that sympathetic neural activation occurs with modest weight gain, plasma norepinephrine concentrations are limited as a measure of sympathetic neural activity because they are influenced by the sampling site, as well as norepinephrine appearance and clearance from the circulation (7). Furthermore, unlike micrography, plasma norepinephrine concentrations do not provide a direct measure of sympathetic neural activity (8). Thus, future studies are necessary to clarify and more directly address this issue.

Accordingly, the purpose of the present study was to determine whether modest weight gain increases directly measured MSNA in healthy nonobese humans. We hypothesized that overfeeding-induced weight gain would increase MSNA in these individuals. Furthermore, in light of our previous observations suggesting that visceral obesity is an important adipose tissue depot linking obesity and sympathetic neural activation (1, 2), we also sought to determine whether the increase in MSNA with weight gain, if observed, would be related to the amount of visceral fat gain.

METHODS

Subjects. Twelve young, nonobese males volunteered for the study. They were non-smokers, active, free of overt chronic disease, and not taking any medications. All subjects ranged from sedentary to recreationally active and were weight stable (± 2 kg) for at least 6 mo prior to beginning the study. The nature, purpose, risks, and benefits were explained before obtaining informed consent. The Virginia Tech, Human Subjects Committee approved all experimental protocols.

Experimental design and protocol. Following baseline testing, subjects were overfed ~1,000 kcal/day for 6–8 wk until a 5-kg weight gain was achieved. Excess calories were provided using a liquid dietary supplement (Boost Plus; Novartis Nutrition Corp; 34% fat, 50% CHO, 16% protein). Progress was assessed by weekly body weight measurements and meetings with a research dietitian (BMD). Each individual was studied at baseline and after 4 wk of weight stability. For all testing sessions, subjects reported to the laboratory between 7 and 11 AM following an overnight fast and having refrained from caffeine and exercise for the preceding 24 h. Following posttesting, subjects were provided with...
dietary and physical activity recommendations and, if desired, meal replacement products to facilitate weight loss.

Body mass and height were measured with a digital balance scale and stadiometer, respectively. Body composition was measured via dual-energy X-ray absorptiometry (GE Lunar Prodigy Advance, Madison, WI) using software version 8.10e. Computed tomography scans (HiSpeed CT/i, GE Medical) were performed to quantify abdominal fat distribution. Maximal oxygen consumption (\(\text{VO}_{2\max}\)) was measured during graded treadmill exercise to exhaustion using open-circuit spirometry (TrueMax 2400, ParvoMedics). Resting blood pressure measurements were made in the seated position via mercury and automated (Colin Press-Mate 8800) sphygmomanometry following a 15-min period of rest. Measurements were obtained on at least three separate visits over a 2-wk period until stability was achieved (±6 mmHg difference on sequential measurements). Recordings of microneurographic technique, as described previously (5). Neurograms were considered acceptable as recordings of efferent MSNA according to previously published criteria (35). During the microneurographic recordings, resting heart rate was determined from lead II of an electrocardiogram; beat-by-beat arterial pressure was measured by finger photoplethysmography (TNO Biomedical Instrumentation), and respiration was monitored using a pneumobelt. Plasma leptin and insulin concentrations were measured using commercially available ELISA kits (LINCO Research). Plasma renin activity was measured by radioimmunoassay (DiaSorin). Urinary sodium concentration was measured from a 24-h collection using a Synchron LX20 Clinical Chemistry Analyzer (Beckman Coulter).

MSNA, heart rate, arterial blood pressure, and respiration were recorded continuously and digitized at 500 Hz for subsequent analysis using signal processing software (Windaq, Dataq Instruments). Neurograms were analyzed in a blinded manner by a single investigator (K. P. Davey). MSNA was quantified as both burst frequency (bursts/min) and burst incidence (bursts/100 beats).

**Statistical analysis.** Differences in subject characteristics and dependent variables before and after weight gain were assessed with paired Student's t-tests. Although normality tests were performed, their validity with small sample sizes is uncertain. Nonetheless, comparisons using Wilcoxon signed rank tests yielded similar outcomes. Relations among variables of interest were assessed using simple and partial correlation analysis. All data are expressed as means ± SE. The significance level was set a priori at \(P < 0.05\).

**RESULTS**

Subject characteristics before and after weight gain are shown in Table 1. Overfeeding resulted in 5.0 ± 0.1 kg (range = 4.2–5.7 kg) of body weight gain in 42 ± 8 days. Total body fat and lean body mass increased 3.2 ± 0.5 kg (\(P < 0.001\)) and 1.4 ± 0.4 kg (\(P = 0.002\)), respectively. Total abdominal fat increased significantly (24 ± 6%, \(P < 0.001\)), due to increases in both subcutaneous (23 ± 6%, \(P < 0.001\)) and visceral fat (25 ± 7%, \(P = 0.002\)). Maximal oxygen consumption expressed relative to body weight declined with weight gain (45.5 ± 1.8 vs. 43.8 ± 1.6 ml·kg\(^{-1} \cdot \text{min}^{-1}\), \(P = 0.017\)), whereas there was no significant change when expressed in absolute terms (3.34 ± 0.23 vs. 3.42 ± 0.24 l/min, \(P > 0.05\)) or relative to fat-free mass (59.5 ± 1.8 vs. 59.7 ± 1.8 ml·FFM\(^{-1} \cdot \text{min}^{-1}\), \(P > 0.05\)). Systolic blood pressure (\(P = 0.02–0.009\)) but not diastolic blood pressure (\(P > 0.05\)) increased with weight gain, whereas resting heart rate tended to increase (62 ± 2 vs. 65 ± 3 beats/min, \(P = 0.09\)). As hypothesized, weight gain elicited increases in MSNA burst frequency (32 ± 2 vs. 38 ± 2 burst/min, \(P = 0.002\), Fig. 1A) and burst incidence (52 ± 4 vs. 59 ± 3, \(P = 0.026\), Fig. 1B).

**Table 1. Subject Characteristics at baseline and following weight gain**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Weight Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>23±2</td>
<td>—</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>73.5±3.1</td>
<td>78.4±3.2*</td>
</tr>
<tr>
<td>Body mass index, kg/m(^2)</td>
<td>23.8±0.7</td>
<td>25.4±0.7*</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>21±1.4</td>
<td>24.1±1.3*</td>
</tr>
<tr>
<td>Total fat mass, kg</td>
<td>14.9±1.2</td>
<td>18±1.1*</td>
</tr>
<tr>
<td>Lean body mass, kg</td>
<td>55.8±2.6</td>
<td>57.2±2.8*</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>83.9±2.1</td>
<td>89.5±2.8*</td>
</tr>
<tr>
<td>Total abdominal fat, cm(^2)</td>
<td>220±22</td>
<td>266±22*</td>
</tr>
<tr>
<td>Abdominal subcutaneous fat, cm(^2)</td>
<td>158±15</td>
<td>187±12*</td>
</tr>
<tr>
<td>Abdominal visceral fat, cm(^2)</td>
<td>63±8</td>
<td>79±12*</td>
</tr>
<tr>
<td>(\text{VO}_{2\max}), ml/kg/min</td>
<td>45.5±1.8</td>
<td>43.8±1.6*</td>
</tr>
<tr>
<td>(\text{VO}_{2\max}), l/min</td>
<td>3.3±0.2</td>
<td>3.4±0.2</td>
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<td>Heart rate, beats/min</td>
<td>62±2</td>
<td>65±3</td>
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<tr>
<td>Automated SBP, mmHg</td>
<td>122±2</td>
<td>125±2*</td>
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<td>Automated DBP, mmHg</td>
<td>67±2</td>
<td>68±2</td>
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<tr>
<td>Manual SBP, mmHg</td>
<td>114±3</td>
<td>118±3*</td>
</tr>
<tr>
<td>Manual DBP, mmHg</td>
<td>75±2</td>
<td>75±2</td>
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<tr>
<td>Leptin, ng/ml</td>
<td>3.28±0.59</td>
<td>5.67±0.80*</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>23.2±4.0</td>
<td>26.7±2.5</td>
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<td>Plasma renin activity, ng/ml/hr</td>
<td>1.2±0.2</td>
<td>2.0±0.3*</td>
</tr>
<tr>
<td>Urinary sodium, mmol/l</td>
<td>157.7±13.9</td>
<td>143.9±16.5</td>
</tr>
</tbody>
</table>

All values are expressed as means ± SE. *\(P < 0.05\) vs. baseline. SBP, systolic blood pressure; DBP, diastolic blood pressure.

Plasma leptin concentrations (3.28 ± 0.59 vs. 5.67 ± 0.80 ng/ml, \(P < 0.001\)) and plasma renin activity (1.2 ± 0.2 vs. 2.0 ± 0.5 ng·ml\(^{-1} \cdot \text{h}^{-1}\), \(P = 0.022\)) both increased following weight gain, whereas plasma insulin concentrations did not increase significantly (23.2 ± 4.0 vs. 26.7 ± 2.5 pmol/l, \(P = 0.271\)). Urinary sodium excretion did not change following weight gain (137.7 ± 13.9 vs. 143.9 ± 16.5 mmol/l, \(P = 0.292\)).

There was no correlation between MSNA burst frequency and blood pressure (all measures) at baseline. However, MSNA burst frequency following weight gain was correlated with diastolic BP measured with mercury (\(r = 0.70, P = 0.011\)) and automated (\(r = 0.61, P = 0.034\)) sphygmonanometry. In addition, MSNA burst frequency at baseline was correlated with the change in MSNA burst frequency with weight gain (\(r = -0.62, P = 0.030\)) but not to the change in systolic or diastolic BP (all measures).

The change in MSNA burst frequency following weight gain was positively correlated with percent change in body weight (\(r = 0.59, P = 0.022\), Fig. 2A), change in body fat (\(r = 0.52, P = 0.043\), Fig. 2B) and percent change in body fat (\(r = 0.51, P = 0.045\), Fig. 2C) and negatively correlated with the change in lean body mass (\(r = -0.56, P = 0.029\)). The correlation between the change in MSNA burst frequency and automated systolic blood pressure did not achieve statistical significance (\(r = 0.43, P = 0.08\)). The change in MSNA burst incidence was correlated only with the percent change in body weight (\(r = 0.56, P = 0.029\)). There was no correlation between the increase in MSNA and changes in plasma leptin or insulin concentrations or plasma renin activity (all \(P > 0.05\)).

**DISCUSSION**

The novel finding of the present study was that modest, diet-induced weight gain increased sympathetic neural activity ~15–20% in healthy, nonobese males. The magnitude of
increase in MSNA was correlated with the magnitude of body weight and fat gain and was accompanied by increases in resting blood pressure. These results are consistent with cross-sectional studies that have reported higher MSNA in obese compared with nonobese individuals, (2, 10, 11, 27, 28, 36) and with intervention studies demonstrating reductions in MSNA following weight loss in obese individuals (9, 29, 32).

We have extended those previous observations by demonstrating that even modest increases in body weight and body fat elicit sympathetic neural activation in nonobese humans. In the present study, the increase in MSNA with weight gain was correlated with the magnitude of body weight and fat gain. Importantly, the increase in MSNA with weight gain was observed after 4 wk of weight stability, thus avoiding the acute aftereffects of overfeeding on SNS activity (22).

The increase in MSNA with weight gain was not obviously related to increases in visceral fat. This latter observation is in contrast to our previous observations suggesting that visceral obesity is an important adipose tissue depot linking obesity and sympathetic neural activation in humans (1, 2). The reasons for this discrepancy are unclear, but our small sample size and inclusion of only nonobese subjects in the present study might contribute. It is also possible that much larger increases in visceral fat with weight gain are necessary to contribute importantly to the sympathetic neural activation associated with weight gain.

Although there is considerable evidence indicating that the SNS plays an important role in the etiology of obesity hypertension (6, 12), the increases in MSNA and blood pressure in the present study were not correlated. Thus, it is possible that increases in MSNA (i.e., sympathetic neural activity to skeletal muscle arterioles) are not causally linked to blood pressure elevation with weight gain. Muscle sympathetic neural activity may not be directly involved in the pathogenesis of obesity hypertension, but rather, may serve as a marker for sympathetic activation to the kidney. It is important to note that renal sympathetic nerve activity is increased in obese compared with lean individuals (25, 33), and activation of renal sympathetic nerve activity is known to play a critical role in long-term blood pressure regulation (13). That plasma renin activity increased with weight gain in the present study is consistent with this view.

Alternatively, Charkoudian et al. (3) have reported that vascular adrenergic responsiveness is reduced in individuals with elevated MSNA. In the present study, we observed a significant inverse relation between baseline MSNA and the change in MSNA with weight gain. Thus, blunted vascular adrenergic responsiveness may have limited the rise in blood

Fig. 1. A: individual responses and average MSNA burst frequency (bursts/min) at baseline and following weight gain. B: individual responses and average MSNA burst incidence (bursts/100 beats) at baseline and following weight gain. (*P < 0.05 vs. baseline).

Fig. 2. A: relation between the change in MSNA (bursts/min) with weight gain and percent change in body weight. B: relation between the change in MSNA (bursts/min) with weight gain and the change in body fat (kilograms). C: relation between the change in MSNA (bursts/min) with weight gain and change in percent body fat. (*P < 0.05 vs. baseline).
pressure with weight gain in individuals with higher MSNA at baseline. Taken together, these observations may provide an explanation, at least in part, for the lack of association between the change in MSNA and the change in blood pressure with weight gain.

The mechanism(s) by which weight gain elicits sympathetic neural activation remains unclear. Landsberg (16) hypothesized that the increase in SNS activity with weight gain serves the homeostatic role of stimulating thermogenesis to prevent further weight gain. Indeed, an increase in energy expenditure is a recognized consequence of weight gain (18) and, taken together, our present findings constitute indirect support of Landsberg’s hypothesis. However, Landsberg further postulated that a diet-induced increase in plasma insulin concentration was the primary mechanism mediating weight gain-induced sympathetic neural activation (17). In contrast, the lack of an increase in plasma insulin concentrations in our study precludes us from drawing the same conclusion. Although the reason(s) for this discrepancy is unclear, the measurement of plasma insulin concentration following a period of weight stability may be important. In an attempt to gain insight into other possible mechanisms of sympathetic activation in the current study, we measured plasma concentrations of leptin and renin activity before and after weight gain. Although both increased significantly following weight gain, the changes were not correlated with the increases in MSNA. Future studies will be necessary to determine the mechanisms mediating sympathetic neural activation following weight gain.

There are some limitations of the present study that should be considered. We did not include a control group and our sample size was relatively small. However, several lines of evidence suggest it is unlikely that the changes we observed in MSNA were random deviations that occurred over time rather than as a result of the imposed weight gain. First, the magnitude of increase in MSNA in the present study is consistent with that which would be predicted from studies involving weight loss (9, 29, 32). Second, the increases in MSNA with weight gain in the present study are considerably larger than the error in measurement (8, 34). Finally, 10 of the 12 subjects (83%) experienced increases in MSNA, and the change in MSNA with weight gain was positively correlated with the manipulated variable (i.e., body weight/body fat). Taken together, these observations are consistent with our conclusion that the increases in MSNA observed in the present study were a direct result of the experimental weight gain.

The subjects were limited to young, nonobese males. The results of previous studies suggest that gender (30) and age (31) may affect the magnitude of blood pressure elevation with weight gain. Therefore, the sympathetic neural adjustments to weight gain may also be different in magnitude in females or older subjects. As such, our findings should not be extrapolated beyond the population studied.

Sympathetic nervous system activity is regulated in a highly region-specific manner (21) and, as such, it is possible that the effects of weight gain on sympathetic outflow to other organs or tissues may be quantitatively or qualitatively different. That heart rate failed to increase significantly in the present study could be interpreted to be consistent with previous data suggesting that cardiac sympathetic activity is not elevated in obese individuals (25, 33). We should emphasize, however, that the increase in heart rate following weight gain is believed to be mediated primarily by a reduction in parasympathetic outflow to the heart (6, 12).

The deliberate weight gain in the present study may not reflect the more gradual changes that occur under free-living conditions in the general population. As such, our findings should be considered with this mind.

In summary, the results of the current study indicate that modest overfeeding-induced weight gain produces sympathetic neural activation in nonobese males and that the extent of sympathetic neural activation is related to the magnitude of body weight and fat gain. These findings suggest that individuals who gain even modest amounts of weight may experience increases in SNS activity regardless of whether they become obese. If left untreated, sustained activation of the SNS may contribute to the development of hypertension and other cardiovascular disorders. Importantly, overfeeding-induced weight gain in humans may provide an insightful model to investigate the mechanism(s) mediating weight gain-induced sympathetic neural activation.

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WEIGHT GAIN AND SYMPATHETIC NEURAL ACTIVATION