Published in final edited form as:

Clin Auton Res. 2014 April ; 24(2): 77–85. doi:10.1007/s10286-014-0235-0.

Relationship of muscle sympathetic nerve activity to insulin sensitivity

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Abstract

Purpose—An association between insulin resistance and activation of the sympathetic nervous system has been reported in previous studies. However, potential interactions between insulin sensitivity and sympathetic neural mechanisms in healthy people remain poorly understood. We conducted a study to determine the relationship between sympathetic activity and insulin resistance in young, healthy humans.

Methods—Thirty-seven healthy adults (18–35 years, BMI $>28 \text{ kg m}^{-2}$) were studied. Resting muscle sympathetic nerve activity (MSNA) was measured with microneurography and

insulin sensitivity of glucose and free fatty acid metabolism was measured during a hyperinsulinemic-euglycemic clamp with two levels of insulin.

Results—During lower doses of insulin, we found a small association between lower insulin sensitivity and higher MSNA ($P > 0.05$) but age was a cofactor in this relationship. Overall, we found no difference in insulin sensitivity between groups of low and high MSNA, but when women were analyzed separately, insulin sensitivity was lower in the high MSNA group compared with the low MSNA group of women.

Conclusions—These data suggest that MSNA and insulin sensitivity are only weakly associated with young healthy individuals and that age and sex may be important modifiers of this relationship.

Keywords

Sympathetic nervous system; Insulin resistance; Glucose clamp technique; Body composition

Introduction

The prevalence of insulin resistance has been increasing with the worldwide increase in overweight and obesity. Sympathetic nervous system activity is elevated in individuals who are insulin resistant, particularly in those whose insulin resistance is associated with abdominal adiposity [9]. The cause of the increase in sympathetic activity in these individuals is not known, but central activation of the sympathetic nervous system by elevated insulin and/or FFA concentrations [23, 36] could play a role. It has also been suggested that greater levels of sympathetic activity lead to insulin resistance [19] and contribute to the development or worsening of comorbidities such as hypertension [23]. This suggests the potential for a “vicious cycle” in which insulin initiates sympathetic activity and leads to insulin resistance thereby further increasing circulating insulin.

The relationship between insulin sensitivity and sympathetic activity in young, lean, healthy subjects has not been previously directly investigated. The present study was therefore conducted to test the hypothesis that greater levels of muscle sympathetic nerve activity (MSNA) are associated with lower insulin sensitivity in healthy men and women.

Methods

The Mayo Clinic Institutional Review Board approved the study, subjects gave written, informed consent, and the studies were therefore performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. The study was a prospective, non-randomized study of the relationship between sympathetic activity and insulin sensitivity in healthy young subjects. A total of 37 subjects were recruited from Rochester, MN and the surrounding area by advertisements (21 female and 16 male). Inclusion criteria were age 18–35 years, BMI $< 28 \text{ kg m}^{-2}$, and healthy, as determined by a medical history and physical examination by a physician. Subjects refrained from exercise, alcohol, and caffeine for at least 24 h prior to the study day. Studies were conducted at the Mayo Clinic Clinical Research Unit (CRU), Rochester, MN.

Body composition measurements were performed prior to the study day. Dual-energy X-ray absorptiometry (DEXA) scans were performed and computed tomography was used to measure visceral and thigh fat content from single slices [17]. A weight-maintenance, balanced diet was consumed for 3 days prior to the study day under the supervision of the CRU research dietitians. Subjects were admitted to the CRU at 1700 the day before the study and fasted overnight except for water.

The study day began at 0630. Subjects were studied in a supine position at rest in a climate controlled room. A 20 gauge brachial arterial catheter was placed for obtaining blood samples and beat-to-beat measurements of blood pressure under aseptic conditions using ultrasound guidance after local anesthesia. An intravenous catheter was placed in the contralateral arm for drug administration. A three-lead ECG was used to measure heart rate.

Muscle sympathetic nerve activity (MSNA) was measured via microneurography of the peroneal nerve posterior to the fibular head [37]. The signal was amplified, bandpass filtered, rectified, and integrated (662C-4 Nerve Traffic Analysis System, University of Iowa, Iowa City, IA) and then recorded at 250 Hz (WinDaq, DATAQ Instruments, Akron, OH). Sympathetic bursts in the integrated neurogram were identified in the recorded data using an automated analysis program [21] that assigns each sympathetic burst to the appropriate cardiac cycle by compensating for latency. The automated analysis was then reviewed and corrected manually by individuals who were blinded to the specifics of the study day. In addition to the criteria included in the automated program [21], a minimum of a 3:1 signal to noise ratio was used for burst confirmation. MSNA is reported as bursts min^{-1} (burst frequency) and bursts 100 heartbeats $^{-1}$ (burst incidence).

After completion of the MSNA measurements, the electrodes were removed and a hyperinsulinemic, euglycemic clamp was performed for measurements of glucose and FFA kinetics [3]. An infusion of [^3H]-palmitate ($\sim 0.3 \mu\text{Ci min}^{-1}$) and a primed ($2.4 \text{ mg kg FFM}^{-1}$) infusion ($0.04 \text{ mg kg FFM}^{-1} \text{ min}^{-1}$) of [^2H]-glucose were started before the clamp at 0900 and 0630, respectively, and were continued throughout. At 0930, an infusion of insulin was started at $0.25 \text{ mU kg FFM}^{-1} \text{ min}^{-1}$ and continued for 3 h. At 1,230, the insulin infusion was increased to $1.0 \text{ mU kg FFM}^{-1} \text{ min}^{-1}$ for 3 h. These doses were chosen as they provide graded physiological responses to insulin in healthy individuals [3]. Plasma glucose was measured every 10–15 min during the infusions (GL5, Analox Instruments, London, UK) and a target plasma glucose concentration of $90 \pm 5 \text{ mg dL}^{-1}$ ($5.0 \pm 0.3 \text{ mmol L}^{-1}$) was maintained using an infusion of 40 % dextrose with [^2H]-glucose.

Blood samples were drawn prior to starting the clamp and during the final thirty min of each dose of insulin (steady state) for measurement of plasma renin, leptin, insulin, aldosterone, catecholamines, total FFA, palmitate, glucose, [^2H]-glucose, insulin, and catecholamine concentrations, and palmitate specific activity. Samples were analyzed by the Immunochemistry Core Laboratory of the CRU of the Mayo Clinic CTSA and the Endocrine Laboratory of the Mayo Clinic Department of Laboratory Medicine and Pathology.

High performance liquid chromatography was used to measure total FFA and palmitate concentrations and palmitate specific activity [27]. Gas chromatography–mass spectrometry was used to determine plasma [^2H]-glucose concentration and enrichment [35]. Glucose rate of disappearance (R_d) and endogenous glucose production (EGP) at steady state were calculated and normalized to lean body mass [39]. Palmitate rate of appearance (R_a) was calculated using mean specific activities [26].

Regression analysis was performed on MSNA and demographic and clinical characteristics including measures of fat distribution and neuroendocrine hormone levels to determine if MSNA was related to any physical characteristics of the subjects. To assess the association of baseline MSNA and baroreflex sensitivity with glucose and FFA kinetics during the clamp, linear or logarithmic regression was performed and Pearson's product moment correlation was calculated. Adjusted regression analyses between MSNA and insulin sensitivity measurements were also performed for age, sex, total body fat, and visceral fat. The subjects were then separated into two groups based on the median of the observed MSNA distribution and analysis of variance (ANOVA) was used to test for differences in glucose and FFA kinetics between groups. Where significant differences were observed, two-sample t tests were used to test for differences between groups. The primary outcome variable of the study was the difference in insulin sensitivity measurements including the glucose infusion rate (GIR) needed to maintain euglycemia, EGP, plasma FFA, and glucose and palmitate R_a . Subgroup analyses of insulin sensitivity were performed between the top and bottom tertiles of subjects based on resting MSNA, as has been previously reported [2, 31]. For all of these analyses, MSNA was analyzed as burst frequency except where specified otherwise. All data are presented as mean $\pm$ SEM and $\alpha = 0.05$ was used for statistical significance. The difference in glucose turnover between lean and upper body obese men has been shown to be $2.9 \text{ mg kg}^{-1} \text{ FFM min}^{-1}$ with a pooled standard deviation of $1.4 \text{ mg kg}^{-1} \text{ FFM min}^{-1}$ [33]. For the present study, we assumed that the standard deviation of glucose turnover would be similar however we expected a smaller difference between those with “high” versus “low” MSNA. Based on this assumption, we determined that a sample size of at least $N = 15$ subjects per group would be needed to provide statistical power in this study (two-tailed, $\alpha = 0.05$) of at least 80 % to detect a difference of glucose turnover of $1.5 \text{ mg kg}^{-1} \text{ FFM min}^{-1}$ between groups.

Results

Thirty-seven subjects (21 female and 16 male; age range 18–45 years) were successfully studied. Subject characteristics, including body composition and fasting laboratory values, are shown in Table 1. There was no difference in age, resting HR, or diastolic or mean blood pressure between men and women. Women had significantly greater total body fat and leptin concentrations. Women had lower visceral fat but there was no difference in trunk to leg fat ratios. Plasma glucose concentrations were slightly lower in females but there was no difference in plasma insulin concentrations.

The average resting MSNA for all subjects was $15 \pm 1 \text{ burst min}^{-1}$ (burst frequency) and $23 \pm 2 \text{ bursts 100 heartbeats}^{-1}$ (burst incidence). There was no difference in MSNA between men and women. There was a small, but significant relationship, between MSNA and

arterial plasma norepinephrine concentration ($R^2 = 0.17$, $P < 0.05$) across individuals. Baseline MSNA was significantly related to age ($R^2 = 0.22$, $P < 0.005$), yet there was no relationship between MSNA and measurements of fatness, including body weight, BMI, percent body fat, trunk fat to leg fat ratio, visceral fat, or subcutaneous abdominal fat, for all subjects. Fasting leptin, insulin, glucose, and total FFA plasma concentrations were also not related to MSNA or to sympathetic and cardiac baroreflex sensitivity. In addition, there was no relationship between MSNA and any of these variables when men and women were analyzed separately.

Steady state conditions were achieved at baseline (fasting) and during the last 30 min of both insulin infusions during the hyperinsulinemic, euglycemic clamp (Fig. 1). Measurements of insulin sensitivity obtained from the results of the glucose clamp at baseline and during the lower and higher doses of insulin are shown in Table 2. Fasting plasma insulin concentration was related to GIR ($R^2 = 0.38$, $P < 0.001$) and EGP ($R^2 = 0.13$, $P < 0.05$) during the higher dose of insulin, but not to EGP at baseline or to GIR or EGP during the lower dose of insulin. Percent body fat but not subcutaneous, visceral or total abdominal fat predicted baseline fasting EGP ($R^2 = 0.22$, $P < 0.01$). During the glucose clamp there was no relationship between body fat, abdominal fat (visceral or subcutaneous), or the leg to trunk fat ratio and FFA, palmitate Ra, GIR, or EGP.

Muscle sympathetic nerve activity was not related to baseline palmitate Ra or EGP. During the lower dose of insulin, there was a significant inverse relationship between MSNA and GIR (Fig. 2), but not between MSNA and EGP ($P = 0.07$), total FFA ($P = 0.12$), or palmitate Ra ($P = 0.07$). During the higher dose of insulin there was no relationship between MSNA and either GIR, EGP, total FFA, or palmitate Ra. Because of the association between age and MSNA [41], we performed analyses with age, sex, and visceral fat as covariate separates and found that the relationship between MSNA and GIR was no longer significant when age was adjusted for but was still significant when sex and visceral fat were included in the model.

The subjects were separated into two groups based on the median value of MSNA: low MSNA ($N = 19$, 9.0 ± 0.8 burst min^{-1}) and high MSNA ($N = 18$, 21.3 ± 1.3 burst min^{-1}). Compared with the low MSNA group, the high MSNA group was older (29.0 ± 0.8 vs. 24.3 ± 0.8 years, $P < 0.001$) and had greater visceral fat (58.3 ± 6.3 vs. 35.9 ± 6.0 cm^2) but total weight, BMI, and total body fat were similar. There was no difference in fasting glucose, insulin, norepinephrine, leptin, palmitate Ra, glucose Rd, or EGP between the groups. During the lower dose of the insulin clamp, there were no significant differences between the two groups in FFA ($P = 0.17$), palmitate Ra ($P = 0.29$), GIR ($P = 0.15$), or EGP ($P = 0.14$). Nor were there differences between the groups during the higher dose of insulin in FFA ($P = 0.08$), palmitate Ra ($P = 0.09$), GIR ($P = 0.51$), or EGP ($P = 0.07$).

As young women generally have lower MSNA compared with young men [32], comparisons between the low and high MSNA groups were also performed for men and women separately (Table 3). Women with high MSNA were slightly older than those with low MSNA (28.9 ± 1.0 vs. 24.0 ± 1.0 years, $P < 0.05$) but there was no difference in total body fat or visceral fat. Men with high MSNA were also older (24.6 ± 1.6 vs. 19.0 ± 1.3

years, $P = 0.05$) and had higher visceral fat (77 ± 9 vs. 40 ± 8 cm², $P < 0.01$). There were no differences in fasting insulin, leptin, FFA, palmitate Ra, glucose Rd, or EGP between low and high MSNA groups in men or women. However, in women, during the lower dose of insulin the high MSNA group had significantly greater FFA, palmitate Ra, and EGP and lower GIR compared with the low MSNA group. These differences remained significant when age and visceral fat were each included in the model separately. In women, during the higher dose of insulin, no differences were found between the lower and higher MSNA groups. In men, the only difference between low and high MSNA groups was a lower GIR in the low MSNA group during the lower dose of insulin.

Finally, to determine if there were any differences between the extremes of the observed MSNA distribution, the tertiles of MSNA distribution were compared. Under baseline conditions, there was no difference in glucose, C-peptide, total FFA, palmitate Ra, or EGP between groups of low MSNA ($N = 12$), moderate MSNA ($N = 13$), and high MSNA ($N = 12$). There was no difference in insulin between the low and high MSNA tertiles at baseline or at steady state during the lower and higher doses of insulin. During the lower dose of insulin, there was no difference in total FFA or palmitate Ra between the groups ($P = 0.10$ and $P = 0.15$, respectively) but GIR was higher and EGP was lesser in the low MSNA group compared to both the moderate and high MSNA groups ($P < 0.05$ for both). During the higher dose of insulin, there was no difference between the MSNA tertiles in total FFA ($P = 0.10$), palmitate Ra ($P = 0.06$), GIR ($P = 0.22$), or EGP ($P = 0.07$).

Discussion

The primary finding of our study is that higher sympathetic activity is associated with a lower rate of glucose infusion during a hyperinsulinemic, euglycemic clamp. However, this association was only found at low doses of insulin and was not present when age was accounted for. In addition, comparisons between groups of individuals with high and low MSNA did not demonstrate any differences in multiple measurements of insulin sensitivity, including suppression of lipolysis and endogenous glucose production and the rate of glucose infusion needed to maintain euglycemia. These results thus indicate that resting sympathetic activity may be related to insulin sensitivity, but that the influence overall is small and may not be consistent among tissue types.

Insulin resistance is associated with sympathetic activation in overweight and obese people with well-controlled diabetes [15] and in non-diabetic offspring of persons with diabetes [14]. Surrogate measurements of sympathetic activity such as heart rate during sleep [4], heart rate at rest, and the high frequency/low frequency ratio of heart rate variability [6] are also related to insulin sensitivity. Because an obese model of insulin resistance was employed in these studies, the relationship between insulin sensitivity and sympathetic activity cannot be understood separately from the effects of obesity itself. Also, factors including obstructive sleep apnea and hypertension make the implications for a healthy human population unclear [10, 13]. The present study thus provides novel insight into the relationships between the sympathetic nervous system and insulin sensitivity in healthy, lean individuals.

It is unclear whether increased sympathetic neural activity is a cause, or a consequence, of a reduction in insulin sensitivity. Fasting insulin has been shown to be associated with MSNA in some studies [34], while others have found a relationship only in hypertensive subjects [11]. Insulin infusion activates the sympathetic nervous system and the increase in MSNA after a meal is related to the increase in insulin concentration [43]. The activation of the sympathetic nervous system by insulin may be due to its central sympathoexcitatory effects [29] or its effects on baroreflex gain [9, 43]. These studies are consistent with the idea that insulin resistance produces sympathetic activation because it results in increased circulating insulin concentrations.

On the other hand, some studies show that an acute increase in sympathetic nervous system activity results in rapid decreases in muscle insulin sensitivity, possibly due to a decrease in tissue blood flow and glucose delivery [16]. A similar reduction in insulin sensitivity is seen during the administration of the α -adrenergic agonist norepinephrine [20] suggesting that the reduction in insulin sensitivity may be mediated by α 1-adrenergic receptors [25]. However, other studies have not found whole body glucose uptake to be affected by sympathetic stimulation [12]. Some prospective studies suggest that sympathetic activation precedes insulin resistance: norepinephrine levels in men predict insulin levels 10 years later [24] and greater sympathetic reactivity is associated with the development of insulin resistance many years later [5]. In our study, we found that MSNA was related to insulin sensitivity during the glucose clamp, but not to baseline plasma insulin concentrations, despite the fact that subjects consumed a controlled meal for 3 days prior to the study and were studied fasting. This is consistent with a direct role for the sympathetic nervous system in modulating insulin sensitivity as opposed to increase sympathetic activity being a consequence of increased plasma insulin. Regardless, our findings do suggest a relationship between insulin sensitivity and MSNA.

Our results demonstrate an age-related increase in MSNA similar to previous studies [37]. When age was controlled for, the relationships between MSNA and insulin sensitivity were no longer apparent. This suggests that even moderate differences in age appear to affect both MSNA and insulin sensitivity. The increase in MSNA with aging has been shown to be associated with increases in body fat, particularly abdominal fat [18]. However, when we controlled for both differences in body composition and the adipose tissue-derived hormone leptin, the relationship between age and MSNA was still significant, suggesting that the increase in MSNA in our subjects with age related to other factors besides body fat. This is also consistent with other studies that have found that leptin is not related to MSNA in healthy individuals [30]. Furthermore, there was no difference in body fat between the groups based on MSNA levels. When we controlled for measures of fatness, the associations we found between MSNA and insulin sensitivity were still significant. Further studies are needed to determine why age appears to be associated with sympathetic activation, and decreased insulin sensitivity, and how these variables are linked.

Sex differences in insulin sensitivity and sympathetic activity are well known but are thought to be largely influenced by body fat distribution [8, 18]. The average MSNA in our groups of men and women was almost identical overall and when we adjusted for sex, we found that the inverse relationship between MSNA and GIR was still significant. However,

when we compared low and high MSNA groups separately for men and women, we found that there were differences present in the group of women that were not present in men. Specifically, women with higher MSNA had lower suppression of lipolysis (higher FFA and palmitate Ra), less suppression of EGP, and less peripheral glucose uptake (higher GIR) in response to insulin infusions. When age and visceral adiposity were controlled for, the differences between the low and high MSNA groups were still present in the women. Interestingly, while not significant, there were directional differences between the men and women with low and high MSNA which may have affected our ability to see a difference between the groups of low and high MSNA overall. This suggests that the autonomic nervous system may regulate insulin sensitivity differently in men compared with women and aging may affect this difference in men and women.

It is possible that the relationship between insulin sensitivity and sympathetic activation is due to factors that independently decrease insulin sensitivity and increase sympathetic activity. A candidate factor is free fatty acids, which cause insulin resistance [1] and increase sympathetic activity [7]. Free fatty acids derive from adipose tissue lipolysis, which may have a variable relationship with the size of the adipose tissue depot [22]. We found a trend for a relationship between MSNA and lipolysis during insulin infusion and lower suppression of lipolysis in women with high MSNA. However, in our healthy lean subjects visceral fat was not associated with sympathetic activity or measures of insulin sensitivity.

The idea that sympathetic hyperactivity, insulin resistance, and the development of hypertension are patho-physiologically linked is an attractive explanation for why these conditions commonly coexist in the metabolic syndrome. We did find that greater diastolic blood pressure and mean arterial blood pressure were associated with lower insulin sensitivity in our young healthy subjects, and it is known that the metabolic syndrome is associated with impaired baroreflex sensitivity [9].

A few experimental considerations and potential limitations of our study are worth noting. In supine, resting humans, it has been shown that MSNA measured in the peroneal nerve correlates to sympathetic activity measured by norepinephrine spillover techniques [40, 42]. However, it may be that there is differential activation of sympathetic traffic to certain tissues that we are not able to detect, such as the liver. Dissociation between systemic and regional sympathetic activation by insulin has been reported previously [11]. The magnitude of the increase in MSNA during hyperinsulinemia, which we did not measure, is important in insulin sensitivity and may vary [23]. Finally, the results of some studies may reflect acute effects of diet and energy balance that were not present in our study because of the use of a controlled diet for 3 days prior to our measurements.

We used two different doses of insulin based on fat free mass in this study because of the distinct sensitivity of various tissues (e.g., muscle vs. visceral fat) to insulin. However, only during the lower dose of insulin did we find any relationship between measures of insulin sensitivity and MSNA. It is possible that the higher dose of insulin was too large for this group of young lean subjects and that a different dose of insulin would allow for a better examination of the relationship of peripheral tissue insulin sensitivity and sympathetic activity.

Sympathetic activity varies widely among individuals but it is difficult to find large number of individuals with very high or very low activity. Subanalysis of the upper and lower tertiles of MSNA activity has been used to test if the extremes of MSNA have implications for blood pressure regulation [2, 31]. When we compared tertiles of MSNA, we did find that high MSNA was associated with lower glucose insulin sensitivity. It may be that individuals with very high MSNA have either lower insulin sensitivity or are at a risk for the future development of insulin resistance and/or diabetes. Prospective studies of the long-term implications of high sympathetic tone are needed.

Finally, we did not control for menstrual cycle or for oral contraceptive use in women and we did not measure sex hormones concentrations in our subjects. Menstrual cycle may influence both sympathetic activity [28] and insulin sensitivity [38]. Based on this and the fact that we showed a difference in insulin sensitivity in women between the low and high MSNA suggests that further studies of the relationship between sex hormones, sympathetic activity, and insulin sensitivity are warranted.

In summary, we found that insulin sensitivity in young lean individuals was related to sympathetic activity and that even in our young population moderate differences in age may have played a role in this finding. In general, there were little differences in insulin sensitivity between individuals with low and high resting MSNA. Secondary analyses suggest that there may be a difference in insulin sensitivity in women with low and high MSNA and further studies are needed to determine how sex, aging, sympathetic activity, and insulin sensitivity are related. Prospective studies would be particularly useful to determine if changes in sympathetic activity that occur with normal aging predict the future development of insulin resistance and if there is a difference in the effects of aging on men and women.

Acknowledgments

Special thanks to D. Schroder of Mayo Clinic Biomedical Statistics and Informatics for support in designing studies and statistical analysis. We also thank S. Roberts, S. Wolhart, K. Edens, C. Johnson, L. Matzek, J. Taylor, B. Walker, P. Engrav, N. Meyer, and D. Vlazny of Mayo Clinic for their assistance in conducting the studies. The project was supported by the Mayo Foundation for Medical Education and Research and NIH Grant Numbers UL1RR24150, UL1TR000135, R01DK50456, K23DK82424, T32AR056950, F32AG 38067, R01DK90541, R01HL67933, R01HL83947. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of Mayo Foundation or the NIH.

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Fig. 1. Steady-state values at baseline and during the hyperglycemic, euglycemia clamp for **a** palmitate specific activities, **b** palmitate concentrations, **c** glucose concentrations, **d** H^2 -glucose enrichment. Samples were drawn at 5 min intervals immediately prior to starting the clamp and at 10 min intervals during the last 30 min of each dose of insulin

Fig. 2. Relationship between muscle sympathetic nerve activity (MSNA) and glucose infusion rate (GIR) needed to maintain euglycemia during the low dose insulin infusion ($P < 0.05$, $R^2 = 0.13$). Nonlinear (logarithmic) regression curve is shown

Table 1

Subject characteristics including fasting blood tests

	Total	Female	Male
<i>N</i>	37	21	16
Age (years)	26.5 ± 0.7	26.3 ± 0.9	26.8 ± 1.2
Weight (kg)	69.3 ± 1.8	63.4 ± 1.7	77.0 ± 2.3*
BMI (kg m ⁻²)	23.2 ± 0.4	23.0 ± 0.5	23.5 ± 0.6
Lean body mass (kg)	29.2 ± 1.2	24.3 ± 0.7	35.7 ± 1.3*
Body fat (%)	27.7 ± 1.1	32.1 ± 1.1	22.0 ± 1.2*
Trunk to leg fat ratio (%)	92.2 ± 3.0	87.6 ± 4.2	98.0 ± 4.9
Total abdominal fat (cm ²)	150 ± 12	154 ± 15	143 ± 18
Subcutaneous abdominal fat (cm ²)	104 ± 9	120 ± 12	85 ± 12*
Visceral abdominal fat (cm ²)	47 ± 5	37 ± 5	59 ± 8*
HR (beats min ⁻¹)	63 ± 1	63 ± 2	63 ± 2
MAP (mmHg)	90 ± 1	90 ± 2	89 ± 2
Cholesterol (mg dL ⁻¹)	166 ± 5	167 ± 7	165 ± 8
Triglyceride (mg dL ⁻¹)	83 ± 6	91 ± 9	72 ± 6
HDL (mg dL ⁻¹)	57 ± 2	60 ± 3	52 ± 3
LDL (mg dL ⁻¹)	93 ± 4	88 ± 6	98 ± 7
Glucose (mmol L ⁻¹)	5.1 ± 0.2	5.6 ± 0.1	5.9 ± 0.1*
Insulin (pmol L ⁻¹)	32.4 ± 2.0	34.9 ± 3.1	29.4 ± 2.5
Epinephrine (ng mL ⁻¹)	39 ± 4	30 ± 3	51 ± 7*
Norepinephrine (mg dL ⁻¹)	206 ± 13	187 ± 17	231 ± 20
Leptin (ng mL ⁻¹)	9.0 ± 1.0	12.8 ± 1	4.2 ± 0.7*
MSNA (bursts 100 heartbeats ⁻¹)	24 ± 2	23 ± 3	24 ± 2
MSNA (bursts min ⁻¹)	15 ± 1	15 ± 2	15 ± 1

BMI body mass index, *HR* heart rate, *MAP* mean arterial pressure, *LDL* low density lipoprotein, *HDL* high density lipoprotein, *MSNA* muscle sympathetic nerve activity

* $P < 0.05$ vs. females

Table 2

Measurements of insulin sensitivity at baseline and at steady state during the low and high doses of insulin during the hyperinsulinemic, euglycemic clamp

	Baseline	LDI	HDI
Insulin (pmol L ⁻¹)	32.4 ± 2.0	54.2 ± 1.9 [*]	176.4 ± 4.8 ^{*,†}
GIR (μmol kg ⁻¹ min ⁻¹)		10 ± 1	54 ± 4 [†]
Free fatty acids (μmol L ⁻¹)	418 ± 23	172 ± 19 [*]	48. ± 9 ^{*,†}
Rd glucose (μmol kg ⁻¹ min ⁻¹)	15 ± 0	17 ± 1	33 ± 2 ^{*,†}
EGP (μmol kg ⁻¹ min ⁻¹)	15 ± 1	10 ± 1 [*]	-6 ± 1 ^{*,†}
Palmitate Ra (μmol min ⁻¹)	103 ± 19	48 ± 7 [*]	19 ± 3 ^{*,†}

LDI low dose insulin, *HDI* high dose insulin, *GIR* glucose infusion rate, *Rd* rate of disappearance, *EGP* endogenous glucose production, *Ra* rate of appearance, *Rd* rate of disappearance

^{*} $P < 0.05$ vs. baseline

[†] $P < 0.05$ vs. LDI

Table 3

Measurements of insulin sensitivity at baseline and at steady state during the hyperinsulinemic, euglycemic clamp according to MSNA, based on the median

MSNA group	Free fatty acids ($\mu\text{mol L}^{-1}$)			Palmitate Ra ($\mu\text{mol min}^{-1}$)			GIR ($\mu\text{mol kg}^{-1} \text{min}^{-1}$)			EGP ($\mu\text{mol kg}^{-1} \text{min}^{-1}$)		
	Baseline	LDI	HDI	Baseline	LDI	HDI	Baseline	LDI	HDI	Baseline	LDI	HDI
All	402 $\pm$ 32	142 $\pm$ 24	36 $\pm$ 3	82 $\pm$ 23	37 $\pm$ 9	15 $\pm$ 2	9 $\pm$ 2	41 $\pm$ 4	15 $\pm$ 1	8 $\pm$ 1	8 $\pm$ 1	-8 $\pm$ 2
High	422 $\pm$ 33	193 $\pm$ 27	45 $\pm$ 4	93 $\pm$ 18	54 $\pm$ 10	16 $\pm$ 2	6 $\pm$ 1	38 $\pm$ 3	15 $\pm$ 1	11 $\pm$ 1	11 $\pm$ 1	-4 $\pm$ 1
Women	412 $\pm$ 35	124 $\pm$ 23	36 $\pm$ 4	45 $\pm$ 34	25 $\pm$ 8	11 $\pm$ 3	14 $\pm$ 2	44 $\pm$ 5	15 $\pm$ 1	5 $\pm$ 1	5 $\pm$ 1	-10 $\pm$ 2
High	480 $\pm$ 40	255 $\pm$ 37*	51 $\pm$ 6*	127 $\pm$ 24	65 $\pm$ 7*	18 $\pm$ 2*	6 $\pm$ 2*	34 $\pm$ 4	16 $\pm$ 1	11 $\pm$ 2*	11 $\pm$ 2*	-6 $\pm$ 2
Men	385 $\pm$ 63	171 $\pm$ 38	35 $\pm$ 4	106 $\pm$ 30	53 $\pm$ 19	21 $\pm$ 3	3 $\pm$ 1	36 $\pm$ 4	14 $\pm$ 1	13 $\pm$ 0	13 $\pm$ 0	-3 $\pm$ 2
High	338 $\pm$ 37	116 $\pm$ 36	37 $\pm$ 4	66 $\pm$ 19	40 $\pm$ 18	14 $\pm$ 3	7 $\pm$ 1*	41 $\pm$ 5	14 $\pm$ 1	11 $\pm$ 2	11 $\pm$ 2	-2 $\pm$ 1

MSNA muscle sympathetic nerve activity, LDI/low dose insulin, HDI high dose insulin, GIR glucose infusion rate, Rd rate of disappearance, EGP endogenous glucose production, Ra rate of appearance, Rd rate of disappearance

* $P < 0.05$ vs. low MSNA group