DISSERTATION

SYMPATHETIC NEURAL ACTIVATION IN HUMAN OBESITY IS PHENOTYPE DEPENDENT

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY GUY E. ALVAREZ ENTITLED SYMPATHETIC NEURAL ACTIVATION IN HUMAN OBESITY IS PHENOTYPE DEPENDENT BE ACCEPTED AS FULLFILING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT

SYMPATHETIC NEURAL ACTIVATION IN HUMAN OBESITY IS PHENOTYPE DEPENDENT

A series of studies focusing on the influence of body fat distribution on sympathetic neural activation (SNA) were conducted to determine 1) if muscle sympathetic nerve activity (MSNA) would be greater in men with higher levels of abdominal visceral fat compared to those with lower; 2) if MSNA would be similar in subcutaneous obese men compared with normal weight peers with similar levels of abdominal visceral fat; and 3) if MSNA would be reduced in men with elevated abdominal visceral fat after weight loss.

To accomplish these aims, body composition was measured by dual energy x-ray absorptiometry, abdominal fat distribution was measured by computed tomography, and post-ganglionic multi-unit MSNA was measured via microneurography in 18-40 year old sedentary non-obese and obese men.

The main findings from these studies were as follows. First, MSNA was elevated in men with higher abdominal visceral fat compared with their age- and total fat mass-matched peers with lower levels (32±4 vs. 21±2 bursts/min, $P<0.05$). Basal MSNA was more closely associated with abdominal visceral fat ($r=0.65$, $P<0.05$) than total body ($r=0.32$, $P=0.05$) or abdominal subcutaneous fat ($r=0.27$,

P=0.05). In addition, the relation between abdominal visceral fat and MSNA was independent of total fat mass ($r=0.61$, $P<0.05$).

Second, MSNA did not differ among subcutaneous obese and normal weight men with similar levels of abdominal visceral fat (22.5±2.7 vs. 23.9±2.3 bursts/min, $P>0.05$). Importantly, abdominal visceral fat was the only significant body composition or abdominal fat distribution-related correlate of MSNA ($r=0.48$, $P<0.05$).

Finally, reductions in total body and abdominal fat with weight loss, via caloric restriction, were associated with a ~23% reduction in MSNA in men elevated with abdominal visceral fat.

The results of these studies suggest that abdominal visceral fat is an important adipose tissue depot linking obesity with sympathetic neural activation in humans. These findings may have important implications for understanding the elevated risk of cardiovascular disease observed in individuals with visceral obesity.

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> *"To acquire knowledge, one must study; but to acquire wisdom, one must observe."* - Marilyn vos Savant

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> *"Teachers open the door. You enter by yourself."* - Chinese Proverb

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> *"In prosperity our friends know us; in adversity we know our friends."* - John Churton Collins

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> *"Be bold and courageous. When you look back on your life, you'll regret the things you didn't do more than the ones you did."* – Anonymous

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> *"There are many things in life that will catch your eye, but only a few will catch your heart ... pursue those."* - Anonymous

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CHAPTER I

INTRODUCTION AND SPECIFIC AIMS

The sympathetic nervous system is a diverse and complex system that plays an integral role in metabolic and cardiovascular homeostasis. There is mounting evidence to suggest that a number of human pathophysiological conditions are preceded by, or associated with, elevated sympathetic neural activation (SNA).

The prevalence of obesity has risen dramatically throughout the industrialized and developing world. The strong association between obesity and numerous cardiovascular and metabolic complications gives cause for concern. Moreover, an association also exists between obesity and increases in muscle sympathetic nerve activity (MSNA), a direct assessment of SNA. However, there is large interindividual variability in MSNA at the same level of adiposity. The reasons for this variability remain unclear, but it has been suggested that regional variations in adipose tissue deposition could be an important contributing factor.

The distribution of body fat has considerable influence on the risks associated with obesity. Interestingly, the level of fat in the abdominal viscera is more closely associated with elevated cardiovascular and metabolic disease risk than obesity *per se*. However, it is presently unknown whether abdominal visceral fat is an important adipose tissue depot linking obesity with elevated MSNA. Therefore, the specific aims of this research are as follows:

SPECIFIC AIMS

1) To determine the relation between abdominal visceral fat and MSNA in humans.

2) To determine the relation between subcutaneous adiposity and MSNA in humans.

3) To determine whether reductions in total body and abdominal visceral fat are associated with reductions in MSNA in men with elevated abdominal visceral fat.

HYPOTHESES

1) We tested the hypothesis that MSNA would be increased in men with higher abdominal visceral fat compared to their age-, total body- and abdominal subcutaneous fat-matched peers with lower levels.

2) We tested the hypothesis that MSNA would not differ in men with subcutaneous obesity and normal weight men with similar levels of abdominal visceral fat.

3) We tested the hypothesis that weight loss would be associated with reductions in MSNA in overweight and obese men with elevated abdominal visceral fat. We further hypothesized that these reductions would be most closely associated with reductions in abdominal visceral fat.

CHAPTER II

SYMPATHETIC NEURAL ACTIVATION IN VISCERAL OBESITY

ABSTRACT

Muscle sympathetic nerve activity (MSNA) is elevated in obese humans. However, the potential role of abdominal visceral fat as an important adipose tissue depot linking obesity to elevated MSNA has not been explored. Accordingly, we tested the hypothesis that MSNA would be increased in men (age=18-40 years, body mass index ≤ 35 kg/m^2) with higher abdominal visceral fat (HAVF, n=13, abdominal visceral fat=118.1±15.8 cm^2) compared to their age- (28.7±2.4 vs. 25.5±2.0 years, P>0.05), total body- (20.6±2.1 vs. 20.8±2.4 kg, P>0.05) and abdominal subcutaneous fat- (230.6±24.9 vs. 261.4±34.8 cm^2, P>0.05) matched peers with lower levels (LAVF, n=13, visceral fat= 73.0±6.0 cm^2). To accomplish this, we measured MSNA (microneurography), body composition (dual energy x-ray absorptiometry), and abdominal visceral and subcutaneous fat (computed tomography) in 37 sedentary men across a wide range of adiposity. MSNA was significantly higher in HAVF compared with LAVF (32±4 vs. 21±2 bursts/min, P<0.05). Furthermore, MSNA was more closely associated with the level of abdominal visceral fat (r=0.65, P<0.05) than total body (r=0.32, P<0.05) or abdominal subcutaneous fat (r=0.27, P=0.05). Therefore, the results of this study

suggest that abdominal visceral fat is an important adipose tissue depot linking obesity with sympathetic neural activation in humans. These findings may have important implications for understanding the increased risk of developing hypertension and other cardiovascular diseases in individuals with elevated abdominal visceral fat.

INTRODUCTION

Obesity is a major public health problem in the United States and other industrialized nations. The prevalence of obesity has increased dramatically in recent decades (13). Currently more than 60% of U.S. adults are considered overweight or obese (14). Obesity is an important risk factor for the development of hypertension and other cardiovascular diseases (10,44). As such, a significant portion of the population is considered at elevated risk.

The distribution of body fat has considerable influence on the risks associated with obesity. In particular, elevated abdominal fat is an independent predictor of cardiovascular morbidity and mortality (26). The accumulation of adipose tissue in the abdominal visceral region is considered to be the important fat depot linking obesity with cardiovascular disease (7,25). This association is mediated in part by the clustering of several cardiovascular disease risk factors that have been frequently referred to as the "Metabolic Syndrome" (32).

Obesity is associated with elevated muscle sympathetic nerve activity (MSNA) (1,15,17,34,36,43); however, there is considerable interindividual variability in MSNA even at the same level of adiposity (i.e., body mass index).

The reason(s) for this variability is (are) unclear. One possibility is that this discrepancy could be attributed, in part, to individual differences in the level of abdominal visceral fat. However, it is presently unknown whether abdominal visceral fat is an important adipose tissue depot linking obesity with elevated MSNA. Accordingly, we tested the hypothesis that MSNA would be increased in men with higher abdominal visceral fat (HAVF, n=13) compared to their age-, total body- and abdominal subcutaneous fat-matched peers with lower levels (LAVF, n=13).

METHODS

Subjects. Thirty-seven men (body mass index ≤35 kg/m^2) volunteered to participate in the present study.. All subjects were normotensive (arterial blood pressure <140/90 mmHg), as determined by casual blood pressure measurements, and free from other overt diseases, as determined from individual health histories. Subjects were further evaluated for the presence of overt cardiopulmonary disease by resting and maximal exercise electrocardiograms. Subjects did not smoke, were non-diabetic (2-hr post glucose load <200 mg/dL), and were not taking medications that could influence autonomic-circulatory function. All subjects were sedentary and were not participating in regular physical activity (defined as >20 min on more than 2 days/wk). The nature, purpose, risks, and benefits were explained to each subject before obtaining informed consent. The Colorado State University Human Research Committee approved all experimental protocols.

Experimental Procedures. Body mass and height were measured on a physician's balance scale and a stadiometer, respectively. Waist and hip circumference were measured (Gulick II Tape Measure, Country Technology Inc.) using procedures recommended by the Arlie Conference (27); the waist-to-hip ratio was calculated. Body composition was measured using dual energy x-ray absorptiometry (DPX-IQ, Lunar Radiation Corp.) using software version 4.5c. Computed tomography scans (HiSpeed Cti, GE Medical) were performed to quantify abdominal visceral and subcutaneous fat levels as previously described (12). A cross-sectional scan 10 mm thick, centered at the L4-L5 intervertebral space, was obtained using 170 mA with a scanning time of 2 seconds and a 512 X 512 matrix. Maximal oxygen consumption 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, beat-by-beat arterial pressure was measured in the finger using photoplethysmography (Finapres Medical Systems, The Netherlands). Respiration was monitored continuously using a pneumobelt. Resting finger arterial blood pressures were "adjusted" to brachial arterial blood pressures with an automated device (Dinamap Pro100, GE Medical Systems) prior to the injection of vasoactive drugs (see below). Recordings of multiunit MSNA were obtained from the right peroneal nerve using the microneurographic technique as described previously (6). The neural activity was amplified, filtered (700-2,000 Hz), full-wave rectified, and integrated (time constant, 0.1 s) (nerve traffic analyzer, model 662C-3, University of Iowa Bioengineering). Neurograms were considered acceptable as recordings of efferent MSNA according to previously published criteria (41).

We have previously reported that men with higher levels of abdominal visceral fat have reduced vagal baroreflex gain (2). Therefore, a secondary aim of the present study was to determine whether sympathetic baroreflex gain was similarly reduced. As such, sympathetic and vagal baroreflex responses were measured using the modifided Oxford technique (9) as described previously (2).

Experimental Protocol. All subjects were studied between 7:00 am and 11:00 am following a 12-hour overnight fast. Subjects were instructed to refrain from caffeine and alcohol consumption 24 hours prior to all testing sessions. Subjects were also instructed to avoid participation in any vigorous activity 24 hours prior to testing.

Subjects were instrumented and were positioned supine in a thermoneutral room. After steady state levels of all variables were achieved, a 10-minute recording of basal MSNA was obtained. Subsequently, a bolus injection of sodium nitroprusside (100 μg) was given intravenously followed 60 seconds later by a bolus injection of phenylephrine HCL (150 μg). These pharmacological perturbations decreased and increased arterial blood pressure ~15 mmHg from baseline levels, respectively, during a 3-minute period. Three trials were completed and each trial was separated by a minimum of 15 minutes quiet rest.

Data Analysis. Abdominal visceral and subcutaneous fat regions were determined using commercially available medical imaging software (SliceOmatic ver. 4.2, Tomovision) as described previously (2). Blinded repeat measurements of

abdominal visceral and subcutaneous fat in a random sample (n=24) were highly correlated ($r=0.99$, $P<0.05$).

MSNA, heart rate, arterial blood pressure, and respiration were recorded continuously and digitized at 500 Hz to a laboratory computer for later analysis using signal processing software (Windaq, Dataq Instruments). Basal MSNA was quantified as both burst frequency (bursts/min) and burst incidence (bursts/100 bts). 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 relationship 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 (19). A 4-parameter sigmoid was fit to the data to determine vagal baroreflex gain. The gain was calculated after systematically removing bin values in the threshold and saturation regions as described previously (2). At a minimum, 2 of the 3 trials performed were used to determine average values for sympathetic and vagal baroreflex gain.

Statistical Analysis. Thirteen pairs of men (26 total) from the larger sample of 37 were selected for comparison based on their similar between-pair levels of total body and abdominal subcutaneous fat but different levels of abdominal visceral fat. The designation of high (HAVF) and low (LAVF) abdominal visceral fat was

then assigned. Differences in subject characteristics and dependent variables between groups were assessed with independent student's t-tests. Relations among variables for all 37 subjects were assessed using bivariate correlation analysis. SPSS statistical software (SPSS v.11.0, SPSS) was used to perform all statistical analyses. Data are expressed as mean±SE. The significance level was set *a priori* at $P<0.05$.

RESULTS

Subject Characteristics. Subject characteristics for LAVF and HAVF men are shown in Table 1. No differences in age, height, body mass, body mass index, waist and hip circumferences, body fat %, total fat mass, lean body mass, abdominal subcutaneous fat, total abdominal fat, systolic blood pressure, diastolic blood pressure, heart rate, R-R interval, and maximal oxygen consumption were observed in the LAVF and HAVF groups (all $P>0.05$). As intended, abdominal visceral fat and the waist-to-hip ratio were higher in HAVF (both $P<0.05$).

Basal MSNA and Baroreflex Responses in LAVF and HAVF. Basal MSNA burst frequency (32±4 vs. 21±2 bursts/min) and burst incidence (51±6 vs. 38±4 burst/100 bts, Figure 1) were significantly higher in HAVF compared with LAVF ($P<0.05$). Sympathetic baroreflex gain was similar in the two groups (-8.9±0.6 vs. -9.1±0.7 aiu/mmHg/beat, $P<0.05$), but vagal baroreflex gain was lower in HAVF compared with LAVF (13.8±1.7 vs. 20.0±2.4, ms/mmHg, $P<0.05$).

Body Composition and Anthropometric Correlates. Basal MSNA was more closely associated with abdominal visceral fat ($r=0.65$, $P<0.05$, Figure 2, top panel) than abdominal subcutaneous fat ($r=0.27$, $P=0.05$, Figure 2, middle panel) or total fat mass ($r=0.32$, $P<0.05$, Figure 2, lower panel). The relation between MSNA and abdominal visceral fat was independent of total fat mass ($r=0.61$ $P<0.05$). Basal MSNA was also correlated with body mass index ($r=0.32$, $P=0.05$), waist circumference ($r=0.32$, $P=0.05$), waist-to-hip ratio ($r=0.38$, $P<0.05$), body fat % ($r=0.35$, $P<0.05$), and total abdominal fat ($r=0.42$, $P<0.05$). Neither vagal nor sympathetic baroreflex gains were correlated with any body composition or anthropometric variables.

DISCUSSION

The major new finding of the present study was that basal MSNA was significantly higher in men with elevated abdominal visceral fat compared to their age-, total body-, and abdominal subcutaneous fat-matched peers with lower levels of abdominal visceral fat. In addition, basal MSNA was more closely associated with abdominal visceral fat than abdominal subcutaneous fat or total fat mass. Thus, our observations are consistent with the idea that abdominal visceral fat is an important adipose tissue depot linking obesity with sympathetic neural activation.

Consistent with our previous finding (2), vagal baroreflex gain was reduced in men with elevated abdominal visceral fat in the present study. However, contrary to our hypothesis, sympathetic baroreflex gain was similar in these men compared with their age-, total body-, and subcutaneous matched peers with lower levels of

abdominal visceral fat. In contrast to the report by Grassi et al. (15), sympathetic baroreflex gain was not related to any measure of adiposity in the present study. The reasons for this discrepancy are unclear but may be related to differences between studies in the degree of obesity studied, in the experimental approach used to assess baroreflex function (Grassi et al. used graded steady state infusions of sodium nitroprusside and phenylephrine HCL), or other factors.

The mechanism(s) responsible for the elevated basal MSNA in men with visceral obesity in the present study remain unclear. However, a number of possibilities exist including alterations in circulating neurohumoral factors and central neuropeptide signaling pathways. Adipose tissue-derived angiotensinongen could be an interesting candidate in this regard. Angiotensinogen is expressed more abundantly in visceral compared with subcutaneous adipocytes (38). Furthermore, plasma angiotensinogen concentrations are elevated in obesity (37) and angiotensin II stimulates central sympathetic outflow (29).

Leptin, the product of the OB gene, is secreted from adipocytes in proportion to fat mass and acts on hypothalamic neuronal targets to alter energy intake and expenditure. Leptin also exerts an important influence on cardiovascular and renal function in animals that is sympathetically-mediated (18,20). Thus, hyperleptinemia could be a potential mechanism contributing to elevated basal MSNA in humans with visceral obesity. However, we believe this is unlikely because leptin expression and secretion is lower in visceral compared with subcutaneous adipocytes (33,39) and circulating concentrations of the protein are lower, not higher, in upper body compared with lower body obesity (5,28). In addition, leptin

concentrations have been associated with basal MSNA in some (30,35), but not all studies (31).

We (21,22) have previously reported that waist circumference (i.e., abdominal adiposity) is an important determinant of the age-related increase in basal MSNA in humans. In addition, the higher basal MSNA observed in males is closely associated with their high level of abdominal adiposity compared with females (23). Taken together with the results of the present study, these finding suggest that elevated abdominal visceral fat is an important determinant of basal MSNA in humans. Future studies will be necessary to confirm the results of previous studies implicating abdominal visceral fat as a factor contributing to age- and gender-related differences in basal MSNA.

Visceral obesity has been hypothesized to be the result of a neuroendocrine disorder associated with hypothalamic-pituitary axis dysregulation and sympathetic nervous system activation (3,4). Grassi et al. (17) have reported that dysregulation of the hypothalamic-pituitary axis may contribute to the elevated basal MSNA observed in obese subjects. Thus, it is possible that hypothalamic-pituitary dysregulation could contribute to the elevated basal MSNA observed in visceral obesity. Future studies will be necessary to determine whether this or other mechanisms are responsible for the elevated basal MSNA observed in visceral obesity.

Wallin et al. (42) reported that basal MSNA is closely related to renal norepinephrine spillover (i.e., renal sympathetic activity) in humans. Renal sympathetic activity is elevated in human obesity (40) and is considered to play an

important role in the pathophysiology of obesity hypertension (18). Therefore, increases in basal MSNA may serve as a marker or surrogate in this regard. Future studies will be necessary to determine whether sympathetic activity is increased to the kidney or other regions in visceral obesity.

The accumulation of fat in the abdominal visceral region is a critical correlate of the metabolic syndrome and an important risk factor for cardiovascular disease-related mortality (10,44). Sympathetic neural activation is considered to be important in the pathogenesis of hypertension (11). Therefore, elevated MSNA may be associated with, or be a marker for, elevated risk of developing hypertension in visceral obesity. In turn, reductions in total body fat, particularly abdominal visceral fat, are associated with reductions in arterial blood pressure (24) and improvements in other cardiovascular disease risk factors (8). Reductions in body weight are also associated with reductions in basal MSNA (16). Future studies will be necessary to determine whether reduction in abdominal visceral fat with weight loss is an important determinant of the corresponding reduction in basal MSNA.

In summary, the results of the present study suggest abdominal visceral fat may be an important adipose tissue depot linking obesity with elevated basal MSNA. The mechanisms responsible for these observations remain unclear. Importantly, these findings may have implications for understanding the elevated risk of developing hypertension and other cardiovascular diseases in men with visceral obesity.

Table 1. Subject characteristics in men with lower (LAVF) and higher (HAVF) levels of abdominal visceral fat.

Variable	LAVF (n=13)	HAVF (n=13)
Age (yrs)	25.5±2.0	28.7±2.4
Height (cm)	181.8±2.8	179.4±2.0
Body Mass (kg)	86.8±4.3	86.5±4.0
Body Mass Index (kg/m^2)	26.3±1.3	27.0±1.4
Waist Circumference (cm)	92.1±3.0	97.0±4.3
Hip Circumference (cm)	106.3±2.4	104.4±2.1
Waist-to-Hip Ratio	0.87±0.01	0.92±0.03*
Body Fat (%)	23.3±2.0	23.1±1.7
Total Fat Mass (kg)	20.8±2.4	20.6±2.1
Lean Body Mass (kg)	62.8±2.5	62.6±2.1
Abdominal Subcutaneous Fat (cm^2)	261.4±34.8	230.6±24.9
Abdominal Visceral Fat (cm^2)	73.0±6.0	118.1±15.8*
Total Abdominal Fat (cm^2)	334.4±37.0	349.0±39.1
Systolic Blood Pressure (mmHg)	116.1±2.5	119.3±2.3
Diastolic Blood Pressure (mmHg)	64.5±1.8	69.3±2.8
Heart Rate (bpm)	60.0±2.9	64.7±2.0
R-R Interval (ms)	1.05±0.04	0.97±0.03
VO_2max (ml/kg/min)	44.8±2.1	44.7±2.2
VO_2max (ml/kg FFM/min)	61.3±2.1	61.0±2.3

All values are mean ± SE. VO_2max = maximal oxygen consumption per kg body weight and fat free mass. * $P < 0.05$ vs. LAVF.

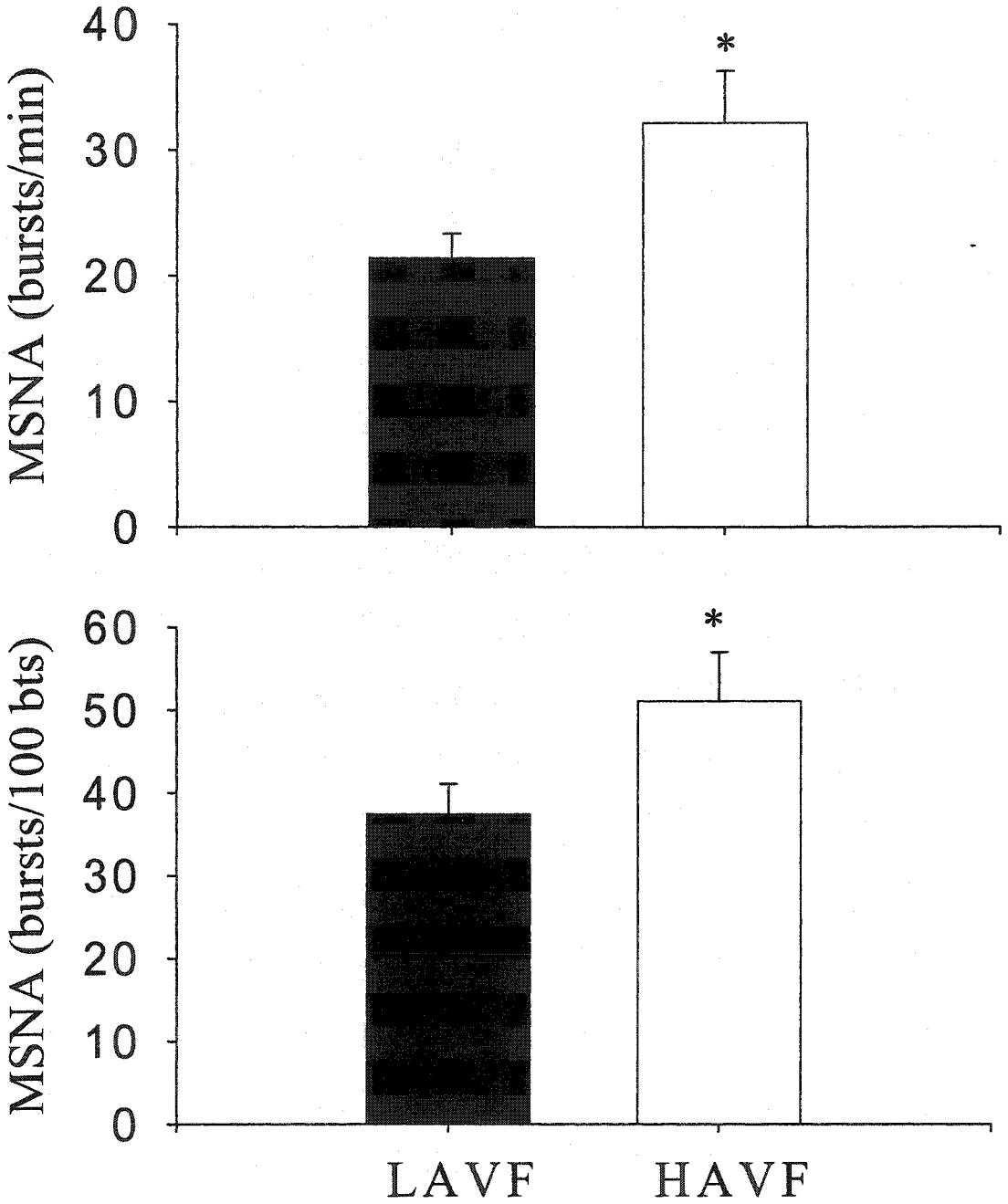

Figure 1. Basal muscle sympathetic nerve activity expressed in bursts/min (top panel) and bursts/100 beats (lower panel) in men with lower (LAVF) and higher (HAVF) abdominal visceral fat. Values are mean±SE. *P<0.05 vs. LAVF.

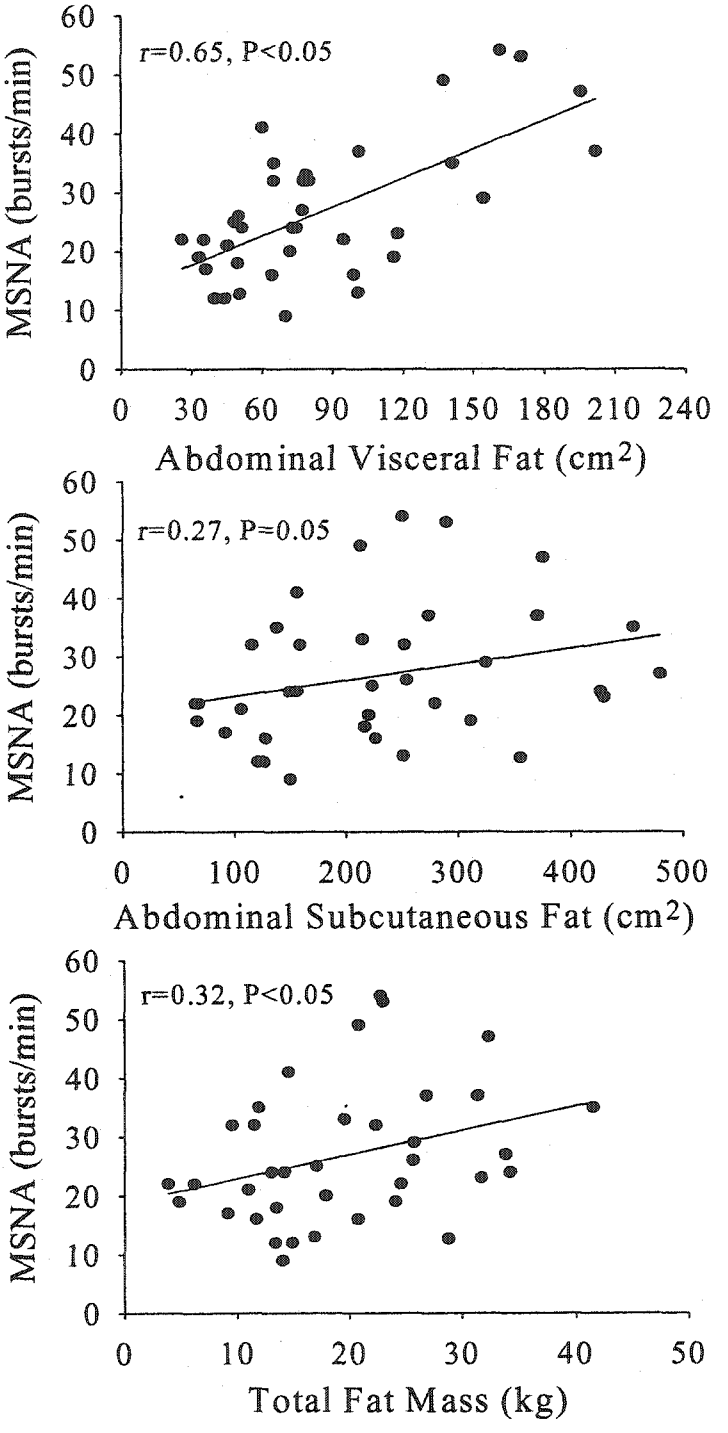

Figure 2. Relations between basal muscle sympathetic nerve activity and abdominal visceral fat (top panel), abdominal subcutaneous fat (middle panel), and total fat mass (lower panel).

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CHAPTER III

ABDOMINAL VISCERAL FAT: KEY ADIPOSE DEPOT LINKING OBESITY AND SYMPATHETIC NEURAL ACTIVATION

ABSTRACT

We have previously reported that men with elevated abdominal visceral fat demonstrate higher levels of muscle sympathetic nerve activity (MSNA) compared with their age- and total fat mass-matched peers with lowers levels. However, it is unknown whether MSNA is elevated in overweight and obese men with a subcutaneous fat phenotype (SUBOB) compared with normal weight (NW) peers. Therefore, the aim of this study was to test the hypothesis that MSNA would not differ in SUBOB men and NW peers with similar levels of abdominal visceral fat. To accomplish this, we measured MSNA (microneurography), body composition (dual energy x-ray absorptiometry), and abdominal visceral and subcutaneous fat (computed tomography) in 9 SUBOB (body mass index $\geq$25 and $\leq$ 35 kg/m^2, 23.4±2.1 yrs) and 15 NW (body mass index $\leq$ 25 kg/m^2, 22.4±1.4 yrs) 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 cm^2) were significantly ($P<0.05$) higher in the SUBOB group compared with NW peers. The level of abdominal visceral fat did not differ in the two groups (55±5 vs. 69±7 cm^2, $P>0.05$). As hypothesized, MSNA was not significantly different between SUBOB and NW (23±3 vs. 24±2 bursts/min, $P>0.05$, respectively). Importantly, abdominal

visceral fat was the only body composition or regional body fat distribution-related correlate ($r=0.45$, $P<0.05$) of MSNA in these individuals. Therefore, the results of this study suggest that MSNA is not elevated in obese compared with non-obese men with similar levels of abdominal visceral fat. Importantly, abdominal visceral fat is a determinant of MSNA even at levels of abdominal visceral fat below the threshold typically associated with significant elevations in cardiovascular and metabolic disease risk. These findings may have important implications for understanding the differences in cardiovascular and metabolic risk with phenotypic variation in adipose tissue distribution.

INTRODUCTION

The sympathetic nervous system (SNS) plays a critical role in the regulation of physiological homeostasis in general, and in both beat-to-beat and long-term regulation of arterial blood pressure. Sustained activation of the SNS has been implicated in various pathophysiological states associated with obesity including hypertension (6,8,12), congestive heart failure (7,31), and sudden cardiac death (5).

Muscle sympathetic nerve activity (MSNA) is elevated in obese compared with non-obese humans (1,14,16,29,33,36). However, there is considerable interindividual variability in the level of MSNA, even at the same level of whole body adiposity. We have previously shown that at least part of this variability may be attributed to abdominal fat distribution pattern; MSNA is higher in men with elevated abdominal visceral fat compared with their age- and total fat mass-matched peers with lower levels (2). However, whether obese individuals with a subcutaneous fat phenotype (SUBOB)

demonstrate higher levels of MSNA compared with normal weight (NW) individuals with similar levels of abdominal visceral fat is unknown.

Accordingly, we tested the hypothesis that MSNA would not differ in SUBOB and NW men with similar levels of abdominal visceral fat. A secondary aim was to determine if the relation between abdominal visceral fat and MSNA (2,20,21) is evident at levels of abdominal visceral fat below the threshold typically associated with significant elevations in cardiovascular and metabolic disease risk (3,18,24,27,37).

METHODS

Subjects. Fifteen normal weight (NW) (body mass index $\leq$25 kg/m^2) and 9 overweight and obese (body mass index $\geq$25 to $\leq$35 kg/m^2) men volunteered to participate in the present study. All subjects were normotensive (arterial blood pressure <140/90 mmHg), as determined by casual blood pressure measurements, and free from overt chronic disease, other than overweight/obesity, as determined from individual health histories. Subjects were further evaluated for the presence of overt cardiopulmonary disease by resting and maximal exercise electrocardiograms. Subjects did not smoke, were non-diabetic (2-hr post glucose load <200 mg/dL), and were not taking medications that could affect autonomic-circulatory function. All subjects were sedentary and did not participate in regular physical activity (defined as >20 min on more than 2 days/wk). The nature, purpose, risks, and benefits were explained to each subject before obtaining informed consent. The Colorado State University Human Research Committee approved all experimental protocols.

Experimental Procedures. A physician's balance scale and a stadiometer were used to measure body mass and height, respectively. Waist and hip circumference were measured (Gulick II Tape Measure, Country Technology Inc.) using recommended procedures (23), and waist-to-hip ratio was calculated. Body composition was measured using dual energy x-ray absorptiometry (DPX-IQ, Lunar Radiation Corp. v.4.5c). A cross-sectional computed tomography scan (HiSpeed Cti, GE Medical), centered at the L4-L5 intervertebral space, was obtained to quantify abdominal subcutaneous and visceral fat (13). Maximal oxygen consumption was measured during graded treadmill exercise to exhaustion using open circuit spirometry (TrueMax 2400, ParvoMedics). Standard criteria for achievement of valid maximal oxygen consumption were met (19). Heart rate was determined from lead II of the electrocardiogram, beat-by-beat arterial pressure was measured in the finger using photoplethysmography (Finapres Medical Systems, The Netherlands). Respiration was monitored continuously using a pneumobelt placed around the upper abdomen. Resting finger arterial blood pressures were "adjusted" to brachial arterial blood pressures with an automated device (Dinamap Pro100, GE Medical Systems) prior to the injection of vasoactive drugs (see below). Recordings of multiunit MSNA were obtained from the right peroneal nerve using the microneurographic technique as described previously (9). The neural activity was amplified, filtered (700-2,000 Hz), full-wave rectified, and integrated (time constant, 0.1 s) (nerve traffic analyzer, model 662C-3, University of Iowa Bioengineering). Neurograms were considered acceptable as recordings of efferent MSNA according to previously published criteria (35).

We have previously reported reductions in vagal, but not sympathetic baroreflex gain, in men with higher levels of abdominal visceral fat compared to 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 NW men.

Sympathetic and vagal baroreflex responses were measured using the modified Oxford technique (11). Briefly, an antecubital venous catheter was placed in the subject's arm for the injection of vasoactive drugs. After a 20-minute rest period and stabilization of baseline arterial blood pressure, heart rate, and respiration, a bolus injection of sodium nitroprusside (100 μg) was given intravenously followed 60 seconds later by a bolus injection of phenylephrine HCL (150 μg). These pharmacological perturbations decreased and increased, respectively, arterial blood pressure ~15 mmHg from baseline levels during a 3-minute period.

Experimental Protocol. All subjects reported to the laboratory between 7:00 am and 11:00 am following a 12-hour overnight fast. Subjects were instructed to refrain from caffeine and alcohol consumption 24 hours prior to all testing sessions. Subjects were also instructed to avoid participation in any vigorous activity 24 hours prior to testing.

Subjects were dressed in light clothing, were instrumented and rested quietly for 20 minutes to achieve steady state levels of all variables prior to a 10-minute recording of basal MSNA, heart rate, arterial blood pressure, and respiration. After

baseline recordings, 3 trials of vasoactive drug infusions were performed. A minimum 15 minutes quiet rest separated each trial.

Data Analysis. Abdominal visceral and subcutaneous fat regions were determined using commercially available medical imaging software (SliceOmatic ver. 4.2, Tomovision) (4). Briefly, total abdominal adipose tissue area was identified by selecting those pixels with an attenuation range of -30 to -190 Hounsfeld units (HU). Abdominal visceral and subcutaneous fat were calculated as the area of pixels within the appropriate HU range outside and within the abdominal wall, respectively. Blinded repeat measurements of abdominal visceral and subcutaneous fat in 24 randomly selected subjects were highly correlated ($r=0.99$, $P<0.05$).

MSNA, heart rate, arterial blood pressure, and respiration were recorded continuously and digitized at 500 Hz to a laboratory computer for later analysis using signal processing software (Windaq, Dataq Instruments). Basal MSNA was quantified as both burst frequency (bursts/min) and burst incidence (bursts/100 bts). MSNA recordings for each subject were also normalized by assigning the largest sympathetic burst under resting conditions to an amplitude of 1,000 (arbitrary integrative units). 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 neural silence between sympathetic bursts. Sympathetic baroreflex responses were determined from the relationship 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 (17). R-R intervals were binned over 3-mmHg

systolic blood pressure for linear regression analysis between R-R interval and systolic blood pressures. A 4-parameter sigmoid was fit to the data and the vagal baroreflex gain was calculated after systematically removing bin values in the threshold and saturation regions as described previously (4). We accepted only regressions with r values ≥ 0.70. A minimum of 2 of the 3 trials performed in each subject were used to determine an average vagal and sympathetic baroreflex gain.

Statistical Analysis. Differences in characteristics of the NW and SUBOB subjects and in the dependent variables were analyzed by independent student's *t*-test. Bivariate correlation analysis was used to assess relations among body composition and abdominal fat distribution variables in the pooled sample. SPSS statistical software (SPSS v.11.0, SPSS) was used to perform all statistical analyses. Data are expressed as mean±SEM. The significance level was set *a priori* at $P<0.05$.

RESULTS

Subject characteristics for SUBOB and NW. Subject characteristics of NW and SUBOB men are presented in Table 1. Body mass, body mass index, waist circumference, hip circumference, waist-to-hip ratio, body fat percent, total fat mass, lean body mass, abdominal subcutaneous, and total abdominal fat were significantly greater in the SUBOB compared to NW men ($P<0.05$). Maximal oxygen consumption was lower in the SUBOB compared with NW men, but not when expressed relative to fat free mass. There were no significant ($P>0.05$) differences in age, height, blood pressure (systolic or

diastolic), heart rate, or R-R interval. As intended, abdominal visceral fat was similar (P>0.05) in the SUBOB and NW men.

Basal MSNA and Baroreflex Responses in SUBOB and NW. There were no differences in basal MSNA burst frequency (22.5±2.7 vs. 23.9±2.3 bursts/min, Figure 1) or incidence (37.0±4.4 vs. 37.9±3.5 bursts/100 bts) in the SUBOB and NW men (P>0.05). Cardiovagal baroreflex gain (17.9±2.8 vs. 14.9±2.0 ms/mmHg, P>0.05) and sympathetic baroreflex gain (–8.1±1.0 vs. -8.5±0.4 arbitrary integrative units/mmHg/beat, P>0.05) were not significantly different in the SUBOB and NW men.

Body Composition, Anthropometric, and Abdominal Fat Distribution-Related Correlates. Abdominal visceral fat was the only significant body composition or abdominal fat distribution-related correlate of basal MSNA ($r = 0.45$, $P<0.05$, Figure 2) and vagal baroreflex gain ($r=0.49$, $P<0.05$) in the pooled sample (n=24). However, there were no significant correlates of sympathetic gain in these men.

DISCUSSION

There are two major new findings from the present study. First, MSNA is similar in men with subcutaneous obesity compared with normal weight men with comparable levels of abdominal visceral fat. 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 is evident even at levels of abdominal visceral fat below the threshold typically associated with elevated

cardiovascular and metabolic disease risk. Taken in conjunction with our previous observations (2), these findings suggest that abdominal visceral fat, but not subcutaneous fat, is the critical adipose tissue-related determinant of MSNA.

Individuals with a subcutaneous fat phenotype have been reported to be more insulin sensitive, have more favorable lipid and lipoprotein concentrations, and have lower cardiovascular disease risk in general compared with their peers with visceral obesity (10,25,34). Sustained activation of the SNS plays an important role in the pathophysiology of hypertension (6,12). In addition, the presence of hypertension or the level of arterial blood pressure appears to be more closely associated with abdominal adiposity than obesity *per se* (28,30,32). Therefore, it is possible that subcutaneous adiposity may be a phenotype that is protected from hypertension development. At least part of this protection may be related to a lack of SNS activation.

We previously observed lower levels of vagal baroreflex gain in men with visceral obesity (2,4). However, abdominal visceral fat was not correlated with vagal baroreflex gain in our previous studies. In the present study, vagal (and sympathetic) baroreflex gain did not differ in SUBOB and NW men. Taken together, these findings might suggest a critical role for abdominal visceral fat. However, vagal baroreflex gain tended to be lower, not higher, in NW men and was positively correlated with abdominal visceral fat. The reason(s) for this apparent discrepancy is (are) unclear; future studies will be necessary to clarify this issue.

There are some limitations of the present study that should be discussed. First, the sample sizes were small. The inclusion of a larger number of subjects in each group may yield a different outcome.

Second, we studied only healthy young men who were free of overt cardiovascular and metabolic diseases. Our observations may be dependent on age, gender, and/or the lack of certain chronic diseases. However, it is important to emphasize that age- and gender-related differences in MSNA appear to depend, at least in part, on age- and gender-related differences in abdominal adiposity (20,22). The potential interaction of abdominal adiposity with chronic diseases on MSNA is unclear.

Finally, 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 (e.g., liposuction) are necessary to confirm or refute our observations.

Taken together with our previous observations (2), these findings suggest that abdominal visceral fat, but not subcutaneous fat, is the critical adipose tissue-related determinant of MSNA. Importantly, the relation between abdominal visceral fat and MSNA is evident below the threshold typically associated with elevations in cardiovascular and metabolic disease risk (18,24,26,27,37). These findings may offer further insight into the complexity of variations in the phenotypic expression of cardiovascular and metabolic disease risk.

Table 1. Subject characteristics of normal weight (NW) and subcutaneous obese (SUBOB) men.

Variable	NW (n=15)	SUBOB (n=9)
Age (yrs)	22.4±1.4	23.4±2.1
Height (cm)	180.6±2.7	181.0±2.9
Body Mass (kg)	70.6±1.8	94.2±4.3*
Body Mass Index (kg/m^2)	21.7±0.5	28.7±0.8*
Waist Circumference (cm)	79.8±1.3	97.1±2.8*
Hip Circumference (cm)	97.3±1.2	110.5±2.5*
Waist-to-Hip Ratio	0.82±0.01	0.88±0.02*
Body Fat (%)	16.0±1.3	26.2±2.1*
Total Fat Mass (kg)	11.5±1.1	24.8±2.4*
Lean Body Mass (kg)	55.9±1.1	66.3±3.0*
Abdominal Subcutaneous Fat (cm^2)	131.7±12.2	306.9±36.0*
Abdominal Visceral Fat (cm^2)	55.1±4.7	68.5±6.7
Total Abdominal Fat (cm^2)	186.8±15.9	375.4±37.4*
Systolic Blood Pressure (mmHg)	114.7±2.0	119.2±2.6
Diastolic Blood Pressure (mmHg)	66.1±1.7	62.7±2.6
Heart Rate (bpm)	63.3±2.1	61.0±3.2
R-R Interval (ms)	1.05±0.05	1.06±0.07
VO_2max (ml/kg/min)	52.4±1.7	45.0±3.0*
VO_2max (ml/kg FFM/min)	65.9±1.9	63.5±3.3

All values are mean ± SE. VO_2max = maximal oxygen consumption per kg body weight and fat free mass. * $P< 0.05$ vs. NW.

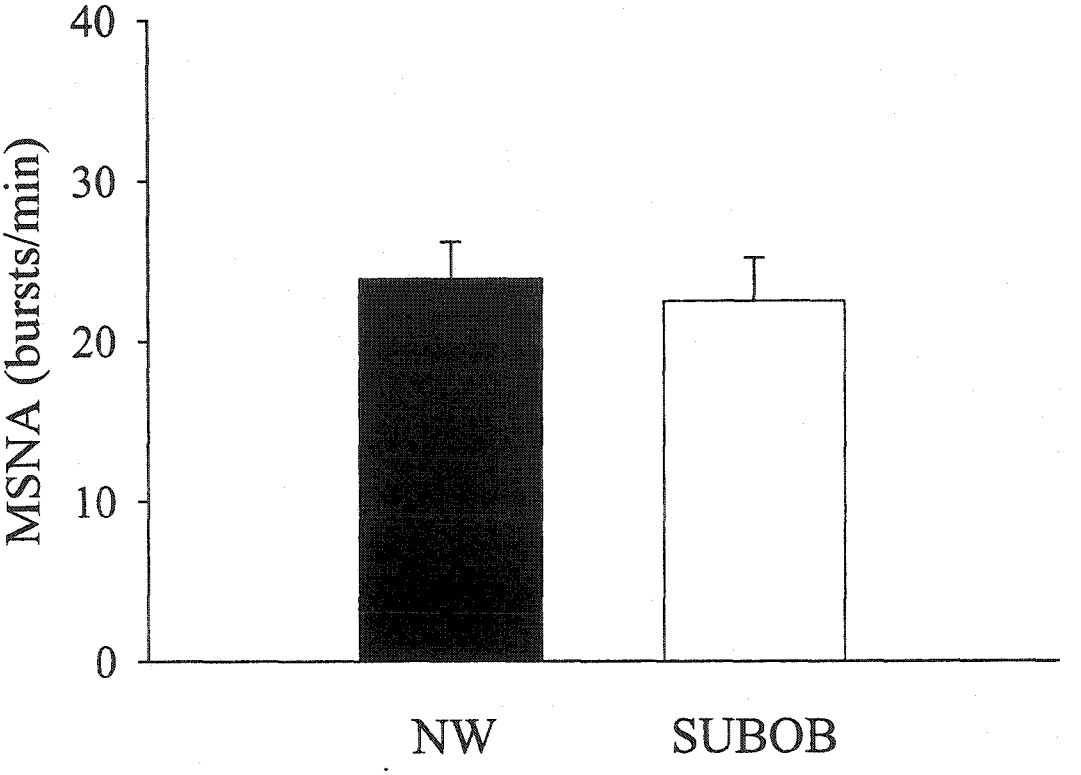

Figure1. Basal muscle sympathetic nerve activity in normal weight (NW) and subcutaneous obese (SUBOB) men. Values are mean±SE. *P<0.05 vs. NW.

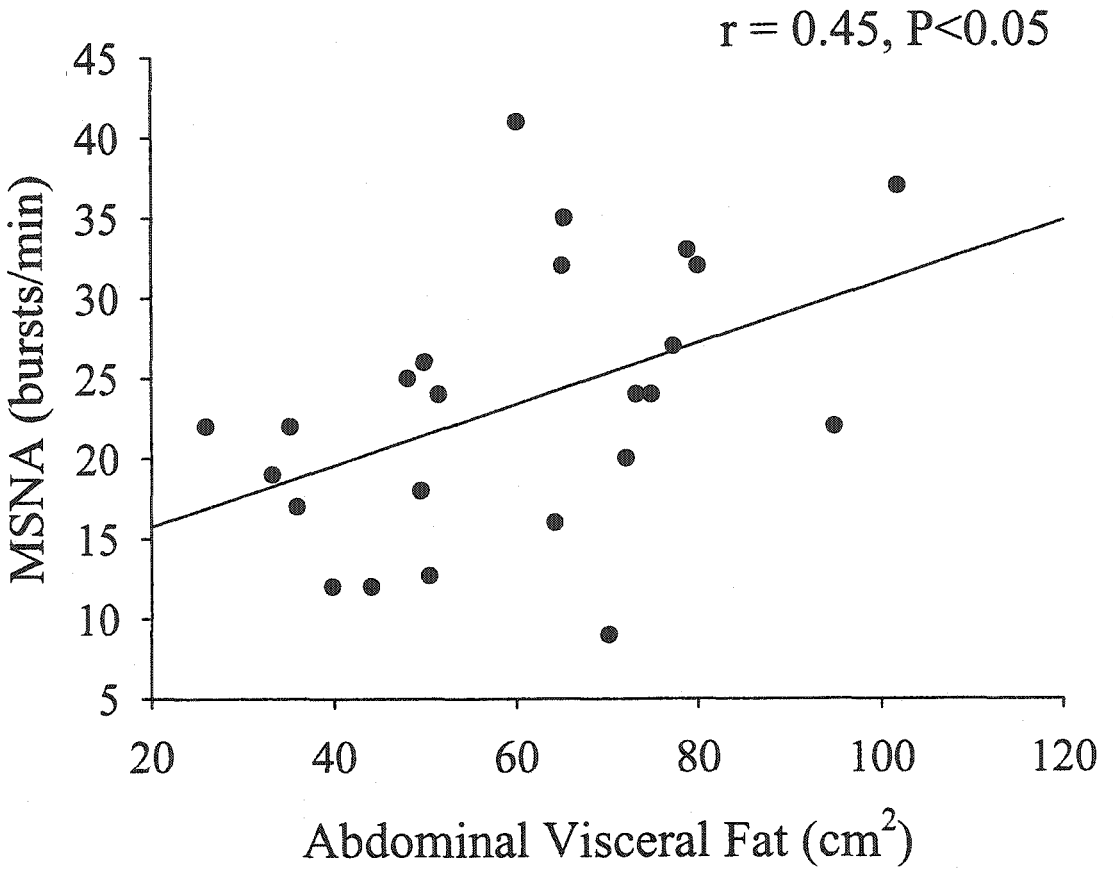

Figure 2. Relation between basal muscle sympathetic nerve activity and abdominal visceral fat in the pooled sample (n=24) of normal weight (NW) and subcutaneous obese (SUBOB) men.

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CHAPTER IV

WEIGHT LOSS REDUCES SYMPTHETIC NEURAL ACTIVITY IN OVERWEIGHT AND OBESE MEN

ABSTRACT

Muscle sympathetic nerve activity (MSNA) is elevated in obese humans and is particularly evident in men with visceral obesity. However, it is unclear whether reductions in total body and abdominal fat are associated with reductions in MSNA in normotensive men. Accordingly, we tested the hypothesis that reductions in total body and abdominal fat, by caloric restriction, would be associated with reductions in MSNA in overweight and obese (body mass index $\geq$25 and $\leq$ 35 kg/m^2, respectively) men with elevated abdominal visceral fat. To accomplish this, we measured MSNA (microneurography), body composition (dual energy x-ray absorptiometry), and abdominal visceral and subcutaneous fat (computed tomography) in 8 sedentary overweight and obese men (mean=33±3 yrs, range=20-40 yrs) before and after 3 months of weight loss. All measurements were performed after 4 weeks of weight stability at each individuals reduced body mass. Body mass (98±5 vs. 91±5 kg), total fat mass (27±3 vs. 23±2 kg), abdominal subcutaneous and visceral fat (324±37 vs. 280±32 and 128±18 vs. 102±15 cm^2, respectively) were reduced (all $P<0.05$) following the weight loss intervention. Arterial blood pressure (SBP=120±4 vs. 121±4, DBP=71±4 vs. 70±3

mmHg), heart rate (64±2 vs. 62±3 bpm), and maximal oxygen consumption (59±3 vs. 62±3 ml/kg FFM/min) did not change significantly with weight loss. As hypothesized, there was an ~23% reduction in MSNA (35±5 vs. 27±5 bursts/min, $P<0.05$) following weight loss. Therefore, the results of this preliminary study support the concept that reductions total body and abdominal fat are associated with reductions in MSNA in men with elevated abdominal visceral fat.

INTRODUCTION

Obesity is a major health concern increasing in epidemic proportions (11,12,22). Approximately 32% of the U.S. population is obese and at elevated risk for developing cardiovascular and metabolic diseases (12,24). There is considerable interindividual variability in the health risks associated with obesity. These risks depend, in part, on the distribution of body fat (7,8,23). Individuals displaying an upper body or abdominal fat distribution pattern demonstrate a clustering of cardiovascular and metabolic disease risk factors (27). The levels of these risk factors increase in proportion to the level of abdominal visceral fat (25).

The sympathetic nervous system plays a critical role in the regulation of cardiovascular homeostasis in general, and in both the beat-to-beat and long term regulation of arterial blood pressure in particular. In addition, activation of the sympathetic nervous system has been implicated in the pathophysiology of a number of cardiovascular diseases (9,14). Subsequently, there is increasing interest in developing therapeutic approaches to lower sympathetic activity, particularly in high-risk individuals (5,21,30,34).

We have previously reported that MSNA is higher in men with elevated abdominal visceral fat compared with their age- and total fat mass-matched peers with lower levels (1). Furthermore, MSNA does not differ in obese men with a subcutaneous fat phenotype compared with normal weight men with similar levels of abdominal visceral fat (unpublished observations). Taken together, these cross-sectional studies suggest that abdominal visceral fat is the critical adipose tissue-related determinant of MSNA.

The results of previous studies suggest that weight loss is associated with reductions in MSNA (2,15,31). However, these previous studies have either focused on hypertensive individuals (2) or failed to establish weight stability following weight loss (15). Therefore, these previous observations may have been specific to hypertensive individuals and due, in part, to their higher baseline levels of MSNA. Moreover, the reductions in MSNA may have been due, in part, to integrative adaptive responses to negative energy imbalance. Therefore, the purpose of the present study was to determine the influence of weight loss on MSNA in obese normotensive weight stable men. We hypothesized that weight loss would be associated with reductions in MSNA in overweight and obese men with elevated abdominal visceral fat. Additionally, we hypothesized that these reductions would be most closely associated with reductions in abdominal visceral fat.

METHODS

Subjects. Eight overweight and obese men (body mass index $\geq$25 to $\leq$35 kg/m^2, 20 to 40 yrs) volunteered to participate in the present study. All subjects were non-

diabetic (2-hr glucose load <200 mg/dL), normotensive (<140/90 mmHg, causal blood pressure recording), and free from other overt cardiovascular disease as assessed by health history questionnaire and by resting and exercise electrocardiograms. Subjects did not smoke and were not taking medications. Participants were sedentary (no regular physical activity >20 min on more than 2 days/wk) as determined by a physical activity questionnaire (29). The nature, purpose, risks, and benefits of the study were explained to each subject before informed consent was obtained. The Colorado State University Human Research Committee approved all experimental protocols.

Weight Loss Program. Participants underwent study sessions to assess basal MSNA before (Baseline) and after (Weight Loss) reductions in total body and abdominal visceral fat via caloric restriction. All participants were weight stable (±2 kg, self reported) for a 6-month period prior to enrollment. Each individual was prescribed a diet (sample menus and meal replacement supplements) consisting of 55-60% carbohydrate, 20-25% fat, and 15-20% protein at a deficit of 500kcal/day of resting energy requirements. Participants had weekly contact with a research dietician throughout the intervention. On each visit subjects were weighed and encouraged to keep daily food diaries to self-monitor intake and enhance adherence to the dietary protocol. The expected weight loss was 5-10% of initial body weight. The intervention was followed by a 1-month (4 weeks) weight stability (±1.5 kg) period prior to repeating experimental testing sessions.

Experimental Procedures. Body mass was measured on a physician's balance to the nearest 0.1kg. Height was measured using a stadiometer. Circumference measures were made according to standard guidelines (26) via tape measure (Gulick II Tape Measure, Country Technology Inc.). Body composition was measure by dual-energy x-ray absorptiometry (DPX-IQ, Lunar Radiation Corp, software v. 4.5c). A 10 mm thick computed tomography scan (HiSpeed CTi, GE Medical) was performed at the L4-L5 interspace of each subject to quantify abdominal subcutaneous and visceral fat levels (10).

Maximal oxygen consumption was measured during graded treadmill exercise to exhaustion using open circuit spirometry (TrueMax 2400, ParvoMedics). Valid maximal oxygen consumption was based on achieving at least three of the following four criteria: 1) plateau in maximal oxygen consumption with increasing work load, 2) attainment of age predicted maximal heart rate, 3) respiratory exchange ratio ≥ 1.10, and/or 4) rating of perceived exertion >18.

Heart rate was determined from a standard lead II of an electrocardiogram, beat-to-beat arterial blood pressure was measured by finger photophethysmography (Finapres Medical Systems, The Netherlands), and respiration was monitored using a pneumobelt (Gould Instruments). Resting finger arterial blood pressures were "adjusted" to brachial arterial blood pressures with an automated device (Dinamap Pro100, GE Medical Systems) prior to vasoactive drug infusions for assessment of sympathetic and vagal baroreflex gain (see below).

Recordings of multiunit MSNA were obtained from the right peroneal nerve using the microneurographic procedure (6). Briefly, external mapping of the peroneal nerve

was performed using low voltage (10-20 volts) passed through the skin to elicit an involuntary foot twitch. Two sterile tungsten microelectrodes (FHC Inc.) were inserted percutaneously. A reference microelectrode was placed near the fibular head (~1-2 cm). The second microelectrode was placed in the mapped region of the peroneal nerve. This electrode was advanced with simultaneous electrical stimulation (~1-3 volts) until the involuntary foot twitch was reproduced. Supporting evidence that the neural recording from the peroneal nerve was sympathetic includes a) afferent discharge upon tapping or stretching the muscle in the innervated area; and b) stimulation of pulse synchronous burst activity during phase II of the Valsalva maneuver or during end- expiratory apnea. Furthermore, there was no evidence of skin sympathetic nerve activity based on the: a) lack of paresthesias with weak intraneural electrical stimulation; b) light stroking of the skin in the innervated area did not elicit afferent discharge; and c) erratic, non-pulse synchronous activity increased by arousal (skin pinch or sudden louse noise) (33).

Experimental Protocol. All experimental testing sessions were between 7:00 am and 11:00 am. Subjects reported to the laboratory following a 12-hr overnight fast, were dressed in light clothing, and were situated supine in a thermoneutral room. Additionally, subjects were instructed to refrain from caffeine and alcohol consumption 24 hours prior to all testing sessions. Subjects were also instructed to avoid participation in any vigorous activity 24 hours prior to testing.

An antecubital venous catheter was placed in the subject's arm for the injection of vasoactive drugs. After a 20-minute rest period and steady state levels of all variables were achieved, a 10-minute recording of basal MSNA was obtained.

Subsequently, a bolus injection of sodium nitroprusside (100 μg) was given intravenously followed 60 seconds later by a bolus injection of phenylephrine HCL (150 μg). These pharmacological perturbations decreased and increased, respectively, arterial blood pressure ~15 mmHg from baseline levels during a 3-minute period. Three trials were completed and each was separated by a minimum of 15 minutes quiet rest.

Data Analysis. Abdominal visceral and subcutaneous fat regions were determined using commercially available medical imaging software (SliceOmatic ver. 4.2, Tomovision) as described previously (4). Blinded repeat measurements of abdominal visceral and subcutaneous fat performed in our laboratory were highly correlated ($r=0.99$, $P<0.05$).

The nerve signal was amplified, filtered (700-2,000 Hz), full-wave rectified, and integrated (time constant 0.1 sec) (nerve traffic analyzer, model 662C-3, University of Iowa Bioengineering). MSNA, heart rate, arterial blood pressure, and respiration were recorded continuously and digitized at 500 Hz to a laboratory computer for later analysis using signal processing software (Windaq, Dataq Instruments). MSNA was expressed as burst frequency (bursts/min) and burst incidence (bursts/100 heart beats). Total activity (arbitrary integration units) was used to determine the reflex changes in MSNA from baseline levels in response to blood pressure perturbations. 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 (17). A 4-parameter sigmoid was fit to the data to determine vagal baroreflex gain. The gain was calculated after systematically removing bin values in the threshold and

saturation regions as described previously (4). The averages of at least 2 of 3 trials performed in each subject were used to determine an average sympathetic and vagal baroreflex gain.

Statistical Analysis. All statistical analyses were performed with SPSS statistical software (SPSS v.11.0, SPSS). Paired-samples *t*-tests were performed to assess changes in subject characteristics and dependent variables from Baseline to Weight Loss. The relationship among the changes in specific subject characteristics and changes in basal MSNA and vagal and sympathetic baroreflex responses for intervention subjects were assessed using bivariate correlation analysis. Data are expressed as mean±SEM. The significance level was set *a priori* at $P<0.05$.

RESULTS

Subject Characteristics. Subject characteristics at Baseline and Weight Loss for overweight and obese men are shown in Table 1. Subjects demonstrated significant decreases in body mass, body mass index, waist circumference, hip circumference, waist-to-hip ratio, body fat, total fat mass, lean body mass, abdominal subcutaneous and visceral fat, and total abdominal fat ($P<0.05$). Maximal oxygen consumption expressed relative to body mass increased ($P<0.05$) after weight loss, although when expressed relative to fat-free mass this observation was no longer present (baseline = 58.9±3.4 vs. weightloss = 62.1±2.6 ml/kg FFM/min, $P>0.05$). There were no significant changes in systolic blood pressure, diastolic blood pressure, heart rate, or R-R interval ($P>0.05$) with the weight loss intervention.

Basal MSNA and Baroreflex Responses. Basal MSNA significantly decreased from Baseline to Weight Loss (35.1±4.7 vs. 26.9±5.2 bursts/min, $P<0.05$), Figure 1. Sympathetic baroreflex gain was similar after intervention (-10.6±1.1 vs. -11.9±1.6 arbitrary integration units/mmHg/beat, $P>0.05$). Vagal baroreflex gain increased after weight loss (12.5±2.2 and 18.5±3.2 ms/mmHg, $P<0.05$, Figure 2).

Correlations. The reductions in MSNA and increases in vagal baroreflex gain with weight loss were not significantly related to baselines levels of MSNA or to changes in body composition or abdominal fat distribution.

DISCUSSION

There were several important findings of the present study. First, reductions in total body and abdominal fat were associated with reductions in MSNA in normotensive overweight and obese men with elevated abdominal fat. Second, reductions in total body and abdominal fat were associated with a significant increase in vagal, but not sympathetic, baroreflex gain following weight loss. Importantly, the reductions in MSNA and increases in vagal baroreflex gain were observed in men who were weight stable for a 4-week period following weight loss. Finally, in contrast to our hypothesis, we did not observe any consistent relation between reductions in total body or abdominal fat and reductions in MSNA or increase in vagal baroreflex gain.

We observed a significant reduction (~23%) in MSNA following an ~8% weight loss in normotensive overweight and obese men. These observations confirm and extend the findings of previous reports that indicated that weight losses ranging from 7-13%

were associated with 15-34% reductions in MSNA (2,15,31). Our findings are important in that MSNA was measured after weight loss following 4 weeks of weight stability. Taken together, these observations would suggest that the reduction in MSNA following weight loss occurs independent of the acute after effects of energy imbalance.

The mechanisms responsible for the reductions in MSNA with weight loss remain unclear. In the present study, the reductions in MSNA were not correlated with reductions in total body or abdominal adiposity. However, the reductions in MSNA with weight loss are more likely to be directly related to reductions in fasting insulin (13,19,20), leptin (16,28), or angiotensin II (18,32). We should emphasize that the sample size of this preliminary study was small. Therefore, future studies with larger numbers of subjects will be necessary to determine whether reductions in total body or abdominal fat or alterations in circulatory neurohumoral factors are related to the reductions in MSNA.

Vagal baroreflex gain, but not sympathetic baroreflex gain, was significantly augmented following weight loss in the present study. The former observation, but not the latter, is consistent with previous reports (15). The mechanisms for the increases in vagal baroreflex gain are also unclear but may be related to increases in arterial compliance with weight loss (3,35). However, the reason(s) for inconsistent effects of weight loss on sympathetic baroreflex gain remain undetermined. One possible explanation contributing to the discrepancies are differences in the methodological approaches employed. For example, Grassi et al (15) utilized graded steady state infusions of phenylephrine HCL and sodium nitroprusside whereas, in the present study,

the modified Oxford technique was chosen for the assessment of baroreflex function. Future studies will be necessary to resolve this issue.

In summary, the results of this preliminary investigation suggest that a modest reduction in total body and abdominal visceral fat are associated with significant reductions in MSNA in men with elevated abdominal visceral fat. These reductions in total body and abdominal fat were also associated with increases in vagal, but not sympathetic, baroreflex gain. Finally, these reductions in MSNA and increases in vagal baroreflex gain were observed in men who were weight stable for a 4-week period following weight loss. These findings may have important implications for understanding the alterations in autonomic-circulatory dysfunction observed in obese humans.

Table 1. Subject characteristics in overweight and obese men before (Baseline) and after (Weight Loss) 3 months of caloric restriction.

Variable	Baseline (n=8)	Weight Loss (n=8)
Age (yrs)	33.3±2.9	-----
Height (cm)	179.1±2.6	-----
Body Mass (kg)	97.7±5.4	90.6±4.7*
Body Mass Index (kg/m^2)	30.4±1.2	28.2±1.0*
Waist Circumference (cm)	103.1±3.7	97.1±3.4*
Hip Circumference (cm)	110.6±2.4	106.7±2.3*
Waist-to-Hip Ratio	0.93±0.02	0.91±0.02*
Body Fat (%)	27.2±1.7	25.3±1.7*
Total Fat Mass (kg)	26.9±2.9	23.2±2.4*
Lean Body Mass (kg)	67.7±2.9	64.5±2.7*
Abdominal Subcutaneous Fat (cm^2)	323.8±37.2	279.8±31.7*
Abdominal Visceral Fat (cm^2)	128.1±17.5	102.3±15.0*
Total Abdominal Fat (cm^2)	451.9±43.6	382.1±38.1*
Systolic Blood Pressure (mmHg)	120.3±3.5	121.0±3.6
Diastolic Blood Pressure (mmHg)	71.1±3.6	70.0±3.2
Heart Rate (bpm)	63.9±1.9	61.9±3.3
R-R Interval (ms)	0.97±0.04	1.00±0.02
VO_2max (ml/kg/min)	41.3±3.1	44.7±2.8*
VO_2max (ml/kg FFM/min)	58.9±3.4	62.1±2.6

All values are mean ± SE. * $P < 0.05$ vs. Baseline.

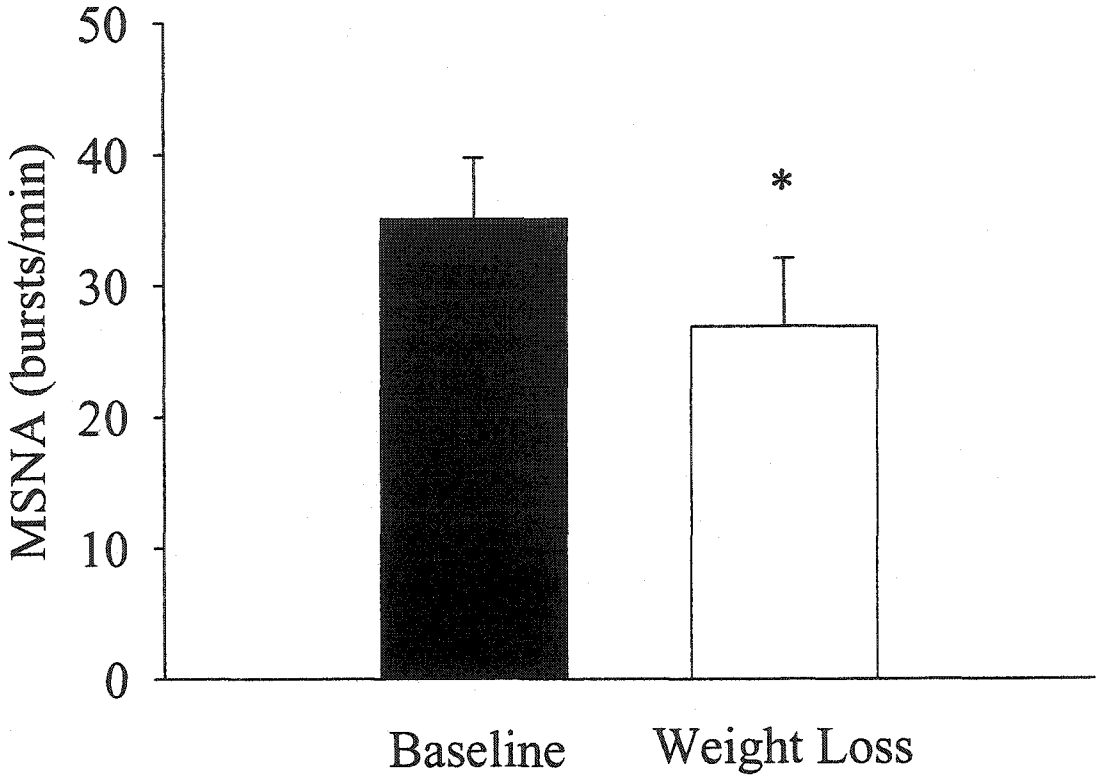

Figure 1. Basal muscle sympathetic nerve activity in men before (Baseline) and following (Weight Loss) 3 months caloric restriction. Values are mean±SE. *P<0.05 vs. Baseline.

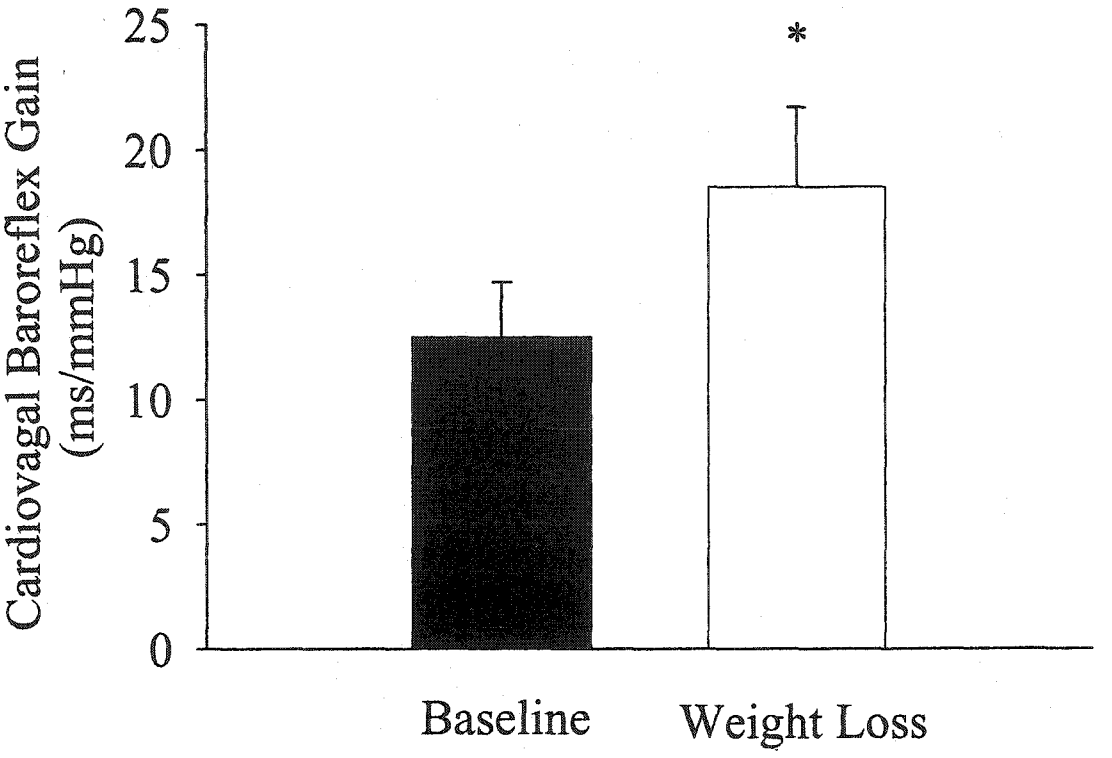

Figure 2. Cardiovagal baroreflex gain in men before (Baseline) and following (Weight Loss) 3 months caloric restriction. Values are mean±SE. *P<0.05 vs. Baseline.

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CHAPTER V

OVERALL CONCLUSIONS

The studies conducted resulted in several important findings. First, muscle sympathetic nerve activity (MSNA) is elevated in men with higher levels of abdominal visceral fat compared to their age- and total fat mass-matched peers with lower levels. Basal MSNA was more closely associated with abdominal visceral fat than other whole body or regional measures of adiposity. Furthermore, the relation between abdominal visceral fat and MSNA was independent of total fat mass.

Second, there were no differences in basal MSNA in subcutaneous obese and normal weight men with similar levels of abdominal visceral fat. Importantly, abdominal visceral fat was the only body composition-related correlate of MSNA in the pooled sample.

Finally, the results of a preliminary investigation indicate that basal MSNA was reduced with weight loss in overweight and obese men with elevated abdominal visceral fat.

Therefore, the findings from these investigations suggest that abdominal visceral fat, but not subcutaneous adiposity, is the critical adipose tissue-related determinant of MSNA in humans. Furthermore, weight loss is associated with reductions in MSNA. Whether these reductions are the result of reductions in abdominal visceral fat remains unclear. These findings may have important

implications for understanding the variations in cardiovascular disease risk related to differences in abdominal fat distribution.

These studies have highlighted the complexity of obesity related disease risk and have identified a number of areas for future study. First, additional studies are needed to confirm the link between abdominal visceral fat and MSNA in men and women of different age and racial/ethnic groups. Second, there is currently no information available on the mechanisms responsible for the association between abdominal visceral fat and MSNA. Third, there is currently no information available on the physiological or pathophysiological consequences of sympathetic neural activation in visceral obesity. Presently, it is unknown whether sympathetic neural activation is an important mechanism for the higher prevalence of hypertension in visceral obesity. Additionally, it is unknown whether sympathetic adrenergic vasoconstrictor tone is increased in visceral obesity. Future studies are needed to address these issues.

The results of future studies will yield important information that may ultimately improve our understanding of the health risks associated with visceral obesity. In turn, this new knowledge may offer insight into new pharmacological therapies for visceral obesity and its associated co-morbidities.

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APPENDIX A:
INFORMED CONSENT

COLORADO STATE UNIVERSITY
INFORMED CONSENT TO PARTICIPATE IN A RESEARCH PROJECT

Project Title: Abdominal Fat and Autonomic-Circulatory Control in Humans
Principal Investigator: Kevin P. Davy, Ph.D.
Co-investigators: Christopher L. Melby, Dr.P.H.
Brenda M. Davy, M.S.
Stacy Beske, M.S.
Guy Alvarez, M.S.
Tasha Phillips, B.S.
Colleen Cooke, M.S.

Contact person and phone number for questions/problems: Kevin P. Davy, Ph.D., Tel: 491-5871

Purpose of the research: To determine how body composition (amount of fat and lean tissue) and fat distribution pattern (apple vs. pear shape) influence the cardiovascular system in humans.

Procedures / Methods to be used: If you agree to participate in this study you will first be required to complete a personal health history questionnaire. Based on our evaluation of the questionnaire you may then be eligible to become a study subject. Eligible candidates will be non-smoking males or females between the ages of 18 and 40 years who are normotensive (blood pressure <140/90), free of dyslipidemia [cholesterol problems], and diabetes as assessed by a medical history or the glucose tolerance test in the case of diabetes (see below for glucose tolerance test). If you have a family history of cardiac sudden death or premature coronary heart disease (heart disease in brother or sister or parent prior to the age of 55 years), you will be required to undergo a physician supervised resting and maximal exercise electrocardiogram to screen for the presence of coronary heart disease. You will not be eligible to participate in the study if you use any medications that could potentially influence your cardiovascular system.

You may be asked or choose to participate in only one component of this study. There are two components: 1) a comparison of subjects with high vs. lower levels of total body fat and abdominal fat and 2) an intervention consisting of underfeeding/weight loss (or control). In addition, you may be asked to or choose to participate in some or all of the sessions which make up this study. For each of these sessions, a check next to yes will indicate that you are being asked to participate in that session. A check next to no will indicate that you are not being asked to participate in that session. If no choice is provided, then participation is a required part of the study.

You will be divided into one of two groups according to your body mass index (calculated from your height and weight): a group whose body mass index is less than 25

or a group whose body mass index is greater than 30 but less than 35. If your body mass index is greater than 30, you may be asked to be randomized to an underfeeding (weight loss) group or a control group who is asked not to change any of their dietary or physical activity habits. If you are assigned to one the weight loss groups, you will be instructed on how to modify your diet to reduce the amount of fat and calories so that you lose 10% of your initial body weight over a 12-week period. You will be asked to come to the CSU campus (Moby Complex or Gifford Building) weekly to have your body weight measured and to discuss with a dietitian any problems you may be experiencing with your weight loss program.

For a one month period (regardless of which group you are in) before and after the weight loss or control group, you will be asked to eat food provided to you and/or your own food so that your body weight remains stable.

You will be asked to come to the CSU campus (either Moby Complex or Gifford Building) every 1-3 days to have your body weight measured.

All of the following sessions and procedures will be performed once for the individuals with a body mass index less than 25 or both before and after the underfeeding (or control) interventions for individuals with a body mass index greater than 30.

Session 1 - Moby Complex, Colorado State University.
(1) Medical History-you will be asked to complete a medical history questionnaire. This procedure is used to screen for pre-existing disease or other reasons you should not participate in this study. Your height and weight will also be measured at this time. Your body weight will be measured on a standard balance scale, and will include the weight of light indoor clothing minus shoes. Your waist, hip, and neck circumference will be measured using a measuring tape.

(2) Cholesterol Levels. A small plastic tube or catheter will be inserted into an arm vein to draw blood (approximately 2 tablespoons) to measure the levels of cholesterol.

(3) Pregnancy Test. If you are female you will be required to have a sample of your urine tested for the presence of human chronic gonadotropin (HCG), a hormone which indicates whether you may be pregnant. This will require approximately 1 cup of your urine. If you are pregnant or the test indicates that you are pregnant you will not be able to participate in this study.

(4) Oral Glucose Tolerance Test. For this procedure you will be asked to drink a very sweet sugar solution (75 grams glucose). Additional blood samples (2 blood samples) to those described above will be taken both before and after you drink this solution. The purpose of this procedure is to determine whether you have a normal glucose response to

drinking this sweet tasting drink (your oral glucose tolerance). If your glucose response is abnormal you may not be able to continue participation and you will be referred to your personal physician. Approximately 3-4 teaspoons of blood will be required for this procedure. This procedure will take approximately an additional 2 hours of your time.

(5) Sodium Excretion. You will collect all of your urine for a 24-hr period in containers that we give you and bring those containers in to the laboratory at the end of the 24-hour period so that the amount of salt in your urine can be measured. You will be asked to return the container provided to you on the following day. This will take approximately 10 min of your time over the course of a day.

Approximate time required: 2.5-3.0 hours

Session 2 – Poudre Valley Hospital, Sleep Laboratory

(1) Sleep Study. You may be asked to have a sleep study to determine whether you have abnormal breathing activity (sleep apnea) while you sleep. If you have this study done, you will be asked to go either to Poudre Valley Hospital (directions will be provided) in the evening close to the time you usually go to sleep and/or to take a portable monitoring system home with you. If the study is performed at the hospital, you will be required to sleep there over night. In either case, you will have the electrical activity in your brain, electrical activity of the muscles in your face, and eye movement, monitored with electrodes attached to your scalp/skin. A sensor attached to your ear or finger will monitor the oxygen level in your blood. A sensor placed just under your nose and mouth will monitor the airflow. Your breathing will be monitored with a plastic belt placed around your chest and abdomen. Your heart rate will be measured from electrodes places on your chest. A physician will determine if the results of your sleep study indicate whether you have sleep apnea. If so, you will be referred to your personal physician or one will be recommended to you. He/she will want to determine whether further tests are needed and if you need medical treatment. If your sleep study is abnormal, you may still be able to participate in this study but we will need written approval from your doctor.

Approximate Time Required: One night or 7-10 hours Yes_____ No_____

Session 3 - Moby Complex, Colorado State University.

(1) Body Composition. To measure your body fat, you will lie on a hospital-type bed and a small amount of x-ray will be passed through your body to determine the amount of bone, muscle and fat in your body. This test takes approximately 45 min and there is no pain associated with the procedure. This procedure will be performed once at the beginning of the study, and a second time at end of your weight loss program. These tests will be performed at the Moby Complex at CSU.

(2) Graded Exercise Test-This procedure involves walking or running on a motorized treadmill while the electrical activity of your heart (electrocardiogram) and blood pressure is monitored. The grade of the treadmill will increase every 2 minutes, and the test will end when you are too exhausted to continue (usually 8-12 minutes). The procedure is used to determine your aerobic fitness level and as a screening test for coronary artery disease.

(3) Diet and Physical Activity Questionnaires. You will be asked to complete three questionnaires. The first two are food intake questionnaires, which will be used to determine your average intake of calories, fat, protein, carbohydrate, fiber, fruits and vegetables during the previous month. You will be asked to remember everything you ate on the previous day. This should take approximately 15-20 minutes. For the second food questionnaire, you will be asked to write down everything you eat for a 4 day period. This should require approximately 10-20 minutes of your time each day. The third questionnaire is to estimate your usual physical activity level, which will require about 15 minutes to complete.

Approximate time required: 1 hr (without Graded Exercise Test); 2 hr (with Graded Exercise Test).

Session 4 - Moby Complex, Colorado State University

(1) Arterial Blood Pressure, Heart Rate, and Breathing. A continuous recording of the blood pressure in your arteries will be made by placing a small cuff around your middle finger while your hand is maintained at
heart level. Heart rate will be measured by placing three electrodes on your chest and reading the electrocardiographic (ECG) signal. You will be asked to pace your breathing to a clock at a rate of 15 breaths per minute. A loose fitting plastic belt will be placed around the upper abdomen to measure your breathing movements.

(2) Sympathetic Nervous System Activity. The measurement of sympathetic nervous system activity involves measuring the activity of one of your nerves on the side of knee or arm. Two small microelectrodes (small needles) will be placed through your skin on the side of your knee or arm. The position of one of the electrodes will be moved back and forth through your skin while a very small electrical impulse (1 -2 volts) is passed through the electrode. This search procedure will continue until the electrode being moved causes your foot or hand to twitch. This procedure will take between 5-60 minutes. When a foot or hand twitch is observed, measurement of the activity of the sympathetic nervous system will begin and continue during the procedure described below.

(3) Catheter and Blood Draw. A small plastic tube will be inserted in your arm to draw blood (approximately 1/3 cup). Norepinephrine, angiotensin II, angiotensinogen, aldosterone, all hormones that influence your cardiovascular system will be measured. We will also measure growth hormone and cortisol, hormones that influence your metabolism and cardiovascular system. The plastic tube (catheter) will be left in your arm for study described below.

(4) Arterial Baroreflex Study. To measure the relationship between changes in blood pressure and change in heart rate and sympathetic nervous activity, you will be given an injection of a drug (sodium nitroprusside 100 to 150 micrograms)-a drug which causes your blood vessels to dilate) into antecubital vein (large forearm vein) which will lower your blood pressure. Sixty seconds later, you will be given an injection of a second drug (phenylephrine HCL (100 to150 micrograms)-a drug which causes your blood vessels to constrict) to raise your blood pressure. This series of injections will be repeated two more times after at least thirty minutes of passed each time. The amount of each drug to be injected will begin with 100 micrograms. The amount may be increased to 150 micrograms if your blood pressure did not drop or increase at least 15 points. In addition, a small, pencil shaped blood pressure measuring device will also be pressed gently against your neck for a short time.

(5) Blood Flow in Heart and Arteries. Blood flow and diameter in your the arteries in your neck, arm, and leg will be measured with an ultrasound machine. The probe used will be pressed gently against an artery in your neck, arm, and leg. You will be asked to squeeze a handgrip device for two-one minute periods at less than half of your maximum ability while the diameter of your neck artery is measured. Your heart rate and blood pressure will also be measured during this time. This will be used to measure the flexibility of your artery. Your maximum handgrip strength will be measured with this handgrip device just before these measurements by having your squeeze this device as hard as you can. The amount of blood that your heart pumps in one beat and in one minute will also be measured with another ultrasound probe. For these measurements, the probe will be pressed gently against two different places on your chest.

(6) Cold Pressor Test. You will be asked to immerse your hand up to wrist level in a bucket of ice cold water for 2 minutes. This will provide additional information about your sympathetic nervous system and circulation not provided by the arterial baroreflex study.

Approximate time required: 3.0 hours

Session 5-Poudre Valley Hospital, Department of Radiology, Fort Collins, CO
(1) Computed Tomography Scan. The amount of total fat, fat around your internal organs, and fat under the skin in the abdominal area will be measured by computed tomography (CT scan). The CT imaging will be performed at Poudre Valley Hospital in Fort Collins.

For this procedure, you will be asked to lie very still on a table. An x-ray machine (the CT scanner) will rotate around you, and the table will moved back and forth slightly making it possible to take x-rays from several angles. The actual x-ray time is approximately less than 2 min. You will be lying on the table for approximately 15 to 30 min.

Approximate time required: 1 hour (Note: a longer period may be necessary due to heavy scheduling and/or emergency use of the CT scan at Poudre Valley Hospital).

Session 6 - Moby Complex, Colorado State University
(1) Resting Energy Expenditure. After fasting for 12 hours you will come to the Moby building Rm. 212C
between 7:00 and 8:00 a.m. You will then lie quietly on a hospital-type bed and after a 15-minute period of
quiet rest a clear bubble-type hood will be placed over your head in order to collect all of the air that you expire. This hood will not disturb your natural breathing pattern.

(2) Intravenous Glucose Tolerance Test. After the completion of the above measurement, two small plastic tubes (catheters) will be placed in each of two arm veins (different arms). The test involves injecting small amounts of glucose (0.3 mg/kg of body weight) and insulin (0.03 unit/kg body weight) into your veins (insulin is a hormone which helps your body's cells metabolize glucose). We will draw blood approximately 28 times. The total amount of blood drawn is equal to about one-half cup or 100 cc. The catheters will remain in your arms throughout the entire test. This test measures your insulin sensitivity.

Approximate time required: 4-5 hours.

Session 7 - Moby Complex, Colorado State University

(1) Arterial Blood Pressure, Heart Rate, and Breathing. A continuous recording of the blood pressure in your arteries will be made by placing a small cuff around your middle finger or wrist while you hand is maintained at heart level. Heart rate will be measured by placing electrodes on your chest and reading the electrocardiographic (ECG) signal. You will be asked to pace your breathing to a clock at a rate of 15 breaths per minute. A loose fitting plastic belt will be placed around the upper abdomen to measure your breathing movements.

(2) Sympathetic Nervous System Activity. The measurement of sympathetic nervous system activity involves measuring the activity of one of your nerves on the side of your knee or arm. Two small microelectrodes (small needles) will be placed through your skin. The position of one of the electrodes will be moved back and forth through your skin while a very small electrical impulse (1-2 volts) is passed through the electrode. This search procedure will continue until the electrode being moved causes your foot or

hand to twitch. This procedure will take between 5-60 minutes. When a foot or hand twitch is observed, measurement of the activity of the sympathetic nervous system will begin and continue during the procedure described below.

(3) Forearm Blood Flow. The amount of blood flow to your arm will be measured in two ways. First, we will place a small flexible piece of plastic around your forearm and blood pressure cuffs around upper and lower arm. The cuffs will be inflated and deflated periodically. Second, we will use an ultrasound machine, which produces sound waves to measure your forearm blood flow. The two techniques will be used together to ensure the most accurate measurement.

(4) Lower Body Negative Pressure. Your lower body (up to your waist) will be sealed in an air tight box which is attached to a vacuum cleaner. When the vacuum cleaner is turned on a negative pressure is created inside the box and this causes some of the blood in your circulation to move to your legs. This procedure will be performed at 3 to 4 different levels (not to exceed –20 mmHg) to increase your sympathetic activity. We will then measure your sympathetic activity and how the blood flow in your forearm and/or leg changes.

(5) Plasma Norepinephrine Concentrations. A small plastic tube (catheter) will be inserted into an arm vein to draw blood (approximately 2 tablespoons) to measure the levels of norepinephrine in your circulation. Norepinephrine is a substance secreted by your sympathetic nerves and causes your blood vessels to constrict.

Approximate time required: 2.5 hours Yes_____ No_____

RISKS INHERENT IN THE PROCEDURES:

Catheters: Some pain or discomfort may be experienced when the catheter is inserted in the vein, but this persists for only a short time. In about 1 in 10 cases, a small amount of bleeding under the skin will cause a bruise. The risk of a blood clot forming in the vein is about 1 in 200, while the risk of infection or significant blood loss is 1 in 1000. Having only people experienced in phlebotomy placing the needle or catheter will minimize these risks.

Oral Glucose Tolerance Test: Because this procedure requires the placement of a catheter in a vein in each arm, the risks here are identical to those stated above. In addition, there is a small risk of low blood sugar occurring during or after the test. If this happens, orange juice (with table sugar) or some other simple-carbohydrate containing food will be given to you.

Sleep Study: There are no known risks associated with sleep studies. However, you may not sleep very well the night of study.

Intravenous Glucose Tolerance Test: Because this procedure requires the placement of a catheter in an arm vein, the risks here are identical to that stated above. In addition, there is a small risk of low blood sugar occurring during or after the test. If this happens, orange juice (with table sugar) or some other simple-carbohydrate containing food will be given to you. A registered nurse will be with you during this test.

Graded Exercise Test: Maximal exercise testing may cause fatigue, muscle strains, an irregular heart beat (arrhythmia's), and a change in blood pressure. There is a 0.01% chance of death, and a 0.02% risk of cardiac arrhythmias requiring hospitalization.

DEXA Scan: The amount of radiation that you will receive in the DEXA exam (combined with the CT scan) is less than the amount permitted by the Food and Drug Administration (FDA) per year. The amount you will receive is equal to 1/20 of a chest x-ray. The more radiation received over the course of your life, the more is the risk of developing cancerous tumors. The radiation in this study is not expected to greatly increase these risks, but the exact increase in such risk is not known.

CT Scan: The amount of radiation that you will receive in the CT scan (combined with the DEXA exam) is less than the amount permitted by the Food and Drug Administration (FDA) per year. The amount you will receive is equal to that produced by a dental x-ray. The more radiation received over the course of your life, the more is the risk of developing cancerous tumors. The radiation in this study is not expected to greatly increase these risks, but the exact increase in such risk is not known.

Sympathetic Nervous System Activity: Some subjects experience a temporary (seconds) pain and discomfort while the microelectrodes are being inserted. After the procedure there is a small risk of numbness, pins and needles type sensations, or pain which lasts 1-3 days. In very rare cases, numbness, pins and needles type sensations, or pain in the leg or arm has lasted several weeks or months (1 -3 in 1000). These problems can be minimized by only having experienced individuals perform this technique. In addition, by minimizing the time to find the nerve to less than 60 minutes, the risk of unpleasant after-effects is reduced even more.

Drug Infusions: Because this procedure requires the insertion of a catheter, the risks here are identical to those described above under catheters. In addition, the infusion of nitroprusside could cause low blood pressure and nausea, sweating, or a sudden elevation in heart rate. These feelings should pass within 1-2 minutes. The infusion of phenylephrine may result in a headache, restlessness, a sudden decrease in heart rate, and/or rarely an irregular heart beat. These feelings or symptoms, if they occur,

usually pass within a few minutes. There is also a small risk that some phenylephrine will leak out from the catheter site causing severe constriction of the surrounding small blood vessels. This may result in an inadequate blood supply to the surrounding tissues and eventual death of that tissue if untreated. This risk is prevented by using a large vein in

your arm. However, if this problem occurs you will be referred to a physician for immediate treatment. These risks are slightly increased because we will repeat both drug infusions two times. It should be emphasized that the amount of these drugs you will be receiving is very small and they are rapidly metabolized by your body. This lowers the risk of any adverse reactions to the drugs. In addition, a registered nurse will supervise this aspect of the study.

Handgrip: Your heart rate and blood pressure will increase only slightly. You will feel some fatigue and discomfort in your hand and forearm while you squeeze the handgrip device but this will pass as soon as you stop.

Lower Body Negative Pressure: There is a very small risk of feeling nausea or fainting. The protocol will be stopped if you begin to feel nauseous, like you may faint, if your heart rate suddenly drops by 15 beats per minute or more, or if your blood pressure suddenly drops by 15 mmHg or more. You will be monitored continuously by investigators to avoid any fainting.

It is not possible to identify all potential risks in an experimental procedure, but the researcher(s) have taken reasonable safeguards to minimize any known and potential, but unknown, risks.

BENEFITS: You will benefit from being informed of your body mass and composition (how much fat and muscle tissue you have), your fitness level, serum lipid and lipoprotein concentrations, your estimated insulin
sensitivity and how many calories your body needs to maintain your body weight. If your BMI is greater than 30 and you are placed in the weight-loss intervention, you will benefit from receiving in depth personal counseling and instruction, and you will receive health benefits from losing weight.

FINANCIAL COMPENSATION: You will receive $100 for completion of the Session #3. If your body mass index is above 30 and you are studied again after weight loss, you will receive another $100 ($200 total) for completion of the same measurements (Session #3).

CONFIDENTIALITY: All of the data obtained on you will be kept in a separate file in a locked cabinet that will be accessible only to the principal and co-investigators listed above. Data will be published as group data; no publication will include any data which can be linked to you as an individual.

LIABILITY: Because Colorado State University is a publicly-funded, state institution, it may have only limited legal responsibility for injuries incurred as a result of participation in this study under a Colorado law known as the Colorado Governmental Immunity Act (Colorado Revised Statutes, Section 24-10-101, et seq.). In addition, under Colorado law, you must file any claims against the University within 180 days after the date of injury. We will attempt to secure emergency medical treatment for you if you are injured or have an adverse reaction or illness as a direct result of this research, but such treatment cannot be guaranteed. Either you or your insurance company will have to pay the costs of the medical treatment. No funds are available from the University. You will need to check with your insurance company to see if they will cover medical expenses arising from participation in a research project, because sometimes they will not cover them. The University cannot pay you
for lost wages, pain, or suffering. Because the University is a state entity, it may have governmental immunity, which may prevent you from recovering damages in a lawsuit. If you think you have been injured because of this research, please contact Celia Walker, CSU Regulatory Compliance Office at (970) 491-1563.

Any other questions concerning treatment of subject's rights also should be directed to Celia S. Walker at (970) 491-1563.

PARTICIPATION: Your participation in this research project is voluntary. If you decide to participate in the study, you may withdraw your consent and stop participating at any time without penalty or loss of benefits to which you are otherwise entitled. Your signature acknowledges that you have read the information stated and willingly signed this consent form. Your signature also acknowledges that you have received, on the date signed, a copy of this document containing 8 pages.

_____	_____
Participant Name (printed)	Date
_____	_____
Participant Signature	Date
_____	_____
Investigator or Co-investigator Signature	Date

During your Session 1 visit described above we would like to draw an extra 5-6 tablespoons of your blood. Part of this sample will be used for genetic testing. This blood sample will be used to make a permanent bank of your white blood cells. DNA or deoxyribonucleic acid (the principle chemical component responsible for your genetic characteristics [for example, hair color] will be extracted from these cells for analysis of genetic factors. These genetic factors may help explain why different people have different levels of heart rate, blood pressure, sympathetic activity, or other variables measured as part of your participation in the above study. The results of the analysis of these genetic factors and any linking to other information measured will be completely anonymous. There will be no way of connecting this information to your name or identity and the results will only be available to the investigators. The DNA extracted from your blood during this project will be done in the strictest confidentiality.

I agree to having my blood drawn for genetic analysis YES ☐ NO ☐

_____ _____

Participant Signature Date

We would also like to store some of your blood for future related research. This blood may be used to measure other variables that may be thought to be important to this project or another new project or to do more genetic testing. Any future project using these samples would have to undergo review by the Colorado State University Human Research Committee. The samples will be handled in a confidential and annonymous way as described above.

I **agree** to having my blood drawn for storage YES ☐ NO ☐

_____ _____

Subjects Signature Date