Effects of Weight Gain on Blood Pressure and Sympathetic Neural Activity in Nonobese Humans

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ABSTRACT

Obesity is associated with sympathetic neural activation and elevated blood pressure(1,2) . However, it is unclear whether modest elevations in body weight are sufficient to induce increases in sympathetic activity (3). Furthermore, there is a large amount of individual variability in the blood pressure response to weight change (4). The reason(s) for this inter-individual variability are still uncertain, but body fat distribution and cardiorespiratory fitness may play a role (5,6). To address these and other issues regarding the relation between adiposity, sympathetic neural activity and blood pressure, we first examined the effects of modest, diet-induced weight gain on muscle sympathetic nervous system activity (MSNA) in healthy, lean, normotensive individuals. We hypothesized that modest weight gain would increase MSNA in these individuals, and that this neural activation would be accompanied by increases in blood pressure. Concordant with this hypothesis, MSNA and resting blood pressure were significantly elevated following weight gain. The increase in MSNA was correlated with the magnitude of body weight and fat gain, but was not obviously related to increases in visceral fat. We next examined the ability of cardiorespiratory fitness (CRF) to modulate the weight gain-induced increase in blood pressure in the same cohort of young, nonobese and normotensive individuals. We hypothesized that the increase in blood pressure would be attenuated in individuals with higher- compared with lower CRF (HCRF and LCRF, respectively). Indeed, we found that HCRF experienced significantly smaller increases in resting and ambulatory blood pressure compared to LCRF. In the pooled sample, baseline fitness was inversely related to the changes in

resting systolic and diastolic pressure, and this relation was not diminished after statistically controlling for changes in abdominal visceral fat. The results of the present investigation suggest that even modest weight gain increases sympathetic activity and blood pressure, which, if left untreated, may contribute to the development of hypertension and other cardiovascular disorders. Maintenance of higher levels of CRF during periods of weight gain may reduce cardiovascular disease risk by mitigating the increases in blood pressure. Collectively, these findings may have important implications for understanding the link between obesity and hypertension.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES:

CHAPTER 1

Introduction

The worldwide prevalence of obesity has increased dramatically in recent decades. In the US, more than 30% of the adult population is currently obese, representing a two-fold increase since 1980 (1). Concomitant with this emerging obesity epidemic has been a significant increase in the prevalence of hypertension, which now afflicts more than 65 million Americans (2). The concurrent increase in obesity and hypertension is not surprising given the close relation between the two morbidities. Data from the Framingham Heart Study suggest that obesity accounts for as many as 70% of newly diagnosed hypertensive cases (3), and that for every 4.5 kg of weight gain, systolic blood pressure increases ~4 mm Hg in both men and women (4). Furthermore, intervention studies have demonstrated that weight loss is consistently associated with reductions in blood pressure (5), whereas diet-induced weight gain in experimental animals invariably leads to increases in pressure (6).

The relation between body weight and blood pressure is linear and persists throughout the nonobese range, suggesting that any increase in body weight, irrespective of obesity status, will likely cause a corresponding increase in blood pressure (7). This notion is of particular relevance given that even small elevations in blood pressure increase the risk for cardiovascular disease, regardless of whether the clinical standard of 140/90 is achieved (8, 9).

Despite the well established relation between body weight and blood pressure, several questions and controversies remain unresolved. One important issue concerns the large individual variability in the blood pressure response to weight change. For example, some obese individuals display blood pressure levels well within the normal range (10), and others experience little blood pressure elevation with weight gain (11, 12). Furthermore, the often cited

1

linear relation between body weight and blood pressure (5, 7) is not supported by many studies (13, 14) The reason(s) for this inter-individual variability are not entirely clear, but likely include a complex interaction of genetic and environmental factors (15, 16). One such factor is the distribution of body fat. Specifically, central or visceral adiposity is more closely related to blood pressure than is peripheral or subcutaneous fat (17-20) In the first study to prospectively examine whether directly measured visceral fat is associated with incident hypertension, Hayashi et al. (21), followed 300 Japanese Americans over a 10yr period and found that visceral adiposity was associated with an increased risk of developing hypertension even after controlling for subcutaneous adiposity, exercise, and fasting insulin.

Cardiorespiratory fitness (CRF) is another factor that may be capable of modulating the blood pressure response to weight change. Epidemiological studies suggest that CRF is an independent predictor of hypertension (22), and vigorous physical activity lowers the risk of hypertension with weight gain (23, 24). Furthermore, prospective studies in animals (25) and humans (26) have demonstrated that CRF and physical activity attenuate the deleterious metabolic effects of diet-induced weight gain. Unfortunately, blood pressure was not measured in these previous studies, and there is a lack of prospective intervention studies examining whether CRF can attenuate the increase in blood pressure that occurs with weight gain.

Another unresolved issue regarding the relation between body weight and blood pressure is the difficulty in distinguishing between the effects of long term changes in body weight and short term changes in caloric balance. For instance, short-term fluctuations in energy intake (i.e. caloric restriction and overfeeding) are associated with directionally similar changes in blood pressure even in the absence of weight change (27-29). Consistent with these findings, it is a common clinical observation that obese-hypertensive patients placed on a low-calorie diet often

display reductions in blood pressure well before any discernable weight loss occurs (30). Furthermore, several long-term studies have demonstrated that the decrease in blood pressure following weight loss is not sustained during subsequent eucaloric conditions, even when body weight is maintained at the reduced level (31, 32). These results suggest that both acute fluctuations in energy intake and chronic changes in body weight have independent effects on blood pressure. Therefore, in order to ascertain the true effects of weight change on blood pressure, it is necessary to control for the effects of energy imbalance by including a eucaloric weight stability period following weight change. Unfortunately, a majority of the available studies have failed to include a period of weight maintenance, which limits the interpretation of the data. (33, 34),

The mechanism(s) that mediate the relation between body weight and blood pressure have not been completely identified. The sympathetic nervous system has been extensively studied in this regard, and several lines of evidence indicate that sympathetic neural activation plays a crucial role in the development and progression of obesity-hypertension. First, the elevation in blood pressure following overfeeding in animals is markedly attenuated following: 1) pharmacological blockade of sympathetic (adrenergic) receptors (35); 2) inhibition of central sympathetic outflow (36), and 3) denervation of the renal sympathetic nerves (37). Second, the reduction in blood pressure following weight loss is significantly blunted in genetically altered mice incapable of producing catecholamines (38). Third, studies in animals (6) and humans (39) have demonstrated that dual α- and β-adrenergic blockade reduces blood pressure to a greater extent in obese compared to lean hypertensives. These data have led to the hypothesis that sympathetic neural activation is involved in the etiology of obesity-hypertension. One requisite for this hypothesis is confirmation that weight gain is accompanied by increases in sympathetic

nerve activity. Indeed, numerous cross-sectional studies have demonstrated that obese

individuals display higher sympathetic activity compared to lean controls (40-46). However,

Huggett et al., recently reported that overweight men displayed no discernable sympathetic

activation compared to lean controls (47). This finding questions whether modest increases in

body weight are sufficient to induce increases in sympathetic activity, or rather, if there is a

threshold of body weight within the obese range which must be achieved before sympathetic

activation occurs. It is important to note that the study by Huggett et al. was not designed to

address this issue, and the cross-sectional design of the study precludes any definitive

conclusions from being drawn. Two prospective studies by Masuo et al. (11, 48) have addressed

this issue more directly by demonstrating that plasma norepinephrine concentrations increase

following weight gain in Japanese men. Again, however, methodological limitations prevent

clear interpretation of the data. For example, plasma norepinephrine concentrations may not

accurately reflect sympathetic neural activity given that they are an indirect measure of

sympathetic function and are limited in their sensitivity and reliability. (49). Furthermore, the

study was observational, and thus confounded by other factors that could affect sympathetic

activity independent of weight gain.

In order to adequately address whether modest weight gain increases blood pressure and

sympathetic activity, overfeeding studies that induce body weight in lean individuals are

necessary. In this regard, several overfeeding studies over the last four decades have greatly

enhanced our understanding of body weight regulation and the consequences of weight gain. (50-

52). However, these studies have surprisingly and disappointingly neglected to include blood

pressure as an outcome variable. Consequently, there is a paucity of information regarding the

effects of modest, diet-induced weight gain on blood pressure in humans, and our understanding

of the relation between body weight and blood pressure is limited to cross-sectional and non-intervention epidemiological studies.

In the context of this background information, we induced a 5kg weight gain in a cohort of healthy, nonobese and normotensive individuals in order to examine several of the unresolved issues regarding the relation between body weight and blood pressure. Our first specific aim was to examine whether this modest weight gain increased sympathetic nervous system activity in these individuals. We hypothesized that modest weight gain would induce sympathetic neural activation in these individuals, and that this neural activation would be accompanied by increases in blood pressure and would be related to the amount of visceral fat gain. Our second specific aim was to examine if cardiorespiratory fitness was capable of modulating the expected weight-gain induced increase in blood pressure. We hypothesized that the increase in blood pressure would be attenuated in individuals with higher- compared with lower cardiorespiratory fitness, and this attenuated response, if observed, would be associated with less visceral fat accumulation in the higher fit individuals. Importantly, we performed all measurements at baseline and following a 4-week weight stability period at each individual's elevated body weight in order to minimize the confounding effects of energy imbalance.

Overall this investigation serves to further define the relation between body weight and blood pressure, and may have important implications for understanding the link between obesity and hypertension. Importantly, overfeeding-induced weight gain in humans may provide an insightful model to investigate the mechanism(s) mediating weight-gain induced sympathetic neural activation. Future studies should further examine the mechanisms responsible for the inter-subject variability in the blood pressure responses to weight gain, as well as the factors that mediate the weight gain-induced sympathetic neural activation.

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CHAPTER 2

Modest Weight Gain Is Associated With Sympathetic Neural Activation

In Nonobese Humans

Abstract

We tested the hypothesis that modest, overfeeding -induced weight gain would increase sympathetic neural activity in nonobese humans. Twelve healthy males (23±2 years; BMI, 23.8±0.7) were overfed approximately 1000 kcals/day until a 5 kg weight gain was achieved. Muscle sympathetic nerve activity (microneurography), resting blood pressure, body composition (dual energy x-ray absorptiometry) and abdominal fat distribution (computed tomography) were measured at baseline and following four weeks of weight stability at each individual's elevated body weight. Overfeeding increased body weight (73.5±3.1 vs. 78.4±3.2 kg, P<0.001) and body fat (14.9±1.2 vs. 18±1.1 kg, P<0.001) in 42±8 days. Total abdominal fat increased (220±22 vs. 266±22 cm^2, P<0.001) with weight gain, due to increases in both subcutaneous (158±15 vs. 187±12 cm^2, P<0.001) and visceral fat (63±8 vs. 79±12 cm^2, P=0.004). As hypothesized, weight gain elicited increases in sympathetic burst frequency (32±2 vs 38±2 burst/min, P=0.002) and burst incidence (52±4 vs. 59±3 bursts/100 heart beats, P=0.026). Systolic, but not diastolic blood pressure increased significantly with weight gain. The change in burst frequency was correlated with the percent increase in body weight (r=0.59, P=0.022), change in body fat (r=0.52, P=0.043) and percent change in body fat (r=0.51, P=0.045). The results of the current study indicate that modest diet-induced weight gain elicits sympathetic neural activation in nonobese males. These findings may have important implications for understanding the link between obesity and hypertension.

Keywords: adiposity; autonomic nervous system; overfeeding

Introduction

The sympathetic nervous system (SNS) plays a pivotal role in cardiovascular and metabolic homeostasis. Sympathetic neural activation is characteristic of numerous cardiovascular disorders, and is associated with adverse clinical outcomes in individuals with chronic heart failure and essential hypertension (1-3). Pharmacologic inhibition of SNS activity is a common therapeutic approach for reducing risk in these populations (4,5).

There has been considerable controversy in the past regarding the effects of obesity on SNS behavior (6). The results of more recent studies (7-12) consistently reveal higher muscle sympathetic nerve activity (MSNA) in obese compared with nonobese individuals. However, Huggett et al (13) reported that MSNA was nearly identical in normal weight and overweight men, whereas obese diabetic men displayed 50% higher levels. Thus, whether modest increases in body weight and body fat are sufficient to induce sympathetic neural activation remains unclear.

Masuo et al. (14, 15) have addressed this issue more directly by demonstrating that plasma norepinephrine concentrations increase following weight gain in Japanese men. While these results suggest that sympathetic neural activation occurs with modest weight gain, plasma norepinephrine concentrations are limited as a measure of sympathetic neural activity because they are influenced by the sampling site as well by norepinephrine appearance and clearance from the circulation (16). Furthermore, unlike microneurography, plasma norepinephrine concentrations do not provide a direct measure of sympathetic neural activity (17). Thus, it remains uncertain whether modest weight gain increases sympathetic neural activity in humans.

Accordingly, the purpose of the present study was to determine if modest weight gain increases directly measured MSNA in healthy nonobese humans. We hypothesized that

11

overfeeding-induced weight gain would increase MSNA in these individuals. Furthermore, in light of our observations suggesting that visceral obesity is an important adipose tissue depot linking obesity and sympathetic neural activation,(7,18) we also sought to determine if the increase in MSNA with weight gain, if observed, is related to the amount of visceral fat gain.

Methods

Subjects

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

Experimental Design and Protocol

Following baseline testing, subjects were overfed approximately 1000 kcal/day for 6-8 weeks until a 5 kg weight gain was achieved. Excess calories were provided using a liquid dietary supplement (Boost Plus; Novartis Nutrition Corp; 34% fat, 50% CHO, 16% protein). Progress was assessed by weekly body weight measurements and meetings with a dietitian (BMD). Each individual was studied at baseline and after 4 weeks of weight stability at their elevated body weight in order to minimize the potential confounding effects of energy imbalance on the primary outcome variables. The liquid dietary supplement was also provided throughout weight stability and post-testing periods. For all testing sessions, subjects reported to the laboratory between 7-11 am following an overnight fast and having refrained from caffeine and exercise for

the preceding 24 hours. Following post-testing, subjects were provided with dietary and physical activity recommendations and, if desired, meal replacement products to facilitate weight loss.

Body mass and height were measured with a digital balance scale and stadiometer, respectively. Body composition was measured via dual energy x-ray absorptiometry (GE Lunar Prodigy Advance, Madison, WI) using software version 8.10e. Computed tomography scans (HiSpeed Cti, GE Medical) were performed to quantify abdominal fat distribution. Maximal oxygen consumption (VO_2max) was measured during graded treadmill exercise to exhaustion using open circuit spirometry (TrueMax 2400, ParvoMedics). Resting blood pressure measurements were made in the seated position via mercury and automated (Colin Press-Mate 8800, San Antonio, Texas) sphygmomanometry following a 15 minute period of rest. Measurements were obtained on at least three separate visits over a two week period until stability was achieved (±6 mmHg difference on sequential measurements). Recordings of multiunit MSNA were obtained from the right peroneal nerve using the microneurographic technique as described previously(19). Briefly, the common peroneal nerve was located by palpation and electrically stimulated by an external probe. A tungsten microelectrode was then inserted and adjusted until the nerve was located. Nerve activity was amplified, filtered (bandwidth700-2,000 Hz), full-wave rectified and integrated (time constant =100ms) to obtain a mean voltage neurogram. Neurograms were considered acceptable as recordings of efferent MSNA according to previously published criteria (20). During the miconeurographic recordings, resting heart rate was determined from lead II of an electrocardiogram, beat-by-beat arterial pressure was measured by finger photoplethysmography (TNO Biomedical Instrumentation), and respiration was monitored using a pneumobelt. Plasma leptin and insulin concentrations were measured using commercially available ELISA kits (LINCO Research, MO). Plasma renin

activity was measured by radioimmunoassay (DiaSorin Inc., MN). Urine sodium concentration was measured from a 24-hr collection using a Synchron LX20 Clinical Chemistry Analyzer (Beckman Coulter, Inc.).

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

Statistical Analysis

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

Results

Subject characteristics before and after weight gain are shown in the Table. Overfeeding resulted in 5.0±0.1kg of body weight gain in 42±8 days. Total body fat and lean body mass increased 3.2±0.5 kg (P<0.001) and 1.4±0.4 kg (P=0.002), respectively. Total abdominal fat increased significantly (24±6%, P<0.001), due to increases in both subcutaneous (23±6%, P<0.001) and visceral fat (25±7%, P=0.002).   Maximal oxygen consumption expressed relative

to body weight declined with weight gain (45.5±1.8 vs. 43.8±1.6 ml/kg/min, P=0.017), whereas

there was no significant change when expressed in absolute terms (3.34±0.23 vs. 3.42±0.24

L/min, P>0.05) or relative to fat free mass (59.5±1.8 vs. 59.7±1.8 ml/FFM/min, P>0.05).

Systolic blood pressure (P=0.02-0.009) but not diastolic blood pressure (P>0.05) increased with

weight gain, whereas resting heart rate tended to increase (62±2 vs. 65±3 b/min, P=0.09). As

hypothesized, weight gain elicited increases in MSNA burst frequency (32±2 vs. 38±2 burst/min,

P=0.002, figure 1A) and burst incidence (52±4 vs. 59±3, P=0.026, figure 1B). Plasma leptin

concentrations (3.28±0.59 vs. 5.67±0.80 ng/ml, P<0.001) and plasma renin activity (1.2±0.2 vs.

2.0±0.5 ng/ml/hr, P=0.022) both increased following weight gain, whereas plasma insulin

concentrations did not increase significantly (3.54±0.57 vs. 3.85±0.36 μU/ml, P=0.271). Urine

sodium excretion did not change following weight gain (137.7±13.9 vs. 143.9±16.5 mmol/L,

P=0.292).

The change in MSNA burst frequency following weight gain was positively correlated with

percent change in body weight (r=0.59, P=0.022, figure 2A), change in body fat (r=.52, P=0.043,

figure 2B) and percent change in body fat (r=0.51, P=0.045, figure 2C), and negatively

correlated with the change in lean body mass (r=-0.56, P=0.029). The correlation between the

change in MSNA burst frequency and automated systolic blood pressure did not achieve

statistical significance (r=0.43, P=0.08). The change in MSNA burst incidence was correlated

only with the percent change in body weight (r=0.56, P=0.029). There was no correlation

between the increase in MSNA and changes in plasma leptin or insulin concentrations or plasma

renin activity (all P>0.05).

Discussion

The novel finding of the present study was that modest, diet-induced weight gain increased sympathetic neural activity ~15-20% in healthy, nonobese males. The magnitude of increase in MSNA was correlated with the magnitude of body weight and fat gain, and was accompanied by increases in resting blood pressure. These results are consistent with cross-sectional studies that have reported higher MSNA in obese compared with nonobese individuals (7-12, 21), and with intervention studies demonstrating reductions in MSNA following weight loss in obese individuals (22-24).

We extend those previous observations by demonstrating that even modest increases in body weight and body fat elicit sympathetic neural activation in nonobese humans. The increase in MSNA with weight gain was correlated with the magnitude of body weight and fat gain in the present study. Importantly, the increase in MSNA with weight gain was observed following 4 weeks of weight stability, thus avoiding the acute aftereffects of overfeeding on the SNS (25).

The increase in MSNA with weight gain was not obviously related to increases in visceral fat. This latter observation is in contrast to our previous observations suggesting that visceral obesity is an important adipose tissue depot linking obesity and sympathetic neural activation in humans (7,18). The reasons for this discrepancy are unclear, but our small sample size and inclusion of only nonobese subjects in the present study might contribute. It is also possible that much larger increases in visceral fat with weight gain are necessary to directly activate the SNS.

The increase in MSNA with weight gain in the present study was accompanied by a significant increase in systolic blood pressure. Although the increases in MSNA and blood pressure were not correlated, there is considerable evidence suggesting that the SNS plays an important role in the etiology of obesity hypertension (26-28). As such, it is possible that MSNA

directly contributes to blood pressure elevation by causing vasoconstriction and by restraining the increase in systemic vascular conductance with weight gain (21). However, the results of a preliminary study by Agapitov et al. suggest that sympathetic vasoconstrictor tone is reduced in obese humans (29). Alternatively, MSNA may not be directly involved in the pathogenesis of obesity hypertension, but rather, may serve as a marker for sympathetic activation to the kidney. Renal sympathetic nerve activity is increased in obese compared to lean individuals (30, 31), and activation of renal sympathetic nerve activity is known to play a critical role in long-term blood pressure regulation(28). That plasma rennin activity increased with weight gain in the present study is consistent with this view.

The mechanism(s) by which weight gain induces sympathetic neural activation remains unclear. Landsberg (32) hypothesized that the increase in SNS activity with weight gain serves the homeostatic role of stimulating thermogenesis to prevent further weight gain. An increase in energy expenditure is a recognized consequence of weight gain (33-35). Taken together, our present findings constitute indirect support of Landsberg's hypothesis. However, Landsberg further postulated that a diet-induced increase in plasma insulin concentration was the primary mechanism mediating weight gain-induced sympathetic neural activation (36). In contrast, the lack of an increase in plasma insulin levels in our study precludes us from drawing the same conclusion. Although the reason(s) for this discrepancy is unclear, the measurement of plasma insulin concentration following a period of weight stability may be important. In an attempt to gain insight into other possible mechanisms of sympathetic activation in the current study, we measured plasma concentrations of leptin and renin before and after weight gain. Although both increased significantly following weight gain, the changes were not correlated with the increases

17

in MSNA. Future studies will be necessary to determine the mechanisms mediating sympathetic neural activation following weight gain.

There are some limitations of the present study that should be considered. First, we did not include a control group and our sample size was relatively small. However, several lines of evidence suggest it is unlikely that the changes we observed in MSNA were random deviations that occurred over time rather than as a result of the imposed weight gain. First, the magnitude of increase in MSNA in the present study is consistent with that which would be predicted from studies involving weight loss (22-24). Second, the increase in MSNA with weight gain in the present study is considerably larger than the error in measurement (17,37). Finally, ten of the twelve subjects (83%) experienced increases in MSNA, and the change in MSNA with weight gain was positive correlated with the manipulated variable (i.e. body weight/body fat). Taken together, these observations are consistent with our conclusion that the changes in MSNA were a direct result of the experimental weight gain.

Second, the subjects were limited to young, nonobese males. The results of previous studies suggest that gender (38) and age (39) may affect the magnitude of blood pressure elevation with weight gain. Therefore, the sympathetic neural adjustments to weight gain may also be different in magnitude in females or older subjects. Thus, our findings should not be extrapolated beyond the population studied. Third, because the SNS is regulated in a highly region specific manner (40), it is possible that the effects of weight gain on sympathetic outflow to other organs or tissues may be quantitatively or qualitatively different. That heart rate failed to increase significantly in the present study could be interpreted to be consistent with previous data suggesting that cardiac sympathetic activity is not elevated in obese individuals (30, 31). We should emphasize, however, that the increase in heart rate following weight gain is believed to be

mediated primarily by a reduction in parasympathetic nervous system activity to the heart (26,28). Finally, the deliberate weight gain in the present study may not reflect the more gradual changes that occur under free-living conditions in the general population. As such, our findings should be considered with this mind.

Perspectives

Sympathetic neural activation is characteristic of several cardiovascular diseases, including hypertension and chronic heart failure. The results of primarily cross-sectional studies suggest that sympathetic neural activation is also present in human obesity. Our current study extends those previous findings by demonstrating that modest overfeeding-induced weight gain in nonobese males increases sympathetic neural activity, and that the extent of sympathetic neural activation is related to the amount of body weight and fat gain. These findings suggest that individuals who gain even modest amounts of weight may experience increases in SNS activity regardless of whether they become obese. If left untreated, activation of the SNS may contribute to the development of hypertension and other cardiovascular disorders. Importantly, overfeeding-induced weight gain in humans may provide an insightful model to investigate the mechanism(s) mediating weight-gain induced sympathetic neural activation.

Acknowledgements

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Table 1. *Subject Characteristics at Baseline and Following Weight Gain*

VARIABLE	BASELINE	WEIGHT GAIN
Age, years	23±2	-----
Body weight, kg	73.5±3.1	78.4±3.2*
Body mass index, kg/m^2	23.8±0.7	25.4±0.7*
Body fat, %	21±1.4	24.1±1.3*
Total fat mass, kg	14.9±1.2	18±1.1*
Lean body mass, kg	55.8±2.6	57.2±2.8*
Waist circumference, cm	83.9±2.1	89.5±1.8*
Total abdominal fat, cm^2	220±22	266±22*
Abdominal subcutaneous fat, cm^2	158±15	187±12*
Abdominal visceral fat, cm^2	63±8	79±12*
VO$_2$Max, ml/kg/min	45.5±1.8	43.8±1.6*
VO$_2$Max, L/min	3.3±0.2	3.4±0.2
Heart Rate, b/min	62±2	65±3
Automated SBP, mmHg	122±2	125±2*
Automated DBP, mmHg	67±2	68±2
Manual SBP, mmHg	114±3	118±3*
Manual DBP, mmHg	75±2	75±2

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

Figure Legends

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

Figure 2. Panel A: Relation between the change in MSNA (bursts/min) with weight gain and percent change in body weight. Panel B: Relation between the change in MSNA (bursts/min) with weight gain and the change in body fat (kg). Panel C: Relation between the change in MSNA (bursts/min) weight gain and change in percent body fat. (*P<0.05 vs. baseline)

Figure 1

Figure 2
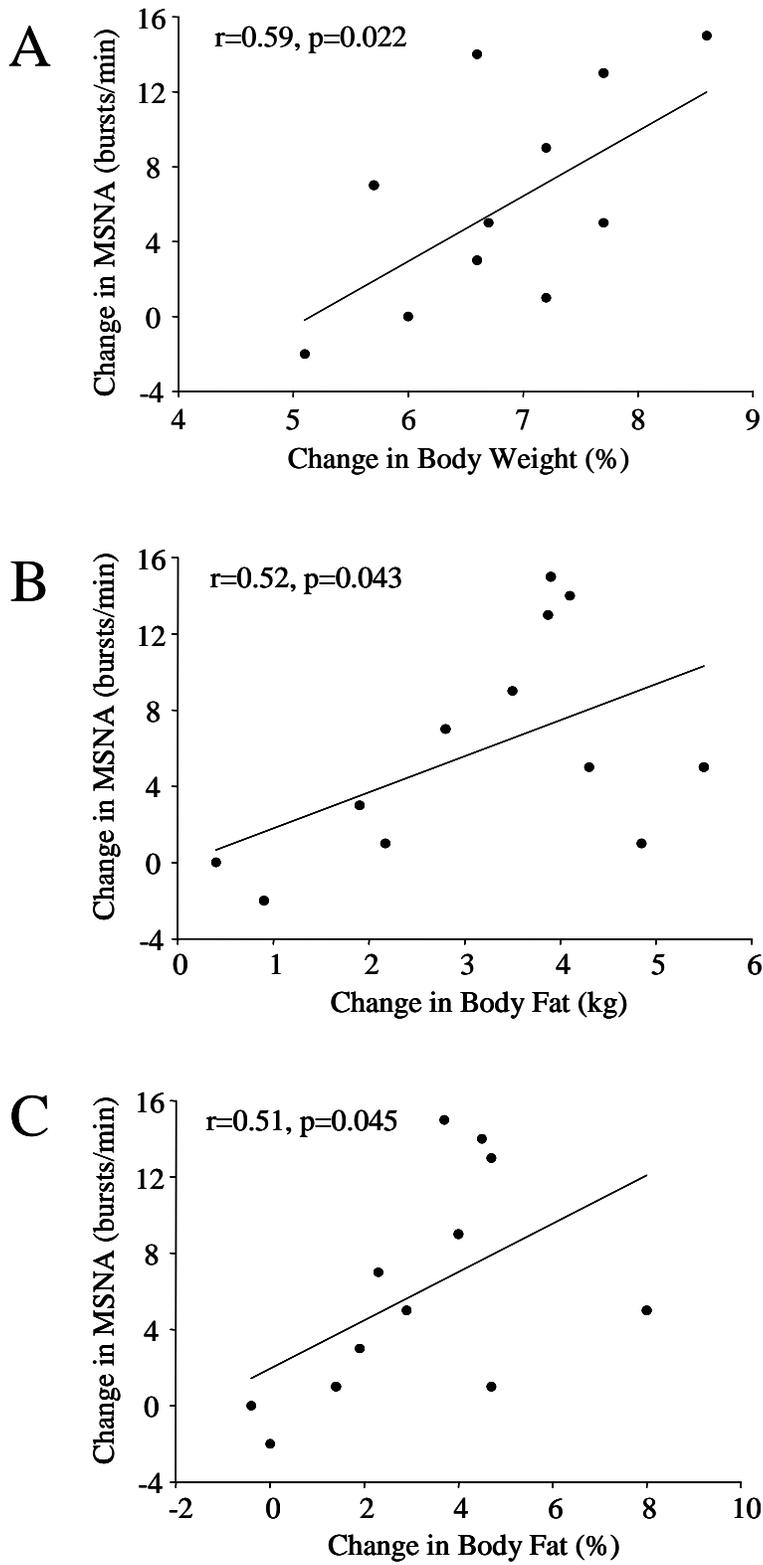
CHAPTER 3

Cardiorespiratory Fitness Is Associated With Smaller Increases in Blood Pressure Following Experimental Weight Gain

Abstract

Objective: We tested the hypothesis that the increase in blood pressure (BP) with weight gain would be smaller in men with higher compared with lower cardiorespiratory fitness (HCRF and LCRF, respectively).

Research Methods and Procedures: Thirteen men (age=23±1, BMI= 24±1) were overfed by ~1000 kcal/d over ~8 weeks to achieve a 5 kg weight gain. Resting BP and 24hr ambulatory BP, body composition, and abdominal fat distribution were measured at baseline and following 4 weeks of weight stability at each individual's elevated body weight.

Results: Cardiorespiratory fitness (CRF) was higher in HCRF compared to LCRF (49.9±1.2 vs. 38.1±1.4 ml/kg/min, P<0.001). At baseline, body weight was similar in HCRF and LCRF, whereas HCRF displayed lower levels of total body fat (13.0±1.7 vs. 16.9±1.3 kg, P=0.049) and abdominal visceral fat (AVF) (49±6 vs. 80±14 cm^2, P=0.032). Resting BP and 24hr ambulatory BP were similar in the two groups at baseline. Following weight gain, body weight increased ~5 kg (P<0.05) in both groups; the changes in body composition and regional fat distribution were similar. As hypothesized, the increases in resting systolic (1±2 vs. 7±2 mmHg; P=0.008) and diastolic BP (-1±4 vs. 5±1 mmHg; P=0.005) were smaller in the HCRF group. CRF was inversely correlated with the increases in resting systolic (r=-0.64; P=0.009) and diastolic BP (r=-0.80; P<0.001). Furthermore, the relation between CRF and BP remained significant after statistically controlling for the changes in the proportion of total abdominal fat gained as visceral fat.

28

Discussion: These findings suggest that higher levels of CRF are associated with a smaller

increase in BP with weight gain independent of changes in abdominal visceral fat.

Introduction

Obesity represents one of the most serious public health issues facing the US and other industrialized nations.   Currently, more than 32% of the US population is obese (1) and at increased risk for the development of cardiovascular diseases (2). Obesity and weight gain are particularly potent risk factors for the development of hypertension (3). Risk estimates from the Framingham Heart Study suggest that approximately 75% and 65% of the cases of hypertension in men and women, respectively, are directly attributable to overweight and obesity (4). Importantly, long duration obesity does not appear necessary to elevate blood pressure (BP) as the relation between obesity and hypertension is evident in children (5) and exists throughout the entire nonobese range (6).

Despite the close relation between obesity and hypertension, there is considerable inter-individual variability in the BP response to weight change (7). This variability is likely due to a complex interaction of genetic and environmental factors (8, 9), and the identification of these factors is essential to understanding the etiology of obesity-hypertension. The results of epidemiological studies suggest that cardiorespiratory fitness (CRF) may be one such factor capable of modulating the BP response to weight change (10, 11). However, the experimental support for this postulate is currently lacking. In addition, the potential impact of CRF on BP throughout an entire 24hr period (i.e., ambulatory BP) has not been studied.

Accordingly, we tested the hypothesis that the BP responses to modest weight gain would be attenuated in individuals with higher- versus lower CRF. In addition, because abdominal visceral fat (AVF) appears to be more closely related to BP than is subcutaneous fat (12, 13), we further hypothesized that the smaller increases in BP in individuals with higher CRF, if observed, would be associated with less visceral fat accumulation.

Subjects

Thirteen nonobese males (age=23±1, BMI= 24±1) participated in the present study and were divided into either a higher cardiorespiratory fitness (HCRF) group or lower cardiorespiratory fitness (LCRF) group based on a median split of cardiorespiratory fitness (VO_2max) of the entire group at baseline. The individual falling on the median was excluded from the group analyses (n=6 per group) but included for the correlation analyses (n=13). All subjects were normotensive, free from overt cardiovascular and metabolic diseases and not taking any medications. In addition, all of the individuals were sedentary-to-recreationally active and weight stable (±2 kg) for six months prior to study entry. The nature and purpose of the study along with potential risks and benefits were explained before obtaining informed consent. The Virginia Tech Human Subjects Committee approved all experimental protocols.

Experimental Design and Protocol

Following baseline testing, individuals were overfed approximately 1000 kcals/day until a 5 kg weight gain was achieved. Excess calories were provided by an over the counter liquid dietary supplement (Boost Plus, Novartis Nutrition Corp; 34% fat, 50% CHO, 16% protein). Progress was monitored via weekly body weight measurements and periodic consultations with a research dietitian (BD). Prior to post-testing, subjects underwent 4 weeks of weight stability at their elevated body weight in order to minimize the potential confounding effects of positive energy balance on the primary outcome variables.

For all testing sessions, subjects reported to the laboratory between 7-11am following a 12hr fast and having refrained from exercise and caffeine for the previous 24 hrs. Body weight was measured on a digital scale (nearest 0.1 kg). Body composition was determined via dual energy x-ray absorptiometry (DXA, GE Lunar Prodigy Advance, Madison, WI) using software version 8.10e. Computed tomography scans (HiSpeed Cti, GE Medical) were performed to quantify abdominal fat distribution. Maximal oxygen consumption (VO_2max) was measured during graded treadmill exercise to exhaustion using open circuit spirometry (TrueMax 2400, ParvoMedics), and minutes of moderate to vigorous physical activity per week were obtained by self report. Resting BP was measured in the seated position via mercury and automated sphygmomanometry following a 15 minute period of rest. Mercury sphygmomanometry measurements were always performed first to avoid bias. The BP measurements were repeated until within-session stability was achieved (±6 mmHg on three sequential measurements) and on at least three separate visits over a two week period to ensure between-session stability. Ambulatory BP (ABP) was measured every 15-30 minutes over a 24-hour period of normal daily activity using a portable device (Spacelabs 90207; Spacelabs Medical Inc., Redmond WA) as described previously (14). Average ambulatory BP values were obtained for 24-hour, daytime (6am-10pm), and nighttime (10pm-6am) systolic and diastolic pressure. Urine sodium concentration was measured from a 24-hr collection using a Beckman Synchron LX20 Clinical Chemistry Analyzer.

Statistical Analysis

Independent-samples t-tests were used to compare subject characteristics and dependent variables at baseline. Repeated measures analysis of variance was used to assess changes in subject characteristics and dependent variables in the HCRF and LCRF groups with weight gain. Differences in the magnitude of change in subject characteristics and dependent variables with weight gain in the two groups were assessed with independent-samples t-tests. Relations among variables of interest were assessed using simple and partial correlation analysis. All data are expressed as mean±SE. The significance level was set *a priori* at $P< 0.05$.

Results

Subject characteristics are displayed in table 1. The HCRF group was older than the LCRF group (P=0.044). At baseline, body weight, BMI, and lean body mass did not differ (all P>0.05) between the two groups, whereas both total body fat and percent body fat were lower in the HCRF group (P=0.049 and P=0.002, respectively). Total abdominal fat and visceral abdominal fat were also lower (P=0.040 and P=0.032, respectively) in HCRF, whereas subcutaneous abdominal fat was similar (P>0.05) in the two groups. As expected, VO_2max was higher (~35%) in HCRF compared LCRF when expressed in absolute terms and relative to body weight (P=0.006 and P<0.001, respectively). Similarly, moderate to vigorous physical activity was higher in HCRF compared to LCRF (172±51 vs. 25±16 min/wk, P=0.011).

Following overfeeding, body weight increased 5.0±0.2 and 5.0±0.2 kg (both P<0.05) in HCRF and LCRF, respectively. Body mass index, percent body fat, total body fat, lean body mass, total abdominal fat, and visceral and subcutaneous abdominal fat increased (all P<0.05) with weight gain, and the magnitude of change was similar between groups. VO_2max decreased

in HCRF when expressed relative to body weight (-2.8±0.8 ml/kg/min, P=0.009), but not when expressed in absolute terms (0.03±0.07 L/min, P>0.05); neither expression changed in LCRF. Heart rate did not change significantly with weight gain in either group (P>0.05).

Manual diastolic pressure, as well as 24hr and daytime ambulatory diastolic pressure were lower in HCRF compared with LCRF at baseline (P=0.005-0.049) (table 1). All other indices of resting and ambulatory BP were similar between groups at baseline (P>0.05) (table 1). Following weight gain, HCRF experienced smaller increases in manual systolic BP (2±1 vs. 7±3 mmHg, P=0.048, figure 1A) and automated systolic (1±2 vs. 7±2 mmHg, P=0.008, figure 1C) and diastolic BP (-1±4 vs. 5±1 mmHg, P=0.005, figure 1D) compared to LCRF. Resting manual diastolic did not change significantly in either group (0±2 vs. 2±2 mmHg, P>0.05, figure 1B).

The increase in 24 hr systolic (3±1 vs. 6±2 mmHg, P=0.081, figure 2A) and diastolic BP (-1±1 vs. 3±2 mmHg, P=0.079, figure 2B) with weight gain tended to be smaller in HRCF compared to LCRF. The magnitude of change in nighttime systolic (-1±3 vs. 8±2 mmHg, P=0.009, figure 2C) and diastolic (-3±3 vs. 4±2 mmHg, P=0.029, figure 2D) pressures were significantly smaller in HCRF. The change in daytime systolic pressure was similar in HCRF and LCRF (5±2 vs. 4±3 mmHg, P>0.05), whereas daytime diastolic pressure remained unchanged in both groups (P>0.05). Urinary sodium excretion was lower in HCRF vs. LCRF at baseline (111.8±15.5 vs. 167.8±17.4 mmol/L, P=0.019) but did not change in either group following weight gain (P>0.05).

As shown in figure 3, CRF was inversely correlated with the increases in resting automated systolic (r=-0.64; P=0.009) and diastolic BP (r=-0.80; P<0.001) in the pooled sample. The change in AVF was not related to changes in BP, although the proportion of total abdominal fat gained as AVF was positively correlated with the changes in resting manual systolic (r=0.50,

P=0.042) and diastolic BP (r=0.56, P=0.023). The relation between CRF and both systolic (r=-0.61, P=0.017) and diastolic BP (r=-0.79, P=0.001) remained significant after statistically controlling for the changes in the proportion of total abdominal fat gained as visceral fat.

Discussion

The major new finding of the present study was that modest, diet-induced weight gain increases resting and ambulatory BP in normotensive, nonobese individuals, but the magnitude of increase is significantly smaller in individuals with higher compared with lower CRF. This protective effect of CRF appears to be independent of changes in abdominal visceral fat.

The results of epidemiological studies indicate that high levels of CRF or physical activity are associated with smaller increases in BP with weight gain (10, 11). The results of the present experimental study extend those findings by demonstrating that higher levels of CRF attenuate the increases in both resting and ambulatory BP following modest, diet-induced weight gain in normotensive, nonobese individuals. In light of recent evidence suggesting that even small elevations in BP within the normotensive range increase cardiovascular and overall mortality (15, 16), the smaller increase in BP in individuals with higher CRF in the present study may be clinically relevant (17).

The results of numerous studies have supported a close relation between abdominal fat and BP (13, 18). In addition, CRF is associated with lower levels of visceral fat independent of total adiposity (19). However, whether visceral fat mediates the relation between CRF and BP is less clear. The results of a recent cross sectional study suggests that high CRF is associated with lower BP at levels of visceral fat typically observed in nonobese individuals (20). Consistent

with this, our current findings suggest that CRF attenuates the increase in BP with weight gain independent of the amount of visceral fat gained.

Cardiorespiratory fitness is a complex phenotype influenced by both genetic and environmental (e.g., physical activity) factors. In this context, it is unclear whether the smaller increase in BP in individuals with higher CRF is a reflection of genetic factors or greater physical activity levels compared to the lower CRF group. Interestingly, rats artificially bred for high aerobic capacity (i.e., CRF) demonstrate lower daytime, nighttime and 24hr mean BP (and several other cardiovascular risk factors) compared with animals with lower aerobic capacity (21). Importantly, the more adverse cardiovascular risk factor profile was observed in 5-week old pups before differences in body weight and visceral fat accumulation were evident. The latter observation is consistent with our current findings as well as the results of exercise training studies indicating that BP lowering is independent of changes in body composition (22, 23).

There are some limitations of the current study that warrant consideration. First, the sample size of our study was small. As such, inclusion of a larger number of subjects may have produced a different outcome. Second, the results of studies in rodents suggest that gender (24) and age (25) can influence the BP response to weight gain. Therefore, our results should not be extrapolated beyond the population studied. Third, our study was not designed or sufficiently powered to determine the mechanisms responsible for the smaller increase in BP in men with higher compared with lower CRF. Future studies will be necessary to determine the potential roles of the sympathetic nervous and renin-angiotensin-aldosterone systems. Finally, as mentioned above, CRF is determined by both genetic and environmental factors, but the design of the present study did not allow us to distinguish the relative importance of these factors in mediating the effect of CRF on the BP response to weight gain. An intervention study designed

to examine the influence of regular aerobic exercise on the BP response to weight gain would be necessary to address this issue.

In conclusion, the results from the current study suggest that higher levels of CRF are associated with smaller increases in resting and ambulatory BP following modest, diet-induced weight gain in healthy males. The beneficial effects of CRF on the BP response to weight gain appear to be independent of changes in visceral fat. Taken together, our findings may have important implications for understanding why weight gain increases cardiovascular disease risk in some individuals more than others, and highlight the importance of maintaining regular physical activity levels even during periods of a positive energy balance.

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Table 1: Subject Characteristics before and after weight gain

VARIABLE	BASELINE		WEIGHT GAIN	
	LCRF	HCRF	LCRF	HCRF
Age, years	26±2	21±1	-----	-----†
Body weight, kg	72.8±4	75.5±5.3	77.8±4.1	80.5±5.4*
Body mass index, kg/m^2	24.2±1	23.6±1.2	25.9±1	25.1±1.2*
Body fat, %	24.4±1.3	17.5±1.3	27.4±1.0	21.0±1.0*†
Total fat mass, kg	16.9±1.3	13±1.7	20.6±1.4	15.6±1.0*†
Lean body mass, kg	52.5±2.9	59.9±3.9	54.3±2.9	61.2±4.6*
Waist circumference, cm	85.3±3	83.3±3.3	91.4±2.8	88±2.5*
Total abdominal fat, cm^2	260±28	183±27	305±28	221±25*†
Abdominal subcutaneous fat, cm^2	180±16	134±23	205±10	163±19*†
Abdominal visceral fat, cm^2	80±14	49±6	100±20	58±8*†
VO$_2$Max, ml/kg/min	38.1±1.4	50±1.2	38.2±1.0	47.1±1.7*†
VO$_2$Max, L/min	2.8±0.2	3.8±0.3	2.8±0.2	3.8±0.3†
Heart Rate, b/min	64±3	61±4	67±4	61±5

Values are mean ± SE; significant effect of time (*) and group (†)

Table 2: Blood Pressure before and after weight gain

VARIABLE	BASELINE		WEIGHT GAIN	
	LCRF	HCRF	LCRF	HCRF
Manual systolic, mmHg	115±4	113±4	122±3	115±4*#
Manual diastolic, mmHg	78±3	71±2	80±4	70±2†
Automated systolic, mmHg	121±3	121±3	127±3	122±3 *#
Automated diastolic, mmHg	69±2	64±2	74±2	63±3*†#
24hr systolic ABP, mmHg	120±2	120±3	126±3	123±2*
24hr diastolic ABP, mmHg	72±3	65±2	74±3	65±3†
Daytime systolic ABP, mmHg	124±3	118±3	127±3	124±3*
Daytime diastolic ABP, mmHg	76±3	65±2	77±3	66±3†
Nighttime systolic ABP, mmHg	118±2	122±4	126±3	121±2*#
Nighttime diastolic ABP, mmHg	70±3	66±3	74±5	63±3#

Values are mean ± SE; significant effect of time (*) and group (†); significant time x group interaction (#); ABP, ambulatory blood pressure;

Figure Legends

Figure 1. Changes in manual systolic (A) and diastolic (B), and automated systolic (C) and diastolic (D) blood pressure in LCRF and HCRF following weight gain.

Figure 2. Changes in 24-hr systolic (A) and diastolic (B) and nighttime systolic (A) and diastolic (B) blood pressure in LCRF and HCRF following weight gain.

Figure 3. Relation between cardiorespiratory fitness at baseline and changes in automated systolic (A) and diastolic (B) blood pressure in the pooled sample.
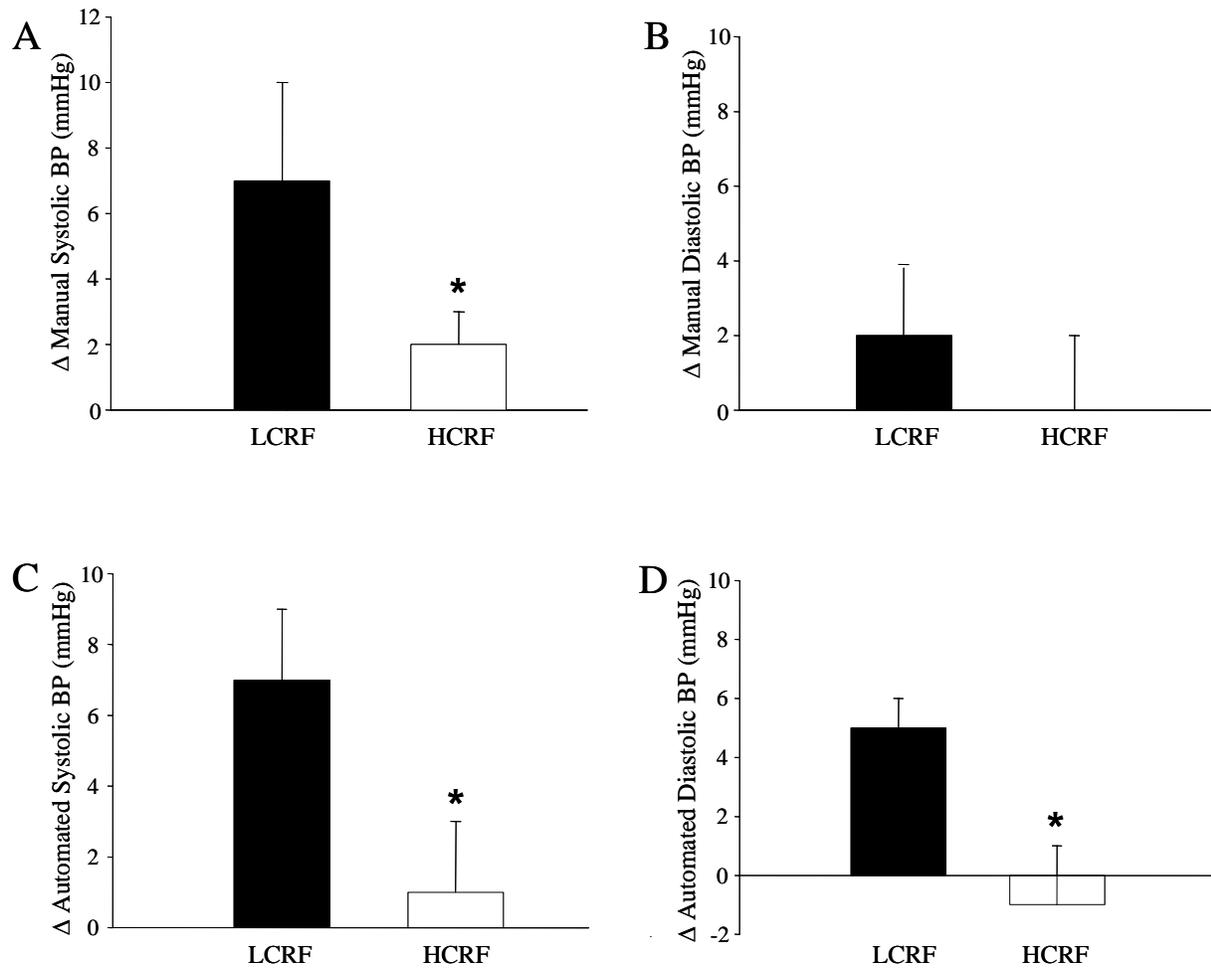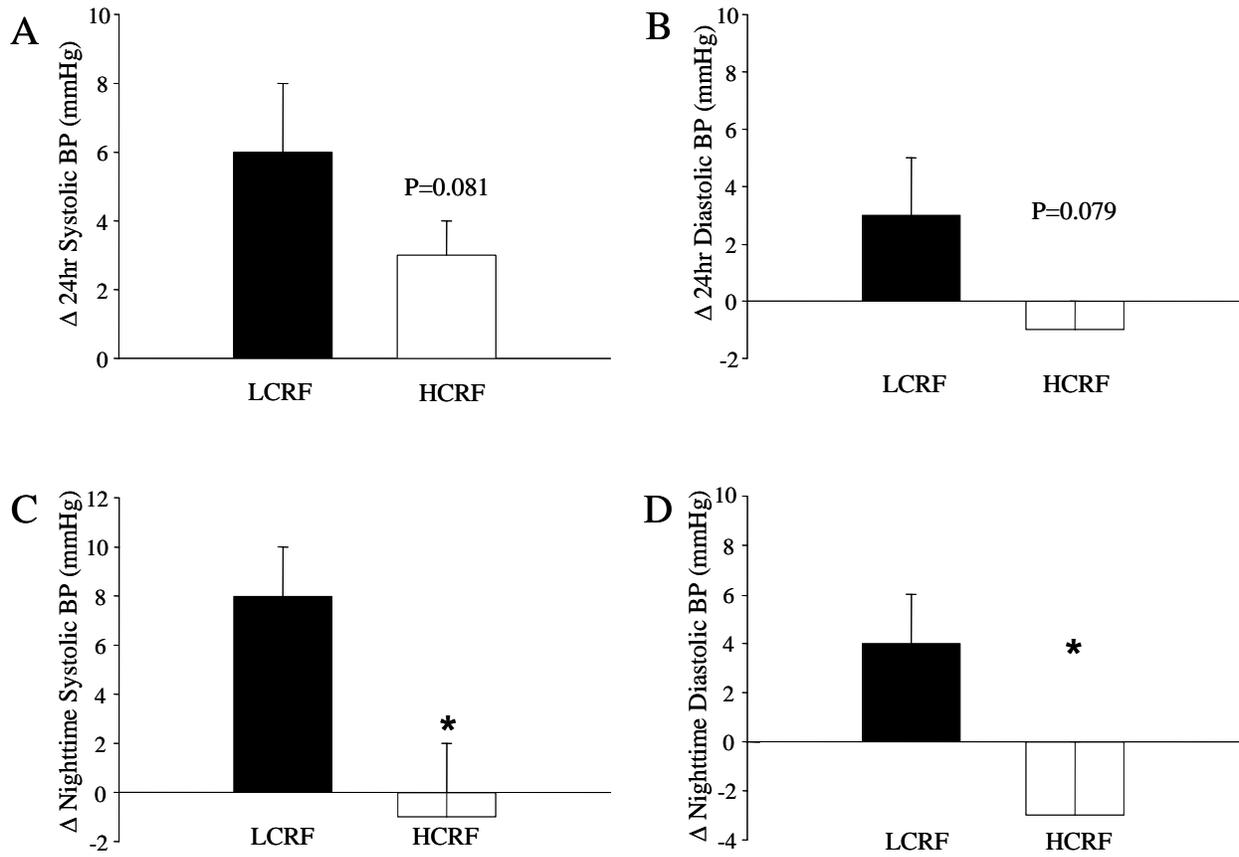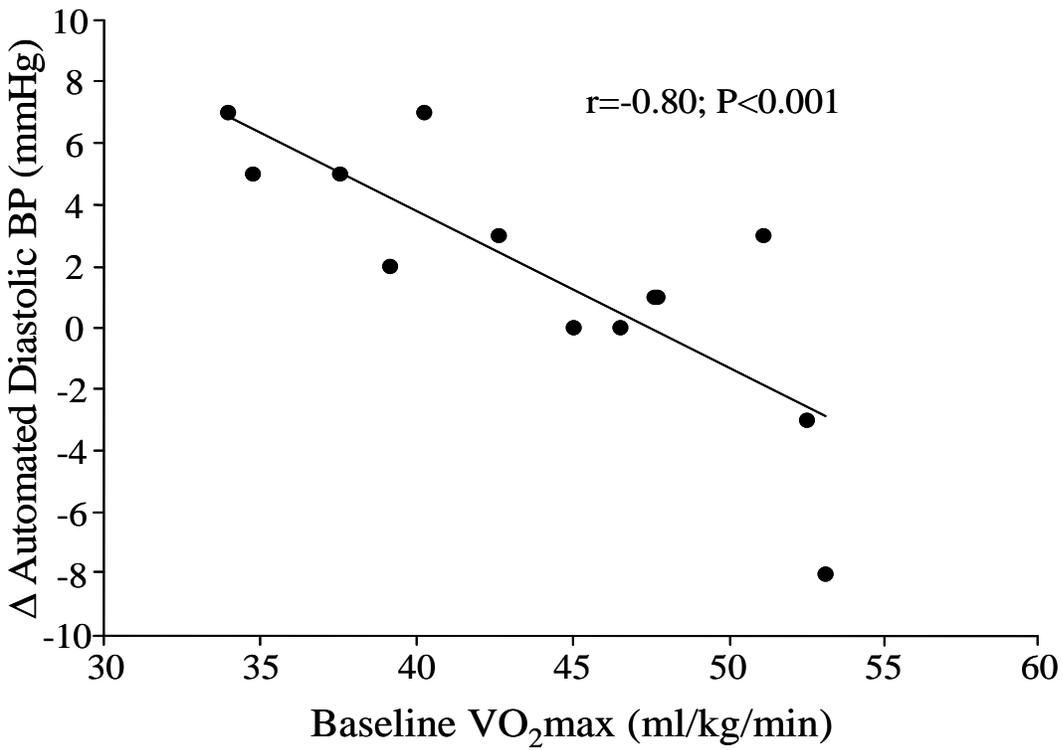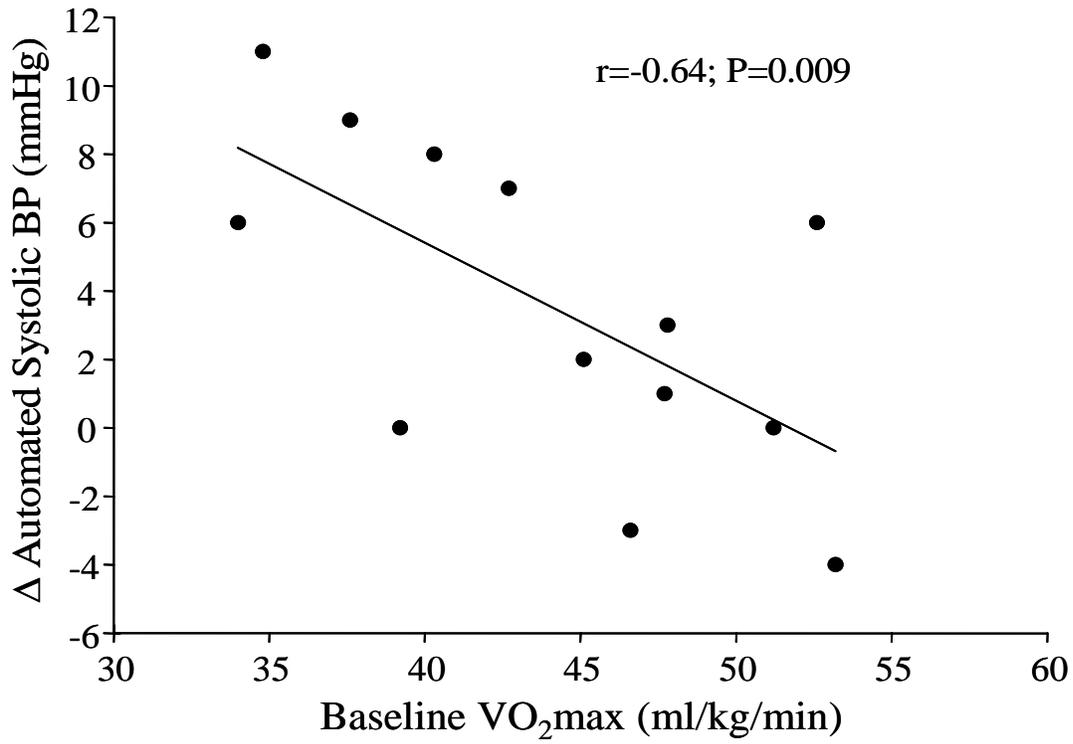
Figure 1

Figure 2

Figure 3

CHAPTER 4

Conclusions

Obesity and hypertension are among the most important public health concerns facing the US in the twenty first century. Despite an abundance of data confirming a strong relation between these two morbidities, numerous issues regarding the nature of the relation remain unresolved. To address these issues, we examined the effects of modest, diet-induced weight gain in healthy, lean, normotensive individuals. Our first specific aim was to examine whether blood pressure and sympathetic nervous system activity increased following weight gain in these individuals. As hypothesized, modest weight gain significantly increased blood pressure, and this hypertensive effect was accompanied by sympathetic neural activation, as measured by muscle sympathetic nerve activity (MSNA). The increase in MSNA was correlated with the magnitude of body weight and fat gain, but was not obviously related to increases in visceral fat. The reasons for the absence of a relation between MSNA and visceral fat are unclear, although our small sample size and inclusion of only nonobese subjects might contribute. It is also possible that much larger increases in visceral fat with weight gain are necessary to directly activate the SNS.

Our second specific aim was to examine if cardiorespiratory fitness (CRF) was capable of modulating the weight-gain induced increases in blood pressure in a similar cohort of young, nonobese and normotensive individuals. In accordance with our hypothesis, the increases in resting and ambulatory blood pressure following weight gain were attenuated in individuals with higher CRF compared to their peers with lower CRF. In the pooled sample, baseline fitness was inversely related to the changes in resting systolic and diastolic pressure, and this relation was not diminished after statistically controlling for changes in abdominal visceral fat; thus suggesting that the protective effects of CRF are independent of visceral adiposity. In light of

recent evidence suggesting that even small elevations in BP within the normotensive range increase cardiovascular and overall mortality, the smaller increase in BP in individuals with higher CRF in the present study may be clinically relevant.

An important aspect of the present study was the inclusion of a 4-week, eucaloric weight maintenance period prior to post-testing. This weight stability period helped minimize the potential confounding effects of energy imbalance on the primary outcome variables, thus allowing for a more accurate examination of the effects of weight gain, *per se*.

The results of the current investigation provide an experimental basis for several future directions. First, the mechanisms of sympathetic activation following weight gain are unclear. In an attempt to gain insight into this question, we measured plasma concentrations of leptin and renin, both of which have been suggested as possible mediators of sympathetic activation. Although both leptin and renin increased significantly following weight gain, the changes were not correlated with the increases in MSNA. Future studies utilizing RAS blockers or leptin administration are needed to more directly address this issue. Second, given that CRF is determined by both genetic and environmental factors, future studies are needed to distinguish the relative importance of these factors in mediating the protective effects of CRF with weight gain. An intervention study designed to examine the influence of regular aerobic exercise on the BP response to weight gain would be necessary to address this issue. Third, the results of studies in rodents suggest that gender and age can influence the BP response to weight gain; thus it is important to determine if the effects of weight gain on blood pressure and sympathetic activity, as well the modulatory effect of CRF, vary among different population. Finally, macronutrient intake can have a dramatic effect on blood pressure, but it is less clear whether the macronutrient composition of the diet can alter the effects of overfeeding on blood pressure and sympathetic

47

activity. Future studies that examine the effects of weight gain using diets of varying macronutrient content on sympathetic activity are needed to address this issue.

In conclusion, the findings of the present investigation suggest that individuals who gain even modest amounts of body weight may experience increases in blood pressure and sympathetic activity regardless of whether they become obese. If left untreated, these alterations may contribute to the development of hypertension and other cardiovascular disorders. Maintenance of higher levels of CRF during periods of weight gain may reduce cardiovascular disease risk by mitigating the increases in blood pressure and sympathetic neural activation. These findings demonstrate that overfeeding-induced weight gain in humans may provide an insightful model to further investigate the relation between weight gain and blood pressure.

Informed Consent for Participants of Investigative Projects

Department of Human Nutrition, Foods and Exercise

Virginia Tech

TITLE: **Weight Gain and Autonomic-Circulatory Control in Humans**

INVESTIGATORS: **Kevin P. Davy, Ph.D. and Brenda M. Davy, Ph.D.**

MEDICAL DIRECTORS: **Jose Rivero, M.D.**
                              Donald Zedalis, M.D.

PURPOSE:
The amount and location of body fat can influence cardiovascular health and function. The build up of fat in the abdominal region is associated with elevated risk of getting high blood pressure. The reason(s) for this elevated risk is/are unclear. However, altered autonomic-cardiovascular function (how the nervous system influences how the heart and blood vessels work) is one possible reason. The general purpose of the present study is to try and learn how the amount and location of body fat influences autonomic-cardiovascular function.

METHODS:
You are being asked to participate in all of the sessions of the study described below. If you agree to participate in this study you will first be required to complete a personal health history questionnaire. The results of your medical history and study tests may be discussed with the study medical director to determine your eligibility. 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 female between the ages of 18 and 70 years who do not have diabetes as assessed by a medical history or the diabetes test. Your body mass index must be less than or equal to 30 and your blood pressure also must be less than 140/90 mmHg. You will not be eligible to participate in the study if you use any medication that might influence your heart, lungs, blood vessels, or kidneys. One hundred people will be included in this study.

You are being asked to participate in a weight gain group or control group. Your participation in one of these groups will be determined by randomization, a procedure similar to flipping a coin. If you are placed in the weight gain group, you will receive instructions on how to modify your diet to increase the amount of calories you eat so that you gain 5-10% of your initial body weight over an 8-10 week period. For example, for someone weighing 170 lbs., the goal would be to gain 8-17 lbs. To do this, you will be asked to increase the amount of snack foods and amount of food eaten at each meal. The individuals in the control group will be asked not to change any of their dietary or physical activity habits. During the weight gain period, some of your food will be provided by the investigators and you may also be given meal vouchers (or credits) for places to eat at Virginia Tech. During this part of the study, you will be asked to return to War Memorial Hall every 1-3 days to be weighed and be given more food (and/or vouchers). You will also be asked to write down the food that you eat each day. After completion of the weight gain aspect of the study, you will be given instructions on how to modify your diet to then reduce the

calories you eat so that you lose the weight previously gained. Additionally, you will have the option to receive a supply of weight loss shakes and meal bars (SLIMFAST) at no cost to you to assist with your weight loss efforts for the duration of the study. During the weight loss phase of the study you will be asked to come to War Memorial Hall weekly to be weighed and to discuss with a dietitian any problems you may be experiencing with your weight loss program. For a one-month period following both weight gain and weight loss you will be asked to eat your normal diet so that your body weight remains the same.

There will be approximately 60 visits if you participate in the weight gain intervention. The actual number and order of visits may depend on your schedule and the availability of the study staff. The order may differ from the order of appearance in this document.

Session 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 or hospital gown without your shoes. Your waist, hip, and neck circumference will be measured using a measuring tape.
- Overnight Fast - You will be asked to avoid eating for 12 hours prior to this visit so that the test results will not be influenced by the food you eat or by the normal digestion process.
- Catheter and Blood Draw – A small plastic tube will be inserted into an arm vein to draw blood (approximately 2 tablespoons) to measure the levels of cholesterol and glucose. An additional 3 teaspoons will be frozen for other blood tests which may include levels of blood hormones which influence your cardiovascular system. The tests will be restricted to those relevant to the research project described. Any blood samples remaining after 10 years will be destroyed.
- Pregnancy Test - If you are female you will be required to have a pregnancy test. This will require 2-3 teaspoons 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.
- Oral Glucose Tolerance Test for Diabetes – For this procedure you will be asked to drink a very sweet sugar solution (75 grams of glucose). Blood samples will be taken both before and four times (total=5 blood samples) after you drink this solution. The purpose of this procedure is to determine whether you have a normal glucose control after drinking this sweet tasting drink (oral glucose tolerance). This procedure will take approximately 2 hours of your time. If your glucose response indicates you may have diabetes you will not be able to continue participation in this study and you will be referred to your personal physician.
- Sodium Excretion – You will collect all of your urine for a 24-hour period. We will give you a container to bring with you for this purpose. We will measure the amount of salt in your urine. This collection will take approximately 10 minutes of your time over the course of a day. You will be asked to return the container provided to you on the following day.

Session 2
- Sleep Test – You may be asked to have a sleep evaluation to determine whether you have abnormal breathing while you sleep. If you have this evaluation done, you will be asked to either come to the sleep laboratory at Sleep Disorders Network Allergy and Asthma Associates of Southwest Virginia located in Christiansburg on an evening close to the time you usually go to sleep. If the evaluation is performed at the sleep laboratory in Christiansburg, you will be required to sleep there overnight. In either case:

- You will have the electrical activity in your brain and the electrical activity of the muscles in your face, and eye movement monitored with small pads with wire that will be attached to your scalp and 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 placed on your chest.

A physician will determine if the results of your sleep study decide if you have sleep apnea. If you do have sleep apnea, you will be referred to your personal physician or a doctor will be recommended to you. Your physician 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 other parts of this study. However, we will need written approval from your personal doctor to continue in the study. The approximate time required is one night or 7–10 hours.

This session will not be required for individuals with a body mass index less than 25 kg/m^2.

Session 3
- Pregnancy Test- If you are female you will be required to have a pregnancy test. This will require approximately 2-3 teaspoons 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.
- Body Composition – This test is 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 unit is called a DEXA scan. This test takes approximately 45 minutes 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 the end of your weight loss program.
- Physical Examination – If you are a male over 45 years of age or female over 55 years of age, you will be required to have a physical examination by a medical doctor. The doctor will ask you questions about your health history and will listen to your heart and lungs with a stethoscope. The doctor may also palpate your pulse in your neck or wrist or feel for swelling in your ankles.
- Graded Exercise Test – This procedure involves walking or running on a motorized treadmill while the electrical activity of your heart (electrocardiogram or ECG – a tracing of your heartbeats) and blood pressure is being monitored. The angle of the treadmill will increase every 2 minutes and the test will end when you are either too exhausted to continue or at approximately 8 – 12 minutes. The test is used to determine your fitness level and as a screening test for heart disease. This test will also be a practice session. You will be asked to return to repeat this test. On the second visit you will also be asked to breathe through a mouthpiece and wear a nose clip so that we can measure how much oxygen you take up during exercise. The physical examination and graded exercise test may take place in either War Memorial Hall at Virginia Tech or at the office of the Heart Specialists of Southwest Virginia (directions wil be provided).
- 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 you eat during the month. You will also be asked to remember everything you ate on the previous day and write it down. This should take approximately 15-20 minute.

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.

- Physical Activity Monitor – You will asked to wear a small monitor to measure your physical activity performed during a 24 hr period.   The monitor is slightly larger than a watch and will clip to your belt or waistband and will not interfere with your normal daily activity.

Approximate time required is 2 hours.

Session 4
- Pregnancy Test - If you are female you will be required to have a pregnancy test. This will require approximately 2-3 teaspoons 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.
- Overnight Fast: You will be asked to avoid eating for 12 hours prior to this visit so that the test results will not be influenced by the food you eat or by the normal digestion process.
- 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 signal of electrical activity from your heart. 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 your waist to measure your breathing movements.
- Sympathetic Nervous System Activity – This test involves measuring the activity of one of your nerves on the side of the knee or arm. Two small needles will be placed through your skin on the side of your knee or arm. The position of one of the needles will be moved back and forth through your skin while a very small electrical impulse (1-2 volts) is passed through the needle. The needles may be inserted up to an inch below the skin of your leg or arm. 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, measurements will begin and continue during the procedure described below.
- Catheter and Blood draw – A small plastic tube will be inserted in your arm to draw blood (approximately 3 tablespoons). We will measure norepinephrine, angiotension II, angiotensinogen, aldosterone, all hormones that influence your cardiovascular system. We will also measure growth hormone and cortisol. These are hormones that influence your metabolism (how your body burns calories and produces body heat) and cardiovascular (the heart, blood vessel and lungs) system. The plastic tube (catheter) will be left in your arm for the study described below.
- Arterial Baroreflex Study – This measures the relationship between changes in blood pressure and the change in heart rate and sympathetic nervous activity. You will be given an injection of a drug - 100 to 150 micrograms sodium nitroprusside - that causes your blood vessels to dilate (get larger), into a large forearm vein that will lower your blood pressure. Sixty seconds later, you will be given an injection of a second drug – 100 to 150 micrograms phenylephine HCL. This drug causes your blood vessels to get smaller and will raise your blood pressure. The amount of each drug to be injected will begin with a small amount, and may be increased if your blood pressure does not change at least 15 points. These two drugs will be injected a total of three times. A time period of at least 20 minutes will separate each series of injections. In addition, a small

pencil shaped blood pressure measuring device will be pressed gently against your neck for a short time.

- Blood Flow in Heart and Arteries – The blood flow and diameter in the arteries in your neck, arm and leg will be measured with an ultrasound machine. An ultrasonic machine is sort-of like radar – a low frequency radio wave that bounces off the tissues and sends a picture back to a "TV-like" screen. A mobile hand unit will be pressed gently against an artery in your neck, arm and leg. The amount of blood that your heart pumps in one beat and in one minute will be measured with another ultrasound probe. For these measurements, the probe will be pressed gently against two different places on your chest.
- Cold Pressor Test – You will be asked to cover your hand up to wrist level in a bucket of iced cold water for 2 minutes. This will provide information about your sympathetic nervous system and circulation not provided by the arterial baroreflex study. Increased activity of the sympathetic nervous system is the normal body response to fear, stress and surprise. The heart rate goes up, the lungs expand for more air intake and you get an energy rush. You may withdraw your hand from the water at any time if the cold water becomes too painful or uncomfortable.
- Handgrip – You will be asked to perform three brief (about 5 seconds) maximal squeezes on a handgrip device and then an isometric handgrip at less than half your maximal grip strength for approximately 2-3 minutes. This will not be exhausting and will provide additional information about your sympathetic nervous system and circulation responses to a different form of stress.

 The approximate time required for these tests will be approximately 3 hours.

Session 5
- Pregnancy Test - If you are female you will be required to have a pregnancy test. This will require approximately 2-3 teaspoons 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.
- Overnight Fast - You will be asked to avoid eating for 12 hours prior to this visit so that the test results will not be influenced by the food you eat or by the normal digestion process.
- Computed Tomography Scan – The amount of total fat, fat around your internal organs, and the fat under the skin in the abdominal area will be measured by computed tomography (CT scan). The CT scan imaging will be performed at Montgomery Regional Hospital. For this procedure, you will be asked to lie still on a table. An x-ray machine (the CT scanner) will rotate around you and the table will move back and forth slightly making it possible to take X-rays from several angles. The actual x-ray time is approximately 2 minutes or less. You will be lying on the table for approximately 15 to 30 minutes. The approximate time required for the entire procedure is one hour. A longer period of time may be required due to heavy scheduling and/or emergency need of the CT scan at the Montgomery Regional Hospital.

Session 6
- Pregnancy Test - If you are female you will be required to have a pregnancy test. This will require approximately 2-3 teaspoons 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.
- Overnight Fast - You will be asked to avoid eating for 12 hours prior to this visit so that the test results will not be influenced by the food you eat or by the normal digestion process.
- Resting Energy Expenditure – After fasting for 12 hours you will come to the clinic between 7:00 and 8:00 a.m. You will 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 breathe out. This hood will not disturb your natural breathing pattern. This test will tell us how many calories you burn at rest.

Session 7
- Pregnancy Test - If you are female you will be required to have a pregnancy test. This will require approximately 2-3 teaspoons 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.
- Overnight Fast - You will be asked to avoid eating for 12 hours prior to this visit so that the test results will not be influenced by the food you eat or by the normal digestion process.
- 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 your hand is maintained at heart level. Heart rate will be measured by placing electrode pads on your chest and reading the electrocardiograph (ECG – a tracing of your heartbeats) 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.
- Sympathetic Nervous System Activity – The measurements 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 move back and forth through your skin while a very small electrical impulse (1-2 volts) is passed through the electrode. The needles may be inserted up to an inch below the skin of your leg or arm. 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.
- Forearm or Leg Blood Flow – The amount of blood flow to your arm or leg will be measured in two ways. First, we will place a small flexible piece of plastic around your arm or leg and blood pressure cuffs around your 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 arm or leg blood flow. The two techniques will be used together to get the most accurate measurement.
- Lower Body Negative Pressure – Your lower body (up to your waist) will be sealed in an airtight 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 body to move into your legs. This causes your sympathetic activity to increase. This procedure will be performed at 3 to 4 different levels of negative pressure to increase your sympathetic activity. We will then measure your sympathetic activity and how the blood flow in your forearm and/or leg changes.
- Catheter and Blood Draw – 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 vessels. Norepinephrine is a substance secreted by your sympathetic nerves and causes your blood vessels to get smaller.

Session 8
- Ambulatory Blood Pressure: Your blood pressure will be measured over the course of an entire day (daytime and nighttime) using a cuff placed around your arm and a small computer type device placed around your waist. Your blood pressure will be measured at 20 minute intervals during the daytime and 30-minute intervals at night. You will be asked to stop and remain still while the cuff inflates around your arm. You will wear this cuff and

computer type device for an entire day and then return it to War Memorial Hall at the end of that period.

The approximate time for this test is 24 hours.

SUMMARY OF SUBJECT RESPONSIBILITIES
- Provide an accurate history of any health problems or medications you use before the study begins.
- Inform the experimenters of any discomfort or unusual feelings before, during or after any of the study sessions.
- Be on time and attend all of the scheduled experiment sessions.
- Follow all participant instructions for each session.
- Record the food you eat and your physical activity as instructed by the study investigators.
- Carefully follow the diet instructions provided by the research dietitian and refrain from starting an exercise program during the study.
- Carefully read the instructions on consuming any food provided to you.
- Inform the study investigators if you are pregnant or become pregnant during the study.

RISKS OF PARTICIPATION
- Catheter and Blood Draw: Some pain or discomfort may be experienced when the catheter is inserted in the vein, but this persists for only a short time. During the blood draws, you may have pain and/or bruising at the place on your arm where the blood is taken. In about 1 in 10 or 10% of the cases, a small amount of bleeding under the skin will cause bruises. The risk of a blood clot forming in the vein is about 1 in 200 (0.005%), while the risk of infection or significant blood loss is 1 in 1000 (0.001%). There is a small risk of the vein becoming inflamed and/or painful in the hours or days after the catheter is removed. If you feel faint during or after a blood draw, you should notify the study doctor or study staff immediately and lie down right away to avoid falling down. Having staff who are experienced in catheter placement and blood draws will minimize these risks.

- HIV/AIDS: Your blood will be tested for the presence of HIV if one of the study investigators is exposed to your blood. There will not be any cost to you for this test. The results will be sent to your primary care physician or the study medical director, Dr. Jose Rivero, if you do not have a primary care physician. He/she will discuss them with you and provide you with the necessary referral for further evaluation and/or counseling if your results are positive. The results of your test will remain confidential.
- Oral Glucose Tolerance: Because this procedure requires the placement of the 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 sugar 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 the study since it is an environment different from your home.

- Graded Exercise Test: Maximal exercise testing may cause fatigue, muscle strains, an irregular heart beat (dysrhythmias) and a change in blood pressure. There is a 0.01% chance of death and a 0.04% risk of heart attack requiring hospitalization. The mouthpiece and noseclip may be uncomfortable to wear.

- 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 from each DEXA is equal to 1/20 of a chest x-ray (total 3/20 of chest x-ray). The more radiation you receive over the course of your lifetime, the more likely your risk increases in developing cancerous tumors. The radiation in this study is not expected to greatly increase these risks, however 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 that the amount permitted by the Food and Drug Administration (FDA) per year. The amount you will receive is less than that received from a chest x-ray. The more radiation you receive over the course of your lifetime, the more likely your risk increases in developing cancerous tumors. The total amount of x-ray exposure from both the DEXA and CT scan is less than a chest x-ray. The radiation in this study is not expected to greatly increase these risks, however the exact increase in such risk is not known. You should know that the CT scan for this study is for research purposes and not for diagnosis. The CT scan will be not be reviewed or saved for future purposes by Montgomery Regional Hospital.

- Sympathetic Nervous System Activity: Some subjects experience a temporary (seconds) pain and discomfort while the microelectrodes are being inserted into the skin. After the procedure, there is a small risk of numbness, "pins and needles"-type of sensation, or pain that may last 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 to 3 in 1000 or 0.001 to 0.003%). It is also possible that permanent nerve damage could occur. The principal investigator of this project has performed this procedure over 300 times and only one individual (0.003%) has experienced pins and needles sensations for 7 to 10 days. All of 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. There is an extremely low risk that your blood pressure would drop so low after the nitroprusside injection that you faint. You should know that a physician will not be on site if this were to occur. Our emergency plan would involve raising your legs to help blood flow return to your head, calling 911 to activate an emergency response from the Virginia Tech Rescue Squad, and continued monitoring of your blood pressure. The Virginia Tech Rescue Squad would provide rapid response to an emergency call at War Memorial Hall. They would initiate emergency treatment which might include intravenous fluids and additional medical treatment to maintain your blood pressure. You would then be driven by ambulance to the emergency room at the hospital. However, the amount of nitroprusside used in the present study was selected to lower blood pressure by approximately 15 points or mmHg. This is similar to how much your blood pressure falls when an individual rises from a lying down to a standing position.

The infusion of phenylephrine may result in a headache, restlessness, a sudden decrease in heart rate, and/or rarely an irregular heart beat. These feeling 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. Using a large vein in your arm minimizes this risk. However, if this problem occurs, you will be referred to a physician for immediate treatment. In the event of an emergency, the Virginia Tech Rescue Squad will be contacted.

There is a remote possibility that you may have an allergic reaction to the medications or its vehicle. If this happens during the study, we will call 911 to initiate an emergency response by the Virginia Tech Rescue Squad. They may give you another medication to treat this allergic response and then transport you to the nearest hospital emergency room. It is important to inform us if you have any known medication allergies before you participate. If you have a history of allergies to phenylephrine or nitroprusside you will be not allowed to participate in this aspect of the study. If you have an allergic reaction to the medications during the study, you will not be allowed to participate further in this aspect of the study.

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 rapidly broken down and eliminated by your body. This lowers the risk of any adverse reactions to the drugs. The principal investigator has performed over 150 of these tests with no adverse events.

- Lower Body Negative Pressure: There is a very small risk of feeling nausea or fainting. The study will be stopped if you begin to feel an upset stomach, like you might faint, if your heart rate or blood pressure suddenly drops. You will be monitored continuously by the study staff to avoid any of these situations.

- Cold Pressor Test: This procedure may be painful and will cause your heart rate and blood pressure to increase.

- Handgrip: This may cause some discomfort and fatigue in your hand (similar to holding a heavy suitcase for several minutes) or forearm and will cause your heart rate and blood pressure to increase. There is a low risk of some temporary soreness in the day or two after the procedure. This test is not meant to continue until exhaustion.

- Pregnancy: You should not become pregnant during this study because of the exposure to x-rays and study drugs. If you are capable of having a child you must have a negative pregnancy test before each session that may pose a risk to an embryo or fetus (x-ray exposure or medication injection). You must agree to use an effective method of birth control, such as abstinence, condom use, oral contraceptives or use of an IUD to ensure that you will not get pregnant. Otherwise, you must be surgically sterile. If you become pregnant during this study, you must notify your study investigator immediately. There may be unforeseen risks to the embryo or fetus in the event that you become pregnant.

- Ambulatory Blood Pressure: The cuff inflation may cause some discomfort and mild bruising in your arm. The cuff inflation may cause you to have difficulty sleeping.

- Permanent weight gain increases your risk of developing diabetes and cardiovascular diseases in the future. Temporary weight gain, as you will experience in this study, is not expected to negatively affect your health. Participation in the weight loss intervention after weight gain should return your weight and BP to beginning levels. In addition, body weight returns to normal in most individuals as a result of normal physiological processes that occur over weeks to months. It may be necessary for you to purchase new clothes as a result of weight gain component of the study. This will be your own financial responsibility and is a potential cost to you that is not covered by the study. Subjects may not be able to easily lose the weight that gained during your participation in the study. They may wish to consider this before agreeing to participate in this study. In addition, if any individuals blood pressure reaches 159/99 (i.e., the leveL at which pharmacotherapy is indicated by JNC VII Guidelines), then the overfeeding phase will be terminated and they will receive necessary instructions and assistance with weight loss. The individual may continue in the weight loss aspect of the study if the Medical Director approves. We should emphasize that we anticipate blood pressure increasing only 5-6 mmHg and decreasing by this amount following weight loss. Thus, the need for terminating the overfeeding for this reason is highly unlikely.

- It is not possible to identify all potential risks in an experiential study, however the study doctors and study staff will take all possible safeguards to minimize any known and potential risks to your well-being. We believe the overall risks of participation are minimal. All of the procedures are well established and used routinely in the study investigators laboratory.

- Side effects are possible in any research study despite high standards of care, and could occur through no fault of your own or the study doctors or the study staff.

BENEFITS OF PARTICIPATION
Your participation will provide you with:
- Information on your body composition and aerobic fitness.
- Information on your blood pressure, cholesterol and glucose tolerance

COMPENSATION
We will pay you $100 each time you complete all the sessions 2-8 described above. If you dropout of the study or are unable to complete the study you will be paid $15 per session for those individual sessions you complete. If you complete each of these sessions three times you will receive a total of $300. You will also receive and additional $100 if you achieve a target weight gain of 5 kg and maintain this for one month as described above. The total amount of compensation you may receive is $400.

CONFIDENTIALITY
The data from this study will be kept strictly confidential. No data will be released to anyone but those working on the project without your written permission. Data will be identified by subject numbers, without anything to identify you by name. In the event that your exercise test indicates you may have a heart problem or if your sleep test indicates that you stop breathing during your sleep, Dr. Rivero or Dr. Zedalis may want to share this information with your doctor but will request your written approval first.

FREEDOM TO WITHDRAW
You are free to withdraw from the study at any time for any reason. Simply inform the experimenters of your intention to cease participation. Circumstances may come up that the researcher will determine that you should not continue as a subject in the study. For example, lack of compliance to instructions, failure to attend testing sessions, or illness could be reasons for the researchers to stop your participation in the study. You may be able to participate in the entire study even if you to choose not to participate in certain sessions/tests. However, if you choose not to participate in Session 1 and/or the graded exercise tests then you will not be able to participate in the entire study. These sessions/tests are included to determine if it is safe for you to participate in the study.

INJURY DURING PARTICIPATION IN THIS STUDY
Neither the researchers, the University nor Montgomery Regional Hospital have money set aside to pay for medical treatment that would be necessary if injured as a result of your participation in this study. Any expenses that you incur including emergencies and long term expenses would be your own responsibility. You should consider this limitation before you consider participating in this study.

APPROVAL OF RESEARCH
This research has been approved, as required, by the Institutional Review Board for Research Involving Human Subjects at Virginia Tech, and by the Department of Human Nutrition, Foods, and Exercise. Montgomery Regional Hospital Institutional Review Board has also approved this research. You will receive a copy of this form to take with you.

SUBJECT PERMISSION
I have read the informed consent and fully understand the procedures and conditions of the project. I have had all my questions answered, and I hereby give my voluntary consent to be a participant in this research study. I agree to abide by the rules of the project. I understand that I may withdraw from the study at any time.

If you have questions, you may contact:
- Principal Investigator: Kevin Davy, Associate Professor, Department of Human Nutrition, Foods, and Exercise. (540) 231-3487; After hours: 540-230-0486
- Chairman, Institutional Review Board for Research Involving Human Subjects: David Moore, (540) 231-4991
- Department of Human Nutrition, Foods and Exercise reviewer: Robert Grange, (540) 231-2725
- Chairman, Institution Review Board, Montgomery Regional Hospital: Chris Riegert: (540) 951-1111

Name of Subject (please print)_____

Signature of Subject_____ Date_____

DATE: September 18, 2006

MEMORANDUM

TO: Kevin P. Davy
              Brenda M. Davy
              Madlyn Frisard

Approval date: 9/13/2006
Continuing Review Due Date:8/27/2007
Expiration Date: 9/12/2007

FROM: David M. Moore

SUBJECT: **IRB Full Review Continuation 3:**   "Abdominal Fat and Autonomic-Circulatory Control in Humans[Part I: Abdominal Fat and Autonomic-Circulatory Control in Humans, Part II:Weight Gain and Autonomic-Circulatory Control in Humans]", OSP #04019808, IRB # 05-457

This memo is regarding the above referenced protocol which was previously granted approval by the IRB. The proposed research, having been previously approved at a convened IRB meeting, required full IRB review prior to granting an extension of approval, according to the specifications authorized by 45 CFR 46.110 and 21 CFR 56.110. The above referenced protocol was submitted for full review continuation and approval by the IRB at its most recent meeting. Pursuant to your request, I, as Chair of the Virginia Tech Institutional Review Board, have, at the direction of the IRB, granted approval for this study for a period of 12 months, effective September 13, 2006.

Approval of your research by the IRB provides the appropriate review as required by federal and state laws regarding human subject research. As an investigator of human subjects, your responsibilities include the following:

1.   Report promptly proposed changes in previously approved human subject research activities to the IRB, including changes to your study forms, procedures and investigators, regardless of how minor. The proposed changes must not be initiated without IRB review and approval, except where necessary to eliminate apparent immediate hazards to the subjects.
2.   Report promptly to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.
3.   Report promptly to the IRB of the study's closing (i.e., data collecting and data analysis complete at Virginia Tech). If the study is to continue past the expiration date (listed above), investigators must submit a request for continuing review prior to the continuing review due date (listed above). It is the researcher's responsibility to obtained re-approval from the IRB before the study's expiration date.
4.   If re-approval is not obtained (unless the study has been reported to the IRB as closed) prior to the expiration date, all activities involving human subjects and data analysis must cease immediately, except where necessary to eliminate apparent immediate hazards to the subjects.

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cc: File
    OSP

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60