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DISSERTATION

Gender and Obesity Effects on Cardiovagal Baroreflex Gain in Humans

Submitted by

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In partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Summer 2001

COLORADO STATE UNIVERSITY

June 29, 2001

WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY STACY D. BESKE ENTITLED GENDER AND OBESITY EFFECTS ON CARDIOVAGAL BAROREFLEX GAIN IN HUMANS BE ACCEPTED AS FULFILLING IN PART THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Co-Advisor

Co-Advisor

Department Head

ABSTRACT

Gender and Obesity Effects on Cardiovagal Baroreflex Gain in Humans

A series of studies focusing on the influence of gender, obesity, and body fat distribution on cardiovagal baroreflex gain were conducted to determine 1) if women would demonstrate lower cardiovagal baroreflex gain compared with men; 2) if cardiovagal baroreflex gain would be reduced in overweight/obese (35>body mass index<25 kg/m^2) individuals compared to their age-matched, non-obese peers (body mass index<25 kg/m^2); and 3) if cardiovagal baroreflex gain would be reduced in men with elevated abdominal visceral fat compared to their peers with lower levels of abdominal visceral fat but similar levels of total body and abdominal subcutaneous. . To accomplish this, we measured cardiovagal baroreflex gain via modified Oxford technique in sedentary, non-obese premenopausal women and men and sedentary non-obese and obese men 18-40 years of age.

The results from these studies suggest that cardiovagal baroreflex gain is lower in non-obese women compared to men (13.2±1.5 vs 20.0±2.8 ms/mmHg; $P<0.05$). The lower cardiovagal baroreflex gain in the women was not related to their lower aerobic fitness and was only marginally related to their higher body fat percentage. This may have important implications for understanding the reduced orthostatic tolerance observed in women.

Furthermore, cardiovagal baroreflex gain is reduced in men with elevated total body and abdominal fat independent of aerobic fitness level (13.2±1.6 vs. 23.1±3.8 ms/mmHg; $P<0.05$). These findings may have important implications regarding the development of cardiovascular diseases in overweight and obese individuals.

Finally, cardiovagal baroreflex gain was ~35% lower in men with elevated abdominal visceral fat compared with total body- and abdominal subcutaneous matched-men with lower levels of abdominal visceral fat (13.0±2.0 vs. 21.4±3.6 ms/mmHg; $P<0.05$). Therefore, reduced cardiovagal baroreflex gain observed in men with abdominal obesity may contribute to the elevated cardiovascular disease-related mortality associated with the metabolic syndrome. Future studies will be necessary to gain insight into mechanisms responsible for the reduced cardiovagal baroreflex gain in women compared with men, in overweight and obese men compared to non-obese men, and in men with elevated abdominal visceral fat compared to men with lower levels of abdominal visceral fat.

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To the countless others who have been influential in guiding me along my journey, your contributions are invaluable.

Two roads diverge in a wood, and I took the one less traveled by, and that has made all the difference. ~ Robert Frost ~

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CHAPTER I

INTRODUCTION AND SPECIFIC AIMS

The cardiovagal baroreflex is responsible for mediating changes in R-R interval in response to beat-to-beat changes in arterial blood pressure. Cardiovagal baroreflex gain is measured by inducing rapid changes in arterial blood pressure and quantifying the relationship with the efferent autonomic response (i.e., changes in R-R interval length). This phenomenon is best illustrated by the sigmoidal relationship between R-R interval length and systolic blood pressure (Figure 1, upper panel). Cardiovagal baroreflex gain is derived from the slope of the linear portion of the sigmoid curve (Figure 1, lower panel). Currently, it is unclear whether there are gender differences in cardiovagal baroreflex gain. In addition, the influence of obesity and regional body fat distribution on cardiovagal baroreflex gain has not been clearly established.

SPECIFIC AIMS

To determine:

1. If women demonstrate reduced cardiovagal baroreflex gain compared to men
2. If the lower cardiovagal baroreflex gain in women compared to men, if observed, can be attributed to the lower aerobic fitness levels and/or higher levels of body fat in women
3. If overweight and obese individuals have reduced cardiovagal baroreflex gain compared to their age-matched, non-obese peers

4. If reduced cardiovagal baroreflex gain in overweight and obese individuals, if observed, was independent of aerobic fitness level
5. If men with elevated levels of abdominal visceral fat have reduced cardiovagal baroreflex gain compared to men with lower levels of abdominal visceral fat when total body and abdominal subcutaneous fat levels are similar between groups

HYPOTHESIS

1. We tested the hypothesis that women would demonstrate reduced cardiovagal baroreflex gains compared with men. If so, we further hypothesized that the reduced cardiovagal baroreflex gains in women would be associated with their lower aerobic fitness and higher body fat percentage compared with males.
2. We tested the hypothesis that cardiovagal baroreflex gain would be reduced in overweight/obese (35>body mass index<25 kg/m^2) individuals compared to their age-matched, non-obese peers (body mass index<25 kg/m^2).
3. We tested the hypothesis that cardiovagal baroreflex gain would be reduced in men with elevated abdominal visceral fat compared to their peers with lower levels of abdominal visceral fat but similar levels of total body and abdominal subcutaneous fat.

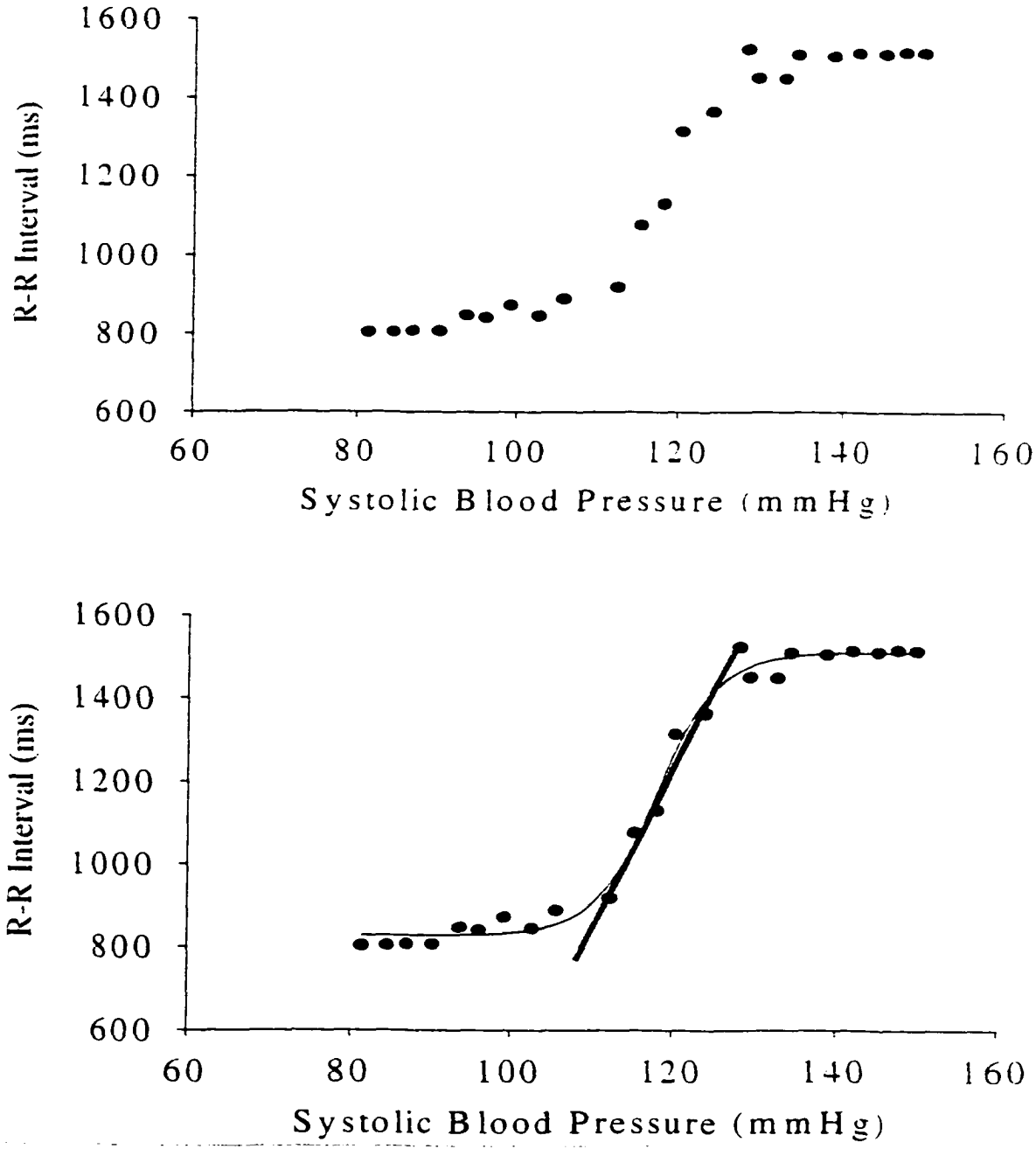

Figure 1. Sigmoidal relationship between R-R interval length and systolic blood pressure (upper panel). Determination of cardiovagal baroreflex gain from the slope of the linear portion of the sigmoid curve (lower panel).

CHAPTER II

GENDER DIFFERENCE IN CARDIOVAGAL BAROREFLEX GAIN IN HUMANS

ABSTRACT

We tested the hypothesis that women would demonstrate lower cardiovagal baroreflex gain compared with men. If so, we further hypothesized that the lower cardiovagal baroreflex gain in women would be associated with their lower aerobic fitness and higher body fat percentage compared with males. To accomplish this, we measured cardiovagal baroreflex gain (modified Oxford technique) in sedentary, non-obese (body mass index<25 kg/m^2) women (age=26.9±1.6 yrs, n=14) and men (age=26.0±2.1 yrs, n=11). Resting R-R interval and diastolic blood pressure were similar in the two groups, but systolic blood pressure was lower (P<0.05) in the women. Cardiovagal baroreflex gain was significantly lower in the women compared with the men (13.3±1.5 vs. 20.0±2.8 ms/mmHg, P<0.05). The lower cardiovagal baroreflex gain in the women was not related (P>0.05) to their lower aerobic fitness and was only marginally related to their higher body fat percentage (r=-0.34, p<0.05). There were no gender differences in the threshold and saturation, operating range, or operating point (all P>0.05), although the operating point fell significantly to left (i.e., at a lower systolic blood pressure) compared with men. Therefore, the findings of this study suggest that the gain of the cardiovagal baroreflex is reduced whereas other parameters were similar in

women compared with men. The mechanisms responsible for the reduced cardiovagal baroreflex gain remain unclear.

INTRODUCTION

Abrupt decreases and increases in systolic arterial blood pressure produce baroreflex mediated shortening and lengthening, respectively, of the R-R interval (13). This phenomenon, otherwise known as the cardiovagal baroreflex, is best described by the sigmoidal relationship between R-R interval length and systolic blood pressure. The linear portion of this relationship is used to derive the slope or gain of the cardiovagal baroreflex. Importantly, lower levels of cardiovagal baroreflex gain have been associated with poor orthostatic tolerance (5-7) and an increased risk of cardiovascular disease-related mortality (24, 25).

The influence of gender on cardiovagal baroreflex gain in humans is controversial, both similar (26, 34, 36) and lower (1, 4, 21, 23, 36) levels have been reported in women compared with men. There are a number of factors that could contribute to the discrepant findings in the past. For example, age (12, 19), hypertension (3, 19), oral contraceptive use (28), smoking (30), obesity (17), and physical activity/aerobic fitness (6-9, 22, 29) all have an important modulatory influence on cardiovagal baroreflex gain. Previous studies that addressed the influence of gender on cardiovagal baroreflex gain have failed to control for these potentially confounding factors. Furthermore, the experimental approaches used to quantify cardiovagal baroreflex gain in previous studies likely differ in their ability to detect differences between men and women.

Accordingly, we tested the hypothesis that women would demonstrate lower cardiovagal baroreflex gain compared with men. Although women generally demonstrate lower aerobic fitness and higher body fat levels compared with men, there is no information available regarding whether these factors contribute to gender differences in cardiovagal baroreflex gain. Therefore, we further hypothesized that the lower cardiovagal baroreflex gain in women, if observed, would be associated with their lower aerobic fitness and higher body fat level compared with males. To accomplish this, we measured cardiovagal baroreflex gain using sequential intravenous bolus injections of sodium nitroprusside followed by phenylephrine HCL, i.e., the modified Oxford technique (11), in sedentary, non-obese men and premenopausal women. In contrast to other approaches, this approach produces robust linear gain estimates and full characterization of the sigmoid relationship between *systolic blood pressure* and R-R interval length. The latter allows the threshold, saturation, operating range, and operating point to be calculated. To date, no information is available regarding gender differences in these other cardiovagal baroreflex parameters. Importantly, to overcome limitations of previous studies, we studied only non-smoking sedentary, non-obese and normotensive individuals 18-40 years of age and women who were not taking oral contraceptives. Our findings indicate that cardiovagal baroreflex gain is lower in sedentary, non-obese premenopausal women compared with men. The lower cardiovagal baroreflex gain observed in women was not associated with their correspondingly lower level of aerobic fitness and only marginally related to their higher body fat percentage. There were no gender differences in the threshold and saturation, operating range, or operating point.

although the operating point fell significantly to left (i.e., at a lower systolic blood pressure) compared with men.

METHODS

Subjects. Fourteen premenopausal women and 11 men participated in the present study. All subjects were sedentary (no regular physical activity >20 min on more than 2 days/wk), non-obese (body mass index <25 kg/m^2), and free from hypertension (<140/90 mmHg) or other overt cardiovascular diseases as assessed by casual blood pressure measurements and health history questionnaire, respectively. Subjects were further evaluated for the presence of overt cardiopulmonary disease by resting and maximal exercise electrocardiograms. All subjects were non-smokers, non-diabetic (2-hr post glucose load <200 mg/dl), and none of the subjects were taking medications that could influence autonomic-circulatory function. None of the women were pregnant (confirmed by pregnancy test) or using oral contraceptives at the time of the study. All the women were eumenorrheic and were studied during the early follicular phase (days 3-5) of their menstrual cycle. The nature, purpose, risks and benefits were explained to each subject before obtaining informed consent. The Colorado State University Human Research Committee approved all experimental protocols.

Experimental Procedures. Body mass was measured on a physician's balance scale to the nearest 0.1 kg. Height was measured using a stadiometer. Body composition was measured using dual energy x-ray absorptiometry (DPX-IQ, Lunar Corporation). Heart rate was measured from lead II of the electrocardiogram. Respiration was measured by a pneumobelt placed around the upper abdomen. Beat-to-beat arterial blood

pressure was measured using finger photoplethysmography (Finapres model 2300, Ohmeda). Resting Finapres arterial blood pressures were "calibrated" to brachial blood pressure during the experiment. Maximal oxygen consumption was measured during graded treadmill exercise to exhaustion. The fraction of expired oxygen and carbon dioxide, and pulmonary ventilation were measured using on-line computer assisted open-circuit spirometry (TrueMax 2400, Parvomedics). The criteria for ensuring a valid maximal oxygen consumption has been described previously (14). The modified Oxford technique was used to quantify cardiovagal baroreflex gain (11).

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

An antecubital venous catheter was placed for the injection of vasoactive drugs. After at least 20 min of quiet rest and achievement of steady state levels of heart rate, arterial blood pressure, and respiration, a bolus injection of sodium nitroprusside (75-100 mcg) was given intravenously followed 60 seconds later by a bolus injection of phenylephrine HCL (150 mcg). In general, these pharmacological perturbations decreased and increased arterial blood pressure ~15 mmHg below and above baseline levels, respectively, during a 3 minute period. During our pilot studies, we observed greater reductions in arterial blood pressure in women with lower body mass and height. Therefore, the 75 mcg dose of sodium nitroprusside was used for 6 of the "smaller"

women in the present study. Three trials were performed and at least two acceptable trials were averaged for each individual (see Data Analysis below). Each trial was separated by at least 15 min.

Data Analysis. Heart rate, blood pressure, and respiration were recorded continuously and digitized at 500 Hz to a laboratory computer for later analysis using signal processing software (Windaq, Dataq Instruments). A 4-parameter sigmoid was fit to the data to calculate the cardiovagal baroreflex gain, saturation, threshold, operating point, and the operating range (33) (See Figure 1). Briefly, systolic blood pressures and R-R intervals were averaged across 3 mmHg systolic blood pressure bins; this averaging reduced variability in the measurements due to ventilation and measurement error. Cardiovagal baroreflex gain was calculated from the regression fitted to the linear region of the sigmoid after bin values in the threshold and saturation regions were excluded. R-R intervals were used because they are more closely related to vagal outflow than heart rate (13). We accepted only regressions with correlation coefficients >0.70 (32). The average of the correlation coefficients for the trials was $r=0.93\pm0.01$ and $r=0.94\pm0.01$ for the women and men, respectively.

Statistical Analysis. All statistical analyses were performed using SPSS statistical software (SPSS v.10.1, SPSS Inc.). Data are expressed as mean±SE. Differences in the subject characteristics and dependent variables between men and women were assessed with independent t-tests. Bivariate correlational analysis was used to assess the relationship between specific subject characteristics and cardiovagal baroreflex gain. Analysis of covariance (ANCOVA) was used to test for differences in

cardiovagal baroreflex after adjusting for body fat percentage. The significance level was set a priori at $P<0.05$.

RESULTS

Subject Characteristics. The characteristics of the subjects are shown in Table 1. There was no significant difference in age or body mass index. Body mass, height, and maximal oxygen consumption were lower in women compared with the men, but body fat percentage was higher ($P<0.05$). Casually determined systolic blood pressure (104±2 vs. 118±3 mmHg) was significantly lower in women, but diastolic blood pressure (66±3 vs. 60±2 mmHg) was similar. In addition, resting R-R interval (1003±41 vs. 1012±51 ms) was not different in the two groups.

Cardiovagal Baroreflex Parameters. Cardiovagal baroreflex gain was approximately 35% lower ($P<0.05$) in the women compared with men (Figure 2). However, the threshold (691 ±41 vs. 772±29 ms), saturation (1295±53 vs. 1217±42 ms), and operating range (565±50 vs. 468±38 ms) were not significantly different in the women (n=12) and men (n=11). The operating point was also not different, but fell significantly to the left in the in the women compared with men.

Correlations. Cardiovagal baroreflex gain was significantly correlated to body fat percentage ($r=-0.34$, $p<0.05$), but not any expression of maximal oxygen consumption ($P>0.05$). The gender difference in cardiovagal baroreflex remained significant after adjusting for body fat percentage (13.3±2.3 vs. 19.5±2.8 ms/mmHg, $P<0.05$).

DISCUSSION

There are several important findings of the present study. First, cardiovagal baroreflex gain was lower in women compared with men. Second, the lower cardiovagal baroreflex gain observed in women was not associated with their correspondingly lower level of aerobic fitness. In addition, only a small portion of the variance (~10%) in cardiovagal baroreflex gain was accounted for by body fat percentage. Finally, the threshold, saturation, operating range, and operating point were similar, although the operating point fell significantly at a significantly lower systolic blood pressure in the women compared with men.

Our findings are consistent with some (1, 4, 21, 23, 36), but not all (26, 34, 36) previous studies. The results of the present study extend previous findings in at least three important aspects. First, we controlled for a number a factors, including age, hypertension, oral contraceptive use, smoking, obesity, and physical activity, that potentially confounded the interpretation of previous studies on this issue.

Second, our findings were unique from previous studies in that the results of our study suggest that the lower cardiovagal baroreflex gain observed in women was not associated with their corresponding lower level of aerobic fitness and only marginally related to their higher body fat percentage compared with men. Therefore, the results of the present study suggest that factors other than lower aerobic fitness and elevated body fat percentage must contribute to the lower cardiovagal baroreflex gain observed in women compared with men.

Finally, an important strength of our study was the use of sequential intravenous bolus injections of sodium nitroprusside followed by phenylephrine HCL to produce

robust gain estimates and characterization of the entire sigmoid relationship between systolic blood pressure and R-R interval. In addition to quantifying cardiovagal baroreflex gain, the threshold and saturation regions, operating point, and operating range could also be derived. To our knowledge, our study is the first to use this experimental approach to investigate gender differences in cardiovagal baroreflex function.

The levels of cardiovagal baroreflex gain obtained in the present study are in the range of that reported previously for healthy, young men and women using similar approaches (i.e, bolus injection of vasoactive drugs) (1, 12). However, we would like to emphasize caution in comparing the values obtained in the present study with those obtained by others. This is particularly important when other experimental approaches to quantifying cardiovagal baroreflex gain are utilized because the gain estimate can vary considerably with the technique used and analysis performed. For example, the inclusion of threshold or saturation regions may produce smaller gain estimates than if they are excluded. Furthermore, the factors mentioned in the preceding paragraphs should also be considered when comparing our values for cardiovagal baroreflex gain with the values reported in the literature.

We can only speculate on the potential mechanisms responsible for the lower cardiovagal baroreflex gain in the women in the present study. First, it is possible that circulating estrogen and/or progesterone was responsible for the observed gender differences in the present study. However, 17-beta estradiol administration enhances cardiovagal baroreflex gain in animals (31) and physiological fluctuations in estrogen and progesterone during the menstrual cycle do not appear to influence cardiovagal

baroreflex gain in humans (27). It is possible that female sex hormones exert an effect on cardiovagal baroreflex that is more chronic in nature.

Second, women have been reported to demonstrate lower carotid artery distensibility compared with men (20, 35). Carotid distensibility has been closely associated with cardiovagal baroreflex gain (2). Therefore, it is possible that lower levels of carotid artery distensibility in women would result in a smaller mechanical transduction of arterial blood pressure into barosensory stretch. This, in turn, would result in an attenuated cardiovagal baroreflex response. Future studies are necessary to address this issue.

There are some important implications of the present study that we would like to emphasize. First, women have been reported to demonstrate reduced orthostatic tolerance compared with men (4, 15, 16). There are a number of factors that have been attributed to the reduced orthostatic tolerance observed in women including reduced cardiovagal baroreflex gain (4-6, 13). Therefore, our findings are consistent with the idea that a lower cardiovagal baroreflex gain in women may contribute to their reduced orthostatic tolerance compared with men. However, future studies will be needed to directly test this hypothesis.

Second, reduced cardiovagal baroreflex gain has been reported to be an important predictor of cardiovascular mortality (24, 25). Thus, the lower cardiovagal baroreflex gain observed in the women in the present study would appear to present a paradox because premenopausal women demonstrate a reduced risk of cardiovascular disease (10). Presumably, more favorable levels of other risk factors (e.g., lipid and lipoprotein concentrations) in women would overwhelm any risk conferred by reduced cardiovagal

baroreflex gain. However, as has been suggested previously (21), it is possible that the lower cardiovagal baroreflex gain in women could contribute to their poorer outcomes after myocardial infarction (18).

Finally, the results our study suggest that gender should be considered separately in future studies focused on cardiovagal baroreflex function. However, an important issue not addressed in the present study is whether women respond differently to interventions directed at modifying cardiovagal baroreflex function. Future studies should consider this possibility.

There are some potential limitations of the present study that should be discussed. We studied only sedentary, non-obese men and women 18-40 years of age who were healthy and free of overt cardiovascular disease. As such, the influence of gender in other groups (e.g., older or obese adults) may differ from those reported here.

In summary, the results of the present study indicate that cardiovagal baroreflex gain is lower in women compared with men. The lower level of cardiovagal baroreflex gain was not associated with aerobic fitness and only marginally related to the body fat content. In addition, the threshold, saturation, operating range, and operating point were similar, although the operating point fell significantly to the left (i.e., at a lower systolic blood pressure) in the women compared with men.

Table 1. Subject Characteristics.

Variable	Men (n=11)	Women (n=14)
Age (yrs)	26.9±1.6	26.0±2.1
Height (cm)	181±3	164±2*
Body Mass (kg)	73.7±2.3	59.5±1.2*
BMI kg/m^2)	23±1	22±1
Body Fat (%)	18.1±1.6	29.7±1.8*
VO_2max (ml/kg/min)	48.0±2.5	37.0±1.6*
VO_2max (ml/kg FFM/min)	61.1±2.5	53.7±3.1*

BMI=Body Mass Index; VO_2max=Maximal Oxygen Consumption. All values are expressed as mean±SE. *P<0.05 vs. Men.

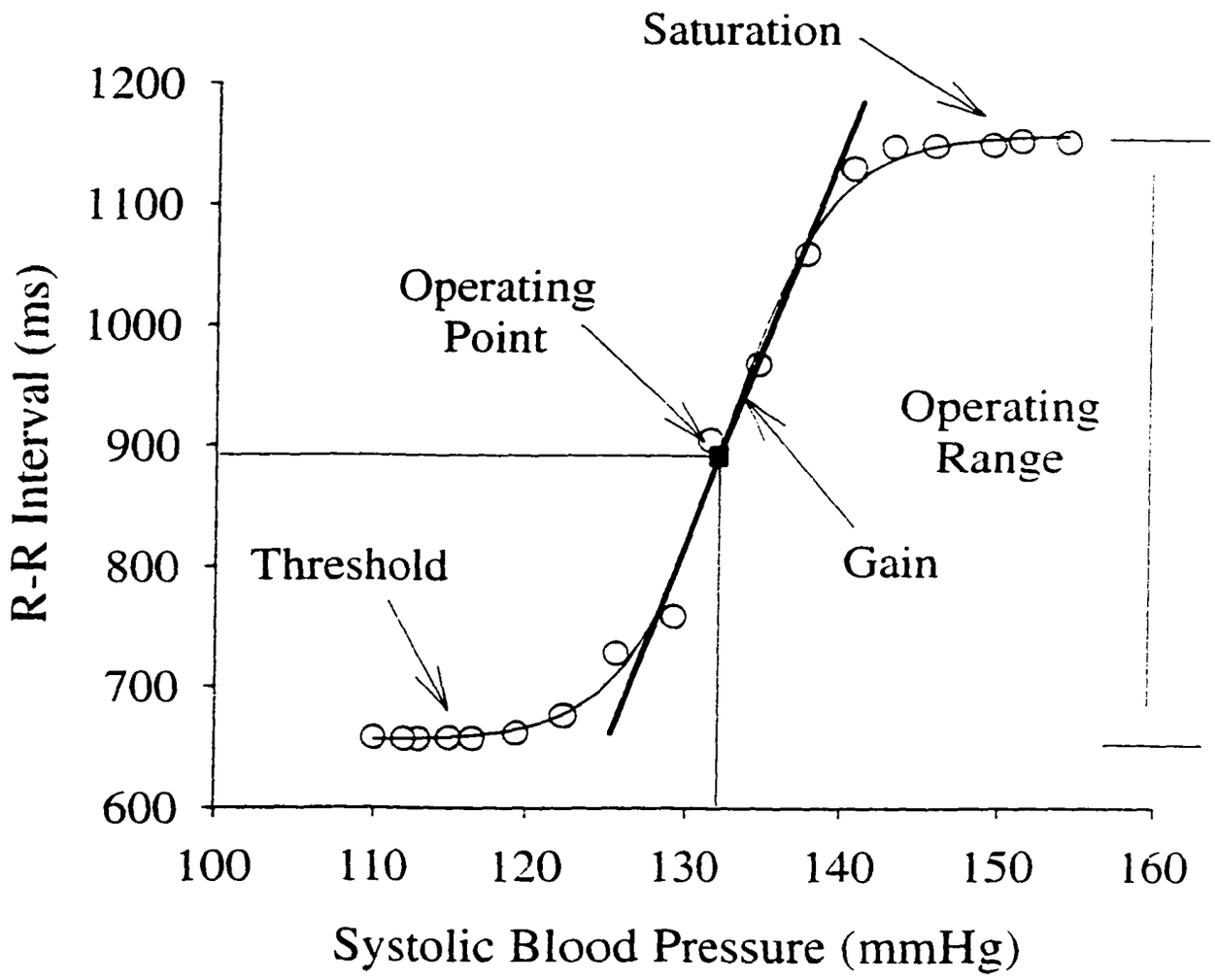

Figure 1. Diagram of the sigmoid relationship between systolic blood pressure and R-R interval during sequential bolus injection of sodium nitroprusside followed by phenylephrine HCL. The calculated parameters include gain, threshold and saturation, operating range, and operating point. Cardiovagal baroreflex gain is calculated from the regression fitted to the linear region of the sigmoid after the values in threshold and saturation regions were removed.

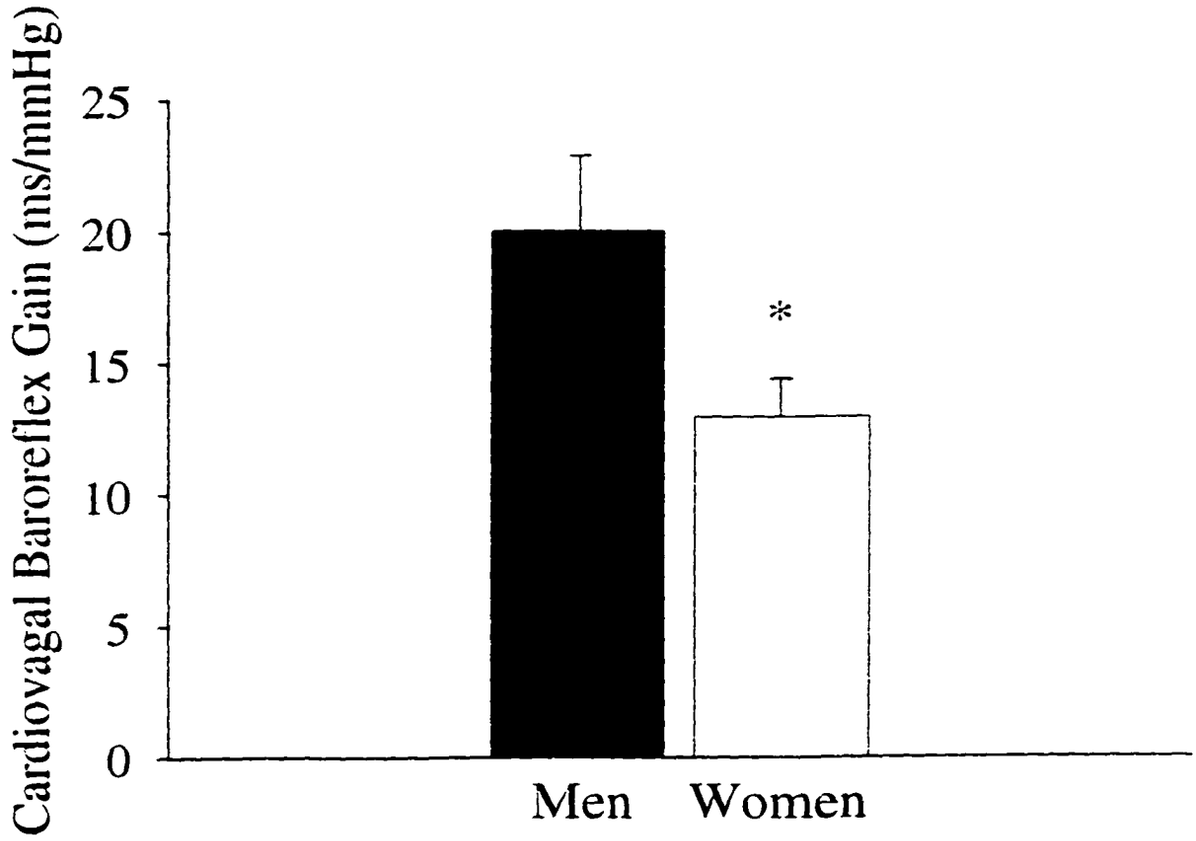

Figure 2. Cardiovagal baroreflex gain in women compared with men. Values are mean±SE. *P<0.05 vs. Men

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CHAPTER III

REDUCED CARDIOVAGAL BAROREFLEX GAIN IN OVERWEIGHT AND OBESE MEN

ABSTRACT

We tested the hypothesis that cardiovagal baroreflex gain would be reduced in overweight/obese (35>body mass index<25 kg/m^2) individuals compared to their age-matched, non-obese peers (body mass index<25 kg/m^2). To accomplish this, we measured cardiovagal baroreflex gain (modified Oxford Technique) in non-obese (BMI, 20.9±0.9kg/m^2, NO) and overweight/obese (28.8±0.7 kg/m^2, OW/OB) sedentary men. Cardiovagal baroreflex gain was significantly lower in OW/OB compared to NO (13.2±1.6 vs. 23.1±3.8, respectively). In addition, cardiovagal baroreflex was reduced in overweight as well as obese men (OW=14.3±2.0, OB=11.3±2.6, both P<0.05 vs. NO). The lower cardiovagal baroreflex gain was observed in a subgroup of OW/OB with aerobic fitness levels similar to their non-obese peers (24.9±3.9 vs. 16.3±2.1, respectively; P<0.05). Thus, the results of the present study indicate that cardiovagal baroreflex gain is reduced in men with elevated total body and abdominal fat independent of aerobic fitness. These findings may have important implications regarding the development of cardiovascular diseases in overweight and obese individuals.

Key Words: Obesity, Baroreflex Gain, Vagal

INTRODUCTION

The accumulation of excess body fat is a major public health problem (5, 30). The prevalence of overweight and obesity has increased dramatically in the last decade (20, 30). Approximately 25% of adults are obese and greater than 50% are overweight in the United States (20). Several epidemiological studies have indicated that obesity is an independent risk factor for sudden cardiac death (17, 24, 35) and cardiovascular disease-related morbidity and mortality (17, 24, 49).

Basal cardiac vagal outflow is reduced in obese compared with non-obese humans (2, 3, 41). However, the influence of obesity on arterial baroreflex control of cardiac vagal outflow (i.e., cardiovagal baroreflex gain) is controversial; both similar (41, 46) and lower (22, 40) levels have been reported. There are a number of factors that could contribute to the discrepant findings observed in the past. For example, hypertension (23, 27) and coronary heart disease (32, 33), both of which are more prevalent in obesity (6, 24, 28, 44), are associated with a reduction in cardiovagal baroreflex gain. Thus, the failure to screen and/or exclude individuals with hypertension and/or coronary heart disease would confound the interpretation of any study focused on determining the influence of obesity on cardiovagal baroreflex function.

Accordingly, we tested the hypothesis that cardiovagal baroreflex gain would be reduced in overweight/obese individuals compared to their age-matched, non-obese peers. To accomplish this, we measured cardiovagal baroreflex gain (modified Oxford technique) in sedentary, normotensive overweight/obese and non-obese men. Our findings indicate that elevated total body and abdominal fat is associated with a reduction in cardiovagal baroreflex gain independent of aerobic fitness.

METHODS

Subjects. Seven non-obese (NO, body mass index<24.9 kg/m^2) and 14 overweight/obese (OW/OB, 25.0<body mass index<34.9 kg/m^2) men volunteered to participate in the study. We excluded subjects with a body mass>35.0 kg/m^2 because 1) severe obesity is associated with greater number of co-morbidities (48) and 2) the prevalence of severe obesity is less than 10% in the general population (20, 30). Thus, our findings should be more generalizable to the majority of overweight/obese adults. To determine whether a reduction in cardiovagal baroreflex gain is observed in both overweight and obese individuals we further divided the OW/OB group according to body mass index WHO criteria (48). Overweight and obesity were defined as a body mass index 25-29.9 and 30-34.9 kg/m^2 , respectively. In addition, only men were studied in the present investigation because gender has an important influence on cardiovagal baroreflex gain (1, 11, 25, 31, 50, Beske et al, unpublished observations). All subjects were normotensive (arterial blood pressure <140/90 mmHg) as determined by casual blood pressure measurements and free from other overt cardiovascular diseases as determined from individual health histories. Subjects were further evaluated for the presences of overt cardiopulmonary disease by resting and maximal exercise electrocardiograms. Subjects were nonsmokers and non-diabetic (2-hr post glucose load <200 mg/dL). Subjects were not taking any medications that could influence autonomic circulatory function. All subjects were sedentary and were not participating in any regular physical activity (<20 min on ≤ 2 days/wk). The nature, purpose, risks and benefits were explained to the subject before obtaining informed consent (See Appendix

A). The Colorado State University Institutional Review Board approved all experimental protocols.

Experimental Procedures. Body mass was measured on a physician's balance to the nearest 0.1 kg. Height was measured using a stadiometer. Waist and hip circumference were measured at the level of the umbilicus and the widest portion of the hip, respectively. Body composition was measured using dual energy x-ray absorptiometry (DPX-IQ, Lunar Corp.). Computed tomography scans were performed to quantify abdominal visceral and subcutaneous fat levels as previously described (38, 47). Briefly, a cross-sectional scan 10 mm thick, centered at the L4-L5 intervertebral disk space, was obtained using 170 mA with a scanning time of 2 s and a 512 X 512 matrix. Heart rate was measured from lead II of an electrocardiogram. Respiration was measured by placing a pnuemobelt around the upper abdomen. Beat-to-beat arterial blood pressure was measured using finger photoplesthmography (Finapres model 2300, Ohmeda). Resting Finapres arterial blood pressures were "calibrated" to brachial blood pressures with an automated device (Dinamap, Critikon Co) prior to the injection of vasoactive drugs (see below). Because aerobic fitness has been reported to exert an important influence on cardiovagal baroreflex gain (12-16, 26, 36), maximal oxygen consumption was measured during graded treadmill exercise to exhaustion using open circuit spirometry (TrueMax 2400, ParvoMedics). Criteria for achievement of a valid maximal oxygen consumption was based on achieving at least three of the following: 1) a plateau in maximal oxygen consumption, 2) a respiratory quotient value of >1.10, 3) rating of perceived exertion >18; and/or 4) achievement of age-predicted maximal heart rate. To determine whether the reduction in cardiovagal baroreflex gain, if observed, was

independent of aerobic fitness, we compared a subgroup of NO and OW/OB with similar levels of aerobic fitness. Cardiac baroreflex gain was quantified using the modified Oxford technique (18).

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

An antecubital venous catheter was placed in the subject's left arm for the injection of vasoactive drugs. After a 20-minute rest period and stabilization of baseline arterial blood pressure, heart rate, and respiration, a bolus injection of sodium nitroprusside (75-100 μg) was given intravenously followed 60 seconds later by a bolus injection of phenylephrine HCL (100-150 μg). These pharmacological perturbations decreased and increased arterial blood pressure ~15 mmHg from baseline levels during a 3 minute period. Three trials were completed and each separated by a minimum of 15 minutes quiet rest.

Data Analysis. Abdominal visceral and subcutaneous fat regions were determined using commercially available medical imaging software (SliceOmatic, Tomovision) (See Appendix C). Adipose tissue was identified by Hounsfeld units (HU) in the range of –30 to –190 HU (38, 47). Abdominal visceral fat was calculated as the area of pixels in the appropriate HU range within the abdominal wall. Abdominal subcutaneous fat was calculated as the pixel area in the appropriate HU range outside the

abdominal wall. Blinded repeat measurements of abdominal visceral and subcutaneous fat in a random sample (n=10) were highly correlated (r=0. 99. P<0.05).

Heart rate. blood pressure. and respiration were recorded continuously and digitized at 500 Hz to a laboratory computer for later analysis using signal processing software (Windaq, Dataq Instruments). A 4-parameter sigmoid was fit to the data to calculate the cardiovagal baroreflex gain and operating range (43) (See Appendix B). R-R intervals were used because they are more closely related to vagal outflow than heart rate (19). Briefly, systolic blood pressures and R-R intervals were averaged across 3 mmHg systolic blood pressure bins. This averaging was performed to reduce variability and error associated with respiration and measurement, respectively. Cardiac baroreflex gain was estimated by calculating the linear gain of the pressure-R-R interval baroreflex responses after the bin values in the threshold and saturation regions were excluded. The average of the three trials was used to determine the average cardiovagal baroreflex gain. We accepted only regressions with correlation coefficients (r)$\geq$0.70 (42). The operating range was calculated as the change in R-R interval between the saturation and threshold regions of the fitted sigmoid curve.

Statistical Analysis. Differences in subject characteristics and dependent variables between NO and OW/OB men were assessed with independent Student's t-tests'. Analysis of covariance was used to gain insight into the potential role of total body fat and abdominal visceral and subcutaneous fat (covariates) in mediating the lower cardiovagal baroreflex gain in OW/OB compared with NO. A one-way analysis of variance with Tukey's post hoc test was used to test for differences in the dependent variables in the non-obese, overweight, and obese groups. Relationships among variables

were assessed using Pearson Product Moment correlations. Stepwise multiple regression analyses were computed to sort out the contribution of total body fat, abdominal visceral and subcutaneous fat mass. Data are expressed as mean ±SE. The significance level was set a priori at $P<0.05$. All statistical analyses were performed using SPSS statistical software (SPSS v.10.1, SPSS Inc.).

RESULTS

Subject Characteristics for Non-Obese and Overweight/Obese Men. Subject characteristics for NO vs. OW/OB are shown in Table 1. No differences in age, height, systolic or diastolic blood pressures were observed in the NO and OW/OB groups ($P>0.05$). Body mass, body mass index, body fat percentage, waist and hip circumferences, abdominal visceral and subcutaneous fat were higher (all $P<0.05$) in OW/OB compared with NO, but VO_2max was lower. In addition, resting heart rate (60±4 vs. 57±2 bpm) and R-R interval (1030±66 vs. 1064±39 ms) were not different between the two groups.

Cardiovagal Baroreflex Gain and Operating Range. Cardiovagal baroreflex gain was significantly lower in OW/OB compared with NO ($P<0.05$, Figure 1). The operating range was also not different in these two groups (NO=486±42 vs. OW/OB=467±48, $P>0.05$).

Subject Characteristics for Non-Obese, Overweight, and Obese Men. Subject characteristics for NO, OW, and OB are shown in Table 2. There was no difference in

age, height, or diastolic blood pressures observed in the three groups (P>0.05). Body mass, body mass index, body fat percentage, waist and hip circumferences, WHR, and abdominal visceral and subcutaneous fat were higher (all P<0.05) in OW and OB compared with NO. All of these variables were significantly higher in the OB compared with OW. Systolic blood pressure was higher (P<0.05) in the OW compared with NO, but the difference between OW and OB was not significant. In addition, resting heart rate (60±4 vs. 57±3 vs. 57±3 bpm) and R-R interval (1030±66 vs. 1068±55 vs. 1055±33 ms) were not different between the NO, OW, OB, respectively.

Cardiovagal Baroreflex Gain and Operating Range. Both OW and OB individuals demonstrated lower (Figure 2) cardiovagal baroreflex gain compared with NO. However, cardiovagal baroreflex gain was not different (P>0.05) in OB compared with OW men. The operating range was also not different in the three groups (NO=486±42, OW=478±53, OB=452±96, P>0.05).

Subject Characteristics for Non-Obese and Overweight/Obese Men with Similar Levels of Maximal Oxygen Consumption. Subject characteristics for NO vs. OW/OB are shown in Table 3. No differences in age, height, systolic or diastolic blood pressures were observed in the NO and OW/OB groups (P>0.05). Body mass, body mass index, body fat percentage, waist and hip circumferences, abdominal visceral and subcutaneous fat were higher (all P<0.05) in OW/OB compared with NO, but VO_2max was similar. In addition, resting heart rate (61±3 vs. 55±2 bpm) and R-R interval (1012±50 vs. 1096±40 ms) were not different between the two groups.

Cardiovagal Baroreflex Gain and Operating Range. Cardiovagal baroreflex gain was lower ($P<0.05$) in the OW/OB compared with NO with similar levels of maximal oxygen consumption (Figure 3). There was no significant difference in the cardiovagal baroreflex operating range in the two groups (501 ± 46 vs. 479 ± 47, $P>0.05$).

Correlational Analysis. Body mass ($r=-.40$, $P<0.05$), body mass index ($r=-0.66$, $P<0.05$), waist circumference ($r=-0.46$, $P<0.05$), WHR ($r=-0.46$, $P<0.05$), body fat percentage ($r=-0.37$, $P<0.05$), abdominal visceral fat ($r=-0.40$, $P<0.05$), abdominal subcutaneous fat ($r=-0.39$, $P<0.05$), and systolic blood pressure ($r=-0.47$, $P<0.05$) were correlated to cardiovagal baroreflex gain. Neither maximal oxygen consumption ($r=0.28$, $P>0.05$), diastolic blood pressure ($r=-0.28$, $P>0.05$) nor hip circumference ($r=-0.33$, $P>0.05$) were correlated to cardiovagal baroreflex gain.

The difference in cardiovagal baroreflex gain between NO and OW/OB was unchanged after adjusting for body fat percentage, fat mass, abdominal visceral and subcutaneous fat, and systolic blood pressure (data not shown). The results of the stepwise multiple linear regression indicate that there were no independent predictors of cardiovagal baroreflex gain in the present study.

DISCUSSION

There are two new findings from the present study. First, cardiovagal baroreflex gain is reduced in overweight/obese men compared with their non-obese peers. Second, the reduction in cardiac baroreflex gain was observed in overweight as well as clinically

obese men. Finally, the reduction in cardiovagal baroreflex gain in overweight/obese men appears to be independent of their lower level of aerobic fitness.

The results from several previous studies suggest that obesity is associated with reduced cardiovagal baroreflex gain (3, 22, 29, 40, 41). Our findings extend these observations in at least two important ways. First, we have shown for the first time that overweight as well as mild-to-moderate levels of obesity are associated with reduced cardiovagal baroreflex gain.

Second, the reduced cardiovagal baroreflex gain observed in the overweight/obese men in the present study was independent of their correspondingly lower level of aerobic fitness. This suggestion is supported by two observations. First, we observed no significant correlation between maximal oxygen consumption and cardiovagal baroreflex gain in the pooled sample of men in the present study. Second, we observed a lower cardiovagal baroreflex gain in OW/OB compared with NO with similar aerobic fitness levels. Taken together, these observations suggest that the lower cardiovagal baroreflex gain observed in overweight/obese men cannot be attributed to their lower level of aerobic fitness.

Total body and abdominal visceral and subcutaneous fat were correlated with cardiovagal baroreflex gain in the present study. Our findings suggest that neither total body fat nor abdominal visceral or subcutaneous fat independently contribute to the inter-individual variation in cardiovagal baroreflex gain. However, we should emphasize that our study was not designed to specifically address the contribution of total or regional body fat to the reduced cardiovagal baroreflex gain observed in overweight and obese men. Further studies will be necessary to gain insight into this issue.

We can only speculate on the mechanisms responsible for the lower cardiovagal baroreflex gain in the OW/OB men in the present investigation. First, arterial stiffness has been reported to be increased with increasing levels of total body and abdominal fat (21, 39, 45). Additionally, reduced cardiovagal baroreflex gains have been associated with increased arterial stiffness (7, 8). Therefore, arterial stiffening may decrease barosensory transduction of the afferent signal in response to changes in arterial blood pressure and alter cardiovagal baroreflex responses in overweight and obese individuals. This possibility is currently being explored in our laboratory.

Second, impaired muscarinic receptor function has been demonstrated in animal models of diet-induced obesity (9, 37). Thus, it is possible the reduction in cardiovagal baroreflex gain observed in the OW/OB in the present study was due, in part, to reduced cardiac muscarinic receptor number and/or sensitivity.

There are some important implications of our findings that should be discussed. Obesity is an important independent risk factor for sudden cardiac death, hypertension, and cardiovascular disease-related mortality (6, 17, 24, 28, 35, 44). Reductions in cardiovagal baroreflex gain have been similarly related to these adverse health outcomes (4, 10, 33, 34). Therefore, the lower cardiovagal baroreflex gain observed in overweight and obese men may contribute to their greater overall risk.

There are some potential limitations of the present study that should be addressed. First, only sedentary normotensive men 18-40 years of age who were healthy and free from overt cardiovascular disease were included in the study. Thus, these findings may not be applicable to other groups (i.e., women, older individuals, etc.).

Second, it is possible that the difference in cardiovagal baroreflex gain observed in NO and OW/OB would be eliminated if the rise in systolic blood pressure were sustained (i.e., there was adaptation of central neural pathways). We do not believe this is likely because Grassi et al. (22) have reported that cardiovagal baroreflex gain is reduced in obese, albeit severely obese, compared with non-obese humans using the graded, steady state infusion technique.

Third, it is possible that genetic factors contributed to the reduced cardiovagal baroreflex gains observed in overweight and obese men. Reduced cardiovagal baroreflex gain has been associated with the –344C polymorphism in the aldosterone synthase gene (50). It is possible that the overweight and obese men in the present study may have had a higher frequency of the –344C allele. However, at this time there is no evidence to support a higher allele frequency in overweight and obese men. It is also possible the other DNA sequence variations may have differed in frequency or interacted with obesity to contribute to lower cardiovagal baroreflex gain observed in the OW/OB men in the present study.

In summary, the results of the present study indicate that cardiovagal baroreflex gains are reduced in men with elevated total body and abdominal fat independent of aerobic fitness. Furthermore, the cardiovagal baroreflex operating range was not altered in men with elevated total body and abdominal fat. This may have important implications regarding the development of hypertension and cardiovascular disease in overweight and obese individuals. Whether these changes in cardiovagal baroreflex sensitivity are present in women or older populations remains to be determined.

Table 1. Subject Characteristics in Non-Obese and Overweight/Obese Men.

	NO (n=7)	OW/OB (n=14)
Age (yrs)	24.7±2.6	28.1±1.8
Height (cm)	183.5±4.2	180.0±2.0
Weight (kg)	70.2±2.8	93.4±3.4*
Body Mass Index (kg/m^2)	20.9±0.9	28.8±0.7*
Waist Circumference (mm)	79.0±1.7	99.3±2.6*
Hip CircumfSerence (mm)	96.3±2.1	109.2±1.7*
Waist-to Hip Ratio (units)	0.82±0.01	0.91±0.02
Body Fat (%)	15.9±1.9	27.0±1.7*
Fat Mass (kg)	11.4±1.8	25.6±2.2*
Abdominal Visceral Fat (cm^2)	53.4±9.4	124.2±16.0*
Abdominal Subcutaneous Fat (cm^2)	123.9±18.2	293.7±25.0*
SBP (mmHg)	114±3.1	121±2.1
DBP (mmHg)	63±2.8	66±2.3
Heart Rate (bpm)	59.7±3.7	57.5±2.3
VO_2max (ml/kg/min)	49.4±3.2	40.5±2.2*
VO_2max (ml/lbm/min)	61.1±2.5	57.0±2.7

All values are mean±SE. * P<0.05 vs. NO.

Table 2. Subject Characteristics Non-Obese, Overweight, and Obese Men.

Variable	NO (n=7)	OW (n=9)	OB (n=5)
Age (yrs)	24.7±2.6	27.4±2.2	29.4±3.5
Height (cm)	183.5±4.2	181.2±2.4	177.8±3.7
Weight (kg)	70.2±2.8	89.6±4.2*	100.3±5.1*^
BMI ($kg.m^2$)	20.9±0.9	27.2±0.61*	31.6±0.64*^
Waist Circumference (mm)	79.0±1.7	95.8±3.0*	105.5±3.6*^
Hip Circumference (mm)	96.3±2.1	107.4±2.1*	112.2±2.8*
WHR (mm)	0.82±0.01	0.89±0.02*	0.94±0.04^
Body Fat (%)	15.9±1.9	24.2±1.7*	32.0±2.6*^
Abdominal Visceral Fat (cm^2)	11.4±1.8	114.6±16.5*	141.4±35.1*
Abdominal Subcutaneous Fat (cm^2)	53.4±9.4	251.4±27.8*	369.9±25.4*^
SBP (mmHg)	123.9±18.2	126±3.5*	120±2.3
DBP (mmHg)	114±3.1	67±2.6	65±2.5
Heart Rate (bpm)	59.7±3.7	57.5±3.3	57.4±2.7
VO_2max (ml/kg/min)	49.4±3.2	44.0±2.6	34.1±1.7*^
VO_2max (ml/lbm/min)	61.1±2.5	60.6±2.7	53.1±3.6

All values are presented as mean±SE. *Significant difference versus lean, ^Significant difference versus overweight subjects, $P<0.05$.

Table 3. Subjects Characteristics of Non-Obese and Overweight/Obese Men with Similar Levels of Maximal Oxygen Consumption.

Variable	NO (n=6)	OW/OB (n=8)
Age (yrs)	25.3±3.0	26.5±1.9
Height (cm)	184.0±4.9	178.2±2.9
Weight (kg)	69.9±3.3	88.4±4.1*
Body Mass Index (kg/m^2)	20.7±1.0	27.8±0.9*
Waist Circumference (mm)	78.8±1.9	96.3±3.4*
Hip Circumference (mm)	96.2±2.4	107.2±2.0*
Waist-to Hip Ratio (units)	0.82±0.01	0.90±0.02
Body Fat (%)	15.6±2.2	25.9±2.5
Fat Mass (kg)	11.2±2.0	23.2±2.9*
Abdominal Visceral Fat (cm^2)	47.8±8.9	98.3±14.4*
Abdominal Subcutaneous Fat (cm^2)	120.9±21.3	287.9±36.4*
SBP (mmHg)	114±3.6	121±2.4
DBP (mmHg)	64±3.0	65±2.1
Heart Rate (bpm)	60.9±4.1	58.2±3.5
VO_2max (ml/kg/min)	47.2±2.8	44.4±2.0

All values are presented as mean±SE. * P<0.05 vs. NO.

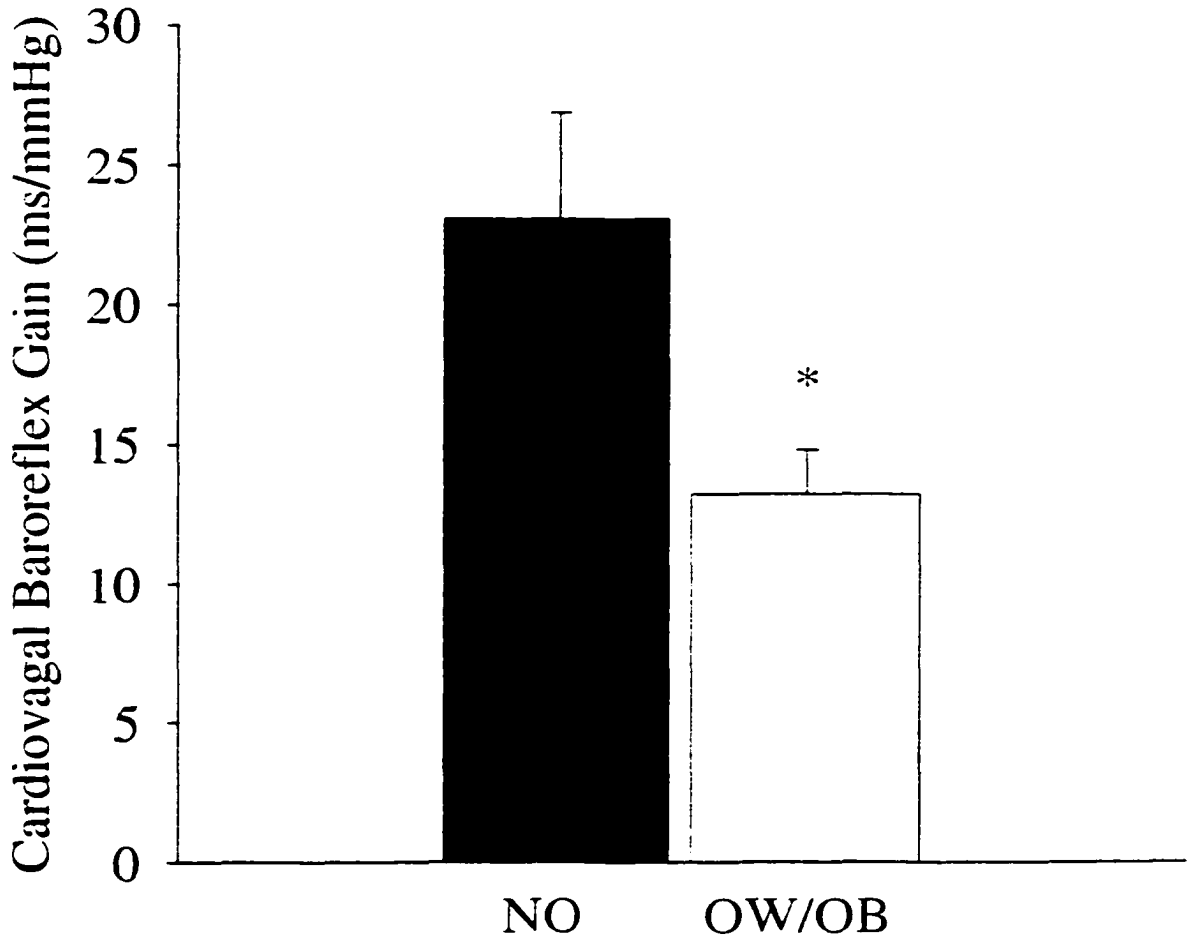

Figure 1. Cardiovagal baroreflex gain in non-obese (NO) and overweight/obese (OW/OB) men. Values are mean±SE. *P<0.05 vs. NO.

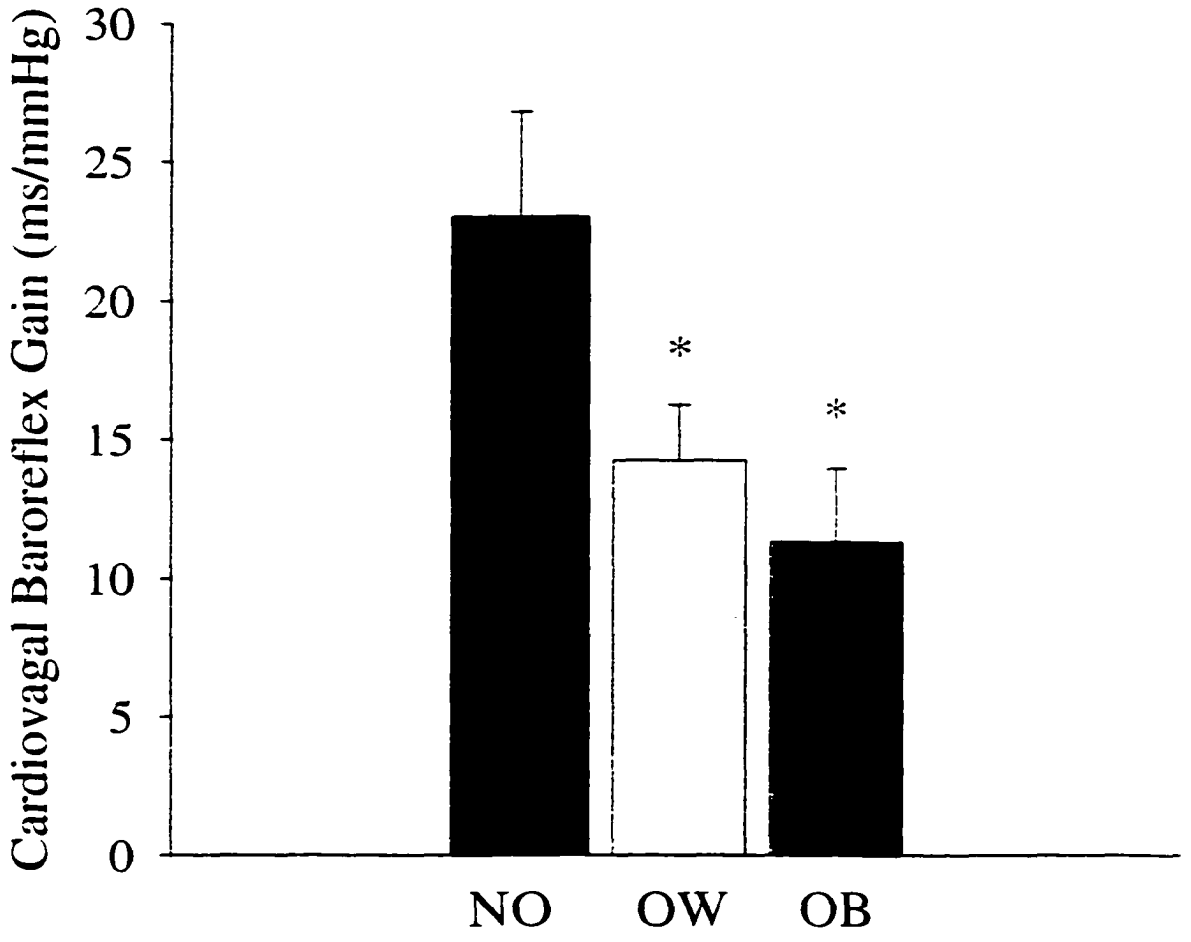

Figure 2. Cardiovagal baroreflex gain in non-obese (NO) and overweight (OW), and obese (OB) men. Values are mean±SE. *P<0.05 vs. NO.

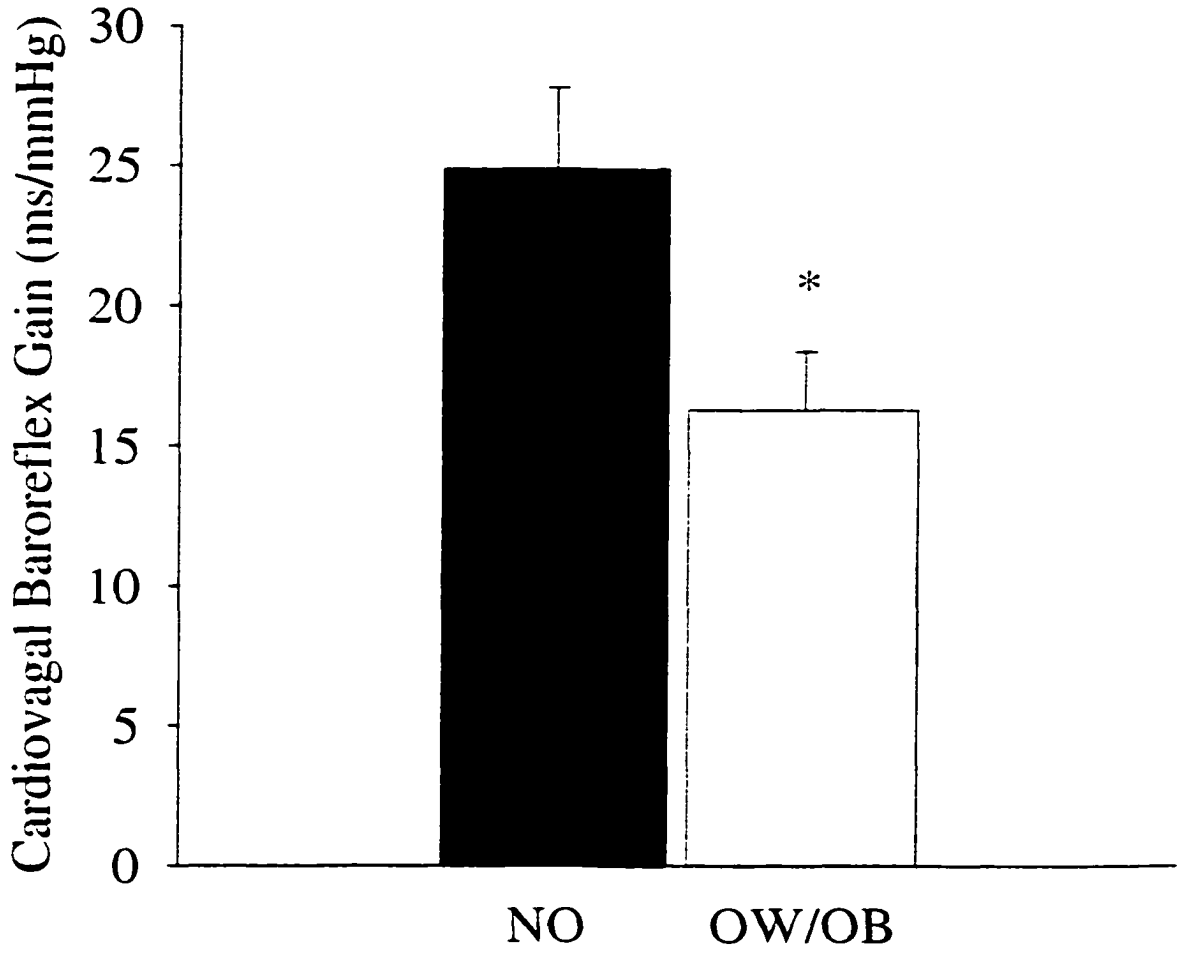

Figure 3. Cardiovagal baroreflex gain in non-obese (NO) and overweight/obese (OW/OB) men with similar levels of maximal oxygen consumption. Values are mean±SE. * $P<0.05$ vs. NO.

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CHAPTER IV

REDUCED CARDIOVAGAL BAROREFLEX GAIN IN VISCERAL OBESITY: IMPLICATIONS FOR THE METABOLIC SYNDROME

ABSTRACT

We tested the hypothesis that cardiovagal baroreflex gain would be reduced in men with elevated abdominal visceral fat compared to their peers with lower levels of abdominal visceral fat but similar levels of total body and abdominal subcutaneous fat. To test this hypothesis, we measured cardiovagal baroreflex gain (modified Oxford technique) in two groups of sedentary, normotensive (<140/90), non-obese (BMI<30 kg/m^2) men with lower (LVF, n=8) and higher (HVF, n=9) levels of abdominal visceral fat (66.2±9.8 vs. 121.7± 19.6 cm^2, P<0.05) matched for total body (20.4±2.4 vs. 23.6±2.4 %, P>0.05) and abdominal subcutaneous fat (215.1±39.1 vs. 219.9±34.8 cm^2, P>0.05). Cardiovagal baroreflex gain was ~35% lower in HVF compared with LVF (13.1± 1.7 vs. 20.4±3.2, P<0.05), but cardiovagal baroreflex operating range did not differ between the two groups (512±48 vs. 477±46 ms, P>0.05). Therefore, reduced cardiovagal baroreflex gain observed in men with abdominal obesity may contribute to the elevated cardiovascular disease-related mortality associated with the metabolic syndrome. Whether reductions in abdominal visceral fat will result in improvements in cardiovagal baroreflex gain remains unclear.

Key Words: Visceral Fat; Metabolic Syndrome; Baroreflex Gain

INTRODUCTION

Elevated abdominal visceral fat is associated with a clustering of several cardiovascular and metabolic risk factors for coronary heart disease including hypertension, dyslipidemia, glucose intolerance and insulin resistance, impaired hemostatic function, and low-grade chronic inflammation (4, 13, 21). Importantly, several prospective studies have demonstrated that abdominal obesity is an independent risk factor for coronary heart disease and total mortality in men and women (14, 23, 26).

Reduced cardiovagal baroreflex gain has been associated with electrical instability of the heart and increased cardiovascular mortality (3, 12, 24, 25). Obesity is associated with reduced cardiovagal baroreflex gain (17, 34). Although previous studies suggest that abdominal obesity is associated with reduced cardiovagal baroreflex gain (37), we are aware of no study that has directly tested this hypothesis.

Recently, Pikkujamsa et al. reported that cardiovagal baroreflex gain was reduced in individuals with components of the metabolic syndrome (30). Insulin resistance and other components of the metabolic syndrome are more closely related to abdominal visceral fat than obesity *per se* (4, 13, 21). Therefore, we hypothesized that cardiovagal baroreflex gain would be reduced in men with elevated abdominal visceral fat compared to their peers with lower levels of abdominal visceral fat but similar levels of total body and abdominal subcutaneous fat. To address this issue, we measured cardiovagal baroreflex gain (modified Oxford technique) in two groups of sedentary, men age 18-40 years of age who differed dramatically in their level of abdominal visceral fat but possessed similar levels of total body and abdominal subcutaneous fat.

METHODS

Subjects. Seventeen non-obese men (body mass index<30 kg/m^2) were included in the present investigation. Obese subjects (body mass index >30 kg/m^2) were excluded in attempt to better isolate the effects of abdominal visceral obesity *per se*. All subjects were sedentary (<20 min ≤2 times/wk), normotensive (<140/90) as determined by casual blood pressure measurements, and free from other overt cardiovascular diseases as determined from health history. Subjects were further evaluated for the presence of overt cardiopulmonary disease by resting and maximal exercise electrocardiograms. Subjects were not taking any medications that could influence autonomic-circulatory function. Subjects were weight stable for at least 6 months prior to any testing. All subjects were non-smokers and non-diabetic (2-hr post glucose load <200 mg/dL). The nature, purpose, risks and benefits were explained to the subject before obtaining informed consent (See Appendix A). The Colorado State University Institutional Review Board approved all experimental protocols.

To address the stated hypothesis, we compared cardiovagal baroreflex gain in subjects with an abdominal visceral-to-subcutaneous fat ratio <0.40 (V/S ratio, LVF, n=8) and a V/S ratio>0.40 (HVF, n=9). A V/S ratio > 0.40 is considered viscerally obese (27). The V/S ratio was determined by dividing abdominal visceral fat area by abdominal subcutaneous fat area (see below). The V/S ratio allowed us to identify two groups of individuals with dramatically (and statistically) different levels of abdominal visceral fat but similar levels of total body and abdominal subcutaneous fat.

Experimental Procedures. Body mass and height were measured using a physicians balance with a stadiometer. Waist (at the level of the level of the umbilicus) and hip (at the widest part of the hips) circumferences were measured to calculate waist-to-hip (WHR) mm. Body composition was measured using dual energy x-ray absorptiometery (DPX-IQ, Lunar Corp.). Computed tomography scans (GE HighSpeed CTi) were performed to quantify abdominal visceral and subcutaneous fat levels as previously described (31, 42). Fasting plasma glucose values were determined using the glucose oxidase method on a glucose analyzer (YSI 2300 STAT Plus, YSI Inc.). Fasting plasma insulin values were determined using an ELISA (DSL Laboratories). Insulin sensitivity was estimated using the Homeostasis Model Assessment (HOMA, 7) and the Quantitative Insulin Sensitivity Check Index (QUICKI, 18) as previously described. Heart rate was determined from Lead II of the electrocardiogram. Respiration was measured by placing a pnuemobelt around the upper abdomen. Finger photoplesthmography (Finapres model 2300, Ohmeda) was used to measure beat-to-beat arterial pressure. Resting Finapres arterial blood pressures were "calibrated" to brachial blood pressures with an automated device (Dinamap, Critikon Co.) prior to the injection of vasoacvtive drugs (see below). Maximal oxygen consumption was measured using open circuit spirometry during graded treadmill exercise to exhaustion (TrueMax 2400, ParvoMedics). Cardiovagal baroreflex gain was quantified using the modified Oxford technique (15).

Experimental Protocol. All experimental procedures were performed in the morning between 7:00 and 11:00 am. Subjects reported to the laboratory following a 12-hour

overnight fast. Subjects were instructed to refrain form caffeine and alcohol consumption for 24 hours prior to all testing sessions. Subjects were also asked to withstand from participating in any vigorous physical activity 24 hours prior to any testing.

For the injection of the vasoactive drugs, an antecubital venous catheter was placed in the subject's left arm. After a 20-minute rest period to ensure that baseline values of arterial blood pressure, heart rate, and respiration were stable, a bolus injection of sodium nitroprusside (75-100 μg) was given intravenously followed 1 minute later by a bolus injection of phenyephrine HCL (100-150 μg). These pharmacological perturbations decreased and increased arterial blood pressure ~15 mmHg from baseline levels during a 3 minute period. The three trials were completed and each separated by a minimum of 15 minutes quiet rest.

Data Analysis. Abdominal visceral and subcutaneous fat regions were determined using commercially available medical imaging software (SliceOmatic, Tomovision) (See Appendix C). Adipose tissue was identified by Hounsfeld units (HU) in the range of –30 to –190 (32, 42). Abdominal visceral fat was calculated as the area of pixels in the appropriate HU range within the abdominal wall. Abdominal subcutaneous fat was calculated as the pixel area in the appropriate HU range outside the abdominal wall. Blinded repeat measurements of abdominal visceral and subcutaneous fat in a random sample (n=10) were highly correlated ($r=0.99$, $P<0.05$).

Arterial blood pressure, electrocardiogram, and respiration were digitized at 500 Hz for subsequent off-line analysis using signal processing software (Windaq, Dataq Instruments). Cardiovagal baroreflex gain and operating range was calculated by fitting a

4-parameter sigmoid to the data (39) (See Appendix B). Briefly, systolic blood pressure and R-R intervals were averaged across 3 mmHg systolic blood pressure bins; this averaging reduced variability in the measurements due to ventilation and measurement error. Cardiovagal baroreflex gain was calculated from the regression line fitted to the linear portion of the sigmoid curve after bin values in the threshold and saturation regions were excluded. R-R intervals were used because they are more closely related to vagal outflow than heart rate (16). Only regressions with correlation coefficients (r) ≥ 0.70 were accepted. The operating range was calculated as the difference in R-R interval (in milliseconds) between the saturation and threshold regions of the sigmoid curve.

Statistical Analysis. Data are expressed as mean$\pm$SE. Differences in subject characteristics and dependent variables between the LVF and HVF were assessed with independent Student's t-tests'. A P level < 0.05 was considered significant. Bivariate correlational analysis was used to assess the relationship between specific subject characteristics and cardiovagal baroreflex gain.

RESULTS

Subject Characteristics. Subject characteristics are shown in Table 1. Age, height, body mass, body mass index, waist and hip circumference, waist-to-hip ratio, body fat percentage, body fat mass, systolic and diastolic blood pressure, heart rate, and maximal oxygen consumption were not significantly different between the two groups. As expected, abdominal visceral fat and the V/S ratio were significantly higher in the HVF compared with LVF. In addition, fasting plasma insulin concentration was higher

(51.40±9.3 vs. 30.46±3.84 pmol/L) and fasting plasma glucose concentration was similar (4.07±0.18 vs. 4.65±0.35 mmol/L) in HVF compared with LVF, respectively. HOMA (1.71±0.4 vs. 1.05±0.2, P<0.05) was higher and QUICKI (0.36±0.01 vs. 0.39±0.01, P<0.05) was lower in HVF compared with LVH, respectively. Both are consistent with a greater degree of insulin resistance in HVF.

Cardiovagal Baroreflex Gain and Operating Range. Cardiovagal baroreflex gain was ~35% lower in HVF compared with LVF (Figure 1, upper panel). However, the operating range for the cardiovagal baroreflex was not different in the two groups (Figure 1, lower panel).

Correlational Analysis. Cardiovagal baroreflex gain was correlated with the following variables: height (r=0.54, P<0.05), body mass (r=-0.41, P<0.05), body fat percentage (r=-0.30, P<0.05), body fat mass (r=-0.36, P<0.05), body mass index (r=-0.68, P<0.05), waist circumference (r=-0.42, P<0.05), WHR (r=-0.41, P<0.05), abdominal visceral fat (r=-0.45, P<0.05), abdominal subcutaneous fat (r=-0.34, P<0.05), systolic blood pressure (r=-0.55, P<0.05). Hip circumference (r=-0.32, P>0.05), maximal oxygen consumption (r=0.16, P>0.05) and diastolic blood pressure (r=-0.31, P>0.05) were not correlated with cardiovagal baroreflex gain.

DISCUSSION

The main new finding of the present study is that men with elevated abdominal visceral fat demonstrate reduced cardiovagal baroreflex gain compared with their total

body- and abdominal subcutaneous fat-matched peers with lower levels. Importantly, these observations were made in men who were non-obese according to the clinical guidelines.

Abdominal obesity has been hypothesized to be a neuroendocrine disorder characterized by dysregulation of the hypothalamic-pituitary-adrenal axis and parallel sympathetic nervous system activation (5, 6, 29, 35). The results of our investigation indicate that abdominal obesity is also associated with reduced cardiovagal baroreflex gain. This finding is consistent with previous reports suggesting that cardiac vagal tone is reduced in obesity (1, 2, 37). Therefore, altered regulation of both the parasympathetic and sympathetic nervous systems may be a common feature of the neuroendocrine disorder, which is characteristic of abdominal obesity. Future studies will be necessary to confirm this theory.

The mechanisms responsible for the lower cardiovagal baroreflex gain in the men with elevated abdominal visceral fat are unclear. However, several possibilities exist. First, elevated abdominal fat is associated with increased arterial stiffness (33, 40). In addition, arterial stiffening is associated with reduced cardiovagal baroreflex gain (8, 10). Therefore, arterial stiffening may impair the ability of arterial blood pressure to deform the barorosensory regions and, in turn, result in reduced cardiovagal baroreflex response as previously reported in older adults (19, 38).

Second, it is possible that central integration of afferent vagal nerve traffic is altered in men with elevated abdominal visceral fat and this, in turn, would result in a proportionally smaller increase in efferent vagal nerve traffic compared with men with

lower levels of abdominal visceral fat. We have no information to support this possibility.

Finally, obese dogs demonstrate a reduction in the number and sensitivity of cardiac muscarinic receptors (11, 28). If this is also observed in obese humans, it is possible that the reduction in cardiovagal baroreflex gain observed in the men with elevated abdominal visceral fat in the present study was due, in part, to reduced muscarinic receptor number and/or sensitivity. Future studies will be necessary to gain insight into mechanisms responsible for the reduced cardiovagal baroreflex gain in men with elevated abdominal visceral fat.

There are important physiological and clinical implications of the present study. First, cardiovagal baroreflex gain is an important physiological determinant of cardiac vagal tone in humans. Therefore, the lower cardiovagal baroreflex gain observed in men with abdominal obesity in the present study may contribute to a reduction in cardiac vagal tone (22) as has been previously reported in obese humans (1, 2, 37).

Second, reduced cardiovagal baroreflex gain is associated with an increased risk of cardiovascular disease-related mortality (3, 12, 24, 25). Thus, the lower cardiovagal baroreflex gain observed in the men with abdominal obesity in the present study may contribute to their higher risk of CVD-related mortality risk. Importantly, the elevated CVD-related risk associated with reduced cardiovagal baroreflex gain is observed in men who are clinically non-obese (i.e., body mass index $< 30\ kg/m^2$).

Last, reductions in total body and abdominal obesity result in improvements in cardiovascular and metabolic risk factors associated with the metabolic syndrome (9, 18, 36, 41). Accordingly, it is possible that weight loss may improve cardiovagal baroreflex

gain in individuals with abdominal visceral obesity. Future intervention studies are needed to address this possibility.

In summary, the results of the present investigation indicate that men with elevated abdominal visceral fat demonstrate reduced cardiovagal baroreflex gain compared to their total body- and abdominal subcutaneous fat-matched peers with lower levels of abdominal visceral fat. Therefore, reduced cardiovagal baroreflex gain observed in men with abdominal obesity may contribute to the elevated cardiovascular disease-related mortality associated with the metabolic syndrome. Whether reductions in abdominal visceral fat will result in improvements in cardiovagal baroreflex gain remains unclear.

Table 1. Subject Characteristics for LVF and VHF groups.

Variable	LVF (n=10)	HVF (n=8)
Age (yr)	24.4±1.6	29.0±2.9
Height (cm)	183.5±3.4	181.5±1.4
Body Mass (kg)	80.8±5.7	89.2±5.2
Body Mass Index (kg/m^2)	24.0±1.5	27.0±1.3
Waist Circumference (mm)	87.9±4.1	93.7±4.2
Hip Circumference (mm)	102.9±3.4	106.3±2.5
Waist-to-Hip Ratio (units)	0.85±0.02	0.88±0.02
Body Fat (%)	20.4±2.4	23.6±2.4
Body Fat Mass	17.5±3.1	21.7±3.5
Visceral Abdominal Fat (cm^2)	66.2±9.8	121.7±19.6*
Subcutaneous Abdominal Fat (cm^2)	215.1±39.1	219.9±34.8
V/S Ratio (units)	0.33±0.02	0.57±0.06*
Systolic Blood Pressure (mmHg)	117.5±2.7	121.5±3.4
Diastolic Blood Pressure (mmHg)	63.2±1.3	68.1±4.3
Heart Rate (bpm)	59±3	58±4
VO_2max (ml/kg/min)	44.9±2.3	44.3±4.2
VO_2max (ml/lbm/min)	58.8±1.8	61.5±4.3

All values are represented as mean±SE. *P<0.05 vs. LVF.

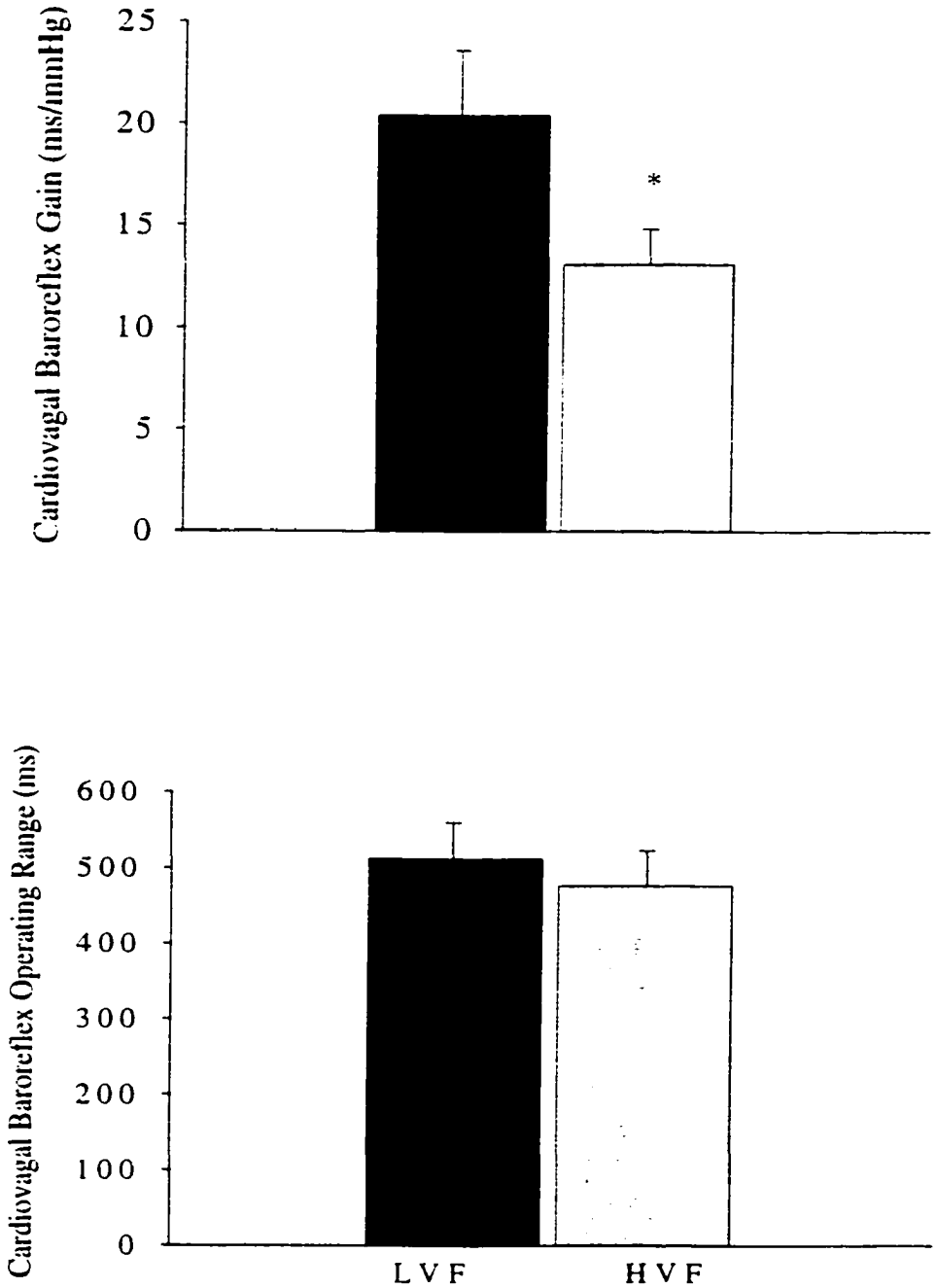

Figure 1. Cardiovagal baroreflex gain (upper panel) and operating range (lower panel) in men with elevated abdominal visceral fat (HFV) compared to men with lower levels of abdominal visceral fat (LVF), but similar levels of total body and abdominal subcutaneous fat. *P<0.05.

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CHAPTER V

OVERALL CONCLUSIONS

Cardiovagal baroreflex gain is reduced in young, non-obese women compared to men. This reduction in cardiovagal baroreflex gain observed in women is independent of aerobic fitness levels and only marginally associated with total body fat. The cardiovagal operating range was similar in both non-obese women and men. The mechanisms responsible for reduced cardiovagal baroreflex gain in women remain unclear. The findings of the present study may have important implications for understanding the reduced orthostatic tolerance observed in women.

A reduction in cardiovagal baroreflex gain is also observed in overweight and obese men compared to non-obese men. Overweight and obese men typically have lower levels of aerobic fitness, which has been shown to influence cardiovagal baroreflex gain. However, cardiovagal baroreflex gain was lower in overweight/obese men compared with aerobic fitness-matched non-obese men. The findings from this study suggest that neither total body fat nor abdominal visceral or subcutaneous fat independently contribute to the inter-individual variation in cardiovagal baroreflex gain. However, this study was not designed to specifically address the contribution of total or regional body fat to the reduced cardiovagal baroreflex gain observed in overweight and obese men.

To address this issue, at least in part, we compared cardiovagal baroreflex gain in men with elevated levels of abdominal visceral fat with total body- and abdominal

subcutaneous fat-matched men with lower levels of abdominal visceral fat. These are the first experimental data to demonstrate reduced cardiovagal baroreflex gain in men with elevated levels of abdominal visceral fat, independent of total body and abdominal subcutaneous fat. The reduced cardiovagal baroreflex gain observed in men with abdominal obesity may contribute to the elevated cardiovascular disease-related mortality associated with the metabolic syndrome.

Follow up studies are needed to determine the mechanisms responsible for reduced cardiovagal baroreflex gain observed in men with mild to moderate forms of obesity and abdominal visceral obesity. In addition, intervention studies are needed to determine if reductions in total obesity and abdominal visceral fat will result in improvements in cardiovagal baroreflex gain.

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APPENDIX A:
INFORMED CONSENT

COLORADO STATE UNIVERSITY
INFORMED CONSENT TO PARTICIPATE IN A RESEARCH PROJECT

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

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

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

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

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

You will be divided into one of two groups according to your body mass index (calculated from your height and weight): a group whose body mass index is less than 25 or a group whose body mass index is greater than 30 but less than 35. If your body mass index is greater than 30, you may be asked to be randomized to an underfeeding (weight loss) group or a control group who is asked not to change any of their dietary or physical

activity habits. If you are assigned to one the weight loss groups. you will be instructed on how to modify your diet to reduce the amount of fat and calories so that you lose 10% of your initial body weight over a 12-week period. You will be asked to come to the CSU campus (Moby Complex or Gifford Building) weekly to have your body weight measured and to discuss with a dietitian any problems you may be experiencing with your weight loss program.
For a one month period (regardless of which group you are in) before and after the weight loss or control group, you will be asked to eat food provided to you and/or your own food so that your body weight remains stable.

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

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

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

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

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

(4) Oral Glucose Tolerance Test. For this procedure you will be asked to drink a very sweet sugar solution (75 grams glucose). Additional blood samples (2 blood samples) to those described above will be taken both before and after you drink this solution. The purpose of this procedure is to determine whether you have a normal glucose response to drinking this sweet tasting drink (your oral glucose tolerance). If your glucose response is abnormal you may not be able to continue participation and you will be referred to your personal physician. Approximately 3-4 teaspoons of blood will be required for this procedure. This procedure will take approximately an additional 2 hours of your time.

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

Approximate time required: 2.5-3.0 hours

Session 2 – Poudre Valley Hospital, Sleep Laboratory

(1) Sleep Study. You may be asked to have a sleep study to determine whether you have abnormal breathing activity (sleep apnea) while you sleep. If you have this study done, you will be asked to go either to Poudre Valley Hospital (directions will be provided) in the evening close to the time you usually go to sleep and/or to take a portable monitoring system home with you. If the study is performed at the hospital, you will be required to sleep there over night. In either case, you will have the electrical activity in your brain, electrical activity of the muscles in your face, and eye movement, monitored with electrodes attached to your scalp/skin. A sensor attached to your ear or finger will monitor the oxygen level in your blood. A sensor placed just under your nose

and mouth will monitor the airflow. Your breathing will be monitored with a plastic belt placed around your chest and abdomen. Your heart rate will be measured from electrodes places on your chest. A physician will determine if the results of your sleep study indicate whether you have sleep apnea. If so, you will be referred to your personal physician or one will be recommended to you. He/she will want to determine whether further tests are needed and if you need medical treatment. If your sleep study is abnormal, you may still be able to participate in this study but we will need written approval from your doctor.

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

Session 3 - Moby Complex, Colorado State University.

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

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

procedure is used to determine your aerobic fitness level and as a screening test for coronary artery disease.

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

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

Session 4 - Moby Complex, Colorado State University

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

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

is passed through the electrode. This search procedure will continue until the electrode being moved causes your foot or hand to twitch. This procedure will take between 5-60 minutes. When a foot or hand twitch is observed, measurement of the activity of the sympathetic nervous system will begin and continue during the procedure described below.

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

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

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

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

Approximate time required: 3.0 hours

Session 5-Poudre Valley Hospital, Department of Radiology, Fort Collins, CO
(1) Computed Tomography Scan. The amount of total fat, fat around your internal organs, and fat under the skin in the abdominal area will be measured by computed tomography (CT scan). The CT imaging will be performed at Poudre Valley Hospital in Fort Collins. For this procedure, you will be asked to lie very still on a table. An x-ray machine (the CT scanner) will rotate around you, and the table will moved back and forth slightly making it possible to take x-rays from several angles. The actual x-ray time is approximately less than 2 min. You will be lying on the table for approximately 15 to 30 min.

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

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

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

Approximate time required: 4-5 hours.

Session 7 - Moby Complex, Colorado State University

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

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

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

to measure your forearm blood flow. The two techniques will be used together to ensure the most accurate measurement.

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

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

Approximate time required: 2.5 hours

Yes_____

No_____

RISKS INHERENT IN THE PROCEDURES:

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

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

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

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

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

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

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

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

Drug Infusions: Because this procedure requires the insertion of a catheter, the risks here are identical to those described above under catheters. In addition, the infusion of nitroprusside could cause low blood pressure and nausea, sweating, or a sudden elevation in heart rate. These feelings should pass within 1-2 minutes. The infusion of phenylephrine may result in a headache, restlessness, a sudden decrease in heart rate, and/or rarely an irregular heart beat. These feelings or symptoms, if they occur, usually pass within a few minutes. There is also a small risk that some phenylephrine will leak out from the catheter site causing severe constriction of the surrounding small blood vessels. This may result in an inadequate blood supply to the surrounding tissues and eventual death of that tissue if untreated. This risk is prevented by using a large vein in your arm. However, if this problem occurs you will be referred to a physician for immediate treatment. These risks are slightly increased because we will repeat both drug infusions two times. It should be emphasized that the amount of these drugs you will be receiving is very small and they are rapidly metabolized by your body. This lowers the

risk of any adverse reactions to the drugs. In addition, a registered nurse will supervise this aspect of the study.

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

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

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

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

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

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

LIABILITY: Because Colorado State University is a publicly-funded, state institution, it may have only limited legal responsibility for injuries incurred as a result of participation in this study under a Colorado law known as the Colorado Governmental Immunity Act (Colorado Revised Statutes, Section 24-10-101, et seq.). In addition, under Colorado law, you must file any claims against the University within 180 days after the date of injury.

We will attempt to secure emergency medical treatment for you if you are injured or have an adverse reaction or illness as a direct result of this research, but such treatment cannot be guaranteed. Either you or your insurance company will have to pay the costs of the medical treatment. No funds are available from the University. You will need to check

with your insurance company to see if they will cover medical expenses arising from participation in a research project, because sometimes they will not cover them. The University cannot pay you
for lost wages, pain, or suffering. Because the University is a state entity, it may have governmental immunity, which may prevent you from recovering damages in a lawsuit. If you think you have been injured because of this research, please contact Celia Walker, CSU Regulatory Compliance Office at (970) 491-1563.

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

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

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

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

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

Participant Signature _____ Date _____

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

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

Subjects Signature _____ Date _____

Page 9 of 9 Subject Initials _____ Date _____

APPENDIX B:
CARDIOVAGAL BAROREFLEX GAIN ANALYSIS

Cardiovagal Barorelfex Gain Analysis

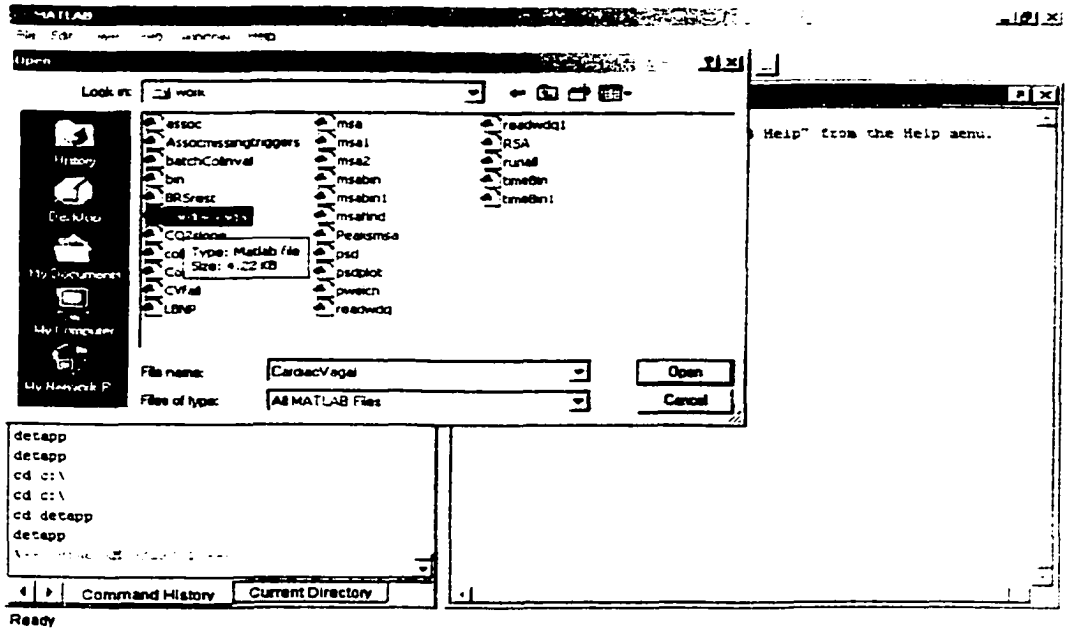
This protocol will walk you through the steps to obtain the linear and sigmoid estimates of cardiovagal baroreflex gain during infusion of phenylephrine and sodium nitroprusside. You will have the ability to calculate the rising and falling gains separately.

Items you will need for this analysis: L2 file (ECG)
L3 file (Blood Pressure)
-These files should be cut from the BRS trials
(See BRS Windaq Analysis instructions)

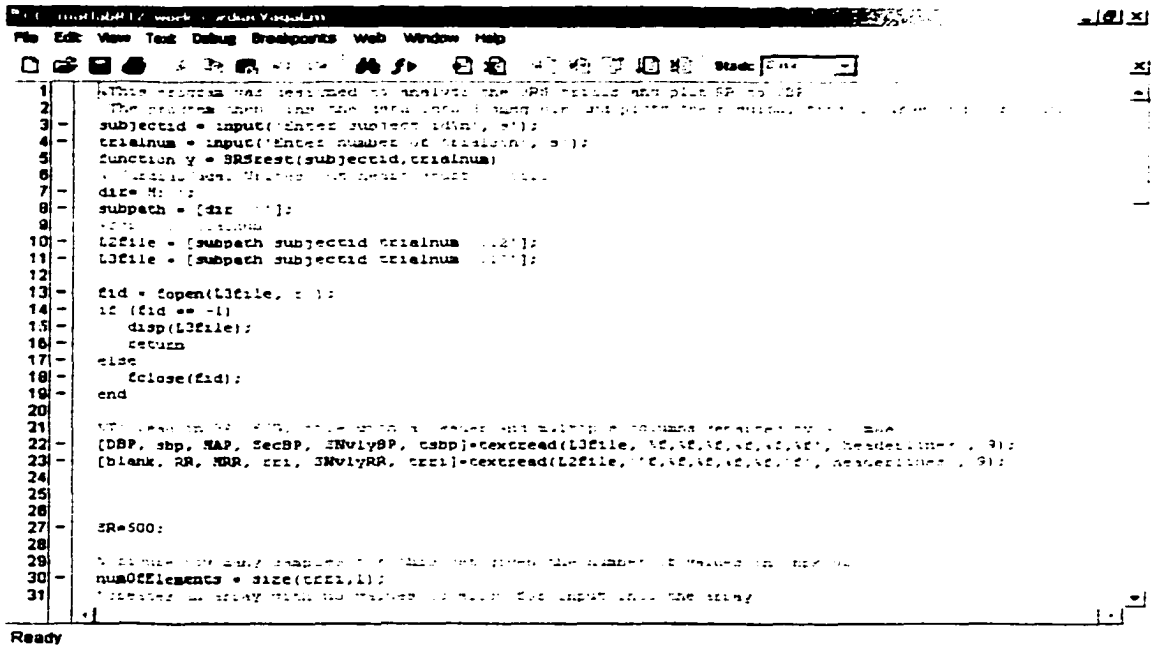
Programs you will need for this analysis: Microsoft Excel
Matlab 12 with Cardiovagal M-file
TableCurve
SigmaPlot

Prior to beginning the analysis, the L-files should be placed in the M:\ drive. Do not place them in a folder.

1. Open Matlab 12
2. Open the Cardiovagal M file
   Go to **File**
   -**Open**, the following screen will appear:

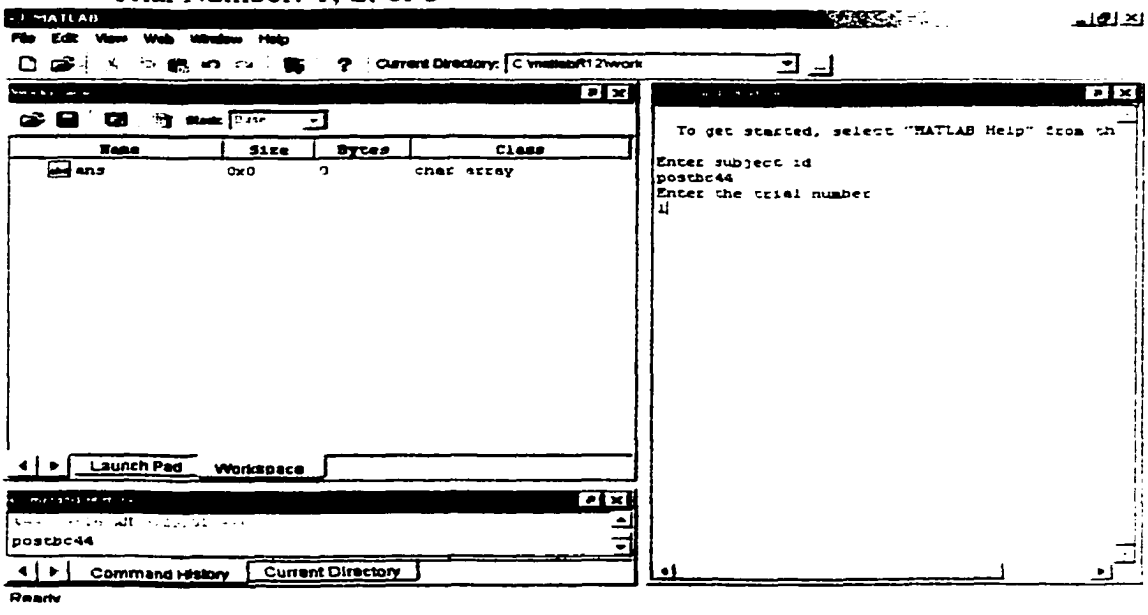


After opening the Cardiovagal M-file, the M-file will appear on your screen. Do not make any changes to this file or your program will not work. This is the working code that commands the processor to run the analysis.
To run the analysis for falling pressures open the Cvfall M-file instead of Cardiovagal.



To initiate the program, press the run key or in the command window type run cardiacvagal after the >> prompt.
You will need to work in the command window from here on out.
You will be prompted for user inputs:

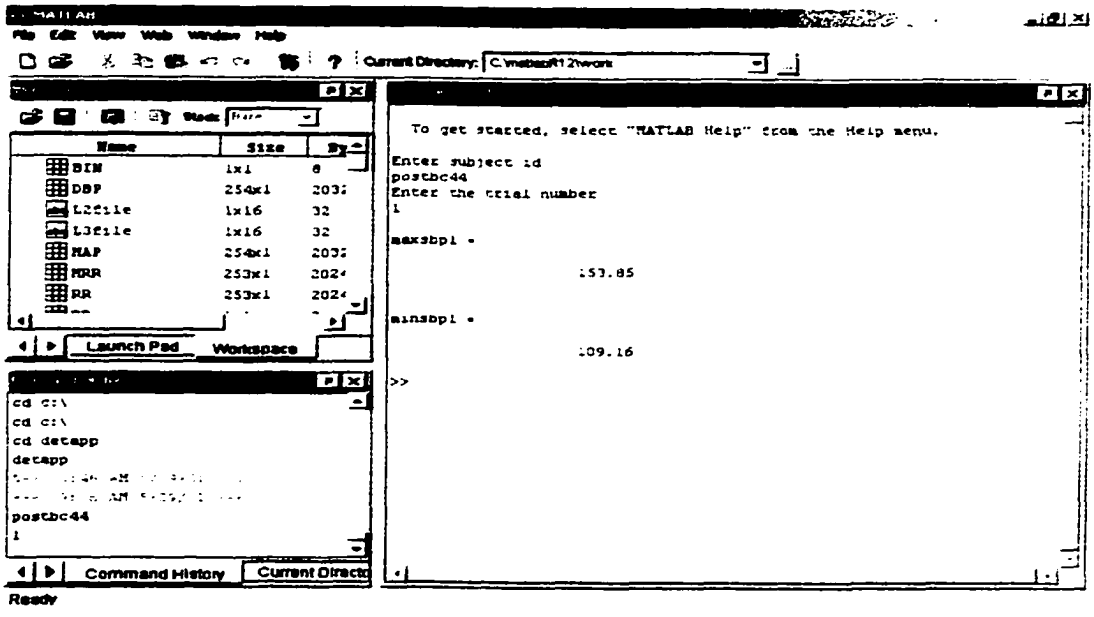
Subject ID: subjectIDbrs (Ex. AA01brs)
Trial Number: 1, 2, or 3



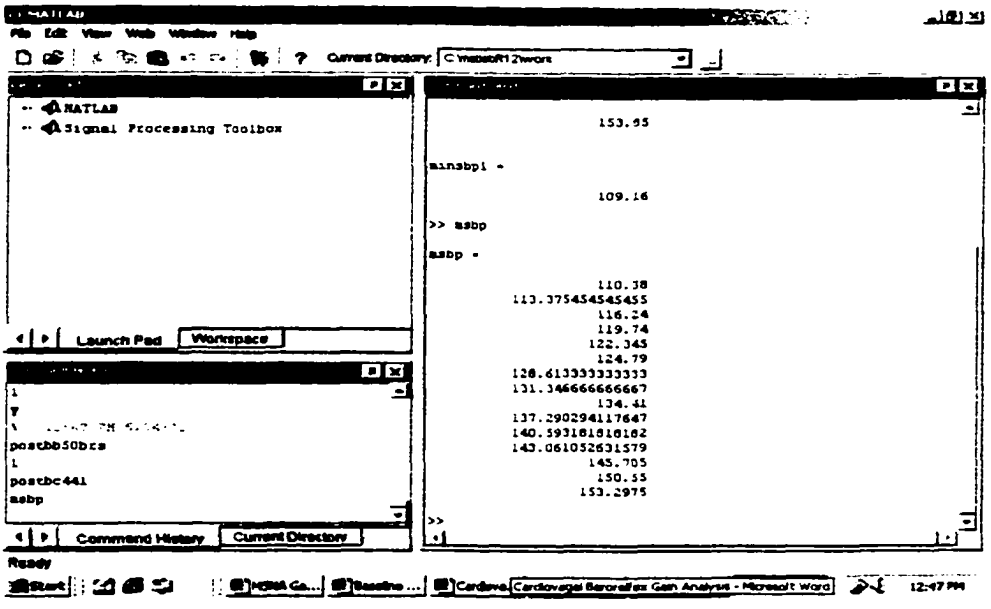
After entering user inputs, hit enter.

First, the minimum systolic blood pressure (minsbp1) and maximum systolic blood (maxsbp1) pressure will appear. You will need to record these in the excel

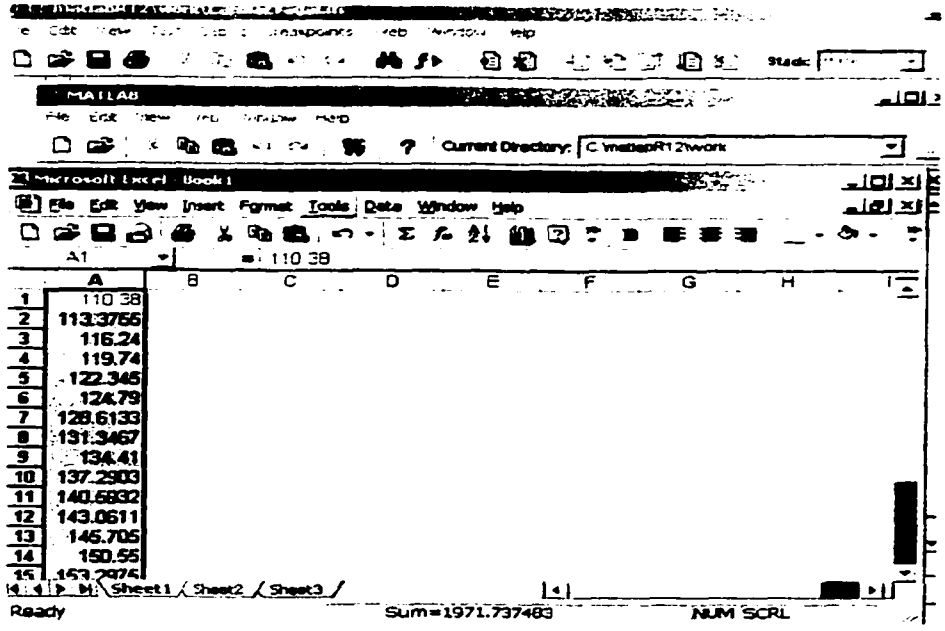
sheet titled CVBRS Trials (M:drive, Datafiles). You can directly enter them into the spreadsheet or you can record them on paper and enter them after you are finished.



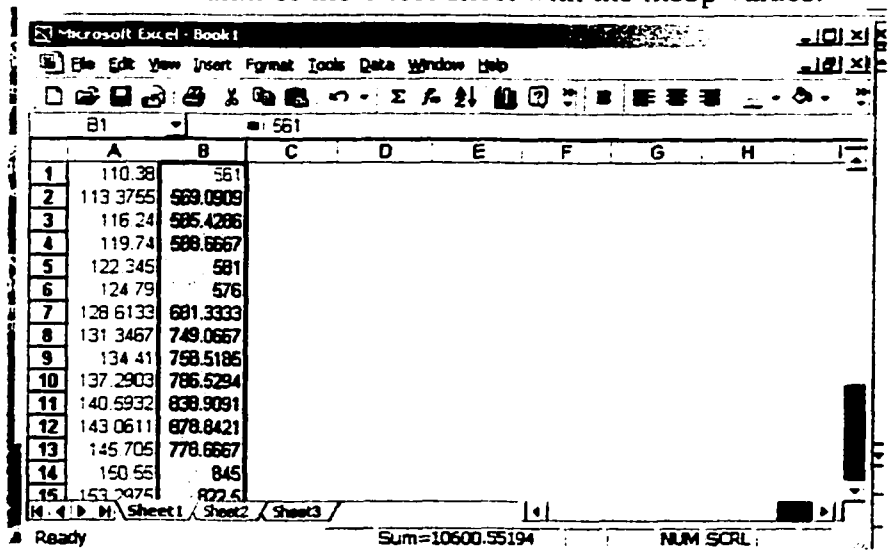
From this point you will need to switch back and forth between excel and Matlab.
In Excel: Open a new excel sheet.
In Matlab: Type msbp after the promt (>>) . The mean sbp for each bin will appear on screen.



Highlight all the msbp values and copy them (control c or right mouse click copy) Paste them in column 1 of the new excel sheet you previously created.



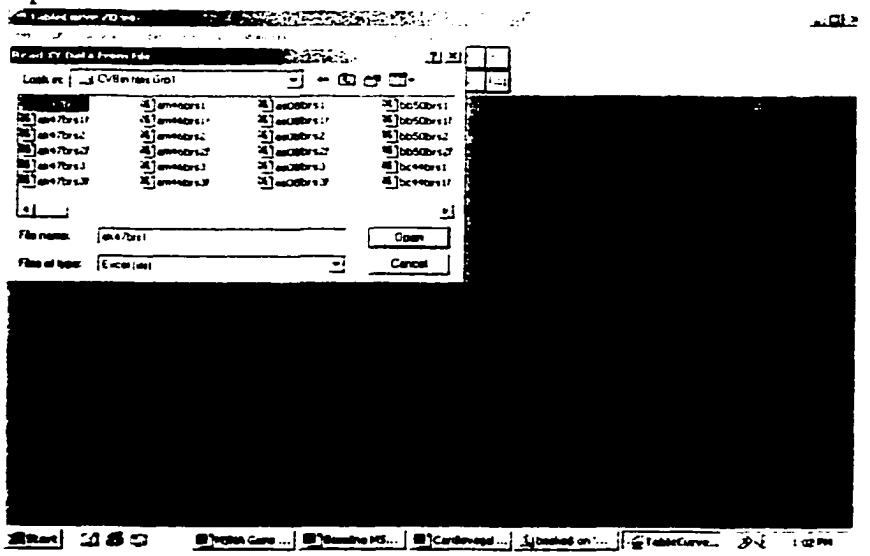
Return to Matlab and type mrr following the prompt (>>). The mean RR interval values will appear on screen. Highlight and copy these values and paste them into the second column of the excel sheet with the msbp values.



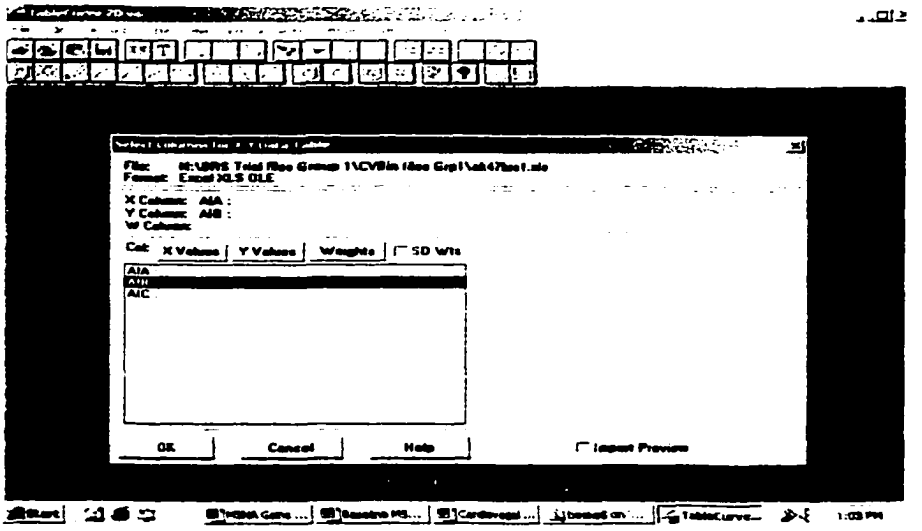
In Excel: You need to alter the excel sheet you just created:

1. Make sure that the blood pressure values are increasing with each advancing row. If this is not the case, then your current file has overlapped your previous file in Matlab. You will need to erase the SBP and RR values from the point that SBP stops increasing to the end of the columns.
2. If any rows have the value –1 in them. Clear these values and leave them blank.
3. Make sure that for every SBP you have an RR interval.
4. In column 3, type in the following formula in row 1:
   =60/A1*1000

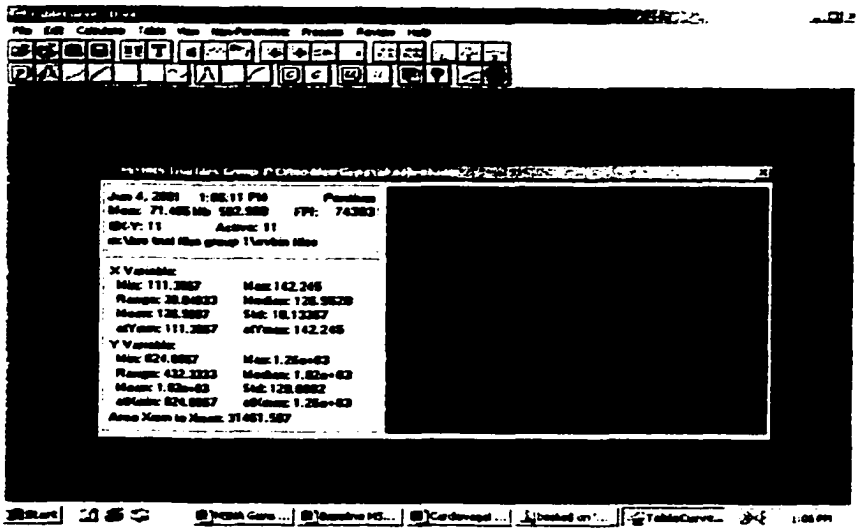
Then highlight the remaining rows and fill the column (ctrl-D) with the formula you just entered. You should now have three columns: SBP. HR. RR)
Once you have checked the file, you can save it as subjectIDbrs# (ex. AA01brs1)
For falling pressures, save the file as subjectIDbrs3f (ex. AA01brs1f)
You can save these to the Documents folder in the C:/drive temporarily.
Close the excel sheet.
Open Table curve.



File-Open the excel sheet you just created.
The Select Columns for X-Y Data Table will appear. You will need to click on AIA (SBP) for the X column and AIB (RR interval) for the Y column.
*AIC is HR is you need to calculate the BRS gains using heart rate. Select AIC in place of AIA for the X-column and proceed as normal.
Click on OK.

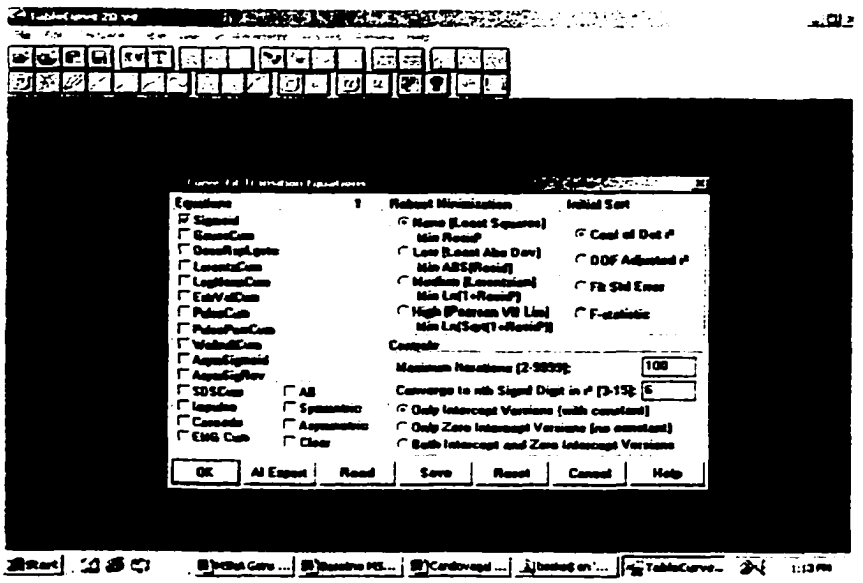


The data description and variable names table will appear. You do not need to make any adjustments here. Click OK.
The following screen will appear:

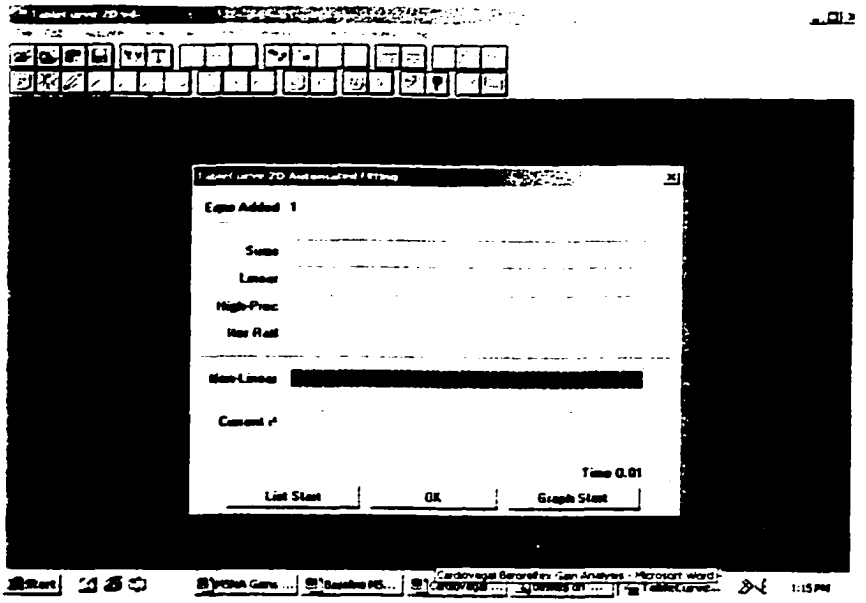


From here you will fit two curve to the data. A linear curve and a sigmoid curve. Lets begin with the sigmoid curve analysis:

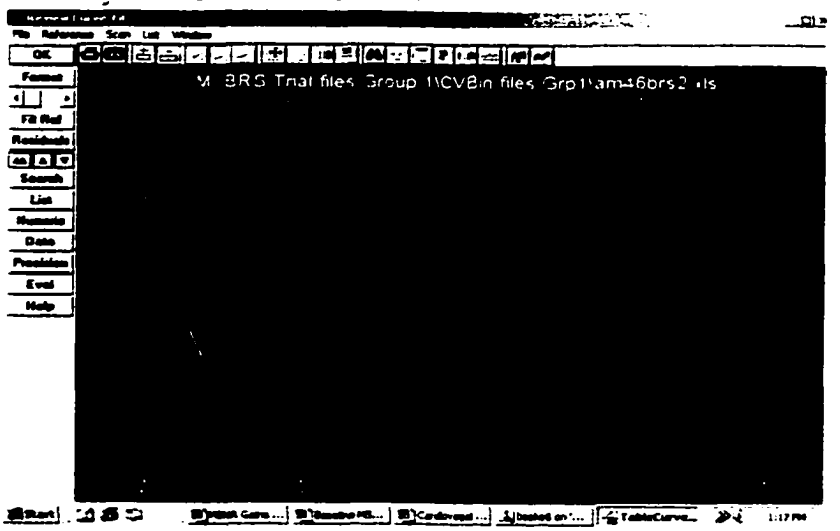
Select the sigmoid curve fit button  . The following screen will appear. Select only the sigmoid equation. None for Robust minimization, and Coef of Det r^2. Select OK.



The following screen will appear as it is processing. When complete select, Graph Start.

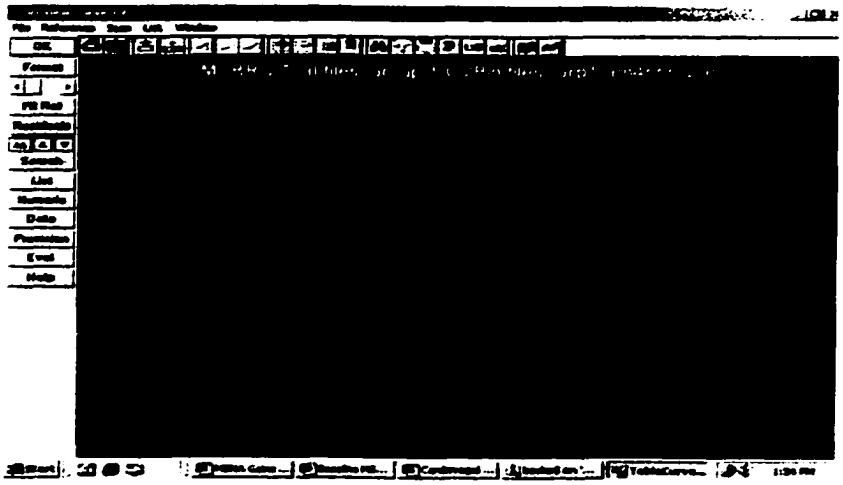


The fitted curve will appear. Now you need to inspect the curve for fit. For the sigmoid analysis, it should be fairly straight forward. Only outliers (red in color) may be eliminated. Otherwise, all values should be included in the analysis.
-To delete a value, use the cursor to click on value. A circle will appear around the data point.
-Then press OK in the top left corner of the screen and rerun the Sigmoid Curve fit analysis. See notes below.

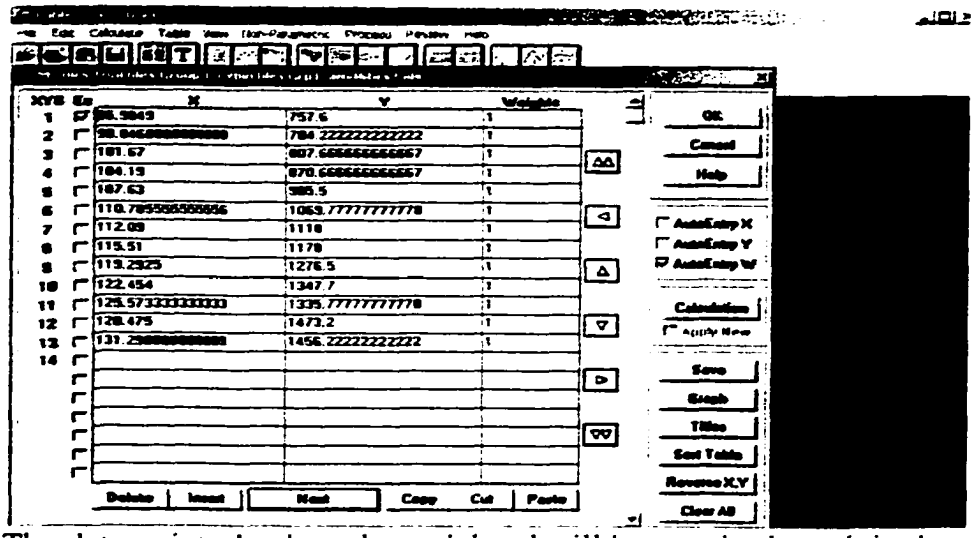


-Once you are satisfied with your curve fit, you need to record the r^2, a, b, c, and d values in the Cvoperatingrange spreadsheet located in the M:\drive, Data Files Folder.
This step is not done for falling pressures.
-Press OK and return to the main TableCurve screen. Now you are ready to do the linear analysis.

-Select the linear analysis key  . The 2D Automated Fitting screen will appear.
-Once analysis is complete, select Graph Start. The linear fitted curve will appear as below:



-For the linear analysis you will need to check the saturation and threshold regions for a clear plateau.
-For linear fits with $r^2 > 0.9$, no adjustments should be made to the linear fit. This should be done with caution as you could easily bias the data by trying to get a good fit. It is always best to delete fewer or no points that to begin to eliminate data points. Data points that are clear outliers (will appear red) may be eliminated from the analysis.
-A plateau is defined as 3 or more data points in a horizontal row. If you observe three or more data points in a horizontal row, you can delete all data points in that row with the exception of the initial data point of the plateau. This should be the point closest or on the linear portion of the curve.
-Select OK and re-run the linear curve fit. Check the r^2 before and after. you eliminate the points.
-If the r^2 improves with the deletion of the data points then you are justified in deleting these points.
-If the r^2 worsens. then you need to undelete the points and include them in the analysis.
-To undelete the data points, select OK to return to the main TableCurve screen. Select View. View Data (or Ctrl-V). The following screen will appear.



The data points that have been deleted will have a check mark in the column labeled Ex. In the example below the first data point has been deleted. To undelete the data point, click in the check mark to remove it.

-Then select OK and re-run the linear analysis. See Notes Below.

-Once you are satisfied with your linear fit, recorded the r^2 and b value (slope) in the excel sheet labeled: CVBBRSTrials located in the M:\drive, Data Files Folder. The falling CV Gain data is entered in the CVBRStrialsfalling excel sheet.

-Once you have completed both the sigmoid and linear curve fits, close TableCurve. You do not need to save any data.

-Use the Explorer to move the excel sheet that you created into the appropriate Cvbindata folder located in the BRS Trials Group# folder. This is a good check to make sure you have completed the analysis.

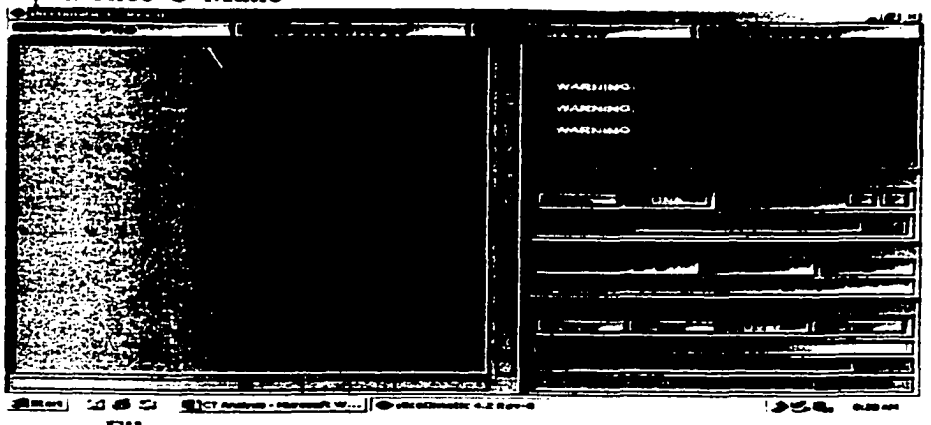
Notes:

-If there are several points that fall on a separate curve, go to the excel sheet and make sure that your blood pressures are increasing with each advancing row.

-If the data points fall on a horizontal line that is not consistent with the saturation or threshold regions, go the windaq file and check to see if the Servo Adjust (Finameter) was on. If so, then you are justified in deleting these points.

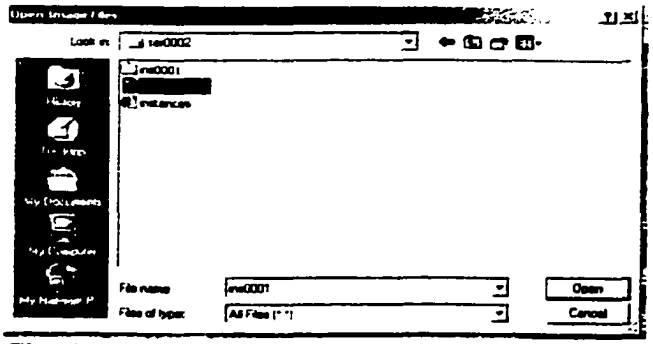
APPENDIX C:
COMPUTED TOMOGRAPHY SCAN ANALYSIS OF ABDOMINAL FAT REGIONS

Open Slice-O-Matic



File

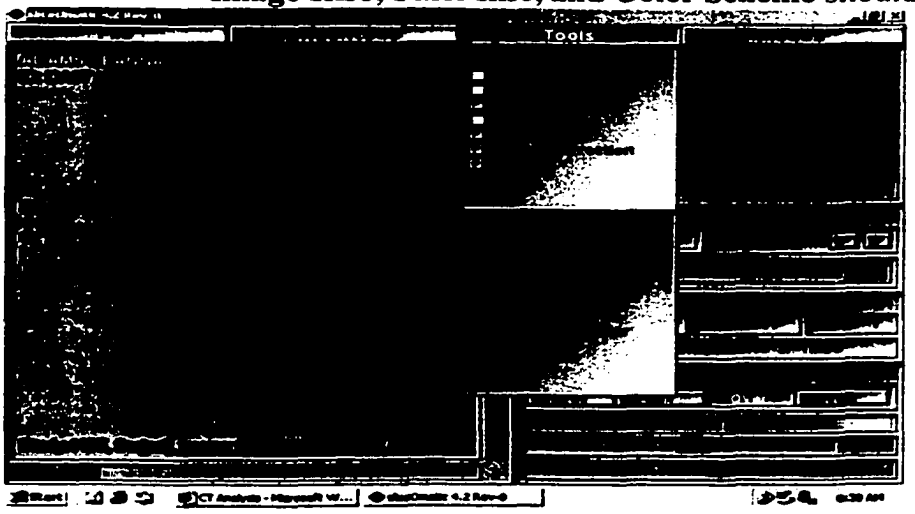
File Open: Select Appropriate File (The files are currently on CD) When opening the file, double click on patient #, then on study 1, then on the last series (either 2 or 3), then select the .dcm file



Check to ensure that the proper tools are activated

Tools

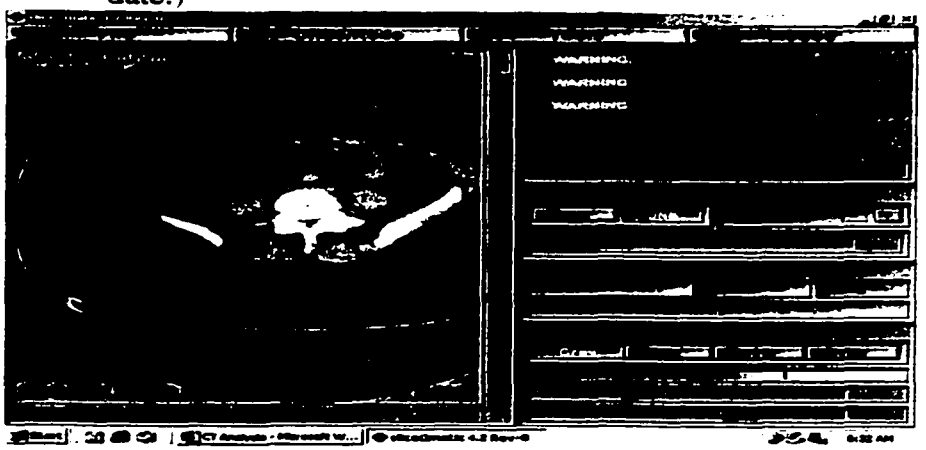
Image Info, Pixel Info, and Color Scheme should all be highlighted



Before entering any of the Modes you must alter the image so that you can view it on the screen and contract the different regions:

-Change Scale from 3 to 1 by clicking on the "-"

-Change the color scheme: Click on **Mix**, slide the black cursor to ~-429 and the white cursor to ~261 (This should allow you to see all of the regions in scan. You may need to adjust these values slightly, but they work for most of the scans to date.)

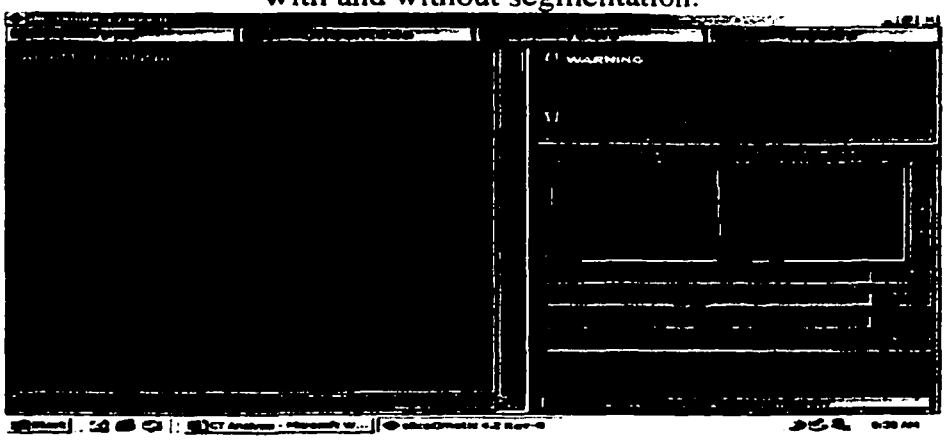


Now you are ready to begin the analysis procedure!

Modes

Segmentation

1. **Threshold 1**: -1024 this should turn the entire scan red, this will allow you to remove the background and the bowel regions from the analysis
2. **Threshold 2**: ~-194, this value will vary slightly (-184 to -197) choose the value closest to -190, if you have to make a decision between a hi an lo value, choose the value above -190, this will be the upper threshold for adipose tissue and will appear green (visceral fat)
3. **Threshold 3**: Between -52 to -27, this value will vary depending on the scan, you will need to select the value that includes all of the adipose tissue, as it is the lower threshold for adipose tissue, this will appear blue (bone, muscle). To check the tissue regions for inclusion of adipose tissue you can toggle between F1 and F2 to view the scan with and without segmentation.



Once you have applied the segmentation regions you must save or "load" the values by clicking on **Compute Segmentation**

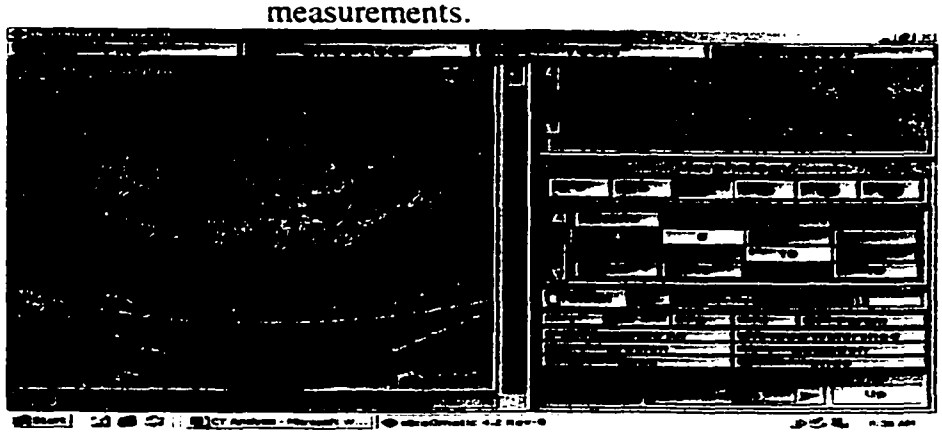
Next you are ready to make the distinction between subQ and visceral fat regions.

Modes

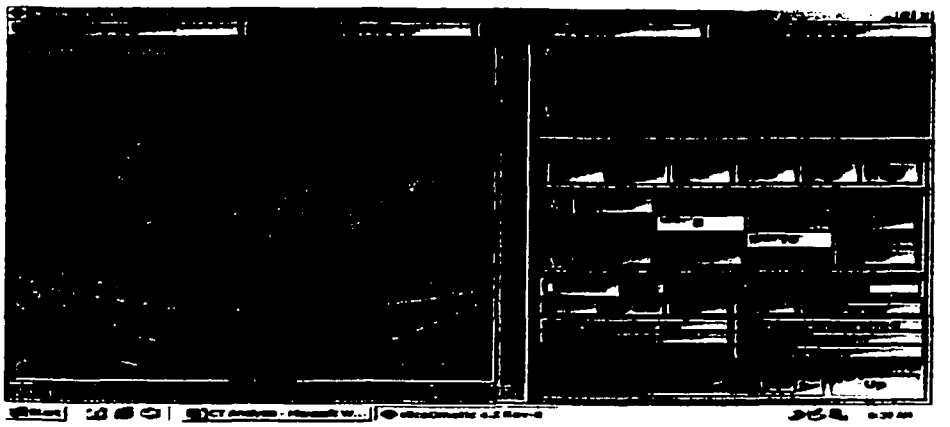
Morpho

1. Press **Compute All** –this will compute your watershed levels 1-4 (1 being the most sensitive, a watershed is a line that is created by discriminating between pixel densities)

2. Select Color #4 (purple) this will be the color assigned to subQ fat and Select a smaller paintbrush (you can choose any size that will allow you to select the appropriate subQ regions)
3. Select the watershed level that delineates between subQ and visceral fat—usually 2 or 3 and then using your purple paintbrush click on the regions that are to be assigned to subQ fat. You can increase your watershed sensitivity to help with precision of the pixel measurements. Include everything outside of the intra-abdominal muscle wall. Do not click on any regions that contain blue and green pixels. You will be able to include these green pixels in the next process. **Load** these measurements.



4. Select the color blue (#3) and a medium size paintbrush. Remove any green pixels that are located within the muscle or bone. You do not want to include these pixels as visceral fat. Only green pixels inside the abdominal cavity should be included as visceral fat.



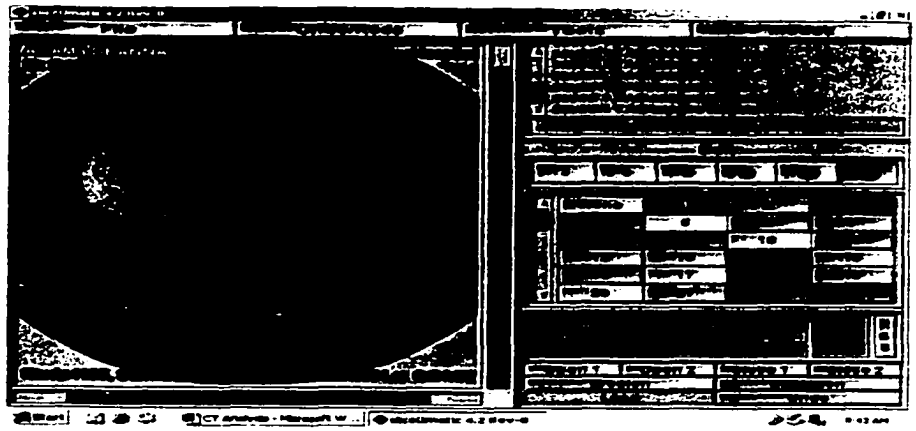
5. Once you are selected all the pixels you can with the different watershed levels, **load** the measurements.

Next you will enter the Edit mode to clean up the measurements and include the pixels that could not be selected from the watershed delineations

Modes

Edit

1. First, select the color red and erase all of the blue and green pixels that fall outside the CT slice, ie. the scan table. **Load** measurements
2. Select Color #4 (purple) and the appropriate size paintbrush that will allow you to select pixels with a high degree of precision. You will usually use the smallest two paintbrushes for this. Now manually highlight (purple) any green pixels that are outside the abdominal muscle wall. If you click on a pixel that you do not want to highlight, use the right mouse click to unhighlight the pixel(s). This is the most tedious of the steps, but it is very important for accurate measurement of both visceral and subQ fat regions. Once you have selected all of the green pixels outside of the abdominal wall, load the measurements.
3. Select the blue paintbrush and do the same for the intramuscular and bone regions of the CT scan. Remember you do not want to include any green pixels within the muscle or bone. **Load** the measurements.
4. Select the red paintbrush. You may have to clean up the edges of the CT scan where the purple extends beyond the CT slice itself. Or you can select color #4 (purple) and press the erosion button. This will retract the purple pixels by one pixel and usually corrects most of the overlap. **Load** the measurements.

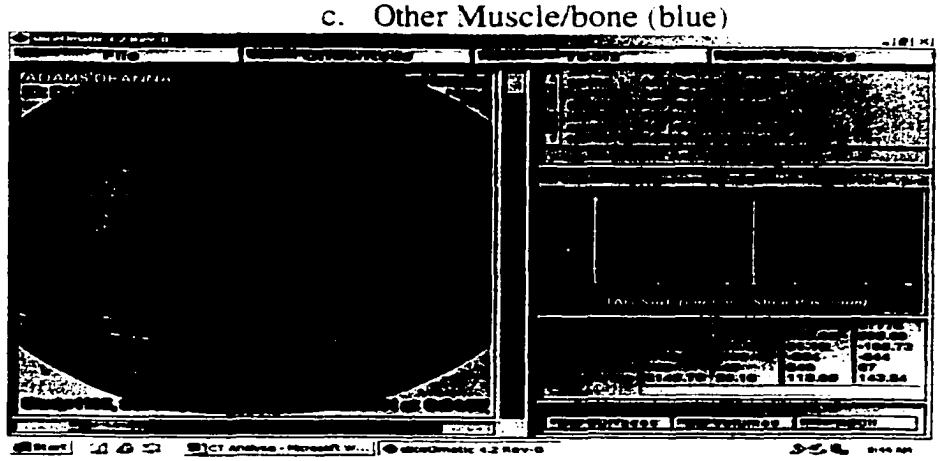


Once you are satisfied with the selected regions. all you have to do is record the values and save your work.

Modes

Surfaces/Volumes

1. Record the area (cm^2) for the following regions:
   a. Visceral Fat (green)
   b. SubQ Fat (purple)
   c. Other Muscle/bone (blue)



2. Save these values to an ASCII file by press the **ASCII** key at the bottom of the page. You cannot save these values to the CD so you will have to select the appropriate destination for the file to be saved. There is a folder in the C: drive labeled CT scans where these files may be saved. The files should be named with the subject ID.

Lastly. you need to save the TAG files so that you can return to the CT scan to make further measurements or to recheck your work without starting from the beginning of the analysis procedure.

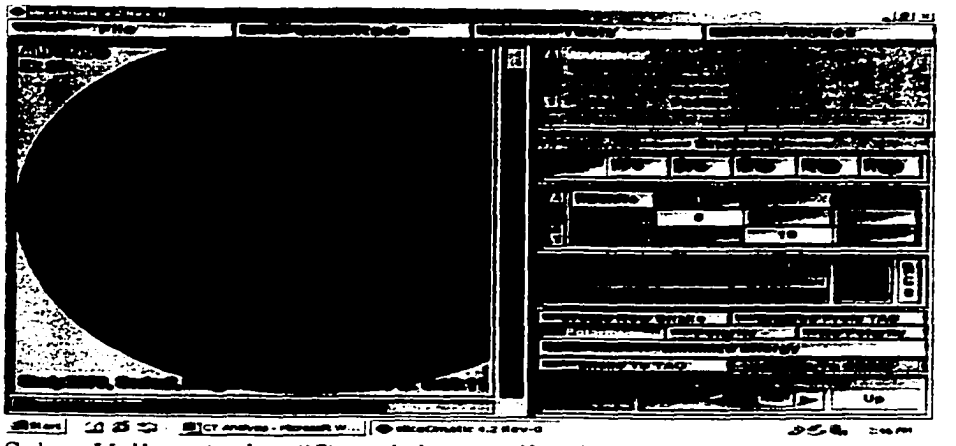
File

Save TAG files

Now you are ready to delineate between the deep and superficial layers of the subcutaneous fat region.

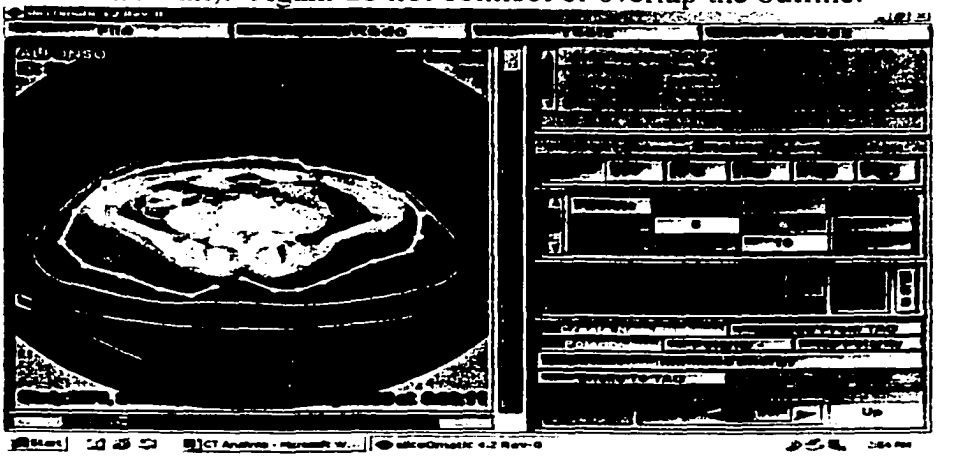
You will use the **Snakes** mode for this portion of the analysis.

Enter the Snakes mode. You should see the following.

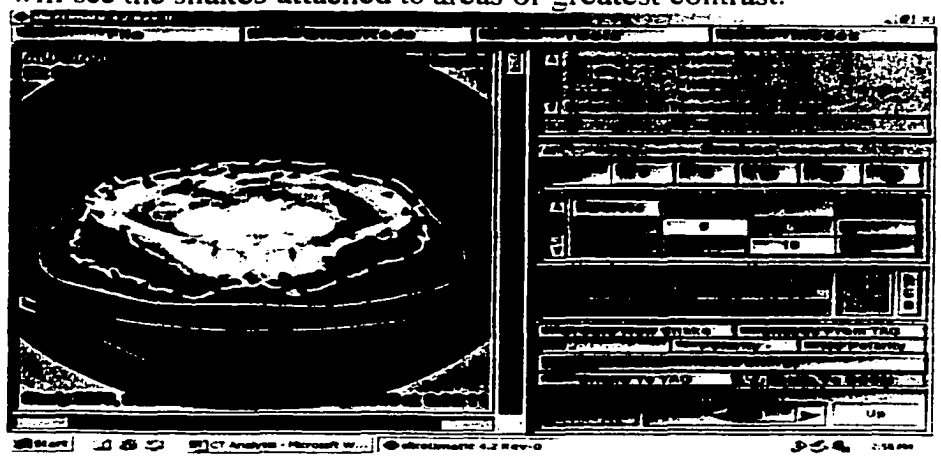


Select Yellow (color #5) and the small paintbrush.
Select "**Create New Snake**".
With the cursor click on the line that separates the two layers of subcutaneous fat. I recommend that you toggle the existing TAG colors off using the F1. This will allow you to visualize the layers more clearly.
-Do not connect or overlap the yellow outline that you have just created.

Now select orange (color #6) and the small paintbrush. (The color layers must increase in number as you move toward the center of the image if you choose other colors)
With the cursor click on the inner boarder of the subcutaneous fat region (or outer abdominal wall). Again do not connect or overlap the outline.



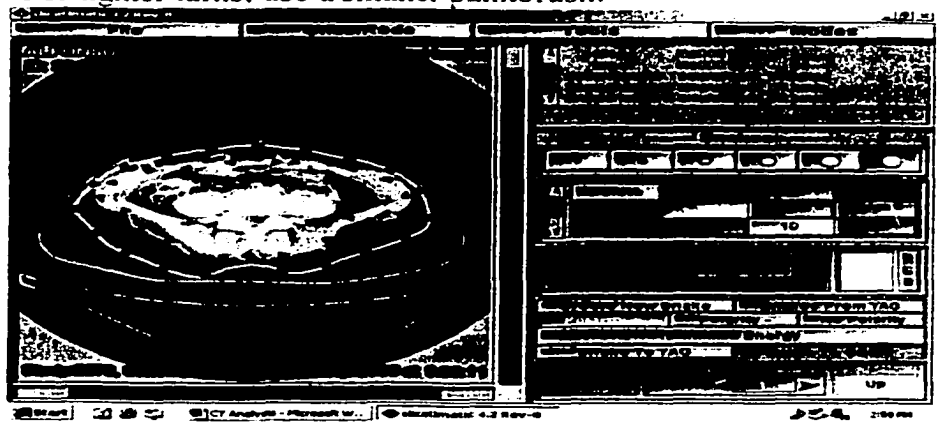
Once you have places both "snakes", select **Minimize Energy**. Do this only once. You will see the snakes attached to areas of greatest contrast.



Now you need to smooth out the snakes and place them on the appropriate boarders. Start with the yellow snake. Select the color yellow and the largest paintbrush. With the cursor you can push/iron out the line and place it where you want it.

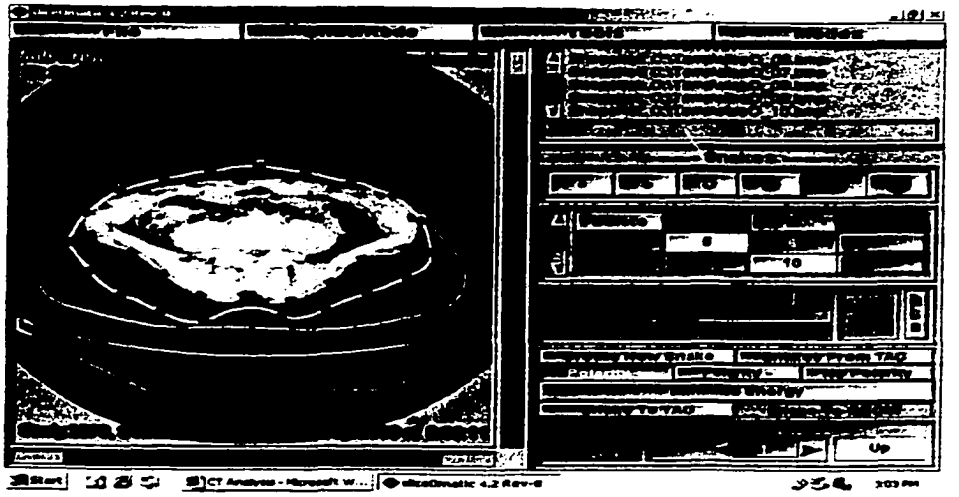
-The best way to get the snake in the most accurate position is to push it out so that you can see the layer and then use the cursor to set it down on the line.

-For tighter turns, use a smaller paintbrush.



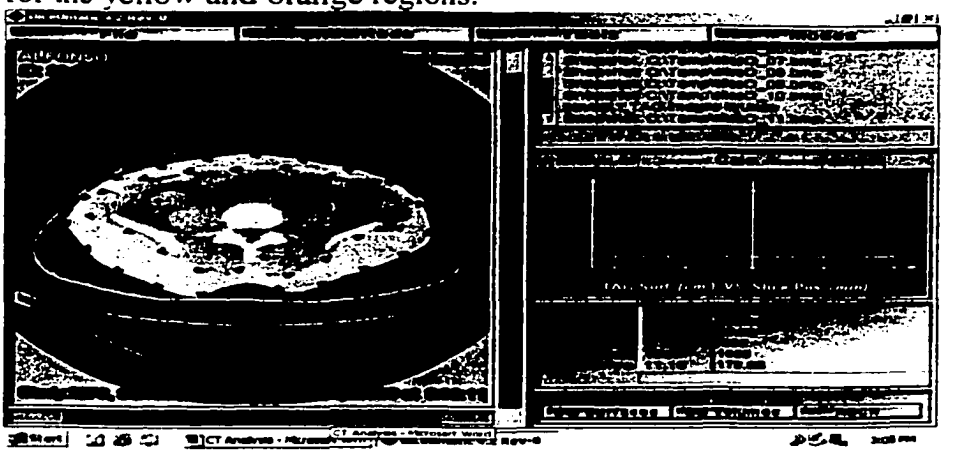
Repeat the same process for the orange snake. Select the color orange and the largest paintbrush. Smooth out the boarder with the cursor and place the snake where you want it.

You final image should look like the following image:



Now select "**Snake To TAG**". You will probably have to press F2 to show the colors.

Now go into the **Surface/Volume** Mode. You will need to record the surf (cm^2) values for the yellow and orange regions.



When you are finished, you will need to **exit** the program to start a new scan.