

AUTONOMIC NERVOUS SYSTEM MODULATION OF THE HEART FOLLOWING  
A HIGH CARBOHYDRATE LIQUID MEAL

A Thesis

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## TABLE OF CONTENTS

ACKNOWLEDGMENTS.....	ii
ABSTRACT.....	v
CHAPTER 1 – INTRODUCTION.....	1
1.1 - Justification for Research.....	3
1.2 - Purpose.....	5
1.3 - Hypothesis.....	5
1.4 – Limitations.....	6
CHAPTER 2 - LITERATURE REVIEW.....	7
2.1 - Overview of Obesity.....	7
2.2 - Sympathetic Nervous System.....	8
2.2.1 - Functions.....	8
2.2.2 - Sympathetic Nervous System Activity in Obese, Debate among Researchers.....	9
2.3 - Thermic Effect of Food.....	14
2.4 – Measurement of Sympathetic Nervous System, Heart Rate Variability.....	16
CHAPTER 3 – METHODS.....	20
3.1 - Subjects.....	20
3.2 - Instrumentation.....	21
3.3 - Design.....	22
3.4 - ECG Data Reduction.....	25
3.5 - Statistical Analysis.....	25
CHAPTER 4 – RESULTS.....	26
4.1 Participant Characteristics.....	26
4.2 Assessment of Carbohydrate Intake.....	26
4.3 Autonomic Reactivity to a High Carbohydrate Meal.....	26
CHAPTER 5 - DISCUSSION.....	31
REFERENCES.....	41
APPENDIX A CONSENT FORM.....	48
APPENDIX B DATA COLLECTION FORMS.....	52
Appendix B.1 Screening visit questionnaire.....	52
Appendix B.2 YMCA sub-max data entry form.....	53
Appendix B.3 Rules for day 2 data collection.....	54
Appendix B.4 Dietary Intake Questionnaire.....	55
APPENDIX C ENSURE.....	57
APPENDIX D TABLE OF LITERATURE.....	58

Table 4 Obese are hyperadrenergic .....	58
Table 5 Obese are hypoadrenergic .....	60
Table 6 Thermic Effect of Food .....	61
Table 7 HRV.....	62
APPENDIX E RAW DATA.....	63
APPENDIX F ADDITIONAL DATA GRAPHS.....	68
APPENDIX G ADDITIONAL DATA RESULTS.....	69
Appendix G.1 BMI - General Linear Model.....	69
Appendix G.2 Waist/Hip - General Linear Model .....	70
Appendix G.3 BEE - General Linear Model.....	71
Appendix G.4 % Body Fat General Linear Model.....	72
VITA.....	73

## ABSTRACT

The involvement of the sympathetic nervous system in the pathology of obesity has long been a debate among researchers. Various methods of capturing the resting sympathetic activity as well as the response of the sympathetic activity elicited by the introduction of a meal or a bout of exercise in both lean and obese individuals have been investigated. This study examined the autonomic modulation of the heart following a high carbohydrate liquid meal in various body types. Twenty-five females with body mass indexes [weight (kg)/height<sup>2</sup>(m)] ranging from 19.3-39.5 were grouped according to relative basal energy expenditure adjusted for lean body mass (High relative basal energy expenditure - HIGH rBEE and Low relative basal energy expenditure -LOW rBEE). Each female consumed *ENSURE with Fiber* at a calorie level equal to forty percent of her basal energy expenditure. Heart rate variability was measured for 15min at rest, immediately following the meal, and hourly for 5hrs postprandial. The LOW rBEE group at baseline as well as postprandial tended to demonstrate an elevated sympathetic activity when compared to the HIGH rBEE group (LOW rBEE - resting LF/HF ratio  $2.36 \pm 2.3$ , peak increase two hours pp LF/HF ratio  $3.09 \pm 2.9$ ; HIGH rBEE – resting LF/HF ratio  $1.93 \pm 1.7$ , peak increase fifteen minutes pp LF/HF ratio  $1.98 \pm 0.9$ ). Meal induced thermogenesis, measured as changes in sympathetic activity, was higher in the LOW rBEE group. In conclusion, this study has shown that in the pre and postabsorptive state, individuals with a LOW rBEE tend to be more hyperadrenergic when compared to HIGH rBEE individuals, which may signify an adverse cardiovascular risk. The evidence supports the assertion that when the SNS response to a meal in various body types is being investigated, a duration of greater than 5hrs post prandial is necessary.

## CHAPTER 1 - INTRODUCTION

The United States ranks high along with other developed countries for the prevalence of obesity. Reaching epidemic proportions, obesity has doubled in the United States within the past two decades (Finkelstein, Fiebelkorn, and Wang, 2003). Today, approximately sixty- five percent of Americans are overweight, and sixty-one percent of adults in the State of Louisiana are overweight (Flegal, Carroll, Ogden & Johnson, 2002). There are serious health consequences associated with obesity, including cancer, cardiovascular disease, and diabetes. Moreover, 280,000 deaths a year in the U.S. are attributed to obesity (Allison et al., 1999). According to the National Institutes of Health, individuals with a BMI of thirty and higher have a 50-100 % increased risk of death from all causes compared with individuals with a BMI between 20 and 25. The total cost of overweight and obesity in 2000 was \$117 billion (Finkelstein et al., 2003).

Overweight and obesity have become such a concern that national goals have been set by Healthy People 2010 for reduction of the prevalence of obesity in the United States. Healthy People 2010 was developed through a broad consultation process. Based on the available scientific evidence, a set of health objectives have been developed for our nation to achieve over the first decade of the new century. The report indicates that between 1988-1994, 11% of children between the ages of 6 and 19 years, and 23% of adults over the age of 20 were either overweight or obese. The suggested target for the year 2010 is an obesity incidence of 5% for children and 15% for adults. To provide some direction in assisting our nation in achieving these goals, Healthy People 2010 includes dietary and physical activity guidelines to prevent and or treat obesity (Healthy People 2010, US Department of Health and Human Services).

The successful management and evaluation of obesity requires an understanding of how obesity is defined, and the specific health risks associated with obesity. In general, obesity is

defined as an accumulation of excess body fat due to an imbalance between energy intake and energy expenditure. This imbalance can be due to excess caloric intake, lack of physical activity, metabolic and endocrine abnormalities, or any combination of these factors, and/or possible unknown mechanisms. Regardless of its cause, obesity is associated with significant health risks including hyperinsulinemia, insulin resistance, cancer, hypercholesterolemia, diabetes, hypertension, and cardiovascular disease. The risk of death from these diseases increases among individuals who are moderately and severely overweight (Calle, 1999).

Because of the large number of comorbidities that tend to exist with obesity, it has been difficult to make strong inferences about cause and effect among these various pathologic conditions. Therefore, while the American Heart Association considers obesity a primary risk factor for heart disease, it remains unclear as to whether this risk exists independent of other risk factors (Scherrer et al., 1994)

As scientists have labored to discover the thread that ties obesity with other health problems, one of the plausible explanations suggests that autonomic nervous system dysfunction is a potential culprit. Autonomic dysfunction appears to be a marker of many disease states, including obesity and other metabolic disorders. Therefore, the autonomic nervous system and its functions have become of particular interest to researchers. Although conflict still remains as to whether the sympathetic tone of obese individuals is enhanced or blunted, there is overall agreement that adverse effects can result from the aberrant sympathetic nervous system. One view, known as the MONA LISA hypothesis: *Most Obesities kNown Are Low In Sympathetic Activity*, suggests that low sympathetic tone leads to hyperphagia, and in the absence of another compensatory mechanism will produce obesity (Somers, 1999; Bray, 2000). However, this position is not universally held insofar as other investigators theorize that augmented

sympathetic activity accompanies obesity and can predispose obese individuals to cardiovascular disease, in particular hypertension (Balka, Strubbe, Bruggink & Steffens, 1993; Grassi, Seravalle, Cattaneo, Bolla, & Lanfrachi, 1995; Troisi et al., 1991; Weyer et al., 2000)

While researchers continue to debate whether sympathetic tone is blunted or increased among obese individuals, there is general agreement that the obese sympathetic response to certain stimulators such as a bout of exercise or a meal is blunted when compared to the sympathetic response observed in lean individuals. Recent research, however, suggests that perhaps such observations may not hold up if adequate time is allowed to fully appreciate the impact of stressors such as the thermic effect of food (Houde-Nadeau, de Jonge & Garrel, 1993; Reed and Hill, 1996; Schutz, Bessar & Jequier, 1984;).

### **1.1 - Justification for Research**

The adverse affects of a blunted sympathetic tone can possibly predispose individuals to obesity; or result in a lower resting metabolic rate and hyperphagia among obese individuals, therefore resulting in a challenging regimen of weight loss. However, if the opposite occurs in obese individuals (i.e. heightened sympathetic tone) the increase risk of mortality due to various diseases may result. Having noted the possible side effects of a malfunctioning sympathetic nervous system it is imperative for researchers to reach a conclusion. Therefore, additional research is needed to reconcile what appears to be a disparate position regarding the involvement of the autonomic nervous system (ANS) in obesity.

While many investigations have examined autonomic activity in lean and obese individuals, fewer studies have examined autonomic reactivity; that is characterizing the reactivity of the autonomic nervous system to various antecedents. Such an approach may provide additional insight into the nature of autonomic dysfunction in obesity. One antecedent

that may be of particular value is the autonomic responses to the introduction of a meal. Upon ingestion of a meal, the digestion and metabolism of nutrients increases the metabolic rate, accounting for 5-10% of the daily energy expenditure. This increase in basal metabolic rate is referred to as the thermic effect of food. The activity of the sympathetic nervous system is heterogeneous. Therefore, the stimulation of activation may occur in some organs, but not others (Patel, Coppack, Rawesh, Miles & Eisenhofer, 2000). It has been documented that the sympathetic nervous system may in fact play a role in the thermic effect of food (Astrup, 1995; Bray, 2000; de Jonge & Bray, 1997; Macdonald, 1995; Ravussin, 1995; Troisi et al., 1991;). The thermic effect of food (TEF) is the increase in basal metabolic rate following food intake (de Jonge & Garrel, 1997; Schwartz, Jaeger, Silberstein & Veith, 1987). While there are multiple factors associated with the stimulation of the TEF, the function of the sympathetic nervous system is responsible for a small percentage of the overall mechanics of TEF; however, the SNS maybe responsible for a feedback system involved in the regulation of food intake (Astrup, 1995; Bray, 2000).

Various methods have been used to measure autonomic activity during rest as well as during a response to stimulators such as diet and exercise. One such autonomic measurement technique that has recently grown in popularity is the assessment of heart rate variability (HRV). Variation in the R-R intervals of the heart can be expressed as a simple time domain index, but can also be described as an accumulation of power occurring throughout a continuum of frequency bands. The physiologic basis for the frequency domain analyses is predicated upon animal studies indicating that the sympathetic and parasympathetic divisions of the autonomic nervous system modulate heart rate at different frequencies (Berntson et al., 1997). Therefore, decomposing HRV into variations occurring at different frequencies allows for some

discrimination between parasympathetic nervous system (PNS) and sympathetic nervous system (SNS) control. More specifically, the PNS, which tends to be operate rather quickly (high-frequency) slowing the rate of the sinoatrial (SA) node through the release of acetylcholine, while the SNS, which speeds up the firing rate of the SA node with the release of norepinephrine, operates more slowly due to second messenger modulated responses (low-frequency). Using HRV to assess the autonomic function in lean and obese may provide additional insight as to the disease process of obesity, and subsequently contribute to successful treatment of the disease.

## **1.2 - Purpose**

The purpose of the present study is to examine the influence of body habitus and basal energy expenditure on changes in HRV observed over a 5-hr period following a high carbohydrate meal. To answer this question women will be assigned to groups using a variety of grouping strategies including BMI (low vs. high), estimated body fat percentage (low vs. high), waist-to-hip ratio (low vs. high), and basal energy expenditure (BEE) corrected for total body weight (low vs. high).

## **1.3 - Hypothesis**

It was hypothesized that individuals in the higher risk categories (high BMI, high body fat, high waist hip, and low BEE) would have a higher sympathetic activity pre- and post-absorption of a meal, but a smaller response in sympathetic activity following a high carbohydrate meal, as evidenced by smaller changes in standard deviation of all normal heart periods (SDNN), square root of the mean square of successful differences (rMSSD), percent of normal R-R intervals that are 50msec greater of its predecessor (pNN50), and high frequency

normalized units (HFnu) in the obese individuals in the fasted state, and a shift towards these low levels in the lean individuals following the meal.

#### **1.4 – Limitations**

Subjects were recruited from the female population attending Louisiana State University. The study was limited to women between the ages of 18-35. Additionally, the use of HRV as a global indicator of autonomic function is a limitation of this study. While HRV is a promising indicator of autonomic activity, there are several limitations to extrapolating HRV to describe autonomic activity of other organ systems or autonomic activity in general. The heterogeneity of the autonomic activity raises questions as to whether the heart is reflecting the rest of the body (Bray, 2000; Patel, 2000; Scherrer, Randin, Tappy, Vollenweider, Jequier and Nicod, 1994, Vaz, Jennings, Turner, Cox, Lambert and Murray, 1996). Therefore, using an end organ response, as a marker of autonomic activity is somewhat suspect, as the behavior of the organ itself, may confound the ability to make inferences specific to the ANS. Moreover, the physiologic correlates of HRV in humans have not been thoroughly elucidated. While animal studies support the use of HRV to describe sympathetic modulation, human studies are less clear. Thus, the ability to draw inference about the sympathetic nervous system *per-se* from HRV is somewhat limited. Despite such limitations, when compared to the Ewing battery, a traditional method to assess various degrees of diabetic autonomic dysfunction HRV was easier to perform and appeared to have better quantifiable results (Pagani, Malfatto, Pierini, Casati, Masu, Poli, Guzzetti, Lombardi, Cerutti, and Malliani, 1988).

## CHAPTER 2 - LITERATURE REVIEW

### 2.1 - Overview of Obesity

While overweight refers to excess body weight compared to set standards, obesity refers specifically to having an abnormally high proportion of fat. An imbalance between the energy value of food intake and the daily energy expenditure results in weight gain. In sedentary individuals approximately 65% of energy expenditure is due to “basal” or “resting” metabolism (Bray, 1999). Basal metabolism includes energy required to maintain body temperature, the contraction of smooth muscles, as well as the conduction of metabolic storage and mobilization processes. Another 10% of energy expenditure is dissipated through thermogenesis, and the final 25% of the body’s energy expenditure is accounted for through activity and exercise. The other side of the energy balance equation is the food we eat. While simply eating an excess of calorie rich foods, the cessation of smoking, and sedentary lifestyle will tip the balance, the influence of genetic factors and or metabolic disorders may predispose some individuals to obesity. The pathology of obesity begins with the imbalance between energy intake and expenditure, which is reflected in increased stored fat. The individual fat cells enlarge as fat stores increase. These enlarged fat cells then produce many peptides and nutrients that produce the disease known as obesity (Bray, 1999).

The most often used method to determine if an individual is overweight or obese is a mathematical calculation known as body mass index (BMI). The National Institutes of Health identifies a BMI of 25-29.9 kg/m<sup>2</sup> as overweight, while a BMI of 30 kg/m<sup>2</sup> or greater as obese. A BMI of 25 kg/m<sup>2</sup> or greater is associated with risk factors such as hypertension, congestive heart failure, coronary heart disease, insulin resistance syndrome, cancer, diabetes, gallbladder disease, osteoarthritis, sleep apnea and other breathing problems (National Institutes of Health, 2004).

According to the guidelines for overweight and obesity, developed by the National Heart, Lung and Blood Institute (1998) there are three general goals of weight loss and management. These goals aim at a minimum to prevent further weight gain; to reduce body weight; and to maintain a lower body weight over a long period of time. The initial target for obese individuals is to reduce weight by ten percent. Once weight loss of ten percent is safely attained additional weight loss may be recommended (NHLBI, 1998). Various treatment strategies, including diet therapy, pharmacological treatment, surgical treatment, exercise therapy, and combined therapy have been implemented by the health industry. While pharmacological and surgical treatment are certainly effective means to accomplish drastic results, there is considerable risk associated with these types of treatments, and the results may be short lived due to a lack of lifestyle change. Perhaps the most successful treatment strategy includes a combination of diet therapy, physical activity, and behavior modification therapy. While this combination of therapies may in fact require surgical or pharmacological assistance, the obese individual learns to make appropriate lifestyle changes to maintain a healthy desirable weight (Collazo-Clavell, 1999). As research on obesity and the related changes in physiological functions continues, treatment strategies can be modified. One physiological function that is receiving attention is the autonomic nervous system, specifically the sympathetic nervous system (SNS). Research has begun to investigate the activity of the SNS and pathology of obesity.

## **2.2 - Sympathetic Nervous System**

### **2.2.1 - Functions**

The sympathetic nervous system combined with the adrenal medulla form the adrenomedullary system. This system functions as one of the main regulators of multiple physiological processes. Principally the maintenance of blood pressure and the regulation of

body temperature are dependent on normal functioning of the sympathetic nervous system. The sympathetic nervous system functions in such a manner that there is selective activation, in other words, the activation of specific tissues or systems can occur while there is little to no effect on other areas (Macdonald, 1995). Evidence from a number of studies indicates the importance of the sympathetic nervous system as a regulator of energy expenditure, specifically resting metabolic rate, the largest component of daily energy expenditure (Ravussin, Tataranni, 1996; Vaz et al., 1997). Circulating catecholamines will stimulate energy expenditure through  $\beta$ -adrenoceptors, and baseline sympathetic nervous system activity exerts a chronic stimulation of energy expenditure. Since the SNS plays a role in energy expenditure, research conducted on the SNS activity and the pathology of obesity has become increasingly popular. However, the consensus to explore obesity and the SNS has left researchers in continuous debate.

### **2.2.2 - Sympathetic Nervous System Activity in Obese, Debate among Researchers**

While researchers examining rodents with hypothalamic obesity or genetic obesity tend to observe a lower sympathetic activity, large inconsistencies remain when the activity of the sympathoadrenal system is compared in lean and obese humans (Tataranni, 1998). Researchers typically ascribe to one of three possible explanations: 1) sympathetic tone is diminished in obese individuals (i.e. *MONA LISA* hypothesis); 2) sympathetic tone is heightened in obese individuals; or 3) lean and obese individuals do not differ in sympathetic activity at rest.

A reduction in sympathetic activity has been implicated in the genesis of animal models of obesity, and body weight gain in humans. While a portion of the research community is in accordance with the genesis of obesity beginning with a reduced sympathetic nervous system, a growing number of researchers believe obese individuals experience an elevation in sympathetic nervous activity. According to these investigators, the sustained sympathetic activation, possibly

related to chronic hyperinsulinemia, and impaired vasodilator responsiveness to insulin are believed to be characteristic of obese individuals. The hyperadrenergic condition in the obese state could contribute to hypertension and cardiovascular morbidity (Vaz et al., 1996).

A number of reviews have been published; however, there does not appear to be a consensus. One group of scientists suggest that while a depressed SA may be an explanation for the development of obesity, it is not a necessarily an origin for the pathology of the disease (Young and Macdonald, 1992). Others have been more committal, suggesting that indeed the overactivity of the SNS is a hallmark of obesity (Sommers, 1999), and finally there are some who suggest that this is not the case at all Grassi (1999). Ravussin and Tataranni (1996) noted that an accumulation of total and central fat is associated with a higher SNS activity; the SNS modulates the resting metabolic rate; and when compared with obese Caucasians, Pima Indians have a low SNS suggesting a role in the development of obesity for this population. Table 4 and 5, which can be found in appendix D on pages 51 and 53, list studies conducted within the last twenty years that address the activity of the sympathetic nervous system among obese individuals.

Hyperinsulinemia, insulin resistance and hypertension are often associated with obesity. Research conducted on obesity-induced rats due to long-term intragastric overfeeding resulted in hyperinsulinemia (Balkan et al., 1993). Insulin is a known stimulator of the SNS; therefore, the hyperinsulinaemic state may be the cause of the elevated sympathetic activity (Emdin et al., 2001; Kush et al., 1986; Park, et al., 2000). However, if the level of insulin secretion is insufficient to overcome insulin resistance perhaps the stimulating effects of insulin will not occur (Astrup, Christensen, & Breum, 1991). In addition, a hyperinsulinemic state can be triggered by an acute increase in sympathetic cardiovascular drive (Grassi, et al., 1996). One may

then be led to believe the high blood pressure associated with obese individuals is actually the culprit of the enhanced cardiovascular sympathetic activity resulting in hyperinsulinemia; however, even in the absence of hypertension, obese individuals have a marked sympathetic activation (Sowers, et al., 1982; Grassi et al., 1995). Perhaps the heterogeneity characteristic of the SNS is to blame for such discrepancies in the research (Bray, 2000; Patel, Coppack, Rawesh, Miles, and Eisenhofer, 2000; Scherrer et al., 1994; Vaz et al., 1996).

The sympathetic nervous system may be stimulated, increasing outflow to one organ, while normal or reduced sympathetic tone occurs in other organs (Bray, 2000; Patel et al., 2000; Scherrer et al., 1994; Vaz et al., 1996). Perhaps this heterogeneity of the sympathetic nervous system will explain why researchers continue to debate the adrenergic state of obese individuals. For instance, Scherrer et al. (1994) failed to notice a correlation between plasma norepinephrine concentrations and body fat; however, an increase in sympathetic firing rate to skeletal muscle was seen with increasing body fat. Therefore, these results offer evidence that sympathetic outflow to various organs may be highly differential (Scherrer et al., 1994). Vaz et al. (1996) also demonstrated the heterogeneity of regional sympathetic outflow; a significant elevation of sympathetic activity occurred in the kidneys, the heart experienced a reduced sympathetic tone, and a normal sympathetic activity of the splanchnic bed. In addition, Patel et al. (2000) noted for the first time an increase in the activity of the sympathetic nervous system postprandial in adipose tissue of the abdomen. The heterogeneity of the SNS is a challenge to researchers; however, the lack of uniform methodology from one study to the next also contributes to the difficulty in reaching a consensus.

When discussing the adrenergic state of obese individuals, the age-old question, “Which came first?” is often asked among researchers. Heightened sympathetic nervous activity offers an

explanation for the cardiovascular complications associated with obesity; however, this elevated activity would offer a protective mechanism to weight gain. A blunted sympathetic tone would explain the weight gain leading to obesity; however, this decrease in sympathetic activity would be cardioprotective. Therefore, the logical design study would be longitudinal. However, how does one predict who will become obese? One interesting approach has been to study a group of individuals who are known to have a high incidence of obesity. The Pima Indians have served as an excellent model in this regard. Findings from studies of the Pima Indians, an ethnic group with a high prevalence of obesity, reveal that these individuals have a low prevalence of hypertension and low sympathetic activity (Ravussin and Tataranni, 1996; Spraul, et al., 1993) possibly associated with resistance to beta-adrenergic stimulation (Christin, O'Connell, Bogardus, Danforth, and Ravussin, 1993).

While the Pima Indians have been of interest as they offer scientists the opportunity to follow a select group over time, the findings may not necessarily extend to other racial/ethnic groups (Macdonald, 1995; Spraul et al., 1993). In fact, studies comparing Caucasians to Pima Indians reveal some clear differences. Spraul and associates observed that muscle sympathetic nerve activity is significantly related to body fatness and energy expenditure in Caucasians; however, not in the Pima Indians. In addition, when compared with Caucasians of similar body weight, body composition, and age matched Pima Indians have a lower fasting muscle sympathetic nerve activity (Spraul et al., 1993).

Another approach to investigating this question has been to follow obese individuals after a period of weight loss. Following a hypocaloric diet resulting in significant weight reduction, multiple studies concluded that obese individuals would experience a significant decrease in postprandial thermogenesis, decrease in sympathetic activity, and ultimately suppression of basal

metabolic rate (Bessard, Schutz & Jequier, 1983; Dulloo & Jacquet, 1998; Grassi, et al., 1998; Sowers et al., 1982). The results of these studies suggest that sympathetic activation accompanying obesity is reversible when weight loss is achieved through dietary treatment. In response to food deprivation the leaner individual had a greater suppression of thermogenesis to conserve energy, while the opposite was true for the obese individual. Depression in thermogenesis with weight loss was directly related to the degree of body fat reduction leading Dulloo and Jacquet (1998) to conclude, "These results suggest the adaptive reduction in basal metabolic rate is partly determined by an autoregulatory feedback control system linking the state of depletion of fat stores to compensatory mechanisms that suppress thermogenesis." However, this reduction in sympathetic activity is not entirely beneficial. While the high prevalence of hypertension, congestive heart failure, and sudden death may be eliminated, the previously obese individual is still predisposed to weight regain (Bessard et al., 1983; Grassi et al., 1998). However, Amatruda et al. (1993) concluded, that although weight loss and weight maintenance were achieved among obese participants, low energy expenditure was not a result. They therefore concluded that abnormally low energy expenditure is not to blame for weight gain.

Most of the research conducted on obesity and sympathetic activity has involved the examination of sympathetic activity at rest. As the health and function of the autonomic nervous system may also be characterized according to one's ability to adapt to changing environments, it may also prove beneficial to examine autonomic reactivity; that is the magnitude of change evoked by changing environments. The regulation of the thermic effect of food is due in part to both the sympathetic nervous system and epinephrine (Astrup, 1995; de Jonge et al., 1997; Park et al., 2000; Schwartz et al 1987; Welle, 1995). Therefore, comparing the sympathetic nervous

system's role during the thermic effect of food among lean and obese individuals will offer additional information to changes in SA during obesity.

### **2.3 - Thermic Effect of Food**

The thermic effect of food can be separated into two components. The energy expended due to digestion, absorption, transportation and storage of the ingested substrates is known as 'obligatory thermogenesis', which can account for two thirds of the entire thermic effect of food. The second component of thermic effect of food is the 'facultative thermogenesis' and while this component plays a small role in the total thermic response to food, it is also very variable, which makes it important in long-term energy balance. Although investigators suggest that the sympathetic nervous system, the sodium/potassium pumps and insulin may be important factors in facultative thermogenesis, the precise mechanisms are unknown (de Jonge et al., 1997; Schwartz et al., 1987).

Meal size, meal composition, and previous diet are all known factors which influence the obligatory component of the thermic effect of food. The meal size or the total caloric content has shown to be positively associated with the thermic effect of food (de Jonge et al., 1997; Welle, 1995). Kinabo et al. (1990) found a significantly greater thermic effect of food following a meal consisting of 1200 kcal as opposed to 600 kcal. The composition of a meal has been known to cause varying responses (Astrup et al., 1992; Labayen, Forga, & Martinez, 1999; Westerterp, Wilson & Rolland, 1999).

The sympathetic stimulation of carbohydrates has been well documented (Astrup et al., 1992). Westerterp et al. (1999) investigated the effect of diets of extreme macronutrient composition on diet-induced thermogenesis in a respiration chamber for one full day, and concluded that a high protein and carbohydrate diet resulted in the greatest thermic response. A

diminished thermic response was found with a high fat diet. When a high carbohydrate liquid meal was compared to a high fat liquid meal in lean young females 20-27 years of age, Labayen et al. (1999) found a similar response. Within the first 30 minutes the metabolic rate increased above resting values following the high carbohydrate meal and remained high for 120min. This sustained increase in metabolic rate can be accounted for by the cost of glucose storage as glycogen and lipid synthesis processes; however, de novo lipogenesis is almost of no importance in humans, especially a net 24H.(Guo, Cella, Baum, Ravussin & Schoeller, 2000). In addition the blood glucose level rose above pre-meal values during the first 30min and began to gradually decline toward the end of the observation period, following the high carbohydrate meal.

Along with determining an increase in thermic effect of food as a result of the carbohydrate concentration of a meal, Labayen et al. (1999) concluded that the carbohydrate load of preceding days would cause an increase in TEF. In addition, glycogen stores may be of importance. When a diet high in carbohydrates is consumed, and skeletal muscle and liver glycogen stores are full, remaining carbohydrates are converted into fat. The utilization of fat as an energy source is less efficient than the use of glycogen. Therefore the energy expended when glucose is transformed into a lipid and thereafter oxidized is greater than when glucose is stored as glycogen before oxidation (de Jonge et al., 1997). Therefore, increasing the dietary ratio of carbohydrate to fat in the post-obese individuals may aid in achieving normal body composition (Astrup et al., 1992).

Although the mechanisms by which facultative thermogenesis operates are not precise, there has been a considerable amount of research conducted examining the influence of sympathetic stimulation. de Jonge et al. (1997) conducted a study in which propranolol was used to inhibit the sympathetic activation following a meal; as a result the thermic effect of food was

decreased. They concluded that the sympathetic influences on the thermic effect of food are similar to those observed in rodents. A higher sympathetic response to a meal will result in a greater thermic effect of food; however this acute response is perhaps blunted in obese individuals.

Matsumoto et al. (2001), using HRV, concluded obese young females have a lower sympathetic response to a mixed meal than that of lean young females. However, the time duration in which Matsumoto et al. (2001) measured the thermic effect of the meal is questionable as these investigators only observed autonomic responses for a period of...while data suggest reference) that as much as 5hrs may be required (Houde-Nadeau et al., 1993; Reed & Hill, 1996; Schutz et al., 1984). In one study that followed participants for 14 hrs following a meal, the findings indicate a blunted response of daily thermogenesis among obese individuals (Schutz et al., 1984). In addition, the thermogenic peak following a meal is different for obese and lean individuals. Non-obese subjects show an earlier and higher peak than obese individuals; therefore, the shorter the duration of measurement the more likely the total thermic effect of food will differ between the two groups (Reed & Hill, 1996). Summaries of these studies and others are compiled in Table 6 of appendix D.

Components of the thermic effect of food have been measured using such techniques as the ventilated hood, metabolic chamber, catecholamine concentrations (plasma and urine) and pharmacological aids. However, a non-invasive technique, used to specifically measure autonomic activity, which is attracting considerable attention, is HRV (Table 7 in appendix D).

#### **2.4 – Measurement of Sympathetic Nervous System, Heart Rate Variability**

Cardiac function is an attractive candidate with which to evaluate autonomic status due to the sensitivity to autonomic influences (Hirsch, 1991). Scientists and clinicians have monitored

the technique of studying heart rate patterns for hundreds of years. This technique has progressed significantly throughout the years, and with the development of the electrocardiograph, the ability to monitor normal and abnormal electrical conduction and to evaluate beat-to-beat changes in heart rate pattern became possible (for a review see Berntson et al., 1997). This technique uses sophisticated mathematical partitioning to identify individual superimposed rhythms producing cyclical variations in heart rate (Grassi & Esler, 1999).

The components of heart rate variability are combined within several frequency bands. The respiratory band, ranging from 0.15 Hz to 0.4 Hz is relatively fast cycling control system, a.k.a. high-frequency band, and is believed to be mediated vagally, thus providing an index of cardiac vagal activity. The frequency band oscillating below 0.15 Hz is known as the low-frequency (LF) band, which ranges as low as .04 Hz. Although the low-frequency band is thought to reflect mainly the sympathetic activity, investigators believe the low-frequency is actually representative of both sympathetic and vagal activity. Therefore, the ratio of low-frequency to high-frequency (HF) provides a measure of sympathovagal balance. The final frequency band is the very low-frequency band, believed to oscillate between 0.003 Hz and .04Hz. While some researchers believe the very low-frequency band reflects thermoregulatory cycles or fluctuations related to plasma renin activity, the precise origin of this band has not been well researched (Berntson et al., 1997).

Heart rate variability offers noninvasive insight into autonomic modulation of the heart, as well as an indirect measurement of autonomic activity in other physiological functions of the body. Heart rate variability can be a marker used in normal subjects as well as among patients with conditions characterized by sympathetic activation, such as heart failure and obesity (Grassi, Vailati, Bertinieri, Servallo, Stella, Dell'Oro and Mancina, 1998).

Table 2.4 HRV parameters

<b>LFnu (.05-.15Hz)</b>	Low Frequency normalized units	Sympathetic & Vagal Origin
HFnu (>.15Hz)	High Frequency normalized units	Primarily vagal origin
<b>LF/HF ratio</b>	Low to High frequency ratio	Primarily sympathetic origin
SDNN	Standard deviation of all normal heart periods	Overall variability estimate
<b>pNN50</b>	Percent of normal R-R intervals that are 50msec greater of its predecessor	Parasympathetic origin
rMSSD	Square root of the mean square of successive differences	Variability estimate, short term
HP	Heart Period	Cardiac rhythms, descriptor when analyses are based on R-R interval data

However, despite the growing confidence in HRV, there is very little research investigating the association between sympathetic activity and obesity. Matsumoto et al. (2001) used HRV to measure autonomic nervous system activity, and concluded obese individuals are lower in sympathetic activity, which perhaps may cause diminished energy expenditure and further weight gain. Matsumoto et al. (2001) evaluated the autonomic activity using the very low frequency (.007 - .035 Hz) band and a ratio of very low to total power (.007 - .5 Hz) as the absolute and relative thermogenic sympathetic nervous system activities, respectively. However, the very low-frequency band is likely representative of a broad array of potential stimuli and conditions, including hemorrhage, aortic constriction, acidosis, posture, congestive heart failure, and slow variations in breathing patterns (Berntson et al., 1997). Therefore, to boldly state the very low-frequency band is representative of the thermogenic sympathetic activity would not be warranted.

The purpose of this study was to compare the sympathetic response to a high carbohydrate meal, for a total duration of five hours, in various body types. The tentative assumption of this study is that the obese individual would be hyperadrenergic, but would experience a blunted sympathetic response to a meal when compared with lean individuals.

## CHAPTER 3 - METHODS

### 3.1 - Subjects

Participants were women between the ages of 18-35. The recruitment pool included students enrolled or working at Louisiana State University. All volunteers with a known food allergy, hypertension, ischemic heart disease, cardiomyopathies, or history of peripheral vascular diseases were excluded. In addition, volunteers reporting current pregnancy, or use of tobacco, or prescription or over-the-counter medications that would potentially interfere with the autonomic nervous system were excluded from the study. In addition, volunteers with a known history of diabetes were excluded from the study.

Twenty-nine females were recruited, and 25 ( $N=25$ ) completed all aspects of the study. The range of body mass index of the participants was 19.3-39.5. The physical characteristics of each group are displayed in Table 3.1. A complete table of the raw data is included in the Appendix E. For the purpose of comparing the autonomic responses of participants of different body habitus, we assigned the participants to groups using various indicators of body habitus. In each case we arbitrarily used median values of certain parameters of interest to establish cutoffs for group assignment. The three methods for classifying individuals into groups were, high vs. low relative basal energy expenditure (rBEE) adjusted for lean body mass; low vs. high BMI; low vs. high body fat; low vs. high waist hip ratio (WHR). With regard to Low and High rBEE the cutoff point was set at **10.5**; for Low and High BMI the cutoff point was set at **19.3-21.8 kg/m<sup>2</sup> (Low) and 25.2-39.5 kg/m<sup>2</sup> (High)**; and with regard to WHR the cutoff point was set at **.74**. Preliminary analyses of the data indicated that the best discriminator for the purpose of this study was rBEE. Therefore, the results presented are illustrations of the females grouped by relative basal energy expenditure, while the analyses based on BMI, body fat percentage, and waist-hip ratio groupings are presented in Appendix F and G.

Table 3.1. Characteristics according to rBEE Groupings

	Low rBEE	High rBEE
<b>n</b>	13	12
<b>BMI</b>	27.80 +/- 4.0 (22.50-35.40)	21.1+/- 1.5* (19.3-23.5)
<b>Age (yr)</b>	24 +/- 3.0 (20-27)	22 +/- 3.8 (18-27)
<b>WHR</b>	.80 +/- .08 (.75-.96)	.73 +/- .03* (.68-.81)
<b>Body Fat (%)</b>	31.88% +/- 6.6% (23.7-42.0)	19.9% +/- 3.9%* (16.4-25.0)
<b>Systolic blood pressure</b>	109 +/- 9.3 (98-120)	98 +/- 8.8 (80-115)
<b>Diastolic blood pressure</b>	73 +/- 9.0 (58-82)	67 +/- 8.6 (65-80)
<b>BEE (Harris Benedict)</b>	1560 +/- 132 (1414-1826)	1364 +/- 56.7* (1294-1512)
<b>rBEE (BEE/weight)</b>	9.52 +/- .7 (8.32-10.44)	11.53 +/- .5* (10.56-12.74)
<b>24hr food recall – CHO intake</b>	180 +/- 86 (95.8-441.5)	200.56 +/- 67.9 (83.9-313.0)

Values are mean +/- SD

\* = group different from low rBEE (p <0.05)

### 3.2 - Instrumentation

Heart Rate Variability- A Biopac MP100 (Santa Barbara, CA) data acquisition system and AcqKnowledge ACK100 (Santa Barbara, CA) software program was used to capture, process, and analyze an electrocardiogram (ECG) signal.

Baecke questionnaire- This questionnaire consists of three sections: work activity, sports activity, and non-sport leisure activity. The answers of each question are scored on a five-point Likert scale, ranging from never to always or very often. The total score of each section are then added together to offer an estimation of the individual's amount of habitual physical activity (Baecke et al., 1982).

The USDA developed the multiple-pass method for the Continuing Survey of Food Intakes by Individuals (CSFII) 1994-1996 (Guenther et al., 1995, 1996). This method of collecting a 24-h food recall consists of three parts: 1. quick list, 2. detailed description, and 3.

review. Food intake data was analyzed using MEnu, Moore's Extended Nutrient Database (Pennington Biomedical Research Foundation).

Blood Pressure - Using a mercury sphygmomanometer, systolic and diastolic blood pressures was measured during both fitness tests and on day two of data collection coinciding with the 15 minute ECG data collection.

Body mass index, percent body fat, and waist-to-hip ratio were measured using standard laboratory techniques. Weight and Height were assessed using a standard physician's scale with stadiometer. Percent body fat was determined using a 4-site skin fold approach. The measurement sites include triceps, biceps, sub-scapular, and suprailiac. Waist to hip measurements were taken using a flexible tape measure. All measurement procedures were followed according to the guides described by Lee and Nieman (1996).

Using the Monark cycle-ergometer, each participant's fitness capacity was determined using the sub-maximal, bicycle ergometer, YMCA protocol. Designed to raise the steady state of heart rate, the YMCA protocol uses two to four, 3-minute stages of continuous exercise. Stage one is initiated with a work rate of 150kgm/min, and the following stages' work rate is set based on the heart rate achieved in the last 15 -30 seconds of the previous stage (ACSM Guidelines for Exercise Testing and Prescription, 2000).

### **3.3 - Design**

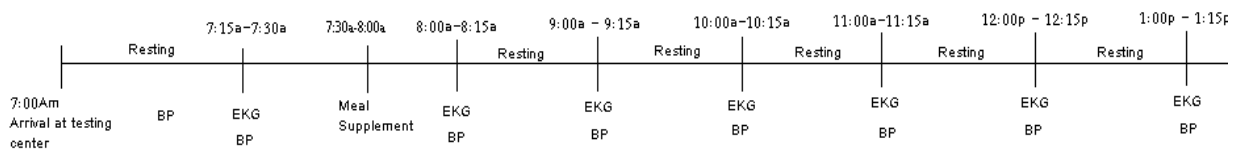
Upon recruitment into the study each participant signed an informed consent, which offered a brief outline of the study design and specific requirements expected of each participant, which was approved by the Louisiana State University Institutional Review Board. Data collection consisted of 2 days.

Day one of data collection required about 1 hour of time and included a questionnaire to assess each participant's routine physical activity, body composition and a submaximal fitness test. Each participant was asked to complete the Baecke Questionnaire of habitual physical activity. Body composition was measured by examining the thickness of skin folds measured at four sites: triceps, biceps, sub-scapular, and suprailiac. In addition, weight and height was obtained to determine the body mass index of each subject. Since weight distribution has been shown to affect the sympathetic activity, waist and hip measurements are included to determine waist to hip ratio (Gao, Lovejoy, Sparti, Bray, Keys, and Partington, 1995). Each participant was required to perform a submaximal cardiovascular test, specifically the YMCA cycle ergometry protocol. Upon completion of day one, each participant scheduled their second data collection day and were given specific instructions to follow prior to testing. Such instructions included, no eating for 12 hrs prior to testing, no alcoholic beverages for 48 hrs prior to testing and no physical activity for 12 hrs prior to testing. Since high carbohydrate loading preceding testing days has been shown to influence the sympathetic nervous system, each subject was instructed to follow the USDA Food Guide Pyramid to encourage a mixed, balanced diet the day before testing (de Jonge et al., 1997; Labayen et al., 1999).

Day two, scheduled to fall within the first two weeks of menstruation for all females, consisted of a 24-h food recall, using the multiple-pass method of data collection (Guenther et al., 1995, 1996). Each participant was asked to consume a high carbohydrate, commercially prepared, meal supplement, specifically ENSURE *Fiber*, with a nutrient breakdown of 66% carbohydrate, 20% fat, and 14% protein, according to the product nutrition label. The high carbohydrate beverage was chosen because of the stimulus effects carbohydrates have on the sympathetic nervous system (de Jonge et al., 1997; Labayen et al., 1999). The total caloric intake

administered to each participant varied. Once establishing the basal energy expenditure using the Harris Benedict Equation, each participant received 40% of his or her calculated BEE (Bessard et al., 1983).

The sympathetic nervous system activity was measured through heart rate variability using an electrocardiograph. Data collection for blood pressure and SNA were taken on seven separate occasions. Each ECG measurement lasted 15 minutes in duration and blood pressure was collected at each 15-minute interval. Participants were asked to arrive at the testing center by 7:00 am. Following a 15-minute latent period, a resting ECG was taken. The participant then consumed the available meal supplement. Immediately following the meal, another ECG was taken for an additional 15 minutes. The duration of day two of testing followed the time line below (figure 3.1).



**Figure 3.1. Day 2 testing time line.**

The participants were instructed to minimize activity, suggesting activities such as studying or watching television, between each administered ECG. Research has indicated that the thermic effect of food can last up to 6 hours (Houde-Nadeau et al., 1993; Reed & Hill, 1996). In addition Reed and Hill (1996) reported the thermic effect of food in obese individuals will peak in the latter part of the 6-hour period, suggesting that measurements lasting less than 4 hours will conclude a false result of differences between lean and obese individuals. However, most individuals will not experience a 6 hour thermic response of food; therefore, Houd-Nadeau, et al. conclude the 5 hour duration is sufficient to justify a single measurement (1993).

### **3.4 - ECG Data Reduction**

HRV was evaluated in accordance with guidelines previously set forth (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). ECGs were visually inspected for non-sinus beats and, if no ectopy was found, were subsequently plotted as a tachogram of heart period. The tachograms were then evaluated for the mean and standard deviation of all normal RR intervals (SDNN). Spectral analysis of HRV was derived via a 1024-point linear fast Fourier Transformation using a Hamming window. The resultant power density spectrum was then analyzed for total power (TP; 0.00-0.40 Hz), LF (0.04-0.15 Hz), and HF (0.15-0.40 Hz). LF and HF were further normalized (LFNU and HFNU) to better quantify sympathovagal balance.

### **3.5 - Statistical Analysis**

The initial group differences in HRV parameters, resting blood pressure, and estimated maximal work capacity were analyzed using a T-test and the possible consideration of these differences as a covariate. Although the power was limited due to the number of repeated measures, a 2x7 mixed model ANOVA examined possible main effects of group, time, and group x time interactions on HRV parameters and arterial blood pressure. Additionally, LSD was used to make post HOC comparisons where indicated. Lastly Pearson product moment correlation was used to report associations between the magnitude of peak change in HRV following the meal and body composition (BMI and % body fat). Alpha was set a-priori at  $p < 0.05$ .

## CHAPTER 4 – RESULTS

### 4.1 Participant Characteristics

The participant characteristics were reported earlier in Table 3.1.

### 4.2 Assessment of Carbohydrate Intake

From the 24-hour food recall an average of 52% of total nutrient intake was from carbohydrates. The group differences were examined according to categorization schemes for BMI, rBEE, percent fat and waist-to-hip ratio and the results indicated no significant group differences for carbohydrate intake according to any of the grouping schemes.

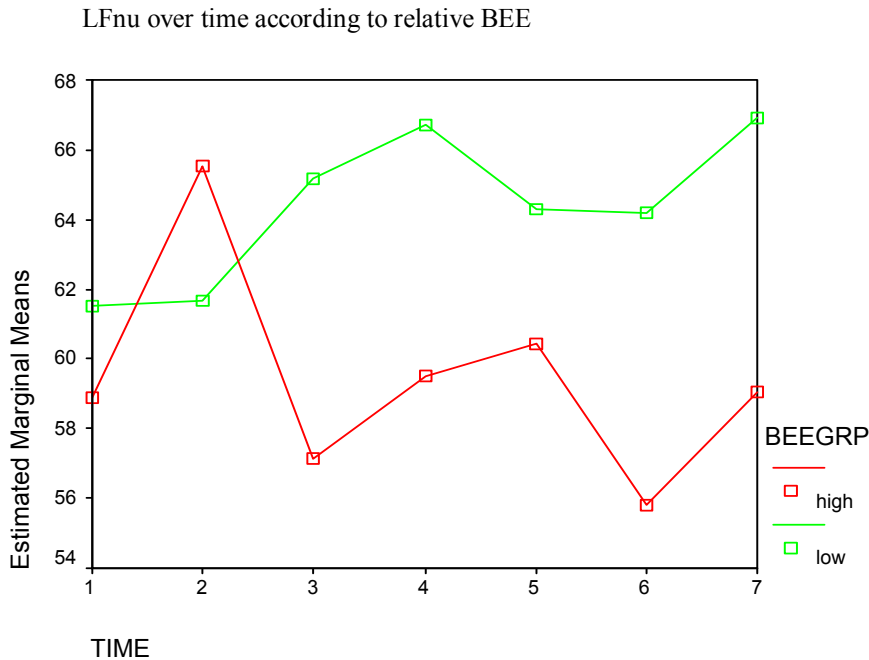
### 4.3 Autonomic Reactivity to a High Carbohydrate Meal

A series of 2x7 repeated measures analyses of variance were used to examine main effects of group and time as well as group by time interactions on heart rate variability parameters. In each case, the seven time points were: immediately pre-fed; 15-minute postprandial; and hours 1 through 5 postprandial. The analyses were applied using various grouping variables (rBEE, BMI, % fat and W/H ratio). We hypothesized that there would be significant interaction effects suggesting that women of differing body habitus would respond differently to the meal.

The results were similar for all grouping variables (rBEE, BMI, body fat percentage, waist-hip ratio). Illustrated in figure 4.1- 4.3 are the responses of LFn<sub>u</sub>, LF/HF ratio, and pnn50, over time according to rBEE grouping. Appendices F and G contain the statistical outputs for all of the grouping variables.

In the case of LFn<sub>u</sub>, the ANOVA indicates no significant effects of time or group, nor a significant interaction effect. However, the interaction term had a p value of 0.15 ( $F = 1.59$ ,  $df = 6$ ). The limited power in this design (high type II error rate) raises doubts as to the confidence with which we can rule out the possibility that a group by time period interaction exists.

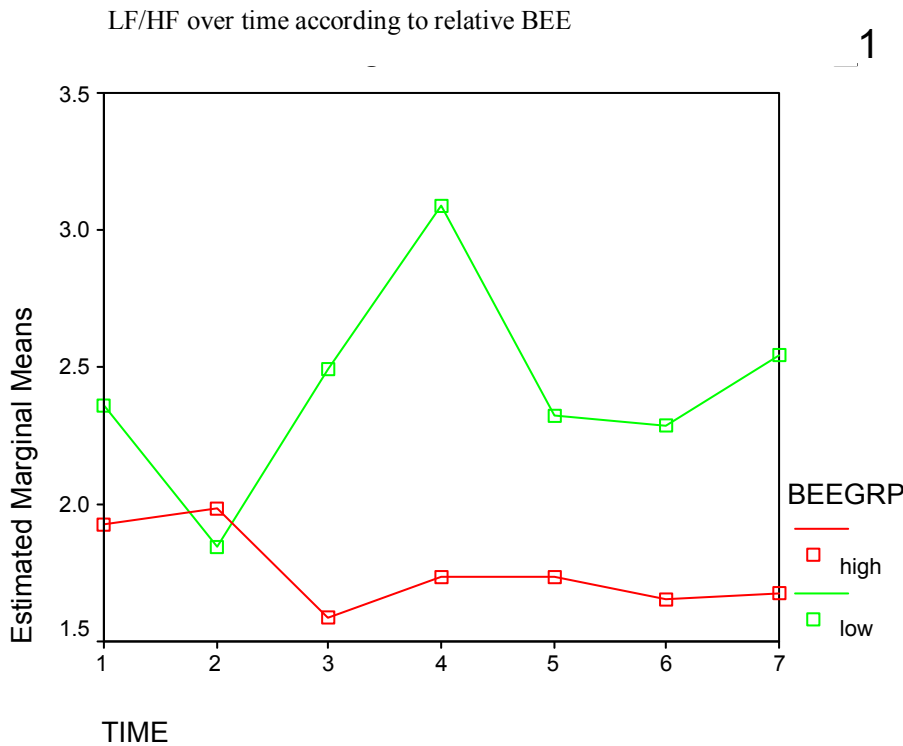
Moreover, separate repeated measures ANOVAS (treating each group separately) indicate that in the high rBEE group, LFnu is significantly elevated 15min post-prandial compared to baseline, and that the LFnu drops back to baseline within 1 hour post-prandial, while in the low rBEE group there was no significant main effect of time. With the addition of more subjects, it would therefore be reasonable to hypothesize that we will observe an interaction characterized by the HIGH rBEE group having an early sympathetic response that returns to baseline within about an hour, and the LOW rBEE group having a delayed response that persists throughout the data collection period.



**Figure 4.1:** Lfnu (p for interaction = 0.15)

The LF/HF ratio data (see figure 4.2) are similar to the LFnu data in that there were no significant main or interaction effects. However, the interaction term was notable ( $F = 1.40$ ,  $p = .22$ ,  $df = 6$ ) and with poor statistical power with these few subjects ( $b = 0.15$ ) it is difficult to rule out the possible influence of body habitus on this response. The data reflect a potential trend towards an increase in sympathetic modulation over the 5-hour post-prandial period in the low

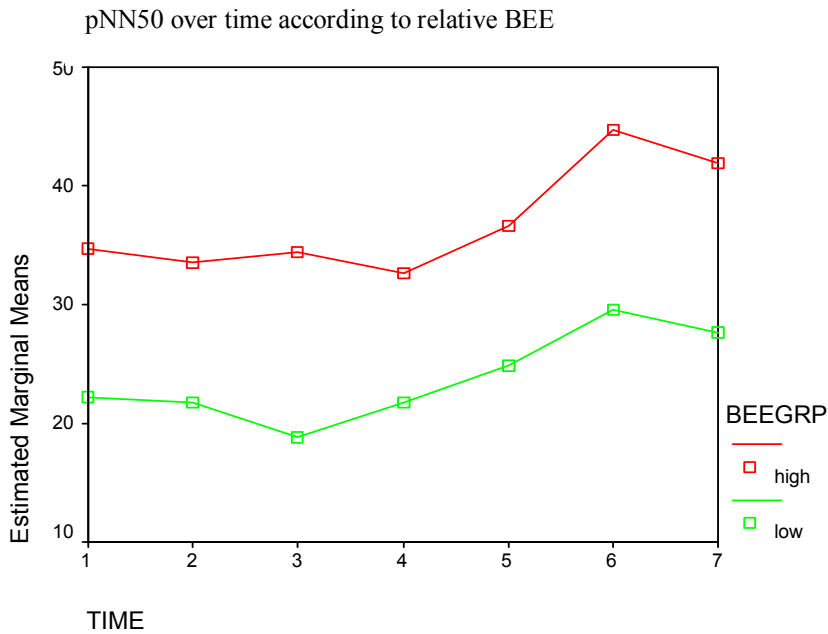
rBEE group, with a small but potentially significant increase in the high rBEE group immediately following the meal that recovers to baseline. Separate repeated measures ANOVAs were run on each rBEE group, and the HIGH rBEE group had a higher LFnu at 15-minute postprandial compared to 1-, 4- and 5-hrs post prandial. The 15-min post LF/HF ratio was not higher than baseline. The LOW rBEE group did not have LF/HF ratios that were significantly different from one another. Nonetheless, the LOW rBEE subjects appear to maintain an elevated sympathovagal balance throughout the testing period, peaking approximately two hours postprandial and not returning to baseline.



**Figure 4.2:** LF/HF Ratio (p for interaction = 0.22)

The pNN50 data are indicative of parasympathetic modulation of the heart. The results (figure 4) indicate a significant main effect of time ( $p < 0.01$ ,  $F = .30$ ,  $df = 6$ ). Follow-up LSD revealed that among both groups the pNN50 values were significantly greater at hours three and

four in comparison to pre-fed and immediate post fed. The data also cannot exclude the possibility of a significant group difference ( $p=0.06$ ) such that the HIGH rBEE group appears to have a greater parasympathetic modulation of the heart throughout the observation period. The data for all of the other heart rate variability indices across time and according to rBEE groups can be found in table 4.1.



**Figure 4.3.** pNN50 Effect of time ( $p < 0.01$ ):Effect of group ( $p = 0.06$ )

Table 4.1. Effect of feeding on time domain measures of heart rate variability

		rest	15' post	1hr post	2hr post	3hr post	4hr post	5hr post
<b>HP</b>	Low	732.97 +/- 206.4	756.75 +/- 109.9	766.82 +/- 105.0	767.57 +/- 82.9	811.88 +/- 124.1	765.85 +/- 514.9	759.2 +/- 217.7
	High	920.70 +/- 171.7	860.80 +/- 143.2	865.10 +/- 141.6	863.7 +/- 152.6	938.57 +/- 169.7	973.42 +/- 117.2	910.80 +/- 198.6
<b>SDNN</b>	Low	103.4 +/- 55.7	102.51 +/- 61.2	78.03 +/- 37.5	88.43 +/- 46.3	89.57 +/- 42.4	100.7 +/- 37.0	109.49 +/- 49.4
	High	98.03 +/- 32.3	128.60 +/- 68.4	106.20 +/- 40.7	104.20 +/- 49.0	112.6 +/- 44.8	109.9 +/- 32.9	134.30 +/- 67.3
<b>Pnn50*</b>	Low	22.20* +/- 15.6	21.72* +/- 17.8	18.88* +/- 19.1	21.10* +/- 19.1	24.8*† +/- 18.4	29.6*† +/- 19.7	27.71* +/- 16.3
	High	34.7* +/- 18.2	33.56* +/- 18.6	34.37* +/- 20.0	32.62* +/- 20.8	36.7*† +/- 18.4	44.7*† +/- 15.9	41.98* +/- 16.9
<b>R-MSSD</b>	Low	82.40 +/- 50.5	89.91 +/- 69.2	61.70 +/- 46.2	64.28 +/- 37.3	67.98 +/- 41.9	81.4 +/- 43.4	83.78 +/- 43.4
	High	68.68 +/- 26.7	99.23 +/- 74.1	87.32 +/- 42.0	89.21 +/- 65.9	87.32 +/- 55.7	94.43 +/- 38.9	100.74 +/- 44.7
<b>LFnu</b>	Low	61.50 +/- 15.6	61.60 +/- 11.5	65.15 +/- 15.2	66.73 +/- 15.5	64.29 +/- 14.3	64.17 +/- 13.4	66.90 +/- 12.2
	High	58.90 +/- 14.4	65.60 +/- 8.8	57.11 +/- 13.8	59.50 +/- 13.5	55.82 +/-15.9	55.80 +/- 15.9	59.04 +/- 12.0
<b>HFnu</b>	Low	38.50 +/- 15.6	38.35 +/- 11.5	34.84 +/- 15.2	39.97 +/- 22.0	35.71 +/- 114.3	35.83 +/- 13.4	33.09 +/- 12.2
	High	41.09 +/- 14.4	36.27 +/- 10.3	42.90 +/- 13.8	40.47 +/- 13.1	39.58 +/- 10.9	44.18 +/- 15.9	40.96 +/- 12.0
<b>LF/HF ratio</b>	Low	2.36 +/- 2.26	1.85 +/- .93	2.49 +/- 1.7	3.09 +/- 2.9	2.32 +/- 1.25	2.29 +/- 1.6	2.55 +/- 1.7
	High	1.93 +/- 1.7	1.98 +/- .91	1.59 +/- .94	1.74 +/- .95	1.73 +/- .86	1.65 +/- 1.3	1.68 +/- .92

\* main effect of time as repeated measure; † different from rest (p<0.05); ‡ different from 4hr (p<0.05)

## CHAPTER 5 - DISCUSSION

This study was designed to examine the autonomic modulation of the heart following a high carbohydrate liquid meal in women with various body types. We hypothesized that overweight and obese individuals would have a higher sympathetic activity pre and post-absorption of a meal while healthy weight individuals would have a greater response in sympathetic activity following the meal.

The study sample included females ranging in BMI from 19.3-35.4kg/m<sup>2</sup> with a percent body fat ranging from 17-40%. The relative basal energy expenditure was determined by dividing their basal energy expenditure by their weight, resulting in a range from 8.32-12.17. Despite using the Harris Benedict Equation as opposed to gas exchange, the basal energy expenditure of the present subjects was comparable to the results of female subjects in other studies (Amatruda, Statt and Welle, 1993; Tentolouris, Tsigos, Perea, Koukou, Kyriaki, Kitsou, Daska, Daifotis, Makrilkis, Rapitis and Katsilambros, 2003). With respect to autonomic nervous system function, the primary outcome variable is the relative low-frequency power, which is of course also related to the low-to-high-frequency ratio. In comparison to previous studies (Bergholm, Westerbacka, Vehkavaara, Seppala-Lindross, Goto and Yki-Jarvinene, 2000; Laitinen, Vaukonen, Niskanene, Hartikainen, Lansimies, Uusitupa and Laakso, 1999; Tentolouris et al., 2003; Zahorska-Markiewicz, Kuagowska, Kucio and Klin, 1993), the values for the low-to-high-frequency ratio were slightly higher (for both the LOW rBEE and the HIGH rBEE groups) in the present study. An explanation for this may have been test anxiety among the participants. Unpublished data from our laboratory indicate that there may be some mild test anxiety that can be resolved by serial exposure. However, within-day reliability, even on the first day of exposure, has been quite high, with intraclass correlation coefficients in the range of 0.8-0.9 for the various HRV parameters.

Despite the high within-day reliability of the outcome variables, there are still a number of factors that are known to influence HRV, and our unpublished reliability data reflect the concern given to experimental control of many such issues including physical activity, caffeine and carbohydrate intake. Therefore, participants were given specific instructions to prepare for day two of testing. The instructions provided to the participants are included in appendix B.1.

In addition to this experimental control, we also attempted to manage individual differences in food intake of the day prior to testing through statistical control. A 24-hour food recall was collected and analyzed to assess CHO load consumed prior to the day of testing. The groups were not statistically different in terms of carbohydrate consumption.

With respect to the purpose of the study, we hypothesized that obese females would have a greater sympathetic modulation of the heart at rest and after the introduction of a high carbohydrate meal; however, the response to the meal would be blunted in comparison to the lean females' response. The results of the study do not support our hypotheses; however, the low power coupled with interaction effects approaching our a-priori alpha ( $p < 0.05$ ) suggest that we cannot rule out possible group by meal interactions.

More specifically, the LOW rBEE females had a greater SNS modulation of the heart at rest and throughout the testing period, which was observed in both the low-frequency band and the ratio of low frequency to high frequency. Although not statistically significant, there was a tendency for an early SNS response observed in the HIGH rBEE group and a more gradual response observed in the LOW rBEE females. Such a finding could be construed as consistent with data from Reed and Hill (1996), which indicate that lean females have an increase in TEF within the first 30 minutes and this response will last up to 120 minutes postprandial and that the thermogenic peak following a meal is different for obese and lean individuals. Non-obese

subjects show an earlier and higher peak than obese individuals. Additionally, they concluded that most people, regardless of weight, have a thermic effect of food lasting greater or equal to six hours; therefore, the shorter the duration of measurement the more likely the total thermic effect of food will differ between the two groups. The results of this project demonstrate that even 5 hours postprandial, the LOW rBEE females had not yet returned to baseline levels (Houde-Nadeau et al., 1993; Reed & Hill 1996). While low-frequency is a measurement of the sympathetic activity it is also a measure of parasympathetic activity; therefore, the ratio of low-frequency to high-frequency (LF/HF) is regarded as providing a better representation of sympathetic modulation. The LF/HF ratio data also suggest that HIGH rBEE females have less modulation from the SNS than do the LOW rBEE at rest. Additionally, the LOW rBEE group, tended to have a greater SNS response than do HIGH rBEE females following ingestion of a high carbohydrate meal; however this group by time interaction did not achieve statistical significance.

In order to assure the responses observed are that of only the SNS, parasympathetic activity must be ruled out. The pNN50 data are indicative of parasympathetic modulation of the heart. In the present study, the HIGH rBEE females had a stronger parasympathetic modulation of the heart throughout; however, unlike the differences observed in LFnu and LF/HF both groups of females had similar vagal responses to the meal. Therefore, the trend towards the group by treatment interaction on LF/HF appears to primarily reflect group differences in sympathetic-mediated responses to the meal.

There have been few studies within the last 3 years that have examined changes in autonomic modulation in response to a meal. Tentolouris et al. (2003) compared 15 lean and 15 obese females' responses to both a high carbohydrate meal and a high fat meal. The conclusions

support the hypothesis of this present study insofar as their obese females had a higher sympathetic modulation of the heart pre- and post- absorption of the meal and, the difference from resting was more predominant in the lean females. However, the study design ceased measurement at 3 hours postprandial, perhaps missing the entirety of the obese individuals' SNS responses.

The most recently published article on this subject was by Nagai et al. (2004), who took into account the duration of time in which the individual was obese. The conclusion was that boys who had been obese for less than or equal to 3 years had a more blunted response to a high carbohydrate meal than lean boys and those who had been obese greater than 3 years. However, this team of researchers chose to measure the SNS activity through the very low-frequency wave, which is representative of a broad array of potential stimuli and conditions, including hemorrhage, aortic constriction, acidosis, posture, congestive heart failure, and slow variations in breathing patterns and should not be the only frequency band observed to conclude the thermogenic SNS response (Berntson et al., 1997).

The present data extend these earlier findings in several different ways. First, we found that categorizing participants according to their estimated rBEE provided the greatest discrimination in autonomic responses to a meal. Uniquely, we observed a tendency for the participants with a low rBEE to have a sympathetic response to the meal by following the participants for a longer time period than used in previous studies. Finally, we observed a tendency for a group x treatment period interaction suggesting that rBEE is related to the autonomic response to a high carbohydrate meal. While projects of similar design concluded obese individuals have a blunted sympathetic activity following the ingestion of a high carbohydrate meal they did not observe the difference in the timing of the response as seen in the

present results and by Reed and Hill (1996). Tentolouris et al. (2003) and Nagai et al. (2004) presented a clean gradual increase in the LF/HF ratio in both the lean and obese subjects; however, the obese remained significantly lower in sympathetic activity for the duration of measurement. Reed and Hill (1996), presented the thermic effect of food is actually a series of peaks and valleys for a duration lasting up to six hours. As to the tendency for the blunted SNS activity in the LOW rBEE, Tentolouris et al. (2003), offer insulin and leptin levels as a potential explanation for the different responses observed in lean and obese females. Nagai et al. (2004) attribute the blunted sympathetic activity to insulin resistance and reduced endogenous carbohydrate oxidation in newly obese young boys.

While there is a debate among researchers as to the activity of the sympathetic nervous system, they do agree there is dysfunction in autonomic modulation. However, despite increasing evidence there is autonomic involvement in obesity, the mechanisms of this association are not understood. Insulin is a known stimulator of the SNS; therefore, the hyperinsulinemic state may be the cause of the enhanced sympathetic activity (Edmin et al., 2001; Kush et al., 1986; Park et al., 2000). Additionally, the response observed in the SNS activity following a meal is specific to the macronutrient content of the meal. The carbohydrate content of a meal is known to positively affect the thermic response to food; therefore, supporting the meal induced response effects of insulin that in turn influences the sympathetic nervous system (Labayen et al., 1999; Westerterp et al., 1998). Additionally, Tentolouris et al. (2003) concluded that sympathetic predominance following a meal is due to hyperinsulinemia shifting the sympathovagal balance, despite a higher (by~45%) increase in plasma insulin concentrations among obese females. Therefore, the blunted sympathetic activity following ingestion of a high carbohydrate meal would not be explained by hyperinsulinemia. Astrup et al. (1991) suggested that if the level of insulin secretion is

insufficient to overcome insulin resistance perhaps the stimulating effects of insulin will not occur. Bergholm et al. (2000) concluded that, independent of obesity, participants who are insensitive to insulin experience no change in sympathetic nervous system response within 120min of insulin infusion. Perhaps the delayed response of the sympathetic activity observed in the low frequency is due in part to the degree of insensitivity to insulin, and as insulin is utilized the sympathetic nervous system begins to respond. Whereas Bergholm et al. (2000) only measured the response to an insulin infusion for up to 2 hours, the results of the present study indicate that the LOW rBEE only begin to experience an increase in sympathetic activity 2 hours postprandial.

Another possibility is that autonomic dysfunction is the early pathophysiological change related to the development of insulin-resistance. Visceral fat is associated with increased blood pressure and insulin insensitivity; however, it appears as though the visceral fat contributes to the higher blood pressure values independently of insulin or leptin levels (Nunes Faria, Filho, Ferreira and Zanella, 2002). Lindmark et al. (2003, 2005) and Laitinen et al. (1999) researched the offspring of type 2 diabetics. Lindmark et al. (2003, 2005) concluded genetically predisposed type 2 diabetics had a larger amount of visceral abdominal fat and lower insulin sensitivity compared with controlled subjects (Lindmark et al., 2003, 2005). According to Laitinen et al. (1999) the offspring of Type 2 diabetics experienced significant changes in total power, HF and the LF/HF ratio of HRV in response to acute hyperinsulinemia. Laitinen et al. (1999) and Lindmark et al. (2003, 2005) both attribute the promotion of insulin resistance to an attenuated parasympathetic activity. This blunted modulation of the parasympathetic nervous system was observed in the present participants. When compared to the HIGH rBEE the LOW rBEE females

had a significantly lower vagal modulation; which may support previous findings described above.

If defects of the autonomic nervous system are the lone culprit of insulin insensitivity, then changes in weight and percent of fat should not be expected to result in changes of autonomic activity. However, Karason et al. (1999) concluded obese individuals have an elevated SNS activity and a withdrawal of vagal activity; however, following weight loss through dietary recommendations and or gastroplasty, these disturbances improve. Additionally, the investigation of the effects of an exercise training program on obese men and women, presented by Amano et al. (2001) revealed a significant increase in both sympathetic and parasympathetic activity, as well as an improved (i.e., lower) LF/HF ratio. Following the exercise program obese individuals experienced a decrease in BMI and percent body fat; however, no significant changes in lean body mass.

This lack of uniformed methodology results in the inability to attain a consistent conclusion among researchers. Therefore, perhaps continued research to determine the optimal methodology is required before clear conclusions can be made. The intention of the present study was not to settle the long lasting debate among researchers; however, despite the lack of power in the results, this project can benefit research of this field. As described in the literature review, the design of this project was developed based on key aspects of previous research. These key points include: providing a meal rich in carbohydrate content to ensure an observation of the thermic effect of food; serving a meal calorically proportional to the individuals' basal energy expenditure; collecting and analyzing the 24-hour food recall to rule out the potential impact of the carbohydrate load from the previous day; and the 5-hour duration in which power spectral

analysis was collected following the meal to make certain all changes in both lean and obese subjects were observed.

The duration of time in which HRV was measured proved essential and demonstrated that a longer duration may be necessary in order for obese individuals to return to the resting state. As noted in previous studies, even after 5 hours, the thermogenic response may not be fully terminated, leaving a portion of the response unmeasured (Houde-Nadeau et al., 1993; Reed & Hill, 1996; Schutz et al., 1984). Additionally, when observing the TEF a meal rich in carbohydrate content should be provided to subjects (de Jonge, et al 1997; Nagai et al., 2004; Tentolouris et al., 2003; Welle, 1995).

Despite clear intentional design, this project is not without limitations. The poor statistical power is the result of inadequate recruitment of obese females. The method that proved most successful was offering financial compensation for the participants' time. Furthermore, it was the experience of this project that females within the ages of 18-35 who were obese refrained from volunteering.

Admittedly the desirable method to determine body composition is the dual-energy x-ray absorptiometry (DEXA); the availability and low cost of skin fold calipers were appealing qualities of this method to determine the participant's percentage of body fat. However, this measurement technique, as well as the difficulty in measuring the skin fold thickness of obese participants, resulted in some degree of error in this data point (Lee & Nieman, 1996). Finally, an acclimatization day may serve to further reduce variability in the data. While within-day reliability, even on the first day of exposure to HRV testing is high, group differences may be difficult to detect if groups are approaching a ceiling effect secondary to test anxiety.

The need for research and answers in the pathology of obesity are of obvious concern. The rate at which obesity is becoming more prevalent, the complications of comorbidities and the overall cost to Americans makes this disease a priority for everyone. Research on the activity and reactivity of the sympathetic nervous system will aid in both lifestyle modification and medicinal therapies. Research that will focus on the resting SNS activity may offer insight into potential medications to treat the disease. Continued research on the SNS activity following the introduction of a meal or a bout of exercise will lead to finding the most effective diet and exercise prescription to promote weight loss. Future studies should include laboratory tests such as fasting blood tests, along with postprandial capillary blood glucose monitoring to allow for the observation of other physiological responses, including any potential glucose intolerance. It would also be of interest to include a detailed inquiry into the participant's family history of obesity and type 2 diabetes as well as the participant's own personal weight history.

The autonomic state of obese individuals remains a question. Further testing of the autonomic nervous system will provide information about the cardiovascular risk among obese individuals. That is, while we have had trouble making a clear direct link between obesity and heart disease, perhaps we can with greater specificity identify risk of heart disease among obese individuals according to their adrenergic state. For now, this area of interest remains a question in research; no clear conclusions have been made to change the medical and therapeutic treatments for obesity.

In conclusion, with an adequate number of participants we theorize that in the pre- and post-absorptive state, individuals with a LOW rBEE are more hyperadrenergic when compared to HIGH rBEE individuals, which may signify an adverse cardiovascular risk, and more so

supports the evidence that when observing the SNS response to a meal in various body types a duration of greater than five hours postprandial is necessary.

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## APPENDIX A CONSENT FORM

### 1. Title

Autonomic modulation of the heart following a high carbohydrate meal in various body types.

### 2. Performance Sites

Data collection will take place on the campus of Louisiana State University in the Huey P. Long building and/or St. James Place.

### 3. Contacts:

Robert H. Wood Principal Investigator 578-9142  
Calynn Davis (project coordinator)  
Home phone – 766-9261  
Work phone – 763-0902  
E-mail address – cdavis5@lsu.edu

### 4. Purpose of the Study:

The purpose of this research study is to observe the different responses to a high carbohydrate meal in varying body types.

### 5. Subjects:

#### A. Inclusion Criteria Subjects must be:

- Between the ages of 18 and 35
- Female
- Not pregnant
- Willing to perform a treadmill fitness test
- Eat a high carbohydrate breakfast of orange sunflower seed bars consisting of 25% fat, 62% carbohydrates, and 13% protein.

#### B. Exclusion Criteria

- Food allergies
- Smokers
- Diabetics
- Pregnant women
- Ischemic heart disease
- Cardiomyopathies
- Peripheral vascular diseases
- Heart problems
- High blood pressure
- Use of prescription or over the counter medications

Maximum number of subjects: 50 subjects

6. Study Procedures:

As a subject you will be requested to come to the laboratory on two occasions, separated by no more than 1 week.

**Day one:** Day one of data collection will require about 1 hour of time and will include a questionnaire to assess your routine physical activity, body composition and a fitness test that will require you to walk on a treadmill. Body composition will be measured by examining the thickness of skin folds measured at four sites: the front and back of the arm, near the shoulder blade, and at the hip. In addition weight, height, and waist and hip measurements will be obtained. For the treadmill test, the speed and incline of the treadmill will begin at a low level and will gradually increase during the test. During the test the investigator will measure heart rate and blood pressure, and you will be asked to rate the difficulty of the exercise. The test will be stopped before the workload reaches what the tester considers to be a "vigorous" level. Additionally, the test may be stopped due to unusual heart rate or blood pressure readings, or if you request to stop. In addition to testing on this day, you will receive instructions for preparation of day 2 data collection.

**Day 2:** You will be asked to report to the testing facility at approximately 7am. Day two will require you to answer questions about the foods eaten the day before, eat a high carbohydrate meal replacement, such as ENSURE *Fiber* and include the collection of an electrocardiogram (ECG). The high carbohydrate meal will consist of 21% fat, 66% carbohydrate and 14% protein. The ECG will require that three foam and gel electrodes be placed on your chest. Data from the ECG will be collected for the ingestion of the meal and for a total duration of 5 hours. Data collection of day two will end at approximately 1:15pm.

7. Benefits:

Participants will gain important information on their fitness level, percent body fat, as well as instruction in the use of the food guide pyramid.

8. Risks/Discomforts:

The potential risks associated with this study include:

1. Exercise is associated with extremely low risks of adverse events. However remote, there is risk of heightened blood pressure, abnormal heart rhythms, heart attack, stroke, and death. In addition physical activity may result in muscle strains, and other injuries as a result of falls. 2. In addition there is a remote possibility of stomach discomfort following the meal.

9. Measures taken to reduce risk

Proper screening, instruction, and supervision, as well as the ability of the subject to stop the test at any time will help to minimize the risks associated with a treadmill exercise test. Specific questions will be asked to determine the risk of discomforting side effects following the breakfast meal.

10. Right to Refuse:

Participation in this study is voluntary, and subjects may withdraw from the study at any time without penalty.

11. Privacy:

All information will be kept confidential. While publications may result from your participation in this study, you will not be identified by name.

12. Financial Information:

There will be neither compensation nor cost to the participant for participating in this study.

13. Withdrawal:

Participation in this study is voluntary, and subjects may withdraw from the study at any time without penalty.

14. Removal: The investigators also have the right to remove you from the study. Examples of conditions for which subjects may be removed include:

- Not following pre-test instructions
- Missing scheduled appointments

Removal from this study will not prejudice my standing with Louisiana State University.

The study has been discussed with me and all my questions have been answered. I may direct additional questions regarding study specifics to the investigators. If I have questions about subjects' rights or other concerns, I can contact Robert C. Mathews, Chairman, LSU Institutional Review Board, (225)578-8692. I agree to participate in the study described above and

acknowledge the researchers' obligation to provide me with a copy of this consent form if signed by me.

\_\_\_\_\_  
Signature of Participant

\_\_\_\_\_  
Date

\_\_\_\_\_  
Witness Signature

\_\_\_\_\_  
Date

APPENDIX B DATA COLLECTION FORMS

**Appendix B.1 Screening visit questionnaire**

Name \_\_\_\_\_

Weight \_\_\_\_\_ Height \_\_\_\_\_

BMI \_\_\_\_\_

Questions:

1. On most days of the week are you usually awake by 7:30am? \_\_\_\_\_
2. Are you currently taking any medications? \_\_\_\_\_
  - a. If so what are you taking?
3. Do you have any known food allergies? \_\_\_\_\_
4. Do you smoke? \_\_\_\_\_
5. Are you pregnant? \_\_\_\_\_
6. Are you between the ages of 18-35? \_\_\_\_\_
7. Do you have any of the following health conditions: Hypertension, Diabetes, Ischemic heart disease, Cardiomyopathies, or history of Peripheral vascular diseases? \_\_\_\_\_
8. What was the date of your last menstrual cycle? \_\_\_\_\_

BEE \_\_\_\_\_

40% BEE \_\_\_\_\_ kcal

**Appendix B.2 YMCA sub-max data entry form**

Subject \_\_\_\_\_

85% HR max \_\_\_\_\_

Age \_\_\_\_\_

1 stage	150 kgm/min (.5kg)
---------	--------------------

	HR < 80	HR 80-89	HR 90-100	HR > 100
2 <sup>nd</sup> stage	2.5 kg	2.0 kg	1.5 kg	1.0 kg
3 <sup>rd</sup> stage	3.0 kg	2.5 kg	2.0 kg	1.5 kg
4 <sup>th</sup> stage	3.5 kg	3.0 kg	2.5 kg	2.0 kg

**Stage 1**

0-1 minute	HR:	RPE:
1-2 minute	BP:	
2-3 minute	HR:	RPE:

**Stage 2**

3-4 minute	HR:	RPE:
4-5 minute	BP:	
5-6 minute	HR:	RPE:

**Stage 3**

6-7 minute	HR:	RPE:
7-8 minute	BP:	
8-9 minute	HR:	RPE:

**Stage 4**

9-10 minute	HR:	RPE:
10-11 minute	BP:	
11-12 minute	HR:	RPE:

**Rest**

12-13 minute	HR:	RPE:
13-14 minute	BP:	RPE:
14-15 minute	HR:	RPE:

### **Appendix B.3 Rules for day 2 data collection**

#### Rules for Day 2 of data collection

1. No caffeine for 24 hrs prior to testing.
2. No alcohol for 48 hrs prior to testing.
3. No food or beverages, other than water, for 12 hrs before testing (7:00pm the night before).
4. Be sure your food intake the day before testing is “typical.”
5. Refrain from vigorous exercise for 24 hrs before testing.
6. Dress comfortably, you may want to pack a sweatshirt.
7. Bring reading material if desired.

Date of Day 2 Data Collection: \_\_\_\_\_ Time \_\_\_\_\_





## APPENDIX C ENSURE

**Vanilla:** -D Water, maltodextrin (corn), sugar (sucrose), sodium and calcium caseinates, high-oleic safflower oil, canola oil, soy protein isolate, oat fiber, corn oil, fructooligosaccharides (FOS), soy fiber, calcium phosphate tribasic, magnesium chloride, potassium citrate, cellulose gel, soy lecithin, potassium phosphate dibasic, sodium citrate, natural and artificial flavors, magnesium phosphate dibasic, choline chloride, ascorbic acid, cellulose gum, potassium chloride, carrageenan, ferrous sulfate, alpha-tocopheryl acetate, zinc sulfate, niacinamide, manganese sulfate, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, pyridoxine hydrochloride, riboflavin, folic acid, chromium chloride, biotin, sodium molybdate, potassium iodide, sodium selenate, phylloquinone, vitamin D3 and cyanocobalamin.

Chocolate flavor contains 2.4 mg of caffeine per 8-fl-oz serving.

<b>Nutrition Facts</b>	
Serving Size 1 can (8 fl oz)	
Amount Per Serving	
<b>Calories</b> 250	Calories from Fat 50
% Daily Value*	
<b>Total Fat</b> 6g	<b>9%</b>
Saturated Fat 0.5g	<b>3%</b>
<b>Cholesterol</b> <5mg	<b>&lt;2%</b>
<b>Sodium</b> 200mg	<b>8%</b>
<b>Potassium</b> 370mg	<b>11%</b>
<b>Total Carbohydrate</b> 42g	<b>14%</b>
Dietary Fiber 3g	<b>12%</b>
Soluble Fiber 1g	
Sugars 12g	
<b>Protein</b> 9g	<b>18%</b>
Vitamin A 25% • Vitamin C 50% • Calcium 35% • Iron 25%	
*Percent Daily Values based on a 2,000 Calorie diet.	



APPENDIX D TABLE OF LITERATURE

Table 4 Obese are hyperadrenergic

Year	Authors	Subjects	Purpose	Method	Results
1982	Sowers	10 borderline hypertensive obese (5 female & 5 male, 23-55yr, BW $\geq 25\%$ IBW) 12 age, sex race matched normotensive nonobese	To compare basal levels of BP, pulse rate, PRA and catecholamines and to examine serial changes in responses to upright posture and isometric handgrip exercise in pt during 8 wk weight reduction of obese and nonobese individuals.	Blood samples taken for measurement of NE, E and PRA	Obese individuals had a higher supine plasma NE, E, and PRA levels as well as greater responses to upright posture and isometric handgrip exercises than nonobese. After wk 2 of weight reduction, supine plasma NE, E and MAP decreased; after 8 wks PRA decreased as well in the obese subjects.
1983	Baessard	6 nondiabetic obese women and 6 control lean women	Compare 24 hr EE and postprandial thermogenesis of obese and lean women. Observe changes in thermogenic response after weight loss in obese women	Respiratory chamber (24-h EE) circuit indirect calorimetry hood (thermogenic response to mixed meal) 5 of the 6 obese were re-tested following a mean weight loss of 12.1 kg. Urinary catecholamines	In absolute terms: 24-h EE and basal EE were significantly greater in obese women. After weight loss both total 24-h EE and basal were significantly lower, however, still greater than controls. Thermogenic response to a mixed meal was significantly reduced in obese when compared to controls. After weight loss thermogenic response remained lower, this unchanged response suggests the thermogenic defect may be a cause rather than consequence of obesity. Epinephrine was similar in both groups. Following weight loss obese E excretion increased although remaining similar to control. NE was significantly elevated in obese b/w weight loss. Weight loss caused a significant reduction in NE excretion.
1991	Troisi	572 Males from the Normative Aging Study (age = 62.2 + 7.9) (BMI = 26.5 + 3.5)	Cross-sectionally assess the relation of obesity, with fat distribution, dietary intake, insulin, glucose levels, and SNA	Anthropometrics; semiquantitative food frequency questionnaire; urine sample; blood pressure	Supports the presence of increased sympathetic activity in obese individuals.
1992	Astrup	8 obese women and 8 matched control women	Study the mechanisms underlying the proposed enhancing effect of high-CHO diet on 24-h EE, substrate oxidation rates, and indexes of SNS activity before and after weight normalization, and to compare these findings to a matched control group	Respiratory chamber (24-h EE) Plasma concentrations of NE Prescribed conventional low fat, high protein, high CHO diet (4.2 MJ/day). Once reached targeted weight, weight stability for 7 wks was required before retest	24-hr EE decreased from obese to post obese; however, remained higher than control group = plasma NE was 50% higher in post-obese women when compared with control. Obese women on high CHO, low fat diet = high SNS activity, which is responsible for higher 24-hr EE, & HR.
1993	Balkan	Male Wistar rats (320-340g, ~12wks old)	Investigate the metabolic consequences of the development of obesity	Infusion of catecholamines & exercise tests performed in beginning & end of 5 wk overfeeding prd. On the 35th day rats were killed and carcass analysis performed	Overfeeding induces changes in sympathetic control of metabolism and insulin secretion. Elevated basal NE reflected increase in EE; however, pattern of nutrient mobilization during exercise is directed toward sparing of fats.
1993	Zahorska - Markiewicz	3 groups of females; 20 obese (23-46); 15 obese (21-57); 18 healthy (22-39)	Compare HRV in obese and lean women	HRV	Obesity is associated with a decrease in PNA and stimulation of the SNA
1993	Spraul	25 Pima Indians (age = 26+6) 19 Caucasian males (age = 29+5)	To measure the sympathetic neural outflow and its relationship to EE and body composition in Caucasians compared with Pima Indians	Underwater weighing, microneurographic recording of MSNA, open circuit indirect calorimetry chamber used to measure 24hEE and sleeping MR.	While MSNA is positively related to body fatness and EE in Caucasians it is not in Pima Indians. In addition Pima Indians have a lower resting MSNA when compared with Caucasians.
1994	Scherer	37 subjects (19 women, 18 men; age 31 + 1yr; BMI= 26.4 + 1.0kg/m <sup>2</sup> ; % body fat = 25.0 + 1.6%)	To examine the potential function of the sympathetic nervous system as the mechanism responsible for obesity related cardiovascular events.	Intraarterial microelectrodes were used to measure sympathetic nerve discharge, calf vascular resistance, and energy expenditure (EE).	Body fat is a major determinant of the resting rate of muscle SNA. The obese subjects (BMI > 27) had almost twice the firing rate of sympathetic discharges when compared to the lean subjects.

1995	Leibel	18 obese, BMI > 28 (11 women, 7 men; 29 ± 10yrs age) 23 nonobese subjects (7 women, 16 men 26 ± 10yrs age)	Examine the components of EE during maintenance of usual and altered BW in obese and nonobese	Ventilated hood	A 10% increase or decrease in the usual weight was accompanied by a 16% increase or 15% decrease, respectively, in 24-h EE. Total EE and resting EE were significantly higher in obese than nonobese, and thermic effect of feeding was lower in obese than nonobese.
1995	Grassi	8 Lean and 10 Obese Normotensive Young Males	Determine if MSNA is modified in obesity in the absence of bp elevation	Microneuro-graphic Technique	Obesity is related to increased activation of the SNS even among normotensive individuals
1996	Gao	16 obese (BMI = 32.9 ± .9) Caucasian women (54.3 ± 1.6yrs age)	Characterize ANS activity using HRV in obese, post-menopausal women	HRV	Cardiac autonomic function varies in women depending on their regional body fat distribution. An increase in cardiac autonomic activity was observed in upper body obesity.
1996	Grassi	Review article	Review evidence that human obesity is characterized by abnormalities in sympathetic cardiovascular control		Sympathetic activity is a common hallmark of the obese state.
1996	Ward	Subjects chosen from the Normative Aging Study: 752 men age 21-80, all Caucasian	Investigate the relationship of insulin level, NE excretion, and obesity to blood pressure.	24-h urine catecholamine concentrations, Fasting and 2h after 100g oral glucose load- serum insulin and glucose	Insulin level and SNA are associated with hypertension. Results consistent with the hypothesis that the interactive effects of hyperinsulinemia and increased SNA may contribute to hypertension.
1997	Vaz	37 Males (age = 18-50) (BMI = 19.6-35.5)	Whole-body NE spillover and regional NE spillover across the heart, hepatomesenteric bed and kidney	Isotope dilution method	Increase SNA in kidneys, reduced SNA in the heart and overall normal tone in the splanchnic bed when comparing lean and obese individuals
1997	Minami	20 pts w/ essential hypertension (53.8 ± 2.3 yrs; 12 men & 8 women)	Evaluate ANS function by HRV in salt-sensitive and non-salt-sensitive pts w/ essential hypertension under low and high salt intakes	HRV (obtained 48x in 24hr)	Salt sensitive pts had a blunted response of the PNS and SNS to dietary salt maneuvers.
1998	Grassi	20 obese (14 men, 6 women; BMI = 30-45 kg/m <sup>2</sup> ; aged 22-41)	The aim was to establish the effect of weight reduction on the SNA, in normotensive individuals. Additionally, the aim to determine whether a loss of body weight will improve the baroreceptor sympathetic reflex, and to determine possible peripheral or central sympathetic deactivation caused by such a loss of body weight.	10 subjects participated in a 16 week weight loss regimen. Blood pressure measured by sphygmomanometer. Sympathetic nerve traffic recorded via efferent postganglionic sympathetic nerve activity. Additional measurements include, plasma norepinephrine, urinary sodium and potassium content, insulin sensitivity, arterial baroreflex and cold pressor test.	Normotensive obese individuals displayed norepinephrine and MSNA values much greater to those age-matched lean normotensive individuals. Accompanying the weight loss due to the hypocaloric diet, individuals experienced a decrease in plasma norepinephrine as well as a lower MSNA when compared to original values. Therefore providing evidence that SNS activation accompanied by obesity is reversible with weight loss.
1999	Grassi	Review article	Debating sympathetic overactivity as a hallmark of human obesity: a pro's position		Obese individuals have a higher sympathetic activity, however, the activity of the SNS is heterogenic. Depending on the method of measuring SNS activity, at first glance one may conclude obese individuals to be hypoadrenergic. While skin and pulmonary circulation can be within a normal adrenergic drive, cardiac, renal, cerebral and muscle sympathetic tone can be enhanced.
2000	Park	Male Wistar rats (70-80g)	Analyze R-R interval variability in controls and diet-induced obese rats.	10wk high-sucrose diet, HRV(5min period), after sacrificing the rats, insulin, glucose, and leptin were withdrawn by cardiac puncture	After diet induced obesity, the rats had high leptin levels and increased SNA; however, when LF/HF is adjusted for leptin, the SNA was lower in the diet induced obese rats, suggesting leptin resistance.
2000	Weyer	42 White Males and 77 Pima Indian Males (age = 18-50)	The interrelation between adiposity, insulinemia, SNS activity, BP may differ between Pima Indians and whites.	Underwater weighing, glucose tolerance test, microneurography	The role of hyperinsulinemia and increased SNS activity as links between obesity and BP can differ between different ethnic groups. An increase in adiposity was associated with increased MSNA which was positively correlated with HR and BP in whites.
2001	Erdin	21 obese and 17 lean	Determine whether the hemodynamic and ANS changes among obesity are due to hyperinsulinemia or insulin resistance. Additionally weight reduction was used to test if ANS abnormalities were reversible.	Electrical bioimpedance, oral glucose tolerance test, Valsalva maneuver, HRV	Among obese: parasympathetic withdrawal and sympathetic activity coincide with hyperinsulinemic hours; LF/HF increased in proportion to hyperinsulinemia independent of BMI; following weight loss a reduction in hyperinsulinemia resulted causing a reversal of hemodynamic and HRV changes.

**Table 5 Obese are hypoadrenergic**

Year	Authors	Subjects	Purpose	Method	Results
1984	Schulz	20 obese women and 8 non-obese (19-44yrs age)	Evaluate the overall thermogenesis response induced by 3 meals over a whole day, continuously measuring EE	Respiration chamber 24hrs, urinary catecholamines	Diurnal urinary NE was lower in obese than controls. An inverse relationship between body fat and diet-induced thermogenesis was found. The magnitude of daily thermogenesis is blunted in obese.
1986	Kush	5 obese & 5 lean male Pima Indians (b/w ages 19-36)	To assess whether thermogenesis or SNS function might differ b/w lean and obese humans	Thermogenesis assessed via indirect calorimetry (TEF = 3.5hrs following 800kcal ensure plus, TEE seated on stationary bike after pedaling for 5 min) and SNA by plasma levels of NE. Thermic responses to NE evaluated during multiple dose infusions (30 min at 4 different doses-.05, .10, .15, .20). The lean and obese were tested on 2 occasions- weight maintenance, overfeeding and only obese following underfeeding	Conclusions apply only to Pima Indians: BEE was equivalent during weight maintenance and increased by 3% in overfeeding in both groups. Thermic responses to exercise and test meal were similar in both groups; however, thermic responses to NE infusion fell during over feeding to a greater degree in obese than lean. Raise the possibility that overfeeding in obese Pima Indians may limit the contribution of sympathetically mediated thermogenesis to EE.
1991	Astrup	50 obese (36.7 ± 1.5yrs age, BMI = 22.3 ± .7), 12 normal wt (32.2 ± 2.2yrs age, BMI = 37.8 ± .8)	Measure the plasma noradrenaline concentration in the fasting state and after 75g of oral glucose in simple obese, NIDDM obese, and normal control.	Blood samples taken for measurement of plasma glucose, insulin and catecholamine concentrations	Obese patients had a 22% lower fasting plasma NA concentration, and an 18% lower NA concentration following oral glucose when compared to control subjects. NA concentration was inversely related to degree of obesity. NA also inversely related to plasma insulin and plasma glucose concentration.
1991	Bray	Review	Identify 4 common pathways involved in each model of obesity		MONA LISA hypothesis
1997	Vaz	37 Males (age = 18-50) (BMI = 19.6-35.5)	Whole-body NE spillover and regional NE spillover across the heart, hepatomesenteric bed and kidney	Isotope dilution method	Increase SNA in kidneys, reduced SNA in the heart and overall normal tone in the splanchnic bed when comparing lean and obese individuals
1999	Somers	Review	Debating sympathetic overactivity as a hallmark of human obesity: an opposing position		Overall SA is not heightened in obese normotensive, and SA is not any higher in obese hypertensive as lean hypertensive

**Table 6 Thermic Effect of Food**

Year	Authors	Subjects	Purpose	Method	Results
1983	Bessard	12 healthy normotensive women, classified into 2 groups (6- nondiabetic obese women with childhood hx. of obesity: 152±6%IBW and 39.1±1.1%fat) (6-lean, no family hx: 99± 2% IBW, 24.2±.7%fat)	compare the 24h EE and postprandial thermogenesis measured for 5h after mixed meal (82kcal/100g formula, 17%PRO, 54% CHO, 29% fat, energy level: 60% BEE) between lean and obese. 5 obese reinvestigated after diet induced weight loss	Ventilated hood Urine catecholamines	The greater 24h EE of obese women is due to their increased BMR. Obese experienced a lower thermogenic response which supports thermogenic defect (nocturnia NE higher in obese, however NE response to meal was lower in obese). Following weight loss obese TEF did not improve suggesting thermogenic defect is a cause not a consequence of obesity.
1984	Schutz	Evaluate the overall thermogenic response induced by 3 meals over entire day.	20 obese women 8 non obese women (age b/w 19-44)	ventilated hood and respiration chamber, urine catecholamine	Thermogenic response blunted in obese when compared to lean. Urinary excretion of NE during the date and the day was significantly greater in obese than controls
1987	Schwartz	20 healthy weight stable males (age 33.3± 8.4) (126±19% IBW)	Investigate the effects of an 800kcal high CHO meal and non-caloric water meal on both plasma NE and EE	Plasma catecholamine; Ventilated hood	EE was significantly greater when compared to baseline at minutes 60 and 75, while NE concentrations was significantly increased at 30 and 45 minutes.
1993	Houde-Nadeau	Assess the reliability and variability of TRF	8 (4 men and 4 women)	Ventilated Hood (6hrs) Standard meal: 3272kJ (44% CHO, 16% PRO, 40%fat)	Intra-individual variability contributes little to the variability in TRF studies, provided measurement period lasts at least 6hrs.
1997	DeJonge	Determine the role of ANS in obligatory and facultative components of TRF	19 lean (age: 18-35; BMI: 18-25)	Ventilated hood, propranolol infusion (inhibit SNS), atropine infusion (inhibit PNS)	SNS necessary for facultative component of TRF. PNA responsible for gastric emptying
1998	Labaven	Examine the influence of high fat meal vs. high CHO meal on substrate oxidation, nutrient induced thermogenesis.	18 women (age 20-27) (BMI: 21.6 ± 4)	HC test meal: 18%PRO, 80%CHO, 2%fat. HF test meal: 17.5% PRO, 15%CHO, 67.5% fat. Ventilated hood, urinary nitrogen, blood glucose test strip, HR (Partner, Cardiosport)	Increase in TEF is mainly due to CHO. Surplus of dietary CHO of preceding days together with large load of CHO can exceed glycogen storage capacity and trigger net lipid synthesis.
1999	Westertep	Investigate the effects of diets of extreme macronutrient composition for an entire day	8 healthy females between ages 23-33 (BMI: 23.3±2.5)	respiratory chamber	Macronutrient composition has a direct impact on dietary induced thermogenesis. High PRO and High CHO induces a greater thermic response
2000	Patel	Compare isotope dilution method with arteriovenous sampling technique to est. NA spillover in subcutaneous adipose tissue in response to meal	2 female 5 male lean healthy Caucasians	Blood samples taken: 30, 20, 10 min immediately b/w meal (15kcal/kg LBM, 50%CHO, 30%fat, 20%PRO) and again at 60, 80, 100, and 120 min after meal	Adrenaline decreased following the meal while NA increased, therefore contributing to TEF. Findings suggest, for the first time, SNS activity increases postprandially in adipose tissue.
2001	Matsumoto	16 age and height- matched obese and non-obese young women	Compare the ANS activities under resting and postprandial conditions in obese and non-obese women	HRV (35min)	Resting SNA is identical in obese and non-obese; however, postprandially obese possess a reduced sympathetic response.

**Table 7 HRV**

Year	Authors	Subjects	Purpose	Method	Results
1991	Hirsch	5 Female 2 Male, mean age 29, BMI range 23.4-49.9 mean BMI 37.3	Measure autonomic function during experimentally induced changes in body weight and energy storage.	HRV	With a 10% increase in baseline weight there was a decline in parasympathetic activity accompanied by an increase in HR. HR declined with weight reduction, but HRV did not significantly change.
1993	Zahorska - Markiewicz	3 groups of females: 20 obese (23-46); 15 obese (21-57); 18 healthy (22-39)	Compare HRV in obese and lean women	HRV	Obesity is associated with a decrease in PNA and stimulation of the SNA
1996	Gao	16 obese (BMI = 32.9 ± .9) Caucasian women (54.3 ± 1.6yrs age)	Characterize ANS activity using HRV in obese, post-menopausal women	HRV	Cardiac autonomic function varies in women depending on their regional body fat distribution. An increase in cardiac autonomic activity was observed in upper body obesity.
1997	Minami	20 pts w/ essential hypertension (53.8 ± 2.3 yrs; 12 men & 8 women)	Evaluate ANS function by HRV in salt-sensitive and non-salt-sensitive pts w/ essential hypertension under low and high salt intakes	HRV (obtained 48x in 24hr)	Salt sensitive pts had a blunted response of the PNS and SNS to dietary salt maneuvers.
2001	Emdin	21 obese and 17 lean (no age or sex given)	Determine whether the hemodynamic and ANS changes among obesity are due to hyperinsulinemia or insulin resistance. Additionally weight reduction was used to test if ANS abnormalities were reversible.	Electrical bioimpedance, oral glucose tolerance test, Valsalva maneuver, HRV	Among obese: parasympathetic withdrawal and sympathetic activity coincide with hyperinsulinaemic hours; LF/HF increased in proportion to hyperinsulinemia independent of BMI; following weight loss a reduction in hyperinsulinemia resulted causing a reversal of hemodynamic and HRV changes.
2001	Matsumoto	16 age and height matched obese and non-obese young women	Compare the ANS activities under resting and postprandial conditions in obese and non-obese women	HRV (35min)	Resting SNA is identical in obese and non-obese; however, postprandially obese possess a reduced sympathetic response.
2003	Tentolouris	15 lean 15 obese (females matched for age)	Compare changes of HRV in lean and obese after consumption of a high CHO and high Fat meals	HRV	Lean females have a greater cardiac SNS activation following a CHO-rich meal than obese females; whereas a high fat meal does not result in any change in either body type.
2005	Nagai	10 obese 13 lean (boys aged 6-11yrs)	Explore the TEF and thermoregulatory SNS activity in response to a high CHO and a high fat meal in obese boys, with respect to duration of obesity.	Spectral analysis, VO2max	Especially during the early phase of obesity, obese boys possessed normal metabolic and sympathetic responses to a high fat meal but showed a diminished thermogenic response to the high CHO meal.

APPENDIX E RAW DATA

Table 8 Participants raw data. Key for abbreviations on table found on page 61.

IDAGE	YMCA sub-max stress test														24-hour Recall							
	Rst sys	Rst dys	WT	HT	BMI	Total BEE	rBEE	CANS	%fat	WS HPStg	HRmax	Sys max	Dys max	RPESTOP	KCALs	PRO	FAT	CHO	% CHO			
1	26	98	58	144	67.00	22.5	1450.00	41.32	2.25	28.80	.79	3	170	140	80	17	85%	1039	37.70	40.10	130.70	
2	25	104	70	141	65.25	23.0	1456.43	44.02	2.25	25.00	.73	4	168	140	82	17	85%	1779	77.70	84.20	184.00	.41
3	22	98	60	149	66.00	24.0	1508.67	43.94	2.50	23.00	.73	4	132	120	80	13	end	1486	63.40	48.20	219.10	.59
4	24	108	82	136	63.00	24.0	1414.00	33.99	2.25	23.75	.71	2	170	150	100	15	85%	3550	109.60	151.80	441.50	.50
5	24	100	60	141	62.75	25.2	1433.00	27.04	2.25	29.90	.76	3	180	140	95	16	85%	1428	86.80	67.50	128.40	.36
6	27	102	75	170	66.00	27.5	1563.00	23.78	2.50	34.00	.86	3	170	150	90	17	85%	2031	75.00	94.70	225.70	.44
7	30	130	80	166	64.50	28.0	1522.00	40.32	2.50	38.67	.79	2	170	142	80	13	85%	1436	73.00	66.10	142.70	.40
8	28	110	82	153	62.00	28.0	1462.00	25.89	2.25	23.70	.92	3	140	150	75	15	quit	829	24.80	29.20	128.90	.62
9	21	120	80	189	67.00	29.7	1683.00	28.14	2.75	34.40	.80	4	170	160	90	15	85%	1451	72.50	69.20	143.70	.40
10	25	110	80	166	62.00	30.4	1607.00	21.86	2.50	34.40	.87	3	108	120	90	19	quit	915	66.00	31.90	95.80	.42
11	23	108	80	209	68.00	31.8	1776.00	22.22	2.75	38.88	.96	4	180	160	85	13	85%	1846	91.70	80.80	183.80	.40
12	21	108	68	170	61.00	32.1	1587.00	21.90	2.50	42.01	.75	3	180	142	80	16	85%	1338	43.20	53.80	174.70	.52
13	20	118	78	220	66.00	35.4	1826.00	17.65	3.00	37.99	.78	3	170	160	90	20	85%	1380	70.20	56.40	153.90	.45
14	21	94	60	109	63.00	19.3	1318.00	60.46	2.00	20.00	.77	3	170	120	80	14	85%	2022	94.40	53.00	313.00	.62
15	20	98	60	114	63.50	19.9	1365.00	63.69	2.00	18.80	.72	4	175	120	80	15	85%	1401	74.70	33.00	223.10	.64
16	19	98	70	118	64.25	20.0	1380.00	68.79	2.25	17.00	.74	4	168	120	80	16	quit	1080	57.20	58.80	83.90	.31
17	18	80	60	111	63.25	20.0	1345.30	57.97	2.00	21.00	.68	4	186	110	60	12	85%	No Data	No Data	No Data	No Data	No Data
18	25	108	75	126	66.00	20.3	1379.00	67.41	2.25	16.30	.73	3	190	135	70	18	85%	1378	48.10	41.20	207.90	.60
19	20	115	80	122	64.00	20.9	1369.00	67.19	2.25	16.70	.74	3	170	120	80	12	85%	1743	52.80	79.10	218.20	.50
20	20	95	65	132	66.50	20.9	1512.00	70.11	2.50	16.40	.68	4	180	120	80	14	85%	1244	44.30	35.70	193.30	.62
21	20	100	60	113	61.75	21.5	1352.00	62.97	2.00	19.00	.81	3	180	150	80	12	85%	1722	87.70	51.70	231.90	.54
22	27	104	68	127	64.00	21.8	1341.00	55.87	2.00	18.90	.74	3	170	140	110	19	85%	1999	76.80	59.80	300.40	.60
23	31	100	80	114	60.00	22.2	1294.00	59.03	2.00	25.80	.74	2	160	150	120	18	85%	1547	113.60	27.70	219.60	.57
24	22	90	60	111	58.00	23.0	1306.30	44.19	2.00	25.00	.72	3	131	120	75	15	quit	1049	21.60	29.70	179.30	.68
25	21	97	60	128	62.00	23.5	1403.50	47.07	2.25	24.00	.72	4	168	160	100	15	quit	993	49.30	44.60	112.40	.45
26	20	98	60	143	65.00	23.5	1487.30	34.96	2.25	23.80	.78	4	144	168	80	14	end	1595	71.60	52.00	215.50	.54
27	21	92	75	124	63.00	22.0	1390.82	43.70	2.25	19.00	.74	4	156	140	70	13	85%	1858	62.00	55.10	289.70	.62
28	20	106	80	142	65.00	23.7	1490.00	39.75	2.25	26.40	.75	3	160	130	90	19	quit	723	25.30	16.40	123.70	.68
29	24	115	80	260	68.00	39.5	1993.00	45.69	3.25	43.43	.81	3	170	168	100	17	85%	2319	112.60	98.50	243.70	.42

LOW rBEE

HIGH rBEE

excluded

ID	BAECKE	RESTLF	RESTHF	REST RATIO	REST SDNN	REST PN50	REST RMSD	RESTHP	LF15	HF15	RATIO _L5	SDNN15	PN5015	RMSD15	HP15
1	5.69	70.40	29.60	2.38	241.74	41.75	127.09	718.32	61.01	38.99	1.56	245.07	49.56	177.31	507.14
2	8.15	42.90	57.10	.75	46.40	23.90	12.50	856.00	53.90	46.10	1.12	43.90	13.80	34.20	813.70
3	9.32	88.00	12.00	7.33	74.60	15.10	37.00	105.30	79.40	20.60	3.86	65.70	6.10	31.10	877.70
4	8.09	53.50	46.50	1.15	99.40	14.81	99.99	722.61	47.39	52.61	.90	152.69	13.22	196.49	762.68
5	6.89	63.35	36.65	1.73	148.75	53.22	122.34	1009.80	70.57	29.43	2.40	144.27	47.71	108.98	964.48
6	7.13	59.35	40.65	1.46	60.85	10.77	62.78	745.82	67.63	32.37	2.09	52.72	9.09	41.47	744.55
7	7.72	37.24	62.76	.59	170.69	43.19	197.80	866.20	40.55	59.45	.68	182.87	41.36	230.49	763.11
8	6.64	50.45	49.55	1.02	126.12	33.44	97.89	761.91	56.15	43.85	1.28	102.68	47.14	79.18	825.98
9	9.14	76.73	23.27	3.30	45.89	5.52	25.31	747.89	53.03	46.97	1.13	62.85	21.31	55.57	729.12
10	5.13	87.63	12.37	7.08	78.89	14.72	40.42	799.59	73.51	26.49	2.77	53.51	3.70	31.80	661.10
11	8.63	59.23	40.77	1.45	84.50	13.90	64.71	759.11	65.76	34.24	1.92	80.81	12.33	58.32	769.02
12	6.25	55.97	44.03	1.27	88.26	11.94	103.32	731.04	74.38	25.62	2.90	57.89	8.73	29.06	677.45
13	6.88	54.76	45.24	1.21	78.15	6.29	79.64	703.78	58.13	41.87	1.39	87.72	8.36	94.82	741.73
14	9.13	47.48	52.52	.90	137.98	57.64	110.66	939.86	49.13	50.87	.97	123.97	41.56	118.23	839.65
15	10.47	58.02	41.98	1.38	96.94	18.09	65.15	782.73	71.92	28.08	2.56	103.37	16.10	59.70	806.54
16	8.13	71.40	28.60	2.49	71.10	15.80	42.30	838.60	75.40	24.60	3.07	57.70	8.20	36.70	799.70
17	8.37	61.40	38.60	1.59	118.40	56.10	103.00	907.90	64.30	35.70	1.80	100.60	40.30	85.90	791.00
18	8.79	87.06	12.94	6.93	64.24	3.52	22.72	794.18	80.01	19.99	4.00	80.18	16.22	37.87	823.81
19	8.13	52.46	47.54	1.10	117.81	27.35	98.81	607.24	68.79	53.01	.89	275.89	43.73	310.12	769.74
20	8.47	38.67	61.33	.63	86.99	40.23	73.93	843.64	64.05	35.95	1.78	182.34	43.70	105.96	670.54
21	7.93	71.73	28.28	2.54	84.76	23.28	58.58	1018.50	69.02	30.98	2.23	114.65	51.79	85.52	905.55
22	8.76	72.38	27.62	2.62	165.15	58.93	47.50	1263.10	68.45	31.55	2.17	158.83	58.63	128.88	1257.80
23	8.19	41.18	58.82	.70	66.74	50.70	74.56	1067.00	64.73	35.27	1.84	219.76	53.57	117.68	865.91
24	7.82	51.10	48.90	1.04	61.60	31.20	47.50	1090.40	53.90	46.10	1.17	62.20	6.20	62.50	950.50
25	9.28	54.10	45.90	1.18	104.70	33.30	79.50	895.30	56.90	43.10	1.32	63.20	22.70	41.70	848.70
26	7.75	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data
27	6.50	74.30	25.70	2.90	68.50	15.20	41.30	847.00	61.20	38.80	1.58	40.40	4.60	24.40	762.30
28	7.64	39.37	60.64	.65	93.64	51.41	85.77	947.47	42.89	57.12	.75	86.21	42.35	73.79	895.66
29	6.75	64.19	35.81	1.79	50.94	7.01	31.19	704.45	No Data	No Data	No Data	No Data	No Data	No Data	No Data

LOW rBEE

HIGH rBEE

excluded

ID	LF1HR	HF1HR	RATI1HR	SDNN1HR	PN501HR	RMSD1HR	HP1HR	LF2HR	HF2HR	RATI2HR	SDNN2HR	PN502HR	RMSD2HR	HP2HR
1	52.23	47.77	1.09	115.76	47.06	78.07	833.19	71.21	28.79	2.47	203.95	40.71	90.77	757.95
2	76.30	23.70	3.23	47.00	4.50	25.10	759.20	77.40	22.60	3.42	36.20	3.50	23.70	766.80
3	86.70	13.20	6.56	56.80	7.50	27.00	903.00	90.20	9.80	9.18	72.60	8.90	32.10	880.00
4	47.28	52.72	.90	116.28	14.39	129.91	708.13	60.50	39.50	1.53	86.76	10.56	90.94	725.62
5	70.57	29.43	2.40	144.27	47.71	108.98	964.48	65.10	34.90	1.87	142.21	53.92	118.68	966.23
6	47.63	52.37	.91	91.67	17.55	103.34	721.76	46.23	53.77	.86	81.08	23.09	92.71	749.28
7	38.52	61.48	.63	134.74	54.78	148.92	851.19	46.73	53.27	.88	102.85	45.91	112.55	779.08
8	62.24	37.76	1.64	68.08	26.90	44.73	845.02	44.32	55.68	.80	83.97	49.00	72.61	816.13
9	75.22	24.78	3.04	48.14	5.04	24.72	648.51	70.04	29.96	2.34	48.67	8.50	28.92	677.26
10	79.50	20.50	3.88	54.44	4.18	36.35	653.73	79.16	20.84	3.80	58.96	8.28	29.22	723.71
11	72.55	27.45	2.64	54.78	12.38	36.94	769.64	55.96	44.04	1.27	124.75	23.93	93.64	777.24
12	57.83	42.17	1.37	42.62	2.37	21.95	652.89	70.35	29.65	2.37	53.32	2.65	24.43	679.51
13	80.40	19.60	4.10	39.78	1.13	16.13	657.95	90.31	96.85	9.33	54.25	4.59	25.42	679.65
14	47.57	52.43	.91	92.54	49.42	88.76	878.51	48.22	51.78	.93	92.98	39.42	88.41	807.68
15	64.63	35.37	1.83	151.01	21.27	93.27	772.53	60.70	39.30	1.54	143.12	16.71	87.96	786.31
16	69.30	30.70	2.25	67.30	8.80	46.90	755.10	79.70	20.30	3.93	59.40	9.60	32.40	773.00
17	41.70	58.30	.72	78.50	42.10	74.00	792.80	64.60	35.40	1.82	62.30	18.60	48.50	727.00
18	79.68	20.32	3.92	66.21	4.72	30.55	797.70	71.60	28.40	2.52	73.78	9.12	45.54	837.83
19	51.82	48.18	1.08	171.02	31.92	189.75	786.51	40.52	59.48	.68	224.12	33.56	280.89	779.65
20	32.16	67.84	.47	102.46	24.47	130.62	695.32	37.83	62.17	.61	85.31	32.84	84.25	676.76
21	68.91	31.09	2.22	96.65	50.15	71.65	875.41	71.54	28.46	2.51	112.98	43.54	75.53	864.22
22	63.66	36.34	1.75	185.64	65.85	106.56	1234.48	59.48	40.52	1.47	92.42	64.38	92.18	1253.92
23	43.76	56.24	.78	103.14	60.86	88.24	977.41	65.01	34.99	1.86	148.33	58.17	104.07	975.39
24	63.80	36.20	1.76	86.50	15.70	74.20	948.00	67.10	32.90	2.04	47.90	6.20	29.00	921.60
25	58.40	41.60	1.40	72.80	37.20	53.40	867.40	48.00	51.90	.92	107.30	59.30	101.80	961.00
26	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data
27	66.40	33.60	1.98	76.20	18.50	49.40	809.30	84.80	18.20	4.49	63.80	6.60	27.10	786.50
28	31.92	68.08	.47	76.60	46.58	70.39	864.07	49.11	50.89	.97	106.58	42.21	82.88	861.52
29	63.38	36.62	1.73	31.53	.75	17.08	679.63	59.30	40.70	1.46	27.71	.53	17.78	670.64

LOW rBEE

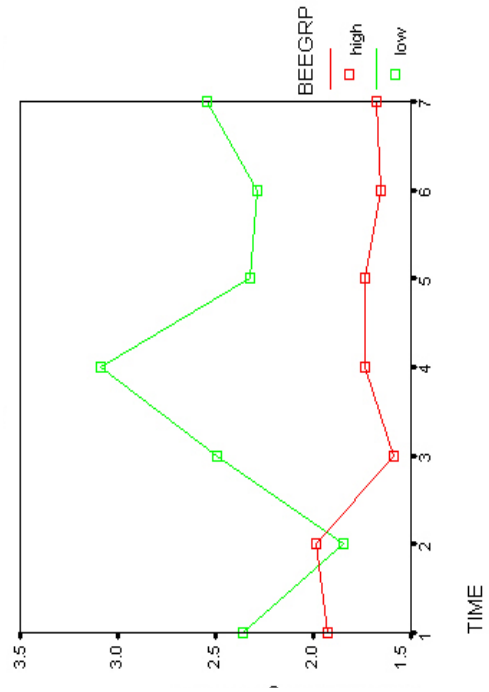
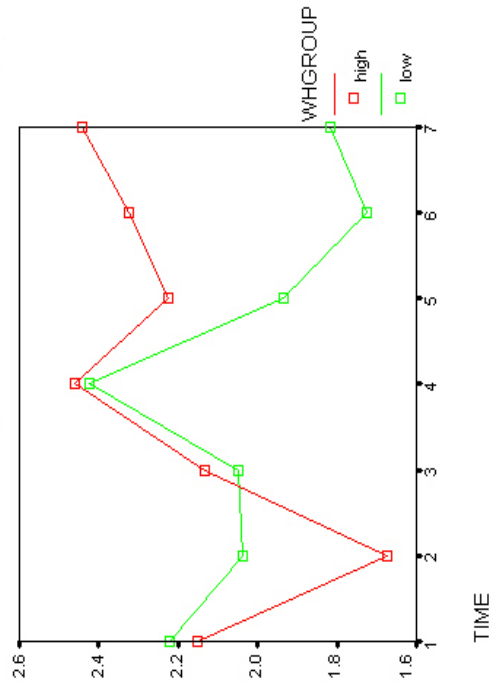
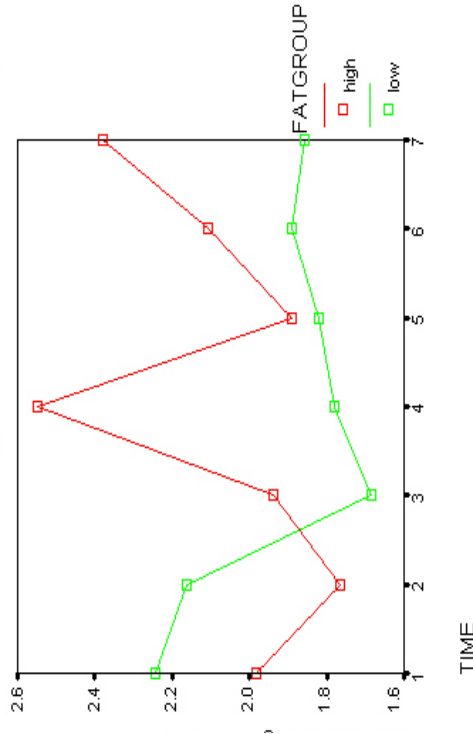
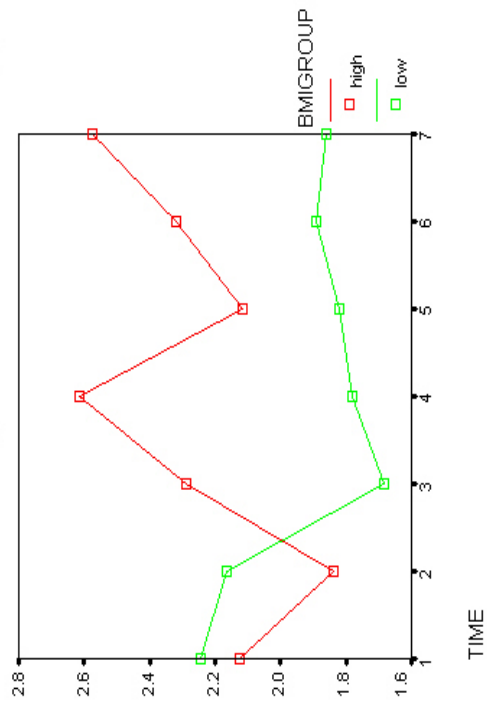
HIGH rBEE

excluded

ID	LF3HR	HF3HR	RATI3HR	SDNN3HR	PN503HR	RMSD3HR	HP3HR	LF4HR	HF4HR	RATIO4HR	SDNN4HR	PN504HR	RMSD4HR	HP4HR
1	66.37	33.63	1.97	175.14	48.76	85.04	No Data	73.87	26.13	2.83	164.52	51.63	83.67	919.17
2	73.80	26.20	2.81	45.70	10.40	32.00	786.40	49.30	50.70	.97	91.20	14.00	109.10	817.40
3	85.20	14.80	5.75	87.70	16.20	42.60	1042.00	81.20	18.80	4.31	81.70	24.70	44.80	105.20
4	38.22	61.79	.62	107.20	13.88	114.15	764.00	43.40	56.60	.77	118.96	19.53	149.71	822.90
5	57.96	42.04	1.38	139.38	60.09	130.50	1028.10	52.02	47.99	1.08	133.14	63.24	132.39	No Data
6	43.07	56.93	.76	113.08	35.75	128.09	780.72	62.09	37.91	1.64	95.46	47.02	82.79	907.44
7	59.61	40.39	1.48	138.81	45.80	122.89	748.26	53.53	46.47	1.15	125.45	46.64	112.47	854.91
8	67.80	32.20	2.11	88.73	36.84	60.60	762.87	72.94	27.07	2.69	105.08	49.69	74.64	845.49
9	68.64	31.36	2.19	49.97	16.36	37.37	757.47	66.77	33.23	2.01	44.73	11.68	31.61	769.45
10	80.49	19.51	4.13	57.18	5.68	25.63	730.70	77.81	22.19	3.51	72.94	8.24	38.46	765.49
11	54.66	45.34	1.21	56.71	20.00	45.36	957.21	53.63	46.37	1.16	153.59	31.94	135.65	842.52
12	58.41	41.59	1.40	47.31	5.44	28.08	699.49	61.83	38.17	1.62	59.33	6.90	31.41	739.10
13	81.47	18.53	4.40	57.52	7.16	31.40	685.33	85.77	14.23	6.03	62.36	9.78	31.46	801.15
14	54.82	45.18	1.21	89.94	50.72	74.64	902.69	48.36	51.64	.94	101.78	49.13	98.43	914.12
15	73.40	26.60	2.76	118.52	16.11	49.41	861.18	67.93	32.07	2.12	134.15	28.67	72.27	846.02
16	72.30	27.70	2.61	67.70	17.70	38.30	873.10	67.70	32.30	2.09	86.90	48.30	66.10	991.40
17	47.30	52.70	.90	99.60	53.30	84.90	889.30	33.20	66.80	.50	100.50	71.00	123.30	934.30
18	64.79	35.21	1.84	93.91	11.38	52.64	806.44	84.27	15.73	5.36	105.65	23.42	47.56	866.74
19	42.46	57.54	.74	199.24	30.38	235.04	802.42	41.94	58.06	.72	174.43	25.77	188.75	842.34
20	50.30	49.71	1.01	173.19	44.31	149.06	685.92	55.86	44.14	1.27	155.46	50.19	118.93	858.85
21	78.39	21.61	3.63	102.25	33.74	61.21	958.20	71.55	28.45	2.51	83.53	24.05	57.57	1019.10
22	62.88	37.12	1.69	96.31	64.26	94.36	1348.07	60.31	39.69	1.52	128.99	62.65	112.21	1201.39
23	57.07	42.93	1.33	162.42	59.93	92.12	1044.58	55.78	44.22	1.26	108.15	61.45	93.91	1068.19
24	58.90	41.10	1.43	42.30	17.10	37.20	1027.00	52.60	47.40	1.11	63.50	26.50	60.50	1013.20
25	62.40	37.60	1.66	105.80	41.00	79.00	1064.00	30.40	69.60	.44	75.50	64.80	93.60	1125.40
26	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data
27	39.60	60.40	.66	82.50	5.80	99.10	829.70	71.80	28.20	2.54	81.80	17.70	40.00	902.30
28	34.95	65.05	.54	86.10	54.20	81.49	947.95	47.05	52.95	.89	119.04	56.22	110.88	950.04
29	61.46	38.54	1.59	40.67	3.25	22.20	712.83	58.75	41.25	1.42	56.94	7.74	38.27	747.15
LOW rBEE														
HIGH rBEE														
excluded														

ID	LF5HR	HF5HR	RATI5HR	SDNN5HR	PN505HR	RMSD5HR	HF5HR
1	65.88	34.12	1.93	236.56	43.44	145.39	748.11
2	43.10	56.90	.76	79.00	6.70	89.60	808.80
3	84.20	15.80	5.33	92.40	26.70	54.70	100.70
4	65.77	34.23	1.92	128.28	19.51	121.54	765.75
5	55.73	44.27	1.26	141.11	55.97	119.33	1038.10
6	64.65	35.35	1.83	95.53	41.64	68.45	812.71
7	53.55	46.45	1.15	160.19	42.39	170.92	819.65
8	61.99	38.01	1.63	116.24	44.53	72.33	857.21
9	73.40	26.60	2.76	46.88	8.56	28.82	752.39
10	76.02	23.98	3.17	74.06	18.46	43.38	815.14
11	64.87	35.14	1.85	105.39	27.59	76.98	928.31
12	73.57	26.43	2.78	71.83	9.53	56.55	676.82
13	87.07	12.93	6.74	75.96	15.22	41.17	746.45
14	49.78	50.22	.99	120.39	52.57	114.89	891.85
15	59.10	40.90	1.45	228.61	43.52	151.42	396.26
16	66.90	33.10	2.02	71.30	30.20	49.90	930.90
27	58.30	41.70	1.40	94.50	49.60	82.70	879.30
17	76.66	23.34	3.28	89.23	12.82	38.12	776.07
18	42.66	57.34	.74	157.33	22.99	164.50	900.39
19	57.30	42.70	1.34	129.38	43.78	77.73	915.59
20	78.01	21.99	3.54	114.77	41.14	66.36	927.73
21	66.32	33.68	1.97	297.11	65.54	180.25	1229.90
22	63.50	36.50	1.74	142.88	51.95	91.47	1029.86
23	40.90	59.10	.69	69.90	23.00	87.80	970.20
24	49.00	51.00	.96	96.60	66.60	103.80	1081.20
26	No Data	No Data	No Data	No Data	No Data	No Data	No Data
28	No Data	No Data	No Data	No Data	No Data	No Data	No Data
25	48.60	51.40	.95	115.67	52.39	103.21	942.84
29	75.85	24.15	3.14	55.84	5.82	35.98	699.75
LOW rBEE							
HIGH rBEE							
excluded							

# APPENDIX F ADDITIONAL DATA GRAPHS



## Appendix G Additional Data Results

### Appendix G.1 BMI - General Linear Model

#### Within-Subjects Factors

Time	Dependent Variable
1	RESTLF
2	LF15
3	LF1HR
4	LF2HR
5	LF3HR
6	LF4HR
7	LF5HR

#### Between-Subjects Factors

BMI Group	N
High	9
Low	9

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	288.543	6	48.090	.681	.665
	Greenhouse-Geisser	288.543	4.013	71.895	.681	.608
	Huynh-Feldt	288.543	5.860	49.239	.681	.662
	Lower-bound	288.543	1.000	288.543	.681	.421
Time * BMI group	<b>Sphericity Assumed</b>	<b>613.693</b>	<b>6</b>	<b>102.282</b>	<b>1.448</b>	<b>.204</b>
	Greenhouse-Geisser	613.693	4.013	152.910	1.448	.228
	Huynh-Feldt	613.693	5.860	104.726	1.448	.206
	Lower-bound	613.693	1.000	613.693	1.448	.246
Error (TIME)	Sphericity Assumed	6780.995	96	70.635		
	Greenhouse-Geisser	6780.995	64.215	105.599		
	Huynh-Feldt	6780.995	93.760	72.323		
	Lower-bound	6780.995	16.000	423.812		

#### Descriptive Statistics

	BMI Group	Mean	Std. Deviation	N
RESTLF	High	60.5233	14.60309	9
	Low	62.2889	14.91795	9
	Total	61.4061	14.34940	18
LF15	High	62.1900	11.14934	9
	Low	67.8967	8.67112	9
	Total	65.0433	10.12427	18
LF1HR	High	64.9400	14.58504	9
	Low	57.7144	15.29858	9
	Total	61.3272	14.96879	18
LF2HR	High	63.1333	16.04395	9
	Low	59.3544	14.50749	9
	Total	61.2439	14.96518	18
LF3HR	High	63.5678	12.36786	9
	Low	60.7378	12.67500	9
	Total	62.1528	12.23543	18
LF4HR	High	65.1544	11.77543	9
	Low	59.0133	15.94121	9
	Total	62.0839	13.95786	18
LF5HR	High	67.8722	10.63319	9
	Low	61.6700	11.62154	9
	Total	64.7711	11.26709	18

## Appendix G.2 Waist/Hip - General Linear Model

### Within-Subjects Factors

Time	Dependent Variable
1	RESTLF
2	LF15
3	LF1HR
4	LF2HR
5	LF3HR
6	LF4HR
7	LF5HR

### Between-Subjects Factors

Waist/hip Group	N
High	11
Low	13

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	185.966	6	30.994	.392	.883
	Greenhouse-Geisser	185.966	4.379	42.465	.392	.830
	Huynh-Feldt	185.966	5.851	31.783	.392	.879
	Lower-bound	185.966	1.000	185.966	.392	.538
Time * Waist/hip group	<b>Sphericity Assumed</b>	<b>817.364</b>	<b>6</b>	<b>136.227</b>	<b>1.723</b>	<b>.120</b>
	Greenhouse-Geisser	817.364	4.379	186.645	1.723	.145
	Huynh-Feldt	817.364	5.851	139.693	1.723	.122
	Lower-bound	817.364	1.000	817.364	1.723	.203
Error (TIME)	Sphericity Assumed	10434.643	132	79.050		
	Greenhouse-Geisser	10434.643	96.343	108.307		
	Huynh-Feldt	10434.643	128.725	81.061		
	Lower-bound	10434.643	22.000	474.302		

### Descriptive Statistics

	BMI Group	Mean	Std. Deviation	N
RESTLF	High	61.6682	14.36704	11
	Low	59.3977	16.11243	13
	Total	60.4383	15.05087	24
LF15	High	60.4082	10.14666	11
	Low	65.3185	10.15407	13
	Total	63.0679	10.23733	24
LF1HR	High	63.2127	14.48254	11
	Low	59.9377	16.08974	13
	Total	61.4388	15.13405	24
LF2HR	High	62.6200	15.37075	11
	Low	63.2800	15.02845	13
	Total	62.9775	14.85502	24
LF3HR	High	64.8436	12.17899	11
	Low	60.6938	13.645810	13
	Total	62.5958	12.88772	24
LF4HR	High	65.3036	12.23481	11
	Low	55.6838	16.63051	13
	Total	60.0929	15.27598	24
LF5HR	High	66.4500	11.33082	11
	Low	59.5162	13.15400	13
	Total	62.6948	12.59171	24

### Appendix G.3 BEE - General Linear Model

#### Within-Subjects Factors

Time	Dependent Variable
1	RESTLF
2	LF15
3	LF1HR
4	LF2HR
5	LF3HR
6	LF4HR
7	LF5HR

#### Between-Subjects Factors

BEE Group	N
High	12
Low	13

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	320.032	6	53.339	.682	.665
	Greenhouse-Geisser	320.032	4.475	71.515	.682	.622
	Huynh-Feldt	320.032	5.931	53.957	.682	.663
	Lower-bound	320.032	1.000	320.032	.682	.417
Time * BEE group	<b>Sphericity Assumed</b>	<b>747.118</b>	<b>6</b>	<b>124.520</b>	<b>1.592</b>	<b>.154</b>
	Greenhouse-Geisser	747.118	4.475	166.953	1.592	.176
	Huynh-Feldt	747.118	5.931	125.963	1.592	.155
	Lower-bound	747.118	1.000	747.118	1.592	.220
Error (TIME)	Sphericity Assumed	10795.163	138	78.226		
	Greenhouse-Geisser	10795.163	102.926	104.883		
	Huynh-Feldt	10795.163	136.418	79.133		
	Lower-bound	10795.163	23.000	469.355		

#### Descriptive Statistics

	BMI Group	Mean	Std. Deviation	N
RESTLF	High	58.9150	14.40201	12
	Low	61.5008	15.56172	13
	Total	60.2596	14.76105	25
LF15	High	65.5500	8.85379	12
	Low	61.6469	11.45996	13
	Total	63.5204	10.27398	25
LF1HR	High	57.1158	13.81798	12
	Low	65.1515	15.21286	13
	Total	61.2944	14.83297	25
LF2HR	High	59.5250	13.15741	12
	Low	66.7315	15.54366	13
	Total	63.2724	14.61681	25
LF3HR	High	60.4175	10.88622	12
	Low	64.2846	14.25963	13
	Total	62.4284	12.64412	25
LF4HR	High	55.8250	15.90112	12
	Low	64.1662	13.39972	13
	Total	60.1624	14.95838	25
LF5HR	High	59.0358	12.03210	12
	Low	66.9077	12.18288	13
	Total	63.1292	12.51703	25

## Appendix G. 4% Body Fat General Linear Model

### Within-Subjects Factors

Time	Dependent Variable
1	RESTLF
2	LF15
3	LF1HR
4	LF2HR
5	LF3HR
6	LF4HR
7	LF5HR

### Between-Subjects Factors

FAT Group	N
High	11
Low	9

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	531.350	6	88.558	1.334	.248
	Greenhouse-Geisser	531.350	4.311	123.250	1.334	.263
	Huynh-Feldt	531.350	6.000	88.558	1.334	.248
	Lower-bound	531.350	1.000	531.350	1.334	.263
Time * %BF group	<b>Sphericity Assumed</b>	<b>588.988</b>	<b>6</b>	<b>98.165</b>	<b>1.478</b>	<b>.192</b>
	Greenhouse-Geisser	588.988	4.311	136.620	1.478	.214
	Huynh-Feldt	588.988	6.000	98.165	1.478	.192
	Lower-bound	588.988	1.000	588.988	1.478	.240
Error (TIME)	Sphericity Assumed	7170.995	108	66.398		
	Greenhouse-Geisser	7170.995	77.601	92.409		
	Huynh-Feldt	7170.995	108.000	66.398		
	Lower-bound	7170.995	18.000	398.389		

### Descriptive Statistics

	BMI Group	Mean	Std. Deviation	N
RESTLF	High	58.6555	15.76492	11
	Low	62.2889	14.91795	9
	Total	60.2905	15.09799	20
LF15	High	61.1082	11.51278	11
	Low	67.8967	8.67112	9
	Total	64.1630	10.65008	20
LF1HR	High	59.1027	17.37856	11
	Low	57.7144	15.29858	9
	Total	58.4780	16.06247	20
LF2HR	High	64.4736	13.96772	11
	Low	59.3544	14.50749	9
	Total	62.1700	14.07579	20
LF3HR	High	60.2455	13.97653	11
	Low	60.7378	12.67500	9
	Total	60.4670	13.05833	20
LF4HR	High	62.7409	12.14992	11
	Low	59.0133	15.94121	9
	Total	61.0635	13.72275	20
LF5HR	High	66.0764	11.08578	11
	Low	61.6700	11.62154	9
	Total	64.0935	11.25199	20

## VITA

Calynn Davis Bunol was born in Metairie, Louisiana, in August of 1978. Her first move from home was to attend Louisiana State University in the fall of 1996. The fall semester of 1998 she ventured off to Greeley, Colorado, for a semester at the University of Northern Colorado. She desired to become well rounded in the area of exercise and nutrition in order to better address the needs of the American population. Therefore, the pursuit of a master's degree in exercise physiology became a goal to achieve. She returned to Baton Rouge to complete her undergraduate degree in dietetics, which was followed by an internship at the University of Houston. Successfully completing the requirements to become a registered dietitian she remained focused on her quest for a master's degree. She recently returned home to Metairie with her spouse, she is employed full time with Touro Infirmary as a Registered Dietitian specializing in diabetes education. Upon completion of her degree she will have accomplished her goal; therefore, becoming a better educator in promoting the development and maintenance of a healthy lifestyle.