Abstract

OBESE CHILDREN HAVE A LOWER LIPOLYTIC RESPONSE TO AN ACUTE BOUT OF EXERCISE WHEN COMPARED TO LEAN

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July, 2010

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Over 17% of American children are obese. These are epidemic proportions considering the associated risk of heart disease and Type 2 diabetes. Excess fat is caused from an imbalance in energy stored versus energy expended. Previous studies of the fat cell in vitro have revealed differences in the lipolytic response to aerobic exercise in subcutaneous abdominal adipose tissue (AAT) from obese and lean adults. There is also evidence that aerobic exercise training can increase subcutaneous AAT lipolysis in obese adults. However, review of the literature revealed no studies examining the training response in subcutaneous AAT of obese children. The purpose of this study was to determine whether there is a lower lipolytic response to aerobic exercise in subcutaneous AAT of obese children. Also, we wanted to know if 16 weeks of aerobic exercise training would increase the lipolytic rates in subcutaneous AAT of obese children. In this study, we recruited children from Greenville, NC and surrounding areas. We used the microdialysis technique to examine the lipolytic rate of subcutaneous AAT in response to aerobic exercise in forty sedentary children ages 8 to 11. All children and parents were made aware of the risks and benefits of the procedure and signed both consent and assent forms. The study was approved by the East Carolina University International Review Board. Children were grouped by body mass index percentile into obese (n =28) or lean (n =12). Children exercised for twenty minutes at 70% of their heart rate max. In response to exercise, dialysate glycerol concentrations (index of lipolysis) increased in the obese less than the lean (8.1 ± 2.1 uM vs 17.1 ± 4.2 uM, respectively;

p <0.05). Changes in subcutaneous AAT blood flow, measured by the ethanol outflow; inflow ratio, was not significantly different between the two groups. We also examined the effects of 16 weeks of aerobic exercise training on subcutaneous AAT lipolytic response in the obese children (n =21). Children were trained (machines, free play) at an average heart rate of 140 bpm for an hour three times a week. The children returned for another microdialysis session after training. The treadmill speed during the exercise portion after training was the same as that used before training. The change in dialysate glycerol during exercise was not significantly different before and after training (8.9 ± 2.5 and 6.2 ± 2.8 uM respectively; *p =0.37*). These results suggest that, like adults, obese children have a lower lipolytic response to an acute bout of aerobic exercise than do lean. In addition, 16 weeks of training does not alter lipolytic response to aerobic exercise in obese children when performed at the same absolute intensity before and after training.

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A Thesis

Presented To

The Faculty of the Department of Exercise and Sports Science

East Carolina University

In Partial Fulfillment of the

Requirements for the Degree

Master of Science in Exercise and Sport Science

By

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July, 2010

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CHAPTER 1: Introduction

The prevalence of obesity has increased in America. To be classified as obese, a person must have a body mass index (BMI) of above 30 kilograms per meter squared (A normal BMI is between 18 and 25 kg/m^2). When a person reaches a large BMI, they are grouped into a high risk category for many diseases. One of the most prevalent is type 2 diabetes. Type 2 diabetes is associated with a poor response to insulin's effect on glucose uptake. Diabetics often have multiple health problems that stem from obesity and insulin resistance. A recent National Health and Nutrition Examination Survey (NHANES) concluded that two thirds of Americans with type 2 diabetes is also obese (Kramer, Caoa, Dugasa, Lukea, Coopera and Durazo-Arvizua 2009). Peripheral arterial disease, hypertension, and death are all highly correlated with obesity (Bjorntorp 1997). Hypertension, also known as high blood pressure, often leads to heart disease and the results from a recent study by Ford, Zhao, Pearson, and Mokdad (2008) concluded that half of hypertensive patients are also obese. Therefore, obesity is an indicator of poor health and diabetes alone is costing billions of dollars. The director for the Centers of Disease Control (CDC) called the rise of diabetes a public health emergency (CDC, 2004). A study funded by the CDC revealed that 32.2 percent of Americans are classified as obese and over 60 percent are overweight (Ogden, Carroll, Curtin, McDowell, Tabak, Flegal 2006). Entrepreneurs have targeted this ever growing population of the obese by creating crash or fad diets to lose this excess weight. These are often unhealthy ways of losing weight and do not always address from where the weight is lost. If weight loss is from fat free mass, as opposed to fat mass, it is not as healthy and therefore should not be as desirable. The truth is that diet is not the only contributing factor to how much someone weighs.

Both diet and exercise contribute to weight management. To maintain weight, a person must remain at a zero energy balance. A zero energy balance requires people to expend as much energy as they consume. The body intakes energy in the form of food and that food is broken up into fat and carbohydrates to be used immediately as fuel (carbohydrate) or stored as potential fuel in adipose tissue (fat). Protein is used mostly for structural purposes and not used for energy to the extent that fat and carbohydrates are used. However, any excess of these three macronutrients, if not used as fuel, will be stored as fat. The main point is that too much food intake causes fat gain. To expend energy, other than the basic function of the organs, the skeletal muscles must be exercised. Only a small amount of energy is devoted to the internal body organ functions. A much greater amount of energy is used during exercise by the working muscle.

Exercise has, for a long time, been viewed as a potential cost effective way to combat weight gain or obesity. In 1996, the surgeon general announced publicly that exercise is good for your health. He also acknowledged that a dose response relationship exists between increased exercise and absence of disease. This means that a higher dose of exercise will bring more benefits. Exercise is an excellent way to burn energy and combat excess fat. Exercise training also has many benefits. These benefits are often present without fat loss, such as strengthening the heart muscle and increasing the effectiveness of the lungs. Exercise training increases the body's response to hormones released during exercise and improves the action of these hormones. Many adults have come to realize that daily exercise is important. Unfortunately, for some, daily exercise may not be enough.

America now has a potentially bigger problem. According to the NHANES 2004 report, 17.1 percent of adolescents are overweight (Ogden, Carroll et al. 2006). These children could be

at risk for many disease states at a very young age. Cardiovascular disease is progressive and these children's mortality risk could be multiplied because of long term exposure to the negative effects of obesity (Coppack 2005).

It is still unclear whether humans are predisposed to be obese. Studies have shown a high correlation between family obesity and childhood obesity (Burns, Moll, Lauer 1989; Whitaker, Wright, Pepe, Seidel, Dietz 1997). Childhood obesity is a potent indicator of obesity in adulthood (Whitaker et al. 2004). It is important to study fat in children at the cellular level to determine if there are defects in the adipocyte (fat cell) that are evident early in life. Also, this population is potentially unaffected by sex hormones which may play a role in metabolism during adolescence.

The review of the literature revealed that studies of lipolysis are dominated by adult subjects or in vitro experiments. The relationship between in vivo lipolysis and obesity in children is still unclear. Few studies have focused on in vivo lipolysis in children. One such study, by Hershberger, McCammon, Garry, Mahar, and Hickner (2004) used the microdialysis technique to measure lipolysis of obese and lean children in vivo during exercise. The authors found no difference in lipolytic rate in response to exercise between the obese and the lean children. However, the rates were not equal and there was a large standard deviation. Unlike Hershberger, our study used a relative intensity based on the of the participants' max hr response during a treadmill test of maximal aerobic capacity (VO_{2max}).

The main question in this study is whether obese children breakdown their fat differently than lean children during exercise. In this study we controlled for caloric intake and fitness to compare obese and lean children's fat breakdown during an aerobic bout of exercise.

Furthermore, this was the first study, to our knowledge, to use microdialysis of subcutaneous abdominal adipose tissue (AAT) in obese children to examine the effects of a sixteen week aerobic training program on lipolysis of the subcutaneous AAT.

Statement of the Problem

The prevalence of obesity has increased dramatically in the past thirty years. Obesity is associated and highly correlated with disease and disease states such as Type 2 diabetes and cardiovascular disease. Public health costs are in the trillions and the national debt is increasing. Obesity is not only a health issue but a fiscal issue as well. The purpose of this study is to determine if obese children breakdown fat, known as lipolysis, at a lower rate than lean, perhaps limiting the availability of fat for energy during exercise.

Purpose

There are two aims of the present study. Aim one examined the response in subcutaneous AAT of obese and lean children to an acute bout of exercise. Aim two examined the effects of 16 weeks of aerobic exercise training on the lipolytic response in subcutaneous AAT of obese children to an acute bout of aerobic exercise.

Research Hypothesis

We hypothesized that (a) obese children would have a lower lipolytic response to an acute bout of exercise when compared to lean children and (b) obese children would raise their lipolytic response to an acute bout of exercise after sixteen weeks of aerobic exercise training.

Definitions

The following terms have been clarified for the purpose of this study:

BMI: the body mass index is calculated from a ratio between height in meters squared and weight in kilograms.

Obese: defined as having a BMI above the 95th percentile when compared to other children of the same age and gender.

Lean: defined as having a BMI below the 80th percentile when compared to other children of the same age and gender.

Lipolysis: metabolic pathway that separates fatty acids from a glycerol "backbone" inside the adipocyte.

Sedentary: children who did not participate in more than 30 minutes of deliberate exercise three or more times a week.

VO_{2max}: the maximal amount of oxygen that can be utilized during maximal exercise intensity. It is the standard criterion for the measurement of health fitness.

Acronyms:

The following acronyms may be used for brevity in this study:

CDC: Centers for Disease Control and Prevention

NHANES: National Health and Nutrition Examination Survey

AAT: Abdominal Adipose Tissue

AR: Adrenoreceptor

FA: Fatty acid

TG: Triglyceride

ATBF: Arterial blood flow

Limitations/Delimitations

The limitations of the study include the following:

1. Neither arterial nor subcutaneous AAT catecholamine concentrations, at rest or exercise, were measured.
2. Arterial glycerol (indicator of whole body lipolysis) concentrations were not measured during exercise.
3. Participants were not fasted during exercise.

Clinical Relevance

It may be that the obese do not oxidize fat, originating from the subcutaneous AAT, as preferably as the lean. Defects may be present in the metabolic pathway of subcutaneous AAT. Knowledge of these defects is necessary to propose strategies to maximize fat utilization. It is possible that alternative treatment, along with exercise and proper diet, is necessary to control weight and over fatness in the obese.

CHAPTER 2: Literature Review

The number of obese children in the United States is reaching epidemic proportions. Now, over 17% of children in America are obese (Ogden, Carroll et al. 2006). This study attempts to investigate this rise in obesity by observing processes inside the adipocyte. This review of literature will focus on research in the following areas: (a) obesity and associated health risk, (b) measuring lipolysis of the adipocyte using the microdialysis technique, (c) adipocyte response to acute exercise and (d) aerobic training.

Obesity and Health

Obesity in America has become a serious public health issue that seems to be getting worse. According to a study by Flegal et al. (1998), obesity increased markedly from 14.1% in 1980 to 22.4% in 1994. The NHANES III report showed that this has increased to 32.2% for adults. This increase is also evident in children. According to the same survey, the incidence of childhood obesity has reached 17.1% (Ogden, Carroll et al. 2006).

Obesity is determined by the Body Mass Index (BMI): the ratio between weight and height. For adults, a BMI measurement above 30 kg/m^2 defines obesity. For children, the BMI is compared with other children of the same age and sex. Above the 95th percentile is classified as obese and below 80th percentile is classified as lean as indicated by Kuczmarski, Ogden, Guo, Grummer-Strawn, Flegal, Mei, et al. (2002). These numbers have been developed from the surveys administered by the National Health and Nutrition Examination Survey (NHANES I, NHANES II, NHANES III), National Health Survey (NHES) II, and NHES III. Though body fat

percentage is probably the best measurement to evaluate over fatness, obtaining the body fat percentage is often expensive or laborious.

Using BMI to determine obesity is easy and non invasive. Must, Dallal, and Dietz, (1991) explored the initial classification of BMI and its reliability in determining obesity. Dietz used NHANES I data collected from 1971 through 1974 to develop percentiles based on age, race, and gender. The authors concluded that children above the 95th percentile were obese and at risk for disease.

Dietz and Bellizzi (1999) investigated the validity of using the BMI to determine excess fat in children. They compared data obtained from previous studies to correlate body fat % measured from hydrostatic weighing or DXA and body fat % measured from BMI. Hydrostatic weighing is the gold standard and techniques such as Dual X ray Absorptiometry (DXA) can distinguish between bone, lean tissue, and fat tissue (Gutin et al. 1996). They found that whether hydrostatic weighing or DXA was used, both were highly correlated with BMI. Though all methods have limitations, Dietz and Bellizi concluded that the use of BMI is an acceptable measure to determine body fatness in children.

Obesity causes a spillover of fatty acids from the adipocyte that circulate in the blood plasma. Spillover begins after the adipocyte fills to capacity with triglycerides (TG) and fatty acids (FA). Previous studies show that these extra TGs and FAs, if not used as fuel, interfere with signaling inside the adipocyte causing physiological changes in the adipose tissue. Obesity, excess FAs, glucose intolerance, and insulin resistance are all central players in the development of type 2 diabetes (Coppack 2005).Type 2 diabetes is becoming more prevalent in America as well. Once thought of as a progressive disease in adulthood, type 2 diabetes is now found in

obese children (Fagot-Campagna, Pettitt, Engelgau, Burrows, Geiss, Valdez, et al. 2000). Insulin resistance is also a central factor in the Metabolic Syndrome. This syndrome is characterized by obesity, insulin resistance, dyslipidemia, hypercholesterolemia, and hypertension. It is a grouping of risk factors that may indicate a high risk of developing diabetes, cancer and/or cardiovascular disease.

Excess FAs may also interfere with the DNA of the cell by altering gene expression. This is true for many different types of cells including the hepatic cells. The liver begins to function differently because of the excess fat storage and this has been linked to non alcoholic fatty liver disease (Malhi, Gores 2008). Recent studies suggest that these excess FAs eventually take a non oxidative pathway and begin to create reactive species that are toxic to the cell. If toxic enough, the cell will undergo apoptosis or death (Kusminski, Shetty, Orci, Unger, Scherer 2009).

These alterations in the adipocyte may start in early childhood as suggested by Whitaker (2004) who examined longitudinal data of obese and lean subjects from childhood through adulthood. The authors concluded that childhood obesity increases the chance of being obese as an adult. This makes childhood obesity a potent indicator when predicting obesity. Other studies have shown an equal contribution of genetics and lifestyle. Burns, Moll, and Lauer (1989) evaluated the relationship between family obesity and childhood obesity. The purpose of the study was to assess the contribution of genes and environment to adult obesity. Subjects were recruited from Muscatine, Iowa and examined over a four year period. BMI and CVD risk markers were evaluated throughout the study. A regression analysis revealed that genetics account for about 50% of the variance in BMI and that environmental factors accounted for the other 50%. The authors concluded that both genetics and environment contribute equally to

childhood and subsequently adulthood obesity. Results from studies examining the contribution of childhood obesity to adulthood obesity remain unclear.

The distribution of fat has also emerged as a consideration when assessing for the Metabolic Syndrome. The reason that obesity is associated with many diseases is because a BMI above the 95th percentile most often indicates a disproportionate amount of fat mass when compared to fat free mass. It is the excess fat, and not muscle, that correlates highly with disease states. Much of this excess fat is stored in the subcutaneous adipose tissue of the body. Some depots can grow very large such as in the abdomen.

Abdominal obesity is recognized as a risk factor in the Metabolic Syndrome. Larsson, Svardsudd, Welin, Wilhelmsin, Bjorntorp, and Tibblin (1984) examined middle aged men chosen by the birth year of 1913 in Gothenburg, Sweden. Anthropometric data was obtained in 1967. 13 years later all men were re evaluated to see who had died from a stroke. Abdominal obesity, as opposed to general obesity, was more associated with risk of having a stroke. The results indicate that abdominal obesity was a strong (and independent) indicator of cardiovascular disease risk. This is also in agreement with a discussion by Per Bjorntorp (1997) that points to central obesity as having a greater correlation with cardiovascular disease than generalized obesity.

Central fat depots are common in obese adults. Is excessive central fat accumulation also common in children? Studies are dominated by the use of adult subjects because children are often disease free. However, children with excess fat are becoming more common. The ones who are obese have a higher incidence of type 2 diabetes and often progress to other diseases that in the past have been more commonly associated with adulthood obesity. However, whether these

alterations in lipolysis of the obese are modifications of the adipocyte or inherent from birth is unclear. Studies in adults have suggested that the obese have lipolytic alterations of the subcutaneous adipose tissue in places such as the abdomen and these central obesity is also seen in children (Arner 2005; Meininger, Brosnan, Eissa, Nguyen, Reyes, Upchurch et al. 2010). The review of literature has not revealed enough information on the lipolytic response of subcutaneous AAT in children.

Measuring Lipolysis

FAs are stored as TGs inside the adipocyte and wait to be released for energy. Before release the adipocyte undergoes a cascade of events known as lipolysis. The FAs are hydrolyzed off of their glycerol backbone by adipose tissue TG lipase (ATGL) and hormone sensitive lipase (HSL) so that they can be moved across the adipocyte membrane. The glycerol is transported directly to the liver via the bloodstream. Here, the glycerol is used to make another TG or to be converted to glucose. The FAs, however, have many possible fates. They may be re-esterified and moved back into an adipocyte for storage but they are also free to move into the blood stream for transport to the muscle or liver. In the muscle, the FA is oxidized for energy used in contraction. The liver stores the FA by creating new TGs (Arner 2005). A consequence of this unidirectional movement of glycerol out of the adipocyte is that it can be obtained and measured as an indicator of lipolysis. The microdialysis technique, developed originally for brain tissue of rats, has been adjusted for use in human tissue. The procedure allows for the measurement of metabolites in the extracellular fluid in muscle or adipose tissue (Lonnroth, Smith 1987; Ungerstedt 1991). This led investigators to a host of questions about local metabolism in tissues of humans. Adipose tissue is not easily accessed by catheterization of a major artery and vein for

traditional arterio-venous measurements, so the microdialysis methodology was often used in this tissue.

Studying fat in vivo in humans is relatively in its infancy. Early work in rats utilized the microdialysis method to measure glycerol released from fat. A study by Arner, Bolinder, Agneta Eliasson, Lundin, and Ungerstedt (1988) used microdialysis probes inserted into the fat tissue of male Sprague-Dawley rats to collect glycerol released into the extracellular space. This study showed successful microdialysis of the subcutaneous fat tissue of rats. Furthermore, it showed that continuous monitoring of the tissue over time was also possible (Arner et al. 1988). These results were found in rats and more studies were needed to assess how well the data translated to humans.

Lonnroth and Smith (1990) wrote an article explaining the usefulness of the microdialysis technique and its implications for measuring metabolites continuously in vivo in humans. Arner, Kriegholm, Engfeldt, and Bolinder (1990) used the microdialysis technique to monitor metabolites in local subcutaneous adipose tissue sites in vivo in humans. Both studies concluded that the technique is valid for in vivo experiments. Wahrenberg, Bolinder, and Arner (1991) wrote a review explaining that the microdialysis technique could be used for continuous monitoring of metabolites such as glycerol from human adipose tissue. The authors explain the non invasive nature of the procedure and the fact that it causes little discomfort. Because of the advances in microdialysis technology, the authors determined this technique to be valid for adipose tissue experimentation. Common sites for insertion of the probe are the abdominal and the femoral region.

Adipocyte Response to Exercise

Increasing lipolysis is necessary to provide energy to meet the physical demands of the body, especially during prolonged exercise lasting over fifteen minutes. The rate of lipolysis is not equal to the rate of fat oxidation, but lipolysis is necessary to move the fat from a stored state to an active one capable of being used to generate ATP for the functions of the cell. Like many other cells in the body, the adipocyte is regulated by hormones. Receptors, expressed on the outside of the cell, are activated like a key in a lock. The control of lipolysis, like many metabolic pathways, is quite complex and the chemistry is not discussed in detail here. However, previous studies have shown that the adipocyte has both stimulatory and inhibitory regulation. Two of the major controls of lipolysis in fat tissue include its response to insulin and catecholamine (Arner 2005; Coppack 2005).

Insulin receptors, when activated, will inhibit lipolysis (Arner, Bolinder, Engfeldt, Ostman 1981; Coppack, Patel, Lawrence 2001). Insulin's antilipolytic effect on the adipocyte is well known and beyond the scope of this review. The focus will remain on catecholamine mediated receptors expressed on the adipocyte and they are the alpha$_2$adrenergic and the beta adrenergic receptors (Alpha$_2$ AR and Beta AR respectively).

Evidence for the alpha$_2$ ARs expressed on animal adipocytes was found in the 1980s (Berlan, Lafontan 1980). Lafontan and Berlan (1980) also explored the nature of the alpha$_2$ ARs and their role in lipolysis. They found that alpha$_2$ ARs in animals had an affinity for catecholamine which is a neurotransmitter released from the nervous system. When catecholamine binds with the alpha$_2$ ARs, lipolysis will slow. Alpha$_2$ ARs antilipolytic effect on lipolysis in adipose tissue is well defined (Lafontan, Berlan 1981).

Alpha$_2$ ARs are counter to the beta ARs. Beta ARs were also discovered to be expressed on human adipocytes. The beta ARs have an affinity for catecholamine much the same as the alpha$_2$ ARs. However, the beta ARs increase lipolysis when bound to catecholamine whereas the alpha$_2$ ARs inhibit lipolysis when bound to catecholamine. In vitro experiments on adipocyte biopsies have revealed the affinity of these two receptors for the catecholamine in humans (Fain, Garcia-Sainz 1982; Mauriege, De Pergola, Berlan, Lafontan 1988).

Keller, Weiss, and Stauffacher (1989) wanted to study the contribution of alpha$_2$ and beta ARs to the lipolytic effects of catecholamine in humans. The investigators used fat from biopsies to compare the alpha$_2$ and beta ARs in vitro. The authors found that both the alpha$_2$ ARs and the beta ARs have an affinity for catecholamine. The importance of the beta ARs in regulation of whole body lipolysis was investigated by Wahrenburg, Lonnqvist, Hellmer, and Arner (1992). Low levels of the catecholamine promote alpha$_2$ inhibition. Higher levels of catecholamine promote beta AR upregulation. These results, found in vitro, were reproduced in vivo in healthy adult males (Barbe, Galitzky, Riviere, Senard, Lafontan, Garrigues, et al. 1993; Lonnqvist, Wahrenberg, Hellstrom, Reynisdottir, Arner, 1992). It is clear that there is a constant battle between the adrenergic receptors because they both have an affinity for catecholamine. During exercise, these catecholamines are released in abundance to speed the heart rate and move the blood rapidly around the body. This is also the way the body signals the adipocyte to release its stores of energy. Fat has approximately double the potential energy when compared to carbohydrates and increasing lipolysis is a favorable response to utilize fat during exercise. Catecholamine binds with the adrenergic receptors of the adipocyte so that it will release its FAs from glycerol. They are then moved to the plasma to be delivered to the working muscle (Horowitz 2003; Stitch, Berlan 2004).

Savard, Despres, Marcotte, Theriault, Tremblay, and Bouchard (1987) examined the effects of acute aerobic exercise on adipocyte lipolysis in fat tissue obtained from biopsy of the subcutaneous adipose tissue. Biopsies of fat were taken from the abdomen and gluteal region before and after aerobic exercise. Adipocytes from both depots were incubated in epinephrine. Glycerol levels post-exercise were significantly higher than pre-exercise in vitro. This study showed that there is an acute adaptation of the adipocyte in response to exercise. These results are in agreement with Wahrenberg, Engfeldt, Bolinder, and Arner (1987) who also showed acute changes in adipocyte lipolysis such as increased glycerol release in response to exercise. These acute adaptations are attributed to the response of the beta ARs to catecholamine release. Since the $alpha_2$ receptors have a higher affinity to catecholamine at lower physiological concentrations, beta ARs are more important during exercise when higher concentrations of catecholamine are present. An admitted limitation to the in vitro experiments is that the adipocyte is removed from its true environment.

Catecholamine is released during exercise relative to the intensity of the exercise. The general consensus is that increasing intensity will increase the amount of catecholamine released. It is clear that higher concentrations of catecholamine are needed to increase lipolysis by activation of the beta ARs. This is also true in local sites such as the subcutaneous AAT (Berlan et al. 2010; Galitzky et al. 1998). Intensity, catecholamine release, lipolysis, and fat oxidation are all linked in the process of metabolism in both healthy and overweight adults (Pillard, Moro, Harant, Garrigue, Lafontan, Berlan, et al. 2007).

Before the microdialysis method, studies used arterial blood plasma samples to examine the effect of intensity on catecholamine release. These studies showed that twenty minutes of

moderate (~50% VO_{2max}) exercise was needed to increase catecholamine concentrations. The increase is moderate at first but rises sharply after about 75% of VO_{2max} (Christensen, Galbo, Hasen et al. 1979; Christensen, Sonne et al. 1985). Blood samples, taken for hormone analysis, were obtained from the arterial bloodstream. Though the catecholamines are delivered via the bloodstream to the adipose tissue, these studies did not answer the question of catecholamine response of lipolysis to exercise specifically in subcutaneous adipose tissue.

Hodgetts, Coppack, Frayn, and Hockaday (1991) examined lipolysis and FA release during exercise at different intensities. They recruited healthy adults to examine exercise intensity and fatty acid mobilization. Subjects exercised at workloads corresponding to 50-70% of their VO_{2max}. Interstitial fluid from the subcutaneous adipose tissue was collected by catheterization as opposed to the microdialysis method. The authors found that glycerol, FAs, and catecholamine all rose similarly in response to different workloads.

Arner et al. (1990) studied the local lipolytic activity of subcutaneous adipose tissue in the abdominal and gluteal region. Dialysate fluid was collected in vivo through the microdialysis technique. Probes were perfused with either saline or a selective blocking agent. Arner also collected data continuously while the subjects transitioned from rest to exercise. The authors found that the abdominal depot was more sensitive to catecholamines than the gluteal depot. They also observed that lipolysis was regulated differently depending on whether the subject was at rest or exercise. Perfusion of a beta-adrenergic receptor blocker had no effect on lipolysis at rest but decreased it during exercise. Perfusion of an alpha AR receptor blocker increased lipolysis during rest but had no effect during exercise. They concluded that $alpha_2$ AR inhibition is dominant at rest but the beta ARs dominate during exercise. These results are in agreement

with later studies that observed the importance of alpha$_2$ and beta ARs in controlling lipolysis in vivo (Barbe, Millet, Galitzky, Lafontan, Berlan 1996; Coppack, Jensen, Miles 1994).

Galitzki et al. (1998) investigated local action of catecholamines on subcutaneous adipose tissue. Subjects were healthy weight and young. Microdialysis of the subcutaneous adipose tissue allowed perfusion of catecholamine directly to the local site. Catecholamines increased the concentration of glycerol in the subcutaneous adipose tissue. The increase in glycerol was shown to be catecholamine concentration dependant. Also, the subjects were re-tested at a later date with no perfusion of drugs. Instead the subjects were asked to move from a supine to an upright position (active tilt) for twenty minutes. Catecholamines were increased during active tilt and interstitial glycerol levels also rose. This study showed that catecholamines do play a role in local tissue depots such as subcutaneous adipose tissue.

A recent study by Glisezinski, Larrouy, Bajzova, Koppo, Polak, Berlan, et al. (2009) used microdialysis of the subcutaneous AAT tissue to investigate the difference in response to catecholamines by the beta ARs of the adipocyte. Subjects underwent an exercise session of sixty minutes at 50% of their VO_{2max}. The authors concluded that adrenaline (epinephrine), and not noradrenaline (norepinephrine), is the main effecter of lipolysis via the beta ARs.

Adipocyte function is altered in the obese. These changes could be evident in physical attributes of the cell such as the size (adipocyte hypertrophy) or in functional attributes of the cell (impaired signaling). In obesity, the adipocyte hypertrophies because it continues to fill with TGs during energy imbalance. These cells will decrease in size in subjects who experience weight loss suggesting that the alterations are a transient effect (Coppack 2005). There is also evidence that there is a heterogeneous number of alpha$_2$ and beta ARs expressed on the adipocyte and

larger adipocytes have been shown to correlate higher with expression of alpha$_2$ inhibitors (Mauriege, Galitzky, Berlan, Lafontan 1987). The obese have more fat mass so it is likely that they will have more whole body lipolysis in absolute terms. When data are expressed per gram of fat, the obese have a lower rate of lipolysis when compared to the lean. Locally, in areas such as the subcutaneous AAT, the lipolytic rates can also differ between obese and lean adults.

Physical differences in adipocytes of obese and lean led investigators to hypothesize that there would be a difference in lipolysis between obese and lean individuals. Jansson, Larsson, Smith, and Lonnroth (1992) measured glycerol concentrations of subcutaneous adipose tissue in lean and obese men. The purpose of the study was to investigate differences of subcutaneous adipose tissue lipolysis in lean and obese men at rest. Subjects underwent the microdialysis procedure so that glycerol could be measured as a rate of lipolysis. The authors concluded that, at rest, the larger adipocytes of the obese had a higher lipolytic rate than the smaller cells of the lean.

Differences in lipolytic rates at rest of the obese and lean, led investigators to hypothesize that the adipocyte of subcutaneous AAT in obese individuals would respond differently than the lean during aerobic. Mittendorfer, Fields, and Klein (2003) recruited both lean and obese subjects to study the rates of lipolysis and fatty acid release during exercise. Subjects were determined by BMI to be lean, overweight, or obese. All subjects performed an aerobic exercise bout on a cycle ergometer at about 50% of VO_{2max}. Results of this study showed an inverse relationship between body fat mass and levels of glycerol (r^2 =.74). There was also an inverse relationship between body fat mass and FA release (r^2 =.69). This study suggests that increased body fat is associated with a reduced lipolytic rate, which limits the availability of FAs to be

used for oxidation. It has been suggested by Madsen, Bulow, and Nielsen (1986) that, because there is a large amount of FAs in the circulating blood, there is no need for the obese to increase lipolysis in subcutaneous AAT during exercise. According to Mittendorfer et al. (2004), the obese also oxidize less fat than the lean during exercise. It is possible that both Madsen et al. (1986) and Mittendorfer et al. (2004) are correct and the obese both (a) use less fat to exercise, and (b) the fat used does not originate from the subcutaneous adipose tissue. Unfortunately, these results do not address in vivo measurement of subcutaneous AAT

Stich, Glisezinski, Crampes, Hejnova, Cottet-Emard, et al. (2000) examined the effect of adrenergic regulation on the lipolytic rate of subcutaneous adipose tissue in lean and obese subjects during exercise. Results showed that the rate of glycerol appearance was lower in the obese subjects when compared to the lean. The authors also explored the relative contributions of the beta ARs and alpha$_2$ ARs during exercise and concluded that the alpha$_2$ AR inhibitors are activated during exercise in the obese. This does not appear to be true with the lean, which seem to have no activation of the alpha$_2$ inhibitors during exercise or their activation is overridden by the overwhelming effects of the beta AR agonist. This activation of alpha$_2$ inhibitors causes a lower lipolytic response to exercise in obese adults. Whether this holds true for children is still unknown.

Hershberger et al. (2004) used the microdialysis technique to measure the lipolytic response of lean and obese children. All children performed an acute bout of exercise at a heart rate of 140 beats per minute for about 20 minutes. Results showed no significant glycerol change between the lean and obese children in response to exercise. However, the study points out that different speeds were needed to elicit the 140 beats per minute in the obese and lean. On average,

the lean required a higher speed than the obese. This difference in speed could indicate a discrepancy in the work intensity between the two groups. This discrepancy could alter the response of subcutaneous adipose tissue to the effects of catecholamines during exercise. Though Hershberger et al. (2004) found no differences in the obese and lean children's lipolytic rate in response to exercise, there is evidence that obese children have a lower plasma catecholamine response to exercise (Eliakim, Alon, Nemet, Zaldivar, McMurray, Culler, Galassetti, Cooper 2006). More studies are needed to investigate these differences in exercise response between obese and lean children.

Training Effects on Lipolysis of Obese

Aerobic exercise training has been shown to cause many physiological changes in humans. The body adapts to stressors like exercise by increasing the availability or the sensitivity of stress controllers (e.g., hormones, catecholamines). This is true for both lean and obese individuals. These adaptations happen acutely as shown by Savard, Despres, Marcotte, Theriault, Tremblay, and Bouchard (1987). They also happen with long term training. Exercise training has been recognized as a positive effecter on fat loss and many mechanisms are utilized to enhance fat loss by increasing lipolysis and fat oxidation for fuel (Bjorntorp 1992; Lafontan, Berlan 1993).

. Another study by Horowitz (1999) examined the lipolytic rate of healthy men at rest both before and after aerobic exercise training. Results indicate that healthy men do not alter their resting lipolytic response after aerobic exercise training. Many in vitro studies have used the adipocyte to examine adipocyte response to exercise training. Despres, Bouchard, Savard, Tremblay, Marcotte, and Theriault (1984) used fat biopsies of subcutaneous adipose tissue both

before and after an aerobic exercise training program. Improved aerobic capacity from exercise training caused adaptations in the adipocyte marked by an increased lipolytic rate found in vitro. Deuster, Chrousos, Luger, DeBolt, Bernier, Trostmann, Kyle, Montgomery, and Loriaux (1989) examined catecholamine responses of trained and untrained men. All participants performed an exercise bout and the hormones from plasma were measured. Results show that exercise trained individuals required less sympathetic activity to perform at any given absolute workload as compared to untrained individuals. Trained individuals were also able to exercise longer.

Despres et al. (1984) also explored the effect of increasing training status of obese men and women to examine the response of the adipocytes to twenty weeks of aerobic exercise training. The results show that the adipocytes of both men and women increased lipolytic rate after exercise training when the cells were exposed in vitro to epinephrine. These results are in agreement with Deuster et al (1989) in that they agree that training status increases lipolysis. It is believed that training increases the response by the beta ARs and perhaps increases the ratio of beta ARs to alpha$_2$ ARs during acute exercise. Some studies have shown a decreased sympathetic response to the same absolute intensity during exercise after aerobic training. Other investigators have reported an increase in sympathetic catecholamine release in response to exercise after aerobic training. Results found by Greiwe, Hickner, Suresh, Cryer, and Holloszy (1998) suggest that higher levels of catecholamine were observed when the participants exercised at the same relative intensity after 10 weeks of training. These studies appear to have differing results. In reality, they all agree that measuring lipolysis or catecholamine response to training is highly dependent on the intensity used during the measurement. Evidence of the effects of training on the ARs of the adipocyte in obese individuals was observed recently in vitro (Stich et al. 1998). In vitro studies built a foundation of knowledge but they remain inept at

describing in vivo situations. The use of microdialysis is unique; allowing in vivo measurement of lipolysis in subcutaneous adipose tissue and specifically the subcutaneous AAT.

Riviere et al. (2003) hypothesized that aerobic training would improve the lipolytic response of subcutaneous adipose tissue in obese men. Subjects were initially sedentary before starting the training period. Subjects trained four months with a protocol that increased exercise intensity as time increased. Lipolysis was measured by the microdialysis technique before and after training. Glycerol release was higher in the obese subcutaneous adipose tissue post training when compared with levels before the intervention. Alpha blockade before training increased glycerol release but had no effect after the intervention. Authors concluded that the obese subcutaneous adipose tissue lipolysis is lower due to increased $alpha_2$ inhibition during exercise (an effect not seen in the lean). With training, the obese were able to reduce the inhibition of $alpha_2$ AR inhibitors and perhaps upregulate the beta AR agonist when performing an acute bout of aerobic exercise. This lack of $alpha_2$ AR inhibitors has been seen in other studies examining lipolysis and training status in normal weight males (Riviere et al. 2001).

Richterova et al. (2004) hypothesized that aerobic training of obese women would alter the lipolysis of subcutaneous adipose tissue. Phentolamine (alpha receptor blocker) infusion allowed examination of the adrenergic response to training. Phentolamine infusion before training resulted in a significant increase in glycerol levels of subcutaneous adipose tissue. This increase was not observed during phentolamine infusion after the increase in aerobic capacity. Results suggest that $alpha_2$ AR inhibition during exercise in obese women can be reduced with aerobic training. However, unlike other studies that suggest a lack of expression of $alpha_2$ ARs in adipocytes after training, this study suggests that training increases the delivery of catecholamine

in the plasma and not the sensitivity to the catecholamine by the adipocyte. Another study showed that exercise training increased the beta AR response to the catecholamine released in subcutaneous AAT in obese males (Stich, Glisezinski, Galitzky, Hejnova, Crampes, Riviere, et al. 1999). These conclusions were also made by Glisezinski, Crampes, Harant, Berlan, Hejnova, Langin, et al. (1998) who also showed that, with training, the obese can increase their responsiveness to catecholamine by the beta ARs while also blunting the antilipolytic effects of the alpha$_2$ ARs. Whatever the mechanism, it is clear that aerobic training causes lipolytic changes in adults. These changes may increase the body's ability to use fat during exercise. There is evidence showing that overweight men can increase their fat oxidation after a four month endurance training program (Crampes, Marion-Latard, Zakaroff-Girard, Glisezinski, Harant, Thalamas, et al. 2003)

Aerobic training adaptations are also evident in children. However, the review of previous literature provided no studies of training changes in lipolysis of the subcutaneous adipose tissue in obese children. This study aims to add to this body of knowledge by measuring the change in glycerol concentration in subcutaneous AAT of obese children and comparing them with the concentration change of lean children when exercising at the same relative intensity. The glycerol changes in response to an acute bout of exericse both before and after a 16 weeks of aerobic exercise training were also measured to determine the effects of aerobic training on subcutaneous AAT lipolysis of obese children.

CHAPTER 3: Methodology

Aim 1

Participants

Forty sedentary children between the ages of 8 and 11 were recruited from Greenville, NC and surrounding areas. Children were classified as obese (n =29) or lean (n =12) when compared to other children their age. Also, some of the obese (n =21) were randomly assigned into an exercise intervention group. Those children participating in more than thirty minutes of deliberate exercise each day for three days a week prior to the procedure were excluded. Children were prepubescent and did not exceed Tanner stage 2. No children were on any medications that targeted blood flow.

Preliminary Testing

The participants initially met at the East Carolina University (ECU) FITT building with their guardian to sign an informed consent (see Appendix B). Because the participants are children, it was necessary for the children and the parents to sign an assent form explaining the risks of the procedure (see Appendix C). Participants were paid a 50 dollar check for the procedure and a 25 dollar gift card from a local Greenville, NC vendor (compensation for the 8 hour monitoring in the FITT lab). This study was approved by the East Carolina University Institutional Review Board (IRB) (see Appendix A).

Body Composition

Height was measured with a stadiometer, and weight was measured using a Cardinal Detecto digital scale (model #708, Webb City, MO, USA). BMI was calculated from the weight in kilograms divided by the height in meters squared. Obese were classified as being above the 95th percentile and lean were classified as being below the 80th percentile when compared to other children their age, gender, and race as indicated by Kuczmarski (2002). The website used to determine percentile ranking can be found at www.cdc.gov/growthcharts. Participants were also evaluated by DXA (QDR-1000/w, Hologic, Inc., Waltham, MA) for the purpose of determining body fat percentage. The DXA machine is a three compartmental system that can distinguish between bone, fat, and lean tissue (Gutin et al. 1996). All obese children had a body fat percentage above 40%. The lean children had a body fat percentage below 28%. On this visit, the participants were also familiarized with the treadmill equipment in anticipation for the maximal oxygen uptake (VO_{2max}) treadmill protocol.

VO_{2max} Protocol

Participants performed a VO_{2max} treadmill protocol (see Appendix D) on a separate visit to the ECU FITT lab. All participants performed at least two VO_{2max} tests. If the results were not within 4%, another test was performed for a maximal of three. Oxygen uptake was determined by indirect calorimetry using the Parvo-Medics metabolic cart. Maximal.VO_2 was calculated by averaging the two closest results. Heart rate was obtained using the Polar Heart Rate Monitor with Cheststrap. Maximal heart rate was determined to be the highest heart rate achieved during any of the three VO_{2max} tests. Children were asked to rate their perceived exertion on a modified Borg Scale. This scale ranged from one as the lowest exertion to ten as the highest exertion. Children were given verbal encouragement to reach exhaustion.

Microdialysis Procedure

Participants arrived at the ECU FITT building on a Saturday morning after an overnight fast. The children were greeted and brought to the exam room and instructed to lay supine on the examination table for a procedure called microdialysis. The microdialysis technique has been used as a minimally invasive surgery with an insertion of a probe into both muscle and adipose tissue (Bolinder, Danielle, Kerckhoffs, Moberg, Hagstrom-Toft, Arner 2000; Lonroth et al. 1990). The probe allows certain sized metabolites such as glycerol to penetrate its semi-permeable membrane. The area 2 cm from the umbilicus is sterilized with iodine swabs. Coldspray (ethyl chloride) is applied directly for topical anesthesia before an 18g catheter, fitted with a probe guide, is inserted into the child's subcutaneous AAT and between the adipocytes. The needle is removed leaving the guide behind. The microdialysis probe (CMA 20 elite 14cm inlet tubing, 10 cm outlet tubing, PAES membrane with 20.000 Dalton cutoff. Solna, Sweden) was then inserted into the guide before the guide itself was removed. All that remains under the skin is the probe. This probe is secured with steri-strips and Tegaderm.

Sample Collection

Saline, and a known amount of ethanol (5 mmol/L), was perfused at a rate of 2 µl/min through the inserted probe using a CMA/Microdialysis 107 pump (Stockholm, Sweden). The fluid was perfused through the probe tubing and collected by a 300 µl CMA/Microdialysis plastic microvial placed on the exit tubing of the microdialysis probe. This fluid in the collection vial (termed dialysate) is measured for concentrations of glycerol.

These collection vials were obtained every hour in a controlled environment at the ECU FITT building. The children remained sedentary until it was time for the acute exercise portion of the day. Activity was be limited to board or video games and movies. Activity was measured by the Actigraph GT1M dual axis accelerometer during the 8 hour microdialysis visit. This accelerometer has been validated as a way to track activity and step count in children (Crouter et al. 2010).

Sample Analysis

After collection, samples were immediately capped and placed into a 4° Celsius refrigerator for storage. After all samples are collected, they were moved from a 4°C refrigerator to a -20°C freezer until they are measured for ethanol and glycerol concentrations. The ethanol outflow/inflow ratio (O:I ratio) was used to monitor changes that may occur in local blood flow (Bernt, Gutmann 1974; Hickner et al. 1991). Glycerol concentration was measured in duplicate by the CMA/600 automated analyzer (Chemical Microdialysis Analyzer, Stockholm, Sweden).

Diet

The children were fed a calorically controlled meal 1.5 hours prior to performing an acute bout of aerobic exercise. The meals were based on percentages of the three macronutrients: carbohydrate, protein, and fat (65%, 15%, and 20% respectively). The total caloric intake was based on the child's body composition, weight, and age. The participants were able to choose from a list of pre packaged meals (Lunchables). They were instructed to finish the entire meal within twenty minutes.

Acute Exercise

The exercise took place one and a half hours after breakfast. The time of exercise was always twenty six minutes on a Lifefitness 93ti Dualshock treadmill. The total minutes included a three minute warm-up and a three minute warm-down. The children walked (or jogged) on a treadmill at an intensity of 70% of their measured maximum heart rate (see Appendix E). This intensity was calculated using the child's maximal heart rate obtained from the VO_{2max} test. During the acute exercise, the treadmill speed was adjusted to maintain a heart rate corresponding to 70 % of the participant's heart rate maximum

Aim 2

Participants

The same obese children (n =21) examined in aim one were also used to test the hypothesis for aim two. These children received 16 weeks of aerobic exercise training. They returned to the FITT building (post testing) after the intervention for another examination of lipolysis.

Training Protocol

Each week the children set up a schedule that included 180 minutes structured activity monitored by an exercise trainer. Children met with trainers at the ECU FITT building for activities that included exercise machines (e.g., treadmill, cycle) and free play (e.g., soccer, jumprope). Participants exercised for an hour three times per week. Their heart rate, measured by a Polar heart rate monitor, was kept at an average of 140 heart beats per minute each hour session. This schedule continued for 16 weeks.

Post Testing

After 16 weeks of aerobic exercise training, the obese children came back in for another body composition and another VO_{2max} test for fitness. Children also came back to the FITT building for another microdialysis procedure. All efforts were made to repeat the day they had during the first microdialysis, including the time of the procedure. The children consumed the same meal they had on the first microdialysis visit during their 16-week follow-up visit. The speed of the treadmill also remained the same as the previous acute exercise protocol.

Statistical Analysis

An independent samples T test was used on descriptive variables to compare the differences in the mean between obese and lean participants. A paired samples T test was used to compare the difference in glycerol concentrations from the pre exercise to exercise timepoints within each group (obese, lean). We used an independent samples T test to compare the mean change in glycerol concentrations from pre exercise to exercise between the obese and lean. To examine the effects of 16 weeks of aerobic exercise training on the obese, we used a paired samples T test on descriptive variables from pre training to post training. Subcutaneous AAT response to training was analyzed by a paired samples T test to compare the mean change in glycerol concentrations during exercise on the two microdialysis days (pre training change vs. post training change). All values are expressed as the mean ± standard error. Significance level was set at $p < 0.05$.

CHAPTER 5: Results

Aim 1

Descriptive characteristics of both obese and lean participants are shown as the mean plus or minus the standard deviation of the sample (*Table 1*). The sample includes 28 obese and 12 lean children.

Table 1

Descriptive Statistics

	Obese (n=28)	Lean (n=12)	Range	
Variable	Mean ± SD	Mean ± SD	Min	Max
Age (yrs)	9.8 ± 0.9	9.5 ± 1.1	8	11
Height (cm)	147.4 ± 8.2	146.1 ± 10.4	133.0	170.0
Weight (kg)	58.5 ± 14.6	36.9 ± 7.1	30.4	85.9
BMI (%)	96.6 ± 4.5	56.1 ± 1.3	15	37
Total FM (kg)	24.4 ± 9.0	7.4 ± 2.3	4.0	41.0

Note: Participants >95th percentile in BMI are classified as obese. Participants <80th percentile in BMI are classified as lean. The obese had a body fat % >40% and the lean had a body fat % <28%.

Oxygen Uptake, Heart Rate, and Rating of Perceived Exertion Results

All participants performed a maximal oxygen uptake protocol on a treadmill for a test of fitness. The same participants also performed an acute bout of moderate exercise on the day of microdialysis. VO_2 and heart rate data obtained are shown as the mean ± the standard deviation (*Table 2*). Maximum heart rate was obtained from the maximal VO_2 protocol. The acute heart rate and Rating of Perceived Exertion (RPE) indicates the averages obtained during the last 15 minutes (steady state) of the acute bout of exercise on the day of microdialysis.

Table 2

VO_2 Statistics

	Obese (n=28)	Lean (n=12)	Range	
Variable	Mean ± SD	Mean ± SD	Min	Max
Weight (kg)	58.5 ± 14.6	36.9 ± 7.1	30.4	85.9
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	38.5 ± 6.8	41.5 ± 7.1	22.4	63.2
Max HR (bpm)	196.7 ± 7.5	201.0 ± 9.5	182	218
Acute HR (bpm)[1]	135.3 ± 6.8	138.3 ± 7.4	120	151
Acute RPE[1]	5.0 ± 3.1	5.2 ± 2.8	0	9

Note: Participants >95th percentile in BMI are classified as obese. Participants <80th percentile in BMI are classified as lean. The obese had a body fat % >40% and the lean had a body fat % <28%.

[1] Denotes statistics obtained from acute exercise during microdialysis day.

Lipolysis Response (Dialysate Glycerol) to Acute Exercise

The change in dialysate glycerol concentrations (μM) from pre exercise to exercise are shown as the mean plus or minus the standard error. (*Figure 1*) A student's T test between the mean change in glycerol dialysate concentration of the obese (8.1 ± 2.1 μM) was significantly (*P=0.039*) less than the lean (17.1 ± 4.2 μM).

FIGURE 1

Figure one shows the change in glycerol concentration of obese (n = 28) and lean (n = 12) children in response to aerobic exercise at 70 % HR_{max}. Dialysate glycerol (µM) was obtained from a microdialysis probe inserted 2 cm from the umbilicus.

*Indicates significance ($p < 0.05$)

Blood Flow Response to Exercise (Ethanol O:I ratio)

The change in blood flow from pre exercise to exercise is shown as the mean ± the standard error (Figure 2). The change in blood flow in the obese was slightly negative (-0.02 ± 0.04) and the change in lean was slightly positive (0.08 ± 0.05). An independent samples t test between the two means revealed no significance ($p = 0.14$).

FIGURE 2

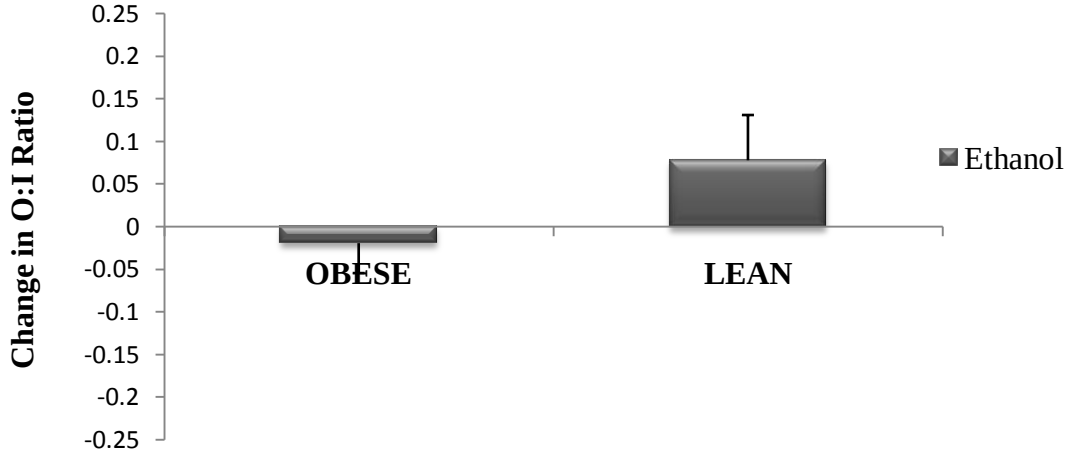

Figure two shows the change in blood flow (O:I) of obese (n = 28) and lean (n = 12) children in response to aerobic exercise at 70 % HR_{max}. Ethanol was obtained after perfusion througha microdialysis probe inserted 2 cm from the umbilicus.

Aim 2

Descriptive characteristics for the obese children who received the exercise intervention are shown as the mean ± the standard deviation (*Table 3*). 21 participants completed the intervention successfully. Participants increased height and weight (6.3 ± 7.7 cm and 2.1 ± 20.2 kg respectively) during the 16 weeks of aerobic training. However, body fat mass did decrease slightly (-0.4 ± 1.7 kg).

Table 3

Participant Characteristics

	Obese (n=21)			
	Pre training	Post training		Paired T test
Variable	Mean ± SD	Mean ± SD	Change	p value
Height (cm)	148.0± 8.0	151.6 ± 7.1	6.3 ± 7.7	0.4559
Weight (kg)	59.5 ± 13.5	61.2 ± 13.8	2.1 ± 20.2	0.6428
Body Fat (%)	41.8 ± 5.6	40.5 ± 6.1	-0.9 ± 2.8	0.2251
Total FM (kg)	24.9 ± 8.1	25.3 ± 7.9	-0.4 ± 1.7	0.3333
BMI (%tile)	97.1 ± 1.7	97.3 ± 1.9	0.8 ± 1.7	0.6605

Note: All participants completed 16 weeks of aerobic exercise training. P values produced from a Paired Samples T test.

Oxygen Uptake and Heart Rate After Training

Descriptive VO_2 data for the 21 obese participants is shown as the mean ± the standard deviation (*Table 4*). Statistical analysis by a paired T test is also represented. VO_{2max} increased from 1.9 ± 0.4 before training, to 2.0 ± 0.3 after training. After the aerobic training, heart rate decreased by 6.7 ± 10.3 beats when compared to the heart rate before training.

Table 4

Obese Intervention VO_2 Statistics

	Obese (n=21)			
	Pre training	Post training		Paired T test
Variable	Mean ± SD	Mean ± SD	Change	P Value
Weight (kg)	59.5 ± 13.5	61.2 ± 13.8	2.1 ± 20.2	0.6428
VO_2 (l/min)	1.9 ± 0.4	2.0 ± 0.3	0.1 ± 0.2	0.0016**
Acute HR (bpm)[1]	134.8 ± 7.0	127.2 ± 11.8	-7.6 ± 10.3	0.0032**
Acute RPE[1]	4.7 ± 3.1	3.3 ± 2.5	-1.3 ± 2.7	0.0531

Note: Participants >95th percentile in BMI are classified as obese. The obese had a body fat % >40%. Pre training refers to testing on the first microdialysis procedure. Post Training refers to testing on the second microdialysis procedure.

[1]Denotes statistics obtained from acute exercise during microdialysis day.

***Denotes significance of P<0.01*

Lipolysis Response (Dialysate Glycerol) to Aerobic Training

Descriptive statistics for the obese participants that received the exercise intervention are shown as the mean ± the standard error (*Table 4*). Twenty-one participants completed the intervention successfully. The average change in glycerol concentration was higher before the exercise intervention (9.5 ± 2.4 μM) than after the intervention (6.6 ± 2.7 μM) in response to aerobic exercise (*Figure 3*).

FIGURE 3

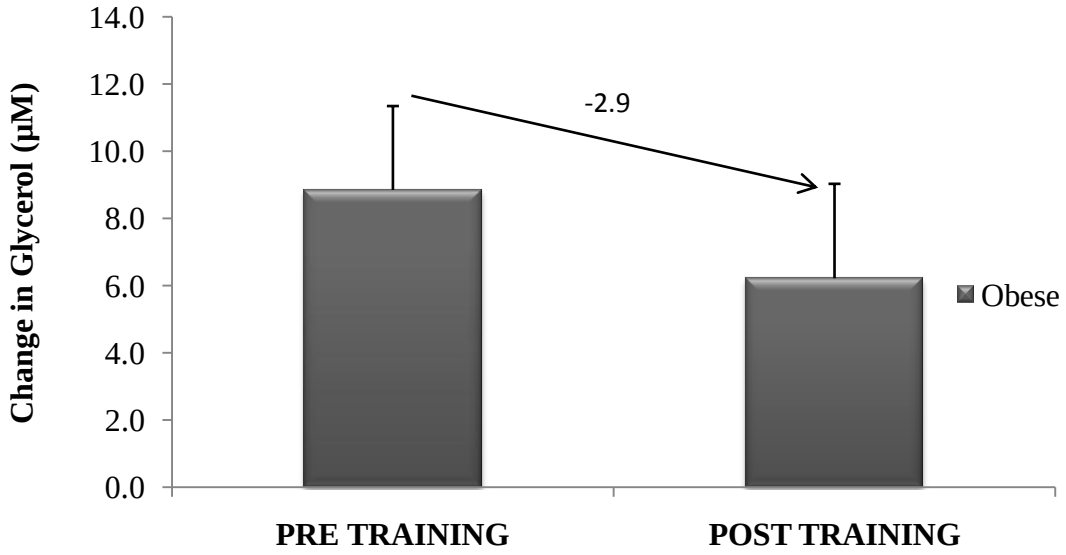

Figure one shows the change in glycerol concentration of obese (n = 21) children in response to aerobic exercise at 70 % HR_{max} both before and after 16 weeks of aerobic exercise training. Dialysate glycerol (µM) was obtained from a microdialysis probe inserted 2 cm from the umbilicus.

CHAPTER 6: Discussion

The review of the literature revealed few studies in lipolysis of subcutaneous AAT in children. In this study we measured the glycerol concentrations of subcutaneous AAT in both obese and lean sedentary children ages 8-11 during an aerobic bout of exercise. Obesity was defined as having a Body Mass Index (BMI) > 95th percentile and the lean was defined as having a BMI < 80th percentile. Sedentary was defined as participating in less than thirty minutes of structured exercise three days per week. The obese (n =28) group had approximately double the amount of participants when compared to the lean (n = 12).

In response to exercise, the obese raised their subcutaneous AAT lipolysis by an average of 8.1 ± 2.1 µM during exercise. The lean raised their lipolysis by an average of 17.1 ± 4.2 µM during the same exercise. Statistical analysis of the changes between obese and lean children in response to exercise revealed that the obese change was significantly less when compared to the lean ($p < 0.05$). These results suggest that lipolysis from subcutaneous AAT is less in obese than lean children in response to an acute exercise bout. These findings are in agreement with other studies involving obese and lean adults (Berlan et al. 2000; Mittendorfer et al. 2004), that found a reduced lipolytic response to exercise in obese adults when compared to lean. However, these results disagree with a childhood study (Hershberger et al. 2004) in which no difference was reported between obese and lean children in response to exercise. These differences may be attributed to the high variability within each group. Our individual glycerol concentrations included both positive and negative changes. Also, this study used a different intensity during the acute bout of exercise before the training intervention. Hershberger et al. (2004) used a heart rate

of 140 beats per minute for all children regardless of fitness level. Our study used intensities relative to that of the child's maximal heart rate (70%).

Though all children recruited in both studies were defined as sedentary, fitness levels may not have been equivalent. As suggested by previous studies (Despres et al. 1984; Hickner et al. 1999; Pillard et al. 2007), fitness level and intensity are major factors in the lipolytic response to catecholamines. Previous studies have also shown an increased catecholamine response when exercising at 50% - 70% VO_{2max} with the latter causing an even greater increase (Hickner et al. 1999; Wahrenburg et al. 1987). Our intensity was chosen to elicit a catecholamine response but one that would not interfere with the substrate utilized during the exercise bout.

Studies in adults suggest that hypertrophy of the adipocyte is related to functional changes in cell maintenance (Arner 2005; Coppack 2005; Large et al. 1998). Excess FAs are the cause of the hypertrophy and may interfere with the cells signaling in response to the catecholamines released by the sympathetic nervous system. It has been hypothesized that these changes may cause $alpha_2$ AR dominance in subcutaneous AAT of obese adults during exercise (Stitch et al. 2000). Evidence exists for resistance to catecholamines by adipocytes in obese adults and obese children (Bougnères et al. 1997; Eliakim et al. 2006; Reynisdottir 1994; Stitch et al. 2000). This resistance was only observed in the subcutaneous adipose tissue and indicates that it is not a whole body resistance but a local one. Unfortunately, due to the delimitations of this study, we were not able to measure the catecholamine response from exercise or the response of the subcutaneous AAT to the catecholamines. More studies are needed to answer whether or not the function of the ARs caused this blunted lipolytic response to exercise in the obese children.

It is unclear whether adipose tissue blood flow (ATBF) played a role in the results of this study. Studies have shown that the obese have a lower blood flow to the subcutaneous AAT. This is according to the outflow/inflow ratio (O:I ratio) which is inversely related to blood flow (Hickner et al. 1991; Hickner et al. 1999). In this study, we found no statistical differences (*p =0.14*) in the change of subcutaneous AAT blood flow between the groups (obese; lean) during the two timepoints (pre exercise and exercise) we measured. We did not get full recovery in our dialysate so the concentrations expressed are not a direct measurement of interstitial glycerol concentration or of lipolytic rate. However, both obese and lean started from the same average glycerol concentration (47.7 ± 6.6 µM; 48.1 ± 3.9 µM respectively) at the pre exercise timepoint. Therefore, a mean change score was the most appropriate analysis. It is unclear if nutrition played a role at any point during the lipolytic response to exercise. The normal response to a meal is antilipolitic (insulin) (Coppack et al. 2001). The children were fed a calorically controlled meal 1.5 hours prior to exercise but stomach emptying or digestion was not controlled. Also, total caloric intake was based on body weight and therefore inherently different in total caloric content between the obese and lean. However, both obese and lean started from the same baseline glycerol concentration (pre exercise) in our first analysis.

The review of literature showed few studies involving microdialysis of subcutaneous AAT in obese children, and no studies focused on the training adaptations of this depot. It is believed that obese adults have a blunted lipolytic response of subcutaneous AAT to aerobic exercise because of the dominance of the antilipolytic effects of the $alpha_2$ ARs (Stitch et al. 2000). This is a trait not evident in the lean or the trained individuals (Arner et al. 1990). Endurance training has been shown to decrease the antilipolytic effect of the $alpha_2$ ARs in obese and overweight men regardless of weight loss (Glisezinski et al. 1998; Glisezinski et al. 2003).

Other investigators have suggested that training increases the sensitivity of subcutaneous AAT to catecholamines (Crampes, Beauville, Riviere, Garrigues 1986). There is consensus in the science community that aerobic training causes changes, whether they are due to the availability of catecholamine or the receptors that bind to it, that are evident in the subcutaneous AAT of obese.

In this study, we also measured the change in lipolytic response of obese children (n =21) ages 8-11 to an acute bout of exercise before and after 16 weeks of aerobic exercise training. All children were ≥ 95th percentile in BMI. Subcutaneous AAT glycerol concentrations from two separate microdialysis procedures (pre training, post training, *Figure 2*) were compared to examine differences in lipolytic rate brought on by aerobic exercise training. A paired samples T test revealed that the mean changes in glycerol concentration before (8.9 ± 2.4 μM) and after (6.2 ± 2.8 μM) training were not significantly different ($p = 0.369$). These results may suggest that 16 weeks of aerobic exercise training decreases lipolytic rate. However, the design of this study did not control for the increased fitness level. A paired samples T test indicated that the obese children raised their aerobic capacity in response to the 16 weeks of training ($p<0.05$). However, the speed of the treadmill was the same during both microdialysis testing days. We used a given relative intensity (70% of maximum heart rate) during the acute exercise on the microdialysis day before the training intervention. However, on the second microdialysis, after training, we used the same treadmill speed (same absolute exercise intensity) during the acute bout of exercise as that used on the previous microdialysis acute exercise bout. If the same relative intensity had been used both before and after aerobic training, it is likely that plasma catecholamine concentrations, and therefore lipolysis, would have been higher after, as compared to before, training (Greiwe et al. 1999). There are also many other regulators of lipolysis that were not controlled for in this study but have been established regulators of lipolysis in adults

(Lafontan et al. 2009). Though we hypothesized that aerobic exercise training would increase the subcutaneous AAT's response to exercise in obese children, we found the opposite. However, these results are in agreement with other studies that have shown that subjects that are trained often have a lower lipolytic rate, catecholamine response, and lipid oxidation in response to exercise at the same absolute intensity before and after training. This decrease is due to adaptations during exercise that allow the body to deal with the stress of exercise as suggested by Wahrenburg et al. (1987).

In conclusion, we found a lower lipolytic response of subcutaneous AAT to acute exercise performed at 70% of the heart rate maximum in healthy obese children when compared to healthy lean children. These findings suggest that obesity is associated with a lower lipolytic response to exercise in subcutaneous AAT of obese children. Whether this is a cause of childhood obesity or an effect is unclear with the current results. Furthermore, we found that 16 weeks of training did not increase the subcutaneous AAT response to an acute bout of exercise in obese children when the same treadmill speed was used both before after the training intervention. More studies are needed to propose clinical strategies for preventing childhood obesity, and subsequently adulthood obesity.

References

Amer, P. (1999). Microdialysis: use in human exercise studies. *Proc Nutr Soc, 58*(4), 913-917.

Arner, P. (1984). Site differences in human subcutaneous adipose tissue metabolism in obesity. *Aesthetic Plast Surg, 8*(1), 13-17.

Arner, P. (1995). Impact of exercise on adipose tissue metabolism in humans. *Int J Obes Relat Metab Disord, 19 Suppl 4*, S18-21.

Arner, P. (2005). Human fat cell lipolysis: biochemistry, regulation and clinical role. *Best Pract Res Clin Endocrinol Metab, 19*(4), 471-482.

Arner, P., Kriegholm, E., & Engfeldt, P. (1990). In situ studies of catecholamine-induced lipolysis in human adipose tissue using microdialysis. *J Pharmacol Exp Ther, 254*(1), 284-288.

Arner, P., Kriegholm, E., Engfeldt, P., & Bolinder, J. (1990). Adrenergic regulation of lipolysis in situ at rest and during exercise. *J Clin Invest, 85*(3), 893-898.

Barbe, P., Galitzky, J., Riviere, D., Senard, J. M., Lafontan, M., Garrigues, M., et al. (1993). Effects of physiological and pharmacological variation of sympathetic nervous system activity on plasma non-esterified fatty acid concentrations in man. *Br J Clin Pharmacol, 36*(1), 25-30.

Berlan, M., & Lafontan, M. (1980). Identification of alpha 2-adrenergic receptors in human fat cell membranes by [3H]chlonidine binding. *Eur J Pharmacol, 67*(4), 481-484.

Berlin, I., Berlan, M., Crespo-Laumonnier, B., Landault, C., Payan, C., Puech, A. J., et al. (1990). Alterations in beta-adrenergic sensitivity and platelet alpha 2-adrenoceptors in obese women: effect of exercise and caloric restriction. *Clin Sci (Lond), 78*(1), 81-87.

Bjorntorp, P. (1997). Body fat distribution, insulin resistance, and metabolic diseases. *Nutrition, 13*(9), 795-803.

CDC. (2004). CDC director calls diabetes public health emergency. *J Miss State Med Assoc, 45*(11), 350, 344.

Coppack, S. W. (2005). Adipose tissue changes in obesity. *Biochem Soc Trans, 33*(Pt 5), 1049-1052.

Coppack, S. W., Patel, J. N., & Lawrence, V. J. (2001). Nutritional regulation of lipid metabolism in human adipose tissue. *Exp Clin Endocrinol Diabetes, 109 Suppl 2*, S202-214.

Crampes, F., Beauville, M., Riviere, D., & Garrigues, M. (1986). Effect of physical training in humans on the response of isolated fat cells to epinephrine. *J Appl Physiol, 61*(1), 25-29.

Crampes, F., Marion-Latard, F., Zakaroff-Girard, A., De Glisezinski, I., Harant, I., Thalamas, C., et al. (2003). Effects of a longitudinal training program on responses to exercise in overweight men. *Obes Res, 11*(2), 247-256.

De Glisezinski, I., Crampes, F., Harant, I., Berlan, M., Hejnova, J., Langin, D., et al. (1998). Endurance training changes in lipolytic responsiveness of obese adipose tissue. *Am J Physiol, 275*(6 Pt 1), E951-956.

De Glisezinski, I., Larrouy, D., Bajzova, M., Koppo, K., Polak, J., Berlan, M., et al. (2009). Adrenaline but not noradrenaline is a determinant of exercise-induced lipid mobilization in human subcutaneous adipose tissue. *J Physiol, 587*(Pt 13), 3393-3404.

De Glisezinski, I., Marion-Latard, F., Crampes, F., Berlan, M., Hejnova, J., Cottet-Emard, J. M., et al. (2001). Lack of alpha(2)-adrenergic antilipolytic effect during exercise in subcutaneous adipose tissue of trained men. *J Appl Physiol, 91*(4), 1760-1765.

De Glisezinski, I., Moro, C., Pillard, F., Marion-Latard, F., Harant, I., Meste, M., et al. (2003). Aerobic training improves exercise-induced lipolysis in SCAT and lipid utilization in overweight men. *Am J Physiol Endocrinol Metab, 285*(5), E984-990.

Despres, J. P., Bouchard, C., Savard, R., Tremblay, A., Marcotte, M., & Theriault, G. (1984). The effect of a 20-week endurance training program on adipose-tissue morphology and lipolysis in men and women. *Metabolism, 33*(3), 235-239.

Deuster, P. A., Chrousos, G. P., Luger, A., DeBolt, J. E., Bernier, L. L., Trostmann, U. H., et al. (1989). Hormonal and metabolic responses of untrained, moderately trained, and highly trained men to three exercise intensities. *Metabolism, 38*(2), 141-148.

Dietz, W. H., & Bellizzi, M. C. (1999). Introduction: the use of body mass index to assess obesity in children. *Am J Clin Nutr, 70*(1), 123S-125S.

Eliakim, A., Nemet, D., Zaldivar, F., McMurray, R. G., Culler, F. L., Galassetti, P., et al. (2006). Reduced exercise-associated response of the GH-IGF-I axis and catecholamines in obese children and adolescents. *J Appl Physiol, 100*(5), 1630-1637.

Fagot-Campagna, A., Pettitt, D. J., Engelgau, M. M., Burrows, N. R., Geiss, L. S., Valdez, R., et al. (2000). Type 2 diabetes among North American children and adolescents: an epidemiologic review and a public health perspective. *J Pediatr, 136*(5), 664-672.

Flegal, K. M., Carroll, M. D., Kuczmarski, R. J., & Johnson, C. L. (1998). Overweight and obesity in the United States: prevalence and trends, 1960-1994. *Int J Obes Relat Metab Disord, 22*(1), 39-47.

Ford, E. S., Zhao, G., Li, C., Pearson, W. S., & Mokdad, A. H. (2008). Trends in obesity and abdominal obesity among hypertensive and nonhypertensive adults in the United States. *Am J Hypertens, 21*(10), 1124-1128.

Galitzky, J., Lafontan, M., Nordenstrom, J., & Arner, P. (1993). Role of vascular alpha-2 adrenoceptors in regulating lipid mobilization from human adipose tissue. *J Clin Invest, 91*(5), 1997-2003.

Greiwe, J. S., Hickner, R. C., Shah, S. D., Cryer, P. E., & Holloszy, J. O. (1999). Norepinephrine response to exercise at the same relative intensity before and after endurance exercise training. *J Appl Physiol, 86*(2), 531-535.

Hershberger, A. M., McCammon, M. R., Garry, J. P., Mahar, M. T., & Hickner, R. C. (2004). Responses of lipolysis and salivary cortisol to food intake and physical activity in lean and obese children. *J Clin Endocrinol Metab, 89*(9), 4701-4707.

Horowitz, J. F. (2003). Fatty acid mobilization from adipose tissue during exercise. *Trends Endocrinol Metab, 14*(8), 386-392.

Horowitz, J. F., Braudy, R. J., Martin, W. H., 3rd, & Klein, S. (1999). Endurance exercise training does not alter lipolytic or adipose tissue blood flow sensitivity to epinephrine. *Am J Physiol, 277*(2 Pt 1), E325-331.

Kuczmarski, R. J., Ogden, C. L., Guo, S. S., Grummer-Strawn, L. M., Flegal, K. M., Mei, Z., et al. (2002). 2000 CDC Growth Charts for the United States: methods and development. *Vital Health Stat 11*(246), 1-190.

Kusminski, C. M., Shetty, S., Orci, L., Unger, R. H., & Scherer, P. E. (2009). Diabetes and apoptosis: lipotoxicity. *Apoptosis, 14*(12), 1484-1495.

Lafontan, M., & Berlan, M. (1981). Inhibitory alpha-2 adrenoceptors in human adipose tissue. *Int J Obes, 5*(6), 651-657.

Lafontan, M., & Berlan, M. (1993). Fat cell adrenergic receptors and the control of white and brown fat cell function. *J Lipid Res, 34*(7), 1057-1091.

Lafontan, M., Berlan, M., Galitzky, J., & Montastruc, J. L. (1992). Alpha-2 adrenoceptors in lipolysis: alpha 2 antagonists and lipid-mobilizing strategies. *Am J Clin Nutr, 55*(1 Suppl), 219S-227S.

Lafontan, M., Betuing, S., Saulnier-Blache, J. S., Valet, P., Bouloumie, A., Carpene, C., et al. (1998). Regulation of fat-cell function by alpha 2-adrenergic receptors. *Adv Pharmacol, 42*, 496-498.

Lange, K. H., Lorentsen, J., Isaksson, F., Simonsen, L., Bulow, J., & Kjaer, M. (2002). Lipolysis in human adipose tissue during exercise: comparison of microdialysis and a-v measurements. *J Appl Physiol, 92*(3), 1310-1316.

Large, V., Hellstrom, L., Reynisdottir, S., Lonnqvist, F., Eriksson, P., Lannfelt, L., et al. (1997). Human beta-2 adrenoceptor gene polymorphisms are highly frequent in obesity and associate with altered adipocyte beta-2 adrenoceptor function. *J Clin Invest, 100*(12), 3005-3013.

Lonnqvist, F., Wahrenberg, H., Hellstrom, L., Reynisdottir, S., & Arner, P. (1992). Lipolytic catecholamine resistance due to decreased beta 2-adrenoceptor expression in fat cells. *J Clin Invest, 90*(6), 2175-2186.

Lonnroth, P., & Smith, U. (1990). Microdialysis--a novel technique for clinical investigations. *J Intern Med, 227*(5), 295-300.

Madsen, J., Bulow, J., & Nielsen, N. E. (1986). Inhibition of fatty acid mobilization by arterial free fatty acid concentration. *Acta Physiol Scand, 127*(2), 161-166.

Mauriege, P., De Pergola, G., Berlan, M., & Lafontan, M. (1988). Human fat cell beta-adrenergic receptors: beta-agonist-dependent lipolytic responses and characterization of beta-adrenergic binding sites on human fat cell membranes with highly selective beta 1-antagonists. *J Lipid Res, 29*(5), 587-601.

Mauriege, P., Galitzky, J., Berlan, M., & Lafontan, M. (1987). Heterogeneous distribution of beta and alpha-2 adrenoceptor binding sites in human fat cells from various fat deposits: functional consequences. *Eur J Clin Invest, 17*(2), 156-165.

Mittendorfer, B., Fields, D. A., & Klein, S. (2004). Excess body fat in men decreases plasma fatty acid availability and oxidation during endurance exercise. *Am J Physiol Endocrinol Metab, 286*(3), E354-362.

Ogden, C. L. (2004). Defining overweight in children using growth charts. *Md Med, 5*(3), 19-21.

Ogden, C. L., Carroll, M. D., Curtin, L. R., McDowell, M. A., Tabak, C. J., & Flegal, K. M. (2006). Prevalence of overweight and obesity in the United States, 1999-2004. *Jama, 295*(13), 1549-1555.

Pillard, F., Moro, C., Harant, I., Garrigue, E., Lafontan, M., Berlan, M., et al. (2007). Lipid oxidation according to intensity and exercise duration in overweight men and women. *Obesity (Silver Spring), 15*(9), 2256-2262.

Richterova, B., Stich, V., Moro, C., Polak, J., Klimcakova, E., Majercik, M., et al. (2004). Effect of endurance training on adrenergic control of lipolysis in adipose tissue of obese women. *J Clin Endocrinol Metab, 89*(3), 1325-1331.

Stich, V., & Berlan, M. (2004). Physiological regulation of NEFA availability: lipolysis pathway. *Proc Nutr Soc, 63*(2), 369-374.

Stich, V., de Glisezinski, I., Berlan, M., Bulow, J., Galitzky, J., Harant, I., et al. (2000). Adipose tissue lipolysis is increased during a repeated bout of aerobic exercise. *J Appl Physiol, 88*(4), 1277-1283.

Stich, V., De Glisezinski, I., Crampes, F., Hejnova, J., Cottet-Emard, J. M., Galitzky, J., et al. (2000). Activation of alpha(2)-adrenergic receptors impairs exercise-induced lipolysis in SCAT of obese subjects. *Am J Physiol Regul Integr Comp Physiol, 279*(2), R499-504.

Stich, V., de Glisezinski, I., Galitzky, J., Hejnova, J., Crampes, F., Riviere, D., et al. (1999). Endurance training increases the beta-adrenergic lipolytic response in subcutaneous adipose tissue in obese subjects. *Int J Obes Relat Metab Disord, 23*(4), 374-381.

Tremblay, A., Coveney, S., Despres, J. P., Nadeau, A., & Prud'homme, D. (1992). Increased resting metabolic rate and lipid oxidation in exercise-trained individuals: evidence for a role of beta-adrenergic stimulation. *Can J Physiol Pharmacol, 70*(10), 1342-1347.

Ungerstedt, U. (1991). Microdialysis--principles and applications for studies in animals and man. *J Intern Med, 230*(4), 365-373.

Wahrenberg, H., Bolinder, J., & Arner, P. (1991). Adrenergic regulation of lipolysis in human fat cells during exercise. *Eur J Clin Invest, 21*(5), 534-541.

Wahrenberg, H., Engfeldt, P., Bolinder, J., & Arner, P. (1987). Acute adaptation in adrenergic control of lipolysis during physical exercise in humans. *Am J Physiol, 253*(4 Pt 1), E383-390.

Wahrenberg, H., Lonnqvist, F., Hellmer, J., & Arner, P. (1992). Importance of beta-adrenoceptor function in fat cells for lipid mobilization. *Eur J Clin Invest, 22*(6), 412-419.

Whitaker, R. C. (2004). Predicting preschooler obesity at birth: the role of maternal obesity in early pregnancy. *Pediatrics, 114*(1), e29-36.

Whitaker, R. C., Wright, J. A., Pepe, M. S., Seidel, K. D., & Dietz, W. H. (1997). Predicting obesity in young adulthood from childhood and parental obesity. *N Engl J Med, 337*(13), 869-873.

APPENDIX A: IRB Approval

University and Medical Center Institutional Review Board
East Carolina University • Brody School of Medicine
600 Moye Boulevard • Old Health Sciences Library, Room 1L-09 • Greenville, NC 27834
Office 252-744-2914 • Fax 252-744-2284 • www.ecu.edu/irb
Chair and Director of Biomedical IRB: L. Wiley Nifong, MD
Chair and Director of Behavioral and Social Science IRB: Susan L. McCammon, PhD

TO: Robert Hickner, PhD, Department of EXSS & Physiology, ECU

FROM: UMCIRB

DATE: July 8, 2009

RE: Full Committee Approval for Continuing Review of a Research Study

TITLE: Reduction in CVD Risk in Children Through Physical Activity

UMCIRB #05-0384

The above referenced research study was initially reviewed by the convened University and Medical Center Institutional Review Board (UMCIRB) on **9/21/05.** The research study underwent a subsequent continuing review for approval on **7/8/09** by the convened UMCIRB. The UMCIRB deemed this **NIH/NIDDK** sponsored study **more than minimal risk** requiring a continuing review in **12 months**. Changes to this approved research may not be initiated without UMCIRB review except when necessary to eliminate an apparent immediate hazard to the participant. All unanticipated problems involving risks to participants and others must be promptly reported to the UMCIRB. The investigator must submit a continuing review/closure application to the UMCIRB prior to the date of study expiration. The investigator must adhere to all reporting requirements for this study.

The above referenced research study has been given approval for the period of **7/8/09** to **7/7/10**. The approval includes the following items:

- Continuing Review Form (dated 6/24/09)
- Protocol Description from Grant Application (dated 3/4/05)
- Protocol Summary (dated 7/26/07)
- Informed consent (dated 7/26/07)
- Minor Assent (dated 7/26/07)
- YRBS (dated 8/1/05)
- Leisure Time Exercise Questionnaire (version 8/1/05)
- 30-Day Physical Activity Recall (version 8/1/05)
- 3-Day Food Record w/Instructions
- Medical History Form
- Physical Activity Logbook
- Peds QL (ver. 4.0)
- Food Frequency Questionnaire
- Physical Perception and Attraction to Physical Activity Scale
- Flyer (dated 1/8/09)

The following UMCIRB members were recused for reasons of potential for Conflict of Interest on this research study:
S. McCammon

NOTE: The following UMCIRB members with a potential Conflict of Interest did not attend this IRB meeting:
R. Hickner

The UMCIRB applies 45 CFR 46, Subparts A-D, to all research reviewed by the UMCIRB regardless of the funding source. 21 CFR 50 and 21 CFR 56 are applied to all research studies under the Food and Drug Administration regulation. The UMCIRB follows applicable International Conference on Harmonisation Good Clinical Practice guidelines.

APPENDIX B: Informed Consent Form

INFORMED CONSENT

Principal Investigator: Robert C. Hickner, Ph.D.
Institution: Human Performance Laboratory
Address: 371 Ward Sports Medicine Building
Telephone Number: (252) 328-4677

TITLE OF PROJECT: Reduction in CVD risk in children through physical activity

INTRODUCTION

Your child has been asked to participate in a research study being conducted by Robert C. Hickner and colleagues. This research is designed to determine the effect of physical activity on cardiovascular disease risk in children.

We will study lean and overweight preadolescent children. Studies will take place in the Human Performance Laboratory of East Carolina University and in Minges Coliseum.

PLAN AND PROCEDURES

Prior to testing, you, as a guardian(s) will read and sign this Informed Consent for research, as well as fill out a medical history questionnaire pertaining to your child.

Your child's participation will involve:

- You will fill out a personal history form that pertains to your child. Your child will fill out forms consisting of a youth risk behavior survey, leisure time exercise questionnaire, a personal history form, a medical form, a 30-day physical activity recall, pediatric quality of life inventory, and a physical self-perception profile and children's attraction toward physical activity scale

- Determination of **body composition** using body mass index (BMI), waist-to-hip ratio (WHR), skinfolds, and a DEXA Scan will be conducted at the Human Performance Laboratory. To calculate BMI, height and weight will be measured. Circumference measures will be taken at the waist and hip to calculate WHR. Finally, skinfold thickness of the tricep, subscapular (shoulder blade), abdomen, thigh, suprailium (hip bone), and calf will be taken on the right side of the body, in duplicate, with a skinfold caliper. Your child will undergo a test of body composition called a DEXA scan. It is like an x-ray of your entire body. During this test your child will be asked to wear minimal clothing (e.g., swimsuit, or shorts and a shirt, or a gown), and to remove all jewelry. He/she will lie still on a padded table for the length of the scan (approximately 6 minutes). The table will move across and up and down to scan his/her body. Your child will not feel anything and can breathe normally during the scan. If your child has metal in his/her body, then your child will not be able to participate in the DEXA scan. Radiation exposure from a DEXA scan is approximately 0.04

mrem. The effective radiation exposure that your child would receive in this study is less than 0.6% of the radiation exposure an individual receives from natural background sources in one year.

- Determination of **Aerobic Capacity**
  Two maximal treadmill tests will be completed to evaluate initial aerobic capacity. Two tests will be performed to assure that there is adequate effort by the children during the maximal treadmill test and to determine day-to-day variability in the test. If these two tests are not very similar, a third test may need to be conducted, so it is important that your child put out a maximal effort for this test. For this test, your child will walk or run on a treadmill for approximately 10-15 minutes. During this test, your child will wear a mouthpiece so the air they breathe out can be collected for analysis of oxygen. At first, your child will walk leisurely on the level treadmill, but the speed and level of hill climbing will become harder until your child can no longer continue.

  You will be asked to complete the n-3 FFQ at baseline, 4 weeks, 8 weeks, 12 weeks, and 16 weeks. This will allow us to determine 1) what your child eats over time and 2) seasonal variations in your childs age group and population.

- Your child will complete a 3 or 4 day food record at baseline (before microdialysis), 4 weeks, 8 weeks, 12 weeks and 16 weeks.

- Your child will wear a **physical activity monitor** (RT3 Triaxial Accelerometer) and a pedometer (Yamax, Japan) five days prior to microdialysis and during the microdialysis portion of the study. Additionally, your child will wear the accelerometer and pedometer for 3 days at 4 weeks, 8 weeks, and 12 weeks.

- A **fasting blood sample** will be obtained from an arm or hand vein. This will involve one to three small needle sticks. The procedure will take place in the Human Performance Laboratory.

- Your child will be given a cotton swab to test for **salivary cortisol levels** and will be instructed to chew on it for 45-60 seconds. Samples will be collected at 7 a.m. (fasting), 1:45 (prior to a standardized 2 p.m. lunch), and 30, 45, and 60 minutes after lunch. Additional samples will be collected every hour on the hour from 9 a.m. to 3 p.m. The collection of samples will take place in the Human Performance Lab.

- At the Human Performance Lab, the insertion of up to <u>three</u> small probes to determine glycerol levels and rates of lipolysis will take place. This probe, called a **microdialysis**

probe, is a small flexible piece of plastic tubing (about an inch long and the width of a needle) that is inserted through the skin, and then through the subcutaneous fat about 1/8 to 1/4 inch below the skin of the stomach. First, to numb the area of insertion, a topical cold spray (ethyl chloride) or a numbing creme (Emla creme) will be applied to the skin. A needle surrounded by plastic tubing will be inserted into the subcutaneous fat of your child's stomach. The needle will be taken out of the fat and replaced with the small piece of tubing. The tubing will not be located in a blood vessel but between fat cells. A Ringer solution (a saline/salt-water solution) will be pumped through the piece of tubing to monitor blood flow and fat break down in the fat tissue. The pumped fluid will be harmless to your child since it is similar to the fluid already present between the cells of the body. The Ringer solution will be pumped at a rate of no more than 5 microliters per minute (equivalent to a very tiny drop). Your child will not feel the presence or effects of this solution. Samples will be collected every hour while your child is at ECU and at home for the remainder of that day until the following morning. Only one overnight sample will be collected when your child wakes up the following morning.

- After the microdialysis pump is set up, your child will participate in activities pre-planned and provided by the study investigators. Activities will take place in Minges Coliseum. Possible activities will include walking on a treadmill, riding a stationary cycle ergometer, roller-skating, and jump roping. Other activities will include watching movies and playing board games. All activities will be monitored by a trained exercise physiologist who is familiar with the usage and safety precautions for each activity. By no means will your child be limited in what he/she can do during the day, except for activities that involve rough physical contact (for example, football).

- The full day monitoring will take place on a day that the child is already out of school (i.e. vacation, weekend).

- If your child is randomized (similar to picking groups by flipping a coin) to the 16-week physical activity program, he/she will undergo all testing described above (preliminary measurements and a separate visit of approximately 8 hours for microdialysis in the lab) <u>before and after</u> the 16 week program. If your child is randomized to the control group that does not participate in the 16 week physical activity program, they will be required to undergo only the preliminary measurements and another visit (microdialysis) of approximately 8 hours in the lab.

RISKS AND DISCOMFORTS

There are certain risks and discomforts that may be associated with this research. They include:

- The total amount of blood drawn for fasting blood draw is negligible. There is an extremely small risk of local hematoma or infection associated with the needle stick.

- Insertion of the microdialysis probe is associated with mild discomfort, similar to that experienced during an intramuscular injection. Your child will not feel discomfort from the substances (for example, Ringer solution) pumped through the microdialysis probe. Risks

associated with this procedure are small, and include hematoma (swelling and bruising) and infection. To minimize the risk of hematoma or infection associated with the insertion of the microdialysis probes into the subcutaneous adipose tissue, these procedures will be performed using sterile techniques. The probes are also made of biocompatible materials.

- There are some risks associated with physical activity such as bumps, bruising, scrapes and other injuries associated with active children.

    - Risks associated with the maximal exercise are dizziness, ventricular arrhythmia (odd heart beats), and in very rare instances death. These risks are very small, with an incidence of fewer than 1 in 10,000 deaths in patients who are known to, or suspected of, having heart disease. The risk is expectedly much smaller than this in a group of young, healthy subjects. To further minimize the risk, faculty and students that have been extensively trained in administering maximal exercise tests will administer the assessments. If during a test a subject complains of dizziness, chest discomfort or other signs of exercise intolerance, the test will be promptly stopped. In the event of loss of consciousness, breathing or heart beat, appropriate CPR and AED administration will be initiated and Greenville Fire/Rescue will be notified via 911.

    - Risks of the body composition assessment are those associated with exposure to low levels of radiation. Risks will be minimized by using an FDA-approved bone density machine (Prodigy, GE Lunar Corp., Madison, WI). This procedure involves a minimal amount of radiation. 1-3 microSieverts) that is within an acceptable range as provided by "North Carolina Regulations for Protection Against Radiation". The amount of radiation (1-3 microSieverts) exposure of one procedure is quite minimal. For example, radiation exposure is approximately 80 microSieverts on a transatlantic airline flight of 8 hours, 50 microSieverts living in Denver, Colorado, at an elevation of 5,000 feet for approximately 4 weeks, or 30 to 40 microSieverts during a typical chest x-ray. There is a potential risk to unborn children for those who are pregnant; therefore, pregnant women must not undergo this procedure.

    - Your child should be aware that there are unforeseen risks involved with this and all research studies.

POTENTIAL BENEFITS

Subjects will be able to participate in supervised physical activity and games. The risks are minimal relative to these benefits and the benefits of gaining knowledge with respect to the role of cortisol in childhood obesity.

TERMINATION OF PARTICIPATION

Your child's participation in this research study may be terminated without your consent if the investigators believe that these procedures will pose unnecessary risk to your child. Your child may also be terminated from the participation if your child does not adhere to the study protocol.

COST AND COMPENSATION
Your child will be paid $50.00 as well as prizes worth $25 for his/her time and inconvenience for completion of each microdialysis procedure.

The policy of East Carolina University does not provide for the compensation or medical treatment for subjects because of the physical or other injury resulting from this research activity. However, every effort will be made to make the facilities of Brody School of Medicine, Pitt County Memorial Hospital available for treatment in the event of such physical injury.

CONFIDENTIALITY
Only the investigators associated with this study will have access to the data obtained. No identifying information will be released. Numeric coding, which only the primary investigator will have access to, will protect the identity of your child and other subjects. Data will be secured in a locked filing cabinet in the office of the primary investigator in the Human Performance Laboratory. The data will be kept for 7 years. Samples will be stored in freezers at the Human Performance Laboratory for a maximum of 7 years. Your child can request destruction (discarded into biohazard containers and disposed of by ECU biohazard personnel) of his/her samples at any time.

VOLUNTARY PARTICIPATION
Your child understands that his/her participation in this study is voluntary. Refusal to participate will involve no penalty or loss of benefits to which your child is otherwise entitled. Furthermore, your child may stop participating at any time he/she chooses without penalty, loss of benefits, or without jeopardizing his/her continuing medical care at this institution.

RESEARCH PARTICIPANT AUTHORIZATION TO USE AND DISCLOSE INFORMATION
Federal laws require that researchers and health care providers protect your identifiable health information. Federal laws also require that researchers get your permission to use collected health information for research. The identifiable information we will collect from subjects in this research project will include:

*General Medical History including: Family health history, medications, nutrition, physical activity levels and body weight history.

*Body composition information, adipose tissue blood flow and metabolism, blood levels of insulin, glucose, free fatty acids, and other compounds related to cardiovascular disease risk.

The members of our research team that will have access to your information will include the Principle investigator, co-investigators, as well as technical and nursing personnel involved in this project. Information about you will be used and released in such a way that will protect your identity as much as possible; however, confidentiality cannot be absolutely guaranteed. We will only share your information with those individuals listed above. If we need to share information with other individuals other than those listed, we will request your permission a second time.

You will be given a signed copy of your authorization to release medical information for your records. You can limit the amount and type of information that is shared and you must

make this request in writing; however, the researcher is able to use any and all information collected prior to the request not to disclose information. Although you can limit the release of your medical information, withholding some information may cause you to become ineligible for this research project. Because research information continues to be looked at after a study is finished, it is difficult to say when the use of your information will stop. There is currently not an expiration date for the use and disclosure of your information for this study.

PERSONS TO CONTACT WITH QUESTIONS

If you have questions related to the sharing of information, please call Robert Hickner at 252-737-4677 (days) or 252-353-5556 nights or weekends or Joseph Garry, M.D. at 744-1953 (days) or 353-2825 (nights and weekends). You may also telephone the University and Medical Center Institutional Review Board at 252-744-2914. In addition, if you have concerns about confidentiality and privacy rights, you may phone the Privacy Officer at Pitt County Memorial Hospital at 252-847-6545 or at East Carolina University at 252-744-2030.

CONSENT TO PARTICIPATE

Your child certifies that he/she has read all of the above information, asked questions, and received answers concerning areas he/she did not understand, and have received satisfactory answers to these questions. Your child willingly consents for participation in this research study. (A copy of this consent form will be given to the person signing as the subject or as the subject's authorized representative.)

Participant's Name (Print)

Authorized Representative's Name (Print) – Guardian #1

Signature of Authorized Representative – Guardian #1 Date

Authorized Representative's Name (Print) – Guardian #2

Signature of Authorized Representative - Guardian #2 Date

AUDITOR WITNESS: I confirm that the contents of this consent/assent form were orally presented.

Auditor's Name (Print)

Signature of Auditor Date

Principal Investigator's Name (Print)

Signature of Principal Investigator Date

FUTURE TESTING OF BLOOD/MICRODIALYSIS SAMPLES

Upon termination of this study, the blood and urine samples collected for this study will be stored for up to 10 years to research scientific questions specifically related to cardiovascular disease risk in children. I will continue to be the owner of the samples and retain the right to have the sample material destroyed at any time during this study by contacting the study principal investigator. During this study the samples will be stored with number identifiers only; however, the number identifier will be linked to a specific name and will be kept on file in the possession of the principal investigator. The linked file will be stored password protected on the Principal Investigator's computer with CD backup. No other individuals will have access to these identifying materials unless the principal investigator is required by law to provide such identifying information. Data will not be publicly available and participants will not be identified or linked to the samples in publication. If a commercial product is developed from this research project, I will not profit financially from such a product.

CONSENT TO PARTICIPATE IN FUTURE TESTING OF BLOOD SAMPLES

I certify that I have read all of the above, asked questions and received answers concerning areas I did not understand, and have received satisfactory answers to these questions. I willingly give my consent for participation in this research study. (A copy of this consent form will be given to the person signing as the subject or as the subject's authorized representative.)

CONSENT TO PARTICIPATE

Your child certifies that he/she has read all of the above information, asked questions, and received answers concerning areas he/she did not understand, and have received satisfactory answers to these questions. Your child willingly consents for participation in this research study. (A copy of this consent will be given to the person signing as the subject or as the subject's authorized representative.)

Participant's Name (Print)

Authorized Representative's Name (Print) – Guardian #1

Signature of Authorized Representative – Guardian #1 Date

Authorized Representative's Name (Print) – Guardian #2

Signature of Authorized Representative - Guardian #2 Date

AUDITOR WITNESS: I confirm that the contents of this consent/assent form were orally presented.

Auditor's Name (Print)

Signature of Auditor Date

Principal Investigator's Name (Print)

Signature of Principal Investigator Date

APPENDIX C: Minor Assent Form

Assent Document for Children

Principal Investigator: Robert C. Hickner, Ph.D.
Institution: Human Performance Laboratory. 371 Ward Sports Medicine Building
Telephone Number: (252) 737-4677

TITLE OF PROJECT: Reduction in CVD risk in children through physical activity

You have been asked by Dr. Robert Hickner and workers in the Human Performance Lab to be part of a research project at East Carolina University. In this project, you will do several different things.

1. You will fill out forms about physical activity habits, including forms consisting of a youth risk behavior survey, leisure time exercise questionnaire, a medical form, a 30-day physical activity recall, pediatric quality of life inventory, and a physical self-perception profile and children's attraction toward physical activity scale

2. You will have your height, weight, and skinfolds and percent body fat measured. Skinfolds are measured by pinching different areas of fat on your body. You may feel a very light pinch. You will then go to another room where we will do a test called a DEXA Scan. It is like an x-ray of your entire body. During this test you will wear shorts and a shirt, or a gown, and you will take off any jewelry. You will then lie still on a padded table for about 6 minutes. The table will move across and up and down to scan your body, but you do not feel anything and can breathe normally during the scan.

3. You will come to the Human Performance Lab for a day (7 a.m. to 3 p.m.), where you will be able to play fun games, watch movies, and make new friends.

4. Someone at the lab will draw blood from a vein in your arm or hand. The needle stick will only hurt for a few seconds, although we may need to try up to three times if we do not get the blood on our first try.

5. You will have a small needle put into the fat under the skin of your stomach. You may feel a slight sting, but Dr. Hickner will try to make sure that this hurts as little as possible by spraying a cold spray or putting a cream on your stomach to numb your skin. A small plastic tube (as thin as a piece of thread) will be put through this needle under your skin. The needle will then be taken out after the plastic tube is in place. The plastic tube will then be hooked up to a little pump (smaller than a Walkman). You will have <u>three</u> of these needle sticks and plastic tubes put under the skin of the stomach. A liquid, called

Ringer's solution, will be pumped through the plastic tubes. This solution will help measure the break down of fat in your tissue. You will wear the pump on a belt while you are at ECU and while you are at home on this day until the next morning. You will have this test done before and after the 16-week physical activity program.

6. You will wear activity monitors, which looks like a pager, for 5 days prior to the day visit, and during the day visit. Additionally, you will wear the activity for 3 days during weeks 4, 8, and 12. You will
wear the monitors on your belt or clothes.

7. You will participate in a maximal exercise test on the treadmill. For this test, you will walk or run on a treadmill for approximately 10-15 minutes. During this test, you will wear a mouthpiece so the air you breathe out can be collected. At first, you will walk on the level treadmill, but the speed and level of hill climbing will become harder until you can no longer continue. You will go through this test on two separate days. If these two tests are not very similar, a third test may be needed, so it is important that you put out a maximal effort for this test.

8. You will participate in a 16-week physical activity program where you will skate, ride bicycles, and play active games. You will need to come to the activity center 3 to 4 times per week for at least one hour per time.
9. You will be asked to complete a 3 or 4 day food records at baseline (before microdialysis), 4 weeks, 8 weeks, 12 weeks and 16 weeks.
10. Your personal information and samples collected will be kept private and safe in the Human
Performance Lab. Only Dr. Hickner and co-workers will have access to your data. If you decide that you want you samples thrown out, your samples will be gotten rid of properly by workers at ECU.

_____ _____

Child's Name (print) (Date)

_____ _____

Child's signature (Date)

PERSONS TO CONTACT WITH QUESTIONS

The investigators will be available to answer your (or your guardian's) questions concerning this research, now or in the future. You or your guardian(s) may contact the investigators, Robert Hickner, Ph.D. at 737-4677 (days) or Joseph Garry, M.D. at 744-1953 (days) or 353-2825 (nights and weekends). Also, if questions arise about your rights as a research subject, you or your guardian(s) may contact the Chairman of the University and Medical Center Institutional Review Board at 252-744-2914 (days).

CONSENT TO PARTICIPATE

You certify that you have read all of the above information, asked questions, and received answers concerning areas you did not understand, and have received satisfactory answers to these questions. You willingly consent for participation in this research study. (A copy of this consent form will be given to the person signing as the subject or as the subject's authorized representative.)

Authorized Representative Name (Print) – Guardian #1

Signature of Authorized Representative – Guardian #1 Date

Authorized Representative Name (Print) – Guardian #2

Signature of Authorized Representative – Guardian #2 Date

AUDITOR WITNESS: I confirm that the contents of this consent/assent form were orally presented.

Objective Third Party Witness Name (Print)

Signature of Objective Third Party Witness Date

Principal Investigator's Name (Print)

Signature of Principal Investigator Date

APPENDIX D: VO_{2max} Treadmill Protocol

Treadmill Tech: _____

Ht._____Wt._____ DOB:_____ Previous(_____) TTE:_____, Max HR_____, Max BP_____

Subject _____ Age _____ Date _____

Age-Predicted Maximum HR _____ 85%_____ 100%

Sitting: HR _____ BP _____/_____/_____

	Stage (min)	SPEED (mph)	GRADE (%)	HEART RATE	RPE	COMMENTS BP
Submax	1	_____	0	_____		_____
(1)	2	_____	0	_____	_____	_____
2.5	3	_____	0	_____	_____	Blood Pressure
	4	_____	0	_____	_____	_____
	5	_____	0	_____	_____	_____/_____
				↓ (140)		
Submax	6	_____	0	_____	_____	_____
(2)	7	_____	0	_____	_____	_____
Hr 140	8	_____	0	_____	_____	Blood Pressure
	9	_____	0	_____	_____	_____
	10	_____	0	_____	_____	_____/_____
$VO_{2\ max}$	11	_____	0	_____	_____	_____
	12	_____	2	_____	_____	_____
	13	_____	4	_____	_____	_____
	14	_____	6	_____	_____	_____
	15	_____	8	_____	_____	_____
	16	_____	10	_____	_____	_____
	17	_____	10	_____	_____	_____
	18	_____	10	_____	_____	_____
	19	_____	10	_____	_____	_____
	20	_____	10	_____	_____	_____
	21	_____	10	_____	_____	_____
	22	_____	10	_____	_____	_____
	23	_____	10	_____	_____	_____
	24	_____	10	_____	_____	_____
	25	_____	10	_____	_____	_____

Total Treadmill Time _____ Max HR _____ Max BP _____ Max RPP_____

Test Terminated Due To:_____

Recovery: Active _____ Supine _____

Time min	Heart Rate	Blood Pressure (if needed)	Comments
1	_____		
2	_____		
3	_____	_____/_____	_____

APPENDIX E: Acute Exercise Protocol

Acute Exercise

Date: **Participant ID:**
Time: **Max HR:**
TM#: **Target HR:**

Warm-Up

Minute	Heart rate	Speed	RPE	Comments
1	_____	_____		_____
2	_____	_____		_____
3	_____	_____	_____	_____

20 minute exercise bout at 70% of HR max

4	_____	_____		_____
5	_____	_____	_____	_____
6	_____	_____		_____
7	_____	_____		_____
8	_____	_____	_____	_____
9	_____	_____		_____
10	_____	_____	_____	_____
11	_____	_____		_____
12	_____	_____	_____	_____
13	_____	_____		_____
14	_____	_____	_____	_____
15	_____	_____		_____
16	_____	_____	_____	_____
17	_____	_____		_____
18	_____	_____	_____	_____
19	_____	_____		_____
20	_____	_____	_____	_____
21	_____	_____		_____
22	_____	_____	_____	_____
23	_____	_____		_____

Warm down

24	_____	_____		_____
25	_____	_____		_____
26	_____	_____		_____

Avg. HR of the last 15 min. of exercise (steady state)_____
Avg. treadmill speed the last 15 min. of exercise (steady state)_____