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# Association of Fat Oxidation and Insulin Resistance in Prepubertal Children

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Association of Fat Oxidation and Insulin Resistance in Prepubertal Children

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**Dedication**

To my Uncle Tony,

Anthony O'Boyle III

December 15, 1941 - October 31, 2005

And to all of the other victims, living and dead, of Hurricane Katrina

been completed.  Thank you for all of the sacrifices you have made in order for me to achieve

this goal. This truly was a family sacrifice.

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**Abstract**

Identifying the relationship between fat oxidation and insulin resistance (IR) may provide vital clues to the mechanisms behind the development of metabolic disease in prepubertal children. The purpose of this study was to examine the association of fat oxidation with insulin resistance (IR) and insulin sensitivity ($S_I$) in prepubertal children. A total of 34 prepubertal 7-9 year olds (18 females, 16 males, 13 non-Caucasian, 21 Caucasian, 8.0±0.8 years, 36.5±12.1 kg) were observed. Subjects participated in indirect calorimetry to obtain respiratory quotient (RQ) and a blood test to obtain fasting insulin and glucose to calculate IR by homeostatic model assessment (HOMA). A subset (n=16) participated in Frequently Sampled Intravenous Glucose Tolerance Testing (FSIGTT) to obtain insulin sensitivity. Pearson correlations between RQ and IR and RQ and $S_I$ were performed. Partial correlations with respect to physical activity, breastfeeding, and birth weight were also performed. A general linear model was used to examine RQ with IR, and separately $S_I$ with respect to physical activity, breastfeeding, birth weight, race and sex. Respiratory quotient and IR were significantly associated when adjusted for physical activity, sex and race and breastfeeding, sex and race. In regards to birth weight, RQ and IR were significantly associated when adjusted for breastfeeding, birth weight, and race, but not when breastfeeding was removed from the model. The results of this study suggest lack of physical activity and breastfeeding may be the most influential risk for factors in the development of IR via a mechanism of impaired fat oxidation. Further research is needed to examine the role of physical activity, breastfeeding, and birth weight on fat oxidation and the development of insulin resistance in prepubertal children, however, the results of this study support the promotion of physical activity, breastfeeding, and good maternal nutrition.

**Chapter One**

**Introduction**

**Fat Oxidation and Insulin Resistance**

Impaired fat oxidation may be related to the pathogenesis of insulin resistance in skeletal muscle and perhaps to the pathogenesis of obesity, however the precise etiology of insulin resistance remains unclear (Kelley, 2005). An important, yet understudied mechanism underlying the development of insulin resistance is the impaired ability of skeletal muscle to oxidize fatty acids as well as the impaired ability to switch easily between glucose and fat oxidation in response to homeostatic signals, known as metabolic flexibility (Cahova, 2007).

Metabolic flexibility is an exciting theory that may provide insight into differences between individuals' muscle oxidative capacity linking fat oxidation with insulin resistance. Metabolic flexibility is characterized by an individual's skeletal muscle response to homeostatic signals. Metabolically healthy skeletal muscle is able to easily switch between glucose and fat oxidation whereas metabolically inflexible skeletal muscle is unable to oxidize fat under appropriate conditions (Cahova, 2007). Whether or not an individual may have impaired fat oxidation may be investigated in individuals by measuring the respiratory quotient (RQ). The RQ is an index of fat to carbohydrate utilization (Maffeis, 2005) and reflects the fuel source being metabolized (Kelley, 2005). A RQ of 0.70 represents fat oxidation whereas a RQ of 1.0 represents carbohydrate oxidation. It is well accepted that an elevated RQ indicates that fat oxidation may be impaired (Zurlo, 1990) leading to a reduced ability to oxidize fat (Ravussin, 2002). This elevated RQ and inability to rely upon fat oxidation demonstrates a metabolic inflexibility in the skeletal muscle (Kelley, 2005). This metabolic inflexibility is related to the development of insulin resistance in skeletal muscle (Cahova, 2007).

Insulin resistance is a critical factor in the development of type 2 diabetes. Type 2 diabetes begins when the body develops a resistance to insulin and no longer uses the insulin properly. Without insulin the body loses the ability to regulate glucose resulting in a build up of glucose in the cells (Perseghin, 2003). This dysregulation causes abnormalities in both carbohydrate and fat metabolism (Scheen, 2003). This dysregulation of fat metabolism begins in the very early stages of insulin resistance and well before the onset of type 2 diabetes. The precise mechanism behind the dysregulation of fat metabolism remains to be determined, however, it may be related to impaired fat oxidation (Lewis, 2002).

**Risk Factors for Impaired Fat Oxidation and Insulin Resistance**

Infancy and the intrauterine environment are considered critical periods for the development of metabolic abnormalities later in life (Sothern, 2004). Lucas (1991) and Jackson et al (1996) suggest there exists a programming response established by the interaction of the infant and their early environment (Hales, 1991; Barker, 1990, 1995). During this sensitive period of early life, long-term changes in physiology and metabolism may take place resulting in biochemical, metabolic and neurological disorders later in postnatal life (Sothern, 2004). There are several factors which may place individuals at risk for overweight and type 2 diabetes. Three of those risk factors include lack of physical activity, lack of breastfeeding, and birth weight.

**Lack of Physical Activity**

Obesity is considered the most prevalent nutritional disease of U.S. children (Hill, 1998). The metabolic changes that accompany excess body fat, especially during critical periods for obesity development in childhood, promote an increased risk for type 2 diabetes in adolescence and adulthood (James, 1999). Impaired growth and development during fetal life and infancy are linked to obesity in both childhood and adult life (Johnson, 2006; Forsen, 2000).

The capacity to oxidize fat may be modified by many factors including physical activity (Ravussin, 2002). Physical activity has been shown to improve body composition and insulin sensitivity (Dumortier, 2003; Ferguson, 1999; Kang, 2002). Moreover, the capacity for fat oxidation by skeletal muscle is increased in lean, aerobically fit individuals (Ukropcova, 2005).

**Lack of Breastfeeding**

Nutrition in both prenatal and early postnatal life may have long-term physiologic effects (Sothern, 2004). Researchers have suggested that breastfeeding may protect against the development of type 2 diabetes (Lucas, 1980; Ravelli, 2000; Pettitt, 1997), however findings from studies regarding this protective effect are inconsistent (Martin, 2005). Studies also observed that breastfeeding during early infancy may provide a protective effect against obesity in both childhood and adult life (Arenz, 2004; Owen, 2005). With this protective effect against obesity the capacity for fat oxidation in skeletal muscle may also be increased, however further research is needed in both children and adults.

**Birth Weight**

Size at birth for gestational age is a marker for fetal growth rate (Ong, 2004). This size at birth is an indicator of maternal and offspring health and of early childhood survival (Ong, 2004). In 1992, Hales and Barker introduced the thrifty phenotype hypothesis. This hypothesis proposed that poor fetal and infant nutrition was associated with an increased risk for the development of type 2 diabetes and the metabolic syndrome in adults. For the fetus, this thrifty way of managing poor nutrition leads to a differential impact on the growth of different organs with selective protection of brain growth.

Compared to the brain, skeletal muscle has a lower priority in nutrient partitioning (Zhu, 2006). Skeletal muscle, however, is the main site for the utilization of fatty acids and glucose

(Petersen, 2002); therefore the effects of poor nutrition in early life produce permanent changes in glucose-insulin metabolism as well as body fat distribution. These changes in glucose-insulin metabolism along with a reduced capacity for insulin secretion and insulin resistance may represent the most important factors in determining type 2 diabetes. Because insulin is a major fetal growth hormone, this glucose-insulin disruption in the fetal environment may affect birth weight (Hales, 2001). Because skeletal muscle is the primary site for fat oxidation and those with low birth weight typically have increased fat mass relative to lean mass (Tappy, 2006), individuals with low birth weight may be at risk for poor fat oxidation.

**Statement of the Problem**

Identifying the relationship between fat oxidation and insulin resistance may provide vital clues to the mechanisms behind the development of metabolic disease in prepubertal children. The reduced ability to oxidize fat is associated with impaired insulin resistance, however, it is unknown whether REE is reduced or RQ is elevated in prepubertal children. Insulin resistance may be detected as early as twenty years prior to the clinical diagnosis of type 2 diabetes (Perseghin, 2003). Therefore, identifying insulin resistance and understanding the association between insulin resistance and fat oxidation prior to the onset of type 2 diabetes may motivate individuals to modify their lifestyle and help prevent type 2 diabetes (Shaibi, 2006).

Insulin resistance plays a critical role in the development of type 2 diabetes. Insulin stimulates glucose uptake into tissues. The tissues of individuals with insulin resistance display a diminished ability to respond to the action of insulin. To compensate for this resistance, the pancreas secretes more insulin, therefore individuals with insulin resistance have high plasma insulin levels (Rao, 2004). These high levels of insulin lead to a decrease in insulin sensitivity. Low insulin sensitivity is a predictor of the development of type 2 diabetes (Ivy, 1999).

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Research examining insulin sensitivity and resistance is an important, yet understudied topic. Treatment of insulin resistance at an early age in life may be crucial for preventing diabetes in adolescence and adulthood (Kahle, 1996).

The prepubertal period represents a critical period of obesity development which is followed by a pubertal, physiologic insulin resistance (Amiel, 1986; Goran, 2001). Therefore, the need to study fat oxidation and insulin resistance prior to puberty is particularly important. The prepubertal period may also be an opportune time to modify risk factors, such as physical activity before the child enters puberty.

Figure 1 provides a model of this programming pathway and the impact that risk factors may have in the development of adult diseases, such as coronary heart disease and type 2 diabetes. These factors begin *in utero* and continue throughout puberty. Understanding the role impaired fat oxidation may play at these critical periods, particularly during the prepubertal period, may help prevent future diseases (Kajantie, 2006).

Maternal nutrition plays a direct role in the development of the fetus. Without proper nutrition, nutrient and oxygen availability are reduced in the fetus. This reduction in essential needs leads to a programming response in the fetus (Barker, 2002). During this fetal programming period, negative changes take place in organ structure that may affect muscle, fat, the pancreas and liver providing a pathway leading to adverse health effects from infancy to prepubertal years and into adulthood (Kajantie, 2006). Breastfeeding during the first year(s) of life, however, may affect the composition of skeletal muscle (Baur, 1998) and may provide a protective effect against the development of overweight and type 2 diabetes (Pettitt, 1997). Low birth weight may be a direct result of maternal nutrient restriction. Because skeletal muscle is not as essential compared to the brain and heart, skeletal muscle development may be impaired

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as a result of poor maternal nutrition. This impaired skeletal muscle may predispose this child to negative health consequences, in particular overweight and type 2 diabetes (Zhu, 2006).

Physical activity in childhood, adolescence, and in adulthood may also help to prevent overweight and type 2 diabetes by enhancing the capacity to oxidize fat, improving insulin sensitivity and body composition (Schmitz, 2002, Ukropcova, 2005).
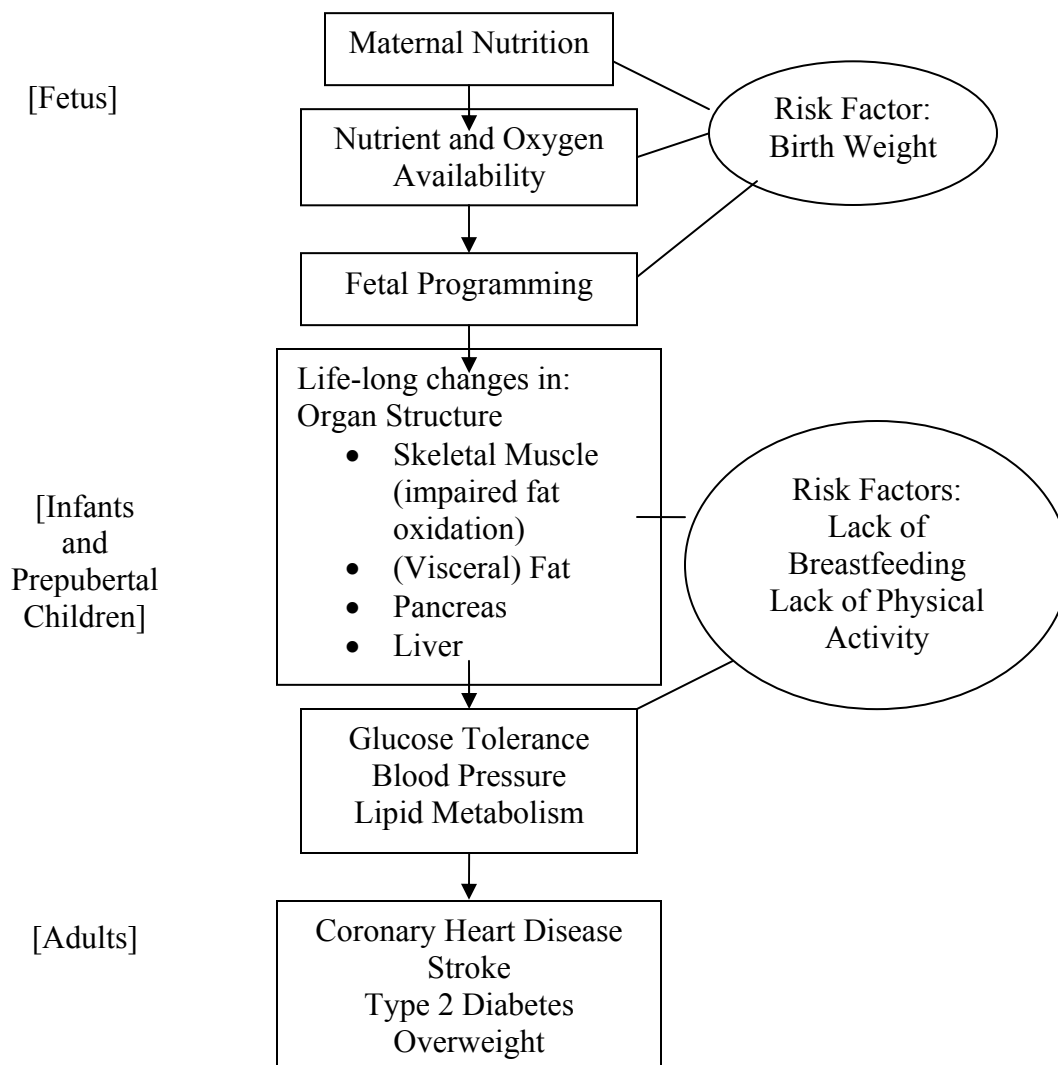
**Figure 1.** Possible pathways of fetal and pubertal programming of adult disease.

(Modified from Kajantie, 2006)

**Specific Aim I:**

Examine the association of impaired fat oxidation with insulin resistance in apparently healthy, prepubertal children.

The RQ was measured by indirect calorimetry and evaluated as an index of fat oxidation. Insulin resistance was calculated from the homeostasis model assessment (HOMA) formula. The following risk factors were controlled for independently and collectively in the analysis: race, sex, physical activity, breastfeeding, and birth weight.

**Hypothesis I:**

Impaired fat oxidation is associated with insulin resistance in apparently healthy, prepubertal children after controlling for race, sex, and the following risk factors:

> a. lack of physical activity
>
> b. lack of breastfeeding
>
> c. birth weight

**Specific Aim 2:**

Examine the association of impaired fat oxidation with insulin sensitivity in apparently healthy, prepubertal children.

The RQ was measured by indirect calorimetry and evaluated as an index of fat oxidation. Insulin sensitivity was assessed using the minimal model technique of Bergman (1987). The following risk factors were controlled for in the analysis: race, sex, physical activity, breastfeeding, and birth weight.

**Hypothesis 2:**

Impaired fat oxidation is associated with insulin sensitivity in apparently healthy, prepubertal children after controlling for race, sex, and the following risk factors:

a. lack of physical activity

b. lack of breastfeeding

c. birth weight

The overall objective of this study was to examine the association between impaired fat oxidation and insulin resistance and impaired fat oxidation and insulin sensitivity in apparently healthy, prepubertal children.

**Definition of Terms**

Respiratory Quotient (RQ):  the ratio of the volume of the carbon dioxide released to the volume of oxygen consumed by an organism or cell in a given period of time.  RQ was determined by ventilated hood indirect calorimetry.

Resting Energy Expenditure (REE): the amount of calories required for a 24-hour period by the body during a non-active period. REE was determined by ventilated hood indirect calorimetry.

Fat Oxidation: the process by which the long fatty acid chains are broken down into 2 carbon units and energy (ATP). RQ was used as the measure of fat oxidation.

Substrates: the material or substance on which an enzyme acts.  Proteins, carbohydrates, and lipids constitute the main substrates for digestive enzymes.

Metabolic Flexibility: skeletal muscle response to homeostatic signals.

Free Fatty Acids: the major lipid transporters in the body.

Insulin Resistance:  a condition when physiologic concentrations of insulin are unable to properly regulate processes necessary for glucose and lipid homeostasis.  Insulin resistance was calculated by the homeostasis model assessment (HOMA) formula.

*ß*-cells: insulin secreting cells in the pancreas which help control blood sugar levels.  *ß*-cell function was derived from the HOMA formula.

Body Mass Index (BMI): used to assess weight relative to height and is calculated by dividing body weight in kilograms by height in meters squared ($kg/m^2$).

Physical Activity: bodily movement that is produced by the contraction of skeletal muscle and that substantially increases energy expenditure.  Physical activity was measured by the Godin-Leisure Time questionnaire and by accelerometer (Godin, 1985).

Programming:  a process by which a stimulus during a critical period of development has lasting or lifelong significance.

Hyperglycemia: a condition in which an excessive amount of glucose circulates in the blood plasma.

Hyperinsulinemia: a condition in which excess levels of insulin circulates in the blood plasma.

Frequently Sampled Intravenous Glucose Tolerance Test (FSIGTT): a method that assesses insulin sensitivity by a computed mathematical analysis of glucose and insulin dynamics.

**FSIGTT measures**:

Insulin Sensitivity ($S_I$): represents the increase in net fractional glucose clearance rate per unit change in serum insulin concentration after the intravenous glucose load. It indicates the net capacity for insulin to promote the disposal of glucose and to inhibit the endogenous production of glucose.

Glucose Effectiveness ($S_g$): represents the net fractional glucose rate due to the increase in glucose itself without any increase in circulating insulin concentration above baseline.  It indicates the capacity of glucose to mediate its own disposal.

Acute Insulin Response to Glucose ($AIR_g$): represents the acute insulin response and is defined as the area under the plasma insulin curve between 0 and 10 minutes.

Disposition Index (DI): a quantitative measure that describes the relationship between ß-cell sensitivity and insulin sensitivity. It is an overall measure of the ability of the islet cells to secrete insulin normalized to the degree of insulin resistance.  DI is the product of $AIR_g$ and $S_I$.  DI = $AIR_g$ x $S_I$

## Chapter Two

## Review of Literature

**Fat Oxidation**

The body exhibits an oxidative hierarchy (Maffeis, 2000). Carbohydrate and protein intake induce carbohydrate and protein oxidation therefore, carbohydrates and proteins are efficiently self-regulated. Fat intake, however, is not able to induce fat oxidation therefore fat balance is affected by the balance of the other two nutrients and depends strictly on them. Independent of fat intake, the body exhibits a preference for the oxidation of carbohydrate and protein rather than fat. With the exception of fat that is oxidized, ingested fat is preferentially stored but is also dependent on the amount of protein ingested and oxidized (Maffeis, 2000). The respiratory quotient (RQ) is an index of fat to carbohydrate utilization (Maffeis, 2005). It is well accepted that an elevated RQ indicates that fat oxidation may be impaired (Zurlo, 1990) leading to a reduced ability to oxidize fat (Ravussin, 2002).

The following RQ values represent the type of nutrient oxidation taking place:

$$RQ = 0.7 = Fat$$

$$RQ = 0.8 = Protein$$

$$RQ = 1.0 = Carbohydrate$$

Fat balance plays a critical role in the regulation of body weight. Fat balance is calculated from the ratio between dietary fat and fat oxidation. A positive fat balance results from the lipid content of the diet being higher than the overall fuel mix oxidized. This positive fat balance leads to a positive fat storage. The perpetuation of this process leads to a progressive increase in body fat mass. These increased fat stores, supported by a high proportion of fat in the diet may promote fat oxidation leading to a lipid balance readjustment. This readjustment then

leads to the maintenance of a new weight equilibrium at a higher level (Maffeis, 1995). Maffeis et al (1995) observed a significantly higher fat oxidation in prepubertal obese children compared to normal weight children. Additionally, a significant association between the postabsorptive fat oxidation rate and fat mass was observed in these obese children, therefore favoring a new equilibrium in fat balance (Maffeis, 1995).

In a study of Pima Indians from Arizona, Zurlo et al (1990) determined that a high RQ (>0.877), indicative of relatively low fat oxidation, was predictive of future weight gain whereas a high fat oxidation rate (low RQ, <0.822) plays a protective role in the risk of weight gain (Maffeis, 2000). The capacity to oxidize fat, however, can be modified by many factors including physical activity (Ravussin, 2002). Physical activity, in particular regular exercise, promotes fat oxidation in the muscle as well as post-exercise oxygen consumption (Maffeis, 2000).

Total daily expenditure consist of three components: 1) resting energy expenditure (REE), 2) food-induced thermogenesis (the energy cost of digestion, absorption, and processing nutrients), and 3) energy expenditure related to physical activity (Tounian, 1999). Resting energy expenditure (REE) is determined by fat free mass (FFM), fat mass and sex (Ravussin, 1986). However, independent of these covariates, a low REE is considered a risk factor for weight gain and impaired insulin sensitivity (Ravussin, 1988). However, the relationship of REE to other risk factors for metabolic disease, such as type 2 diabetes, is unclear.

|  |  |  |
|---|---|---|
| Good Fat Oxidation | → | High REE |
|  |  | Low RQ |
| Poor Fat Oxidation | → | Low REE |
|  |  | High RQ |

Skeletal muscle is a major determinant of resting metabolic rate (Blaak, 2005) and is the main site of the manifestation of insulin resistance (Cahova, 2007). Skeletal muscle consists of two types of fibers. The first is Type I, also known as a slow twitch fiber. Type I fibers are known for their high oxidative potential and their excellent capacity for using lipids as fuel. Studies have observed an inverse relationship between body fat and percentage of Type I fibers. Type II fibers are also known as fast twitch fibers, however, they can be broken down into Type IIa and Type IIb. Type IIb fibers almost exclusively rely on glucose and glycogen for fuel whereas Type IIa fibers may be intermediate and overlap between the oxidative capacity between Type I and Type IIb (Blaak, 2002).

Skeletal muscle is the largest compartment in the body and the one most capable of using fatty acids for energy generation (van Baak, 1999). Skeletal muscles are responsible for the greatest amount of oxidized fat (Maffeis, 2005) and are the primary site for glucose uptake (Ivy, 1999). At rest, skeletal muscle plays a major role in carbohydrate and fat metabolism. During exercise, metabolic flux in the skeletal muscle dominates whole body energy flux. Therefore any impairment of muscle oxidative metabolism will lead to the accumulation of bodily energy stores.

Metabolic flexibility refers to a concept that skeletal muscle adapts to two opposite physiological conditions: reduced energy intake and a reliance on fat oxidation during fasting and an increased energy expenditure during sustained exercise and insulin stimulated conditions (Kelley, 2005). Metabolically healthy skeletal muscle is able to switch between fat oxidation during fasting conditions and glucose uptake and oxidation in postprandial conditions, with a suppression of fat oxidation. Metabolic inflexible skeletal muscle is unable or has a diminished capacity to switch between fuels (Blaak, 2005).

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Free fatty acids are the major lipid transporters in the body. They are the only form of fat released from adipose tissue and are the primary energy source for the heart and skeletal muscle (Raz, 2005). Fat storage and mobilization from fat storage sites is abnormal at a very early stage in insulin resistance syndrome. In insulin sensitive tissues, such as skeletal muscle and in the liver, free fatty acids impair glucose metabolism (Lewis, 2002) and greatly reduce metabolic flexibility (Cahova. 2007). Figure 2 represents the metabolic fate of fat intake. Ingested fat is preferentially stored, however, carbohydrate and protein intake and oxidation as well as exercise can impact the metabolic storage of fat (Maffeis, 2000).
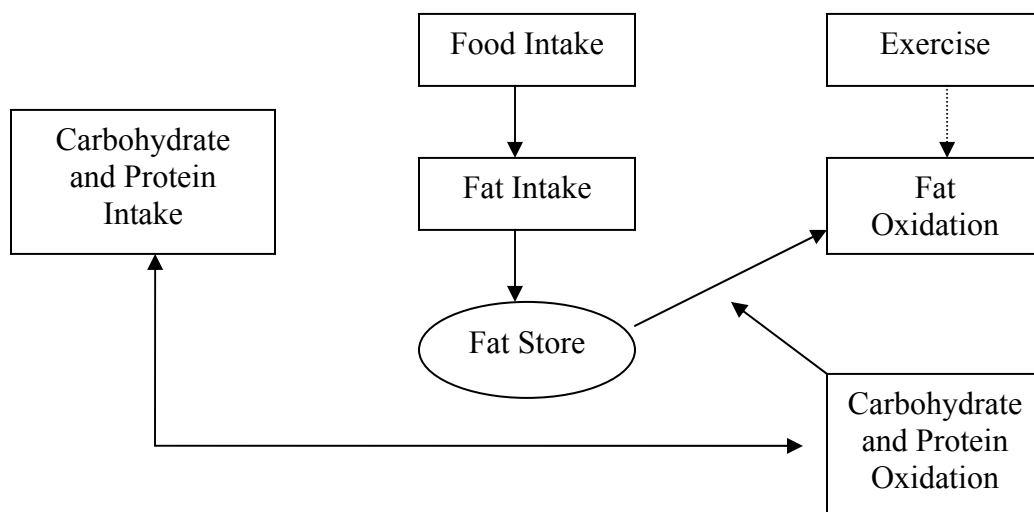
**Figure 2**. Metabolic fate of fat intake and relationship to carbohydrate and protein oxidation and skeletal muscle activity (Maffeis, 2000).

Metabolic inflexibility of skeletal muscle is an important mechanism underlying the development of insulin resistance. Those with type 2 diabetes and obesity display a great reduction of metabolic flexibility (Cahova, 2007). The skeletal muscle of diabetic and overweight individuals demonstrates a lower reliance on fat and a greater reliance on glucose oxidation and an impaired suppression of fat oxidation by insulin (Kelley, 2005). During development of insulin resistance, skeletal muscle displays a diminished ability to oxidize fatty

acids as a consequence of elevated glucose oxidation in the situation of hyperglycemia and hyperinsulinemia (Cahova, 2007).

Obesity, in particular visceral adiposity, is associated with increased concentrations of circulating free fatty acids. In obese adults with visceral fat, skeletal muscle fatty acid oxidation was shown to be impaired during post-absorptive conditions, whereas glucose uptake and glucose oxidation were increased (Blaak, 2002). Fat free mass is known as the metabolically active tissue and the main determinant of REE (Tounian, 2003). Although obese individuals have an increased fat mass, their FFM is increased as well. Therefore, the problem with fat oxidation therein does not appear due to a lack of fatty acids (Blaak, 2002). Under stimulated (or exercise) conditions, delivery of FFA via the blood to the muscle may be decreased in obese individuals and this deficient delivery does not appear to normalize with weight loss (van Baak, 1999). Furthermore, the increase observed in glucose uptake in obese individuals does not appear to normalize after weight loss (Blaak, 2002). This impaired capacity to use fat as a fuel may explain why obese individuals actually develop and/or maintain an overall increase fat storage or why so many individuals regain weight after weight loss (Blaak, 2002). This impairment in skeletal muscle oxidative capacity may play a role in the pathogenesis of obesity (Blaak, 2005).

Molnar and Schutz (1998) investigated fat oxidation in obese and non obese prepubertal and pubertal children. The obese children displayed a significantly higher fat oxidation compared to the normal weight children even after adjusting for lean body mass. However, the difference in fat oxidation disappeared after adjusting for fat mass. Moreover, an increase in fat oxidation was observed with the onset of puberty. After adjusting for lean and fat mass during

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pubertal development, no change was observed in fat oxidation leading to the effect of puberty on fat oxidation being solely related to changes in body composition (Molnar, 1998).

**Fat Oxidation Before, During, and After Physical Activity in Obese Children**

Tounian et al (2003) investigated REE and substrate utilization in 16 obese children (mean age $12.2 \pm 1.7$ years). REE and substrate utilization were measured via indirect calorimetry after an overnight fast. Compared to the control children, REE was significantly higher (18%, $p<0.03$) in the obese children. REE was also significantly correlated with FFM ($r = 0.89$, $p<0.0001$). Controlling for FFM, the measured REE in the obese children was similar to the predicted REE. In all analyses performed, REE was consistently the most important contributor to the variance of fat oxidation. This increased REE caused by the increased FFM is believed to eventually offset the increased energy intake. Conversely, a decrease in energy intake results in a decrease in REE to a level below that predicted by the decrease in FFM, therefore making weight loss only achievable by reducing the energy intake even more or by increasing the level of physical activity. FFM was positively correlated with carbohydrate, fat, and protein oxidation rates whereas fat mass was positively correlated only with fat oxidation rate. This correlation between fat mass and fat oxidation rate in the obese children suggests that in addition to a positive fat balance producing increases in body fat stores, another effect may be taking place. An increase in fat oxidation leading to a decrease in fat storage may lead ultimately to a restoration of a neutral fat balance at a higher percentage of body fat. This also suggests that when the fat balance is negative, the fat stores may decrease only until fat oxidation is equal to that of fat intake. This would make the energy balance near identical to fat balance, therefore supporting a usual higher fat intake in obese children.

Tounian (1999) also examined REE and carbohydrate-induced thermogenesis (CIT) in ten massively obese girls before and after weight loss (baseline mean age $15.4 \pm 1.1$ years). Significant weight loss ($p<0.005$) was observed after an intervention of a controlled diet of low-energy meals and physical activity. Baseline results were collected 2 to 5 weeks after the intervention began whereas the post results were collected 4.5 to 11.5 months later. The results were compared to eight healthy age-matched girls. As observed in the previous study, not controlling for FFM, the obese girls displayed a REE value significantly higher (16%) than the normal weight girls. Using a regression model that allowed the researchers to match each obese girl with a theoretical control having the same FFM, after only two to five weeks on a low-energy diet, the obese girls displayed lower REE values compared to the controls. These lower REE values persisted even after significant weight loss. The researchers concluded that weight restriction rather than weight loss appeared to be the major cause of decrease in REE for FFM. Other studies have observed no differences in REE adjusted for FMM from before weight loss to after weight loss in both children and adults (Maffeis, 1992; Larson, 1995). CIT was found to be similar between the obese girls and the controls, however, the obese girls displayed considerable interindividual variability and were therefore separated into two groups, one with low CIT and the other with normal CIT values. Compared to the obese girls with normal CIT, the obese girls with low CIT had a significantly higher area under the plasma glucose response curve, however, a similar area under the plasma insulin response curve. After weight loss, the area under the plasma glucose response curve was similar between the two groups. The area under the plasma glucose response curve indirectly reflects glucose uptake by the tissues. Therefore, these findings suggest that impaired CIT was secondary to decreased glucose utilization due to insulin

resistance.  It can be concluded that this impairment of glucose storage was induced by obesity-associated insulin resistance resulting in a low CIT (Tounian, 1999).

Brandou et al (2003) examined the effect of a two month diet and exercise program on substrate utilization in obese adolescents.  Fourteen obese (BMI >97th percentile) were measured after two weeks in a specialized institute and then after a home-continuation six weeks later.  Subjects were provided a personalized hypocaloric diet and participated in cycle ergometer exercise sessions for two weeks.  After the two weeks, all subjects continued to participate in the hypocaloric diet, but only half continued to participate in exercise sessions.  Therefore, the group was divided into two, the trained group and the non-trained group.  At the time of the second exercise test, the trained group displayed a significantly lower RER ($p<0.005$) than the non-trained group.  The trained group demonstrated a significant increase ($p<0.05$) in lipid oxidation after the two months whereas lipid oxidation did not significantly change in the non-trained group.  After only two months of diet and exercise an increased ability to oxidate fat during both rest and exercise was demonstrated (Brandou, 2003).

Maffeis et al (2005) investigated nutrient oxidation during moderate intensity exercise in obese prepubertal boys.  Additionally, the researchers sought to determine the walking speed associated with the highest fat oxidation in these boys.  Twenty-four, sedentary, obese prepubertal, Caucasian boys, aged $10 \pm 1$ year were observed.  The boys were divided into three groups based on their BMI.  All participants participated in a graded maximal treadmill test.  Both the energy expenditure and the RQ significantly increased as walking speed increased ($p<0.001$).  Fat oxidation, however, did not change significantly when walking speed increased.  Also observed was a significant correlation between adiposity and energy expenditure, adjusted for FFM.  Additionally observed was a significant associated between adiposity and RQ at each

walking speed, adjusted for exercise intensity.  Therefore, with increased walking speed, the

carbohydrate oxidation rate increased whereas fat oxidation did not change significantly by the

increased workloads.  Based on the results of this study, the researchers concluded that a boy

with a body weight of 70 kg or a BMI of 29 who walked for 40 minutes at only 4 km/hour would

burn approximately 600 kJ.  During this amount of exercise, the carbohydrate oxidation would

be 18 g and the fat oxidation would be 6 g.  While a boy of the same stature walking at 6

km/hour for 27.5 minutes would burn 600 kJ, his carbohydrate oxidation would be 24 g, but his

fat oxidation would only be 3.2 g.  For that reason it would be more reasonable to prescribe

lower exercise intensity for obese children rather than higher exercise intensity.  Because fat

oxidation did not increase with the intensity of the exercise, the lower exercise intensity would

allow the children to participate in the exercise longer and would not affect their fat oxidation.

In fact, working at the higher intensity may contribute to earlier exhaustion and a higher

carbohydrate oxidation leading to an increased appetite and therefore greater food consumption

(Maffeis, 2005).

Recently, Brandou et al (2005) investigated the impact of low and high intensity exercise

training on the type of substrate utilization in obese boys.  Similar to Brandou's previous study

(2003), the boys were measured after two weeks in a specialized institute that combined both a

hypocaloric diet and exercise and were then split into two groups for the remaining six weeks.

All of the boys continued with a hypocaloric diet, however, seven of the boys continued with

low-intensity exercise training and the other eight continued with high-intensity exercise

training.  After two months, both fat and carbohydrate utilization were unchanged in the low-

intensity exercise group whereas fat oxidation significantly decreased and carbohydrate

utilization significantly increased ($p<0.02$) in the high-intensity exercise group.  Although an

increase in fat oxidation was not observed in the low-intensity group, both fat and carbohydrate oxidation were maintained.

In a study of overweight or obese adults with the metabolic syndrome, Dumortier et al (2003) investigated the effects of low-intensity exercise on insulin sensitivity. Subjects participated in eight weeks of the low-intensity exercise training program with no diet intervention. In addition to the low-intensity exercise significantly improving lipid oxidation ($p<0.001$) overall, a significant decrease in insulin resistance and body fat was observed (both $p<0.05$). In summary, the researchers concluded that after only eight weeks of low-intensity training, the ability for overweight or obese adults with metabolic syndrome to oxidize lipids increased. Moreover, a decrease in insulin resistance, a marker of the metabolic syndrome, was displayed (Dumortier, 2003).

Based on the results observed by both Maffeis (2005) and Brandou (2005), low or moderate intensity exercise in obese children may either maintain or promote fat oxidation whereas high-intensity exercise decreased fat oxidation and increased carbohydrate oxidation. Results from the previous studies demonstrate the need to prescribe low to moderate intensity exercise for overweight and obese children for several reasons. Compliance with low intensity exercise programs has been shown to be higher in the obese children compared to high intensity exercise programs (Epstein, 1995). There is no increased benefit in fat oxidation at the high intensity. At the higher intensity, carbohydrate oxidation increases, but fat oxidation does not (Maffeis, 2005).

The studies reviewed provided evidence for the promotion of physical activity to help regulate and offset harmful co-morbidities of obesity and the metabolic syndrome in children. Physical activity has been shown to increase overall metabolic health in overweight children by

improving blood lipids, blood pressure, and body composition.  Physical activity was also found to have a positive effect on fat oxidation.  Moreover, this effect was observed during both low- to moderate-physical activity in overweight children.  This is an encouraging finding given that overweight children may be more capable of performing physical activity at a lower intensity (Epstein, 1995; Dumortier, 2003).   A failure to increase physical activity in response to weight gain may promote obesity in preadolescence (Salbe, 2002); therefore, physical activity promotion should start when children are at a young age to decrease the risk of overweight from childhood to adulthood.  Further investigations are needed to study the impact physical activity may have on obese children with metabolic syndrome, in particular children with abdominal obesity (Wabitsch, 1994).  Additionally, now that the prevalence of children with the metabolic syndrome is rising (Molnar, 2004), more research is needed to fully understand substrate utilization in these children at rest, during physical activity, and after long-term weight loss (Brandou, 2003).

**Insulin Resistance**

Insulin resistance can be defined as a condition when physiologic concentrations of insulin are unable to properly regulate processes necessary for glucose and lipid homeostasis (Decsi, 2003).  With this condition, normal amounts of insulin are inadequate to produce a normal insulin response from fat, muscle, and liver cells.  Obesity leads to insulin resistance and increased circulating insulin concentrations over time (Decsi, 2003; Shalitin, 2005).  This situation decreases insulin sensitivity and impairs pancreatic $\beta$-cell function.  This reduction in insulin sensitivity and impaired $\beta$-cell function are the two main components in the pathogenesis of type 2 diabetes (Shalitin, 2005).  High levels of insulin indicate that the individual is at high

risk for type 2 diabetes (Edelstein, 1997). Furthermore, low insulin sensitivity is a predictor of the development of Type 2 diabetes mellitus (Ivy, 1999).

Impaired glucose tolerance and impaired fasting glucose form an intermediate stage in the natural history of diabetes mellitus and are frequently associated with the metabolic syndrome (Rao, 2004; Shalitin, 2005). In a recent study by Sinha et al (2002), the prevalence of impaired glucose intolerance in obese children aged 4-10 years was found to be 25%.

Little is known about the origin of insulin sensitivity and resistance in children. There are opposing views as to when insulin sensitivity may begin to develop. For a decade or more, poor nutrition during gestation, expressed as low weight at birth, was held to be the factor responsible for insulin resistance later in life. However, birth weights are rising and insulin-resistant states, such as diabetes, are still rising fast. Puberty may play a major role in the development of type 2 diabetes (ADA, 2000). Studies have observed that during puberty, a physiologic pubertal insulin resistance occurs. This insulin resistance occurs with pubertal progression and resolves by the end of puberty. This pubertal insulin resistance is associated with decreased insulin sensitivity and increased insulin secretion (Hannon, 2006). This decrease in insulin sensitivity is independent of changes in body fat (Hannon, 2006; Moran, 1999). Much is yet to be learned about the development of obesity and insulin resistance in children (Wilkin, 2004).

A study by Wilkin et al (2004) attempted to find out exactly which children develop insulin resistance and why. They observed 307 healthy school children (mean age 4.9 years) at school entry, 12, and 24 months. Clear correlations were found between insulin resistance and weight at 5 years. Also found were females throughout life are intrinsically more insulin

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resistant than males and there is dissociation in young children between fatness and insulin resistance (Wilkin, 2004).

Goran et al (2006) examined insulin sensitivity and β-cell function in overweight Hispanic children (mean age 10.9±1.8 years) over the course of one year during the pubertal transition. One hundred and thirty-two children participated in body composition measures, physical exams, and a modified intravenous glucose tolerance test. Over the period of one year, there was a decrease of 24% in insulin sensitivity accompanied by an increase in fasting glucose and insulin. Children in the early stages of maturation (Tanner stage 1 or 2 to 3) displayed appropriate β-cell compensation whereas children in the latter stage of maturation (Tanner stage 3 to 4 or 5) displayed poor β-cell compensation. Goran et al (2006) suggested that the expected rebound of insulin sensitivity after Tanner stage 3 was not observed due to possible overwhelming effects of obesity and insulin resistance.

The prevalence of insulin resistance and impaired glucose tolerance in obese Italian children and adolescents was examined by Valerio et al (2006). One hundred and fifty obese and normal weight children and adolescents participated in blood measures as well as an oral glucose tolerance test. In the children, insulin resistance was found in 40.8% of the obese children and only 3.0% in the normal weight children. Insulin resistance was observed in 41.2% of the obese adolescents whereas none of the normal weight adolescents displayed insulin resistance. Four out of the 100 obese subjects demonstrated impaired glucose tolerance, however, no subjects had type 2 diabetes (Valerio, 2006).

Similar to the previous study by Valerio (2006), Shalitin (2005) determined the prevalence of insulin resistance and impaired glucose tolerance in obese children and adolescents in Israel. Two hundred and fifty-six obese children and adolescents participated in methods

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similar to those performed by Valerio's group. Impaired glucose tolerance was observed in 13.5% of the subjects and insulin resistance was detected in 81.2% of the obese children and adolescents.

The prevalence of type 2 diabetes and impaired glucose regulation was also studied in obese children and adolescents in Germany. The methods used to determine the prevalence of diabetes and impaired glucose regulation was similar to those performed by Valerio (2006) and Shalitin (2005). Overall, 6.7% of 520 subjects displayed impaired glucose regulation or type 2 diabetes. These individuals also demonstrated a higher insulin resistance than those with normal glucose regulation.

**Risk Factors for Insulin Resistance**

Both impaired glucose tolerance and insulin sensitivity are features of the insulin resistance syndrome (Macor, 1997). Impaired glucose tolerance as well as insulin sensitivity may affect substrate utilization, both at rest and during physical activity (Shaibi, 2006). Additionally, obesity has been associated with an impaired utilization of fat as a fuel (Blaak, 2002). Understanding the role impaired insulin sensitivity and impaired glucose tolerance play during rest and exercise is vital in particular, in preventing type 2 diabetes. Figure 3 provides a model of functions linked to diseases and their complementary interactions (Riccardi, 2004). It is essential to understand the role physical activity has on substrate utilization in the body in particular, in children with insulin resistance syndrome. Unfortunately, few studies have examined the effects of training on substrate utilization (van Baak, 1999).

| Functions | Diseases |
|---|---|
| Body fat deposition → | Overweight |
| Insulin sensitivity/resistance | Insulin Resistance |
| Regulation of blood glucose → | Diabetes |
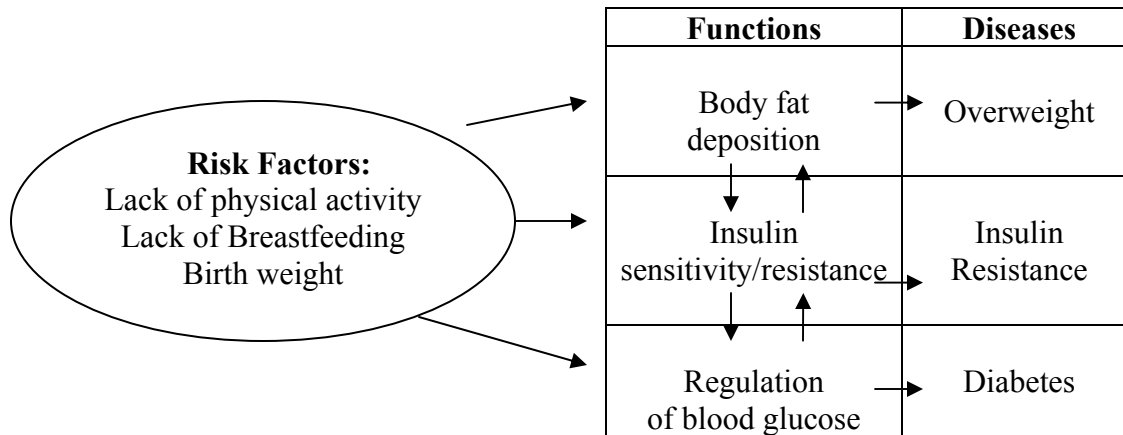
**Figure 3**.  Interactive functions linked to diseases (adapted from Riccardi, 2004).

## Lack of Physical Activity as a Risk Factor

Physical activity is correlated with lower fasting insulin and greater insulin sensitivity in childhood (Schmitz, 2002). Physical activity may improve insulin sensitivity by increasing the expression and activity of notable enzymes that are important to glucose uptake (Corcoran, 2007).  These results are consistent with the findings below that increasing physical activity among youth may reduce the incidence of type 2 diabetes in children and adolescents (Schmitz, 2002).  Nassis et al (2005) examined the effects of an aerobic exercise training program on insulin sensitivity in overweight and obese 9-15 year olds.  After 12 weeks of training, insulin sensitivity increased without changes in body weight and percent fat mass.  Moreover, after the training, lower limb fat free mass increased by 6.2% and this change was significantly associated with enhanced insulin sensitivity (Nassis, 2005).

Pinhas-Hamiel (1996) examined the medical records of 1027 consecutive patients from birth to age 19 years with a diagnosis of diabetes from 1982 to 1995 at a regional, university-affiliated pediatric diabetes referral center. The number of patients (from birth to age 19 years) diagnosed with type 2 diabetes rose 12% in two years from 4% to 16%.  The highest percentage, 33% was found among children aged 10-19 years. In this study, obesity and strong family

histories of type 2 diabetes were found to be important risk factors for children developing type 2 diabetes (Pinhas-Hamiel, 1996). This was also observed in a study by Cruz et al (2004) which examined the metabolic syndrome in Hispanic children. Moreover, Cruz (2004) found that the overweight Hispanic children were at risk for cardiovascular disease and type 2 diabetes due to insulin sensitivity.

Few studies have examined the effect of physical activity on fasting glucose. In Woo's examination (2004) of 82 obese 9-12 year old children, only the children that participated in diet + exercise displayed a significant decrease in fasting glucose ($p<0.002$) after six weeks. No significant difference was observed in the diet only group. Albeit a smaller population than in Woo's study, Kahle (1996) observed a significant decrease in fasting glucose in his sample of seven obese adolescent males after only 15 weeks of mild intensity training.

Alternatively, two studies have not observed any significant differences in fasting glucose after diet and/or physical activity. Ferguson (1999) did not observe any significant changes in fasting glucose in his 79 obese 7-11 year olds over four months. Moreover, over the course of eight months, Kang (2002) did not witness any significant changes in fasting glucose in any one of his three intervention groups.

A similar trend, however, observed with fasting glucose was observed in studies that examined fasting insulin. While some studies demonstrate significant decreases in fasting insulin (Wabitsch, 1994; Ferguson, 1999; Kahle, 1996), others studies have not (Kang, 2002). In Wabitsch's study (1994), the 116, 14-16 year old girls displayed significantly reduced fasting insulin after six weeks of diet and exercise. However, different from that previously reported for LDL, HDL, and TC, the girls with abdominal obesity did not exhibit any greater significant reduction in fasting insulin compared to the girls with gluteal-femoral obesity.

Ferguson (1999) observed significantly decreased (p<0.001) fasting insulin in 79 obese 7-11 year old children after four months of exercise training.   Moreover, in the group that participated in exercise training for the first four months, a rebound effect was seen in fasting insulin at eight months, after the exercise training had been ceased.  Kahle (1996) also observed a significant decrease (p<0.02) in fasting insulin in obese adolescent males after 15 weeks of mild intensity training.

In contrast, Kang (2002) did not observe significant changes in fasting insulin over eight months of moderate or vigorous physical training in 80 obese youths.  Additionally, no significant reductions in fasting insulin were observed by Treuth et al (1998) over five months of resistance training in 12 obese prepubertal girls.

Mensink et al (2005) investigated the effects of a diet and physical activity intervention program on fatty acid oxidation in glucose-intolerant adults. After one year, no significant differences were observed at rest, however during exercise, the intervention group displayed an increase in total fat and plasma free fatty acid oxidation.  Therefore, a diet and physical activity program may prevent further deterioration of impaired fatty acid oxidation during exercise in glucose intolerant individuals (Mensink, 2005).

Additionally, physical activity positively impacts cardiorespiratory fitness in overweight youth.  Loftin et al. (2003) observed that $HR_{max}$ negatively correlated with body weight, percent fat, and BMI (r = -0.53, -0.54, -0.57 respectively) in obese female youth.  Therefore, as body fat increased, $HR_{max}$ decreased (Loftin, 2003).

**Lack of Breastfeeding as a Risk Factor**

The methods of infant feeding may have a profound effect on adult disease development later in life.  Breastfeeding may help protect individuals against allergic disorders, development

of obesity, cardiovascular disease, and type 2 diabetes.  There are several theories as to why breastfeeding may protect individuals from the development of obesity later in life, however the most favored in the literature is that breastfed infants are able to regulate their food intake internally rather than by external cues.  With breastfeeding a precise and dependable point of satiety is regulated by the infant according to their needs for growth and maintenance (Bergmann, 2003).

A study by Baur et al (1998) examined infant feeding and the fatty acid composition of skeletal muscle in infants less than two years old.  Infant feeding information from the mother along with muscle biopsies and fasting blood samples from the children were obtained.  Compared to the non breastfed infants, the breastfed infants had a significantly higher percentage of long-chain polyunsaturated fatty acids (LCPUFAs) and docosahexaenoic acid (DHA), important components of cell membrane structural lipid.  Moreover, the breastfed children had significantly lower plasma glucose levels compared to the non breastfed children (Baur, 1998).

The effects of formula feeding versus breastfeeding can be observed as early as one year of age.  In a study by Dewey et al (1993), one year old formula-fed infants were significantly fatter compared to those who were breastfed for their first year of life.  Moreover, this significant difference remained between the groups even at 24 months of age (Dewey, 1993).

In a longitudinal birth cohort study, Bergmann et al (2003) investigated whether breastfeeding for more than 2 months had preventive effects against overweight in six year olds. At one month of age, those who were breastfed were slightly fatter than those either exclusively or partially bottlefed.  By the second and third month, however, the bottle-fed infants had a significantly higher BMI and thicker skin-folds than the breastfed infants.  This trend was consistently observed from six months on up to six years of age (Bergmann, 2003).   Similar

findings were also observed by Mayer-Davis et al (2006) in girls and boys aged 9-14 years. Breastfeeding was inversely related to childhood overweight regardless of maternal weight status or maternal diabetes (Mayer-Davis, 2006).

In case-control studies of over 1100 overweight and healthy weight subjects aged 12 to 18 years, Kramer (1981) found that breastfeeding provided a significant protective effect against obesity at least through adolescence.  Pettitt et al (1997) examined the association between breastfeeding and type 2 diabetes in Pima Indians.  Of 720 adult Pima Indians, 325 who were exclusively bottlefed for the first two months had significantly higher body weight compared to those who were exclusively breastfed or partially breastfed for the two months.  Moreover, those individuals who were breastfed for those first two months of life had a significantly lower rate of type 2 diabetes than those who were bottlefed (Pettitt, 1997).

Liese et al (2001) also examined the relationship of breastfeeding with the prevalence of overweight in 9-10 year olds.  They found the relationship of breastfeeding and overweight in the study followed a marked-dose-response-like pattern.  The lowest likelihood of being overweight was associated with the longest duration of breastfeeding.  This finding was similar to most of the aforementioned studies cited with increased duration of breastfeeding associated with lower risks of overweight (Grummer-Strawn, 2004; Toschke, 2002; Dewey, 1993; Kramer, 1981).

Unfortunately, not all studies have observed positive effects of breastfeeding on obesity and type 2 diabetes risk factors.  Davis et al (2007) examined the relationship between breastfeeding, adiposity and type 2 diabetes risk factors in overweight Latino youth with a family history of type 2 diabetes.  No significant effects of breastfeeding on adiposity or insulin sensitivity were observed in these youth prior to puberty or across advances in maturation (Davis, 2007).

Racial differences may also play a role in the decision whether or not to breastfeed. A smaller number of African American mothers compared to Caucasian mothers choose not to breastfeed. This racial difference was observed by Forste et al (2001) even after controlling for socioeconomic background. The decision made by African American mothers not to breastfeed, however may be a behavioral one. Forste (2001) found the primary reason African American women chose not to breastfeed was that they "preferred to bottle-feed". Other reasons African-American mothers provided were public embarrassment and concerns of pain (Hannon, 2000). Several studies found that the Women, Infants, and Children (WIC) program facilitated bottle-feeding among African-American mothers with the availability of free formula, however personalized promotion of breastfeeding by WIC employees encouraged positive breastfeeding decisions for almost half of informed mothers (Cricco-Lizza, 2005).

**Birth Weight as a Risk Factor**

Maternal nutrient restriction *in utero* may affect fetal growth and development. Other than the fetal period, there are no other times in an individual's life where the number of muscle fibers increases, therefore the fetal period is crucial for skeletal muscle development. Skeletal muscle is not as a high of a developmental priority as the brain and heart, therefore skeletal muscle may suffer as a result of maternal nutrition restriction and lead to low birth weight. Because skeletal muscle is the main site for the utilization of fatty acids and glucose, this restriction may predispose the infant to long-term consequences including obesity and diabetes (Zhu, 2006).

Low birth weight is associated with type 2 diabetes, insulin resistance and cardiovascular disease later in life (Phillips, 2006; Kajantie, 2006). Low birth weight children tend to have alterations of their body composition with increased fat mass relative to lean body mass. This

increased fat storage contributes to insulin resistance (Tappy, 2006). Several studies have demonstrated an association between low birth weight and the later development of type 2 diabetes (Hales, 1991; McCance, 1994; Lithell, 1996), however all of these studies observed individuals ranging in age from 30 to 64 years. Hypponen et al (2003) investigated the risk of diabetes and its association with low birth weight affected by accumulation of body mass from childhood to adulthood. It was observed that those in whom diabetes developed weighed less at birth and had higher BMIs than others. Moreover, risk of diabetes was found to be significantly higher for obese and overweight participants (Hyponnen, 2003).

Frontini et al (2004), however, examined the relationship between low birth weight and cardiovascular risk factors and glucose homeostasis from childhood to adolescence. During childhood (4-11 years) HDL was significantly lower and LDL cholesterol significantly higher in the low birth weight group compared to the normal weight group. In adolescents (12-18 years), glucose levels were significantly higher in the low birth weight group compared to the normal birth weight. Furthermore, low birth weight was related independently and adversely to longitudinal trends in triglycerides and glucose, regardless of race or gender (Frontini, 2004).

Jaquet et al (2000), however, observed insulin sensitivity in 25 year old low birth weight adults compared to normal birth weight adults. Even as young adults, those born with low birth weight were shown to be hyperinsulinemic, an indication of insulin resistance whereas those born normal birth weight showed no indication of insulin resistance. Moreover, the effect of low birth weight on insulin sensitivity was observed even after adjustment for BMI (Jaquet, 2000).

## Chapter Three

## Methods

**Subjects**

Apparently healthy children, 7-9 years of age, were recruited for participation into the SILLY (Study of Insulin sensitivity in Low-birth weight Louisiana Youth) study. The SILLY study is a large, ongoing, National Institutes of Health (NIH) funded study. The current study is a substudy of SILLY therefore recruitment, inclusion, and exclusion criteria described below are those of SILLY. Recruitment involved medical chart reviews by study staff, advertisement distributions to area pediatricians and family physicians, distribution of study flyers to schools throughout southeast Louisiana, presentations to parent/teacher organizations, participation in community health events, and mass media announcements. Incentives for study participation included a certificate for the participant, athletic equipment (or an age-appropriate toy), and $75 dollars per day for time and travel. Subjects provided several reasons for their willingness to volunteer for the study. Reasons included the opportunity to help sick children or children with diabetes, an interest in science, and their family history of diabetes.

**Inclusion Criteria** – Pre-screening measures included the following:

a.     Phone screening

b.     Family and medical history and physical examination by a physician.

c.     Hematologic and biochemical measurements.

d.     Psychosocial screening

All apparently healthy 7-9 year olds were encouraged to participate regardless of birth weight and current weight status.

**Exclusion Criteria**

Children with evidence of significant cardiovascular disease, cardiac arrhythmias, liver disease, or the chronic use of medications including diuretics, steroids and adrenergic-stimulating agents were excluded. Children whose mothers reported gestational diabetes were excluded. Children with emotional problems such as clinical depression or other diagnosed psychological condition and those currently using prescription medication for psychological conditions were excluded. Children with evidence of family and/or medical neglect or physical, mental or sexual child abuse were excluded. Children whose mothers have a history of alcohol or drug abuse were excluded. Children born premature (< 37 weeks of pregnancy) were excluded. Children who are >9 years of age and children with a mature (Tanner, 1976) level >2 were excluded.

A total of 60 apparently healthy children were enrolled in the study; however, 34 subjects were included in the analyses. Twelve children were deemed ineligible by a physician during their physical exam due to maturation. Six subjects were classified as withdrawn from the study. These children were eligible, however did not participate in all study visits and therefore have missing information. Eight completed subjects were not included in the analyses due to missing blood values. Of the 34 subjects, only 16 subjects' FSIGTT data was included in the analyses. Not all subjects completed the FSIGTT, therefore only subjects with complete FSIGTT data (n=16) were included. The FSIGTT is an invasive procedure that requires experienced personnel to perform. Several subjects were not able to complete the FSIGTT due to the study nurses inability to successfully place an IV into each arm. Furthermore, several subjects completed the FSIGTT, however, lab errors prevented their blood values from being included in the analyses. Additionally only 14 subjects' accelerometry data was included. The distribution of accelerometers was a substudy that began after the study's initiation, therefore not all subjects

received an accelerometer.  Additionally, only subjects who wore the accelerometer for 3 consecutive days (2 weekdays and 1 weekend day) were included in the analyses.

**Consent Procedures**

All methods and testing procedures were previously approved by the Institutional Review Boards of the Louisiana State University Health Sciences Center, the University of New Orleans, the University of Wyoming, Children's Hospital in New Orleans and the Pennington Biomedical Research Center.  At the baseline visit, after a phone survey verified that eligibility criteria were assured, the medical team met individually with each family. An explanation of all procedures, potential risks, temporary side effects, anticipated benefits, and alternative methods were given to both the child and parent(s).  Confidentiality was assured as well as the right not to participate or to withdraw at any time. Signatures of both parent and child were obtained on the consent and assent forms. A copy of the forms was given to each study participant. Assurance that all questions would be answered about the study testing was obtained. Names and telephone numbers of contact persons on the medical team were given to each study participant for use if future questions arise.

**Confidentiality**

All study volunteers were assigned an individual subject identification number (ID #) used on all data recording documents. A code sheet was prepared listing the name and ID # of each study participant. Every effort was made to maintain the confidentiality of the study participant's study records.  However, the members of the Data, Safety, and Monitoring Board and staff from the LSUHSC School of Public Health and LSUHSC/Children's Hospital Clinical Trials Unit were allowed to inspect and/or copy the medical records related to the study.  Any and all adverse events were reported to the principal investigator.

Subject data was collected by physicians, pediatric nurses, and trained study staff at Children's Hospital Clinical Trials Unit (GCRC) in New Orleans and Pennington Biomedical Research Center in Baton Rouge, LA. Anthropometric measures including weight, height, resting heart rate and blood pressure were obtained by a pediatric nurse. A medical screening blood test was performed on all subjects to ensure the child's eligibility to participate in the study. Baseline blood samples including lipid profiles were obtained in the morning after a 12 hour fast. Blood samples were collected by the pediatric nurse in a private, short stay unit room at Children's Hospital. Urine samples were obtained for ketone analysis and yime of last meal was recorded.

**Measurement of Resting Metabolism (Resting Energy Expenditure and Respiratory Quotient):**

The resting energy expenditure (REE) and the respiratory quotient (RQ) were measured to determine resting metabolism. The REE and RQ was measured under standardized conditions in order to observe both resting metabolism and fuel mix oxidation.

Resting energy expenditure was determined by ventilated hood indirect calorimetry with the study participant lying supine on a bed. Study participants fasted for 12 hours prior to the test and were instructed not to engage in vigorous physical activity for 24 hours prior to the test. Study participants were allowed to drink water prior to the test and rested in a recumbent position for 30 minutes prior to the start of each testing session (Salbe, 1997). This included a washout period of 5 minutes before and 5 minutes at the end. Metabolic measurements were continuously taken during the test. Expired gases were sampled and analyzed for O2 and CO2 concentrations using a Delatrac Metabolic Measurement Cart for a 30 minute period. Resting energy expenditure was then calculated for each study participant based on inspired and expired

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O2 and CO2 concentration (Salbe, 1997; Fontvieille, 1992).  Respiratory Quotient was also

evaluated as an index of fat oxidation.

**Measurement of Insulin Resistance**

The homeostasis model assessment (HOMA) formula was used to calculate insulin

resistance.  HOMA was calculated as follows:

$$\frac{\text{Fasting glucose (mmol/L) x fasting insulin (mIU/L)}}{22.5}$$

HOMA was used to calculate $\beta$-cell function (%) using the following formula:

$$\frac{20 \text{ x fasting insulin (mIU/L)}}{\text{fasting glucose (mmol/L)} - 3.5}$$

The fasting insulin and glucose values for HOMA were the mean values of the three baseline

samples of the FSIGTT or the glucose and insulin values obtained at minute 0.

**Measurement of Insulin Sensitivity**

**Frequently Sampled Intravenous Glucose Tolerance Test (FSIGTT)** (Bergman, 1987;

Cutfield, 1990)

Insulin sensitivity was assessed using the minimal model technique of Bergman (1987).

The minimal model estimates insulin sensitivity through a computer-based mathematical analysis

of the glucose-insulin dynamics during a FSIGTT.  The minimal model technique was selected

for this study because a) it is relatively easy to perform and significantly reduces the participant

burden; b) is more practical and applicable to younger study participants; c) is more applicable

for a large cross-sectional study; and d) is shown to be accurate in pre-pubertal youth (Goran,

2006).  The FSIGTT was conducted the morning following a 12 hour overnight fast. The

parent(s) and study participant were escorted to the short stay outpatient unit at Children's

Hospital in New Orleans and participated in the blood test and FSIGTT. The pediatric nurse and

study coordinator supervised the testing and assured the child's safety and comfort. A non-violent children's movie was shown during the test to help the child to relax.

One IV cannula was inserted into each arm of the study participant. Three baseline blood samples were drawn at 15-min intervals, designated –30,--15, and 0 min. At zero time, 0.3g/kg 25% dextrose was injected over 120 seconds followed by 0.005-0.03 units of insulin per kg body weight 20 minutes later (Cutfield, 1990). Additional blood samples (1.1 ml) were then collected at the following times relative to glucose administration at 0 min: 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 24, 25, 30, 40, 50, 70, 90, 100, 120, 140, 160, and 180 min (Gower, 1999). Each 1.1-mL sample was placed in a red top tube. Glucose was assayed using a Yellow Springs Instruments analyzer, which uses a membrane bound glucose oxidase technique. Insulin was assayed using an EIA kit from ALPCO. A physician was in attendance during each procedure to evaluate study participants for signs and symptoms of hypoglycemia. Insulin and glucose levels were measured in all timed samples. Insulin sensitivity ($S_I$), glucose effectiveness ($S_g$), the acute response to glucose ($AIR_g$), and disposition index (DI) was calculated from glucose and insulin data by the minimal model computer program (MIN-Mod Millennium computer program Version 6.02; Richard N. Bergman, Los Angeles, CA). All study participants were resting supine throughout the testing.

**Measurement of Percent Fat (% fat), Bone Mineral Density (BMD), Fat and Lean Body Mass (FBM, LBM) by Dual-Energy X-ray Absorptiometry (DEXA)**

Dual-Energy X-ray Absorptiometry DEXA was used to determine % fat, FBM, LBM, and BMD in the study participants. DEXA utilizes an x-ray source to generate photons to scan study participants. Bone-mineral content measurements previously calibrated against secondary standards with ashed bone sections are used to help calculate LBM (Friedl, 1992; Mazess, 1990).

Percentage of fat and LBM can be predicted with accuracy by observing the ratio of absorbency of the different-energy-level photons, which are linearly related to the percentage of fat in the soft tissues of the body (Friedl, 1992). The coefficient of variation of fat-free tissue measurement has been calculated at 2%, which is comparable to that obtained by hydrodensitometry.

The study participants received total body scans with a Hologics QRX 2000 DEXA. They were instructed to remove all metal and jewelry and were dressed in a gown before they were correctly positioned on the scan table, lying down on their back with their arms to their side. The DEXA measured X-rays as they were transmitted through the study participant's body.

**Measurement of Weight**

An electronically calibrated scale (Indiana Scale, Terahuat, IN) was used to obtain the weight in kilograms of each study participant. The study participant removed his or her shoes and stepped on the scale. The study participant remained as still as possible. The study participant's weight in kilograms was recorded once the digital reading was constant. The study participant remained on the scale until the weight was recorded. The process was repeated two additional times. The average weight in kilograms of each measure was calculated and recorded on the data statistical form.

**Measurement of Height**

A calibrated stadiometer (Holtain, Ltd, UK, Dyfed) was used to obtain the height in centimeters of each study participant. The study participant removed his or her shoes and stepped onto the floor platform facing in an outward direction with the heels together. The scapula and buttocks remained in contact with the back of the stadiometer during the measure. The head was positioned in a horizontal plane. The clinician moved the headboard onto the most superior aspect of the study participant's head. This procedure was repeated two additional times. The

average height in to the nearest tenth of a centimeter (0.1 cm) was calculated and recorded on the data statistical form.

**Measurement of Body Mass Index**

Body Mass Index (BMI) was determined using the following formula: Weight in kilograms/height in meters$^2$ . According to the Center for Disease Control (CDC, 2000), the healthy weight range in children and teens is the 5$^{th}$ percentile to less than the 85$^{th}$ percentile, at risk for overweight is the 85$^{th}$ to less than the 95$^{th}$ percentile, and overweight is equal to or greater than the 95$^{th}$ percentile. Subjects classified according to their BMI percentile are presented in Table 1.

**Table 1**. Classification of subjects according to BMI percentiles

|  | Females (n) | Males (n) | Total (n) |
|---|---|---|---|
| **Healthy Weight** | 7 | 7 | 14 |
| **At Risk of Overweight** | 3 | 3 | 6 |
| **Overweight** | 8 | 6 | 14 |

**Measurement of lipid profiles and biochemical parameters**

Biochemical markers, total cholesterol, triglycerides, high-density lipoproteins, and low-density lipoproteins (LDL) were examined by using samples of whole blood obtained during the baseline sample of the Frequently Sampled Intravenous Glucose Tolerance Test (FSIGTT) in a certified laboratory. Study participants were required to fast for 12 hours prior to the test. Venous blood was collected in the morning after a 12 hour fast with the study participant seated. Blood was collected in tubes containing 1.5 mg/ml EDTA for procedures requiring plasma, in tubes with no additives for serum measures and citrate tubes for homeostasis endpoints. All routine chemistry and lipid analyses were performed on a Beckman Synchron CX5 automated chemistry analyzer and the LDL cholesterol was calculated using the Friedewald equation.

**Measurement of Physical Activity**

Physical activity was assessed by 2 methods, the Godin-Leisure Time Questionnaire (Godin) and accelerometer (Godin, 1985). The Godin is a 4-item self-reported recall of usual physical activities during a typical week. Children were asked to write how many times per week they engaged in vigorous, moderate, and light intensity activity for 15 minutes or more. Examples of the types of activity were provided on the form. A total score is derived by multiplying the frequency of each category by a standard metabolic equivalent (vigorous x 9, moderate x 5, light x 3). Children were also asked to report how many times they engaged in activity long enough to make them sweat. Adequate reliability and validity were reported (Sallis, 1993) with children as young as the 4th grade.

Physical activity was also measured by the GT1M ActiGraph uniaxial accelerometer (Manufacturing Technologies Inc. Health Systems, ActiGraph, Fort Walton Beach, FL). The ActiGraph accelerometer is a small, electro-mechanical device that records acceleration and deceleration of movement. The ActiGraph recorded these accelerations and decelerations as activity counts. These counts are linearly related to the intensity of the movement performed by the participant and were summed over a set period of time (60 second epoch). The ActiGraph monitor was used in several studies to assess physical activity in children, even as young as preschoolers (Eisenmann, 2004; Janz, 1995; Pate, 2004; Trost, 2003). Taking multiple days of accelerometer measurements and averaging them was reported to increase reliability from r=0.42 for one monitored day to r = 0.7 when three or more days of monitoring are used (Janz, 1995).

The accelerometer was worn by the study participant for a minimum of 3 consecutive days (72 hours) which included 2 weekdays and 1 weekend day. Individual accelerometer data was sorted into 3 consecutive days and then sorted by activity counts. Accelerometer activity

counts were converted to their metabolic equivalent (MET) value using the following equation developed by Treuth et al (2004):

$$MET = 2.01 + 0.000856 \text{ (counts/60 seconds)}$$

The total intensity-weighted minutes (MET-weighted minutes) of total physical activity (TPA) and moderate to vigorous physical activity (MVPA) were then computed by summing the MET values above a light intensity threshold to determine TPA and summing the MET values above a moderate intensity threshold to determine MVPA. The count threshold (counts/60 seconds) was set at 101-2999 for light physical activity, 3000-5200 for moderate physical activity, and >5200 for vigorous activity (Treuth, 2004). The moderate activity count represented approximately 4.6 METs which seperates slow (4.6 METs) and brisk ($\geq$4.6 METs) walking (Webber, 2008).

**Measurement of Breastfeeding**

Breastfeeding information was obtained from a questionnaire completed by the mother of the study participant. The questionnaire was administered by a member of the study staff during the history and physical exam.

**Measurement of Birth Weight**

Birth weight was obtained from the mother and recorded during the medical history examination by a member of the study staff.

**Statistics**

The current study is a substudy of two larger, federally funded studies sponsored by the National Institute of Child Health and Human Development (NICHD 41071, NICHD 49046). Therefore, all statistical analyses were performed by Julia Volaufova, PhD, at the School of Public Health, Louisiana State University Health Sciences Center. Dr. Volaufova is a Co-

Investigator and the biostatistician for the NICHD studies.  She is also a member of the Data and Safety Monitoring Board for the studies and manages the studies' databases containing the subjects' data.

All statistical procedures were performed in SAS, version 9.1.3.  Significance was set at the 0.05 level. Descriptive statistics including means and standard deviations (SD) was computed for all of the data.  To test the hypotheses that impaired fat oxidation is associated with insulin resistance and insulin sensitivity, Pearson correlations were performed.  Partial correlations with respect to race, sex, physical activity, breastfeeding, and birth weight were also performed.  For both correlation analyses, total Godin scores and TPA and MVPA (measured by accelerometer) were examined separately for the variable of physical activity. A two-way analysis of variance (ANOVA) was used to compare the dependent variables [body composition, blood results, physical activity (measured by both total Godin score and TPA and MVPA by accelerometer)] with respect to sex and race.  A one-way ANOVA was performed to compare the dependent variables with respect to breastfeeding.  A chi-square analysis was performed to examine the association of race and breastfeeding and sex and breastfeeding.  A two-factor ANOVA was performed with respect to RQ levels (low, medium, high).  A multifactor ANOVA with respect to race, sex, breastfeeding, and birth weight was performed for all variables examined.  A general linear model was used to examine RQ with insulin resistance, and separately insulin sensitivity, fasting glucose and fasting insulin adjusted for sex, race, physical activity (measured by total Godin score), breastfeeding, and birth weight.  The significance of an effect after adjustment was established by comparing the full model and restricted model, in which the effect in question and its corresponding interactions were left out. The general linear model was selected because it considers interaction terms therefore allowing for different slopes and

intercepts. An example of the general linear model is provided in the appendix.  No analyses were adjusted for current weight status.

The following power analysis was performed for partial correlations.  This power analysis is applicable between any two variables adjusted for five different variables.  The partial correlations in this study were adjusted for five variables: race, sex, physical activity, breastfeeding, and birth weight.  The smallest number of subjects included in any one partial correlation analysis was 28.  Therefore, if the partial correlation were 0.6 or higher, then with 28 subjects, adjusted for 5 additional variables, we would have 80% power to detect it as significant on 5% significance level.

**Table 2.**  Power analysis for any two variables adjusted for five variables

| Partial Correlation | Power | Total n |
|:---:|:---:|:---:|
| 0.1 | 0.800 | 787 |
| 0.2 | 0.800 | 198 |
| 0.3 | 0.801 | 89 |
| 0.4 | 0.803 | 51 |
| 0.5 | 0.801 | 33 |
| 0.6 | 0.818 | 24 |
| 0.7 | 0.826 | 18 |
| 0.8 | 0.840 | 14 |
| 0.9 | 0.864 | 11 |

# Chapter Four

## Results

Subjects' characteristics grouped by sex (mean±SD) are presented in Table 3 and subjects' characteristics grouped by race (mean±SD) are presented in Table 4.

**Table 3.** Physical characteristics of prepubertal males and females (mean±SD)

| | Females n=18 | Males n=16 | F | p value |
|---|---|---|---|---|
| Age (years) | 8.1±0.8 | 7.9±0.7 | 0.5 | 0.492 |
| Body Weight (kg) | 38.8±13.8 | 34.0±9.5 | 5.1* | 0.032 |
| Height (cm) | 134.0±8.6 | 132.2±10.3 | 1.2 | 0.283 |
| BMI (wt(kg)/ht(m)$^2$) | 21.2±5.3 | 19.2±3.4 | 5.5* | 0.026 |
| Birth Weight (kg) | 3.2±0.5 | 3.5±0.7 | 2.7 | 0.110 |
| Percent Fat (%) | 31.0±7.7 | 22.9±8.0 | 11.5* | 0.002 |
| Fat Body Mass (kg) | 12.6±7.2 | 8.3±5.0 | 8.8* | 0.006 |
| Lean Body Mass (kg) | 25.7±7.0 | 25.9±5.5 | 0.9 | 0.349 |
| Total Body Mass (kg) | 38.3±13.8 | 34.2±9.6 | 4.3* | 0.047 |
| Bone Mineral Density (g/cm$^2$) | 0.8±0.0 | 0.8±0.1 | 1.95 | 0.173 |
| Total Physical Activity (MET weighted minutes) | 428.2±198.6 n=6 | 366.3±124.2 n=8 | 2.5 | 0.142 |
| Moderate to Vigorous Physical Activity (MET weighted minutes) | 24.1±20.5 n=6 | 17.6±10.0 n=8 | 0.0 | 0.994 |
| Godin score | 67.2±25.3 n=17 | 89.4±61.5 n=15 | 2.1 | 0.160 |

*$p<0.05$

**Table 4.** Physical characteristics of prepubertal Non-Caucasian and Caucasian (mean±SD)

| | Non-Caucasian n=13 | Caucasian n=21 | F | p value |
|---|---|---|---|---|
| **Age (years)** | 8.0±0.8 | 8.0±0.8 | 0.0 | 0.870 |
| **Body Weight (kg)** | 40.9±15.5 | 34.0±8.8 | 5.4* | 0.027 |
| **Height (cm)** | 138.0±9.6 | 130.2±8.1 | 7.0* | 0.013 |
| **BMI (wt(kg)/ht(m)$^2$)** | 21.1±5.9 | 19.8±3.6 | 2.0* | 0.170 |
| **Birth Weight (kg)** | 3.2±0.4 | 3.4±0.7 | 1.7 | 0.204 |
| **Percent Fat (%)** | 25.0±10.7 | 28.6±7.2 | 0.3 | 0.569 |
| **Fat Body Mass (kg)** | 11.4±8.9 | 10.1±4.8 | 1.8 | 0.189 |
| **Lean Body Mass (kg)** | 29.2±7.4 | 23.6±4.3 | 9.0* | 0.005 |
| **Total Body Mass (kg)** | 40.7±15.5 | 33.7±8.6 | 5.2* | 0.030 |
| **Bone Mineral Density (g/cm$^2$)** | 0.8±0.1 | 0.8±0.1 | 3.9 | 0.058 |
| **Total Physical Activity (MET weighted minutes)** | 239.3±113.1 n=5 | 478.1±102.4 n=9 | 28.6* | 0.000 |
| **Moderate to Vigorous Physical Activity (MET weighted minutes)** | 19.4±11.2 n=5 | 20.9±17.4 n=9 | 0.3 | 0.630 |
| **Godin score** | 67.6±25.9 n=12 | 83.5±55.2 n=20 | 1.9 | 0.185 |

**\*p<0.05**

Significant differences (p<0.05) between females and males were observed in body weight, BMI, %fat, FBM, and total body mass (Table 3). No significant difference was found in age, birth weight, height, LBM, and BMD between the sexes (Table 3). Significant differences (p<0.05) between Non-Caucasians and Caucasians were observed in body weight, height, BMI, LBM, and total body mass (Table 4). No significant differences between races were found in age, birth weight, %fat, FBM, and BMD (Table 4). Significant sex by race interactions (p<0.05)

were observed in weight ($F_{(3,30)}=5.4$, $p<0.05$), BMI ($F_{(3,30)}=8.6$, $p<0.01$), %fat ($F_{(3,30)}=5.3$, $p<0.05$), FBM ($F_{(3,30)}=7.7$, $p<0.01$), and total body mass ($F_{(3,30)}=5.6$, $p<0.05$).

The females in this study displayed a mean %fat of 31.0% and males 22.9%. Fat body mass in the current study in the females was 12.6kg whereas the males' FBM was 8.3kg. Lean body mass of the females and males was, 25.7kg and 25.9kg, respectively.

Subjects' breastfeeding information is presented in Table 5. A total of 16 mothers reported breastfeeding their child in the study. A total of 13 mothers reported not breastfeeding their child in the study. A total of 5 mothers provided no response when asked whether or not they breastfed the child in this study. No significant differences between breastfed and nonbreastfed children were observed in any of the variables examined in this study. A chi-square analysis compared the three breastfeeding groups, breastfed, nonbreastfed, and no response. With all three groups included, no significant association between race and breastfeeding was observed ($p=0.086$). However, when the breastfed were compared to the non breastfed groups, excluding the non-responders, a significant association between race and breastfeeding ($p<0.05$) was observed. No significant associations between sex and breastfeeding were observed when all three groups were included ($p=0.923$) nor when the non-responders were excluded ($p=0.837$).

**Table 5.** Reporting of breastfeeding

| | Breastfed (n) | Nonbreastfed (n) | No response (n) | Total (n) |
|---|---|---|---|---|
| Females (n) | 8 | 7 | 3 | 18 |
| Males (n) | 8 | 6 | 2 | 16 |
| Total (n) | 16 | 13 | 5 | 34 |
| | Breastfed (n) | Non-breastfed (n) | No response (n) | Total (n) |
| Non-Caucasian (n) | 3 | 7 | 3 | 13 |
| Caucasian (n) | 13 | 6 | 2 | 21 |
| Total (n) | 16 | 13 | 5 | 34 |

Results grouped by sex from the resting energy expenditure, blood tests, and the FSIGTT are presented in Table 6. Similar results grouped by race are presented in Table 7.

**Table 6.** Values from resting energy expenditure, blood tests, and the FSIGTT grouped by sex

| | Females n=18 | Males n=16 | F | p value |
|---|---|---|---|---|
| **Resting Energy Expenditure (kcal/day)** | 1122.6±295.0 | 1180.7±331.7 | 0.0 | 0.973 |
| **Respiratory Quotient ($VCO_2/VO_2$)** | 0.85±0.0 | 0.84±0.0 | 0.1 | 0.766 |
| **Fasting Glucose (mmol/L)** | 4.3±0.5 | 4.2±0.7 | 0.4 | 0.550 |
| **Fasting Insulin (uU/mL)** | 7.1±5.8 | 2.2±2.0 | 20.6* | <0.001 |
| **Insulin Resistance (mM.mu/l^2)** | 1.4±1.3 | 0.4±0.4 | 15.4* | 0.001 |
| **β-cell Function** | 220.2±123.3 | 56.2±100.7 | 19.7* | 0.001 |
| **Insulin Sensitivity (X $10^{-4}$ min$^{-1}$/(µIU/ml)** | 13.8±13.2 n=7 | 15.8±5.8 n=9 | 0.4 | 0.540 |
| **Glucose Effectiveness** | 0.02±0.0 n=7 | 0.03±0.01 n=9 | 3.9 | 0.073 |
| **Disposition Index** | 3530.7±1198.0 n=7 | 2741.0±1628.0 n=9 | 0.3 | 0.062 |
| **Acute Insulin Response to Glucose** | 384.3±258.0 n=7 | 203.2±132.2 n=9 | 1.5 | 0.240 |

**\*p<0.05**

**Table 7.** Values from resting energy expenditure, blood tests, and the FSIGTT grouped by race

| | Non-Caucasian n=13 | Caucasian n=21 | F | p value |
|---|---|---|---|---|
| **Resting Energy Expenditure (kcal/day)** | 1224.9±342.6 | 1103.5±285.6 | 1.2 | 0.285 |
| **Respiratory Quotient (VCO$_2$/VO$_2$)** | 0.83±0.0 | 0.86±0.0 | 3.9 | 0.058 |
| **Fasting Glucose (mmol/L)** | 4.4±0.4 | 4.1±0.7 | 1.6 | 0.223 |
| **Fasting Insulin (uU/mL)** | 5.9±7.2 | 4.1±2.9 | 5.6* | 0.025 |
| **Insulin Resistance (mM.mu/l$^2$)** | 1.2±1.6 | 0.8±0.6 | 4.8* | 0.036 |
| **β-cell Function** | 137.0±127.9 | 146.8±148.5 | 0.6 | 0.430 |
| **Insulin Sensitivity (X 10$^{-4}$ min$^{-1}$/(μIU/ml)** | 13.2±6.5 n=7 | 15.8±10.7 n=9 | 0.5 | 0.502 |
| **Glucose Effectiveness** | 0.03±0.0 n=7 | 0.02±0.01 n=9 | 0.0 | 0.999 |
| **Disposition Index** | 3632.9±1784.0 n=7 | 2838.1±1317.9 n=9 | 0.5 | 0.488 |
| **Acute Insulin Response to Glucose** | 306.5±121.0 n=7 | 271.5±246.1 n=9 | 0.2 | 0.631 |

**\*p<0.05**

Significant differences ($p<0.05$) between females and males were observed in fasting insulin, insulin resistance, and β-cell function (Table 6). No significant differences comparing females and males were found in REE, RQ, fasting glucose, insulin sensitivity, Sg, DI, and AIRg (Table 4). Significant differences ($p<0.05$) were found between Non-Caucasians and Caucasians fasting insulin and insulin resistance (Table 7). No significant differences between races were found in REE, RQ, fasting glucose, β-cell function, insulin sensitivity, Sg, DI, and AIRg (Table 7).

Significant sex by race interactions ($p<0.05$) were observed in fasting insulin ($F_{(3,30)}=6.7$, $p<0.05$) and insulin resistance ($F_{(3,30)}=5.4$, $p<0.05$). A two-way ANOVA revealed significant sex by breastfeeding ($p<0.05$) and race by breastfeeding ($p<0.05$)

interactions on REE.  A significant sex by breastfeeding interaction ($p<0.05$) was observed in RQ.  A significant sex by birth weight interaction ($p<0.001$) was observed on insulin sensitivity.

**Specific Aim 1**

**Impaired Fat Oxidation and Insulin Resistance**

In all subjects observed (n=34) Pearson correlation results indicated no significant association between RQ and insulin resistance (r=0.02, p=0.90) (figure 4), however further analyses (general linear model) demonstrated RQ and insulin resistance were significantly associated when adjusted for race, breastfeeding, birth weight ($F_{(8, 14)}=6.1$, $p<0.01$), sex, race, physical activity ($F_{(7, 17)}=5.1$, $p<0.01$), and sex, race, breastfeeding ($F_{(8, 15)}=17.7$, $p<0.0001$). All of the general linear models performed are presented in Table 8. When the following variables, i.e. physical activity, breastfeeding, and birth weight were entered into the model collectively, there was no significant association between RQ and insulin resistance, even after adjusting for race and sex.  Moreover, each of the variables were examined independently in a general linear model with no significant association between RQ and insulin resistance observed. However, after race, sex, and breastfeeding ($p<0.0001$) and race, sex, and physical activity ($p<0.01$) were entered into the model, RQ and insulin resistance were significantly associated. When birth weight was entered into the model, RQ and insulin resistance were only significantly associated when breastfeeding, race, and sex were entered as well ($p<0.01$).  These findings suggest breastfeeding and physical activity affect RQ and help to explain why insulin resistance is related.  Partial correlations (adjusted for physical activity, TPA, MVPA, breastfeeding, birth weight, race, and sex) between RQ and insulin resistance ranged from 0.19 to -0.32, however no correlations were significant despite the fact that there was adequate statistical power.

**Table 8**. General linear models for the dependent variable of insulin resistance and RQ (table cont.)

| Variables considered for adjustment | Dependent Variable | dffull | dfrestricted-dffull | F | p |
|---|---|---|---|---|---|
| race  birth weight | Insulin resistance | 26 | 4 | 0.6888 | 0.60625 |
| breastfeeding  birth weight | Insulin resistance | 22 | 6 | 0.6861 | 0.66287 |
| sex  totalgodin | Insulin resistance | 24 | 4 | 0.0760 | 0.98888 |
| race  totalgodin | Insulin resistance | 24 | 4 | 0.0599 | 0.99292 |
| breastfeeding  totalgodin | Insulin resistance | 21 | 5 | 0.3538 | 0.87400 |
| birth weight  totalgodin | Insulin resistance | 24 | 4 | 0.4975 | 0.73769 |
| sex  race | Insulin resistance | 26 | 4 | 0.5012 | 0.73507 |
| sex  breastfeeding | Insulin resistance | 23 | 5 | 0.1072 | 0.98962 |
| race  breastfeeding | Insulin resistance | 22 | 6 | 0.7257 | 0.63368 |
| sex  birth weight | Insulin resistance | 26 | 4 | 0.1974 | 0.93748 |
| sex  race  birth weight | Insulin resistance | 19 | 7 | 1.1402 | 0.38031 |
| sex  breastfeeding birth weight | Insulin resistance | 14 | 8 | 1.7352 | 0.17537 |
| race  breastfeeding  birth weight | Insulin resistance | 14 | 8 | 6.0734* | 0.00175 |
| sex  race  totalgodin | Insulin resistance | 17 | 7 | 5.1469* | 0.00274 |
| sex  breastfeeding totalgodin | Insulin resistance | 13 | 7 | 2.3942 | 0.08263 |
| race  breastfeeding totalgodin | Insulin resistance | 13 | 7 | 2.4741 | 0.07523 |
| sex  birth weight totalgodin | Insulin resistance | 17 | 7 | 0.1022 | 0.99745 |
| race  birth weight totalgodin | Insulin resistance | 17 | 7 | 0.4114 | 0.88209 |
| breastfeeding  birth weight totalgodin | Insulin resistance | 13 | 7 | 0.2495 | 0.96333 |

| sex  race  breastfeeding | Insulin resistance | 15 | 8 | 17.7087* | $< 10^{-5}$ |
|---|---|---|---|---|---|

**\*p<0.05**

One outlier was identified in the group with a fasting insulin of 26 uU/ml (normal fasting insulin range for a child 0-8 years old is 0-13 uU/ml).  While this fasting insulin value was outside of the normal range, there was no biological basis to exclude her from the analyses since there was no documentation in the subject's chart indicating that she was not fasted for the blood test.  Moreover, her fasting glucose (94 mg/dL) was within normal range (65-110mg/dL).  A review, however, of the subject's family history indicated her paternal and maternal grandmothers were type 2 diabetics.   Further analyses excluding this subject were performed to examine whether or not excluding this subject would influence the results.  Pearson and partial correlations were performed between RQ and insulin resistance excluding this subject.  The results excluding this subject did not differ from the previous results reported.  No significant correlation (r=0.08, p=0.65) or partial correlation (r=0.13, p=0.55) between RQ and insulin resistance was observed when excluding this subject.

**Figure 4.** The association of insulin resistance and fat oxidation in all subjects (n=34)
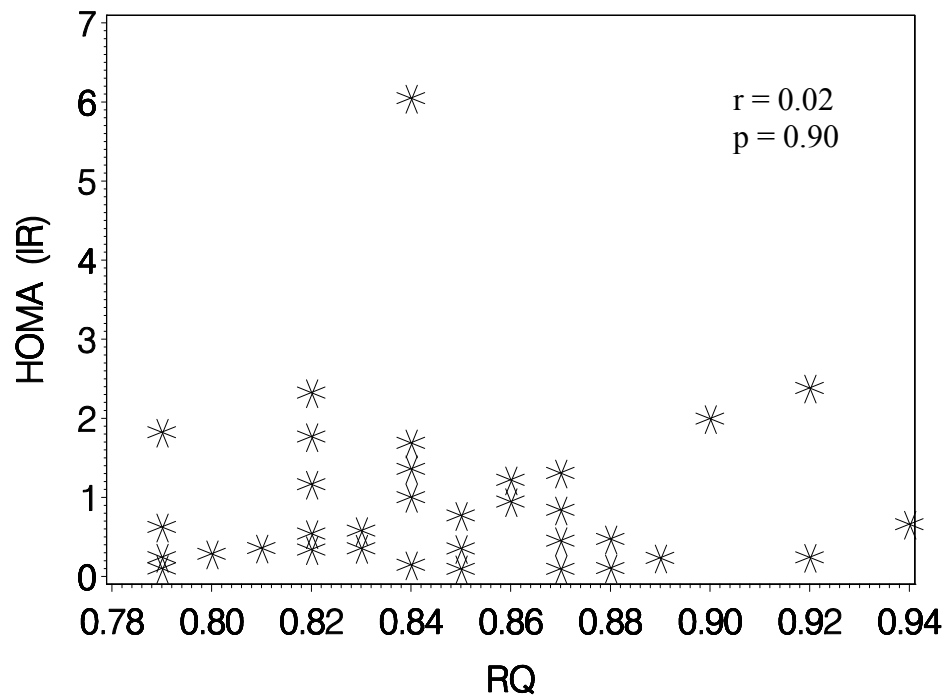
**Figure 5.** The association of insulin resistance and fat oxidation
in females (n=18) and males (n=16)
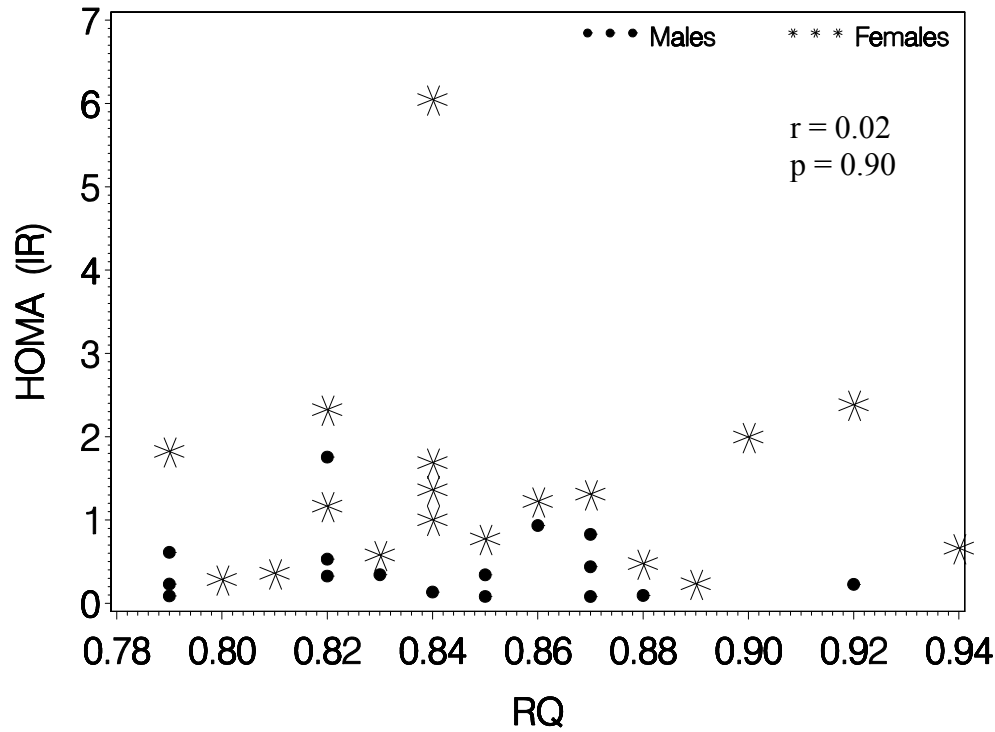


**Specific Aim 2**

**Impaired Fat Oxidation and Insulin Sensitivity**

In all subjects observed who participated in the FSIGTT (n=16), Pearson correlation

coefficients revealed no significant association between RQ and insulin sensitivity (r=-0.04,

p=0.88) (figure 6).  Further analyses (general linear model) did not display any significant

associations between RQ and insulin sensitivity when adjusted for physical activity,

breastfeeding, birth weight, race, and sex, independently or collectively.  Partial correlations

(adjusted for physical activity, TPA, MVPA, breastfeeding, birth weight, race, and sex) between

RQ and insulin sensitivity ranged from -0.08 to -0.41, however no correlations were significant

despite the fact that there was adequate statistical power.

**Table 9**. General linear models for the dependent variable of insulin sensitivity and RQ

| Variables considered for adjustment | Dependent Variable | dffull | dfrestricted-dffull | F | p |
|---|---|---|---|---|---|
| sex | Insulin Sensitivity | 12 | 2 | 0.0123 | 0.98775 |
| race | Insulin Sensitivity | 12 | 2 | 0.2078 | 0.81522 |
| breastfeeding | Insulin Sensitivity | 12 | 2 | 0.7340 | 0.50035 |
| totalgodin | Insulin Sensitivity | 12 | 2 | 0.0965 | 0.90873 |
| race totalgodin | Insulin Sensitivity | 9 | 3 | 0.0878 | 0.96495 |
| breastfeeding totalgodin | Insulin Sensitivity | 9 | 3 | 0.3159 | 0.81372 |
| sex race | Insulin Sensitivity | 9 | 3 | 0.1260 | 0.94233 |
| sex breastfeeding | Insulin Sensitivity | 9 | 3 | 0.6694 | 0.59188 |
| race breastfeeding | Insulin Sensitivity | 9 | 3 | 0.3644 | 0.78049 |
| sex totalgodin | Insulin Sensitivity | 9 | 3 | 0.0115 | 0.99819 |
| sex race totalgodin | Insulin Sensitivity | 5 | 4 | 0.1830 | 0.93752 |
| sex breastfeeding totalgodin | Insulin Sensitivity | 5 | 4 | 0.6886 | 0.63033 |
| race breastfeeding totalgodin | Insulin Sensitivity | 5 | 4 | 0.1243 | 0.96741 |
| sex race breastfeeding | Insulin Sensitivity | 6 | 4 | 0.3125 | 0.85998 |
| sex race breastfeeding totalgodin | Insulin Sensitivity | 2 | 4 | 0.2860 | 0.86761 |

*p<0.05

**Figure 6.** The association of insulin sensitivity and fat oxidation (n=16)

r = -0.04
p = 0.88

Insulin Sensitivity

RQ
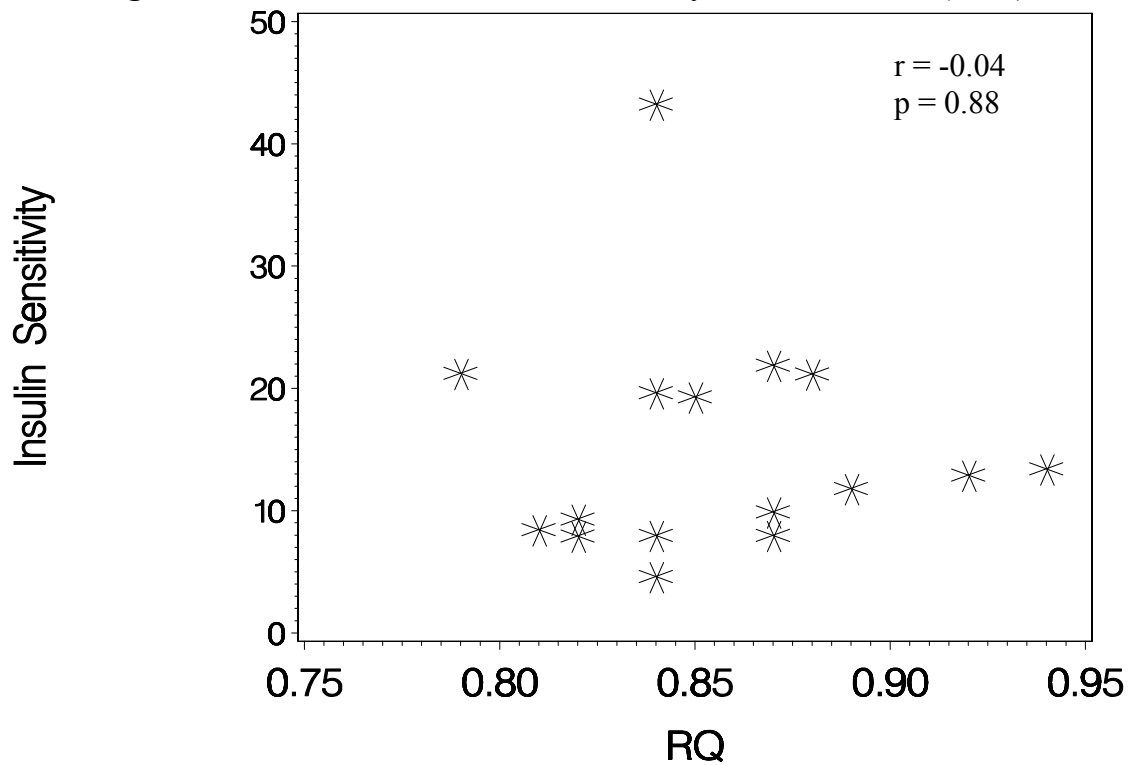
**Figure 7.** The association of insulin sensitivity and fat oxidation
in females (n=7) and males (n=9)

• • • Males    * * * Females

r = -0.04,
p = 0.88

Insulin Sensitivity

RQ
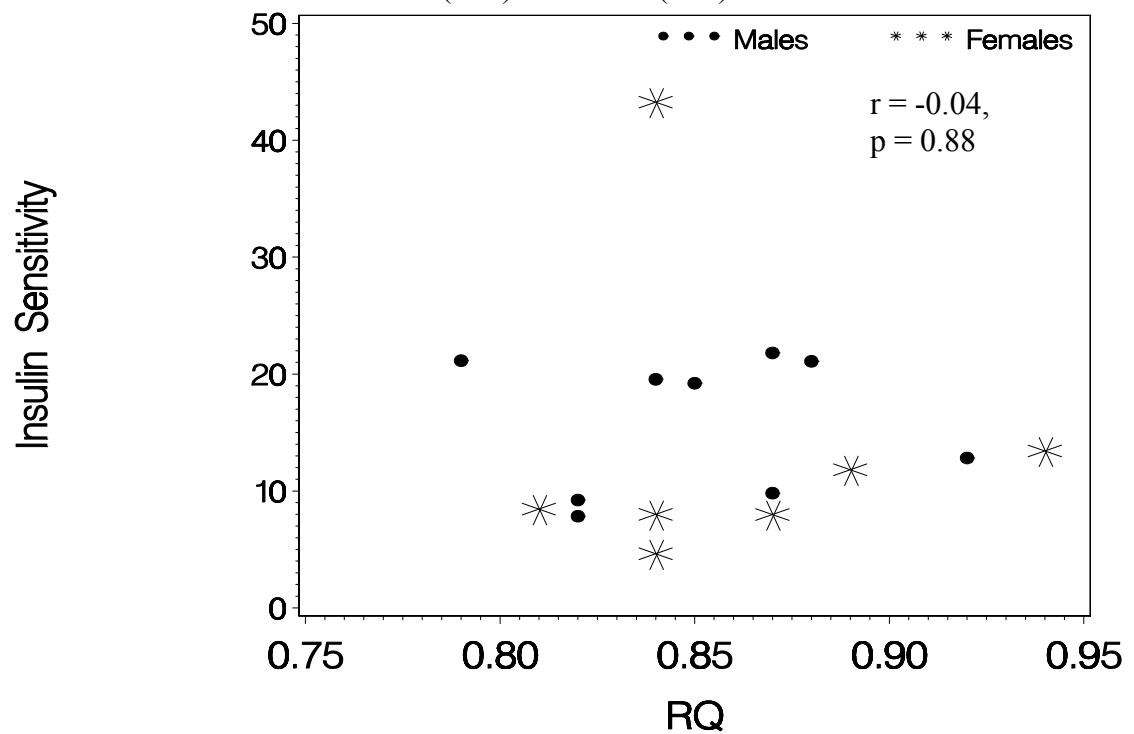
**Respiratory Quotient**

In all subjects observed (n=34), significant, Pearson correlations were identified between RQ and weight (r=-0.38, p<0.05), RQ and LBM (r=-0.41, p<0.05), RQ and total body mass (r=-0.36, p<0.05), and RQ and TPA (r=0.54, p<0.05). These significant, negative correlations were unexpected and do not reflect the majority of findings in other research studies. These correlations may be due to the small sample size, however, further investigation is warranted. A multifactor ANOVA model examining RQ was not significant ($F_{(14,19)}=1.60$, p=0.17), however a significant sex by breastfeeding interaction within the model ($F_{(2,19)}=4.75$, p<0.05) was observed on RQ. Partial correlation coefficients adjusted for physical activity, TPA and MVPA, breastfeeding, birth weight, race and sex revealed no significant associations in regards to RQ.

In subjects with FSIGTT information (n=16), no significant Pearson correlations or correlations adjusted for physical activity, TPA and MVPA, breastfeeding, birth weight, race and sex were observed in regards to RQ.

The 34 subjects were classified into the following 3 groups according to their RQ (Zurlo, 1990):

Low RQ: <0.822 (n=11)

Medium RQ: 0.822 – 0.877 (n=16)

High RQ: >0.877 (n=7)

Fasting glucose, fasting insulin, insulin resistance, β-cell function, and %fat were examined between the groups. No significant differences were observed in any of the variables examined.

**Insulin Resistance**

In all subjects observed (n=34), significant, Pearson correlations were observed between IR and BMI (r=0.63, p<0.001) (Figure 8), IR and %fat (r=0.55, p<0.001) (Figure 9), IR and FBM (r=0.73, p<0.001) (Figure 10), IR and LBM (r=0.61, p<0.001) (Figure 11), and IR and total

body mass (r=0.72, p<0.001) (Figure 12), however, a multifactor ANOVA on insulin resistance

adjusted for breastfeeding, birth weight, race and sex was not significant (F(14,19)=1.29,

p=0.30). Partial correlation displayed a significant association between IR and BMI (r=0.61,

p<0.01), IR and %fat (r=0.52, p<0.05), IR and FBM (r=0.71, p<0.001), IR and LBM (r=0.72,

p<0.001), IR and total body mass (r=0.74, p<0.001), and IR and total cholesterol (r=0.42,

p<0.05).

**Figure 8.** The association of insulin resistance and body mass index (n=34)
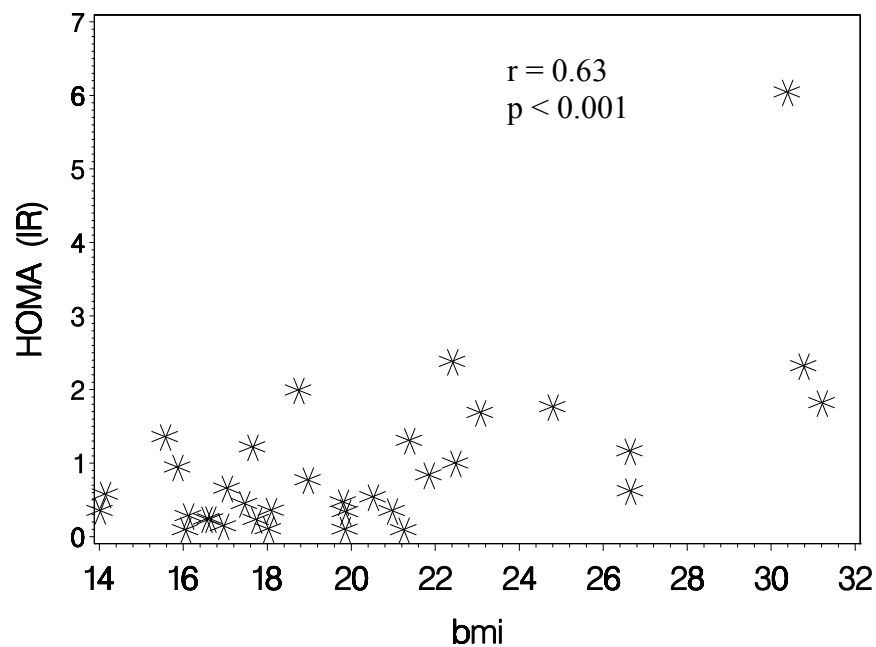
**Figure 9.** The association of insulin resistance and percent fat (n=34)
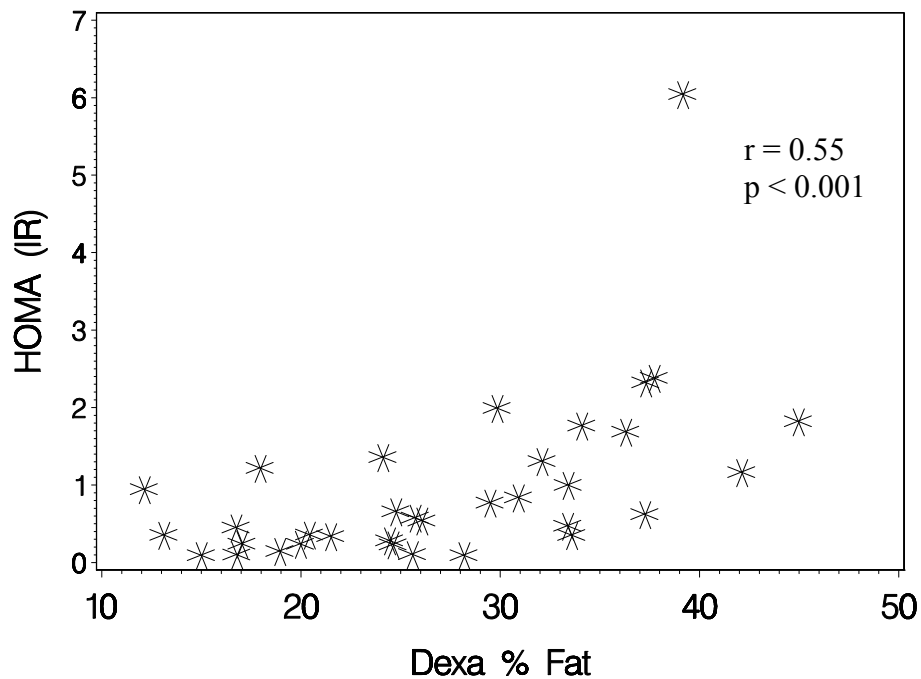


**Figure 10.** The association of insulin resistance and fat body mass (n=34)
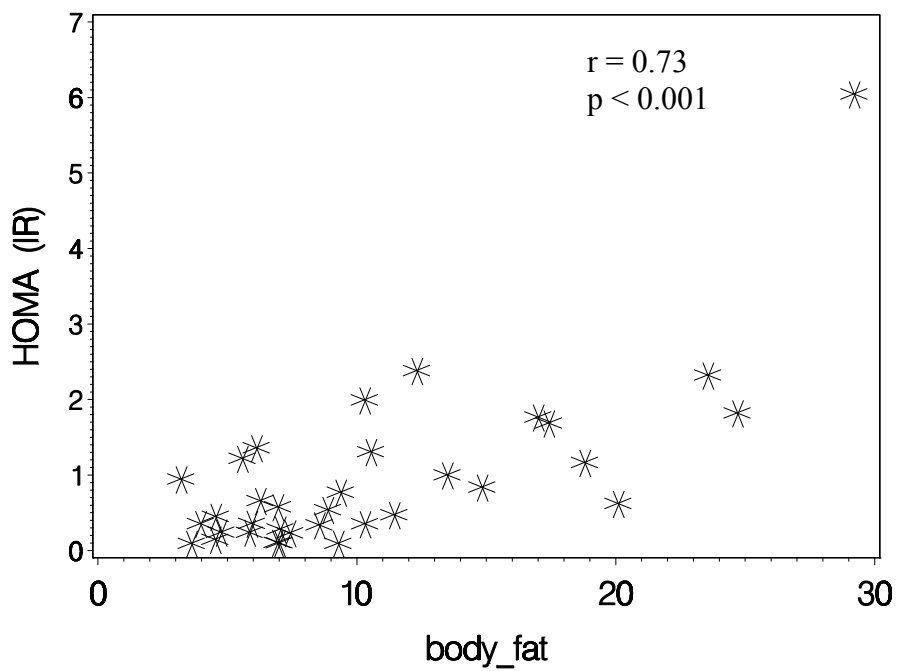
**Figure 11.** The association of insulin resistance and lean body mass (n=34)
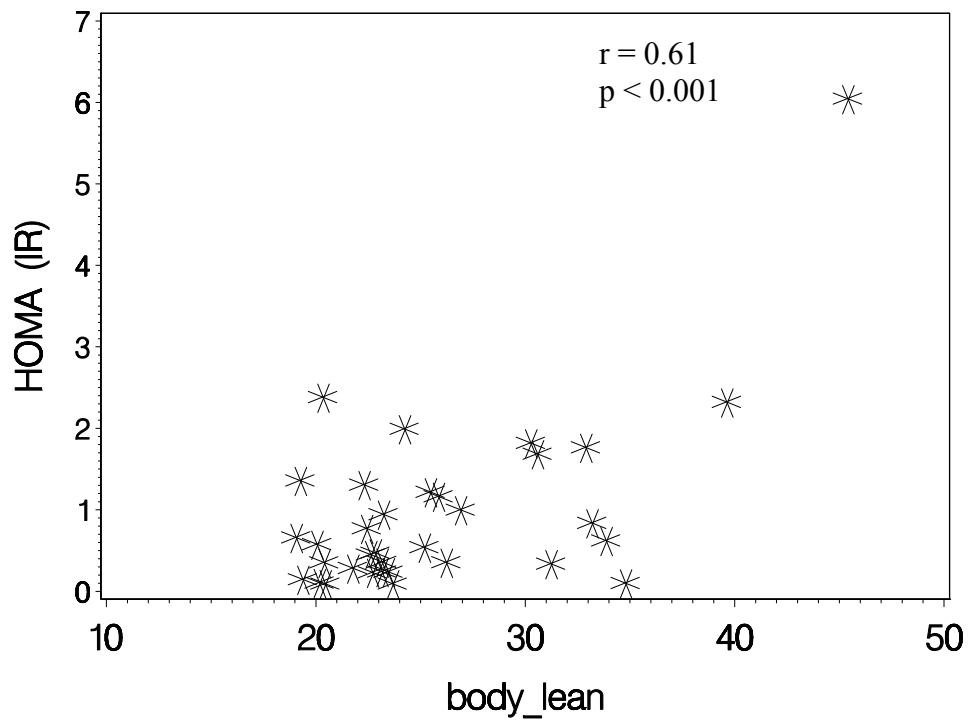


**Figure 12.** The association of insulin resistance and total body mass (n=34)
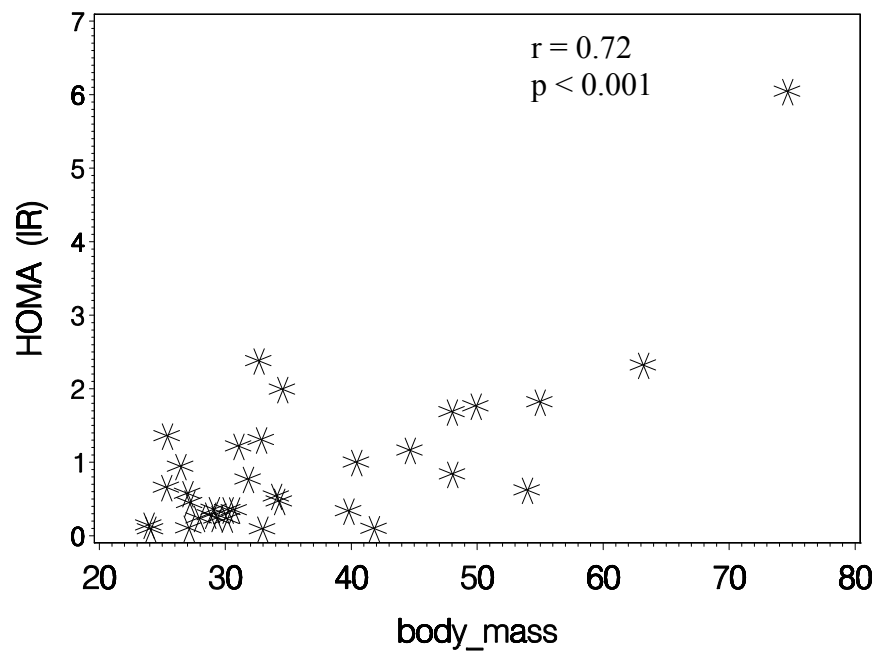
In subjects who participated in the FSIGTT (n=16), a significant, negative Pearson correlation was observed between IR and $S_g$ (r=-0.55, p<0.05). Partial correlations adjusted for physical activity, breastfeeding, birth weight, race and sex displayed significant correlations between IR and LBM (r=0.82, p<0.05), IR and total body mass (r=0.83, p<0.05), IR and TPA (r=0.87, p<0.05), and IR and FBM (r=0.84, p<0.05).

**Insulin Sensitivity**

Examining the results of those who participated in the FSIGTT (n=16), a significant, negative association was observed between insulin sensitivity and BMI (r=-0.53, p<0.05) (Figure 13). A multifactor ANOVA on insulin sensitivity adjusted for breastfeeding, birth weight, sex and race was significant (F(9,6)=7.44, p<0.05). The $R^2$ was 0.92; therefore 92% of the variance in insulin sensitivity may be attributed to breastfeeding, birth weight, sex, and race. Moreover, a significant main effect of sex (F(1,6)=45.38, p<0.001) and a significant sex by birth weight interaction (F(1,6)=48.57, p<0.001) was observed.

**Figure 13.** The association of insulin sensitivity and body mass index (n=16)
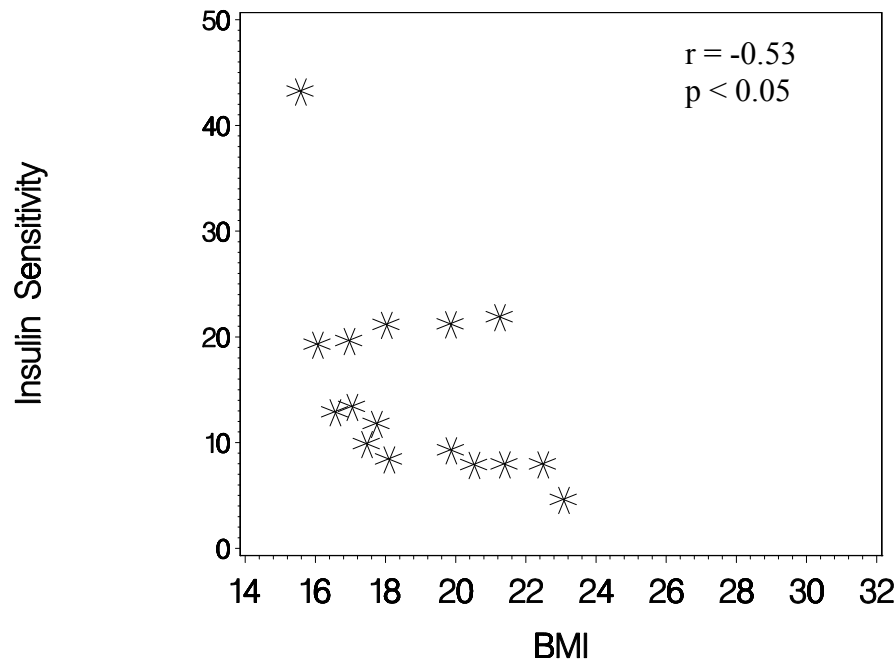


## Resting Energy Expenditure

Examining all subjects (n=34) Pearson correlation coefficients displayed significant associations between REE and BMI (r=0.65, p<0.0001), REE and %fat (r=0.37, p<0.05), REE and FBM (r=0.61, p<0.0001), REE and LBM (r=0.75, p<0.0001), and REE and total body mass (r=0.72, p<0.0001).  A multifactor ANOVA on REE adjusted for breastfeeding, birth weight, sex, and race was not significant, however significant sex by race (F(1,19)=9.67, p<0.01), sex by breastfeeding (F(2,19)=3.88, p<0.05), and race by breastfeeding (F(2,19)=4.50, p<0.05) interactions were observed within the model.  Partial correlations displayed significant associations between REE and BMI (r=0.68, p<0.001), REE and %fat (r=0.61, p<0.01), REE and FBM (r=0.71, p<0.001), REE and LBM (r=0.74, p<0.001), REE and total body mass (r=0.75, p<0.001), REE and insulin (r=0.44, p<0.05), and REE and β-cell function (r=0.53, p<0.01).  Correlation coefficients adjusted for TPA, breastfeeding, birth weight, race and sex revealed a

significant association between REE and fasting glucose (r=0.69, p<0.05). Partial correlation coefficients adjusted for MVPA, breastfeeding, birth weight, race and sex revealed a significant association between REE and fasting glucose (r=0.80, p<0.01).

In subjects who participated in a FSIGTT (n=16), no significant Pearson correlation coefficients were observed in regards to REE. Correlation coefficients adjusted for physical activity, breastfeeding, birth weight, race and sex displayed a significant association between REE and fasting glucose (r=0.88, p<0.05). No significant partial correlations adjusted for TPA, breastfeeding, birth weight, race and sex were observed in regards to REE. A significant partial correlation coefficient adjusted for MVPA, breastfeeding, birth weight, race and sex was observed between REE and fasting glucose (r=0.82, p<0.05).

**Fasting Glucose**

No significant Pearson correlations were identified when examining all subjects (n=34). A multifactor ANOVA adjusted for breastfeeding, birth weight, sex, and race displayed no significance on REE. Correlations adjusted for physical activity, breastfeeding, birth weight, race and sex displayed a significant association between fasting glucose and total cholesterol (r=0.47, p<0.02) and fasting glucose and HDL (r=0.47, p<0.05). A significant association adjusted for TPA, breastfeeding, birth weight, race, and sex was observed between fasting glucose and REE (r=0.69, p<0.05). A similar, significant correlation adjusted for MVPA, breastfeeding, birth weight, race, and sex was found between fasting glucose and REE (r=0.80, p<0.01).

In subjects who participated in the FSIGTT (n=16), no significant Pearson correlations between fasting glucose and other variables was observed. A significant association was found between fasting glucose and REE (r=0.88, p<0.02) adjusted for physical activity, breastfeeding,

birth weight, race and sex.  A significant association was observed between fasting glucose and REE (r=0.80, p<0.05) adjusted for MVPA, breastfeeding, birth weight, race and sex, however no significant associations were observed between fasting glucose and any other variables adjusted for TPA, breastfeeding, birth weight, race and sex.

**Fasting Insulin**

In all subjects observed (n=34), significant, Pearson correlations were found between fasting insulin and BMI (r=0.66, p<0.001), fasting insulin and %fat (r=0.58, p<0.001), fasting insulin and FBM (r=0.75, p<0.001), fasting insulin and LBM (r=0.62, p<0.001), fasting insulin and total body mass (r=0.73, p<0.001), and fasting insulin and fasting glucose (r=0.45, p<0.01). A multifactor ANOVA on fasting insulin adjusted for breastfeeding, birth weight, sex, and was not significant.  Significant associations adjusted for physical activity, breastfeeding, birth weight, race and sex were displayed with fasting insulin and BMI (r=0.67, p<0.001), fasting insulin and %fat (r=0.57, p<0.01), fasting insulin and FBM (r=0.77, p<0.001), fasting insulin and LBM (r=0.76, p<0.001), fasting insulin and total body mass (r=0.79, p<0.001), fasting insulin and REE (r=0.44, p<0.05), and fasting insulin and fasting glucose (r=0.43, p<0.05).  A significant association was observed between fasting insulin and body weight (r=0.70, p<0.05) adjusted for MVPA, breastfeeding, birth weight, race and sex, however no significant associations were observed between fasting glucose and any other variables adjusted for TPA, breastfeeding, birth weight, race and sex.

In the findings of FSIGTT participants (n=16), a significant Pearson correlation was observed between fasting insulin and $S_g$ (r=-0.57, p<0.05).  Significant associations were observed between fasting insulin and body weight (r=0.91, p<0.05), fasting insulin and LBM (r=0.93, p<0.05), and fasting insulin and total body mass (r=0.96, p<0.01) adjusted for physical

activity, breastfeeding, birth weight, race and sex. Significant associations between fasting insulin and body weight ($r=0.86$, $p<0.05$), fasting insulin and FBM ($r=0.85$, $p<0.05$), and fasting insulin and total body mass ($r=0.90$, $p<0.05$) adjusted for TPA, breastfeeding, birth weight, race and sex. Significant associations between fasting insulin and body weight ($r=0.82$, $p<0.05$), fasting insulin and BMI ($r=0.86$, $p<0.05$), fasting insulin and FBM ($r=0.92$, $p<0.01$), and fasting insulin and total body mass ($r=0.84$, $p<0.05$) adjusted for MVPA, breastfeeding, birth weight, race and sex.

**Total Physical Activity**

In all subjects with accelerometry results (n=14), a significant association was observed between TPA and RQ ($r=0.54$, $p<0.05$) and TPA and HDL ($r=-0.61$, $p<0.05$). These correlations were an unexpected finding and do not reflect findings in published literature. These findings may be due to the small sample size. Future research will examine these variables in a larger number of subjects. A multifactor ANOVA on TPA adjusted for breastfeeding, birth weight, sex, and race was significant ($F(10,3)=13.76$, $p<0.05$). Also a significant main effect of sex ($F=10.71$, $p<0.05$), race ($F=18.53$, $p<0.05$), and a significant interaction of sex and birth weight ($F=10.87$, $p<0.05$) and race and birth weight ($F=12.00$, $p<0.05$) was observed. In FSIGTT subjects with accelerometry results (n=10), no significant correlations were observed.

**Moderate to Vigorous Physical Activity**

In all subjects with accelerometry results (n=14), a multifactor ANOVA on MVPA adjusted for breastfeeding, birth weight, sex, and race was not significant. In FSIGTT subjects with accelerometry results (n=10), no significant correlations were observed.

**Chapter Five**

**Discussion**

Two hypotheses were examined in the current study. Hypothesis 1.a stated that impaired fat oxidation was associated with insulin resistance in prepubertal children when adjusted for race, sex, and physical activity. This hypothesis was supported. Respiratory quotient and insulin resistance were significantly associated when adjusted for race, sex, and physical activity collectively, however there was no significant association between RQ and insulin resistance when physical activity was examined independently. Race and sex were considered in all analyses. Respiratory quotient and insulin resistance were not significantly associated after adjusting for only race and sex.

When physical activity was entered into the model with race and sex, RQ and insulin resistance were significantly associated. Therefore physical activity may be a modifiable risk factor which may help improve metabolic flexibility and improve insulin action. In adults, both low-intensity endurance training and weight training were shown to improve the capacity for fat oxidation and decrease insulin resistance in adults (Schrauwen, 2002, Dumortier, 2003) and in children (Shaibi, 2006).

In lean subjects, physical activity is shown to stimulate fat oxidation during and after exercise (Blaak, 2002). Therefore in overweight subjects, physical activity may be able to compensate for their impaired ability to oxidize fat, in turn, improving metabolic flexibility (Blaak, 2002). In the current study 21% of the prepubertal children displayed a high RQ indicating poor fat oxidation. Moreover 47% were classified into a medium fat oxidation group. Therefore introducing or increasing physical activity in these two groups, may improve fat oxidation which in turn may play a protective role in the risk of weight gain (Maffeis, 2000). In

particular, prescribing physical activity targeting type 1, slow-twitch fibers, which have a high oxidative potential and an excellent capacity for using lipid as a fuel, may be most beneficial (Blaak, 2002). Prescribing endurance or low-intensity physical activity may be most effective in increasing fat oxidation in these prepubertal children. Maffeis et al (2005) found that increasing the exercise intensity in obese prepubertal boys did not promote fat oxidation. In fact, the highest fat oxidation rate was during low intensity physical activity (Maffeis, 2005).

The prepubertal period may be an opportune time to increase physical activity in children at-risk for insulin resistance. Early pubertal, overweight, impaired glucose tolerant children do not display lower cardiorespiratory fitness or physical activity levels compared to overweight, normal glucose tolerant children. However, since impaired glucose tolerance represents an early stage in the progression to insulin resistance and type 2 diabetes, it may be possible that as their disease state progresses, impairment in physical activity may become evident as well (Shaibi, 2006). As observed in figure 8 (page 58) of the current study, as BMI increases, insulin resistance increases. This significant association supports the need for increased physical activity in prepubertal children, and in particular, overweight, prepubertal children.

Regardless of weight change, however, physical activity may improve components of insulin resistance. In obese adolescents, mild intensity physical activity for 15 weeks decreased insulin levels even without weight loss (Kahle, 1996). In obese children, four months of physical activity training was shown to decrease %fat and fasting insulin. However, after the physical training ceased in these obese children, the benefits of the physical activity were lost over the course of four months (Ferguson, 1999).

In the current study, females displayed a higher RQ and a significantly higher insulin resistance compared to the males. This finding was similar to those by Zurlo et al (1990). Zurlo

found a significant and independent effect of sex on RQ with the adult females displaying a higher RQ than the males. Also similar to the present study, Zurlo (1990) found that on average, the females were fatter than the males. Moreover, only when the sexes were considered separately did the degree of obesity positively correlate with RQ. Perhaps the existence of different patterns of fat utilization and/or storage may help to explain the higher relative fat mass in women (Zurlo, 1990). Overweight in childhood is associated with early physical maturation therefore it may be suggested that the females in this study had advanced maturation. However, all of the subjects included in this study were examined by a medical doctor and classified as prepubertal with a maturation level <2 (Tanner, 1976).

Although the prepubertal females in the current study had significantly higher weight, total body mass and %fat than the males, the females had relatively the same LBM as the males (25.7 vs. 25.9, respectively). This lower amount of LBM places the females at a disadvantage for fat oxidation. Since skeletal muscle is responsible for the greatest amount of oxidized fat in the body, a smaller amount of skeletal muscle leads to a smaller amount of fat oxidation. Additionally, with a smaller amount of skeletal muscle, there is a greater reliance on glucose oxidation rather than fat oxidation leading to metabolic inflexibility (Cahova, 2007). This smaller amount of fat oxidation will eventually lead to the accumulation of fat stores (Maffeis, 2000) in both adipose tissue and in muscle. This fat, accumulating inside of the muscles, is negatively associated with insulin resistance in adults (Schrauwen, 2002); however, this association is unknown in children.

In the current study, females compared to males and non-Caucasians compared to Caucasians displayed significantly higher fasting insulin values and significantly higher insulin resistance. In regards to physical activity, there was no significant difference between sexes in

TPA or MVPA. There was also no significant difference between races for MVPA, however, the non-Caucasians participated in significantly less TPA than the Caucasians. Fat oxidation and insulin resistance were significantly associated when adjusted for sex, race, and physical activity. Therefore encouraging physical activity in these prepubertal children in particular would target the modifiable risk factor in this model.

Hypothesis 1.b stated that impaired fat oxidation was associated with insulin resistance when adjusted for breastfeeding, race, and sex. This hypothesis was supported. Respiratory quotient and insulin resistance were significantly associated when adjusted for breastfeeding, birth weight, and race collectively as well as breastfeeding, sex, and race collectively. When breastfeeding was examined independently on RQ and insulin resistance, no significant association was observed.

Similar to the findings of physical activity on RQ and insulin resistance, when breastfeeding was entered into the model with birth weight and race and sex and race, RQ and insulin resistance were significantly associated. Therefore, similar to physical activity, breastfeeding may be a modifiable risk factor which may help improve metabolic flexibility and improve insulin action. Baur et al (1998) found breastfed infants displayed a significantly greater amount of LCPUFAs and DHAs and a significantly lower fasting glucose than non breastfed infants. In the current study, however, no significant differences were observed in blood values or body composition between breastfed and non breastfed children.

In the current study, RQ and insulin resistance were significantly associated when adjusted for breastfeeding, birth weight, and race collectively as well as breastfeeding, sex, and race collectively. When comparing sex characteristics, the females had significantly higher weight, BMI, %fat, FBM, and TBM compared to the males. Moreover, the females had a higher

RQ and significantly higher fasting insulin and insulin resistance compared to the males. Although the effect of breastfeeding was not compared between the males and females in the current study, breastfeeding may be a modifiable risk factor that may help prevent overweight in children, in particular females. Liese et al (2001) found that the prevalence of overweight was significantly higher in 9-10 year olds compared to those who were never breastfed. This protective effect of breastfeeding on childhood and adult overweight was also observed in several other studies (Arenz, 2004, Owen, 2005, Young, 2002, Mayer-Davis, 2006, Bergmann, 2003).

In the current study there were no differences in the number of males versus females whose mothers reported breastfeeding. However a significant association between race and breastfeeding was observed. A similar association was found by Forste (2001). In the current study, 62% of Caucasian subjects' mothers reported breastfeeding while only 23% of the non-Caucasian mothers reported breastfeeding. There were no significant differences in body composition variables in the current study in breastfed versus non breastfed.

In 50 year olds, Ravelli et al (2000) found those who were at least partially bottle fed displayed a lower glucose tolerance, and a more atherogenic lipid profile than those who were exclusively breastfed. Furthermore, the deleterious effect of non breastfeeding on lipid profiles was more pronounced in women than in men (Ravelli, 2000). In Pima Indian adults, Pettitt (1997) observed a significantly lower rate of type 2 diabetes in those who were breastfed for the first two months of life compared to non breastfed (Pettitt, 1997). In 5-19 year old Pima Indians, fasting insulin in childhood was a significant predictor of diabetes in later adolescence and adulthood (McCance, 1994). While sex may be a risk factor for poor fat oxidation and insulin

resistance, it is obviously not a modifiable one; therefore the recommendation of breastfeeding may be important, particularly to mothers of female children.

Hypothesis 1c stated that impaired fat oxidation was associated with insulin resistance when adjusted for birth weight, race, and sex independently and collectively. This hypothesis was not supported. Respiratory quotient and insulin resistance were significantly associated when adjusted for breastfeeding, birth weight, and race however, without breastfeeding included, birth weight would not be considered a significant risk factor. There was also no significant association between RQ and insulin resistance observed when birth weight was adjusted for independently or alone with race and sex. This finding suggests that breastfeeding may have a protective effect on the development of insulin resistance regardless of birth weight.

Hyponnen et al (2003) observed adults born with low birth weight were at an increased risk for type 2 diabetes. However, low birth weight was followed by a relatively high weight gain in the adults who eventually developed diabetes. This trend of excessive weight gain subsequent to low birth weight was observed in several other studies (Whincup, 1997, Barker, 2002, Bavdekar, 1999). Low birth weight children tend to have increased fat mass relative to lean body mass with the increased fat storage contributing to insulin resistance (Tappy, 2006). Similar increased fat mass was also observed in data collected for the Bogalusa Heart Study by Frontini et al (2004). In low birth weight children and adolescents, the rate of yearly increase in BMI (which also included muscle mass) was significantly lower compared to the control group. However, the low birth weight group displayed an increased rate of subscapular skinfold, a measure of truncal fat suggesting a priority towards gaining truncal fat over muscle mass (Frontini, 2004).

In the current study, as BMI increased, insulin resistance increased as well. The same trend was observed in the entire group between %fat, FBM, LBM, TBM and insulin resistance. Regardless of birth weight, these results demonstrate the crucial need to prevent childhood overweight (and later development of insulin resistance) in these low and normal birth weight babies.

Hypothesis 2 stated that impaired fat oxidation and insulin sensitivity were associated when adjusted for race, sex, physical activity, breastfeeding, and birth weight. This hypothesis was not supported. There was no significant association between RQ and insulin sensitivity when physical activity, breastfeeding, and birth weight were controlled for independently or collectively.

Physical activity was not identified as a potential modifiable risk factor for fat oxidation and insulin sensitivity in the current study, however, other studies reported a positive association between physical activity and improved insulin dynamics (Schmitz, 2002, Nassis, 2005). In 10-16 year old children, significant, positive correlations were observed between physical activity and both fasting insulin and insulin sensitivity (Schmitz, 2002). In overweight and obese 9-15 year old girls, 12 weeks of aerobic training improved insulin sensitivity and glucose metabolism (Nassis, 2005). These improvements were observed without changes in body weight or fat which suggests that the improvement in insulin sensitivity may be caused by changes in the ability of the muscles to metabolize glucose. Nassis et al (2005) also observed increased lower limb fat free mass which may at least in part explain the enhanced insulin sensitivity in these children. These findings support previous studies in adults that found physical training-induced improvements in insulin sensitivity without changes in body fat (Dengel, 1998). Mensink et al (2005) demonstrated a stabilization of fatty acid oxidation in a group of glucose-intolerant men

who underwent a year of diet and physical activity lifestyle intervention. These men may have been able to prevent further impairment in fatty acid use, while the control group, displayed a decrease in fatty acid oxidation (Mensink, 2005).

While physical activity is recommended to help increase insulin sensitivity, resistance training, specifically may provide the most beneficial results. Compared to endurance training, resistance training may substantially affect skeletal muscle hypertrophy and strength. Resistance training may increase skeletal muscle mass therefore increasing whole-body glucose disposal capacity (Corcoran, 2007). In overweight, Latino, adolescent males, 16 weeks of resistance training significantly increased insulin sensitivity independent of changes in body composition (Shaibi, 2006).

Although no significant association between fat oxidation and insulin sensitivity adjusted for physical activity, breastfeeding, and birth weight were observed in the current study does not mean an association does not exist. This non significant finding may be due to the low sample size of FSIGTT participants. Moreover, only one study to date has examined the protective effects of breastfeeding on type 2 diabetes using FSIGTT. Davis et al (2007) examined 8-13 year old overweight Latino youths and found no significant protective effects of breastfeeding on adiposity or insulin sensitivity (Davis, 2007). However, the effect of breastfeeding on both fat oxidation and insulin sensitivity are not clearly understood and should be investigated further.

In the current study, a significant association between fat oxidation and insulin sensitivity was not observed controlling for physical activity, breastfeeding, and birth weight, however, a complex multifactor ANOVA on insulin sensitivity identified that 92% of the variance in insulin sensitivity was due to race, sex, breastfeeding, and birth weight. Both the females and non-Caucasian displayed lower insulin sensitivity and lower birth weight than the males and

Caucasians. Poor insulin sensitivity is highly correlated with defects in skeletal muscle oxidative capacity and fat metabolism (Kelley, 1999). Therefore, the findings of the current study may support the view that these children, in particular, the females, and non-Caucasian children may have a defect in their skeletal muscle oxidative capacity.

Findings from Baur et al (1998) demonstrated that breastfed infants had a significantly greater amount of LCPUFAs in their skeletal muscle compared to nonbreastfed infants. The breastfed infants displayed a muscle fatty acid composition similar to that of insulin sensitivity adults whereas the nonbreastfed infants displayed a muscle fatty acid composition similar to that of insulin-resistant adults with a deficiency in DHA and LCPUFAs. This examination demonstrates the early beneficial effects of breastfeeding on skeletal muscle composition. Such early alterations in skeletal muscle as witnessed by Baur et al (1998) may play a role in the subsequent development of diseases associated with insulin resistance.

The positive effect of breastfeeding on skeletal muscle composition may be most beneficial to those born with low birth weight. In 25 year olds, low birth weight adults were hyperinsulinemic compared to normal birth weight adults. Furthermore, the effect of low birth weight on insulin sensitivity was observed even after adjustment for BMI (Jaquet, 2000). Low birth weight children are typically born with a smaller amount of skeletal muscle, therefore breastfeeding may enhance the composition of the skeletal muscle leading to an improved fat oxidation and decreasing the risk for insulin resistance. In the current study, 92% of the variance in insulin sensitivity was attributed to race, sex, breastfeeding, and birth weight, therefore it may be worthwhile to target two of the four modifiable factors, breastfeeding and birth weight, to positively influence insulin sensitivity. Moreover, in the current study, any negative implications that low birth weight may have on fat oxidation appear to be mediated by breastfeeding.

There are several limitations of this study including the classification of the subjects according to RQ. In the current study, RQ was divided into 3 groups, high, medium, and low. There are no studies in prepubertal children that identified high and low RQ cutoff points therefore, the high and low group cutoff points applied in this study were derived from research examining Pima Indian adults (Zurlo, 1990). However, based on previous literature, prepubertal children may have slightly higher fat oxidation (lower RQ) rates. Higher fat oxidation rates were observed in obese prepubertal children compared to normal weight children (Maffeis, 1995) and both Jones (1998) and Kostyak (2007) identified a higher fat oxidation rate in 5-10 year old children compared to adults. Therefore, the cutoff points applied to RQ in the current study may actually need to be shifted slightly lower to reflect this higher rate of fat oxidation in prepubertal children. This may also explain why significant differences were not observed in any of the insulin resistance or sensitivity variables between the RQ groups.

The current findings are limited by a small sample size and should be interpreted with caution, especially since so few African American mothers breastfed. Moreover, all breastfeeding and birth weight information was obtained through self-reported questionnaires and are subject to recall bias. Another limitation of the study is that HOMA is an estimate of insulin resistance whereas assessment of insulin sensitivity by FSIGTT is a more precise measure. The FSIGTT is an accurate and valid technique for the measurement of insulin sensitivity in adults, adolescents, and children (Cutfield, 1990, Bergman, 1987), however, it is time consuming, invasive, labor intensive, and requires experienced personnel (Conwell, 2004) to perform the procedure.

In the current study, lack of physical activity and lack of breastfeeding appear to be the most influential risk factors in the development of insulin resistance via a mechanism of

impaired fat oxidation.  Impaired fat oxidation may be related to decreased physical activity and perhaps a lack of breastfeeding.  Breastfeeding may also influence RQ through the improvement of body composition, especially in youth who lack sufficient skeletal muscle.  Lack of physical activity, lack of breastfeeding, and low birth weight may collectively create a phenotype for poor metabolic flexibility leading to an increased risk for insulin resistance.  The findings of this study however are promising, because the risk factors, physical activity, breastfeeding, and birth weight, which may potentially improve fat oxidation and, therefore, prevent insulin resistance are modifiable.  Increased physical activity in this age group may improve skeletal muscle flexibility by enhancing the ability of the muscles to metabolize glucose while increasing muscle mass (Nassis, 2005, Corcoran, 2007).  This combination may lead to an improved fat oxidation resulting in improved insulin action.  Physical activity and breastfeeding may work together by improving both skeletal muscle and overall body composition.  And although birth weight was not specifically identified as a significant risk factor in this study, previous research supports efforts to encourage good prenatal care among expectant mothers.

Two hypotheses were examined in this study.  The first hypothesis was supported. Respiratory quotient and insulin resistance were significantly associated after controlling for race, sex, physical activity, breastfeeding, and birth weight.   The second hypothesis was not supported.  Respiratory quotient and insulin sensitivity were not significantly associated after controlling for race, sex, physical activity, breastfeeding, and birth weight. Although a significant association between fat oxidation and insulin sensitivity was not observed in the current study due to a small sample size, further analyses demonstrated that the majority of variance in insulin sensitivity was due to race, sex, breastfeeding, and birth weight.  Therefore

these findings warrant the modification of potential risk factors for poor fat oxidation and insulin resistance and sensitivity.

Physical activity may increase the oxidative capacity of skeletal muscle which may improve metabolic flexibility therefore, improving insulin dynamics (Bruce, 2004). Early pubertal, overweight, impaired glucose tolerant children do not display lower cardiorespiratory fitness compared to overweight, normal glucose tolerant children (Shaibi, 2006), therefore, the prepubertal period may be an opportune time to increase and encourage physical activity. Moreover, physical activity may enhance insulin sensitivity in children independently of changes in body mass (Nassis, 2005).

Breastfeeding may have a protective effect on childhood and adult overweight as well as a positive effect on skeletal muscle composition. The positive effect of breastfeeding on markers of fat oxidation (via skeletal muscle) and insulin resistance was demonstrated in infants (Baur, 1998), and adults (Pettitt, 1997). Moreover, the current findings suggest breastfeeding may provide a protective effect regardless of birth weight.

Individuals with low birth weight tend to have increased fat mass relative to skeletal muscle (Tappy, 2006). This smaller amount of skeletal muscle may limit the oxidative capacity, however in the current study breastfeeding appears to mediate poor fat oxidation. Breastfeeding may alter skeletal muscle composition leading to an improved fat oxidation, therefore protecting against insulin resistance (Baur, 1998, Jaquet, 2000).

An important finding in the current study was the important role of breastfeeding as a potential protective factor for impaired fat oxidation and insulin resistance. Obviously, however, it is too late at the prepubertal period to modify the lack of breastfeeding. Therefore, the most important finding of this study may be the ability of physical activity to modify for a lack of

breastfeeding.  It is not too late to modify physical activity during the prepubertal period.

Increasing physical activity is especially important prior to the onset of puberty at which time a

physiological insulin resistance occurs.  Additionally, a recent review (Corcoran, 2007) of the

effects of exercise on skeletal muscle and insulin resistance supports the initiation of a physical

activity program.  Corcoran et al (2007) suggests a long-term physical activity program

comprised of both endurance and strength training (along with reductions in saturated fat intake)

would prevent the occurrence of insulin resistance in the general population and improve insulin

sensitivity in the obese population (Corcoran, 2007).

This study evolved as emerging research continued to examine the mechanisms behind

impaired fat oxidation and insulin resistance.  Initially, the literature proposed insulin resistance

as a mechanism for poor fat oxidation, however recent research suggests otherwise.  The concept

of metabolic flexibility and its effect on insulin resistance and sensitivity continues to be

examined.  Furthermore, the role physical activity, breastfeeding, and birth weight may play on

fat oxidation and insulin resistance remains unclear in the literature, in particular in children.

The number of subjects in this current substudy represents ~10% of the participants proposed for

the larger, NIH funded study.  Therefore, the role physical activity, breastfeeding, and birth

weight may play on fat oxidation and the development of insulin resistance in prepubertal

children will continue to be examined.

The results of this study support the promotion of physical activity, breastfeeding, and

good maternal nutrition in order to positively influence birth weight.  Fortunately, these three

risk factors may be modified by healthy habits of mothers during the pre- and postnatal periods

as well as an increase in physical activity in children.

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# Appendix

**Phone Screening and Information Form**

Date:_____               Phone #:_____

Child's Name:_____

Sex:           M       F

Age:_____

DOB:_____/____/_____

Height (approx., ft./in.):_____

Current weight (approx., lbs.):_____

Birth weight (approx., lbs.):_____

Name of Mother:_____

Name of Father:_____

Address:_____

Relation of caller to child:_____

Child's pediatrician, family physician, or referring physician:_____

Is the child currently taking any medications?          Y       N

If so, please provide the names of any current medications:

_____

Are any of the child's family members overweight?      Y       N

Does the child's mother have diabetes?                       Y       N

Does the child's father have diabetes?                         Y       N

Does the child have any present serious illnesses?      Y       N

If so, what type?_____

Has the child experienced any past serious illnesses?                    Y        N

If so, what type?_____

Has the child undergone any periods of hospitalization?    Y        N

If so, for what and for how long?_____

Was the child born premature (<37 weeks of pregnancy)?   Y        N

If not, what was the term of the pregnancy? :_____

Does the child have any allergies to? :

        Food:                    Y        N

        If so, which foods? :_____

        Medications:             Y        N

        If so, which medications? :_____

        Other:_____

Has the child ever been diagnosed with any of the following? :

Depression                       Y        N

Personality disorder             Y        N

Anxiety                          Y        N

Schizophrenia          Y        N

Attention Deficit Disorder    Y        N

Other (Describe)                 Y        N        _____

Has the child been prescribed medication for any of the above?    Y        N

If yes, please describe:_____

Does the child have any of the following conditions?:

AIDS or HIV                                      Y        N

| | | | |
|---|---|---|---|
| Anemia | Y | N | |
| Anorexia | Y | N | |
| Arthritis | Y | N | |
| Bleeding/Clotting Problem | Y | N | |
| Blood Circulatory Problems or Clots | Y | N | |
| Bulimia | Y | N | |
| Cancer (past or present) | Y | N | Type:_____ |
| Depression | Y | N | |
| Diabetes | Y | N | Type:_____ |
| Drug or Alcohol Abuse | Y | N | |
| Epilepsy or Seizures | Y | N | |
| Gallbladder Disease | Y | N | |
| Glaucoma | Y | N | |
| Gout requiring medication | Y | N | |
| Gynecologic Problems | Y | N | |
| Heart Problems or Cardiovascular disease | Y | N | |
| Hepatitis or Liver Disease | Y | N | |
| High Blood Pressure | Y | N | |
| Impaired Vision | Y | N | |
| Impaired Hearing | Y | N | |
| Kidney Disease or Stones | Y | N | |
| Lung Problems | Y | N | |
| Lupus | Y | N | |

Mental Illness                                    Y       N       Specify:_____

Migraine Headaches                                Y       N

Multiple Dystrophy or Sclerosis                   Y       N

Prostatitis/ Prostate Enlargement                 Y       N

Sickle Cell Anemia                                Y       N

Stomach/ Intestinal Problems                      Y       N

Stroke                                            Y       N

Thyroid Disorders                                 Y       N

Condition requiring steroids/cortisone            Y       N

      (Sinus infections, Joint problems, etc.)

Other (specify):_____

Does the child use of any of the following medications? :

Diuretics:                                        Y       N

Steroids:                                         Y       N

Adrenergic-stimulating agents:                    Y       N

Does the child have a history of any of the following? :

Family or medical neglect:                        Y       N

Physical, mental or sexual abuse:                 Y       N

Did the mother of the child have gestational diabetes during the pregnancy?       Y       N

Did the mother drink alcohol while pregnant with the child?

Y       N       If yes, what type_____ Amount per week_____

Was any medication taken by the mother while pregnant with the child?

Y       N       If yes, please describe:_____

DEPARTMENT OF PSYCHOLOGY                                              Page 1 of 1

**Laura Scaramella**

**From:** Laura Scaramella
**Sent:** Wednesday, January 24, 2007 3:16 PM
**To:** April Bedford; Connie Lynn Vanvrancken
**Subject:** 02jan07 approval letter

*University Committee for the Protection*
*of Human Subjects in Research*
*University of New Orleans*

*Campus Correspondence*

April Bedford, PI
Connie Tompkins
ED 342B

1/24/2007

RE:      Fat oxidation and insulin resistance and insulin sensitivity in normal weight and
          overweight 7-9 year olds

IRB#:   02jan07

Your project is eligible for expedited review because the research involves use of previously
collected data. The described research and procedures are compliant with the University of
New Orleans and federal guidelines.

Please remember that approval is only valid for one year from the approval date. Any changes
to the procedures or protocols must be reviewed and approved by the IRB prior to
implementation.

If an adverse, unforeseen event occurs (e.g., physical, social, or emotional harm), you are
required to inform the IRB as soon as possible after the event.

Best of luck with your project!
Sincerely,

Laura Scaramella, Ph.D.
Chair, University Committee for the Protection of Human Subjects in Research

1/24/2007

*University Committee for the Protection*
*of Human Subjects in Research*
*University of New Orleans*

**Form Number:**     02jan07

*(please refer to this number in all future correspondence concerning this protocol)*

**Principal Investigator:**  April Bedford          **Title:** Associate Professor

**Department:**  Curriculum & Instruction          **College:** Education

**Project Title:**  Fat oxidation and insulin resistance and insulin sensitivity in normal weight and overweight 7-9 year olds

**Dates of Proposed Project Period**     From          10.1.2004          to          5.1.2010

**Approval Status:**

☐ Full Board Review                    ☒ Approved  Date:  1-24-0?

☒ Expedite                              ☐ Deferred  Date:

☐ Exempt                                ☐ Disapproved  Date:

☐ Project requires review more than annually.  Review every _____ months.

*\*approval is for 1 year from approval date only and may be renewed yearly.*

1ˢᵗ continuation  Signature of IRB Chair                    **Date:**

2ⁿᵈ continuation  Signature of IRB Chair                    **Date:**

3ʳᵈ continuation  Signature of IRB Chair                    **Date:**

4ᵗʰ continuation  Signature of IRB Chair                    **Date:**

Committee Signatures:

Laura Scaramella, Ph.D. (Chair)

James Evans, LCSW

Pamela Jenkins, Ph.D.

Isabelle Maret, Ph.D.

Ann O'Hanlon, Ph.D.

Richard B. Speaker, Ph.D.

Kari Walsh

Kathleen Whalen, LCSW

**INFORMED CONSENT FORM**

1.  **Study Title***: Mechanisms for the Metabolic Syndrome in Prepubertal African American and Caucasian Youth*

2.  **Performance Sites**

    Clinical Trials Center

    Children's Hospital

    200 Henry Clay Avenue

    New Orleans, LA   70118

    24-hour Telephone:  (504) 896-9511


    Pennington Biomedical Research Center

    6400 Perkins Road

    Baton Rouge, LA 70808-4124

    24-hour Telephone:  (225) 763-2500


    Children's Hospital, Baton Rouge Clinic

    720 Connell Park Lane

    Baton Rouge, LA 70808

    24-hour Telephone:  (225) 615-7332


3.  **Names and Telephone Numbers of Investigators**:


Principal Investigator:      Melinda S. Sothern, PhD
                             Associate Professor
                             Director, Section of Health Promotion
                             LSUHSC, School of Public Health
                             (504) 568-3051

Medical Director            Stuart Chalew, M.D.
and Co-Investigator:        Day Phone: (504) 896-9441
                            24-hr. Emergency Phone No

(504) 899-9511 (Children's Hospital)

| Co-Investigators: | Stewart Gordon, MD |
| | (225) 358-1310 |
| | Claude Bouchard, PhD |
| | (225) 763-2500 |
| | Eric Ravussin, PhD |
| | (225) 763-2686 |
| | Ennette Larson-Meyer, PhD |
| | (307) 766-4378 |
| | William Cefalu, MD |
| | (225) 763-2658 |

| Supervising Physicians: | Alfonso Vargas, MD |
| | (504) 896-9572 |
| | Flavia Jung, MD |
| | (504) 896-9238 |
| | Arlette Soros, MD |
| | (504) 896-9572 |

## 4. Purpose of Study

This is a research study to determine whether children who were born with a weight less than 5.5 pounds are more likely to have a higher risk for developing Type 2 diabetes than children born with a weight more than 5.5 pounds. Type 2 diabetes is a disease that individuals get when they become very overweight and their body is unable to properly use insulin. This study will also determine whether this risk is higher in African American children than in Caucasian children.

Four hundred healthy children ages 7 to 9 are expected to participate in this study. Researchers are studying African-American children and Caucasian children in particular. But children of all ethnic and racial backgrounds can participate.

## 5. Description of the Study

In this study, parents will be interviewed by phone to determine if their child is eligible for the study. If so, the parent/guardian will be asked to bring the child to the Clinical Trials Center at Children's Hospital in New Orleans and/or the Children's Hospital clinic in Baton Rouge and /or the Pennington Biomedical Research Center in Baton Rouge on two or three separate days so that the child may participate in testing. This study will not involve any long-term follow-up, long-range testing, or re-contact.

All the tests to which the child will be subjected are approved diagnostic procedures.

First day:

- Candidate screening session (about 1 ½ hours)
    - Medical and family history
    - Physical activity questionnaire (Include copy)
    - Nutrition questionnaire (Include copy)
- Physical examination
    - Height
    - Weight
    - Resting Heart Rate
    - Blood Pressure
    - Tanner Staging (sexual maturity staging)
- Medical Screening Blood Test (A)
- Physical Activity Accelerometer Measurement (B)

First or Second day:

- Magnetic Resonance Imaging (C)
    - Leg muscle testing
    - Liver and abdominal scan

Second or Third day:

- Resting metabolism measurement (D)
- Frequent Sampling Intravenous Glucose Tolerance Test (E)
- Body Composition (F)

**Description of Testing Procedures:**

In all tests that require the child to sit/lay quietly for a length of time, every effort will be made to keep the child comfortable and entertained. When appropriate, a non-violent cartoon video will be shown to the child, or a headset playing children's books-on-tape will be provided. Cushions and pillows will be used to ensure that the child is as comfortable as possible.

A.     Medical Screening Blood Test (about 20 minutes):

The medical screening blood test will be performed to ensure your child's eligibility for this study. The pediatric nurse will use a small needle to obtain a small portion of blood (1.5 mL) from your child's arm. The test will determine whether your child is healthy prior to participation in the other testing measures.

B.     Physical Activity Accelerometer Measurement (about 10 minutes):

The accelerometer is a small device that measures your child's activity level.  It is a device that is worn as a belt around their waist.  It should be worn at all times except while bathing, showering, or swimming.  The device should be worn for at least 3 days (72 hours), but no more than 7 days (168 hours).  Your child will be able to start wearing the accelerometer at the end of the first day of testing.  Arrangements will be made for you to bring back the accelerometer at the end of the testing period or for you to mail back the accelerometer.

C.      Magnetic Resonance Imaging (MRI, about 30 minutes):

The Magnetic Resonance Imaging (MRI) test will take pictures of your child's leg muscles, stomach, and liver so that we will be able to will determine how much fat is contained in each.  During the MRI leg muscle testing your child's right leg will be positioned inside a long tube and images will be taken of the leg muscle. Then, the child will be asked to lie on his/her stomach so that the same sort of images can be taken of his/her stomach area and liver.

D.      Resting Metabolism Measurement (about 30 minutes):

This test will measure your child's resting energy expenditure to help determine your child's resting metabolism (how many calories your child uses while resting).  Your child will lie quietly on a hospital bed and breathe normally toward a plastic hood, which will collect and analyze the air that the child exhales.

E.       Frequent Sampling Intravenous Glucose Tolerance Test (FSIGTT, about 3 hours):

This test will determine how your child's body handles sugar.  The pediatric nurse will supervise the testing and assure your child's safety and comfort.  A flexible needle (called an IV cannula) will be placed into the vein in each of your child's arms and taped down for the duration of the test.  Three baseline blood samples (1.1 mL) will be drawn through one of the needles.  From one of these samples, a small portion (about ½ teaspoon) of your child's blood will be saved and stored at -70 degrees Celsius in a freezer at Children's Hospital for future genetic analysis.  This blood sample will then be shipped to Pennington Biomedical Research Center for genetic analysis.  No additional blood will be drawn for this genetic analysis.

I give my permission for a sample of my child's blood to be saved and stored for future genetic analysis.

_____Yes        _____ No          _____ Initials

A sugar solution (25% dextrose) will then be injected into your child through the same needle.  Then, 20 minutes later, a small amount of insulin will be injected.

After the insulin is injected, additional blood samples (1.1 mL each, less than ½ a teaspoonful) will be drawn at short intervals for 180 minutes.  The needles will then be removed.  Before the blood samples are drawn, your child will be required to provide a small amount of urine in a cup to test for the presence of ketones.  Ketones are substances that are made when the body breaks down fat for energy.

F.  Body Composition (DEXA scan, about 20 minutes)

DEXA stands for dual energy x-ray absorptiometry. The DEXA machine will scan your child from head to toe as they lie perfectly still on their back. The DEXA machine is like an X-ray but it is much safer than having a tooth x-ray because the x-ray beam is much weaker. In fact, when your child flies in an airplane several hours or watches an hour of colored TV their body gets more radiation than doing this procedure. The DEXA scan will tells us how solid your child bones are and estimates how much muscle and fat your child has. The test is completely painless and takes about 10- 20 minutes.

**6.   Benefits to Subject**

There may be no benefits to the subject.  Information collected in this study may provide information to help determine whether your child has a risk for developing Type 2 diabetes or obesity.  The results of these tests can be provided to your child's primary care physician for follow-up if any abnormalities are detected.

7. **Risks to the Subject**

**a.**  Physical Activity Accelerometer Measurement:

There is no risk associated with this measurement other than possible discomfort the child may feel while wearing the accelerometer.

b.  Magnetic Resonance Imaging (MRI):

MRI scanning is a safe and routine medical procedure. Standard FDA-approved clinical protocols will be followed. The magnetic field and radio waves used for MRI scans are considered too weak to do any biological damage. No needles will be used, and no X-ray exposure will occur. However, your child should not participate in this study if your child has a cardiac (heart) pacemaker, neural (brain) pacemaker, cochlear or eye implants, metal fragments, or other sources of metal (such as surgical clips, bone pins, or metal iron filings) in or near the brain, eye, or blood vessels because the MRI could disrupt the function of these objects or cause them to move. This will be reviewed and decided for each subject. Localized heating of tissue may also occur near implanted metal objects (including some tattoos) or in the rare case of an improperly programmed MRI sequence or an equipment malfunction. Your child will be given an alarm button to press to enable him/her to immediately alert the MRI operator to stop the scan if any heating occurs.

Taking part in a laboratory experiment and being inside a scanner is an unusual experience.  Some people are also bothered by the noise of the scanner and become restless while they are being scanned. If your child is uncomfortable with closed-in places, your child may experience some anxiety (nervousness) during scanning because the walls of the MRI cylinder are close to the face. Usually, conversation and reassurance will make people less nervous. However, your child should not participate in the study if your child has been diagnosed with claustrophobia (fear of being in a confined space) by a doctor. Some people find the scanning environment to be chilly in temperature. Your child will be offered a sheet or light blanket to keep warm. Occasionally, a person may experience slight dizziness or other minor sensations during a MRI scan due to the magnet or the sounds that are part of the MRI process. If your child experiences discomfort during the study, you or your child can end the session. You or your child will be able to discontinue the scanning procedure at any time, if it becomes uncomfortable in any way.

c.  Resting Metabolism Measurement:

There is no risk associated with this test other than discomfort the child may feel while lying still.

d.  Frequent Sampling Intravenous Glucose Tolerance Test (FSIGTT):

During this test sugar (glucose) and insulin are given into the bloodstream through a vein. This test measures how glucose and insulin are used in the body.

Beforehand, EMLA cream is used to numb the skin where the needle is being placed. An IV needle is placed in a vein both arms, one side is used to deliver glucose and insulin. The other needle is used to draw blood during the test.  Your child may feel some discomfort when the needle is inserted into the vein. Some bleeding and/or bruising may occur at the site of the needle stick.   Rarely, after the test, an infection can occur later at the site where the needle has been.

When the glucose is flowing into the vein there may be feelings of pressure, coldness or burning at the vein for several moments. Once glucose is in the blood stream there may be feelings of tiredness, dizziness, headache, or upset stomach. These feelings usually disappear by themselves by the end of the test.

In the middle of the test a small amount of insulin is also given into the vein. Insulin lowers blood sugar (glucose). Sometimes lowering blood sugar levels may cause feelings of hunger, dizziness, sweating, tiredness, headache, irritability or confusion. Your child's blood glucose levels will be closely watched during the test. If blood glucose drops too low and/ or your child has very uncomfortable feelings, the test will be stopped and your child will be given food or medicine to raise the blood glucose level. Uncomfortable feelings from lowered blood glucose usually disappear once blood glucose levels are increased.

e. Body Composition (DEXA scan):

No discomfort will be felt with DEXA scan but the test will expose your child to a very small amount of radiation. The levels emitted during a whole body scan are typically less than 0.2 uSv, which in practical terms is much less than the amount received during a cross-country airplane trip or approximately equal to the amount your child would normally be exposed to while watching color television for one hour.

## 8. Alternatives to Participation in the Study

An alternative to participation in the study is not to participate. This study is not a treatment study and therefore will not provide treatment. You may choose to have tests for diabetes and other health issues done at the clinics or by your child's primary care physician.

## 9. Subject Removal

Subjects will be removed from the study if they fail to attend the scheduled visits.

## 10. Subject's Right to Refuse to Participate or Withdraw.

Your decision to participate in this research study is voluntary. You can decide not to participate or you can withdraw from the study at any time without penalty or loss of benefits or treatment to which you are entitled. Your choice will not affect your present or future medical care or treatment at Children's Hospital or LSUHSC. You will be informed of any significant new findings that become available during the study that may influence your willingness to continue in the study. If you or your child decide to withdraw from the study, you are asked to call Dr. Melinda Sothern at 504 568-3051.

**11. Subject's Right to Privacy**

The results of the study may be released to the National Institutes of Health and the Food and Drug Administration. The results of the study may be published. The privacy of the subjects will be protected and their names will not be used in any manner.

**12. Release of Information**

The medical records related to the study are available to both the National Institutes of Health and the Food and Drug Administration. Upon your request, we can release your child's medical records related to the study to your child's primary care physician.

**13. Financial Information**

    A.    Participation in this study will not result in any extra charges above and beyond those routinely incurred by patients with similar illnesses.

    B.    The costs of all drugs, visits, procedures and study related and unforeseen complications must be met by the subjects

    C.    Subject Payment

        i.    You will receive $75.00 as reimbursement for time and travel for your child's participation in each testing session for a total of $150.00 for the two testing sessions.

        ii.    Your child will be given recognition for his/her participation in the form of sports equipment or clothing at each testing session valued at approximately $10.00 for each testing session for a total of approximately $20.00 for the two testing sessions.

**14. Signatures**
The study has been discussed with me and all my questions have been answered. I have been informed that additional questions regarding the study should be directed to investigators listed on page 1 of this consent form. I have been informed that if I have questions about subjects' rights, or other concerns, I can contact the Chancellor of LSU Health Sciences Center, at (504) 568-4801 or Dr. Druby Hebert, Chairman of the Children's Hospital IRB (504) 899-9511. I agree with the terms above, acknowledge I have been given a copy of the consent form and agree to participate in this study. I have been informed that I have not waived any of my legal rights by signing this form.

_____     _____
Signature of Subject                                              Date

_____     _____
Signature of Witness                                             Date
The study subject has indicated to me that the subject is unable to read.  I certify that I have read this consent form to the subject and explained that by completing the signature line above the subject has agreed to participate.

_____     _____
Signature of Reader                                              Date
**Child Assent (if study subject is seven years old or older)**

I have been told that I am being asked to take some tests for a research study.  I have been told that I will have to lie still for a little while.  I have been told that a nurse will put a needle in my arm to take blood for one of the tests and that might hurt a little bit, but it won't hurt for long.

_____     _____     _____
Child's Name and Age                Child's Signature                      Date

Reason for not obtaining child assent:
_____

_____
The study subject is a child and I certify that I am his/her legal guardian.

_____     _____     _____
Legal Guardian Name               Legal Guardian Signature              Date

_____     _____
Signature of Person Administering                      Date
Consent

_____     _____
Signature of Principal Investigator                      Date

**LOUISIANA STATE UNIVERSITY HEALTH SCIENCES CENTER AND CHILDREN'S HOSPITAL**
**NEW ORLEANS**

**INSTITUTIONAL REVIEW BOARD/ADMINISTRATIVE REVIEW COMMITTEE**

- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

**AUTHORIZATION FOR USE AND DISCLOSURE OF PROTECTED HEALTH INFORMATION FOR RESEARCH PURPOSES**

**Name of Research Project:**  Mechanisms for the Metabolic Syndrome in Prepubertal African American and Caucasian Youth

 **Sponsor Name and Protocol Number if applicable:**  NIH: 1 R01 HD49046

**Principal Investigator:**  Melinda S. Sothern, PhD          **IRB/ARC Number:** 6297

I hereby request and authorize the LSUHSC-New Orleans and Children's Hospital to use and disclose and protected health information from the record(s) of:

**Patient's Name/Address:**  _____

**Birth Date:** _____          **Social Security or CPI Number:** _____

Specifically, I request and authorize any part of my health information relevant to the research project, identified above and in the Informed Consent document, to be used and/or disclosed to or by the Principal Investigator identified above or his/her designee, in connection with the research project.  I understand that this may include information relating to:  Human Immunodeficiency Virus ("HIV") infection or Acquired Immunodeficiency Syndrome ("AIDS"); treatment for or history of drug or alcohol abuse; and/or mental or behavioral health or psychiatric care.

I understand that copies of the records indicated above will be:

- Used by employees of LSUHSC-New Orleans and/or Children's Hospital including treatment providers, and/or other members of its workforce.

- Disclosed by LSUHSC-New Orleans and/or Children's Hospital to government officials or government agencies, study sponsors, study monitors, or others responsible for oversight of the research project.

- Sent to collaborating researchers outside LSUHSC-New Orleans and/or Children's Hospital if and to the extent indicated in the attached Informed Consent document(s).

I understand that by signing this form, I am allowing Children's Hospital, LSUHSC-New Orleans and their researchers to use or disclose my health information in connection with the

attached Informed Consent and for the purpose of the research that is described in the Informed Consent. For example, the researchers may need the information to verify that I am eligible to participate in the study, or to monitor the results, including expected or unexpected side effects or outcomes. Other University, hospital and government officials, safety monitors, and study sponsors may need the information to ensure that the study is conducted properly. Also, I understand that my health information may be disclosed to insurance companies or others responsible for my medical bills in order to secure payment.

I understand that any privacy rights not specifically mentioned in this Authorization are contained in the Notice of Privacy Practices that I received or will receive from the Principal Investigator or at the facility that I attend.

I understand that I may revoke this authorization at any time, (except to the extent that LSUHSC-New Orleans and/or Children's Hospital has already relied on the authorization), by sending or transmitting a facsimile of a written notice to the contact person listed in the attached Informed Consent document(s).

I understand that if my information already has been included in a research database or registry as described in the attached Informed Consent document(s), LSUHSC-New Orleans and Children's Hospital considers themselves to have relied on it, and therefore my information will not be removed from those repositories, unless I request that it be removed. Unless otherwise revoked, I understand that this authorization will not expire during the length of the research study. I understand that if I do not sign this form, I will not be able to participate in the above research study or receive the study-related interventions, but that LSUHSC-New Orleans or Children's Hospital cannot otherwise condition treatment on my signing this form.

While the research study is in progress, my right to access any research records or results that are maintained by the facility in the designated record set, may be suspended until the research study is over. If my access is denied, I understand that it will be reinstated at the end of the research study.

I understand the information disclosed by this authorization may be subject to re-disclosure by the recipient and no longer be protected by the Health Insurance Portability and Accountability Act. The LSUHSC facility, Children's Hospital, their employees, officers, and physicians are hereby released from any legal responsibility or liability for disclosure of the above information to the extent indicated and authorized herein.

I UNDERSTAND THAT THIS AUTHORIZATION SUPERSEDES ANY CONTRARY INFORMATION IN ANY OTHER DOCUMENTS I HAVE SIGNED RELATED TO THE ATTACHED STUDY.

Signature of Subject or Subject's Legal Representative: _____ Date: _____

Printed Name of Legal Representative (*if any*):

_____

Representative's Authority to Act for Subject (*e.g., relationship to patient*):
_____

Verification of Representative's Authority:  (  ) viewed driver's license    (  ) viewed Power of Attorney

(  ) viewed other _____ (specify)

Subject Initials/Study #:_____

<center>**SILLY Study**</center>

<center>**"Insulin Sensitivity in Prepubertal African American and Caucasian Youth"**</center>

<center>HISTORY AND PHYSICAL EXAMINATION</center>

<center>**Historical Data**</center>

*Health history of subject:*

Birth Weight_____                    Birth Length_____

Head Circumference:_____ If obese, age at onset_____

Term of Pregnancy_____

Problems during delivery and/or prenatal and childbirth_____

Family members who are overweight_____

List attempts at weight reduction:_____

List any present or past serious illness:_____

List any hospitalization:_____

Gynecological History/Sexual History

1.  Menarche:_____

2.  Last menstrual period_____

3.      Breast development:     Yes_____     No_____

        Age of onset:           _____Yrs.

        Pubic hair:             Yes_____     No_____

        Age of onset:           _____Yrs.

        Birth control:          Yes_____     No_____

        Birth control method:_____

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Medications:        Yes, Describe_____                No_____

Nonprescription Drugs: Yes, Name and/or describe _____

        No_____

Allergy:        Yes_____    No_____

        Food:_____

        Medications:_____

        Other:_____

## B.  Dietary History

    **1.**    Participant

    **2.**     Family

Problem foods:_____

Problem Places:_____

Relative's:homes_____friends_____TV_____

**Dietary History: Food preparation**

% Fried_____  % Broiled _____%Boiled_____

%Baked_____    %Grilled _____%Other_____

List the Common Meats Eaten in Your Family: _____

Indicate with or without skin:_____

Types of fat-cooking:

Vegetable oil _____ Margarine_____ Butter_____ Lard_____ Other_____

### C. Physical Athletic History

**1.** Participant

**2.** Family

Athletic activities:_____ None_____ Walk_____ Ride bike_____

Skating (Ice and/or roller)_____ Swim _____ Run _____ Jump

Rope_____ Dance_____ Other_____

The amount of time each session:

3times/week_____2times/week_____Other____>20min_____<20 min _____

### D. Psychosocial History

**1 .** Participant

**2.** Family

### SOCIAL HABITS:

Smoking or tobacco:     Yes          No

How many pack(s) per day?_____

Drinking:       Yes          No          How long?_____

Which type:     Beer             Wine          Liquor

Has the subject ever been diagnosed with any of the following?

|  |  |  |
|---|---|---|
| Depression | Yes___ | No___ |
| Personality Disorder Yes___ | No___ | |
| Anxiety | Yes___ | No___ |
| Attention Deficit Disorder | Yes___ | No___ |
| Other (Describe) | Yes___ | No___ |

Has the subject been prescribed medication for any of the above?
If yes, describe._____

What is the combined annual household income for your family? $_____

How many years of education have you completed?_____

Your spouse?_____

| PHYSICAL EXAMINATION | WNL ← | PF↑ |
|---|---|---|
| *GENERAL APPEARANCE* | | |
| **SKIN, HAIR, NAILS** | | |
| **LYMPH NODES** | | |
| **HEAD, FACE, EARS, EYES, NOSE, THROAT, MOUTH** | | |
| **NECK/ THYROID** | | |
| **CHEST, BREAST, LUNGS**<br>**TANNER  STAGE:** | | |
| **HEART** | | |
| **ABDOMEN** | | |
| **GENITALIA TANNER STAGE: (if applicable)**<br>**Pubic Hair- TANNER STAGE:** | | |
| **ADDITIONAL DESCRIPTION OF POSITIVE FINDINGS:** | | |

**Physician Signature: _____      Date: _____**

**←WNL = within normal limits**          **↑PF = positive finding**

**Signature**_____ **Date:** _____

**Additional Information for Mother**

1.  Have you ever used tobacco products?  __**Yes**     __**No**

2.  Did you smoke or use tobacco while pregnant for the child participating in the study?

     __**Yes**  __**No**  If yes, what type/amount?_____

3.  Do you consume alcohol?  __**Yes**  __**No**

          If yes, what type:_____          Amount (per week):_____

4.  Did you drink alcohol while pregnant for the child participating in this study?

          __**Yes** __**No**  If yes, what type_____ Amount per week_____

5. Did you take any medication while pregnant for the child enrolled in this study?

     __**Yes**  __**No**  If yes, describe._____

6.  Did you breast-feed the child participating in this study?  __**Yes** __**No**

          If yes, answer questions 7 – 10.  If no, skip these questions.

7.  How long did you breastfeed the child participating in this study?_____

8. Did you smoke or use tobacco while breastfeeding the child participating in this study?

     __**Yes**  __**No**  If yes, what type/amount?_____

9.  Did you consume alcohol while breastfeeding the child in this study?

     __**Yes**  __**No**  If yes, what type:_____Amount (per week):_____

10.  Did you take any medication while breastfeeding the child participating in this study?

     __**Yes**  __**No**  If yes, describe._____

11. Does anyone in your household smoke?          __**Yes** __**No**

12. Did anyone smoke in the home during your pregnancy with this child?  __**Yes** __**No**

13. Did anyone smoke in the home during your child's infancy?     __**Yes** __**No**

14. Did anyone smoke in the home during your child's preschool years?     __Yes __No


15.  Did you have a Cesarean Section for the child enrolled in this study? __Yes __No

16.  Did you receive any pain medication prior to, during or after delivering the child enrolled in this study? If so, list all medications and how long you were on this medication:
_____
17.  Did you receive any other medication prior to, during or after delivering the child enrolled in the study? If so, list all medications and how long you were on this medication:

_____

18.  Did the child to be enrolled in this study experience any of the following after birth during the first 3 months of life (check all that apply):
___ jaundice
___ infection that required medication, if so, list medications received:_____
___ other medical problems, please list_____

19.  Please check all vaccinations that your child has received since birth:
___ Hepatitis B

___ Rotavirus

___ Diphteria, Tetanus, Pertussis

___ Haemophilus influenzae type b

___ Pneumococcal

___ Inactivated Poliovirus

___ Influenza

___ Measles, mumps, rubella (MMR series)

___ Varicella

___ Hepatitis A

___ Meningococcal

___ Human Papillomavirus

Other:_____


20. What is your current weight? _____  height?_____


21. What is the most you have weighed (non-pregnant)? _____lbs.  when?_____

22. What was your highest weight while you were pregnant for the child participating in

     this study? _____

23. Are you currently employed by LSU or have you been employed by LSU in the last 2 years?

                **Yes**  **No**  If yes, List college: _____List division:_____

**I have completed the above questions to the best of my knowledge and understand that this information will be used to ensure my child's safety should he or she be accepted into the study.**

_____ **Signature** _____**Date**

**Godin Leisure-Time Exercise Questionnaire**

1. During a typical **7-Day period** (a week), how many times on the average do you do the following kinds of exercise for **more than 15 minutes** during your free time (write on each line the appropriate number).

*Times Per Week*

a) **STRENUOUS EXERCISE**
   **(HEART BEATS RAPIDLY)**                                    _____

   (e.g., running, jogging, hockey, football, soccer,
   squash, basketball, cross country skiing, judo,
   roller skating, vigorous swimming,
    vigorous long distance bicycling)

b) **MODERATE EXERCISE**
   **(NOT EXHAUSTING)**                                         _____

   (e.g., fast walking, baseball, tennis, easy bicycling,
   volleyball, badminton, easy swimming, alpine skiing,
   popular and folk dancing)

c) **MILD EXERCISE**
   **(MINIMAL EFFORT)**                                         _____

   (e.g., yoga, archery, fishing from river bank, bowling,
   horseshoes, golf, snow-mobiling, easy walking)

2. During a typical **7-Day period** (a week), in your leisure time, how often do you engage in any regular activity **long enough to work up a sweat** (heart beats rapidly)?

| OFTEN | SOMETIMES | NEVER/RARELY |
|-------|-----------|--------------|
| 1. ☐ | 2. ☐ | 3. ☐ |

Example of general linear model
Race, birth weight, and breastfeeding with the dependent variable of RQ

Full model:
$\beta_0 + \beta_1 race + \beta_{2,1} bf_1 + \beta_{2,2} + bf_2 + \beta_3 bwt + \beta_4 RQ + \beta_{5,1} race * bf_1 + \beta_{5,2} race * bf_2 + \beta_6 race * bwt + \beta_7 race * RQ + \beta_{8,1} bf_1 * RQ + \beta_{8,2} bf_2 * RQ + \beta_{9,1} bf_1 * bwt + \beta_{9,2} bf_2 * bwt + \beta_{10} bwt * RQ + \beta_{11,1} race * bf_1 * bwt + \beta_{11,2} race * bf_2 * bwt + \beta_{12,1} race * bf_1 * RQ + \beta_{12,2} race * bf_2 * RQ + \beta_{13,1} bf_1 * bwt * RQ + \beta_{13,2} bf_2 * bwt * RQ + E$

Restricted model:
$\beta_0 + \beta_1 race + \beta_{2,1} bf_1 + \beta_{2,2} + bf_2 + \beta3,1 race * bf1 + \beta3,2 race * bf2 + \beta4 race * bwt + \beta5,1 bf1 * bwt + \beta5,2 bf2 * bwt + \beta6,1 race * bf1 * bwt + \beta6,2 race * bf2 * bwt + E$

Run both models then

F = [(SSrestricted – SSfull) / (dfrestricted – dffull)] / Mean square from full model

p value calculated from F (dfrestricted – dffull, dffull)

**Vita**

Connie VanVrancken Tompkins was born on October 11, 1976, in Metairie, Louisiana. She is a graduate of Archbishop Chapelle High School, Metairie, Louisiana. She graduated from the University of New Orleans on May 14, 1999, with a Bachelor of Science degree in Human Performance and Health Promotion and on December 15, 2000, with a Master of Arts degree in Human Performance and Health Promotion. She will graduate from the University of New Orleans with a Doctor of Philosophy degree in Human Performance and Health Promotion on May 17, 2008.