Abstract
Substrate Utilization During Submaximal Exercise in Children with a Severely Obese Mother and Response to Exercise Training
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The aim of this study was to compare fat metabolism during submaximal exercise in children with a severely obese mother (BMI ≥ 35 kg/m²) to children with a lean, non-obese mother (BMI ≤ 30 kg/m²). All participants (n=15) completed two maximal exercise tests as well as a submaximal exercise test at a relative (65%VO₂Max) and absolute (15W) workload and a respiratory exchange ratio (RER) was measured via indirect calorimetry. After initial testing, subjects performed an exercise intervention consisting of three hour-long visits a week for four weeks. There were no differences in RER between groups prior to the exercise intervention during submaximal exercise, however, there was a significant time effect following the exercise intervention. Regardless of maternal obesity status, both groups had a significant (p<0.05) increase in fat utilization at the relative workload of 65%VO₂Max following the exercise intervention. This finding is supportive of earlier data that is indicative of exercise being able to treat and correct decrements in FAO. However, there was not enough evidence in the current study to suggest that there is a genetic or epigenetic component responsible for the reduction in FAO in children with a severely obese mother.
Substrate Utilization During Submaximal Exercise in Children with a Severely Obese Mother and Response to Exercise Training

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Chapter I: Introduction

Obesity is one of the most widespread and severe health concerns facing the world today (6). In 2000-2005, self-reported BMI over 30 increased by 24%, while self-reported BMI over 40 increased by 50%, and BMI over 50 increased by 75% (13). The results of this study suggest that severe obesity is increasing at a much faster rate than moderate obesity and puts more of an emphasis on the health concerns that coincide with severe obesity (13).

Much like adult obesity, childhood obesity is increasing as well (3,12). In 2012, more than one-third of the children and adolescents in the United States were overweight or obese (3). One study revealed that 70% of a sample of children and adolescents 5-17 years of age already had one positive risk factor for heart disease (3). Much like adult obesity, childhood obesity has immediate and long-term effects associated with the disease including type 2 diabetes, hypertension, stroke, certain cancers, psychological and social problems, and excess mortality (3,12,13).

Being that adult and childhood obesity are both skyrocketing collectively, research is being done to investigate the possibility of an inherent component. Whitaker et al. explored the likelihood of a genetic component or predisposition that would increase the probability of a child with obese parents becoming obese in young adulthood. It was found that childhood obesity is a significant predictor of adulthood obesity, regardless if the child has obese parents or not; however, the probability of a child becoming obese more than doubles if parental obesity is present. These results suggest that while an environmental component is present, there is also a genetic susceptibility factor as well linking childhood obesity and adult obesity (16).
While there are different evidence-based views of why there is such a massive rise in the prevalence of severe obesity, lipid oxidation is one of the many mechanisms being investigated to explain this increase. Research has shown that there is a reduction in lipid oxidation levels in obese individuals, suggesting that a defect in the oxidation of lipids can lead to weight gain (10,11,14).

Researchers are interested to see if the defect in lipid oxidation seen in the obese is also seen in vitro and whether the phenotype is preserved after massive weight loss. Analyses such as these allow researchers to determine if massive amounts of weight loss, such as gastric bypass surgery, can change the inherent nature of the cell itself, or if the original traits are preserved, and therefore potentially passed on to their children. Thyfault et al. (14) found a significant decrease in the percentage of fat oxidation in extremely obese and weight-reduced women when compared to lean controls, and also that the same decrement in free fatty oxidation is found in vitro skeletal muscle incubations as well as in vivo tracer methodology. These findings are indicative of weight-reduced and obese women possessing similar inherent attributes, and that the development of extreme obesity could be related to a degraded ability to oxidize plasma fatty acids (14).

Clinical interventions and their effect on fat oxidation were another subject of investigation. Berggren et al. (2) studied the effects of clinical interventions on lipid oxidation by exercising women for one hour per day over ten consecutive days and found that the defect in lipid oxidation in obese individuals which persists after massive weight loss can be improved and corrected with exercise training. Another study by Eaves et al. studied the effects of an exercise program on substrate utilization in
children with a familial disposition for obesity, and it was found that the children with a severely obese parent experienced an increase in respiratory exchange ratio (RER), implying a decreased reliance on lipid oxidation, when compared to children with 2 non-obese parents. This data supports previous results that are suggestive of a genetic component that enhances a reduction in lipid oxidation in adults with severe obesity (4).

However, this particular study only examined the effect of exercise training on 3 children (4); the purpose of the proposed study is to expand the number of subjects in order to determine if exercise training is truly an effective intervention.

**Purpose**

The purpose of the present study was to continue investigating the effects of an exercise program on substrate utilization in children with lean, non-obese parents versus at least one severely obese parent. We will be utilizing a study design from previous work that also investigated substrate utilization during submaximal exercise in children with a severely obese parent (4).

The main outcome measure of the study will be RER measured by indirect calorimetry after a four-week exercise program. Prior to the exercise intervention, each participant will undergo 2 maximal cycle ergometer exercise tests as well as an submaximal cycle ergometer exercise test at similar relative and absolute intensities to account for any discrepancies in cardiorespiratory fitness. Following pre-testing, the subjects will participate in a four-week endurance based exercise program aimed at increasing lipid oxidation levels. The children will exercise three times a week under the supervision of an exercise mentor and activities will consist of walking on a treadmill, elliptical, stationary cycling, and other recreational activities. After four weeks of
exercising, the children will have another maximal and submaximal cycle ergometer test, and height and weight measurements.

**Hypothesis**

It is hypothesized that, compared to the lean parent control group, children with a severely obese parent will exhibit a decrement in lipid oxidation, and that impairment can be corrected with four weeks of exercise.

**Delimitations**

- The study will be limited to prepubescent boys and girls ages 8-12
- Participants will be excluded if are on any medication that would alter their metabolism, ability to exercise or put them at risk when exercising.
- Children will also be excluded if they are regularly involved in sports or other recreational activities (i.e. endurance based sports).

**Limitations**

- Analysis is limited to the accuracy of the metabolic cart and DEXA scanner.
- Fatigue may occur during pre and post testing due to low levels of cardiorespiratory fitness.

**Assumptions**

- The equipment used for maximal and submaximal testing will not interfere with participant performance
• Indirect calorimetry is an appropriate and accurate way of measuring respiratory exchange ratios.

• Participants will be compliant to study guidelines and expectations.

Operational Definitions

• Maximal Exercise Testing - an exercise stress test in which subjects increase their heart rate during exercise to 80% to 90% of the maximal rate, which is estimated from each subject's age and sex.

• Submaximal Exercise Testing – a measurement to determine the heart rate response to one or more submaximal work rates (i.e. relative or absolute workloads)

• Indirect Calorimetry- the measurement of the amount of heat generated in an oxidation reaction by determining the intake or consumption of oxygen or by measuring the amount of carbon dioxide released and translating these quantities into a heat equivalent

• Respiratory Exchange Ratio - the ratio of the amount of carbon dioxide produced to the amount of oxygen consumed or taken up

• Lipid Oxidation – Biological process of burning of fat to create and release energy to the rest of the body

• Cardiorespiratory Fitness - ability of the circulatory and respiratory systems to supply oxygen to skeletal muscles during sustained physical activity.
Chapter II: Literature Review

Obesity Trends

Obesity is one of the most prevalent and serious health concerns in the United States (6). In 2000-2005, self-reported BMI over 30 increased by 24%, while self-reported BMI over 40 increased by 50%, and BMI over 50 increased by 75% (13). This finding suggests that severe obesity is progressing at a much faster rate than moderate obesity, causing a significant shift in weight distribution among populations (13). With morbid obesity increasing at such a rapid rate, there has been an increase of health concerns that are associated with obesity. Type II diabetes, hypertension, dyslipidemia, stroke, heart disease, arthritis, and certain cancers are all linked to obesity (6). Higher levels of obesity are also associated with certain cancers, and excess mortality, which is mostly due in part to cardiovascular disease (6).

Obesity and Genetics

Not only is obesity affecting adults, it is affecting children and adolescents as well (3,12). The percentage of children, ages 6-11, who were obese increased from 7% in 1980 to almost 18% in 2012 (3). Likewise, the percentage of obese adolescents, ages 12-19, increased from 5% to 21% in the same amount of time (3). Much like adult obesity, childhood obesity has immediate and long-term health effects and concerns. In a sample of 5-17 year olds, 70% were already positive for at least one risk factor for cardiovascular disease (3).

With adult and childhood obesity on the rise, researches have explored the possibility of obesity being genetic (7, 8, 9, 15, 16). Whitaker et al. investigated the risk
of obesity in young adulthood associated with both childhood obesity and obesity in one or both parents (16). To do this, they followed close to 900 subjects born between the years of 1965 and 1971 until they were 21 years old and reviewed their parents’ medical history (16). Using height and weight measurements, they tracked the children’s obesity rates by BMI. It was found that the probability of becoming obese increases with the age of the obese child, regardless if the parents are obese or not. However, it was also found that parental obesity more than doubles the risk of adult obesity among obese and non-obese children less than 10 years of age (16).

Treuth, Butte, and Sorkin had similar findings when exploring the causal factors underlying the development of obesity in children. They studied young girls with lean and obese parents and measured their height, weight, and body composition one and two years after the baseline assessment (15). They determined that non-obese girls with two obese parents had a high risk of developing obesity, having a low free-living total energy expenditure, and low muscle oxidative capacity, all of which predict high rates of fat gain (15). In a review, Herrera and Lindgren concluded that the development of obesity has an obvious environmental contribution, but a genetic susceptibility component is also present (9). They found that a similarity for obesity decreases correspondingly with the degree of relatedness, which points to an inherent component in obesity susceptibility (9). Likewise, when investigating adoptees, the BMI of the adopted individual was closer correlated with the biological parent’s BMI versus the adoptive parents’, further implicating the presence of a genetic factor (9).
Lipid Oxidation and Obesity

One of the mechanisms being investigated to try and help explain the current increase in severe obesity is fatty acid oxidation (FAO). Fat oxidation is the metabolic pathway that breaks down stored fats to create and release energy to cells, which primarily takes place in the mitochondria of skeletal muscle cells. The majority of that released energy comes from fatty acid chains stored in adipose tissue. Our bodies are constantly metabolizing fats and other molecules to create energy to sustain life and homeostasis.

Researchers have found that FAO is reduced in obese individuals (10,11,14). Kim et al. found a significant negative relationship between adiposity and fat oxidation (11). There was also a 50% reduction in CO$_2$ production in muscle from fat, which is indicative of an increased reliance on carbohydrate oxidation versus lipid oxidation (11). Relying on the oxidation of carbohydrates can be predictive of obesity and can also prompt previously obese individuals to store fats instead of metabolizing them (11).

When Hulver et al. studied skeletal muscle fatty acid oxidation in individuals of varying weights it was found that palmitate oxidation was 58% and 83% lower in skeletal muscle from extremely obese individuals when compared to normal-weight and overweight/obese individuals (10). Long-chain fatty acyl CoA content was also found to be high in both overweight/obese and morbidly obese patients when compared to their normal-weight counterparts even though FAO was found to be decreased only in extremely obese individuals (10). These findings imply that severe obesity may be due to genetic defects in skeletal muscle lipid metabolism (10).
Another way of assessing FAO and muscle metabolism is through a twenty-four-hour respiratory quotient (RQ). This type of test represents the ratio of carbon dioxide exhaled versus the amount of oxygen consumed by the individual and helps interpret energy expenditure and substrate utilization. Zurlo et al. measured RQ in non-diabetic Pima Indians that were fed a weight-maintenance diet (5). The RQ values varied from 0.799 to 0.903; about 18% of that variance was attributed to prior changes in body weight, sex, 24-hour energy balance, and percent body fat (5). In subgroup of 66 siblings from 28 families, family membership explained 28% of the 24-h RQ remaining variance (5). When follow up data was obtained from the participants, those with a higher 24-h RQ were associated with subsequent increases in body weight and fat mass (5). Subjects with a higher 24-h RQ were at a 2.5 higher risk of gaining at least 5kg of body weight when compared with those with a lower 24-h RQ (5). Zurlo et al. concluded that family membership is the principal determinant of the ratio of fat to carbohydrate oxidation and that a low ratio of fat to carbohydrate oxidation is associated with subsequent weight gain (5).

As previously stated, several observations suggest that muscle lipid oxidation is reduced with obesity. While the exact cellular mechanisms responsible are not apparent, one contender is the reduction in the activity of carnitine palmitoyltransferase (CPT-1), which is an enzyme that regulates the transport of long-chain fatty acids across mitochondrial membranes (11). Kim et al. measured CPT-1 levels to determine if it played a role in the reduction in lipid oxidation in obese human skeletal muscle. It was found that lipid oxidation and CPT-1 activity in the vastus lateralis were depressed with obesity (11). This was reinforced by the negative correlation between muscle CPT-1
activity and BMI (11). It was concluded that fat oxidation was depressed at the mitochondrial level in obese individuals, as the rate of oxidation is dependent on transport across the membranes via CPT-1 (11). The reduction in CPT-1 activity is significant due to the fact that it appears to remain even after weight loss, further insinuating a genetic component driving the obesity epidemic.

The recent research done on lipid oxidation suggests that FAO impairment is associated with obesity and can lead to a positive fat balance in the development of obesity (5). FAO is reduced at the whole body level, specifically in skeletal muscle, in the severely obese, suggesting that those with lower a rate of fat oxidation are prone to weight gain (4).

Fatty Acid Oxidation and Obesity: The Influence of Weight Loss and Exercise

As previously stated, obesity can be associated with a decrement in the ability to oxidize lipids from skeletal muscles (10,11,14). Researchers have become interested in investigating the effects of weight loss and exercise on FAO in obese, severely obese, and previously severely obese individuals. Guesbeck et al. studied severely obese women after weight loss surgery and compared them with their weight-matched controls to establish if there was a difference in substrate utilization between the two groups during fasting and submaximal exercise (8). VO$_{2\text{max}}$, relative to body mass, was significantly lower in the weight loss group, despite there being no difference in body mass (8). Respiratory exchange ratio (RER) was higher in the weight loss group during exercise at the same workload when compared to the control group which reflects a
lower fat utilization in the weight loss group (8). This defect in substrate utilization could lead to increased fat mass deposition, especially during positive energy balance (8).

While it is documented that severely obese individuals exhibit a reduction in lipid oxidation compared to lean controls, it is not known whether this effect is also seen in vivo and whether the phenotype is preserved after massive weight loss. Thyfault et al. published a study that compared free fatty acid oxidation during rest and exercise in female subjects that were lean (BMI <23), severely obese (BMI> 41), or post-gastric bypass patients that had lost greater than 45kg (14). By using tracer methodology and indirect calorimetry to measure the percentage of plasma FFA uptake, it was found that there is a significant decrease in the percent of uptake oxidized in the extremely obese and weight-reduced subjects when compared to the lean controls (14).

The research team went on to compare data from the current study with data from a previous study to demonstrate that a similar decrement in FFA oxidation is found both in in-vitro skeletal muscle incubations as well as in- vivo using tracer methodology (14). As previously stated, it has been shown that previously severely obese women have reduced lipid oxidation during exercise compared to healthy women with the same BMI (10,14). Therefore, the weight-reduced group seems to possess different attributes than the lean subjects with a similar BMI. These results suggest that the development of severe obesity could be related to a reduced ability to oxidize plasma fatty acids (14).

Berggren et al. studied the effects of clinical interventions (i.e. weight loss via surgery or exercise training) on FAO, and whether or not the defect could be reversed (1). It was found that there was no difference in FAO between the severely obese and the weight loss group, and FAO was decreased when compared to the lean subjects.
(1). FAO did not improve following a year of weight loss for those who underwent weight loss surgery, suggesting that the cause of excess lipid storage was independent from weight loss itself and was more genetic in nature (1).

It was found, however, that after ten consecutive days of exercise training, FAO increased significantly in the skeletal muscle of lean, obese, and previously obese participants (1). These data suggest that the defect in the ability to oxidize lipid in skeletal muscle that is seen in the obese and persists after massive weight loss, can be improved, and even corrected, with exercise (1).

Fatty Acid Oxidation and Exercise: What Happens in Children?

With the documentation of FAO impairments in severe obesity, researchers have begun to investigate the possibility of a genetic influence from one generation to the next. Eaves et al. studied children, ages 8-12, with a severely obese parent, or two lean/non-obese parents to determine if a reduction in FAO is evident in the children with an obese parent and if exercise training could remedy the ailment (4). At a low workload (15 watts) RER was significantly lower in the lean parent (LP) group versus the obese parent (OP) group with no significant differences in body composition, fitness level, age, or race between the 2 groups (4). This suggests a reduced dependence on FAO for energy production in the OP group (4). When the children exercise at a higher intensity, however, there were no differences in substrate consumption between the two groups (4). After exercise training, a very small number of subjects (N=3 per group), displayed a trend of decreasing RER, implying an increased utilization on FAO,
regardless of familial disposition. The findings of this study suggest that a FAO impairment does have an inherent component that may be overcome with exercise (4).

Summary

Due to the amount of research on fatty acid oxidation and obesity, it is apparent that FAO impairment is affiliated with severe obesity and can be improved and corrected with exercise training. This evidence opens the door for more research to be done to evaluate the effects of clinical interventions on lipid oxidation impairment in children and adolescents.
Chapter III: Methods

Experimental Design and Research Subjects

The purpose of this study was to compare substrate utilization during submaximal exercise in children, ages 8-12, with a severely obese mother (BMI ≥35 kg/m²) versus children with a lean, non-obese mother (BMI <35 kg/m²). The children were examined after a 4-week endurance based exercise program to see if substrate utilization was altered.

Children with a severely obese mother were recruited by contacting individuals who were contemplating, or had weight-loss surgery and by contacting children who had previously participated in other studies. Children with lean, non-obese parents were recruited by flyers and newspaper advertisements.

Exclusion criteria included children on any medication or affected by any medical condition that would alter metabolism, limit ability to exercise, or put them at risk for exercise. Children were also excluded from the study if they regularly participated in sports or physical activity or if they were past stage 3 on the Tanner scale.

Subject Characteristics

Initial screening involved at least one parent and the child. Questionnaires were used to determine Tanner stage for the children. The children and parents’ physical activity patterns and health history were also documented. Height and weight were assessed for each child and mother. Each child was made familiar with the exercise and testing equipment and was given an accelerometer to assess baseline activity levels in the participants.
Maximal Exercise Test

A cycle ergometer was used for the maximal exercise test, and oxygen consumption was measured using indirect calorimetry. The maximal test protocol was based off of work by Arngrimsson, Sveinsson, and Johannsson, which examined 9 and 15 year old adolescents. Initial workload and the stepwise increments were 20 W if the child’s body mass was less than or equal to 30kg, or 25 W if body mass was above 30kg. Workload was increased every third minute until voluntary exhaustion or a pedal rate of 60 rpm could not be sustained. The results of the maximal exercise test were used to determine the intensity of the submaximal exercise test.

Submaximal Exercise Test

The submaximal test was also done on a cycle ergometer, and the protocol was based upon a previous study that also studied substrate utilization with severe obesity. Participants exercised for 10 minutes at an identical absolute workload (15W) as well as at a relative workload (65% of VO2peak) in order to account for possible differences in cardiovascular fitness between the groups. A 5-minute break was given in between the 2 workloads if requested by the participant. The specific workloads were chosen due to the fact that they were easy to maintain and relied on a variety of substrates for energy production. Likewise, the 10 minute bouts of exercise were used to exhibit a steady-state response, which allowed for a more accurate depiction of substrate utilization.

Exercise Training
All participants partook in a four week exercise intervention that consisted of supervised physical activity that was aimed at increasing lipid oxidation during moderate intensity exercise. Training was three times a week at 65% of VO\textsubscript{2peak} for 60 minutes. All exercise sessions were supervised and each session began with a warm-up of stretching and walking, followed by 30 minutes on an exercise machine (i.e. walking on a treadmill, stationary cycling, or the elliptical) and finish up with other recreational activities. After four weeks of training participants underwent another submaximal and maximal exercise test and height and weight were be measured.

**Statistical Analysis**

Data were compared between the lean/non-obese and obese parent groups before and after training with a between groups and repeated measures ANOVA, respectively. Statistical analyses were completed using SPSS 16.0 (SPSS Inc., Chicago, IL) and significant differences accepted at $P \leq 0.05$. Values are expressed as mean ± the standard error (SE).
Chapter IV: Results

Maternal Characteristics

Participants were divided into 2 groups based on their corresponding maternal characteristics (lean/non-obese mother or obese mother) which are presented in Table 1. There were no significant differences between the two groups in age or stature, while the obese mother group, understandably, was significantly heavier and had a higher BMI than the lean/non-obese mother group. Body mass prior to gastric weight loss surgery was used for the mothers in the obese group if applicable.

Table 1 Maternal Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Lean Parent</th>
<th>Obese Parent</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>38.7 ± 1.8</td>
<td>39.8 ± 2.3</td>
<td>.693</td>
</tr>
<tr>
<td>Mass, kg</td>
<td>71.4 ± 5.3</td>
<td>105.6 ± 9.9</td>
<td>.012</td>
</tr>
<tr>
<td>Height, cm</td>
<td>171.8 ± 1.8</td>
<td>161.8 ± 5.3</td>
<td>.113</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.2 ± 1.7</td>
<td>39.6 ± 1.9</td>
<td>.000</td>
</tr>
</tbody>
</table>

Characteristics of the mothers of the participants. Pre-bariatric surgery characteristics were used in the obese mothers when applicable. Mean ±SE. *P≤0.05 for a difference between groups.

Subject Characteristics

As presented in Table 2, there were no differences in age, stature, or Tanner stage between the 2 groups. The gender distribution was as follows, 10 males and 5 females, with a racial distribution of 12 Caucasians and 3 African Americans. As seen in Table 2, there were significant differences in body composition of the participants between the obese mother and lean/non-obese mother groups. Data analyses were performed to attempt to match and balance BMI z-score and percentile across groups; however, this manipulation did not alter the interpretation of the findings.
Table 2 Descriptive characteristics of the participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>OM (n=8)</th>
<th>LM (n=7)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>10 ± .423</td>
<td>9.57 ± .528</td>
<td>.533</td>
</tr>
<tr>
<td>Mass, kg</td>
<td>55.71 ± 5.598</td>
<td>35.46 ± 3.288</td>
<td>.010</td>
</tr>
<tr>
<td>Height, cm</td>
<td>147.49 ± 3.578</td>
<td>142.18 ± 3.902</td>
<td>.334</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.09 ± 1.483</td>
<td>17.34 ± .947</td>
<td>.001</td>
</tr>
<tr>
<td>BMI Z Score</td>
<td>1.87 ± .149</td>
<td>.06 ± .395</td>
<td>.003</td>
</tr>
<tr>
<td>BMI Percentile</td>
<td>95.80 ± 1.508</td>
<td>50.70 ± 13.095</td>
<td>.014</td>
</tr>
<tr>
<td>Tanner Stage</td>
<td>2 ± .378</td>
<td>1 ± .143</td>
<td>.06</td>
</tr>
</tbody>
</table>

OM, children with a severely obese mother; LM, children with a lean/non-obese mother. Mean ± SE. *P≤0.05 for a difference between groups.

Maximal Exercise

Each participant completed two maximal exercise tests with the test obtaining the highest value used in data analyses. Mean data for the maximal exercise tests can be seen in Table 3. There were no significant differences between the groups.

Table 3 Maximal Exercise characteristics of the children

<table>
<thead>
<tr>
<th>Variable</th>
<th>LM</th>
<th>OM</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vo2peak, ml/kg/min</td>
<td>29.71 ±1.53</td>
<td>27.16 ±1.80</td>
<td>.307</td>
</tr>
<tr>
<td>Vo2peak, L/min</td>
<td>1.25±.24</td>
<td>1.475±.08</td>
<td>.366</td>
</tr>
<tr>
<td>Peak Watts</td>
<td>66.43±11.2</td>
<td>98.88±6.2</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Table 3 Characteristics obtained during a maximal exercise test on a cycle ergometer. LM, children with a lean/non-obese mother; OM, children with a severely obese mother. Mean ± SE. There was a significant difference in peak watts achieved during the maximal exercise test between groups.

Submaximal Exercise

Exercise Training

Each participant partook in the exercise intervention, which consisted of 3 hour-long sessions a week for a total of 4 weeks. Heart monitors were used to gauge exercise intensity, and participants were encouraged to stay at or above 65% of their
predicted maximum heart rate. Exercise sessions included walking on a treadmill, working out on an elliptical, and endurance-based activities such as soccer, racquetball, tennis, and circuit training; each participant had 100% compliance in completing the pre-testing, exercise intervention, and post-testing.

There were no significant changes in RER with exercise training at the absolute workload (Figure 1A). Following the exercise intervention, regardless of maternal obesity status, there was a decrease (p=.013) in RER during the relative workload portion of the submaximal exercise test (Figure 1B, 1C). There was no change in substrate utilization at the absolute workload with exercise training (Figure 2A). A decrease in RER at the relative workload was also reflected by an increase in the percentage of fat oxidation contributing to the total energy demand during exercise (Figure 2B).

![Substrate Utilization at the Absolute Workload (15W)](image)
Figure 1
Respiratory exchange ratio (RER) (Panel A) during submaximal exercise test at an absolute workload of 15W. (Panel B) during a cycling exercise test at a workload of 65% VO\textsubscript{2}Max. Both show pre and post training results following a 4 week endurance-oriented exercise training program in children with a lean/non-obese mother (n = 7) or a severely obese mother (n = 8). Pre and post exercise intervention data, regardless of maternal obesity status (C).
Figure 2
Substrate utilization in the form of percentage of fat utilized during a cycling exercise test of 15W (A) as well as at a relative workload of 65% VO2Max (B).
Chapter V: Discussion

Being surrounded by an obesogenic environment is, without a doubt, a pivotal component when it comes to contributing to the advancement of obesity status. Nevertheless, genetic susceptibility also plays a part as revealed by studies that estimate heritability being between 40 to 70% for the obese state (9). One research group has found a decrement in fat oxidation at the whole-body level as well as in skeletal muscle with severe obesity, both of which may stimulate weight gain in the affected individuals and lead to severe obesity (1, 8, 11, 14).

The main finding of the present study was that regardless of maternal obesity status, both groups of children exhibited a significant decrease in RER at the submaximal workload of 65% VO\textsubscript{2}Max following the four week exercise intervention, which depicts a shift from utilizing predominantly carbohydrates during exercise to utilizing fat as the primary energy source. Secondly, it is also noted that there was no significant difference in RER between the two groups, with either workload, prior to beginning the exercise intervention. This finding differs slightly from a prior study with a similar protocol. Eaves et al. found that children with a severely obese parent displayed a decrement in fat oxidation during mild exercise (4). Another study, also with a similar protocol, reported that fat oxidation was reduced in severely obese women who had lost weight via gastric bypass surgery when compared with weight-matched controls who were never severely obese (8).

The differences in the findings of the current study with others with similar protocols bring about questions of possibly confounding conditions concerning obesity and fat oxidation. The first is the difference between maternal and paternal obesity
status and how it affects the children. While there were no significant differences between the participants in the previous study (4) and the current, Eaves et al. did include maternal and paternal parent obesity status for each participant, whereas the current study focused solely on maternal obesity status. This alteration in study methods could explain the difference between the results of the previous and the current study, specifically being that children receive their cellular respiration DNA, which is controls their substrate utilization, from their birthmothers rather than their fathers.

Another topic of discussion is participant age. Previous studies that have investigated fat oxidation in subjects have primarily used middle-aged women as their subjects (7, 8, 11, 14) and have found that obese women, as well as weight-reduced women, exhibit a decrement in fat utilization when compared to their lean, non-obese counterparts. However, this effect was not significantly seen in the current study; there was no significant difference in substrate utilization between the two groups prior to beginning the exercise intervention. These findings could suggest that there could be a gap in the process of fat utilization seen earlier in life, making the differences more noticeable and evident in an adult participant. There could also be an underling inherent component which increases its expression and/or environmental influences that accumulate during the development of the severely obese state, beginning in adolescence and leading into adulthood.

Differences in participant body composition could also be a factor that would explain different results among studies. The previous study of Eaves et al. (9) had an average participant BMI z-score of $0.455 \pm 0.16$ and $1.031 \pm 0.29$ for the lean parent
and obese parent group, respectively. The current study’s participants’ body composition differed greatly from the previous with a BMI z-score of 0.06±.40 and 1.87±.15 for the lean mother group and the obese mother group. The lean mother group was much smaller and the obese mother group was much bigger; this difference suggests that the participants’ weight drove the results of the present study, i.e. that both the lean and obese groups were quite different from population means. However, it could also be said that if we propose that obese parents breed obese children, and vice versa for lean non-obese parents, then having a significantly higher BMI z score in the obese mother group, as well as a lower BMI z score in the lean, non-obese mother group, would be expected.

While the sample size was relatively small (n=15), a four-week exercise intervention consisting of hour long sessions three times a week proved to be successful in increasing fat utilization during submaximal exercise at a relative workload of 65% of VO₂Max, regardless of maternal obesity status. A comparable exercise protocol also lowered RER and improved the rate of fat oxidation during submaximal cycling exercise at workloads ranging from 30 to 70% VO₂peak (17). In the study by Duncan and Howley (17), children trained exclusively on a cycle ergometer for 30 minutes a day, 3 times a week for 4 weeks as opposed to the more common exercise program applied in the current study. This lack of precise training could be responsible for the absence of change at our absolute (15W) workload compared to their results [19]. However, we tried to create an exercise program that was pleasurable as well as clinically pertinent for children; the finding of a possible enhancement in FAO offers potential for this intervention. To corroborate the effectiveness of exercise, we have
found that FAO in skeletal muscle improved in formerly severely obese individuals after only 10 days of endurance-oriented training for 1 hour per day [2].
Chapter VI:

In the current study there was no significant difference in RER in children with a severely obese (BMI ≥ 35 kg/m²) mother versus children with a lean, non-obese mother. However, both groups, regardless of maternal obesity status, exhibited a significantly lower RER and a higher dependence on lipid oxidation during submaximal exercise at a relative workload (65% VO2peak) after completing a 4 week exercise intervention consisting of hour long sessions 3 times a week. This finding is supportive of earlier data that is indicative of exercise being able to treat and correct decrements in FAO at the submaximal workload of 65% VO2max. However, there was not enough evidence in the current study to suggest that there is a genetic or epigenetic component responsible for the reduction in FAO in children with a severely obese mother. With a reasonably brief course of endurance-oriented exercise training, 4 weeks, FAO tended to increase at the relative workload, which is suggestive of the potential effectiveness of exercise for prevention/intervention.
References


Notification of Continuing Review Approval

From: Biomedical IRB
To: Joseph Houmard
CC: Gabriel Dubis
Date: 3/24/2016
Re: CR00004173
UMCIRB 15-000332
Fat Metabolism in Children During Exercise

I am pleased to inform you that at the convened meeting on 3/23/2016 at 12:15 PM of the Biomedical IRB, this research study underwent a continuing review and the committee voted to approve the study. Approval of the study and the consent form(s) is for the period of 3/23/2016 to 3/22/2017.

The Biomedical IRB deemed this study Greater than Minimal Risk.

Changes to this approved research may not be initiated without UMCIRB review except when necessary to eliminate an apparent immediate hazard to the participant. All unanticipated problems involving risks to participants and others must be promptly reported to the UMCIRB. The investigator must submit a continuing review/closure application to the UMCIRB prior to the date of study expiration. The investigator must adhere to all reporting requirements for this study.

Approved consent documents with the IRB approval date stamped on the document should be used to consent participants (consent documents with the IRB approval date stamp are found under the Documents tab in the study workspace).

The approval includes the following items:

<table>
<thead>
<tr>
<th>Document</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assent Script &lt;12 year old children(0.02)</td>
<td>Consent Forms</td>
</tr>
<tr>
<td>Adult Questionnaire of Child Health and Physical Activity Habits(0.01)</td>
<td>Surveys and Questionnaires</td>
</tr>
<tr>
<td>Assent Version 1(0.05)</td>
<td>Consent Forms</td>
</tr>
<tr>
<td>Data Collection Sheet(0.01)</td>
<td>Data Collection Sheet</td>
</tr>
<tr>
<td>Fat Metabolism in Children During Exercise Advertisement(0.05)</td>
<td>Recruitment Documents/Scripts</td>
</tr>
</tbody>
</table>
The following UMCIRB members were recused for reasons of potential for Conflict of Interest on this research study: None

The following UMCIRB members with a potential Conflict of Interest did not attend this IRB meeting: None
APPENDIX B: INFORMED CONSENT

Title of Research Study: “Fat Metabolism in Children during Exercise”

Principal Investigator: Joseph Houmard, PhD
Institution/Department or Division: Human Performance Lab
Address: 371 Ward Sports Medicine Building
Telephone #: (252) 737-1288

Researchers at East Carolina University (ECU) study problems in society, health problems, environmental problems, behavior problems and the human condition. Our goal is to try to find ways to improve the lives of you and others. To do this, we need the help of people who are willing to take part in research.

The person who is in charge of this research is called the Principal Investigator. The Principal Investigator may have other research staff members who will perform some of the procedures. The person explaining the research to you may be someone other than the Principal Investigator. Study Coordinators, Graduate Students and Research Technicians may be asking you to take part in this study.

You may have questions that this form does not answer. If you do, feel free to ask the person explaining the study, as you go along. You may have questions later and you should ask those questions, as you think of them. There is no time limit for asking questions about this research.

You do not have to take part in this research. Take your time and think about the information that is provided. If you want, have a friend or family member go over this form with you before you decide. It is up to you. If you choose to be in the study, then you should sign the form when you are comfortable that you understand the information provided. If you do not want to take part in the study, you should not sign this form. That decision is yours and it is okay to decide not to volunteer.

This form explains why this research is being done, what will happen during the research, and what your child will need to do if your child decides to volunteer to take part in this research.

**Why is this research being done?**
The purpose of this research is to determine how children respond to exercise, specifically in terms of the energy it takes to exercise. During exercise people use fat and carbohydrates. The decision to take part in this research is your child’s to make. By doing this research, we hope to learn what types of fats and
carbohydrates you use when you exercise. We are trying to determine if there is a relationship between paternal obesity and the fat metabolism during exercise in children.

Why is my child being invited to take part in this research?
Your child is being invited to take part in this research because he/she is a healthy volunteer. Child participants for the control group must have biological parents with a BMI of <28 kg/m². Participants in the experimental group must have a biological parent that has a BMI > 35 kg/m². Children of gastric bypass patients are included as long as the patient's BMI was greater than 40 prior to surgery. If your child volunteers to take part in this research, he/she will be one of about 60 people to do so.

Are there reasons my child should not take part in this research?
Your child should not volunteer for this study if he/she is on any medication or has any health condition that would affect his/her ability to exercise or pose additional risk.

What other choices does my child have if he/she does not take part in this research?
Your child has the choice of not taking part in this research study.

Where is the research going to take place and how long will it last?
The research procedures will be conducted at the Fitness, Instruction, Testing and Training Facility (F.I.T.T.). Your child will need to come to F.I.T.T. during the study. Each visit will take approximately 1 hour. The total amount of time your child will be asked to volunteer for this study is approximately 20 hours over the next 2 to 3 month period. After the initial screening, if your child is still eligible for enrollment, their participation will involve:

What will my child be asked to do?
- Your child is being asked to do the following: You will fill out personal history forms that pertain to you and your child. Participation in this study will require medical history such as body weight, height and history of diabetes from at least one biological parent. Your child will fill out forms relating to his/her health status and physical activity.

- (Visit 1): Determination of Body Composition and Aerobic Capacity. Body mass index (BMI) and skinfolds will be conducted at the Human Performance Laboratory. To calculate BMI, height and weight will be measured. Seated height and minimum waist girth will also be measured. Skinfold thickness of the triceps and calf will be taken on the right side of the body, in duplicate, with a skinfold caliper.

A maximal test on a stationary cycle (cycle ergometer) will be completed to evaluate aerobic capacity. For this test, your child will cycle on the stationary bicycle for approximately 10-15 minutes. During this test, your child will wear a mouthpiece so the air they breathe out can be collected for analysis of oxygen. At first, your child will cycle at a very low resistance (low workload) but the resistance will become harder until your child can no longer continue. Your child will perform two (2) of these tests to insure that the measurements are correct. If the measurements the researchers obtain indicate that the effort was not maximal, a second test may be performed.

- (Visit 2) Your child will perform a submaximal exercise test which will consist of cycling for 20 min at two relatively low resistances. For this test, your child will warm-up on the cycle for 3-5 min. After the warm-up, your child will cycle at 10 minutes at one resistance. After this 10 minute period,
your child will rest for 5-10 min and then perform another 10 min exercise session. During this test, your child will wear a mouthpiece so the air they breathe out can be collected for analysis of oxygen. This test will take place in the morning after your child has fasted for 8-12 hours (overnight fast). Your child can drink all the water they want prior to the test but cannot consume any foods and drinks containing energy (calories).

- **(Between Visits 1 and 2)** Your child will wear a **physical activity monitor** (RT3 Triaxial Accelerometer) and a pedometer (Yamax, Japan) for three days. The parent or an adult may need to assist the child in recording the data, resetting the monitors and other similar procedures. The purpose of this test is to determine how much physical activity your child is performing. You/your child can elect to not perform this aspect of the study.

- **Exercise Training**: After the completion of the testing visits your child will exercise in the Human Performance Laboratory (HPL) for 4 consecutive weeks. The exercise training will involve your child exercising for one hour per day for 3 days a week; the exercise will consist of riding a stationary cycle, walking uphill on a treadmill and performing recreational activities such as basketball and racquetball. The exercise will be supervised by HPL personnel and performed at workloads that will increase the heart rate to rates prescribed for effective exercise training. The tests described above (body composition, evaluation of aerobic capacity, submaximal exercise test) will be repeated approximately 2-4 days after completing the final exercise session of the 4 weeks.

**What possible harms or discomforts might my child experience if my child takes part in the research?**

There are always risks (the chance of harm) when taking part in research. We know about the following risks or discomforts you may experience if you choose to volunteer for this study. These are called side effects. The following side effects are known to occur in some people:

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

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

  - The risks associated with exercise training are musculoskeletal injury (i.e. sore/pulled muscles, connective tissue injuries). These risks will be minimized by having the exercise performed under adult supervision and using the appropriate exercise intensities as determined by the exercise stress tests.

It is important for you to tell us as quickly as possible if you experience a side effect.

**Are there any reasons you might take my child out of the research?**

During the study, information about this research may become available that would be important to your child. This includes information that, once learned, might cause your child to change his/her mind about wanting to be in the study. We will tell you as soon as we can. This might include
information about the side effects that are caused by taking part in this study. If that happens, we can tell you about these new side effects and let you decide whether you want your child continue to take part in the research.

There may be reasons we will need to take your child out of the study, even if your child want to stay in. We may find out that it is not safe for your child to stay in the study. It may be that the side effects are so severe that we need to stop the study or take your child out of the study to reduce his/her risk of harm. If we find that the research might harm your child or that it is not providing enough of a benefit to justify the risks your child is taking, we will take your child out of the study and provide the results of tests/data that has deemed him/her ineligible. If those things are found to be true, we will need to take your child out of the study.

**What are the possible benefits my child may experience from taking part in this research?**

We do not know if your child will get any benefits by taking part in this study. This research might help us learn more about how parental characteristics play a role in determining how your child utilizes carbohydrate and fat. Your child will also be exposed to exercise testing and training which may encourage an active lifestyle. There may be no personal benefit from your child’s participation but the information gained by doing this research may help others in the future.

**Will I or my child receive any compensation for taking part in this research?**

Your child will be paid $80 if he/she completes all the required tests and exercise training for the study. Pre-testing ($15 for each testing visit); $20 for completing the exercise training; Post testing ($15 for each testing visit). Pre and Post testing will consists of 4 total visits.

**What will it cost my child and me to take part in this research?**

It will not cost you or your child any money to be part of the research.

**Who will know that your child took part in this research and learn personal information about your child?**

To do this research, ECU and the people and organizations listed below may know that your child took part in this research and may see information about your child that is normally kept private. With your permission, these people may use your private information to do this research:

- Any agency of the federal, state, or local government that regulates human research. This includes the Department of Health and Human Services (DHHS), the Food and Drug Administration (FDA), the North Carolina Department of Health, and the Office for Human Research Protections.
- The University & Medical Center Institutional Review Board (UMCIRB) and its staff, who have responsibility for overseeing your welfare during this research, and other ECU staff who oversee this research.

**How will you keep the information you collect about me and my child secure? How long will you keep it?**

Only the investigators associated with this study will have access to the data obtained. No identifying information will be released. Numeric coding, which only the primary investigator will have access to, will protect the identity of your child and other subjects. Data will be secured in a locked filing cabinet in the office of the primary investigator in the Human Performance Laboratory. The data will be kept for a minimum of 3 years.
What if my child decides he/she does not want to continue in this research?
If your child decides he/she no longer wants to be in this research after it has already started, he/she may stop at any time. Your child will not be penalized or criticized for stopping.

What if my child gets sick or hurt while he/she is in this research?
We do not expect your child to become sick or hurt as a result of being part of this research. However, people respond differently to things and sometimes accidents do happen. Therefore, if your child needs emergency care call 911. If possible, take a copy of this consent form with you when you go.

Call the principal investigator as soon as you can. He/she needs to know that your child is hurt or ill. Call Joseph Houmard at (252) 737-4617.

If you do NOT need emergency care, but have been hurt or get sick:
Contact Joseph Houmard at 252-737-4688 or 252-737-4617. Call the principal investigator as soon as you can. As necessary, go to your regular doctor. It is important that you tell your regular doctor that you are participating in a research study. If possible, take a copy of this consent form with you when you go.

The ECU Medical Clinics may be able to give you the kind of help you need. However, you may need to get help from a different type of medical facility and your Principal Investigator will know best what you should do.

If your child is harmed while taking part in this study: If your child believes he/she has been hurt or if he/she gets sick because of something that is done during the study, you should call Joseph Houmard, Ph.D. at 252-737-4617 immediately. There are procedures in place to help attend to your child’s injuries or provide care for your child. Costs associated with this care will be billed in the ordinary manner, to you or your insurance company. However, some insurance companies will not pay bills that are related to research costs. You should check with your insurance about this. Medical costs that result from research-related harm may also not qualify for payments through Medicare, or Medicaid. You should talk to the Principal Investigator about this, if you have concerns.

Who should I contact if I have questions?
The people conducting this study will be available to answer any questions concerning this research, now or in the future. You may contact the Principal Investigator, Joseph Houmard at (252) 737-4617 (days, between 8am-5pm).

If you have questions about your rights as someone taking part in research, you may call the ECU Office of Research Integrity & Compliance (ORIC) at phone number 252-744-2914 (days). If you would like to report a complaint or concern about this research study, you may call the Director of ORIC, at 252-744-1971.
My child has decided to take part in this research. What should I do now?
The person obtaining informed consent will ask you to read the following and if you agree, you should sign this form:

- I have read (or had read to me) all of the above information.
- I have had an opportunity to ask questions about things in this research I did not understand and have received satisfactory answers.
- My child understands that he/she can stop taking part in this study at any time.
- By signing this informed consent form, I am not giving up any of my rights.
- I have been given a copy of this consent document, and it is mine to keep.

___ As parent or legal guardian I have read all of the above information, asked questions, and received answers concerning areas I did not understand, and have received satisfactory answers to these questions. I willingly consent for the participation of my child in this research study. (A copy of this consent form will be given to the person signing as the subject or as the subject’s authorized representative.)

___ Please contact me concerning similar studies in the future concerning myself/my child.

___ Please do not contact me concerning similar studies in the future.

Participant’s Name (Print)

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

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

Phone numbers in case of injury

Signature of Authorized Representative – Guardian #1

Date

Person Obtaining Informed Consent: I have conducted the initial informed consent process. I have orally reviewed the contents of the consent document with the person who has signed above, and answered all of the person’s questions about the research.

Person Obtaining Consent (PRINT) Signature Date

Person Obtaining Verbal Assent

If the child is age 7-11 and has given verbal assent to participate in the study

“By initialing in the following places, the parent/guardian and investigator indicate their opinion that the patient is too young or otherwise not able to give consent/assent.”

_____Parent/Guardian

_____Investigator
Appendix C: Informed Assent

East Carolina University
Assent Form
Things You Should Know Before You Agree To Take Part in this Research

IRB Study # 15-000332

Title of Study: Fat Metabolism in Children During Exercise

Person in charge of study: Joseph A. Houmard, Ph.D
Where they work: East Carolina University
Other people who work on the study: Jenna Rouse and Gabe Dubis

Study contact phone number: 252-737-1288
Study contact E-mail Address: dubisg@ecu.edu, rousej10@students.ecu.edu

People at ECU study ways to make people’s lives better. These studies are called research. This research is trying to find out how children respond to exercise. During exercise people burn fat in their bodies. The purpose of this study is to study how much fat you use when you exercise and how exercising for 4 weeks may affect how your body burns fat.

Your parent(s) needs to give permission for you to be in this research. You do not have to be in this research if you don’t want to, even if your parent(s) has already given permission.

You may stop being in the study at any time. If you decide to stop, no one will be angry or upset with you

Why are you doing this research study?
The reason for doing this research is to study how much fat you use when you exercise and how exercising for 4 weeks may affect how your body burns fat.

Why am I being asked to be in this research study?
We are asking you to take part in this research because you are a healthy normal child.

How many people will take part in this study?
If you decide to be in this research, you will be one of about 60 people taking part in it.

What will happen during this study?
Visit 1: Measure Height, Weight, Waist size and your skinfolds. You will also answer some questions about your health and how much you exercise
You will ride an exercise bike for about 12 minutes. The exercise bike will become harder to pedal every few minutes until you are too tired to turn the pedals. This is called a maximal exercise test. During the test you will be breathing through a special mask.

**Visit 2:** (1 hour) You will be riding the same exercise bike as before but this time the pedals will not get harder to push. You’ll ride the bike for 10 minutes and then take a break and ride for another 10 minutes. You will have to do this test in the morning and not eat any breakfast before you come and ride the bike.

**Exercise:** (1 month) You will exercise at the FITT building for 1 month. You will need to come 3 days a week for 1 hour. You will play tennis, walk on the treadmill ride the exercise bike or play other games.

**Final visits:** After you have exercised for 1 month you will have to come back and do the same bike tests that you did at the beginning of the study.

This study will take place at FITT building.

**Who will be told the things we learn about you in this study?**

Your personal information will be kept safe and private at the Human Performance Laboratory. Only the people performing the study can see your information.

**What are the good things that might happen?**

Sometimes good things happen to people who take part in research. These are called “benefits.”

Here are some benefits that you might have from this study.

1. You will learn more about exercise and physical activity.
2. You will learn about your body and how healthy you are.

**What are the bad things that might happen?**

Sometimes things we may not like happen to people in research studies. These things may even make them feel bad. These are called “risks.”

The risks in this study are:

1. Sometimes people feel sick (for example like throwing up) for a short period of time when they exercise so hard

To make sure you do not get sick during exercise, we will watch how your heart responds to the exercise and will stop the exercise if you feel sick.

2. When you start the 4 weeks of exercise training your muscles may get sore and you may be tired.

You will be supervised by people in the laboratory to make sure you do not get hurt or exercise too hard.

You may or may not have these things happen to you. Things may also happen that the researchers do not know about right now. You should report any problems to your parents and to the researcher.
**Will you get any money or gifts for being in this research study?**

You will receive $80 for participating in this study. If you drop out of the study you will only be paid for the tests you have completed. Pre-testing ($15 for each testing visit); $20 for completing the exercise training; Post testing ($15 for each testing visit). Pre and Post testing will consists of 4 total visits.

**Who should you ask if you have any questions?**

If you have questions about the research, you should ask the people listed on the first page of this form. If you have other questions about your rights while you are in this research study you may call the Institutional Review Board at 252-744-2914.

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

If you decide to take part in this research, you should sign your name below. It means that you agree to take part in this research study.

__________________________
Sign your name here if you want to be in the study

Date

__________________________
Print your name here if you want to be in the study

__________________________
Signature of Person Obtaining Assent

Date

__________________________
Printed Name of Person Obtaining Assent
APPENDIX D: Subject Recruitment and Screening Questions

Hi, my name is _________________________________, and I am a researcher with the Human Performance Laboratory at East Carolina University.

I am calling to see if you might be interested in participating in a study we are currently conducting. We are studying fat metabolism during exercise in children and studying if there is a relationship between parents and children.

You will be compensated for your participation.

We are looking for children between the ages of 8 and 12 years of age to participate who have parents within a certain weight range.

They will come into the FITT building located on ECU’s campus and exercise on a bike for us. There are no invasive procedures like a blood draw involved.

If you are interested, I have a few questions to ask you.

Do you have any children between the ages of 8 and 12?  

Y                N

If yes, what are their names and ages? _____________________________________________________

Has your child started puberty yet (menstral cycle for girls)?  

Y                    N

Are you the biological parent of the child or children?  

Y                N

Will it be possible to get both parents’ height and weight information?  

Y                      N  

*If in the experimental group, okay if no – as long as parent has or had (prior to gastric bypass) a BMI ≥40

Does your child have any medical conditions?  

Y                 N

Is your child on any medications?  

Y                N

Have you or the child’s other parent ever undergone gastric bypass or a similar procedure due to obesity?  

Y                        N

What is your height? _________________________

What would you guess is your current weight? ____________________________

Calculated BMI ________________________

Will you be able to make 4 visits about an hour each to the FITT building on ECU’s campus?
APPENDIX E: Tanner Staging Questionnaires

Tanner Staging

Please read the descriptions below and circle the stage that best describes the development of your child.

I. Girls

<table>
<thead>
<tr>
<th>Tanner Stage</th>
<th>Stage of development</th>
<th>Pubic Hair</th>
<th>Breasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>Early adolescence</td>
<td>Preadolescent</td>
<td>Preadolescent</td>
</tr>
<tr>
<td>Stage 2</td>
<td></td>
<td>Sparse, straight</td>
<td>Small mound</td>
</tr>
<tr>
<td>Stage 3</td>
<td>Middle adolescence</td>
<td>Dark, curl</td>
<td>Bigger; no contour separation</td>
</tr>
<tr>
<td>Stage 4</td>
<td></td>
<td>Coarse, curly, abundant</td>
<td>Secondary mound of areola</td>
</tr>
<tr>
<td>Stage 5</td>
<td>Late Adolescence</td>
<td>Triangle; medial thigh</td>
<td>Nipple projects; areola part of breast</td>
</tr>
</tbody>
</table>

I. Boys

<table>
<thead>
<tr>
<th>Tanner Stage</th>
<th>Stage of development</th>
<th>Pubic Hair</th>
<th>Penis</th>
<th>Testes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>Early adolescence</td>
<td>None</td>
<td>Preadolescent</td>
<td>Preadolescent</td>
</tr>
<tr>
<td>Stage 2</td>
<td></td>
<td>Scanty</td>
<td>Slight increase</td>
<td>Larger</td>
</tr>
<tr>
<td>Stage 3</td>
<td>Middle adolescence</td>
<td>Darker, curls</td>
<td>Longer</td>
<td>Larger</td>
</tr>
<tr>
<td>Stage 4</td>
<td></td>
<td>Adult, coarse, curly</td>
<td>Larger</td>
<td>Scrotum dark</td>
</tr>
<tr>
<td>Stage 5</td>
<td>Late adolescence</td>
<td>Adult - thighs</td>
<td>Adult</td>
<td>Adult</td>
</tr>
</tbody>
</table>

Adapted from Medical College of Georgia Department of Pediatrics
Please rate yourself on pubic hair development according to the description and pictures below.

Circle the stage that best describes your development.

**Pubic Hair Development**

<table>
<thead>
<tr>
<th>Pubic Hair</th>
<th>[CIRCLE ONE]</th>
</tr>
</thead>
</table>

1. No pubic hair.

2. Scanty, long slightly darker in color.

3. Darker, begins to curl, small amount

4. Resembles adult type, but less in quantity; coarse, curly
5. Adult distribution, spread to inside of thighs.
To help us determine the stage of sexual maturation for your daughter, please rate your daughter on breast development according to the descriptions and pictures below.

Circle the stage that best describes your daughter’s development.

### Stages of Sexual Maturation

#### Breast Development  [CIRCLE ONE]  [FAMS1501]

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Breasts flat</td>
<td><img src="image1.png" alt="Image" /></td>
</tr>
<tr>
<td>2.</td>
<td>Breast buds (small mounds beneath areola)</td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>3.</td>
<td>Increased breast size</td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>4.</td>
<td>Areola projects above breast plate.</td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>5.</td>
<td>Mature stage</td>
<td><img src="image5.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Form 15 1:2 Version 1 (09/06)
To help us determine the stage of sexual maturation for your daughter, please rate your daughter on pubic hair development according to the descriptions and pictures below.

Circle the stage that best describes your daughter’s development.

**Stages of Sexual Maturation**

<table>
<thead>
<tr>
<th>Pubic Hair</th>
<th>[CIRCLE ONE] [FAMS1502]</th>
</tr>
</thead>
</table>

Stage

1. No pubic hair.

2. Scant growth of long, straight or slightly curled hair along labia.

3. Hair darker, more abundant, curled, and coarser.

4. Adult pattern but pubic hair does not extend to medial surface of thighs.

5. Mature stage; pubic hair extends to medial surface of thighs

Form 15 2:2 Version 1 (04/06)
APPENDIX F: Adult Questionnaire of Child Health and Physical Activity Habits

Adult Questionnaire of Child Health and Physical Activity Habits
- Adapted from Amherst Health and Activity Study

Child Name: __________________________ Date: ______________

This survey should be completed by the adult in the home who is most familiar with this child’s daily activities.

This survey will help us understand physical activity and other health habits of children and teenagers. Your answers are very valuable. Please take a few minutes to complete the survey, and then return it immediately to the administrator of the survey. Feel free to ask any questions.

All the questions on this survey are about the child whose name is at the top of this page.

________________________________________________________________________
Adult’s signature

GENERAL INFORMATION ABOUT THIS CHILD

Q1. What is this child’s birth date? ___________ month ________ day __________ year

Q2. What was this child’s birth weight? ___________ lbs __________ oz

Q3. Was this child born preterm? (circle one)   No Yes - How many weeks________

Q4. What is this child’s gender? (circle one) Male Female

Q5. What is this child’s height? ________ feet ________ inches

Q6. What is this child’s weight? __________ pounds

Q7. Does this child have any medical conditions or disabilities that limit his or her physical activity? (circle one number)
   0. No 1. Yes, please specify: __________________________

Q8. How do you identify your child’s racial or ethnic background? (circle one number only)
   1. Asian Pacific Islander
   2. African American
   3. Native American Indian
   4. Latino/Hispanic
5. Caucasian
6. Multi-racial/ Multi-ethnic
7. Other (please specify) ___________________________________________________

THIS CHILD'S ACTIVITIES

Circle the number of the answer that you feel is correct for this child.

Q9. On how many of the past 7 days did this child exercise or participate in sports activities for at least 20 minutes that made him/her sweat and breathe hard, such as basketball, jogging, swimming laps, tennis, fast bicycling, or similar aerobic activities? Circle one number

0 1 2 3 4 5 6 7

Q10. On how many of the past 7 days did this child do exercises to strengthen or tone his/her muscles, such as push-ups, sit-ups, or weight lifting? Circle one number

0 1 2 3 4 5 6 7

Q11. On how many of the past 7 days did this child walk, jog, or bicycle for at least 30 minutes at a time? Circle one number

0 1 2 3 4 5 6 7

Q12. On an average WEEK day, how many hours per day did this child watch television and videos or play computer or video games? Circle one number

0 1 2 3 4 5 6 7

Q13. On an average WEEKEND day (Saturday and Sunday), how many hours per day does this child watch television and videos or play computer or video games? Circle one number

0 1 2 3 4 5 6 7

Q14. Compared to others of the same age and sex how much physical activity does this child get?

Circle one number

1 2 3 4 5
Much less than others The same as others Much more than others
Q15. Compared to others of the same age and sex how do you rate this child’s athletic coordination?  **Circle one number**

1. Much less coordinated than others
2. The same as others
3. Much more coordinated than others

Q16. How much does this child enjoy physical activity?  **Circle one number**

1. Very unenjoyable
2. Neutral
3. Very enjoyable

Q17. How much does this child enjoy physical education classes at school?  **Circle one number**

1. Very unenjoyable
2. Neutral
3. Very enjoyable

Q18. In your opinion, how often is this child physically active?  **Circle one number**

1. Frequently
2. A moderate amount
3. Rarely

Q19. What does this child do when she or he has a choice about how to spend recreational time?  **Circle one number**

1. Almost always chooses activities like TV, reading, listening to music, computers
2. Usually chooses activities like TV, reading, listening to music, computers
3. Just as likely to choose active as inactive recreation
4. Usually chooses activities like bicycling, dancing, outdoor games or active sports
5. Almost always chooses activities like bicycling, dancing, outdoor games or active sports

Q20. Within the past year, how many organized sports and/or physical activities did this child participate in?  **Circle one number**

0  1  2  3  4 OR MORE
Q21. Within the past year, how often did this child participate in organized sports and/or physical activities? **Circle one number**

1. Never  
2. 1-2 times per month  
3. 3-7 times per month  
4. 8-14 times per month  
5. 14 or more times per month

Q22. Where does this child go most often after school?

1. After-school program at school  
2. After-school program at another location  
3. Home, with supervision  
4. Home, without supervision  
5. Home of a relative or friend  
6. Other, please specify: ___________________

Q23. In the past seven (7) days, how many days did this child do physical activity or sports at these locations?

<table>
<thead>
<tr>
<th>Location</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. School grounds (after-school only)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. Park or playground</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. Neighborhood</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. After-school care</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. Commercial Facility (YMCA, B&amp;GC, health club, dance studio)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. Public recreation Center</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G. Other, Please specify:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**FAMILY HISTORY FOR THIS CHILD**

Please check any of the conditions that a family member of this child has been diagnosed to have by a physician.
The following questions ask about **your own** health and physical activity habits.

Q1. What is **your** height? _____ feet _____ inches

Q2. What is **your** weight? ________ pounds

Q3. On how many of the past 7 days did **you** exercise or participate in sports activities for at least 20 minutes that made you sweat and breathe hard, such as basketball, soccer, running, swimming laps, fast bicycling, fast dancing, or similar aerobic activities? **Circle one response**

   0 days 1 day 2 days 3 days 4 days
   5 days 6 days 7 days

Q4. On how many of the past 7 days did **you** participate in physical activity for at least 30 minutes that did NOT make you sweat or breathe hard, such as fast walking, slow bicycling, skating, pushing a lawn mower, or mopping floors? **Circle one response**

   0 days 1 day 2 days 3 days 4 days
   5 days 6 days 7 days

<table>
<thead>
<tr>
<th>CONDITION</th>
<th>YES</th>
<th>NO</th>
<th>RELATION TO CHILD</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Blood Pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 2 Diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart Disease</td>
<td></td>
<td></td>
<td>Please specify</td>
</tr>
<tr>
<td>Bone or Joint Problems</td>
<td></td>
<td></td>
<td>Please specify</td>
</tr>
<tr>
<td>Asthma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Cholesterol</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Q5. On how many of the past 7 days did you do exercises to strengthen or tone your muscles, such as push-ups, sit-ups, or weight lifting? Circle one response

<table>
<thead>
<tr>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
</tr>
</tbody>
</table>

Q6. When you are at work, which of the following best describes what you do? Circle one number

1. Mostly sitting or standing
2. Mostly walking
3. Mostly heavy labor or physically demanding work

<table>
<thead>
<tr>
<th>During a typical week how often do you:</th>
<th>Never</th>
<th>Once</th>
<th>Sometimes</th>
<th>Almost daily</th>
<th>Daily</th>
</tr>
</thead>
<tbody>
<tr>
<td>Encourage your child to do physical activity or play outside?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Play outside or do physical activity with your child?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Provide transportation to a place where he or she can do physical activity or play?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Watch your child participate in physical activities or outdoor games?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Tell your child that physical activity is good for his or her health?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>
PERSONAL HISTORY FORM

Please read each of the following questions and circle YES or NO regarding your child.

1. YES  NO  Does your child ever have pains in his/her heart and chest?
2. YES  NO  Does your child often feel faint or have any spells of severe dizziness?
3. YES  NO  Has your child’s doctor ever said his/her blood pressure was too high?
4. YES  NO  Has your child’s doctor ever told you he/she has a bone or joint problem such as arthritis that has been aggravated by exercise or might be made worse with exercise?
5. YES  NO  Does your child have asthma?
6. YES  NO  Does your child have Diabetes or any other metabolic disease?
7. YES  NO  Is there a good physical reason not mentioned here why your child should not participate in an activity program even if he/she wanted to?
8. YES  NO  In the past two weeks, has your child followed a normal and regular sleep pattern?
9. YES  NO  Is your child currently taking any medication?
10. YES  NO  Has your child undergone surgery?

If you answered YES to any of the following questions (with exception to #8), please explain:

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

In case of an emergency, please contact:
Name:  
________________________________________________________________________
Address:  
________________________________________________________________________
Phone Number:  

APPENDIX H: Data Collection Sheet

Fat Metabolism in Children Data Collection Sheet

Check offs

_________ Informed Consent
_________ Assent
_________ Tanner Staging
_________ Questionnaire

Subject Identifier____________________________       Group       E               C       (Circle one)
Name _______________________________________________________
Age:_____________ years   Date of Birth __________________________
Height ____________ cm
Weight ___________ lbs ______________ kg

Skinfold Measurements

<table>
<thead>
<tr>
<th>Measurement 1</th>
<th>Measurement 2</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tricep</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial Calf</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Check Offs

_________ Introduction to metabolic cart equipment and bike
_________ Instructions for next visit

Scheduled dates and times for next visits

Max Test 1 ________________________________________________________
Max Test 2 ________________________________________________________
Submax Test _______________________________________________________
Data on Parents

Mother

Age_______________________ Date of Birth _________________________
Height____________________ cm
Weight____________________ lbs
BMI________________________ kg/m²
Gastric Bypass or other       Y               N
APPENDIX I: Maximal Exercise Test Protocol

Date: 
Predicted Max HR: 
85% HR: 
Height (cm): 
Weight (kg): 

<table>
<thead>
<tr>
<th>Minutes</th>
<th>Watts</th>
<th>Heart Rate</th>
<th>Blood Pressure</th>
<th>RPE</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>25</td>
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<td>3</td>
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<td>4</td>
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<tr>
<td>7</td>
<td>75</td>
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<td></td>
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<tr>
<td>8</td>
<td>75</td>
<td></td>
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</tr>
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<td>100</td>
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<td>13</td>
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<td>14</td>
<td>125</td>
<td></td>
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<td>15</td>
<td>125</td>
<td></td>
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<td>20</td>
<td>175</td>
<td></td>
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</tr>
<tr>
<td>21</td>
<td>175</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total Exercise Time: 
VO2 Max (ml/kg/min): 
Max HR: 
Max RER: 
Max BP:
APPENDIX J: Submaximal Exercise Test Protocol

**IRB 15-000332**

**Submax Fat Met Test Form**

<table>
<thead>
<tr>
<th>ID:</th>
<th>Date of Submax Test:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Check One: □ Baseline □ Post</td>
</tr>
</tbody>
</table>

**Gender:**

**DOB:**

**Height of subject:**

**Weight of subject:**

**Previously determined VO₂max:**

**Regression equation:**

**65% of VO₂max:**

**Estimated Watts**

**Confirm 12 hours + fast:**

---

**EXERCISE MEASUREMENTS**

At t = 10 min, adjust workload to 65% of VO₂max as determined from the regression equation from previously determined VO₂max. Any adjustments to workload needs to occur by t = 12 min. Cadence should remain constant, and at a minimum of 60 rpm. If necessary, the subject may have a 2 min rest upon completion of the absolute workload at t = 10 min. Please check the box if a rest period is taken, □

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Resistance (watts)</th>
<th>Cadence (rpm)</th>
<th>Heart rate (bpm)</th>
<th>RPE</th>
<th>Time (min) to insert mouthpiece and connect participant to metabolic cart</th>
</tr>
</thead>
<tbody>
<tr>
<td>REST 0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-5</td>
<td>15</td>
<td>0</td>
<td></td>
<td></td>
<td>3-5</td>
</tr>
<tr>
<td>5-10</td>
<td>15</td>
<td>0</td>
<td></td>
<td></td>
<td>8-10</td>
</tr>
<tr>
<td>10-15</td>
<td>(65% VO₂max)</td>
<td></td>
<td></td>
<td></td>
<td>10-15</td>
</tr>
<tr>
<td>15-20</td>
<td>(65% VO₂max)</td>
<td></td>
<td></td>
<td></td>
<td>18-20</td>
</tr>
</tbody>
</table>

**Technician:**

**Comments:**