The Effect of Omega-3 Fatty Acids on Energy Metabolism, Energy Intake, and Metabolic Response in Normal Weight and Overweight and Obese School Aged Children (8-12 years)

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The Effect of Omega-3 Fatty Acids on Energy Metabolism, Energy Intake, and Metabolic Response in Normal Weight and Overweight and Obese School Aged Children (8-12 years)

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of the requirement for the degree of
Master of Science in Human Environmental Sciences

by

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Abstract

**Background:** Obesity is a major health concern in the United States. Omega-3 fatty acids (O3FA) have been observed to improve metabolic health and therefore might be useful in treatment of obesity. However, little is known regarding the effect of O3FA on school aged normal weight and overweight children.

**Objective:** The objective of this thesis was to determine if habitual intake of O3FA at breakfast improves energy metabolism, appetite, and metabolic response in overweight and obese school-aged children.

**Design:** Twenty healthy, normal weight (NW; n = 11) and overweight (OW; n = 9) children aged 8-12 years were randomly assigned to receive either a vegetable oil based (Control) breakfast drink or a O3FA based breakfast based drink to observe postprandial effects of each treatment. Anthropometrics, appetite, resting energy expenditure (REE), substrate oxidation, and food intake were evaluated for each treatment.

**Results:** Body weight ($P < 0.001$), and BMI percentile ($P < 0.001$) were higher in the OW group. Fat mass and free fat mass were higher in the OW group ($P < 0.001$) and ($P < 0.05$), respectively. There was an effect of breakfast type ($P < 0.05$) on carbohydrate oxidation after O3FA consumption. There was an effect of time and body weight on hunger ($P < 0.001$). There was also an effect of breakfast over time on feelings of fullness ($P < 0.05$). There was no difference in leptin or adiponectin in response to breakfast. There was no statistical significance of total food (kcal) intake in Control or O3FA

**Conclusion:** Taken together, these data suggest that increasing O3FA in the diets of school-aged children may have beneficial effects on EE, satiety, and metabolic responses throughout the day.
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Dedication

This thesis is dedicated to my husband, André Mitchell, children, AJ, Halle, and Hezekiah, my parents, Charles and Elaine, and grandmother Lou Della as without them I would not be who I am or where I am today.
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Introduction

In the United States rates of childhood obesity have doubled from \(~7\%\) to \(17.2\%\) in the last thirty years [15, 16]. Moreover, in Arkansas, childhood obesity rates are \(14.4\%\) in children aged 2-4 and \(20\%\) in children aged 10-17 [17]. Obesity is associated with body mass index (BMI). The BMI of children is calculated in percentiles based on height and weight in comparison with other children with in their same sex and age group (\(<85^{\text{th}}\) percentile, normal weight; \(\geq 85^{\text{th}}\) percentile, \(<95^{\text{th}}\) percentile, overweight; and \(\geq 95^{\text{th}}\) percentile, obese). With this increase obesity comes health consequences such as, cardiovascular (dyslipidemia), and endocrine (type 2 diabetes mellitus (T2DM) disorders [24].

Obesity is a metabolic disorder with multiple contributors. Factors such as genetics [25], environment [26], and energy balance (energy intake versus energy expenditure) are among the contributors to the prevalence of obesity [27, 28]. However, when there is a constant state of energy imbalance, e.g. energy intake exceeds energy expended, weight gain and/or obesity may occur [30]. The concept of energy balance has been the focus of obesity research for decades [31] with a specific focus on decreasing energy intake and increasing energy expenditure (EE) in order to enhance weight loss [32]. Whole body EE has three primary components; resting energy expenditure (REE), the energy needed to maintain bodily functions at rest; the thermogenic effect of feeding (TEF), the energy increase associated with digestion and absorption of macronutrients; and activity induced thermogenesis, energy associated with physical activity [29, 33]. Thermogenic effect of feeding is unique because it can change with dietary macronutrient composition making it an ideal target for obesity treatment and/or prevention.

Diets higher in dietary fats, have proven to be successful strategies to improve metabolic health disorders associated with obesity [38]. In addition, O3FA supplementation has been
shown to sustain appetite reduction, resulting in increased satiation and a subsequent decrease in food intake [14]. Ebrahimi et al [39], conducted a six-month intervention where individuals were provided O3FA capsules that contained 180mg eicosapentaenoic acid (EPA) and 120mg docosahexaenoic acid (DHA) per day for six months resulting increased weight loss (2.63 kg or 3.8% body weight) compared to a control group who had the same protocols, but were not given supplementation. Taken together, data suggests that supplementation of O3FA could be an effective intervention and treatment for obesity [36, 41-49].

Breakfast has been suggested to be the most important meal of the day [4-7, 50, 51]. However, according to 47 observational review studies within the United States and Europe, breakfast skipping has been a frequent lifestyle behavior and 10%-30% of children and adolescents do not consume breakfast [52]. Consequently, breakfast skipping is linked to obesity due to it having a strong correlation with overeating at subsequent meals [57, 58]. Recent research has found that there is a positive effect on satiety of breakfast type with increased dietary fat at breakfast [59-64]. However, more research is needed to define what a quality breakfast consists of to guide optimal macronutrient composition of breakfast, which may lead to a strategy to decrease the prevalence of obesity.

Therefore, the objective of this thesis was to determine if habitual intake of O3FA at breakfast improves energy metabolism, appetite, and metabolic response in overweight and obese school-aged children. We hypothesized that increasing O3FA intake at breakfast would improve energy metabolism and reduce energy intake throughout the day in overweight/obese school aged children.
Literature Review

Childhood Obesity

Childhood obesity has become the number one health concern to parents in the United States [71]. The prevalence of obesity in youth aged 2-19 years has doubled over the last 30 years [72]. Arkansas is currently ranked 6th in adult obesity and 6th in childhood obesity [73]. Obesity is more likely to occur in children between the ages of 5 and 14 years [74]. Obese individuals have an increased risk for developing chronic diseases such as type 2 diabetes, hypertension, dyslipidemia and nonalcoholic fatty liver disease [75]. Once restricted to adults, these diseases are now being diagnosed in children [75]. A population sample survey conducted by the Centers for Disease Control and Prevention showed that 70% of children between the ages of 5-17 years had at least one risk factor of cardiovascular disease [76]. In addition, children who are obese are more likely to be obese in adulthood [72]. There are several nutritional behaviors that contribute to obesity such as high intakes of energy dense, nutrient poor foods and diets rich in fats that do not offer a varied meal composition or satiation [77, 78]. With the increase in the prevalence of obesity and the morbidity attached to it, there is a need to not just treat the symptom, but to find the cause so that preventative measures can be taken.

Contributors to Childhood Obesity

The development of childhood obesity has multiple contributing factors, such as environment, genetics, and lifestyle behaviors [74, 79-81]. Increased energy intake has been identified as playing the largest functional role in obesity [29, 82-86] and has been attributed to environmental changes such as increased portion size, increased energy dense convenient foods, and decreased physical activity [84, 86]. Rolls et al [84] showed that a 50% increase in portion sizes can sustain increased energy intake for up to 11 days, resulting in increased intake of 423
kcal in excess over the daily recommended value [84]. Consistent findings by French et al [82] showed that when individuals are provided a meal with increased portion size, energy intake will increase overtime when compared with individuals provided meals with smaller portions [82]. Although these studies were conducted in adults they provide evidence that environments that when larger meal portions are provided, energy intake will increase overtime [87]. Behaviors, such an increased intake of energy dense convenient foods can modulate the development of obesity. Researchers observed over three shopping occasion where a total of 399 products were purchased that over half of the product’s audience were targeted to children, two-thirds fit into five categories: cereal, fruit snacks, meal products, frozen desserts, and candy [88]. Thus, most of the products marketed as healthy, however fell into the low nutritional value category.

Additionally, physical activity has decreased and sedentary behavior has increased. For example, recent data demonstrates that sedentary behavior is linked to T2DM and cardiovascular disease [89]. The increase in technological advances (e.g. video games and smart phone) has also been associated childhood obesity [90]. In a nationally representative survey, Kann et al [91] observed that 77% of children 9-13 years of age reported participating in physical activity, however as they enter high school only 29% report to participate in any physical activity.

**Obesity Link to Chronic Diseases**

Obesity is linked to serious health consequences and independently increases the risk of developing chronic diseases such as type 2 diabetes, heart disease, stroke, and certain types of cancer [1, 92]. Moreover, these metabolic disorders once only associated with adults now are being diagnosed in children. Diabetes mellitus is characterized as a disease that prevents the human body from efficiently utilizing glucose for energy [93]. Currently, type 2 diabetes (T2DM) accounts for ninety to ninety-five percent of cases of diabetes diagnosed [94]. T2DM is
a common metabolic disorder, whereas in the past it has been associated with the aging population it is now a common diagnosis in overweight and obese individuals [89, 95-97]. There is also an increased incidence of children being diagnosed with T2DM in the United States [98, 99]. The increase in the incidence of T2DM was first observed in children and adolescents in minority groups, such as Pima Indians in Arizona [100]. Within the Pima Indian population the frequency of the disease was 22.3:1000 in children and adolescents 10-14, and 51:1000 in adolescents 15-19 years of age [100]. T2DM also increases the risk of developing other chronic diseases, such as heart disease [101].

Coronary heart disease is the primary form of heart disease and is one of the leading causes of death in the United States [102, 103]. Recent research has shown that individuals with a higher BMI have an increased risk for developing heart disease [104]. In addition, previous research revealed that individuals with a higher BMI not only had an association with heart disease, but having a higher BMI could be a predictor of heart disease [105-107]. Obesity has been linked to heart disease and Kang et al [104] provided evidence that individuals with higher BMI showed signs of coronary syndrome. Research has also shown that overweight or obese children have an increased risk of developing diseases such as high blood pressure and high cholesterol which increase the risk of developing heart disease [108]. In addition, several studies have revealed that childhood obesity is associated with early onset of type I lesions in the artery walls, which are a precursor to the advancement of coronary atherosclerosis [109-113].

Nutrition interventions are an inexpensive method for treating and/or preventing chronic diseases associated with obesity [114-116]. For example, research observed that changing the macronutrient distribution in the diet (e.g. the ratio of protein, carbohydrate and fat), resulted in successfully decreasing food intake and increased EE, likely due to the satiating effect of
supplementation of O3FA [117]. Decreasing food intake is directly related to health status; over consumption of energy dense foods result in weight gain, increasing the risk for metabolic related diseases, such as T2DM, and cardiovascular disease [101, 118]. In addition, increasing EE is a mechanism for decreasing weight gain and regulating energy balance [30, 119]. Furthermore, the satiating effects of O3FA have also been observed [14]. The result of one study showed that the supplementation of O3FA in men and women aged 20-41 years over an eight week period randomized to either sunflower oil capsules, lean fish 150 g portion of cod 3 times per week, fatty fish 150 g portion of salmon 3 times per week, and, fish oil capsules (DHA/EPA) observed a significant decrease in body weight with O3FA intake (.45 times loss from baseline to midpoint), suggesting that diets with O3FA result in weight loss may be associated with increased satiety [120]. Taken together, this suggests that nutrition interventions at breakfast could be a potential preventative and treatment strategy.

**Energy Metabolism and Energy Expenditure**

Increasing energy expenditure through macronutrient composition is a well-established method to decrease fat mass [33, 35, 121-124]. Total body EE has three primary components; resting energy expenditure (REE), the energy needed to maintain bodily functions at rest; the thermic effect of feeding (TEF), the energy increase associated with digestion and absorption of macronutrients; and activity induce thermogenesis, energy associated with physical activity [29, 33]. REE represents 60% - 70% of whole body EE and is the component of EE that represents energy produced for the body to function at rest. Experts have linked body composition as being a major factor that determines REE and have found a disproportionally large span of kcal/d per individual [29]. AEE is the second largest component of EE, costing the body 20%- 30% of energy expended through physical activity (PA), and has the most variability of the three
measurements of energy expenditure [29]. The increase of metabolic rate after food consumption is the component of energy expenditure where the energy expended to breakdown food during thermic effect of food (TEF) [125].

Several studies have shown a relationship between the regulation of energy balance and obesity [126-128]. The regulation of energy balance is dependent upon calories consumed and is influenced by macronutrient composition [125]. TEF is considered one of the smaller components of energy expenditure, but could play a large role in the energy balance and assist in fighting obesity [129]. Maffeis et al [130] reported that the role of TEF and nutrient composition especially high fat intake in children may lower postprandial thermogenesis than a diet isoenergetic, isoproteic and lower in fat.

Dietary fat has been viewed as a contributor to obesity and not as treatment or preventative mechanism for obesity. Moreover, literature provides evidence that meal composition is a major contributor to metabolic homeostasis [131-133]. Thus, indicating that may be the fat source should be examined versus fat alone. Energy imbalance results in multi-metabolic dysfunctions, however O3FA’s have shown the reversal effects in health status, such as increased energy expenditure, subsequently inducing weight loss [134]. Logan et al [135] demonstrated that the supplementation of 3 g/d of O3FA over a 12 week intervention significantly increased REE by 14%. Therefore, supporting that consumption of O3FA may be a strategy to decrease fat mass. Previous and recent studies provide evidence that increased EE through modulating macronutrients at breakfast is an effective strategy prevent and treat obesity [4, 35, 136, 137]. However, there is a gap in knowledge in the effects of O3FA on EE in relation to school-aged children.

The Importance of Breakfast
The benefits of breakfast consumption have been extensively studied [6, 7, 136, 138], especially breakfasts higher in protein [35, 44, 53, 58, 139]. Breakfast has been defined across literature as being the first meal of the day consumed prior to 10:00 am [138]. In the literature, breakfast skipping has been defined as missing the breakfast meal more than five more times per week [138]. Eating habits such as breakfast skipping are also associated with diet quality [55, 140]. There is a negative association between breakfast skipping, the nutrient quality of the meal, and energy intake throughout the day [4, 35, 36, 44, 53, 58, 68, 69, 136, 141-144]. Data shows that children who do not regularly consume breakfast do not meet two-thirds of the recommended dietary allowance (RDA) for vitamins A, E, D, and minerals calcium, phosphorus, magnesium and riboflavin [5]. Affenito et al [145] observed the eating behaviors of 2,379 girls aged 9-19 and found that as the frequency in breakfast consumption decreased there was a reduction in calcium and fiber intake.

Childhood is a crucial period in life that requires adequate nutrition. A review of literature by Affenito et al [7] revealed that breakfast is a missed opportunity and between 10-30% of American’s and Europeans skip breakfast, particularly children aged 1-10 years and adolescents aged 11-18 years [7]. Moreover, there is an association between breakfast skipping and obesity [57, 146-148]. Literature has shown a correlation between increased BMI in individuals who skip breakfast compared with those who regularly consume breakfast [65, 66, 68, 149, 150], and this is a trend that continues through adulthood [56, 148, 151]. In addition, studies have shown that girls skip breakfast more often than boys, typically as means of weight loss [150, 152]. However, girls who skip breakfast are 15% more likely to be overweight or obese [149].
Breakfast intake has been observed to improve cognitive thinking and academic performance in children [150, 153]. Breakfast consumption is also associated with improved appetite response [44, 70, 139]. Leidy et al [44] conducted a study in breakfast skipping adolescents and found that breakfast intake increased the feeling of fullness compared with breakfast skipping. This observation is further supported throughout literature [5, 68, 70, 151, 154]. A review by McCrory et al [155] summarized similar findings from three acute studies (less than one day) where breakfast consumption increased the feeling of fullness and decreased energy intake (EI). In longer-term studies (more than seven days), Leidy et al [139] found that the intake of breakfast increased the feeling of fullness compared to 7 days of breakfast skipping.

Data has also shown that regularly consuming breakfast could be a successful strategy for improving metabolic health. Frid et al [54] observed in fourteen adults diagnosed with T2DM that meals supplemented with whey protein at breakfast or lunch increased insulin response and blood glucose reduced by -21%. Additionally, one study showed that the intake of breakfast has an acute response on appetite control resulting in reduced energy intake [139]

Macronutrient intake at breakfast is correlated with health status [132, 156, 157]. It is well established that omega-3 fatty acids (O3FA) decrease the risk of heart disease and improve brain development in children. Moreover, there is recent evidence suggesting that diets with omega-3 fatty acids (O3FA) supplementation may be a possible treatment for metabolic disorders such as obesity [39, 46, 158, 159]. In addition, there is still a gap in the scientific literature regarding the role of O3FA supplementation in children.

**Omega-3 Fatty Acids**

In previous years, diets high in fat have been suggested to increase chronic disease and risk of obesity in individuals [160-162]. However, the Dietary Guidelines for Americans 2015-
2020 [163], suggest that not all fats should be avoided, with the exception of trans and saturated fats. Trans and saturated fats are linked to metabolic disorders, such as CVD, high cholesterol, coronary heart disease, T2DM, and high blood pressure [161, 164, 165]. Willet et al [166] suggest in a review of short- and long-term studies that fat intake and obesity are not directly correlated. A review of the short term studies revealed a trend of modest weight reduction on a low fat diets and in longer term studies of a year or more there was a daily fat consumption between 18-40, which is suggested to have little effect on adiposity [166].

Fatty acids play an important role as metabolic substrates, involved in fat metabolism, and increasing lipogenesis a process mainly in the adipose tissue where the body converts excess glucose into fatty acids [167]. Polyunsaturated fats (PUFAs) are a classification of O3FA, and are described as an essential fatty acid due to our inability to synthesize them in the body, therefore they must be obtained through the diet. These fats are typically found in fatty fish (e.g. salmon) and nuts (e.g. walnuts) [168]. Another benefit of O3FA is the beneficial role they play in preventing and treating chronic diseases due to their anti-inflammatory properties and modulation of lipid metabolism [169, 170].

Omega-3 fatty acid supplementation has become of research interest due to its effects on body fat loss through the mechanisms of adipogenesis, lipid homeostasis, and reduced inflammation [38, 40, 171]. There is much supported evidence that O3FA are associated with decreasing the effects of cardiovascular disease (CVD) and that O3FA play an important role in providing DHA for growth and brain development [172-178]. Less is known regarding the effects of O3FA in weight loss and management, especially in children.

There are contradicting conclusions regarding fat intake in the literature. For example, a critical review by Anderson el al [179] describes the negative impact of fats on health, (e.g. ...
CVD, insulin resistance, increased levels of low-density lipoprotein). However, more recent data suggests the opposite [117, 135, 180, 181] The harmful effects of fat appear to be due to the type of fat (e.g. trans fat and saturated fat), and not the quantity of fat in the diet [182]. Therefore, the dietary guidelines have shifted focus regarding recommendations for dietary fat intake, by recommending an increase in the intake of healthy fats such as monounsaturated fat (O6FA; e.g. olive oil, avocados, and peanut butter) and polyunsaturated fat (O3FA; e.g. sunflower oil, salmon, trout, and walnuts), and decreasing the intake of saturated fats (e.g. hydrogenated vegetable oil and animal fats), in order to help lower the risk of CVD, a leading cause of death in the United States [163, 182-186]. However, there is recent evidence that reveals the positive effects on metabolic disorders such as CVD, insulin resistance, vitamin D deficiency associated with obesity, and non-alcoholic fatty liver disease [38, 47, 158, 187-190].

In a randomized, cross sectional study Maffeis et al [77] observed in ten obese prepubertal boys that a moderate fat meal (pasta, olive oil, grana cheese, and ice cream with 27% energy from fat) improved postprandial triglyceride levels. Additionally, providing evidence that meal composition is important in satiety, a meal with moderate fat reduced appetite, and was more effective in increasing TEF [77]. A recent study by Logan et al [135], where women ≥ 60 years of age were randomly assigned to either 3 g/d eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) or a placebo (olive oil) for 12 weeks. The diets with O3FA supplementation increased REE 14%, EE during PA 27%, lowered triglyceride levels by 29%, and increased lean mass by 4%. This supports previous findings by Gerling et al [117], who conducted a randomly assigned single-blind were active males were supplemented with 3.0 g/d EPA and DHA or olive oil for 12 weeks that found O3FA supplementation increased resting metabolic rate (RMR) and reliance on fat oxidation. However, as described above, dietary fat is
linked to slower gastric emptying which may result in postprandial satiation [191]. Furthermore, it is argued that the satiating effects of fats be due to a brain-gut connection such as gastrointestinal hormones secreted from the that control feeding (leptin, a satiety hormone and ghrelin, a hunger hormone) that circulate in the bloodstream relaying neural signals to the brain stem [192].

The regulation of fatty acid and triglyceride synthesis plays an important role in controlling metabolism, which influences energy storage and the development of obesity [193, 194]. There may be a strong association between O3FAs and the regulation of hepatic glucose output expressed in diabetes and obesity [195, 196]. Extensive reviews by Jump [194, 197] defined dietary fat as an important essential component in the diet, however dietary fat could result in energy imbalance and metabolic dysfunctions if consumed in excessive amounts [197]. Obesity is associated with excessive amounts of fat deposit in the tissue [198] Furthermore, diets high in fat (e.g. trans and saturated) have been shown to increase adiposity or amount of body fat an individual has compared with lean mass [199, 200]. There is also an associated increase in serum cholesterol with diets high in fat, which is linked to coronary heart disease [164, 201]. However, there is also evidence that these metabolic diseases can be prevented or treated with supplementation or consumption of foods high in O3FA [12, 158, 186, 189, 202].

A recent review summarized the effects of O3FA and omega-6 fatty acids (O6FA) and their ratio on obesity in the human diet; because of the increased intake of O6FA in the Western diet, changing the ratio from 1:1 to 20:1 or higher increased the prevalence of metabolic disorders associated with obesity, such CVD [46, 203, 204]. Randomized controlled trials examining the relationship between O3FA supplementation and health outcomes observed
positive changes in body composition, such as weight reduction, although not measured the weight loss may have been result of the effects of O3FA in appetite regulation.

Consumption of O3FA is also associated with improved appetite response. In an eight-week nutritional intervention by Thorsdottir et al [120], 320 men and women were randomized to four isocaloric diets; control capsules (6 times/day), cod 150 g (3 times/week), salmon 150 g (3 times/week), and fish oil capsules (6 times/day). Health outcomes measured included weight loss (6.5 kg ± 3.3 kg) in men and (4.2 kg ± 2.4 kg) in women. There was a significant interaction of EI by diet in groups that received diets with O3FA compared to those that received lean fish. Furthermore, supporting the previous findings, Parra et al [14] examined, in an eight-week nutritional intervention, how the supplementation of O3FA modulated satiety in overweight and obese males and females aged 20 – 40. Participants were randomized into four diet types; control capsules (6 times/day), cod 150 g (3 times/week), salmon 150 g (3 times/week), and fish oil capsules (6 times/day). At baseline, there was no difference in EI. However, immediately after consuming the fatty fish group had an immediate feeling of fullness compared with the lean fish, in addition the fatty fish group had more fullness compared with the lean fish group 2 hours postprandial. Concluding that O3FA supplementation of 6 fish oil capsules/day or the intake of 150 g of food rich in O3FA could be an effective weight loss strategy modulating postprandial satiety in overweight and obese individuals. In the same comparison study, correlation analysis showed significant correlations between circulating hormones leptin, ghrelin, and insulin with appetite response, a positive effect of O3FA consumption.

Hormones play a key role in appetite regulation [205]. Moreover, leptin is released from the adipose tissue and is link to satiety [206]. However, the amount of leptin circulating in the body increases with fat mass [207]. Therefore, overweight and obese individuals have an
increased amount of leptin compared to normal weight individuals, however overweight and obese individuals have a decreased responsiveness to leptin signaling [208]. Leptin acts on the hypothalamus; however, the hormone response is decreased with obesity and is known as leptin resistance [209].

Furthermore, Itoh et al [210] provided evidence in a single blind study that parallel to increased leptin signaling O3FA also increases circulating adiponectin, a hormone secreted from the adipose tissue. In 52 men and women that satisfied characteristics for metabolic syndrome it was found after a three-month purified EPA treatment of 1.8 g/day that plasma concentrations of adiponectin were increased compared to control (e.g. diet alone), in addition there was also a significant decrease in blood serum triglyceride concentration. Furthermore, these findings in humans supported previous finds in rodent and rat models where O3FA supplementation increased leptin [211-213]. Therefore, showing that there is a correlation between obesity and hormone regulation.

There is also emerging evidence that O3FA assist with muscle protein synthesis [47]. Smith et al [47] provided evidence that there is an interaction between muscle protein and lipid metabolism. In a study of healthy 25-45-year-old individuals showed an acute response, that with O3FA supplementation increased muscle protein synthesis [47]. Lastly, the effects of O3FA in insulin resistance has also proved positive [214]. Farsi et al [215] observed improved insulin sensitivity in a randomized study of four-forty T2DM participants after a 4 g/day supplementation of O3FA in gel capsule form over a 10-week period. In addition, supporting these finding Albert et al [216] assessed red blood cell EPA concentrations twice, 16 weeks apart found a 45% increase in insulin sensitivity in men who had a high O3FA index compared with
those who had a lower O3FA index. Therefore, these findings may further emphasize the importance of which source of fats are consumed and the health benefits associated with O3FAs.

Positive outcomes from O3FA supplementation and increased protein intake have been observed in both acute and chronic conditions. Although there is a lack of health outcomes with supplementation at breakfast, there is evidence of increased REE, and metabolic health, with similar findings in protein intake. However, additional information is needed in school-aged children. Furthermore, data is needed regarding the adaptive response to EE, and appetite hormones leptin and adiponectin.

**Objective and Hypothesis**

The objective of this proposed research is to determine if habitual O3FA intake at breakfast improves energy metabolism in overweight and obese school-aged children. We hypothesize that increasing O3FA intake at breakfast will improve energy metabolism and reduce energy intake throughout the day in overweight/obese school aged children.
MATERIALS AND METHODS

Participant Characteristics

This protocol was approved by the Institutional Review Board IRB approval # 15-09-094) and the Institutional Biosafety Committee (IBC) #16002 at the university of Arkansas. School age children, 8-12 years whom resided in Northwest Arkansas (Fayetteville, Bentonville, Rogers, Springdale and surrounding areas) were recruited to participate in this study. The participants were recruited through a University of Arkansas Newswire advertisement and via flyers in the Food Science Department building. Only participants who had no known food allergies, diet restrictions, ate breakfast habitually, were not picky eaters, were females who had not started menstruation, and did not have any other diet related conditions were recruited to participate in the study. Written consent was obtained from the legal guardian of the children as well as written assent obtained from the child to participate in this study. Male and female, normal weight and overweight/obese subjects were recruited to participate aged 8-12 years. Upon completion of the study the participants were compensated with $400 cash.

Two screenings were required to participate in the study. The first screening occurred via phone conversation with the parent or legal guardian of the child to determine if the minimum qualifications were met. The second screening occurred at the Health, Human, Performance, and Recreation (HHPR) building and included baseline height, measured to the nearest 0.1cm, and weight 0.1 kg for weight. The participants were then placed into one of two intervention classifications: 1) normal weight (NW) with BMI ≤85 percentile for age, sex, and height; or 2) overweight with BMI ≥85 percentile according to the CDC BMI percentile growth charts ages 2-19 years [217]. Body composition (fat-free mass and fat mass) was assessed with the use of Dual Energy X-Ray Absorptiometry (DEXA) (Lunar Prodigy, GE Healthcare).
Study Design

The study was a double-blind randomized, crossover design. Each participant in the study acted as their own control. Subjects were randomly assigned to one of the four treatment groups (NW control, NW O3FA (n=11), OW control, OW O3FA (n=9)) selected using Excel©, Microsoft 365 randomizing function to control for treatment type. All participants completed four study day visits over a six to ten-week period to the Energy Metabolism Laboratory in the Department of Food Science (refer to Appendix A for an outline of the study day). At each laboratory visit the participant had anthropometric measurements (height and weight) taken. On study day one (D1) each participant was randomly assigned to one of two color-coded isocaloric and macronutrient-matched breakfast beverage drink groups: breakfast beverage #1 omega-3 fatty acids-based or breakfast beverage #2 vegetable oil-based. Participants were given fifteen minutes to consume their assigned beverage. Following the first test day, each participant consumed the assigned breakfast beverages before 10AM and prior to consuming any other food item daily for two weeks. On study day 14 (D14) each participant returned to the Food Science Department around 7:30 AM in a fasted state to have blood drawn and REE measured. A minimum of a 7-day washout period occurred between each treatment to attain dietary adaptation control to eliminate residual diet from previous treatment [218]. This protocol was repeated for the second test beverage. All the breakfast beverages were provided to the participants throughout the duration of the study. Participants with the guidance of their parents were asked to complete a 3-day food record and physical activity levels during each week of the study, consecutive food record days were assigned to the participants to be recorded one-week day and two weekend days, however this is not standard protocol (two weekdays and one weekend day) and to correct this error we averaged the three-day food intake over the two-week
period per treatment. Day 8 and day 14 of each intervention subjects returned their empty beverage containers for ensured compliance.

**Test Breakasts**

Participants were assigned to one of two breakfast beverages presented in Table 1. All breakfast beverages were supplied by SmartFish (Oslo, Norway). Participants were provided containers by our lab and were instructed to shake the beverages to ensure that contents of the drink that may have separated were well mixed. Participants were instructed to place the straw that came with beverage into the container to assist with consuming it, then place their lips on the straw and begin to drink on the count of three so that they were ready to consume the beverage. At the first sip of the breakfast beverage the four–hour testing period began. The participants were required to finish the breakfast beverage within fifteen minutes or they were removed from the study.

**Anthropometric Measurements**

Body height was measured to the nearest 0.01 cm using a stadiometer (Detecto, St. Louis, MO) with participants barefoot, in a freestanding position. Body weight was measured in the fasting state with participants barefoot to the nearest 0.01 kg using a calibrated balance scale (Detecto, St. Louis, MO. BMI percentile was calculated using CDC Child and Teen calculator, [https://www.cdc.gov/healthyweight/assessing/bmi/childrens_bmi/about_childrens_bmi.html](https://www.cdc.gov/healthyweight/assessing/bmi/childrens_bmi/about_childrens_bmi.html). Body composition was assessed by dual energy x-ray absorptiometry (DXA; Lunar Prodigy, GE Healthcare, Belgium) in the Human Performance Laboratory at the University of Arkansas.

**Satiety Hormone Measurements**

On D1 of each dietary intervention, each participant had three individual blood draws repeated at time-points baseline (prior to breakfast treatment), 90, and 240 minutes, post
consumption of breakfast beverage. Blood collection was conducted by a licensed phlebotomist. Blood (10ml) was collected using one 10ml BD Vacutainer EDTA, Spray-Dried tubes (Becton, Dickinson and Company ©, Franklin New Jersey, USA) per time-point where three 10ml tubes totaling 30 ml of blood per participant D1 of each dietary intervention. Blood was centrifuged at 1,800 x g for 10 minutes at 4°C in an Allegra™ X-22R Centrifuge (Beckman Coulter, Inc., Brea, California, USA). Plasma was collected and stored at -80°C. The plasma collected was used to measure biomarkers of satiety (adiponectin and leptin), using 96 wells enzyme immunometric assay (EIA) kits, (Caymen Chemicals) and the Biotech Plate Reader (absorbance read at 450 nm) was used to read the intensity of color within each well to determine the immunological response of human adiponectin and leptin over time.

Energy Expenditure and Substrate Oxidation

The TrueMax 2400 metabolic cart (Parvomedics, Sandy, Utah) was used to measure resting energy expenditure (REE) in a supine, reclined position, and used the method of indirect calorimetry, a non-invasive measurement of metabolic rate in humans with the use of the ventilation hood technique that uses the heat produced by living organisms by measuring their production carbon dioxide [35, 219]. D1 REE (kilocalories per minute) was measured at baseline for 30 minutes [219]. After breakfast treatment consumption REE was measured in 20 minute increments starting at time-point 60 minutes, which is the first time-point being used after the breakfast beverage was consumed and to provide time in between each REE measurement so that the participant was not continuously under the metabolic hood and subsequently at 120, and 240 minutes to assess the thermic effect of feeding (TEF) [122, 220]. Respiratory quotient (RQ), the ratio of carbon dioxide expelled (VCO₂ liters per minute) to oxygen consumption (VO₂ liters per
minute) was calculated, providing a parameter of measurement for substrate oxidation rates [219, 221].

**Appetite and Palatability Assessment**

Appetite and palatability were assessed on D1 of each dietary intervention using visual analog scale (VAS) questionnaires. The questionnaires asked the participants to rate their perception at that moment of hunger, fullness, and desire to eat using a 100mm VAS with opposing anchors such as; not full or extremely full [222, 223]. There were 7 questions asked at baseline and subsequently at 15, 30, 60, 120, 180, and 240 minutes. Participants were asked to place an “X” along the line in the order of: “how hungry do you feel at this moment,” “how full do you feel at this moment,” “how strong is your desire to eat at this moment,” “how much food could you eat at this moment,” “how strong is your desire for something salty,” “how strong is your desire for something sweet,” “how strong is your desire for a snack.” Participants were also asked to evaluate palatability, “how much do you like the taste of the drink,” with opposing anchors “dislike extremely” or “like extremely” once they had consumed at least half of the breakfast beverage on D1.

**Dietary Assessment**

After the completion day 7 of each treatment participants returned weighed, food records to the laboratory. Participants were provided blank 24-hour food records and a digital scale with weight units in grams, ounces, pounds, and milliliters (Amir Direct®, Amazon.com, Seattle, WA). Participants were given a tutorial on how to record food consumed on their first laboratory visit and provided an example of the level of detail needed when completing the food records. The participants measured, weighed, and recorded each item consumed as well as the time of day with the assistance of their parent or legal guardian. To ensure compliance each food record
entry was reviewed with the parent and child to ensure each section was descriptive and complete. Food records were analyzed using Genesis R&D nutrient analysis software (ESHA Research, Salem, OR).

**Statistical Analysis**

Statistical summaries were performed by using net incremental area under the curve (niAUC) to calculate REE, protein and fat substrate oxidation, appetite ratings, adiponectin and leptin values [44]. Two-sample independent test were used to determine differences between NW and OW and to analyze participant characteristics, breakfast palatability, and comparison of niAUC between treatments (control versus O3FA). When significant differences were found two-sample independent t-test was used to determine the degree of significance. One-Way analysis of variance (ANOVA) were used to calculate differences in niAUC of treatment groups for appetite response, palatability, adiponectin, leptin, REE and to measure weighed food record macronutrient intake. Two-way ANOVA was used to examine significant differences between treatments and weight groups intake of diet over time for appetite responses, adiponectin, leptin, energy expenditure, and substrate oxidation. One-way ANOVA was used to analyze differences macronutrient intake and beverage palatability. When significant differences were found two-sample independent t-test was used to determine the degree of significance. Net increment under the curve (niAUC) was calculated for appetite rankings, REE, substrate oxidation, leptin and adiponectin concentrations. Where significance was observed the Tukey correction for multiple comparisons was applied within the analyses to two-sample independent t-test was used to determine the degree of significance. Results were expressed as ± standard error of the mean (SEM). GraphPad Prism 7 © (GraphPad Software, Inc. La Jolla, California, USA) was used to
analyze data and determine statistical significance. $P$-value of less than 0.05 was considered statistically significant.
Results

Participant Characteristics

After screening and participant selection, data from 20 children were collected. The physical characteristics of the participants are presented in Table 2. There was no statistical difference in age and height between NW and OW groups, however body weight \((P < 0.001)\), and BMI percentile \((P < 0.001)\) were higher in the OW group. Fat mass and free fat mass were higher in the OW group \((P < 0.01)\) and \((P < 0.05)\), respectively.

Energy Expenditure and Substrate Oxidation

Energy expenditure and substrate oxidation are expressed as line graphs (individual time points) and bar graphs (niAUC) in Figure 1. The overall analysis showed that O3FA trended to slightly increase REE, carbohydrate oxidation, and fat oxidation. However, there was no statistically significant effect of breakfast type in NW and OW, where we hypothesized O3FA would have an effect. Overall, there was no observed statistical significance in energy expenditure and substrate oxidation O3FA statistically significant increased. The results for RQ, \(\text{VO}_2\), \(\text{VCO}_2\) are presented in Table 3.

Appetite and Palatability Response

Based on the participants’ self-reported appetite and palatability ratings measured at each time interval using a VAS for perceived hunger, fullness, desire to eat, and prospective food consumption (how “much” food a participant perceived they could consume) are presented in line graphs (individual time points) and bar graphs (niAUC) Figure 2. For each appetite response, there was an effect of time between NW and OW groups \((P < 0.001)\). However, for fullness there was a significant breakfast over time interaction \((P < 0.05)\). The results for food cravings are presented in Figure 3. There was an effect of time \((P < 0.001)\). The perceived taste
response to each breakfast beverage was measured after the first beverage was consumed. There
was no significant difference in taste between control or O3FA breakfasts. Results are presented
in Table 2.

Satiety Hormone Variables

The results for leptin responses are presented in Figure 4. Leptin levels significantly
differed ($P < 0.05$) at time point 0 (prior to breakfast consumption) between NW control and NW
O3FA. In addition, OW control leptin values at time point 0 minutes were significantly higher
than at time point 90 minutes compared with OW O3FA. However, adiponectin did not result in
a significant difference as seen in Figure 5. There was no difference in niAUC leptin or
adiponectin concentrations between control or O3FA breakfast.

Dietary Assessment

The results for average daily energy intake are presented in Table 4. There was no
statistical significance of total food (kcal) intake in NW and OW groups consuming the Control
or O3FA treatments. However, there was a slight trend in both NW and OW participants
consuming O3FA to consume less food. In addition, there was a trend for both NW and OW
O3FA group to consume more protein and less carbohydrate. However, the OW, O3FA group
tended to consume more fat than in the OW, Control group.
Discussion

To our knowledge, this is one of the first studies to examine the effect of O3FA and protein supplementation on energy metabolism, energy intake, and appetite response in NW and OW school-aged children. It was hypothesized that increasing O3FA at breakfast would improve energy metabolism and reduce energy intake throughout the day in OW children. The primary finding was that breakfast consumption (both control and O3FA beverages) had an effect over time. Secondly, O3FA tended to slightly improve energy metabolism by increasing REE, presented in Table 3.

Furthermore, it has been suggested that dietary fats in particularly O3FA have metabolic effects as increased EE, satiety, and a decrease in the risk of certain metabolic disorders (e.g. T2DM and cardiovascular disease) in adults. Several studies have examined the effects of protein beverages on EE [144, 224, 225], however few of studies have examined the effects of O3FA on EE, especially in children [226]. The current study is unique because it is the first to compare the effects of O3FA intake on energy expenditure in 8-12-year-old children. In addition, many studies examining the effect of O3FA have used capsules as a supplement. The current study is unique because it uses O3FA incorporated into a breakfast beverage rather than as capsule. One study examined five male and five females who consumed either encapsulated (1 g) or emulsified (5 g ~ teaspoon) O3FA over a 48-hour timeframe. At the end of the intervention emulsified O3FA had an enhanced and extended rate of absorption compared to the encapsulated O3FA [226]. Although this study did not investigate the absorption rate of O3FA it did provide findings that OW participants had an increased REE over an acute period compared with the control. However, Logan et al [135], supported the findings in this study that O3FA increases EE in individuals in a study of older women where EE was increased by 14% with 3g/d O3FA
supplementation for 12 weeks. These findings provide evidence that increased O3FA in the diet increases EE, similarly to the results of this study. Although not examined directly at breakfast these studies did demonstrate that O3FA have a positive effect on increasing EE. The present study observed that O3FA tended to decrease carbohydrate and fat oxidation when consumed at breakfast, suggesting that there may be an association between O3FA and increased substrate oxidation. However, a longer data collection period is needed.

In terms of appetite regulation, a study where rats were injected with monounsaturated oleic acid (OA) or polyunsaturated O3FA docosahexaenoic acid (DHA) it was demonstrated that rats injected with O3FA had reduced energy intake and a decrease in body weight for 48 hours following injections [38]. In addition, Parra el al [14] showed that appetite control plays a major role in obesity in a study of 248 volunteers that suggested that O3FA intake modulates postprandial satiety in OW individuals. Participants in the current study had a significant increase in fullness following breakfast consumption, with no effect of either protein or O3FA. However, this could be because macronutrient percentage and caloric content were the same between breakfast beverages. It could also be due to the short duration of the appetite response period since appetite was assessed four hours postprandial on the first study day and not following the 14-day adaptation period.

Several studies focus on the effect of O3FA intake on increased leptin signaling in adults [213, 227, 228], there is no data on the effect of O3FA in children. Kazmi et al [229] studied 40 NW and 50 OW participants and found that OW adults had a much higher serum leptin levels, which is consistent with the finding in this study. Leptin concentrations are strongly associated with BMI and long-term regulation of leptin is dependent upon adiposity, due to leptin’s role in acting as a gauge to energy reserves that signals the central nervous system to adjust food intake
to assist with energy balance [230, 231]. However, breakfast skipping can interrupt energy balance and decrease leptin signaling [208, 232].

Children who consume breakfast regularly tend to have improved nutritional balance (intake of all needed calories, macronutrients, and micronutrients from diet to increase health status), and are less likely to have micronutrient deficiencies such as calcium and iron [149, 151, 233]. Children who consume breakfast are also less likely to be overweight or obese compared with those who skip breakfast [147]. However, data suggest that the macronutrient composition of breakfast is also important. Breakfast macronutrient composition is associated with improved health benefits such as improved circulating adiponectin and regulation of leptin signaling [208, 213, 227, 228, 234]. Our current study observed an increase in leptin concentration in both NW and OW children who consumed the O3FA breakfast compared with the control breakfast. There was a similar effect of O3FA was observed in a study using rat models fed demonstrated that the presence of O3FA in a sucrose–rich diet (SRD), prevented adiposity and increased circulating plasma leptin [235].

There were several limitations to this study. The first limitation was the duration, this study only focused on the postprandial effects of the first day of each dietary treatment. Future studies should examine the effects of O3FA supplementation after a longer adaptation period since most of the literature reports an effect of O3FA supplementation following several months of supplementation [117, 135]. However, O3FA content was comparable to levels published in literature. Several studies show the cardio-metabolic benefits of increasing O3FA intake in the diet [135, 236-238]. Another limitation was the difficulty in recruiting overweight and obese subjects. A longer recruiting period for future studies will be needed to increase the number of obese and overweight participants. The low sample size meant that this study may not have been
adequately powered to see significance. Finally, there was difficulty with taking blood samples from some subjects due either to anxiety from the participant or the inability to find a vein, which resulted in a lower sample size for some of the plasma analysis.

**Conclusion**

In conclusion, dietary interventions have shown promising results for the treatment of obesity. Breakfast consumption of O3FA tended to increased EE. In addition, there was an increase in carbohydrate oxidation in O3FA compared to control. Omega-3 fatty acid supplementation influenced satiety with and observed increase of fullness over time. Additionally, O3FA supplementation was observed to have a biochemical response on leptin in OW children. Taken together, these data suggest that increasing O3FA in the diets of school aged children has beneficial effects on EE, satiety, and metabolic responses throughout the day, however it may be beneficial to conduct more interventions in school aged children to have more comparative data.
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Figure 1. Day 1 postprandial EE and substrate oxidation after ingestion of either a control or O3FA breakfast in NW and OW children. Data are means ± SEMs; NW n = 11, OW n = 9. EE overtime per weight group and niAUC for each for each breakfast type combined (A). CO over time per weight group and niAUC with groups combined for CO each breakfast type (B). FO over time per weight group and niAUC with weights combined for FO for each breakfast type (C). O3FA, Omega-3 fatty acid based breakfast; REE, resting energy expenditure; CO, carbohydrate oxidation; niAUC, net increment under the curve, NW, normal weight; OW, overweight/obese.
Figure 2. Ratings of appetite after consumption of either control or O3FA in NW or OW children with the use of visual analog scales. Data are ± SEMs; NW n = 11, OW n = 9 children. Perceived hunger over time per weight group and breakfast type and net incremental area under the curve (niAUC) per breakfast type, with weight groups combined (A). Perceived fullness over time per weight group and niAUC per breakfast type, with weight groups combined (B). Perceived desire to eat over time per weight group and niAUC per breakfast type, with weight groups combined (C). Perceived much over time per weight group and niAUC per breakfast type, with weight groups combined (D).
Figure 3. Ratings of food cravings after consumption of either control or O3FA in NW or OW children with the use of visual analog scales. Data are ± SEMs; NW n = 11, OW n = 9 children. Perceived sweet craving over time per weight group and net incremental area under the curve (niAUC) per breakfast type, with weight groups (A). Perceived salty craving over time per weight group and niAUC per breakfast type, with weight groups combined (B). Perceived snack craving over time per weight group and niAUC per breakfast type, with weight groups combined (C).
Figure 4. Changes in leptin concentrations after consumption of either control or O3FA in NW or OW children. Data are means ± SEMs; NW *n* = 11, OW *n* = 9. Leptin concentrations net incremental area under the curve (niAUC) per breakfast type, with weight groups combined. Data points with a letter indicate a difference between breakfast types (control versus O3FA), *P* < 0.05. O3FA, omega-3 fatty acid, niAUC net incremental area under the curve; NW, normal weight; OW, overweight/obese.
Figure 5. Changes in adiponectin concentrations after consumption of either control or O3FA in NW or OW children. Data are means ± SEMs; NW n = 11, OW n = 9. Adiponectin concentrations niAUC per breakfast type, with weight groups combined. ns. O3FA, omega-3 fatty acid, niAUC, net incremental area under the curve; NW, normal weight; OW, overweight/obese.
Table 1. Dietary Characteristics of Test Breakfast

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>O3FA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy content, kcal</td>
<td>320</td>
<td>320</td>
</tr>
<tr>
<td>Total Protein, g</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Total Carbohydrate, g</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>Fiber, g</td>
<td>7.6</td>
<td>7.6</td>
</tr>
<tr>
<td>Total fat, g</td>
<td>17.2</td>
<td>17.2</td>
</tr>
<tr>
<td>Total O3FA, g</td>
<td>0</td>
<td>5000</td>
</tr>
<tr>
<td>DHA, mg</td>
<td>0</td>
<td>2000</td>
</tr>
<tr>
<td>EPA, mg</td>
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<td>2000</td>
</tr>
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</table>

Breakfast palatability, mm³  

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>O3FA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>61.3 ± 10.3</td>
<td>74.3 ± 9.3</td>
</tr>
</tbody>
</table>

1 Values are means ± SEMs unless otherwise indicated, n = 20. Control, protein + vegetable oil based-breakfast (fat source unknown); O3FA, protein + O3FA based-breakfast.

2 Total O3FA = 5000 g; 1000 mg fat source unknown

3 Units are in millimeters (mm) according to the traditional 100-mm visual analog scale
Table 2. Participant Demographics

<table>
<thead>
<tr>
<th></th>
<th>NW</th>
<th>OW</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Participants, n</strong></td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Age, y</td>
<td>9.8 ± 0.4</td>
<td>10.2 ± 0.5</td>
</tr>
<tr>
<td>Height, cm</td>
<td>144.4 ± 3.8</td>
<td>151.0 ± 4.5</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>34.9 ± 2.6</td>
<td>56.3 ± 4.8***</td>
</tr>
<tr>
<td>BMI Percentile, %</td>
<td>50.0 ± 10.0</td>
<td>100.0 ± 0.0***</td>
</tr>
<tr>
<td>Fat Mass, kg</td>
<td>7.3 ± 1.2</td>
<td>19.6 ± 2.7**</td>
</tr>
<tr>
<td>Free Fat Mass, kg</td>
<td>28.0 ± 2.7</td>
<td>36.2 ± 3.8*</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
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<td>Male</td>
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<tr>
<td>Female</td>
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<td>4</td>
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<tr>
<td><strong>Ethnicity</strong></td>
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<tr>
<td>African American</td>
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<td>3</td>
</tr>
<tr>
<td>†Biracial</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

1 Age, height, weight, BMI percentile, fat mass, and free fat mass are expressed as mean ± SEM or n. *, **, *** Different from NW *P < 0.05, **P < 0.01, ***P < 0.001. NW, normal weight; OW overweight/obese.

†Indicates one parent as Caucasian and one parent as African American
Table 3. Metabolic Variables following consumption of either control or O3FA- based test breakfast

<table>
<thead>
<tr>
<th>Time Following Breakfast, min</th>
<th>NW D1 (n=11)</th>
<th>OW D1 (n=9)</th>
<th>Effect of Time</th>
<th>Effect of Breakfast Type</th>
<th>Time x Breakfast Type Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>group</td>
<td>Control</td>
<td>O3FA</td>
<td>Control</td>
<td>O3FA</td>
<td>ns</td>
</tr>
<tr>
<td>RQ</td>
<td>0</td>
<td>0.79 ± 0.10</td>
<td>0.91 ± 0.02</td>
<td>0.92 ± 0.02</td>
<td>ns</td>
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<tr>
<td></td>
<td>60</td>
<td>0.79 ± 0.10</td>
<td>0.96 ± 0.01</td>
<td>0.93 ± 0.01</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>0.92 ± 0.02</td>
<td>0.92 ± 0.01</td>
<td>0.91 ± 0.02</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>240</td>
<td>0.78 ± 0.10</td>
<td>0.87 ± 0.02</td>
<td>0.86 ± 0.02</td>
<td>ns</td>
</tr>
<tr>
<td>VO₂ mL/min</td>
<td>0</td>
<td>127.28 ± 16.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>157.60 ± 8.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>204.22 ± 10.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>207.968 ± 14.54&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>60</td>
<td>150.67 ± 17.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>168.10 ± 18.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>224.47 ± 11.90&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>120</td>
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<td>240</td>
<td>145.29 ± 17.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>166.37 ± 7.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>208.09 ± 12.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>214.86 ± 19.13&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>VCO₂ mL/min</td>
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<td>145.44 ± 18.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>140.23 ± 7.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>185.49 ± 9.02&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
<td>240</td>
<td>125.96 ± 15.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>141.51 ± 6.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>182.48 ± 10.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>184.14 ± 17.57&lt;sup&gt;a&lt;/sup&gt;</td>
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</table>

<sup>a</sup>Values are means ± SEMs. Labeled means within a treatment column without a common upper case letter differ, P &lt; 0.05. Labeled means within a row without a common lower case differ P &lt; 0.05. If a row does not contain a lowercase letter, there is no difference within that row. Respiratory Quotient RQ.
**Table 4. Energy and macronutrient composition of food intake D1 in NW and OW children after Control or O3FA\(^1\)**

<table>
<thead>
<tr>
<th></th>
<th>NW (n=11)</th>
<th></th>
<th>OW (n=9)</th>
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<tr>
<td></td>
<td>Control</td>
<td>O3FA</td>
<td>Control</td>
<td>O3FA</td>
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<td><strong>Energy intake, kcal</strong></td>
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<tr>
<td>Total</td>
<td>867.7 ± 126.6</td>
<td>840.8 ± 68.8</td>
<td>963.1 ± 130.4</td>
<td>920.5 ± 117.2</td>
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<tr>
<td>Protein</td>
<td>87.1 ± 13.5</td>
<td>100.1 ± 10.8</td>
<td>80.5 ± 11.8</td>
<td>92.8 ± 12.9</td>
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<tr>
<td>Carbohydrate</td>
<td>349.8 ± 105.1</td>
<td>320.4 ± 54.3</td>
<td>489.0 ± 156.6</td>
<td>369.6 ± 93.0</td>
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<tr>
<td>Fat</td>
<td>430.8 ± 89.5</td>
<td>420.3 ± 76.7</td>
<td>393.6 ± 73.5</td>
<td>458.1 ± 116.0</td>
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<td><strong>Macronutrient intake, % energy</strong></td>
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<tr>
<td>Protein</td>
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<td>12</td>
<td>8</td>
<td>10</td>
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<tr>
<td>Carbohydrate</td>
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<td>38</td>
<td>51</td>
<td>40</td>
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<tr>
<td>Fat</td>
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\(^1\) Values are means ± SEMs. Data obtained from D1 (weekday or weekend), day 1 weighed food records. Control, vegetable oil based-breakfast (fat source unknown); NW, normal weight; OW, overweight/obese; O3FA, O3FA based-breakfast.
## Appendix A

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<th>Mins.</th>
<th>DEXA</th>
<th>Anthropometrics</th>
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<th>3-day food log</th>
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January 20, 2016

MEMORANDUM

TO: Jamie Baum
   Brianna Neumann
   Stephanie Shouse
   Charlayne Mitchell

FROM: Ro Windwalker
      IRB Coordinator

RE: New Protocol Approval

IRB Protocol #: 15-09-094

Protocol Title: Nutrition Intervention to Improve Energy Metabolism, Energy Intake, and Metabolic Response in Overweight and Obese School-Aged Children

Review Type: ☒ FULL IRB

Approved Project Period: Start Date: 01/19/2016 Expiration Date: 01/18/2017

Your protocol has been approved by the IRB. Protocols are approved for a maximum period of one year. If you wish to continue the project past the approved project period (see above), you must submit a request, using the form Continuing Review for IRB Approved Projects, prior to the expiration date. This form is available from the IRB Coordinator or on the Research Compliance website (https://vpred.uark.edu/units/rscp/index.php). As a courtesy, you will be sent a reminder two months in advance of that date. However, failure to receive a reminder does not negate your obligation to make the request in sufficient time for review and approval. Federal regulations prohibit retroactive approval of continuation. Failure to receive approval to continue the project prior to the expiration date will result in Termination of the protocol approval. The IRB Coordinator can give you guidance on submission times.

This protocol has been approved for 200 participants. If you wish to make any modifications in the approved protocol, including enrolling more than this number, you must seek approval prior to implementing those changes. All modifications should be requested in writing (email is acceptable) and must provide sufficient detail to assess the impact of the change.

If you have questions or need any assistance from the IRB, please contact me at 109 MLKG Building, 5-2208, or irb@uark.edu.
MEMORANDUM

TO: Dr. Jamie Baum

FROM: Ines Pinto, Biosafety Committee Chair

RE: Protocol Modification

PROTOCOL #: 16002

PROTOCOL TITLE: The impact of nutrient quantity, quality and timing on metabolic health throughout the lifecycle

APPROVED PROJECT PERIOD: Start Date August 13, 2015  Expiration Date August 12, 2018

The Institutional Biosafety Committee (IBC) has approved your request, dated March 6, 2016, to modify Protocol # 16002, “The impact of nutrient quantity, quality and timing on metabolic health throughout the lifecycle” on the condition that you do not ship any fecal samples until DOT (Department of Transportation) training is obtained and proof provided to the IBC.

The IBC appreciates your assistance and cooperation in complying with University and Federal guidelines for research involving hazardous biological materials.