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Effect of a Low Starch/Low Dairy Diet on Fat Oxidation in Overweight and Obese Women with Polycystic Ovary Syndrome

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Abstract

Background—Polycystic ovary syndrome (PCOS) affects between 4%- 18% of reproductive-aged women and is associated with increased risk of obesity and obesity-related disease. PCOS is associated with hyperinsulinemia which is known to impair fat oxidation. Research shows that carbohydrates from dairy and starch-based foods cause greater postprandial insulin secretion than carbohydrates from non-starchy vegetables and fruits.

Objective—To determine whether an *ad libitum* 8-week low starch/low dairy diet would improve fasting and postprandial fat oxidation after a high saturated fat liquid meal (HSFLM) in overweight and obese women with PCOS.

Methods—Prospective 8-week dietary intervention using a low starch/low dairy diet in 10 women (BMI ≥25kg/m^2 and ≤45kg/m^2) with PCOS. Indirect calorimetry was used at fasting and for 5 hours following consumption of the HSFLM to determine respiratory exchange ratio (RER), macronutrient oxidation, and energy expenditure (EE) at week 0 and week 8.

Results—Participants had a reduction in body weight (−8.1±1.8kg, p<0.05) and fasting insulin (−19.5±8.9μg/mL, p<0.05) after dietary intervention; however, these were not significantly correlated with improved fat oxidation. There was a reduction in fasting RER, and fasting and postprandial CHO oxidation, and an increase in fasting and postprandial fat oxidation after adjusting for body weight. There was also significant difference in incremental area under the

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The authors of this study have nothing to disclose, and they declare no conflict of interest.

curve (iAUC) from pre- to post-diet for fat (0.06±0.00 g/kg/5hour; $p<0.001$) and carbohydrate oxidation (−0.29±0.06 g/kg/5hour; $p<0.001$), but not for RER or EE.

Conclusions—An 8-week low starch/low dairy diet increased fat oxidation in overweight and obese women with PCOS.

Keywords

PCOS; hyperinsulinemia; respiratory exchange ratio; RER; insulin resistance

Introduction

Polycystic ovary syndrome (PCOS) affects between 4% and 18% of women of reproductive age worldwide and is associated with increased risk of type 2 diabetes mellitus, obesity, cardiovascular disease, cancer and infertility (Teede *et al.* 2010; Yildiz *et al.* 2012). Hyperinsulinemia is believed to contribute to or worsen all of these conditions (Barber *et al.* 2006; Mehran *et al.* 2012). Between 38% and 88% of women with PCOS are also overweight and obese (Barber *et al.* 2006). In addition to the genetic predisposition to obesity, insulin resistance and compensatory hyperinsulinemia makes weight loss difficult and worsens hyperandrogenemia in women with PCOS (Barber *et al.* 2006).

Metabolic inflexibility, or the incapacity to switch back and forth from lipid oxidation to glucose oxidation under insulin-stimulated conditions, has been shown to be a key feature in women with PCOS, which may be related to insulin resistance and compensatory hyperinsulinemia (Di Sarra *et al.* 2013; Moghetti *et al.* 2013). One study found that testosterone administration to female rats was followed by alterations in muscle morphology associated with insulin resistance (Holmang *et al.* 1990). After testosterone administration, there was an increase in plasma insulin levels and a redistribution of muscle fibers towards fewertype 1 fibers with lower density of capillaries; red (type 1) fibers are more insulin sensitive than white (type 2) fibers because of a higher density of insulin receptors (Holmang *et al.* 1990). Therefore, the resultant hyperinsulinemia and muscle fiber redistribution after testosterone treatment could potentially reduce skeletal muscle lipid oxidation (Kelley *et al.* 1999; Tanner *et al.* 2002). Di Sarra, et al (Di Sarra *et al.* 2013) found that serum free testosterone may also have an impact on the metabolic inflexibility in this population, possibly because of the influence of testosterone on insulin resistance. Research on metabolic flexibility has demonstrated the reduced capacity of insulin-resistant individuals to effectively oxidize fatty acids because of elevated insulin levels, leading to tissue accumulation of lipids as triglycerides in skeletal muscle and further impaired insulin signaling (Galgani *et al.* 2008). This reduced capacity to oxidize fatty acids is manifested as an impaired drop or lack of drop in overnight respiratory exchange ratio (RER), which has been associated with a diminished ability to switch from glucose to lipid oxidation during overnight fasting and is often seen in obese and insulin resistant individuals (Galgani *et al.* 2008).

Research has shown that insulin-mediated suppression of fatty acid oxidation (Bonadonna *et al.* 1990) can be increased with weight loss in insulin resistant individuals (Corpeleijn *et al.* 2009); however, recent research in mice also shows that hyperinsulinemia drives weight

gain and can make it difficult to lose weight (Mehran *et al.* 2012). The study by Mehran et al., (2012) used mice that were lacking the *Ins1* gene, which contributes to approximately one-third of secreted insulin, to determine whether these mice would be incapable of high-fat diet-induced hyperinsulinemia and obesity. The I*ns1* deficient mice were protected from diet-induced adult-onset weight gain, suggesting that pancreatic insulin hyper-secretion is required for diet-induced obesity and suppressed weight loss. A postprandial rise in insulin increases carbohydrate (CHO) oxidation acutely through the activation of glycolytic enzymes (i.e., phosphofructokinase); these glycolytic enzymes strongly decrease fatty acid oxidation by inhibiting fatty acid transport into mitochondria (Whigham *et al.* 2013). Lipoprotein lipase (LPL) is an enzyme responsible for lipolysis and is regulated by substrate availability; LPL activity is high after a high fat mealand low in the post-absorptive state. Hyperinsulinemia decreases expression of LPLin skeletal muscle, but increases LPL expression in adipose tissue, which drives lipolysis in the adipocyte leading to high levels of circulating fatty acids that further impair insulin signaling and lipid oxidation in skeletal muscle (Guilherme 2008). This reduced activity of skeletal muscle LPL, and subsequently reduced fat oxidation, has been linked with greater weight gain in several prospective studies (Zurlo *et al.* 1990; Seidell *et al.* 1992; Galgani *et al.* 2008). Thus, dietary modification that leads to a reduction in hyperinsulinemia may have important implications in improving fatty acid oxidation, facilitating weight loss, and preventing further weight gain in women with PCOS (Kopp 2003; Mehran *et al.* 2012).

Studies indicate that there is dissociation between the glycemic response and insulinemic response to certain CHO foods (Bao *et al.* 2009; Nimptsch *et al.* 2011), which may be even more evident in insulin resistant populations (Galgani & Valentino 2013). The specific carbohydrate foods that have shown to have an increased insulin response are starch-based foods and dairy foods, as well as foods with added sugars (Gannon *et al.* 1998; Hoyt *et al.* 2005; Nuttall & Gannon 2007; Hoppe *et al.* 2009). The purpose of this study was to determine whether an 8-week low starch/low dairy diet would improve fasting and postprandial fat oxidation in response to a high saturated fat liquid meal (HSFLM) challenge in women with PCOS. We hypothesized that an 8-week low starch/low dairy diet would decrease circulating insulin levels via the removal of insulinemic foods, resulting in an increase in both fasting and postprandial fat oxidation in overweight and obese women with PCOS.

Materials and Methods

Subjects

Ten overweight or obese women (mean body mass index (BMI) of 38.5±4.2 kg/m^2) with a confirmed diagnosis of PCOS were recruited from a gynecological/obstetrical and fertility clinic under the supervision of a reproductive endocrinologist (REI). Eligible women were between 18–45 years of age with a BMI ≥25kg/m^2 and ≤45 kg/m^2. Diagnosis of PCOS was based on oligomenorrhea and/or amenorrhea and the presence of hyperandrogenism (clinical and/or biochemical), consistent with the Rotterdam criteria (Rotterdam 2004). Oligomenorrhea was determined by cycle length (>35d), and amenorrhea was determined as lack of a menstrual period ≥12 months. Clinical hyperandrogenism (hirsutism, severe acne,

or androgenic alopecia) and/or biochemical hyperandrogenism (testosterone > 55 ng/dl) was assessed by the REI. All participants had at least 1 polycystic ovary by ultrasound. Women with adrenal enzyme defects such as Cushing's Syndrome or adrenal virilizing tumors, participants with type 2 diabetes, evidence of late onset 21-hydroxylase deficiency, or any other medical condition requiring supervision were excluded from the study. Women who were nursing during the length of the study, women with a confirmed eating disorder, and women with gastrointestinal absorption issues were excluded as well. Subjects were also expected to discontinue insulin sensitizers, oral contraceptives, and cyclic progesterone for 1 month prior to the study. All subjects gave their informed written consent before the study, which was conducted in accordance with the guidelines in the Declaration of Helsinki and approved by Texas Tech University Health Science Center's institutional review board. Further approval was granted by the University of Texas Medical Branch's institutional review board for data analysis and manuscript development.

Protocol

Weight, height, waist circumference (WC), hip circumference (HC), waist-to-hip ratio (WHR), and body fat percentage were measured on week 0 (pre-intervention) and week 8 (post-intervention). Weight and height were used to calculate BMI. Body weight and body composition was measured using the BOD POD (Cosmed Chicago, Illinois). Fasting and 2-h glucose and insulin were measured via a 75g 2-h oral glucose tolerance test (OGTT) with blood samples taken at 0 and 120 minutes pre- and post-intervention. HemoglobinA1c (HbA1c), a measure of long term glucose control, and total and free testosterone were measured via a fasting blood sample at pre- and post-intervention.

Indirect calorimetry was used for the metabolic measurements 1 week prior to beginning the study (pre-intervention) and within 1 week after the conclusion of the intervention (post-intervention). Subjects were asked to schedule a day to conduct these measurements as soon as possible following anthropometric and laboratory measurements on week 8, and were asked to continue to follow the diet until these final metabolic measurements were taken. Metabolic measurements were conducted before and after the diet intervention to ensure that it did not interfere with other laboratory measurements on week 8.

For the metabolic measurements, participants arrived at the Human Nutrition Lab at 0700 hours following a 12-h fast and at least 24-h without strenuous exercise or consumption of alcohol. Resting metabolic rate (RMR) was measured for 30 minutes using the ParvoMedicsTrueOne 2400 Canopy System (ParvoMedics, Sandy, Utah). For RMR testing, participants were asked to lie on a bed, avoid any movements, and remain awake during the test. Respiratory gases were used to calculate energy expenditure (EE) using the Weir equation (Weir 1949).

Following the baseline RMR measurement, subjects drank a HSFLM, which had a base of 8 floz (237 ml) of chocolate Ensure (Abbott Laboratories, Chicago, Illinois) containing a total of 6g of fat (1g of saturated fat (SFA), 3g of polyunsaturated fat, and 2g of monounsaturated fat), 40g of CHO, and 9g of protein. To increase the proportion of SFA to make the liquid meal a SFA-rich high-fat meal, we added 32g of butter, 5g of coconut oil, and 19g of palm oil to the chocolate Ensure. After the addition of dietary fat and lecithin, total grams of fat in

the high-fat meal equaled 56g which constituted 68% of total calories (Table 1). The subjects were instructed to finish the liquid meal within 5 minutes. A SFA-rich meal was chosen because of studies suggesting that SFAs may worsen insulin resistance in insulin resistant individuals (Riccardi *et al.* 2004). In addition, there have been some studies that support the idea that SFAs are implicated in weight gain and lower levels of fat oxidation (Lee *et al.* 2006; Casas-Agustench *et al.* 2009). Based on the fact that SFA-rich high-fat meals and diets are thought to contribute to weight gain, we decided to use a SFA-rich as a meal challenge in order to measure these important metabolic responses.

Following ingestion of the HSFLM, indirect calorimetry was used to measure respiratory gases for each participant in 30 min increments for a total of 330 min (30 min for fasting measurements during RMR plus 300 min (5 hours) for postprandial measurements). Respiratory gases were used to calculate macronutrient oxidation using standard equations developed by Frayn (Frayn 1983):Fat (g/min) = (1.67*VO_2 (L/min)) − (1.67*VCO_2 (L/min)); Carbohydrate (g/min) = (4.56*VCO_2 (L/min)) − (3.21*VO_2 (L/min). For these calculations, data was collected for 20 min and then subjects were allowed a 10 min break from measurement during each 30 min interval. Additionally, the first 5 min of each 20min segment of data was discarded to allow subjects to enter into a steady state. Therefore, the data presented here represents 15 min segments in each 30 min interval. Area under the curve (AUC) was calculated according to the trapezoidal rule. The metabolic cart was calibrated against methanol burns throughout the duration of the study (Schoffelen *et al.* 1997). The percentage recoveries from each burn were used to develop correction factors for the corresponding metabolic cart data from each study visit. The average recovery percentages for O_2 and CO_2 were 100.2% and 99.4% respectively. Diet induced thermogenesis (DIT) was calculated from EE (postprandial EE subtracted by baseline EE) (Clevenger *et al.* 2014).

Intervention

Subjects were instructed to follow a low starch/low dairy diet throughout the 8-week study. They received intensive diet education with a Registered Dietitian and were provided with written materials. Subjects did not meet with the Registered Dietitian again until week 8. The diet included *ad libitum* consumption of lean animal protein (meat, chicken, turkey, other fowl, fish, shellfish, and eggs), non-starchy vegetables, fruits (including fatty fruits, such as avocado and olives), nuts, seeds, and oils. Subjects older than 21 years were allowed one 6 oz (177.44 mL) glass of red wine per day, and all subjects were allowed up to 1 oz (28.35 g) of prepared or fresh, full-fat cheese per day. Cheese has not been found to be as insulinemic as other dairy products because of its low whey content and was allowed in restricted amounts to aid in dietary compliance. The diet excluded all grains (refined and whole), beans, pulses, dairy products (low-fat and whole milk and milk products), and sugar (including fruit juice from concentrate, cane sugar, beet sugar, raw turbinado sugar, evaporated cane juice, brown rice syrup, high-fructose corn syrup, corn sugar, honey, or a gave nectar) because of their insulinemic properties. Non-nutritive sugar substitutes were allowed for participants that wished to use them. Participants were not advised to count calories or CHOs and were encouraged to eat until they were satisfied, but not to overeat.

Participants were instructed not to change their level of physical activity throughout the intervention.

Three day food records were collected at weeks 1, 4, and 7 to determine both dietary compliance and each participant's estimated food quotient (FQ) using Black's Formula (Black 1986). FQ has been used to predict fasting RER in previous studies (Black 1986), which provides data to determine whether macronutrient oxidation matches macronutrient intake in study participants.

Assays

Glucose, insulin, and HbA1c were run by one laboratory (University Medical Center, Lubbock, Texas), and testosterone and free testosterone assays were run by another laboratory (Quest Diagnostics Nichols Institute, San Juan Capistrano, California). All assays performed on the Roche Diagnostics Cobas 6000. Glucose was performed by enzymatic reference method with hexokinase, HbA1c by turbidimetric inhibition immunoassay, insulin by electrochemiluminescence immunoassay, and testosterone by electrochemiluminescence immunoassay. Free testosterone was calculated by taking the concentrations of total testosterone and sex hormone binding globulin into account and assuming a fixed albumin concentration of 43 g/L, as described elsewhere (Vermeulen *et al.* 1999).

Statistical Analysis

Two-tailed paired *t* tests were used to test for statistical significance of anthropometric and biochemical outcomes variables. Simple and partial correlations were analyzed by Pearson's correlation analysis. A repeated measures ANOVA was used to analyze diet and diet-time interaction in resting (preprandial) and postprandial measurements, and results were compared with the associated paired *t*-test for pre- and post-intervention AUC and incremental AUC (iAUC). In the case of significant repeated measures ANOVAs on both the raw data and change in substrate oxidation over the time course (each time point minus fasting), Bonferroni and Tukey's adjusted and unadjusted post-hoc tests were compared to see which time points were significantly different. All data analyses were performed using SAS version 9.3 (SAS institute, Cary, North Carolina). A p value ≤ 0.05 was used to determine statistical significance for all analyses, with the Bonferroni correction used in the case of testing multiple time points following ingestion of the HSFLM.

From the food logs, we were able to calculate pre- and post-diet measurements for all dietary outcome variables. From this data, paired *t* tests were employed to probe for statistically significant changes. Some of the outcomes of interest were significantly non-normally distributed, as determined by a battery of normality tests. However, as the paired *t* test is robust against the violation of the assumption of normality, we only departed from the parametric *t* test in cases of extremely high outlying observations (such as 2 h insulin > 1000 μg/mL).

Results

Participants had an average age of 29.6 ± 4.6 years. Thirteen participants were initially recruited and tested at baseline; one of those participants was not compliant with the diet,

one could not schedule follow-up testing because of work conflicts, and one experienced claustrophobia during testing. Of the 10 participants that completed the study, 6 were White, 3 were Hispanic, and 1 was Native American. All participants had a reduction in total body mass, fat mass, percent fat, BMI, WC and HC (Table 2), despite the fact that the intervention was not designed as a weight loss diet (i.e., participants were not allowed to increase physical activity levels and were instructed to eat approved foods *ad libitum* without calorie or CHO counting). While participants were instructed to eat *ad libitum,* nutritional analysis of food records showed that participants consumed approximately 1400 kcal, 71g fat (20g saturated, 33g monounsaturated, 18g polyunsaturated), 90g carbohydrate, 23g fiber, and 94g protein per day. According to food records, all participants were compliant with dietary recommendations. The average total daily energy expenditure (TDEE) using a physical activity level of 1.65 (IOM 2005) was approximately 2814 kcal/day for our study participants. Therefore, participants were following a hypocaloric diet despite the fact that this was not designed to be an energy restrictive diet.

There was a significant reduction in fasting insulin ($p < 0.001$) from pre- to post-intervention. Two-hour insulin was found to be significantly reduced ($p = 0.02$) using the Wilcoxon Signed Rank because of non-normally distributed data (Table 2). There was no significant changes in WHR, percent fat free mass, lean body mass, fasting or 2 h glucose, HbA1c, or free testosterone from pre- to post-intervention. There was, however, a significant correlation between change in weight and change in free testosterone ($\rho=0.862$, $p=0.001$), but not change in fasting insulin or change in total testosterone.

Respiratory Exchange Ratio

A repeated measures ANOVA showed that the low starch/low dairy diet was significant in reducing mean fasting RER from 0.85±0.05 to 0.77±0.02 from pre- to post-diet, which resulted in a change of −0.08±0.01 ($p < 0.001$) (Table 2). Post-hoc analyses revealed Bonferroni significant differences ($p<0.05/11=0.0045$) between pre- to post-intervention in RER at all 11 time points. Since fasting values from pre- to post-diet were significantly different, we calculated the change in substrate oxidation (RER, fat, and CHO oxidation) over the postprandial period (each time point minus fasting) in order to isolate the meal response. There was no significance in the change in postprandial RER at any time point ($p = 0.66$) although the overall effect of time and time x diet interaction in the change in postprandial RER were found to be significant ($p = <0.001$ and $p = <0.001$, respectively) (Figure 1). While a paired *t* test found a significant reduction in AUC RER ($p < 0.001$) after the low starch/low dairy diet (Table 2, Figure 1), the comparable test for iAUC was not significant ($p=0.70$) (Figure 1). This lack of significance suggests that the major effect of the diet served in decreasing fasting RER rather than having a large effect on the postprandial RER responses. The partial correlation between the pre-to post-diet change in RER and change in fasting insulin after adjusting for change in weight ($\rho=0.067$, $p=0.86$), BMI ($\rho=0.079$, $p=0.84$), free testosterone ($\rho=0.0784$, $p=0.84$), and total testosterone ($\rho=0.110$, $p=0.78$) were all found to be non-significant. The correlations between change in fasting insulin and fat and CHO oxidation indices were also not statistically significant.

While fasting RER post-intervention was reflective of the food quotient (FQ) that was analyzed using Black's Formula (Black 1986), a small, albeit statistically significant, difference was found between the 2 measurements (Table 3). The FQ was calculated after averaging the nutrition analysis of food logs provided by the subjects undergoing metabolic testing on the Thursday, Friday, and Saturday days that corresponded with weeks 1, 4, and 7. The average FQ for the 3 weeks of food logs was observed to be higher than fasting RER (0.80 vs. 0.77, respectively) and the associated paired *t* test found the difference between FQ and RER to be significantly different (mean difference = 0.03±0.02, p=0.004). In addition, the partial correlation (after adjusting for diet) between free testosterone levels and reduction in RER after adjusting for diet was found to be non-significant (ρ=0.139, p =0.570)

Energy Expenditure

A paired *t*-test showed that AUC EE was significantly reduced from 114.5±13.1 to 105.7±9.8 kcal from pre- to post-diet, resulting in a change of −8.7±3.2 kcal (p = 0.005) from pre- to post-diet; however, the comparable test for iAUC was not significant (p=0.84). Since all study participants lost a significant amount of weight, an EE that was adjusted for body weight (EE/kg) analysis was also conducted; however, a battery of normality tests suggested the change in AUC EE/kg was not normally distributed (p<0.05). As such, we found the median change in AUC EE/kg to be <−0.01 kcal with inner quartile range of 0.65 (p=1.000, Wilcoxon Signed Rank).

For the repeated measures ANOVA, the exponential transformation was applied to EE/kg to assure the assumption of normality was not violated. In this analysis, the time of the measurement was a highly significant factor (p<0.001) even after the Huynh-Feldt correction for violations of sphericity, but the time-diet interaction was insignificant (p = 0.389), suggesting that the diet factor did not affect EE/kg over time. There was no difference in fasting EE/kg from pre- to post-intervention (Table 2). There was a significant time effect (p <0.001) but no time-diet interaction when analyzing change in postprandial EE/kg (p = 0.50). This suggests that diet factors alone did not change EE, but rather the resulting weight change was responsible for the lower EE at post-intervention (Figure 2). Furthermore, AUC for the DIT showed no difference (+0.02 ± 0.05 kcal/kg, p = 0.439).

Fat Oxidation

The repeated measures ANOVA revealed that the low starch/low dairy diet was significant in increasing fasting fat oxidation after adjustment for bodyweight in kg (Table 2) as well as postprandial fat oxidation (p< 0.001). Cross-sectional tests for time using the Bonferroni method found a significant increase in baseline to post-intervention fat oxidation for all intervals after adjustment for bodyweight in kilograms. A paired *t* test showed a significant increase in AUC fat oxidation values from 0.05±0.01 to 0.08±0.01g/kg per 5hr (p<0.001) from pre to post diet which resulted in a change of 0.03±0.00g/kg per 5hr (p< 0.001). However, since fasting fat oxidation was significantly different pre- versus post-diet (Table 2), we looked at the change data to determine meal response differences. There was a significant time, diet and time-diet interaction in the change in postprandial fat oxidation (p <0.001, p<0.001 and p< 0.001, respectively) (Figure 3). The increases in the change in postprandial fat oxidation post-intervention were at all intervals between 60 min and 150

min. The comparable test for iAUC was also significant (0.06±0.03 to 0.12±0.03g/kg per 5hour; $p<0.001$).

CHO Oxidation

Not surprisingly, the repeated measures ANOVA showed that the low starch/low dairy diet was significant in reducing fasting CHO oxidation (Table 2) and postprandial CHO oxidation after adjustment for bodyweight in kilogram ($p< 0.001$). Cross-sectional tests of time concluded that there was a significant decrease in CHO oxidation from baseline to post-intervention at all intervals using the Bonferroni method, after adjustment for body weight per kilogram. A paired *t* test showed a significant reduction in adjusted AUC CHO values from 0.16±0.02 to 0.08±0.02g/kg per 5hour, a change of −0.08±0.01g/kg per 5hour ($p< 0.001$) after the 8-week low starch/low dairy diet. However, since fasting CHO oxidation was significantly different pre- versus post-diet (Table 2), we looked at the change data to determine meal response differences. Diet was also found to be significant after analyzing change in postprandial CHO oxidation ($p<0.001$), as well as time and time-diet interaction ($p< 0.001$ and $p< 0.001$, respectively). The decreases in the change in postprandial CHO oxidation post intervention were at all intervals between 30 minutes and 180minutes (Figure 4). The comparable test for iAUC was also significant (0.07±0.07 to −0.23±0.13g/kg per 5hour, $p<0.001$) (Figure 4).

Discussion

This is the first study to assess fasting and postprandial metabolic responses to a high-fat meal challenge in women with PCOS before and after an 8-week low starch/low dairy diet intervention. Our data suggest that at baseline, women with PCOS have elevated fasting and postprandial RER, indicating an impaired ability to oxidize fats after an overnight fast and a high fat meal challenge. The 8-week low starch/low dairy diet led to a reduction in fasting RER, a reduction in both fasting and postprandial CHO oxidation and an increase in fasting and postprandial fat oxidation. This was true both before and after adjustment for body weight. There was also a significant reduction in weight, BMI, WC, HC, fat mass, percent fat mass, fasting and 2h insulin, and total testosterone after the 8-week dietary intervention. While an improvement in markers of insulin resistance may be related to the reduction in BMI in our study participants, it is worth noting the difficulties this population faces in losing weight. We believe that because of the *ad libitum* nature of the diet (no calorie or carbohydrate counting), it was less restrictive and required less education (i.e., portion size, calorie counts, carbohydrate grams) for the participants. In addition, participants naturally ate fewer calories despite being allowed to eat to satiety, which could have been due to the high protein content or high fiber content. Therefore, this dietary approach is potentially a clinically therapeutic option. Future research on the long term sustainability of this type of diet in a PCOS population is warranted.

Hyperinsulinemia has been shown to be strongly associated with obesity, but whether hyperinsulinemia drives obesity or is simply a compensatory response to obesity-driven insulin resistance remains unknown and is a topic of great debate (Sigal *et al.* 1997; Mehran *et al.* 2012). Recent research has shown that diet-induced pancreatic insulin over-secretion

by the consumption of a high glycemic/high insulinemic diet promotes obesity and its associated complications (Heller 1994; Sigal *et al.* 1997; Isken *et al.* 2010; Mehran *et al.* 2012). According to Mehran et al (2012), there is a critical need to investigate approaches to reduce hyperinsulinemia for the prevention and treatment of obesity. Our findings demonstrate for the first time that reduction of insulinemic foods during a hypocaloric diet leads to weight loss and a reduction in serum insulin levels, as well as an increase in fasting and postprandial fat oxidation in women with PCOS. However, the improved anthropometric and biochemical outcomes and increase in fat oxidation could be due to the weight loss, the diet, a reduction in overall energy consumption, or a combination of the 3. Since all subjects lost weight, and body weight and diet are associated, separating the effects of diet and weight loss on these metabolic outcomes is difficult. In addition, the partial correlation between change in RER and change in fasting insulin after adjusting for change in weight, BMI, free testosterone, and total testosterone were all found to be non-significant. In addition, change in fasting insulin was not significantly correlated with fat and CHO oxidation indices. It is therefore unknown whether the concomitant reduction in fasting insulin levels after the low insulinemic diet is associated with an improvement in fat oxidation.

The association between insulin resistance and metabolic inflexibility has been previously demonstrated in women with PCOS and other populations exhibiting insulin resistance, such as individuals with obesity and type 2 diabetes (Zurlo *et al.* 1990; Galgani *et al.* 2008; Di Sarra *et al.* 2013). Interestingly, previous findings by Di Sarra et al (2013) found that serum free testosterone levels were an additional predictor of metabolic inflexibility in women with PCOS, independently of muscle and adipose tissue insulin resistance and body fat. Since a non-significant reduction in serum free testosterone after dietary intervention was observed in the present study (Table 2), it is not surprising that the partial correlation (after adjusting for diet) between free testosterone levels and reduction in RER after adjusting for diet was found to be non-significant. There was, however, a significant correlation between change in weight and change in free testosterone.

Research is conflicting on whether obesity, insulin resistance, or another component of PCOS is causing impaired fat oxidation. Previous research has shown that hyperinsulinemia decreases lipolysis in skeletal muscle, but increases lipolysis in adipose tissue, leading to high levels of circulating fatty acids that further impair insulin signaling and lipid oxidation in skeletal muscle (elevated RER)(Guilherme 2008; Whigham *et al.* 2013). At baseline, the fasting RER for the participants in our study was quite high (0.85±0.05 CO_2/O_2), which provides evidence of impaired fasting fat oxidation in our overweight and obese women with PCOS. Conversely, in the study by Di Sarra et al. (2013), hyperandrogenemic women had a fasting RER of 0.73. The BMI in our study participants, however, was much higher than those in the Di Sarra et al study (38.5 kg/m^2 versus 32.9 kg/m^2)(Di Sarra *et al.* 2013). This could indicate that the impaired fat oxidation we observed was due more to the fact that the women were overweight or obese, rather than the PCOS. In a different study, researchers found no metabolic abnormalities in obese women with PCOS compared with obese women without PCOS (Adamska *et al.* 2013). There are also several other studies that would support the notion that it is BMI, rather than PCOS, that is negatively affecting fat metabolism (Adamska *et al.* 2013). In a review by Kelley et al,(Kelley & Mandarino 2000),

they showed that fasting RER values of obese subjects without PCOS from several different studies ranged from 0.80 to 0.90. However, it is currently unknown whether the PCOS condition (i.e., hyperinsulinemia and hyperandrogenemia) is associated with impaired fat oxidation or whether obesity is the main factor. Further studies are needed that directly compare fasting RER in women with PCOS to other BMI-matched and hyperinsulinemic populations without PCOS.

Our study revealed a significant reduction in fasting RER after the diet intervention, indicating an increase in fasting fat oxidation. Further, the average FQ was slightly higher than the fasting RER at post-intervention, indicating that the subjects had been able to adjust fat oxidation to match, and actually exceed, dietary fat intake. This indicates that participants were actually in a negative fat balance. Pre-intervention food records were not obtained, however, so it is unclear whether improvements in metabolic flexibility occurred. While we found differences in both fasting and post-meal fat oxidation after adjusting for changes in body weight, we found no improvements in post-meal RER (no weight adjustment is made because RER is a ratio which may be why we found differences in fat oxidation but not RER). An increase in fat oxidation at rest and after consumption of a high fat meal would suggest an improvement in metabolic flexibility; however, considering the insignificant change in post-meal RER, this association is likely more complex. Additional studies are needed to determine whether a low starch/low dairy diet improves metabolic flexibility in women with PCOS, as well as studies designed to determine the length of time necessary for women with PCOS to adjust to substrate flux.

We found that after 8-weeks of eliminating insulinemic foods, women increased their fasting and postprandial fat oxidation. While an increase in fat oxidation with a lower insulinemic diet is somewhat predictable and has been shown in previous low calorie (Hainer *et al.* 2000; Tittelbach *et al.* 2000) and low carbohydratedietary intervention studies, the low starch/low dairy diet presented here was less restrictive than both low calorie and low carbohydrate diets. This is especially important considering the large body of evidence that suggests a reduced ability to oxidize fat predicts future weight gain (Zurlo *et al.* 1990; Seidell *et al.* 1992; Ravussin & Swinburn 1993; Marra *et al.* 1998). Further research is needed using this type of dietary approach in populations with a reduced capability to oxidize fat, as well as long term follow-up studies to determine whether increased ability to oxidize fat protects against future weight gain.

This study has several limitations including small sample size, lack of a control group and lack of pre-intervention dietary intake information. Since there was no control group, subjects served as their own controls in this pre/post intervention design. In order to analyze differences in metabolic flexibility in the metabolic measurements, a food frequency questionnaire or 3-day food record could have been administered to estimate pre-intervention dietary intake to provide a pre-diet FQ. Additionally, biomarkers or additional food logs could have provided increased confidence in dietary compliance. Urinary nitrogen excretion was not measured, thus, all metabolic calculations assume that protein oxidation did not change from pre- to post-diet. Metabolic measurements depended on subject availability, thus, timing of these measurements (i.e., 1 day versus 7 days) prior to or post-intervention may have influenced the observed effects. Finally, additional time

measurements during the OGTT could have provided more information regarding post-prandial insulin levels (i.e., 30, 60, 90, and 180 min).

In conclusion, we found that an 8 week low starch/low dairy diet resulted in increased fasting and postprandial fat oxidation in overweight and obese women with PCOS, but this was not significantly correlated with change in BMI, fasting insulin, or testosterone. As previously mentioned, there is conflicting research on whether hyperinsulinemia, obesity, or another component associated with PCOS is causing impaired fat oxidation. However, hyperinsulinemia has been shown in previous studies to be strongly associated with obesity, and methods for reducing serum insulin levels may lead to weight loss and increased fat oxidation (Mehran *et al.* 2012). It is important to explore whether a reduction in insulin levels leads to weight loss and subsequently increases fat oxidation, or whether reduced insulin levels lead to increases fat oxidation and subsequent weight loss. Further studies using a low starch/low dairy dietary approach should be designed where subjects meet calorie goals to prohibit weight loss in order to determine whether this dietary approach maintains improvement in metabolic outcomes via reduction in serum insulin levels, independent of weight loss. Considering the chronic disease risks associated with insulin resistance, hyperinsulinemia, and metabolic inflexibility, future prospective studies are needed to (*i*) determine to what extent a low starch/low dairy diet improves metabolic flexibility in other insulin resistant and metabolically inflexible populations and (*ii*) determine whether a low starch/low dairy diet improves metabolic flexibility independent of weight loss.

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References

Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril. 81:19–25.

Adamska A, Karczewska-Kupczewska M, Nikolajuk A, Otziomek E, Górska M, Kowalska I, Straczkowski M. Normal metabolic flexibility despite insulin resistance women with polycystic ovary syndrome. Endocr J. 2013; 60:1107–1113. [PubMed: 23801024]

Bao J, de Jong V, Atkinson F, Petocz P, Brand-Miller JC. Food insulin index: physiologic basis for predicting insulin demand evoked by composite meals. Am J Clin Nutr. 2009; 90:986–992. [PubMed: 19710196]

Barber TM, McCarthy MI, Wass JA, Franks S. Obesity and polycystic ovary syndrome. Clin Endocrinol (Oxf). 2006; 65:137–145. [PubMed: 16886951]

Black AE. Use of food quotients to predict respiratory quotients for the doubly-labelled water method of measuring energy expenditure. Human nutrition. Clinical nutrition. 1986; 40:381–391. [PubMed: 3771290]

Bonadonna RC, Groop LC, Zych K, Shank M, DeFronzo RA. Dose-dependent effect of insulin on plasma free fatty acid turnover and oxidation in humans. American Journal of Physiology - Endocrinology and Metabolism. 1990; 259:E736–E750.

Casas-Agustench P, López-Uriarte P, Bulló M, Ros E, Gómez-Flores A, Salas-Salvadó J. Acute effects of three high-fat meals with different fat saturations on energy expenditure, substrate oxidation and satiety. Clinical Nutrition. 2009; 28:39–45. [PubMed: 19010571]

Clevenger HC, Kozimor AL, Paton CM, Cooper JA. Acute effect of dietary fatty acid composition on postprandial metabolism in women. Exp Physiol. 2014

Corpeleijn E, Saris WHM, Blaak EE. Metabolic flexibility in the development of insulin resistance and type 2 diabetes: effects of lifestyle. Obesity Reviews. 2009; 10:178–193. [PubMed: 19207879]

Di Sarra D, Tosi F, Bonin C, Fiers T, Kaufman JM, Signori C, Zambotti F, Dall'Alda M, Caruso B, Zanolin ME, Bonora E, Moghetti P. Metabolic inflexibility is a feature of women with polycystic ovary syndrome and is associated with both insulin resistance and hyperandrogenism. J Clin Endocrinol Metab. 2013; 98:2581–2588. [PubMed: 23596136]

Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. Journal of Applied Physiology. 1983; 55:628–634. [PubMed: 6618956]

Galgani JE, Moro C, Ravussin E. Metabolic flexibility and insulin resistance. American Journal of Physiology - Endocrinology and Metabolism. 2008; 295:E1009–E1017. [PubMed: 18765680]

Galgani JE, Valentino G. Should insulin resistance degree be taken into account for assessment of glycemic index? The American Journal of Clinical Nutrition. 2013; 97:902–903. [PubMed: 23515393]

Gannon MC, Nuttall FQ, Westphal SA, Fang S, Ercan-Fang N. Acute Metabolic Response to High-Carbohydrate, High-Starch Meals Compared With Moderate-Carbohydrate, Low-Starch Meals in Subjects With Type 2 Diabetes. Diabetes Care. 1998; 21:1619–1626. [PubMed: 9773720]

Guilherme A. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nature reviews. Molecular cell biology. 2008; 9:367–377.

Hainer V, Stunkard AJ, Kunešová M, Parízková J, Štich V, Allison DB. Intrapair resemblance in very low calorie diet-induced weight loss in female obese identical twins. International Journal of Obesity & Related Metabolic Disorders. 2000; 24:1051. [PubMed: 10951545]

Heller RF. Hyperinsulinemic obesity and carbohydrate addiction: the missing link is the carbohydrate frequency factor. Med Hypotheses. 1994; 42:307–312. [PubMed: 7935072]

Holmang A, Svedberg J, Jennische E, Bjorntorp P. Effects of testosterone on muscle insulin sensitivity and morphology in female rats. American Journal of Physiology - Endocrinology and Metabolism. 1990; 259:E555–E560.

Hoppe C, Molgaard C, Dalum C, Vaag A, Michaelsen KF. Differential effects of casein versus whey on fasting plasma levels of insulin, IGF-1 and IGF-1/IGFBP-3: results from a randomized 7-day supplementation study in prepubertal boys. Eur J Clin Nutr. 2009; 63:1076–1083. [PubMed: 19471293]

Hoyt G, Hickey MS, Cordain L. Dissociation of the glycaemic and insulinaemic responses to whole and skimmed milk. Br J Nutr. 2005; 93:175–177. [PubMed: 15788109]

IOM. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Washington DC: The National Academies Press; 2005.

Isken F, Klaus S, Petzke KJ, Loddenkemper C, Pfeiffer AFH, Weickert MO. Impairment of fat oxidation under high- vs. low-glycemic index diet occurs before the development of an obese phenotype. American Journal of Physiology - Endocrinology and Metabolism. 2010; 298:E287–E295. [PubMed: 19934403]

Kelley DE, Goodpaster B, Wing RR, Simoneau JA. Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. Am J Physiol. 1999; 277:E1130–1141. [PubMed: 10600804]

Kelley DE, Mandarino LJ. Fuel selection in human skeletal muscle in insulin resistance: a reexamination. Diabetes. 2000; 49:677–683. [PubMed: 10905472]

Kopp W. High-insulinogenic nutrition--an etiologic factor for obesity and the metabolic syndrome? Metabolism. 2003; 52:840–844. [PubMed: 12870158]

Lee JS, Pinnamaneni SK, Eo SJ, Cho IH, Pyo JH, Kim CK, Sinclair AJ, Febbraio MA, Watt MJ. Saturated, but not n-6 polyunsaturated, fatty acids induce insulin resistance: role of intramuscular accumulation of lipid metabolites. Journal of Applied Physiology. 2006; 100:1467–1474. [PubMed: 16357064]

Marra M, Scalfi L, Covino A, Puente AE-D, Contaldo F. Fasting respiratory quotient as a predictor of weight changes in non-obese women. International Journal of Obesity & Related Metabolic Disorders. 1998; 22:601. [PubMed: 9665683]

Mehran AE, Templeman NM, Brigidi GS, Lim GE, Chu KY, Hu X, Botezelli JD, Asadi A, Hoffman BG, Kieffer TJ, Bamji SX, Clee SM, Johnson JD. Hyperinsulinemia drives diet-induced obesity independently of brain insulin production. Cell Metab. 2012; 16:723–737. [PubMed: 23217255]

Moghetti P, Tosi F, Bonin C, Di Sarra D, Fiers T, Kaufman JM, Giagulli VA, Signori C, Zambotti F, Dall'Alda M, Spiazzi G, Zanolin ME, Bonora E. Divergences in insulin resistance between the different phenotypes of the polycystic ovary syndrome. J Clin Endocrinol Metab. 2013; 98:E628–637. [PubMed: 23476073]

Nimptsch K, Brand-Miller JC, Franz M, Sampson L, Willett WC, Giovannucci E. Dietary insulin index and insulin load in relation to biomarkers of glycemic control, plasma lipids, and inflammation markers. Am J Clin Nutr. 2011; 94:182–190. [PubMed: 21543531]

Nuttall FQ, Gannon MC. Dietary management of type 2 diabetes: a personal odyssey. J Am Coll Nutr. United States. 2007:83–94.

Ravussin E, Swinburn BA. Metabolic predictors of obesity: cross-sectional versus longitudinal data. International journal of obesity and related metabolic disorders: journal of the International Association for the Study of Obesity. 1993; 17(Suppl 3):S28–31. discussion S41–22.

Riccardi G, Giacco R, Rivellese AA. Dietary fat, insulin sensitivity and the metabolic syndrome. Clin Nutr. 2004; 23:447–456. [PubMed: 15297079]

Schoffelen PF, Westerterp KR, Saris WH, Ten Hoor F. A dual-respiration chamber system with automated calibration. J Appl Physiol (1985). 1997; 83:2064–2072. [PubMed: 9390982]

Seidell JC, Muller DC, Sorkin JD, Andres R. Fasting respiratory exchange ratio and resting metabolic rate as predictors of weight gain: the Baltimore Longitudinal Study on Aging. International journal of obesity and related metabolic disorders: journal of the International Association for the Study of Obesity. 1992; 16:667–674.

Sigal RJ, El-Hashimy M, Martin BC, Soeldner JS, Krolewski AS, Warram JH. Acute Postchallenge Hyperinsulinemia Predicts Weight Gain: A Prospective Study. Diabetes. 1997; 46:1025–1029. [PubMed: 9166675]

Tanner CJ, Barakat HA, Dohm GL, Pories WJ, MacDonald KG, Cunningham PRG, Swanson MS, Houmard JA. Muscle fiber type is associated with obesity and weight loss. American Journal of Physiology - Endocrinology and Metabolism. 2002; 282:E1191–E1196. [PubMed: 12006347]

Teede H, Deeks A, Moran L. Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. BMC Med. 2010; 8:41. [PubMed: 20591140]

Tittelbach TJ, Mattes RD, Gretebeck RJ. Post-Exercise Substrate Utilization after a High Glucose vs. High Fructose Meal During Negative Energy Balance in the Obese. Obesity Research. 2000; 8:496–505. [PubMed: 11068955]

Vermeulen A, Verdonck L, Kaufman JM. A Critical Evaluation of Simple Methods for the Estimation of Free Testosterone in Serum. The Journal of Clinical Endocrinology & Metabolism. 1999; 84:3666–3672. [PubMed: 10523012]

Weir JB. New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol. 1949; 109:1–9. [PubMed: 15394301]

Whigham, LD.; Butz, DE.; Dashti, H.; Tonelli, M.; Johnson, LK.; Cook, ME.; Porter, WP.; Eghbalnia, HR.; Markley, JL.; Lindheim, SR.; Schoeller, DA.; Abbott, DH.; Assadi-Porter, FM. Current Metabolomics. 2013. Metabolic Evidence of Diminished Lipid Oxidation in Women With Polycystic Ovary Syndrome; p. 269-278.

Yildiz BO, Bozdag G, Yapici Z, Esinler I, Yarali H. Prevalence, phenotype and cardiometabolic risk of polycystic ovary syndrome under different diagnostic criteria. Hum Reprod. 2012; 27:3067–3073. [PubMed: 22777527]

Zurlo F, Lillioja S, Esposito-Del Puente A, Nyomba BL, Raz I, Saad MF, Swinburn BA, Knowler WC, Bogardus C, Ravussin E. Low ratio of fat to carbohydrate oxidation as predictor of weight gain: study of 24-h RQ. Am J Physiol. 1990; 259:E650–657. [PubMed: 2240203]

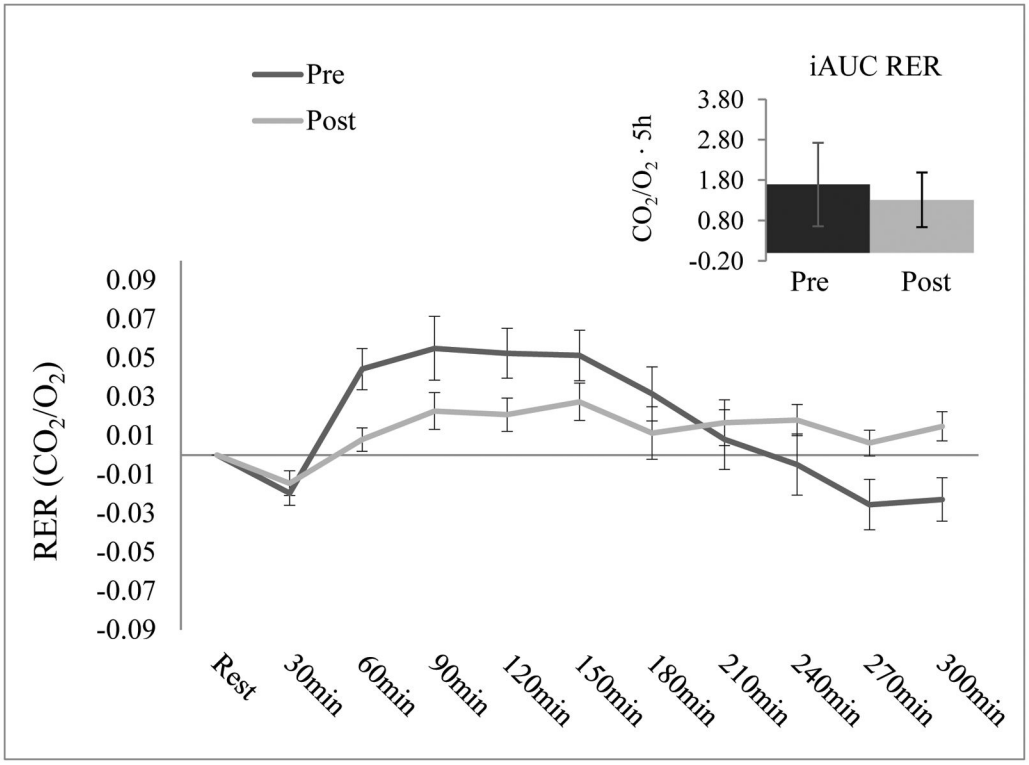

Figure 1.

Respiratory exchange ratio (RER). Change in RER and increamental area under the curve (iAUC) RER from pre- to post-diet. Rest indicates fasting measurements and all subsequent intervals represent postprandial measurements. Data shown here represent 15 min measurements per 30 min intervals. iAUC RER is reported in per hour units of time so that values represent physiological values. No significant differences in the change in RER were found from baseline to post-intervention.

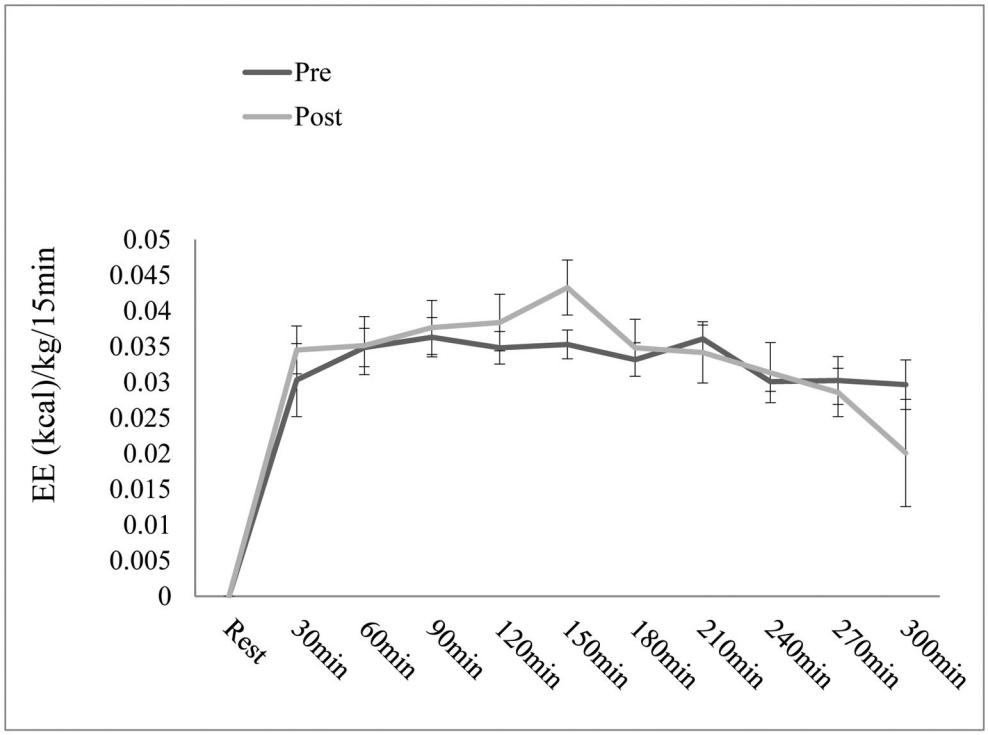

Figure 2.

Energy expenditure (EE). Change in EE from pre- to post-diet. Rest indicates fasting measurements and all subsequent intervals represent postprandial measurements. Data shown here represent 15 min measurement per 30 min interval. No significant differences in EE were found from baseline to post-intervention.

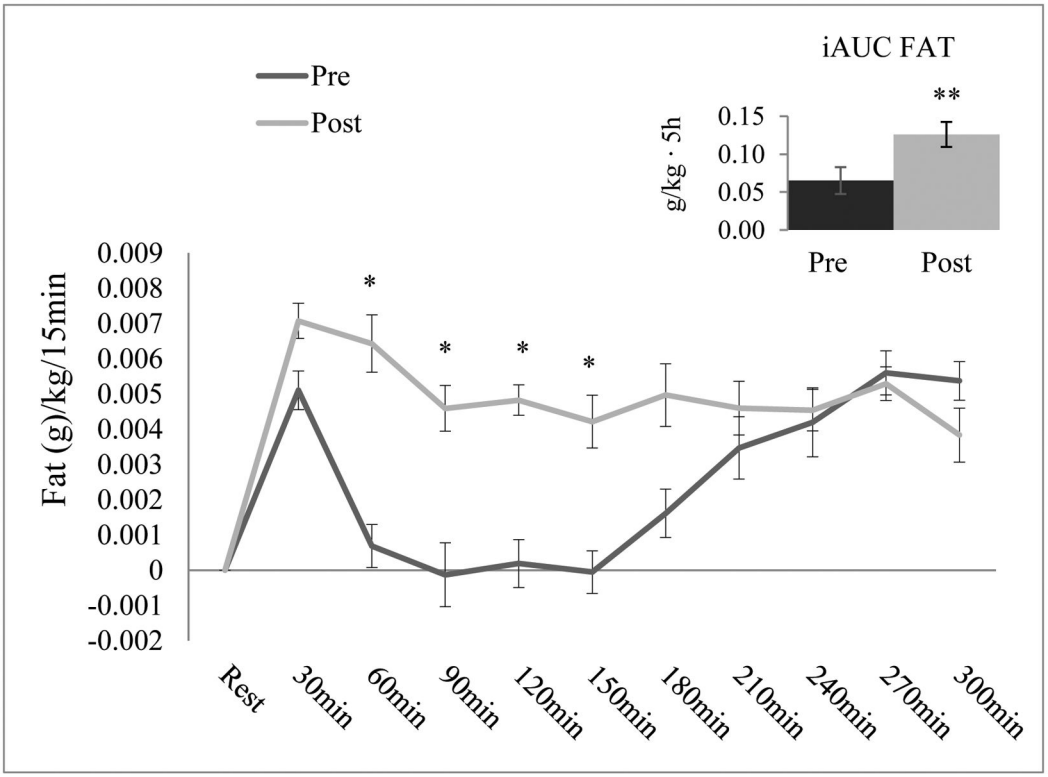

Figure 3.

Fat oxidation **(FAT).** Change in FAT and increamental area under the curve (iAUC) FAT from pre- to post-diet. Rest indicates fasting measurements and all subsequent intervals represent postprandial measurements. Data shown here represent 15 min measurements per 30 min intervals.

*$p < 0.05$

**significance for iAUC at $p < 0.05$

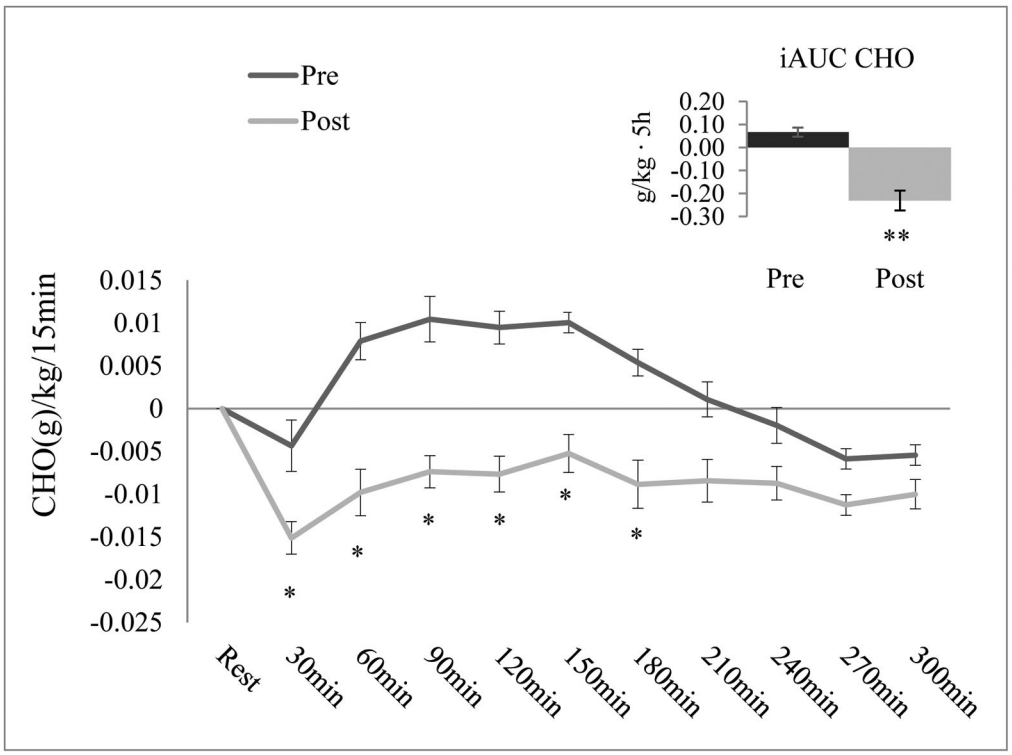

Figure 4.

Carbohydrate oxidation **(CHO).** Change in CHO and increamental area under the curve (iAUC) CHO from pre- to post-diet. Rest indicates fasting measurements and all subsequent intervals represent postprandial measurements. Data shown here represent 15 min measurements per 30 min intervals.

*$p < 0.05$.

**significance for iAUC at $p < 0.05$

Table 1

Nutrient breakdown for the high saturated fat liquid meal

	Total
Total Energy (kcals)	691
Energy from fat (kcals)	471
Energy from saturated fat (kcals)	311
Protein (g)	8.9
Carbohydrate (g)	43.0
Saturated Fat (g)	34.5
Monounsaturated Fat (g)	10.6
Polyunsaturated Fat (g)	3.8
% of total energy from fat	68.1%
% of total energy from saturated fat	44.9%

kcals: kilocalories; g: grams; %: percent

Table 2

Change in anthropometric, biochemical, and metabolic outcomes at baseline and after the 8-week dietary intervention ($n = 10$).

	Baseline ($x \pm SD$)	Week 8 ($x \pm SD$)	Change ($x \pm SD$)
Weight (kg)	105.4± 14.5	97.3± 13.9	−8.1 ± 1.8 *
BMI (kg/m^2)	38.5 ± 4.2	35.5 ± 4.5	−3.0 ± 0.6 *
WC (inches)	44.6 ± 3.7	41.5 ± 2.9	−3.0 ± 1.3 *
HC (inches)	51.5 ± 4.1	48.9 ± 4.3	−2.5 ± 1.3 *
WHR	0.87 ± 0.04	0.85 ± 0.04	−0.02 ± 0.04
Fat Mass (kg)	52.4 ± 14.8	45.7 ± 12.5	−6.6 ± 3.8 *
Lean Mass (kg)	52.3 ± 10.7	50.5 ± 11.4	−1.8 ± 2.5
FM (%)	49.3 ± 4.7	47.0 ± 4.8	−2.3 ± 2.5 *
FFM (%)	50.7 ± 4.7	52.5 ± 4.8	+1.8 ± 2.9
$Glucose_{fasting}$ (mg/dl)	91.6 ± 9.4	88.6 ± 5.8	−3.0 ± 8.9
$Glucose_{120\ min}$ (mg/dl)	131.7 ± 45.2	120.9 ± 20.4	−10.8 ± 47.6
HgbA1c (%)	5.5 ± 0.4	5.3 ± 0.3	−0.2 ± 0.3
$Insulin_{fasting}$ (μg/mL)	35.3 ± 7.0	15.8 ± 6.0	−19.5 ± 8.9 *
$Insulin_{120\ min}$ (μg/mL)	271.6 ± 285.0	132.3 ± 89.3	−139.3 ± 230.1 *
T_{total} (ng/dl)	53.2 ± 27.6	41.3 ± 19.6	−11.9 ± 14.0 *
T_{free} (pg/dl)	6.6 ± 3.1	6.1 ± 2.0	−0.51± 3.0
$RER_{fasting}$ (CO_2/O_2)	0.87 ± 0.03	0.78 ± 0.02	−0.09 ± 0.01 *
$EE_{fasting}$ (kcal/kg)	0.21 ± 0.02	0.21 ± 0.02	0.00 ± 0.00
$FAT_{fasting}$ (g/kg)	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00 *
$CHO_{fasting}$ (g/kg)	0.03 ± 0.01	0.01 ± 0.00	−0.02 ± 0.00 *

BMI: body mass index; WC: waist circumference; HC: hip circumference; WHR: waist-to-hip ratio; FF: fat mass; FFM: fat free mass; T: testosterone, RER, respiratory exchange ratio; EE, energy expenditure; FAT, fat oxidation; CHO, carbohydrate oxidation.

* = $p < 0.05$.

Table 3

Food quotient and post-intervention fasting respiratory exchange ratio (n=10)

Subject	FQ	RER	(RER – FQ)
1	0.79	0.78	−0.01
2	0.80	0.77	−0.03
3	0.79	0.75	−0.04
4	0.77	0.79	0.02
5	0.81	0.80	−0.01
6	0.78	0.79	0.01
7	0.82	0.80	−0.02
8	0.80	0.80	0.00
9	0.83	0.79	−0.04
10	0.80	0.77	−0.04
Total, mean (±SE)	0.80 (±0.01)	0.78 (±0.01)	−0.02 (±0.01) *

FQ, food quotient; RER, respiratory exchange ratio.

* p = < 0.05