RESEARCH REPORT
HUMAN NUTRITION

Title: A Randomized, Controlled, Crossover Trial to Assess the Effects of a Lean Pork-containing, High-protein Breakfast on Indices of Satiety and Metabolic Health In Men and Women with Prediabetes **– NPB #16-104**

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Industry Summary:
Experimental studies in humans designed to test the impact of pork consumption on glucose homeostasis and other elements of the cardiometabolic risk factor profile are limited. Previous research on pork intake and type 2 diabetes, metabolic syndrome and related components concluded that, compared to other commonly consumed protein sources including chicken, beef and shrimp, pork consumption did not alter markers of carbohydrate metabolism. Furthermore, results from the OmniHeart study, which compared three diets that emphasized carbohydrates, protein, or unsaturated fatty acids, indicate that a diet rich in protein from mixed sources reduces appetite compared to carbohydrate-rich or unsaturated fatty acid-rich diets (1). Previous studies completed by our group have also shown that replacing refined carbohydrates with lean protein reduces hunger, increases satiety, and reduces glucose and insulin excursions after a breakfast meal (2). However, whether the replacement of refined dietary carbohydrate (e.g., sugars and refined starches) with lean pork foods, a rich source of high-quality protein, at the breakfast meal will improve parameters appetite control and carbohydrate metabolism is not known. Thus, this research was conducted to provide a next step in evaluating the relationship between increased pork intake, appetite control, and glucose metabolism in subjects with pre-diabetes and thus at increased risk for the development of type 2 diabetes mellitus.

The primary objectives of this study were to assess the effects of consumption of a lean pork-containing, high protein breakfast versus a refined carbohydrate-rich breakfast for 2 weeks on satiety and metabolic factors in overweight or obese adults with pre-diabetes. Twenty-one adults completed the study, which involved consuming either the lean-pork containing breakfast or refined carbohydrate-containing breakfast daily for 2 weeks. The satiety effect of consuming the breakfasts and carbohydrate and lipid metabolism outcomes were determined at the end of each 2-week intervention.

The results show that intake of a lean-pork containing breakfast may have a favorable effect on some aspects of appetite, including less hunger and less desire to eat over the following 4 hours, but this effect does not appear to be robust enough to impact food intake at the following lunch meal, compared to a refined carbohydrate-containing breakfast. Results also suggest that eating a lean-pork breakfast meal may have a favorable affect on circulating glucose and insulin levels for the following 4 hours and circulating triglyceride levels for the following 2 hours, however, most other areas of carbohydrate or lipid metabolism were not impacted by breakfast meal type. Since this was a short-term study, further research on the long-term effect of consuming lean pork as a breakfast meal is warranted.

Keywords: pork; protein; refined carbohydrate; satiety; pre-diabetes; insulin sensitivity; lipid metabolism; glucose metabolism

Scientific Abstract:
BACKGROUND: Pre-diabetes is a common condition in the U.S. and places individuals at a higher risk for developing diabetes. Replacing refined carbohydrates (CHO) with protein in the diet may impact satiety and glucose and lipid metabolism.

OBJECTIVE: The objectives of this study were to assess the effects of consumption of a lean pork-containing, high protein (pPro) breakfast versus a refined CHO-rich breakfast for 2 weeks on satiety and cardiometabolic parameters in overweight or obese adults with pre-diabetes.

METHODS: In this crossover study, overweight or obese men and women with pre-diabetes were provided with either a pPro breakfast meal or a refined CHO breakfast meal (2-week intervention, ≥2-week washout). Visual analog scales (VAS) were used to determine satiety and related outcome measures; fasting glucose, insulin, lipids and related markers of glucose and lipid metabolism were assessed.

RESULTS: A total of 21 (13 females and 8 males) were included in the efficacy evaluable sample and had a mean (± standard error of the mean; SEM) age of 44.4 ± 3.1 y and a mean BMI of 30.4 ± 0.9 kg/m^2. Mean hunger net incremental area under the curve from pre-meal to 240 min post-meal ($niAUC_{0\text{-}240min}$) was significantly ($p = 0.041$) lower following the pPro breakfast intake compared to the refined CHO breakfast intake; mean desire to eat $niAUC_{0\text{-}240min}$ was also significantly ($p = 0.040$) lower following the pPro breakfast intake compared to the refined CHO breakfast intake. No other assessed markers of satiety, including mean $niAUC_{0\text{-}240min}$ for fullness and prospective consumption, daily VAS average scores for hunger and fullness and *ad libitum* lunch energy intake and food intake, were significantly affected by diet condition. Energy $niAUC_{0\text{-}240min}$ and focus $niAUC_{0\text{-}240min}$ were also not significantly affected by intake of a pPro breakfast or refined CHO breakfast. The mean incremental AUC for glucose and insulin were significantly lower, $p = 0.003$ and $p = 0.001$, respectively, following the pPro breakfast intake versus the refined CHO breakfast intake. The mean percent change from baseline for triglycerides (TG) at 120 min was significantly ($p = 0.006$) less pronounced following intake of the pPro breakfast (10.0 ± 6.8% increase) compared to intake of the refined CHO breakfast (32.3 ± 7.7% increase). No other significant differences were observed related to the assessed lipid parameters.

CONCLUSIONS: Intake of a lean-pork containing breakfast may have a favorable effect on some acute aspects of satiety, and circulating glucose, insulin and TG levels. Evaluation of the longer-term effects of some of the acute differences observed between consuming lean pork versus refined CHO at the breakfast meal is warranted.

Introduction

Pre-diabetes is a common condition affecting 86 million Americans in 2012 (3). Individuals with pre-diabetes are at higher risk for developing diabetes, a major risk factor for cardiovascular disease and other adverse outcomes (4, 5). Lifestyle changes, including diet and physical activity behavioral counseling targeting moderate (7%) body weight loss and at least 150 minutes per week of moderate-intensity physical activity, are recommended for the prevention or delay of type 2 diabetes (6). Data regarding the dietary patterns associated with a lower metabolic risk profile are still emerging, but diets rich in refined carbohydrates (CHO), including refined starches and added sugars, with a high glycemic load, have been shown to exacerbate metabolic risk factors (7-10), and to be associated with increased risk for the development of type 2 diabetes mellitus in prospective cohort studies (11).

Replacing CHO with protein has been shown to have favorable a metabolic effect, including improving the lipoprotein lipid profile (8). Results from dietary intervention studies indicate that partial replacement of refined CHO foods with foods containing protein and/or unsaturated fatty acids may improve insulin sensitivity and other aspects of the cardiometabolic profile (12-14). In addition, higher protein intake has been associated with greater satiety (2, 15-17), which may have implications for body weight regulation. Results from the OmniHeart study, which compared three diets that emphasized carbohydrates, protein, or unsaturated fatty acids, indicate that a diet rich in protein from mixed sources reduces appetite compared to CHO-rich or unsaturated fatty acid-rich diets (1).

Some evidence also indicates that the timing of energy and macronutrient intake may also have important physiological consequences that have the potential to impact dietary behavior and/or metabolic outcomes (2, 15, 18, 19). Eating a protein-rich breakfast may increase satiety and reduce appetite later in the day compared with a lower protein breakfast (2, 15, 20), which may be important for weight and metabolic management in overweight or obese, premenopausal women. Results from Rabinovitz et al. (2014) indicate that consuming a protein-rich, fat-containing breakfast (33% of total daily energy) improves glucose metabolism and markers of satiety compared to consumption of a smaller, refined CHO-rich breakfast (12.5% of total energy) in overweight or obese adults with type 2 diabetes following an isocaloric diet for 3 months (18).

Lean pork is a source of high-quality protein, and its effects have not been investigated among those with pre-diabetes, a group who may benefit from the metabolic and appetite effects observed with higher protein and lower refined CHO intakes. However, the effects of consuming a lean pork-containing, high protein (pPro) breakfast, versus a refined CHO-rich breakfast on satiety and markers of cardiometabolic risk in overweight or obese adults with pre-diabetes has not been previously investigated.

Objectives

The primary objectives of this study were to assess the effects of consumption of a lean pork-containing, high protein (pPro) breakfast versus a refined carbohydrate (rCHO)-rich breakfast for 2 weeks on satiety and cardiometabolic parameters in overweight or obese adults with pre-diabetes.

Materials & Methods:

Design

This randomized, crossover trial was conducted in accordance with Good Clinical Practice Guidelines, the Declaration of Helsinki (2000) (21) and the United States 21 Code of Federal Regulations. The institutional review board at Illinois Institute of Technology (Chicago, IL) approved the protocol before initiation of the study and subjects provided written informed consent before any study procedures were performed.

Subjects

Overweight or obese men and women, 18-74 years of age, with pre-diabetes (glycated hemoglobin of 5.7-6.4% or fasting capillary glucose of 100-125 mg/dL) and body mass index (BMI) of 25.0-39.9 kg/m^2 were recruited. Subjects had to be regular breakfast consumers, (at least 4 d/week), be willing to comply with study food

consumption and maintain their habitual physical activity patterns throughout the trial. Individuals were excluded if they had fasting glucose ≥126 mg/dL or known diabetes mellitus; had fasting triglycerides (TG) levels ≥500 mg/dL; reported a recent weight change of ±4.5 kg; used medications or supplements known to treat or alter lipid metabolism, CHO metabolism, weight loss or appetite; was a vegetarian or vegan; had an allergy, sensitivity or intolerance to any of the study foods or ingredients; or had a recent history of alcohol or drug abuse. Subjects with a history or presence of clinically important endocrine; cardiovascular (including, but not limited to, atherosclerotic disease, history of myocardial infarction, peripheral arterial disease, stroke); pulmonary; biliary; or gastrointestinal disorders or cancer (in the last 2 years); or had uncontrolled hypertension were excluded from the study.

Study Test Food Procedures

Eligible subjects were randomly assigned, at baseline, to consume one of two diet condition daily, either a lean pork protein-containing breakfast (500 kcal and 35/39/26% kcal from protein/carbohydrate/fat, respectively; pPro condition) or a refined CHO-rich breakfast (500 kcal and 8/66/26% kcal from protein/carbohydrate/fat, respectively; CHO condition) (**Table 1**). Subjects were provided with their respective pPro condition or CHO condition breakfast study foods, packaged into daily servings, and instructed to consume each day's assigned study foods during their usual daily breakfast period at home. Subjects were required to maintain their habitual diets for all other meals and snacks during each 2-week intervention period, and report back to the clinic on the last day of each 2-week intervention period.

On the last day of each 2-week intervention period, subject returned to the clinic fasted (12 ± 2 hours, water only) and were provided with 8 oz of water (t = -60 min). At t = 0 min, subjects were provided with their assigned pPro condition or CHO condition breakfast study foods, which they consumed in their entirety within a 15-min period. Following study food intake, subjects were provided with another 8 oz of water or other non-caloric beverage (i.e. caffeinated, non-caloric cola, caffeinated coffee, or tea with non-caloric sweetener), and a Palatability Questionnaire was completed immediately. Appetite assessments and clinical assessments were conducted. After the last day of the first intervention period, subjects were instructed to return to their habitual diets for ≥2-week washout period, after which subjects crossed over to the other diet condition.

Three-day diet records and the Stanford 7-day Physical Activity Questionnaire were completed at baseline and the end of each diet condition (Stanford ref). The nutrient profiles of both the pPro condition and refined CHO condition study foods, total dietary intakes and baseline dietary intakes were analyzed using Food Processor® Nutrition Analysis software (version 11.0.124, ESHA Research, Salem OR) using the 3-day diet records. Dietary compliance was assessed by calculating the servings of uneaten study foods provided and returned by the subject and dividing it by the total number of servings of study foods provided. Compliance of 100% indicated that 100% of all study foods provided for the diet condition intervention period were fully consumed.

Appetite Assessments

Visual Analog Scales (VAS)

On the last day of each intervention period, in-clinic VAS assessments for hunger, fullness, desire to eat, prospective consumption, focus and energy were completed at t = -15, 30, 60, 90, 120, 150, 180, 210, and 240 ± 3 min. Additionally, during each intervention period, an at-home VAS diary was dispensed to and collected from subjects to rate perceived fullness and hunger each evening prior to retiring.

Ad Libitum Lunch Intake

A tortellini and sauce lunch was provided to subjects on the last day of each intervention period at t = 240 ± 5 min. Subjects were allowed at least 25 min for lunch and were instructed to eat until comfortably full. Food was weighed to the nearest gram prior to and following consumption.

Clinical Assessments

Clinic visit procedures, including body weight and systolic and diastolic blood pressure measurements, were conducted at each clinic visit. Fasting (12 ± 2 hours, water only) blood draws were collected for measurement of plasma glucose, insulin and lipoprotein lipids at the baseline visit and at the end of each intervention period. Blood samples were collected for analysis of plasma glucose and insulin at t = -15, 30, 60, 120 and 240 ± 3 min and at t = 120 and 240 ± 3 min for fasting lipoprotein lipids.

Lipid Profile

Fasting lipids, including total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), non-high density lipoprotein cholesterol (non-HDL-C) and TG, levels were analyzed according to the Standardization Program of the Centers for Disease Control and Prevention and the National Heart, Lung and Blood Institute using enzymatic colorimetric methodology. LDL-C concentrations (mg/dL) were calculated according to the Friedewald equation as follows in mg/dL: LDL-C = TC – HDL-C – TG/5 (22). Non-HDL-C concentrations (mg/dL) were calculated by subtracting the HDL-C concentration (mg/dL) from the TC concentration (mg/dL) for each subjecct: non-HDL-C = TC – HDL-C.

Carbohydrate Metabolism

Plasma glucose was assessed using an enzymatic colorimetric method (Randox, Laboratories, Crumlin, UK), and Plasma insulin was assessed using an immunoturbidimetry assay (Kamiya Biomedicals, Tukwila, WA). At least 20 min prior to consumption of the study food breakfast, an indwelling intravenous catheter was inserted for obtaining blood samples. In order to maintain patency, the catheter was flushed with at least 10 mL normal saline solution at least hourly or normal saline was infused as a slow continuous intravenous drip. Fasting insulin sensitivity (HOMA2-%S) and beta-cell function (HOMA2-%B) indices were estimated using average glucose and insulin values according to homesostasis model assessments (www.dtu.ox.ac.uk/homacalculator/index.php) (23).

Statistical Analysis

The primary outcome variable was difference between test conditions in hunger (net incremental areas under the curve (niAUC) from pre-consumption (t = -15 min, used as t = 0 in the niAUC calculation) to post consumption (t = 240 min). Power calculations indicated that an evaluable sample of 18 subjects was expected to provide 80% power to detect a difference between treatment conditions of 0.67 standard deviations (8.4 mm) for the average postprandial hunger VAS rating, calculated as the net incremental area under the curve divided by 240 min (2). A sample of 22 subjects was randomized to allow for possible non-compliance and attrition. All tests of significance, were performed at alpha = 0.05, two-sided.

The primary analysis utilized data from all subjects who provided data during both diet conditions. A per protocol analysis was also completed in which subjects with inadequate compliance or other major protocol deviations were excluded. Because results did not differ materially between the two analyses, only those from the primary analysis are presented. Statistical comparisons were made for differences between conditions for some variables (e.g., nutrient intakes), and changes or percent changes from baseline for others. Statistical modeling was completed using a mixed model repeated measures analysis of variance using IBM SPSS Statistics, versions 22 and higher (IBM, North Castle, NY). The initial models contained terms for diet condition, sequence and, where applicable, baseline value as a covariate, with subject modeled as a random effect.

Assumptions of normality of residuals were investigated for each model. If significant non-normality for a variable or class of variables was detected using the Shapiro-Wilk test with an alpha level of 0.01, an analysis using rank transformation was applied. Diet condition by sequence interaction was modeled statistically and results in the two sequence groups were examined to identify evidence of carryover effects. Because no statistically significant carryover (treatment by sequence interaction) was noted, results are presented for the pooled sequence groups.

Results

A total of 38 volunteers were screened and 22 eligible subjects were randomized to a diet condition sequence and included the safety population. The primary reasons for screen failure was not meeting the fasting blood glucose requirements (n = 7) or not meeting blood glycated hemoglobin levels (n = 2). Other reasons included not meeting body mass index (BMI) requirements (n = 2), scheduling or work conflicts (n = 3), not being identified as a "regular breakfast consumer" (n = 1) and having extreme dietary habits (n =1).

An additional subject was removed because the subject withdrew consent; thus, 21 (13 females and 8 males) subjects provided data for both treatment conditions, and were included in the efficacy evaluable (EE) population resulting in a mean age of 44.4 ± 3.1 y and a mean BMI of 30.4 ± 0.9 kg/m^2 (**Table 1**); baseline characteristics of the EE sample are summarized in **Table 1**. Satiety and assessed cardiometabolic outcome results for subjects included in the EE sample are summarized in the associated **Tables and Figures**. Median compliance with study food intake was 100% during both the pPro condition and the refined CHO condition ($p > 0.05$ between conditions).

Appetite Parameters

Hunger

Mean VAS ratings (mm) for hunger, presented as changes from pre-meal value, are shown in **Figure 1** and niAUC results are presented in **Figure 2**. Hunger VAS ratings dropped after the test meal consumption (t = 0 min), relative to the pre-meal value (t = -15 min) and rose progressively throughout the 240 min test period during both diet conditions. The refined CHO diet condition resulted in a significantly greater $niAUC_{0\text{-}240\ min}$ for hunger versus the pPro diet condition ($p = 0.041$, **Figure 2**).

Fullness

Mean VAS ratings (mm) for fullness dropped progressively after the test meal consumption (t = 0 min), relative to the pre-meal value (t = -15 min) throughout the 240 min test period during both diet conditions **(Figure 3)**. There were no significant differences in the $niAUC_{0\text{-}240\ min}$ for fullness between the refined CHO and pPro diet conditions ($p = 0.257$, **Figure 4**).

Desire to Eat

As with hunger, mean desire to eat VAS ratings dropped after the test meal consumption (t = 0 min), relative to the pre-meal value (t = -15 min) and rose progressively throughout the 240 min test period during both diet conditions **(Figure 5)**. The refined CHO diet condition resulted in a significantly greater $niAUC_{0\text{-}240\ min}$ for desire to eat versus the pPro diet condition ($p = 0.040$, **Figure 6**).

Prospective Consumption

Mean VAS ratings (mm) for prospective consumption dropped immediately after test meal consumption (t = 0 min) and rose progressively thereafter, relative to the pre-meal value (t = -15 min) throughout the 240 min test period during both diet conditions **(Figure 7)**. There were no significant differences in the $niAUC_{0\text{-}240\ min}$ for prospective food consumption between the refined CHO and pPro diet conditions, but there was a trend for higher $niAUC_{0\text{-}240\ min}$ for prospective food consumption with the refined CHO diet condition relative to the pPro diet condition ($p = 0.061$, **Figure 8**).

Energy

Mean VAS ratings (mm) for energy rose after the test meal consumption (t = 0 min), relative to the pre-meal value (t = -15 min) and tapered slightly by the end of the 240 min test period for both diet conditions **(Figure 9)**. There were no significant differences in the $niAUC_{0\text{-}240\ min}$ for energy between the refined CHO and pPro diet conditions ($p = 0.139$, **Figure 10**).

Focus

Mean VAS ratings (mm) for focus slightly rose after test meal consumption (t = 0 min), relative to the pre-meal value (t = -15 min) and remained constant thereafter throughout the 240 min test period during both diet conditions **(Figure 11)**. There were no significant differences in the $niAUC_{0\text{-}240\ min}$ for focus between the refined CHO and pPro diet conditions, but there was a trend for a lower $niAUC_{0\text{-}240\ min}$ for focus with the refined CHO diet condition relative to the pPro diet condition (p = 0.055, **Figure 12**).

Daily Hunger and Fullness
No significant differences existed for mean daily VAS ratings (mm) for hunger between the refined CHO and pPro diet conditions, when averaged during week 1 (p = 0.596) and week 2 (p = 0.649) **(Appendix).** No significant differences existed for mean daily VAS ratings (mm) for fullness between the refined CHO and pPro diet conditions, when averaged during week 1 (p = 0.903) and week 2 (p = 0.454) **(Appendix).**

Ad Libitum Lunch Energy Intake
No significant differences existed in the amount of grams consumed of the *ad libitum* lunch, provided 240 min post-test meal intake, between the refined CHO and pPro diet conditions (p = 0.912; **Appendix**).

Lipoprotein Lipids Assessments
Fasting Lipids
Mean (SEM) percent changes from baseline for fasting TC, non-HDL-C, LDL-C, and HDL-C are presented in **Table 2**; median (interquartile range limits, IQL) percent changes from baseline for fasting TG are also presented in **Table 2.** No significant differences exist for the percent changes from fasting values at baseline to fasting values at the end of the 2-week diet condition for TC, non-HDL-C, LDL-C, HDL-C and TG between the diet conditions (p > 0.05).

End of Treatment Lipids: Baseline to 120 min
No significant differences exist for the percent changes from baseline fasting values to 120 min post-meal consumption values for TC, non-HDL-C, LDL-C and HDL-C between the refined CHO and pPRO diet conditions (p > 0.05) **(Appendix)**. However, the percent change from baseline fasting TG values to 120 min post-meal consumption TG values were significantly (p = 0.006) higher after the refined CHO breakfast intake (32.3 ± 7.7%) compared to the pPro breakfast intake (10.0 ± 6.8%) **(Figure 13)**.

End of Treatment Lipids: Baseline to 240min
No significant differences exist for the percent changes from baseline fasting values to 240 min post- meal consumption values for TC, non-HDL-C, LDL-C, HDL-C and TG between the refined CHO and pPRO diet conditions (p > 0.05) **(Appendix)**.

Glucose Metabolism Assessments
Plasma Glucose
Indicators of glucose metabolism are presented in the **Appendix**. No significant differences exist for the percent changes from fasting plasma glucose values at baseline to fasting plasma glucose values at the end of the 2-week diet condition between the diet conditions (p = 0.190). Mean plasma glucose rose after the test meal consumption (t = 0 min), relative to the pre-meal value (t = -15 min) and dropped progressively throughout the 240-min test period during both diet conditions **(Figure 14)**. The refined CHO diet condition resulted in a significantly greater $iAUC_{0\text{-}240\ min}$ for plasma glucose levels versus the pPro diet condition (p = 0.003, **Figure 15**).

Plasma Insulin
No significant differences exist for the percent changes from fasting plasma insulin values at baseline to fasting plasma insulin values at the end of the 2-week diet condition between the diet conditions (p = 0.243). Like plasma glucose, mean plasma insulin rose after the test meal consumption (t = 0 min), relative to the pre-meal value (t = -15 min) and dropped progressively throughout the 240 min test period during both diet conditions

(Figure 16). The refined CHO diet condition also resulted in a significantly greater $iAUC_{0\text{-}240\ min}$ for plasma insulin levels versus the pPro diet condition ($p = 0.001$, **Figure 17**).

HOMA-%S and HOMA-%B
No significant differences exist in the percent change from baseline to the end of the 2-week diet condition for HOMA-%S ($p = 0.346$) and HOMA-%B ($p = 0.828$) between the refined CHO and pPRO diet conditions **(Appendix)**.

Discussion
Consuming a lean pork-containing breakfast daily for two weeks resulted in less hunger and less desire to eat compared to consuming an isocaloric, fat-matched high refined CHO-containing breakfast, suggesting a potentially beneficial effect on some aspects of satiety, in metabolically at-risk overweight and obese men and women. However, other markers of satiety, including fullness, prospective consumption and subsequent *ad libitum* food and energy intake following the breakfast test meal were not affected by diet condition. VAS focus and energy ratings were also not affected by diet condition.

Compared to the refined CHO diet condition, the pPro diet condition reduced both the glucose and the insulin response to breakfast intake, indicating that the glucometabolic burden is alleviated with a protein-rich meal, e.g. lean pork, compared to a refined-CHO meal. This is an important consideration since all of the subjects had pre-diabetes, and reducing the glucose and insulin load may be a favorable option to help manage a metabolically compromised system. However, fasting plasma glucose, fasting plasma insulin and HOMA-%S and HOMA-%B, markers of insulin sensitivity and beta-cell function, respectively, were not affected by diet condition. These results suggest that an intake of a protein-rich breakfast, e.g. a lean-pork containing breakfast, may exert an acute beneficial effect on glucose metabolism but a longer-tem impact on carbohydrate metabolism and insulin sensitivity is not clear.

The effect of a two-week intake of lean pork at breakfast, compared to a refined CHO breakfast, may also be limited on lipid metabolism. The pPro diet condition had no effect on fasting levels of TC, LDL-C, non-HDL-C and HDL-C, compared to the refined CHO diet condition. However, the percent change from baseline of circulating TG was significantly and markedly decreased at 120 min following intake of the pPRO breakfast (10% increase) compared to intake of the refined CHO breakfast (32% increase), indicating a more favorable acute metabolic effect and reduced lipid burden with protein intake. The acute effect was no longer evident after 240 min since the percent change from baseline TG levels was 32% with the pPro breakfast and 33% with the refined CHO breakfast, and no longer significantly different between the two conditions.

In conclusion, the intake of a lean-pork containing breakfast may have an acute favorable effect on some aspects of appetite but this did not impact *ad libitum* food intake at a lunch meal, compared to a refined CHO-containing breakfast. Consuming a lean-pork meal as the breakfast meal may also have a favorable metabolic impact on both glucose and TG metabolism. Although acute, the diminished glucose and insulin excursions observed with the pPRO condition, compared to the refined CHO condition, when repeated habitually, may have beneficial physiological consequences long-term, particularly in a population of individuals with pre-diabetes for whom reducing dietary glycemic load may help to slow the progression of pancreatic beta-cell function (24). Similarly, although the attenuation of increased TG levels post-meal intake after the pPro condition versus the refined CHO condition was transient (only observed up to 120 min), the repeated attenuation of post-meal TG elevation may also have long-term metabolic benefits.

Taken together, the results of this trial are generally consistent with previous research results indicating that higher protein intake in place of refined CHO may be of benefit in appetite control and improve some aspects of the metabolic profile, particularly postprandial glucose, insulin and triglyceride concentrations (2, 12, 14). Further research on the effects of consuming lean pork over more extended periods as part of a breakfast meal on appetite and indicators of metabolic health is warranted.

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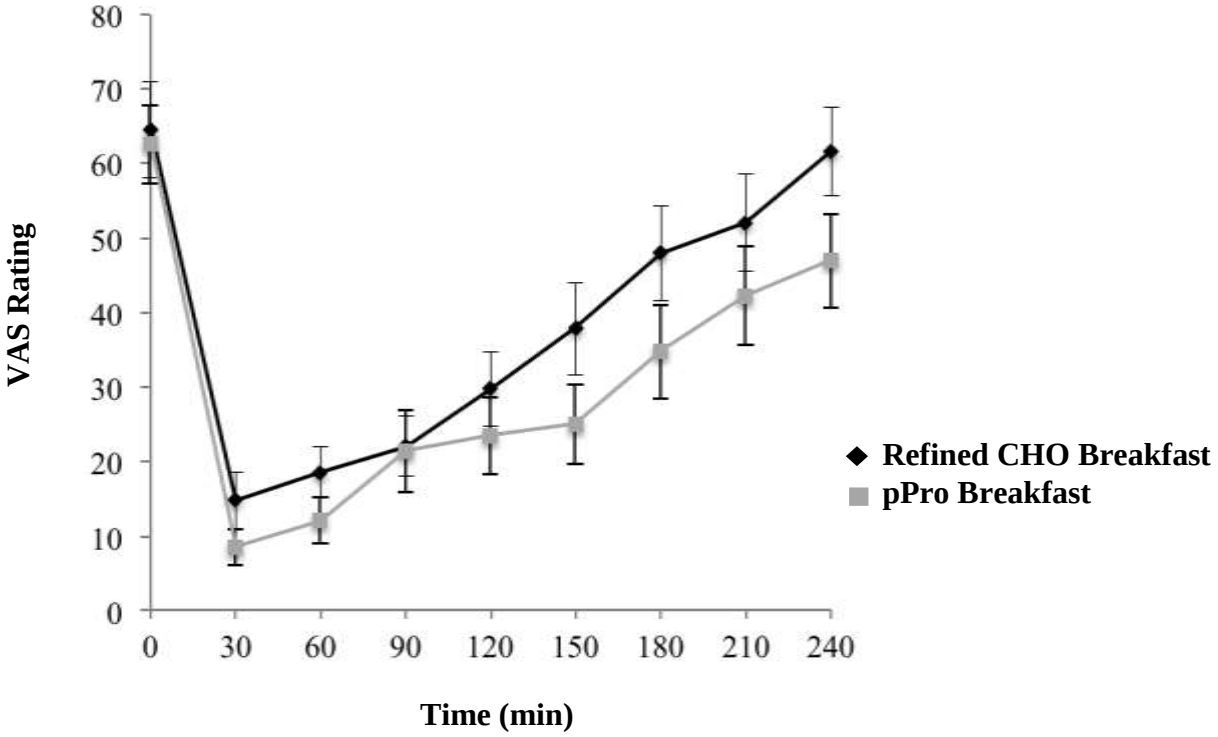
Tables and Figures

Table 1. Subject baseline characteristics

Characteristic	Efficacy Evaluable Sample (N = 21)
	n (%)
Gender	
Male	8 (38%)
Female	13 (62%)
Race	
Black/African American	8 (38%)
Other	7 (33%)
White/Caucasian	6 (29%)
Ethnicity	
Not Hispanic/ Latino	17 (81%)
Hispanic/ Latino	4 (19%)
Current Smoker	0 (0%)
Current Alcohol Consumer	15 (71%)
	Mean (SEM)
Age (y)	44.4 (3.1)
BMI (kg/m^2)	30.4 (0.9)
Fasting Plasma Glucose (mg/dL)	109 (2.0)
Systolic Blood Pressure	112 (1.9)
Diastolic Blood Pressure (mm Hg)	71 (1.3)

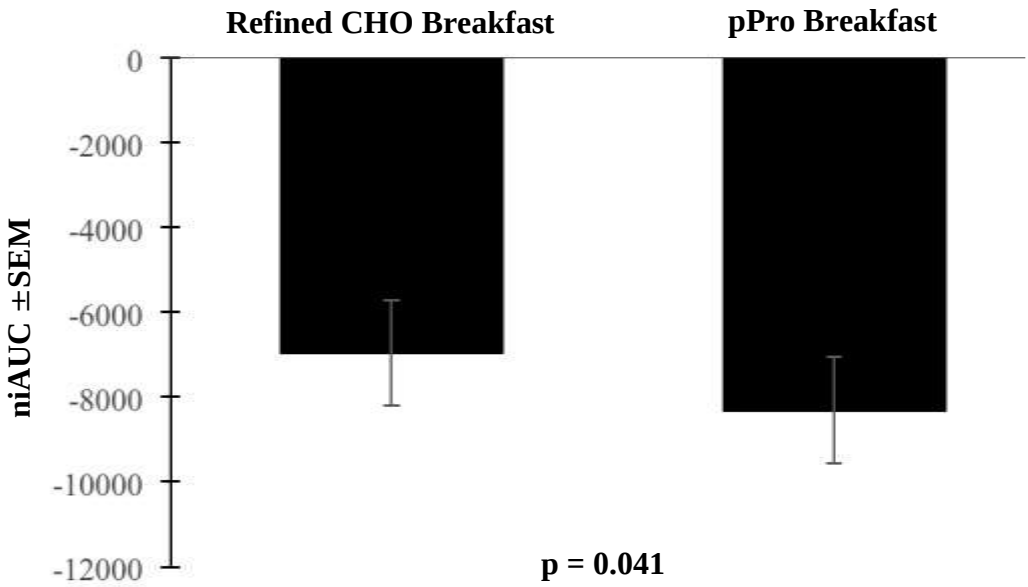
Abbreviations: BMI, body mass index; SEM, standard error of the mean

Figure 1. Mean (SEM) hunger VAS



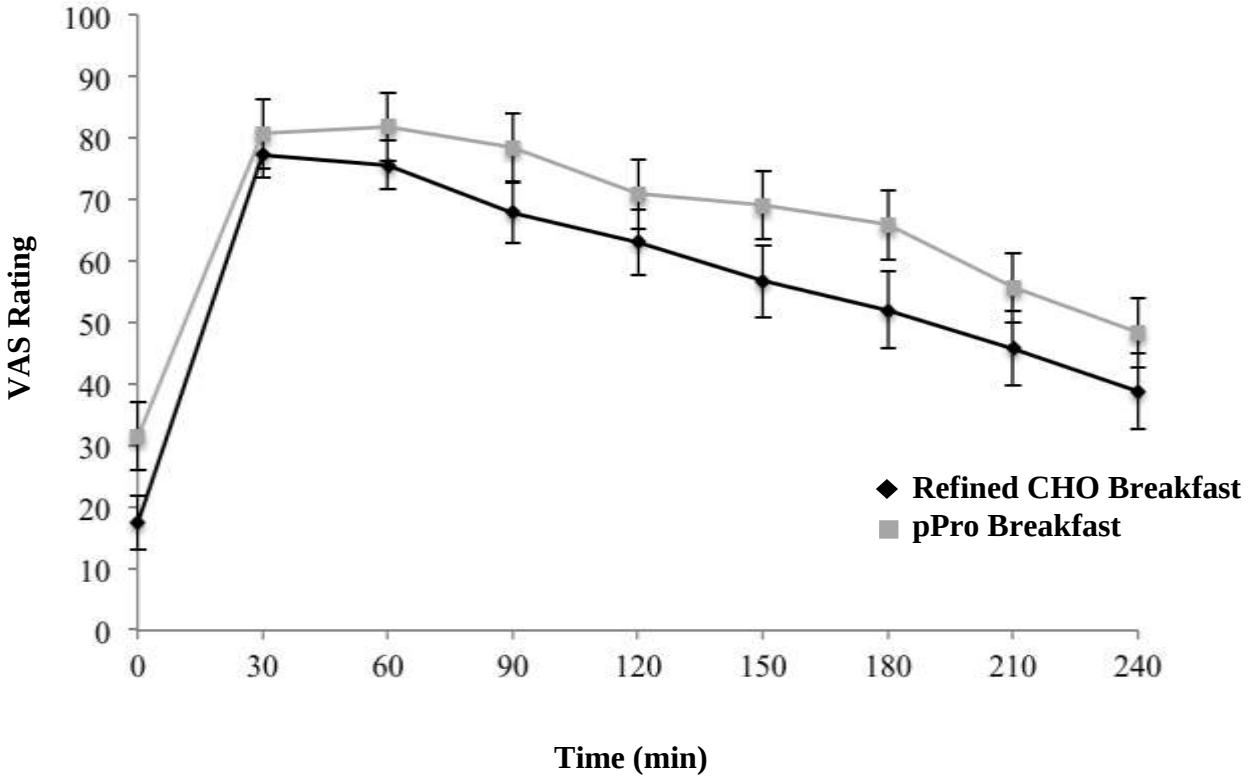
Abbreviations: CHO, carbohydrate; pPro, lean pork, high-protein; SEM, standard error of the mean; VAS, Visual analog scale

Figure 2. Mean (SEM) hunger niAUC from pre-meal (t = -15 min) to 240 min post-meal



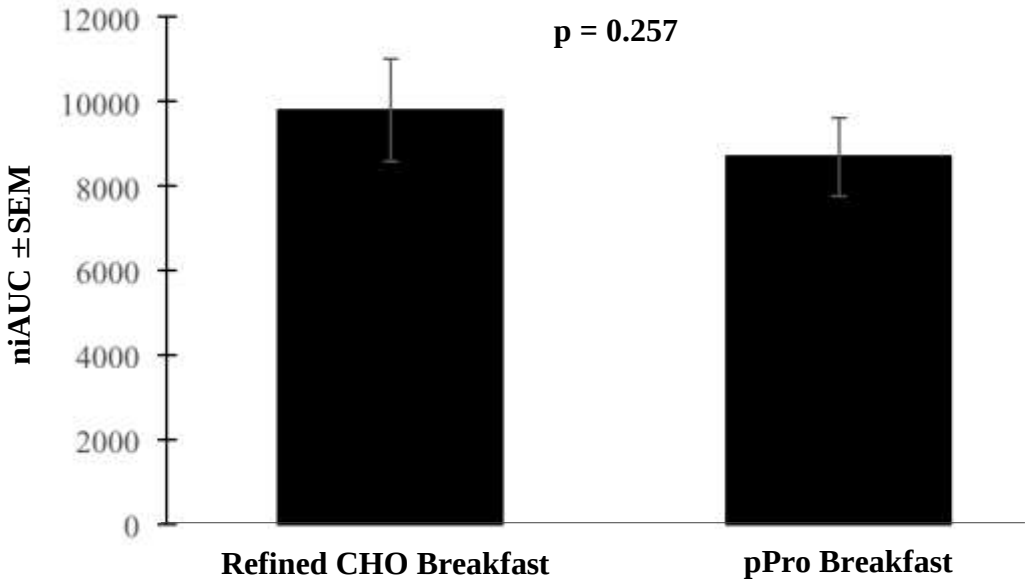
Abbreviations: CHO, carbohydrate; niAUC, net incremental area under the curve; pPro, lean pork, high-protein; SEM, standard error of the mean

Figure 3. Mean (SEM) fullness VAS



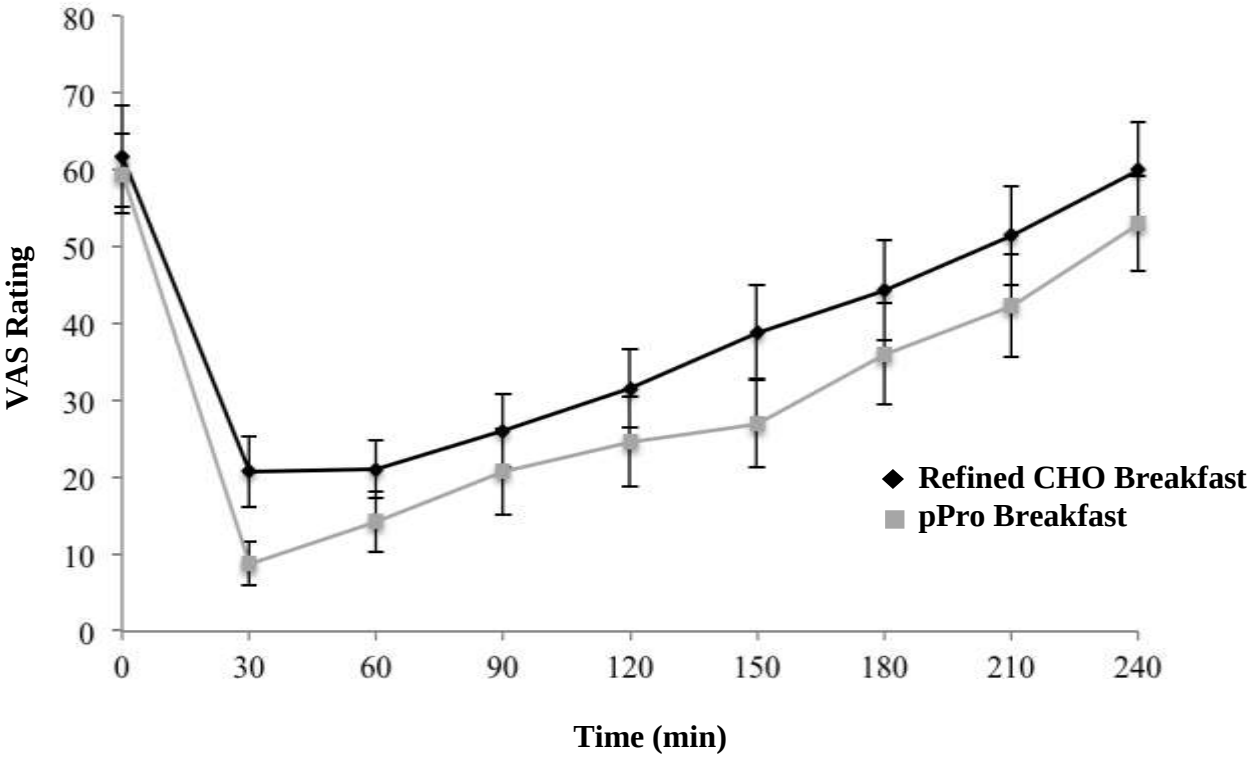
Abbreviations: CHO, carbohydrate; pPro, lean pork, high-protein; SEM, standard error of the mean; VAS, Visual analog scale

Figure 4. Mean (SEM) fullness niAUC from pre-meal (t = -15 min) to 240 min post-meal



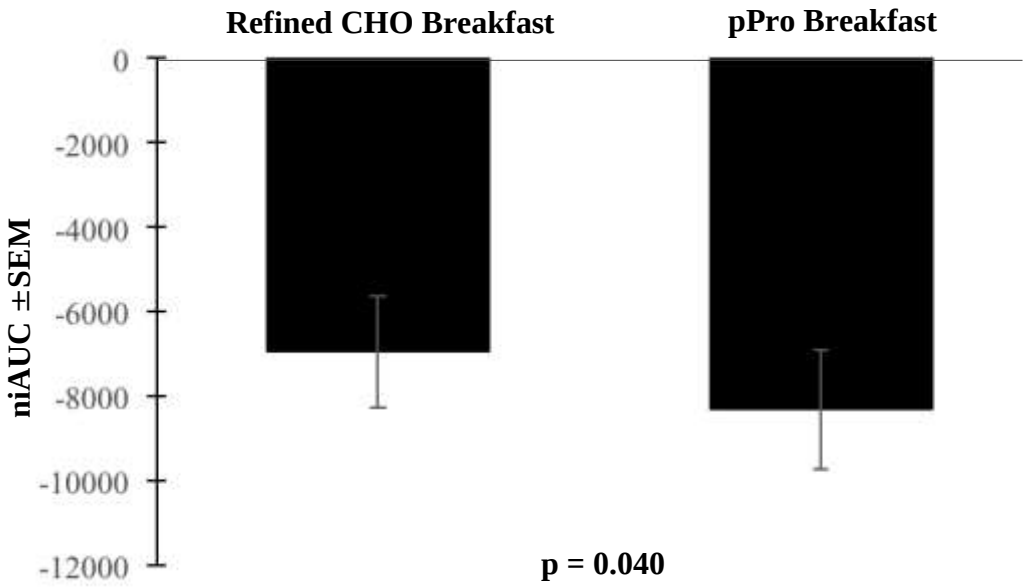
Abbreviations: CHO, carbohydrate; niAUC, net incremental area under the curve; pPro, lean pork, high-protein; SEM, standard error of the mean

Figure 5. Mean (SEM) desire to eat VAS



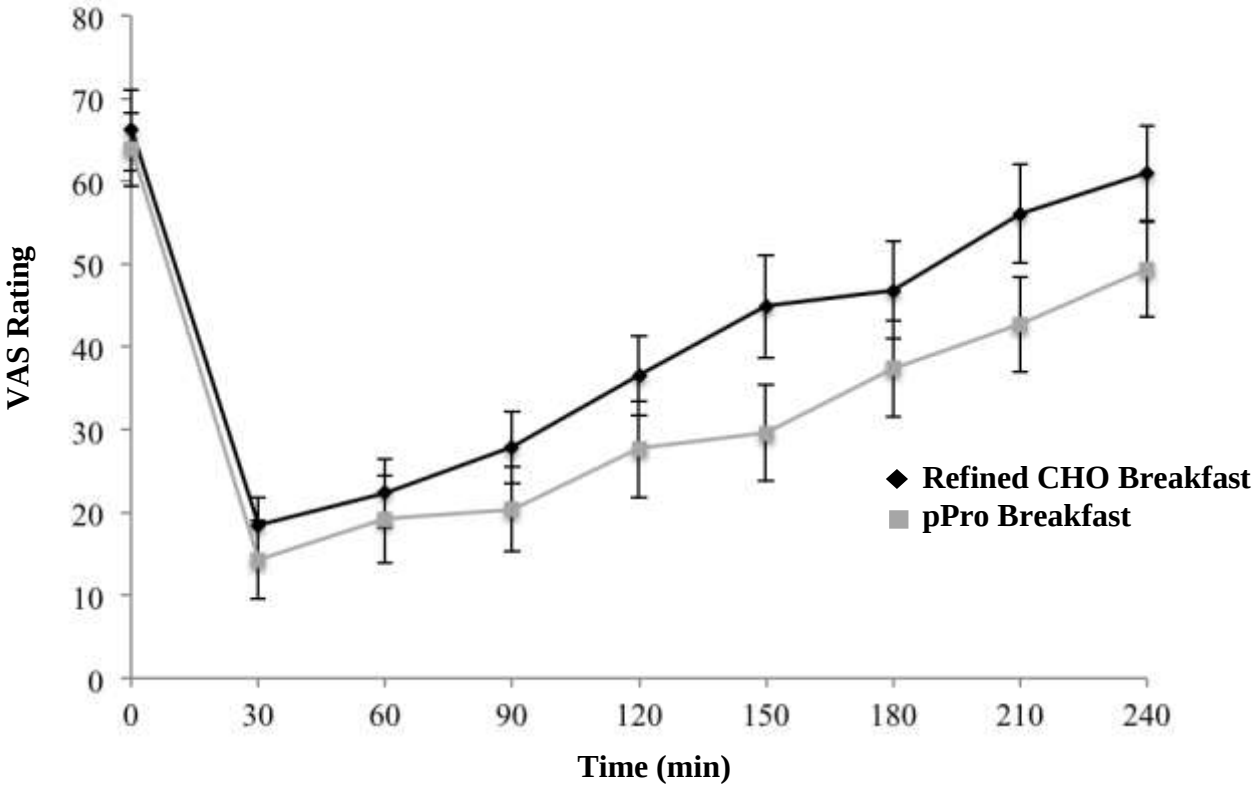
Abbreviations: CHO, carbohydrate; pPro, lean pork, high-protein; SEM, standard error of the mean; VAS, Visual analog scale

Figure 6. Mean (SEM) desire to eat niAUC from pre-meal (t = -15 min) to 240 min post-meal



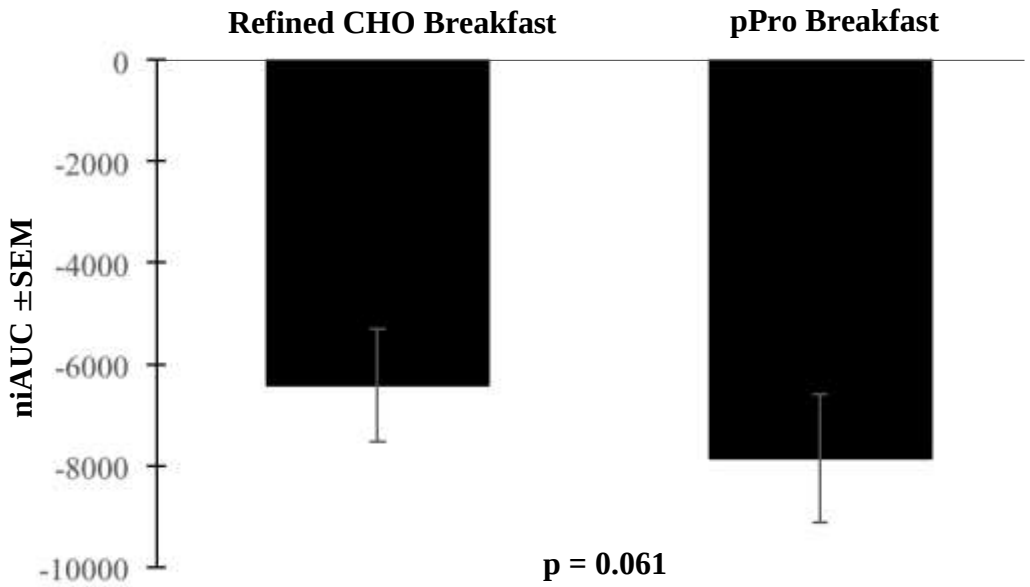
Abbreviations: CHO, carbohydrate; niAUC, net incremental area under the curve; pPro, lean pork, high-protein; SEM, standard error of the mean

Figure 7. Mean (SEM) prospective consumption VAS



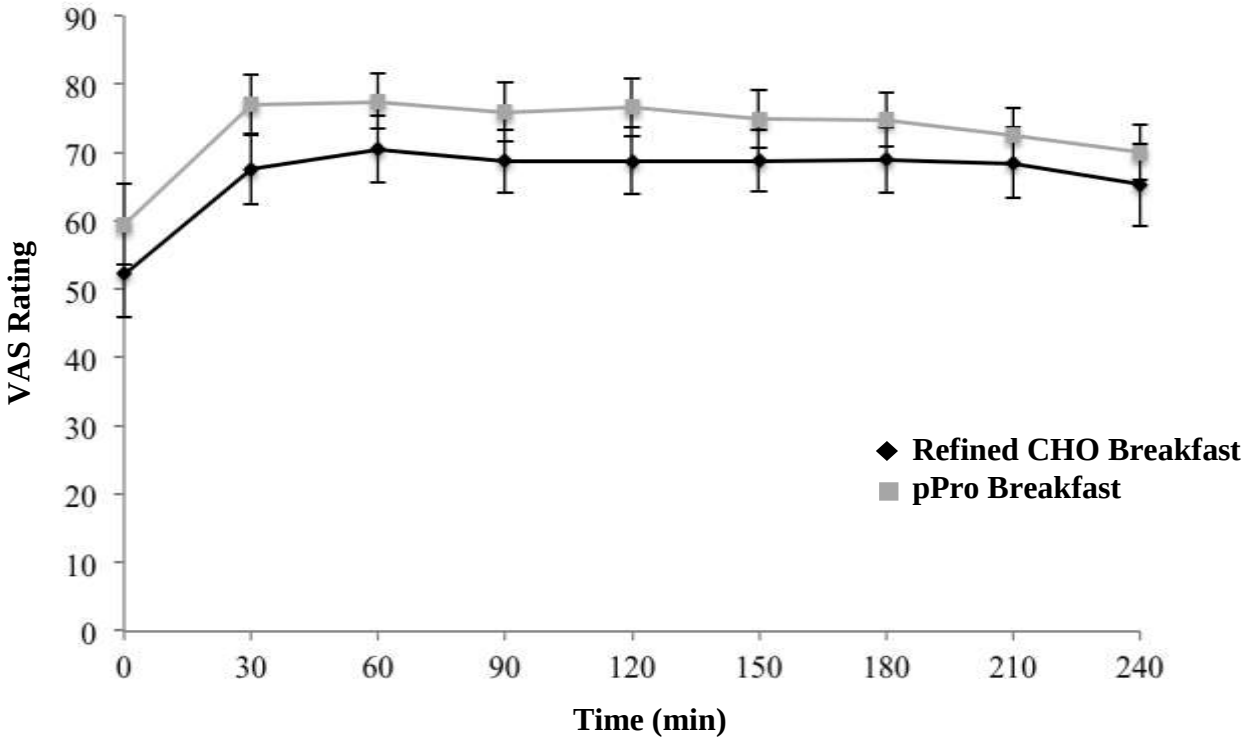
Abbreviations: CHO, carbohydrate; pPro, lean pork, high-protein; SEM, standard error of the mean; VAS, Visual analog scale

Figure 8. Mean (SEM) prospective consumption niAUC from pre-meal (t = -15 min) to 240 min post-meal



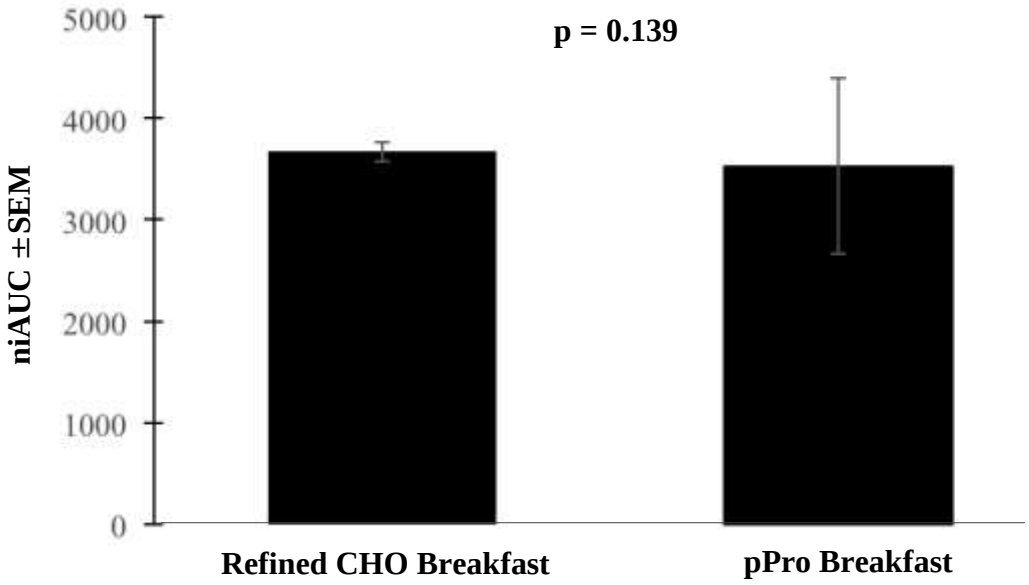
Abbreviations: CHO, carbohydrate; niAUC, net incremental area under the curve; pPro, lean pork, high-protein; SEM, standard error of the mean

Figure 9. Mean (SEM) energy VAS



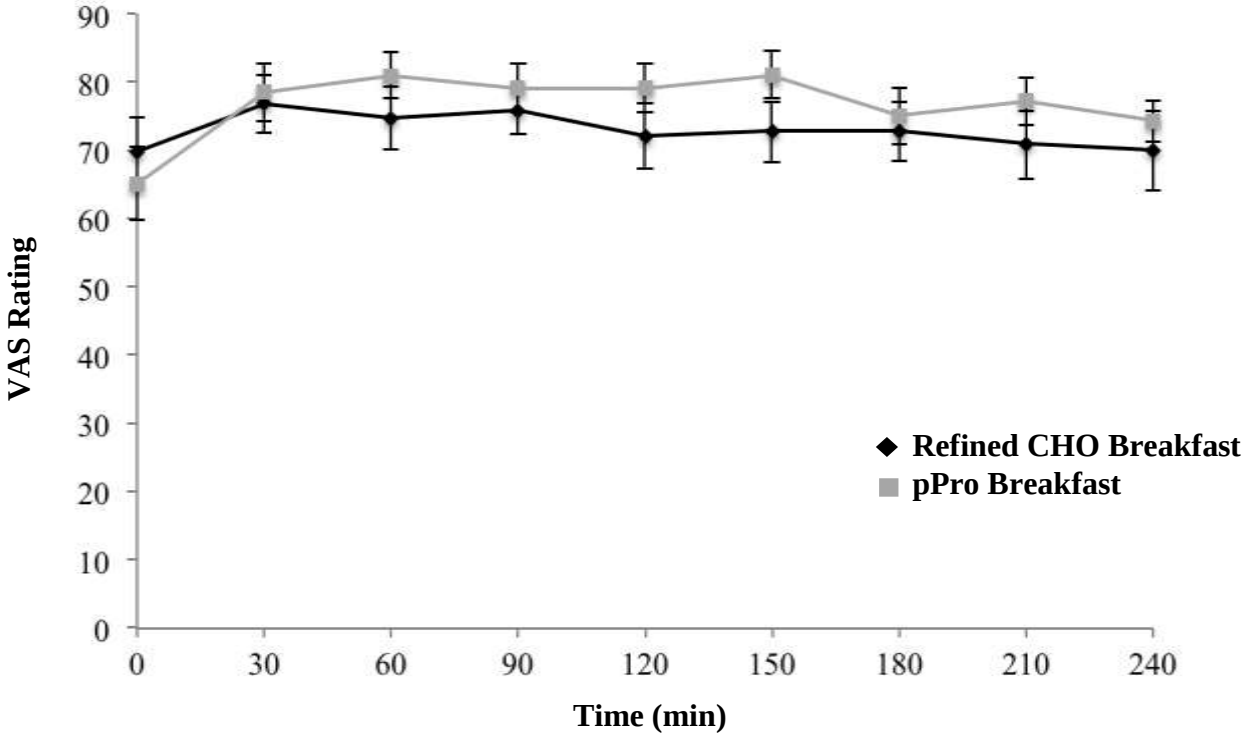
Abbreviations: CHO, carbohydrate; pPro, lean pork, high-protein; SEM, standard error of the mean; VAS, Visual analog scale

Figure 10. Mean (SEM) energy niAUC from pre-meal (t = -15 min) to 240 min post-meal



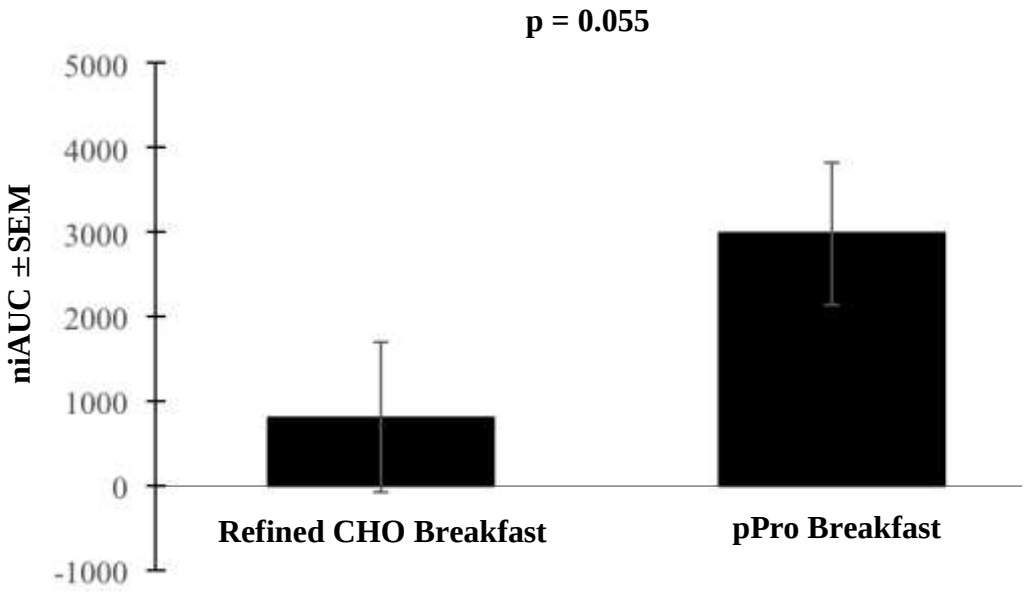
Abbreviations: CHO, carbohydrate; niAUC, net incremental area under the curve; pPro, lean pork, high-protein; SEM, standard error of the mean

Figure 11. Mean (SEM) focus VAS



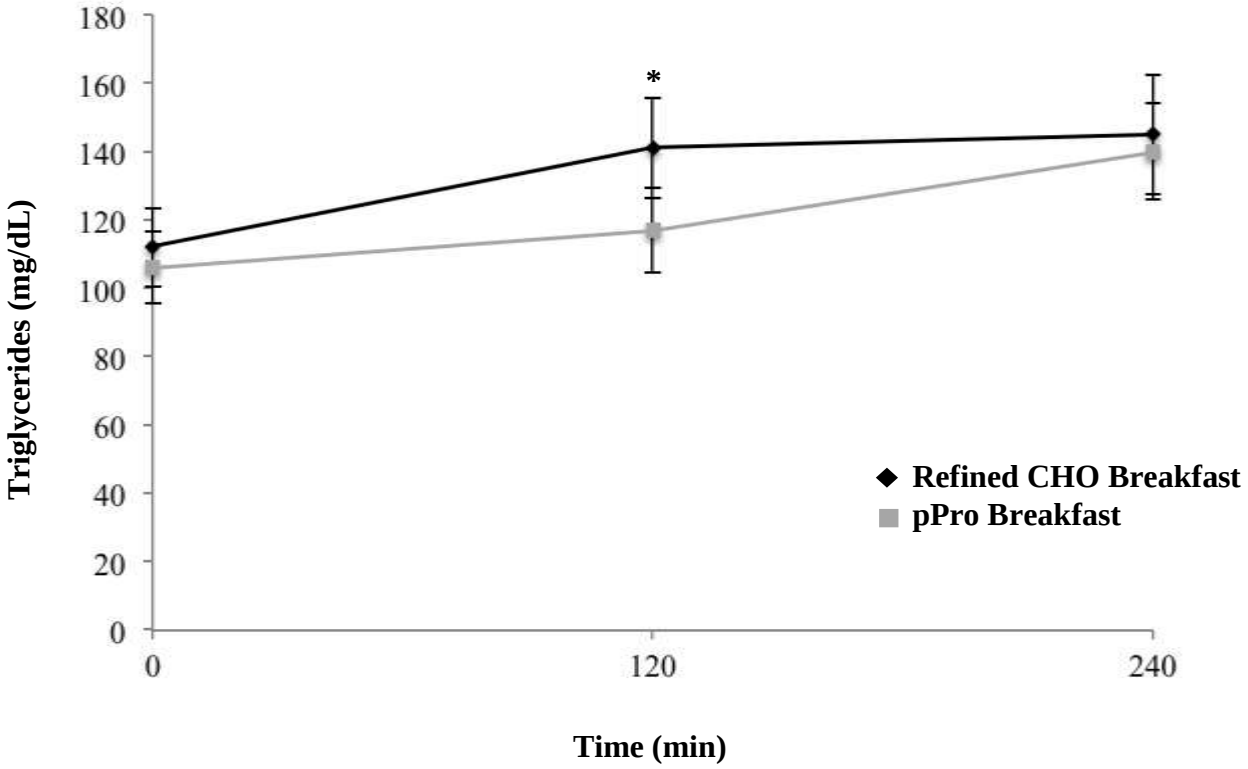
Abbreviations: CHO, carbohydrate; pPro, lean pork, high-protein; SEM, standard error of the mean; VAS, Visual analog scale

Figure 12. Mean (SEM) focus niAUC from pre-meal (t = -15 min) to 240 min post-meal



Abbreviations: CHO, carbohydrate; niAUC, net incremental area under the curve; pPro, lean pork, high-protein; SEM, standard error of the mean

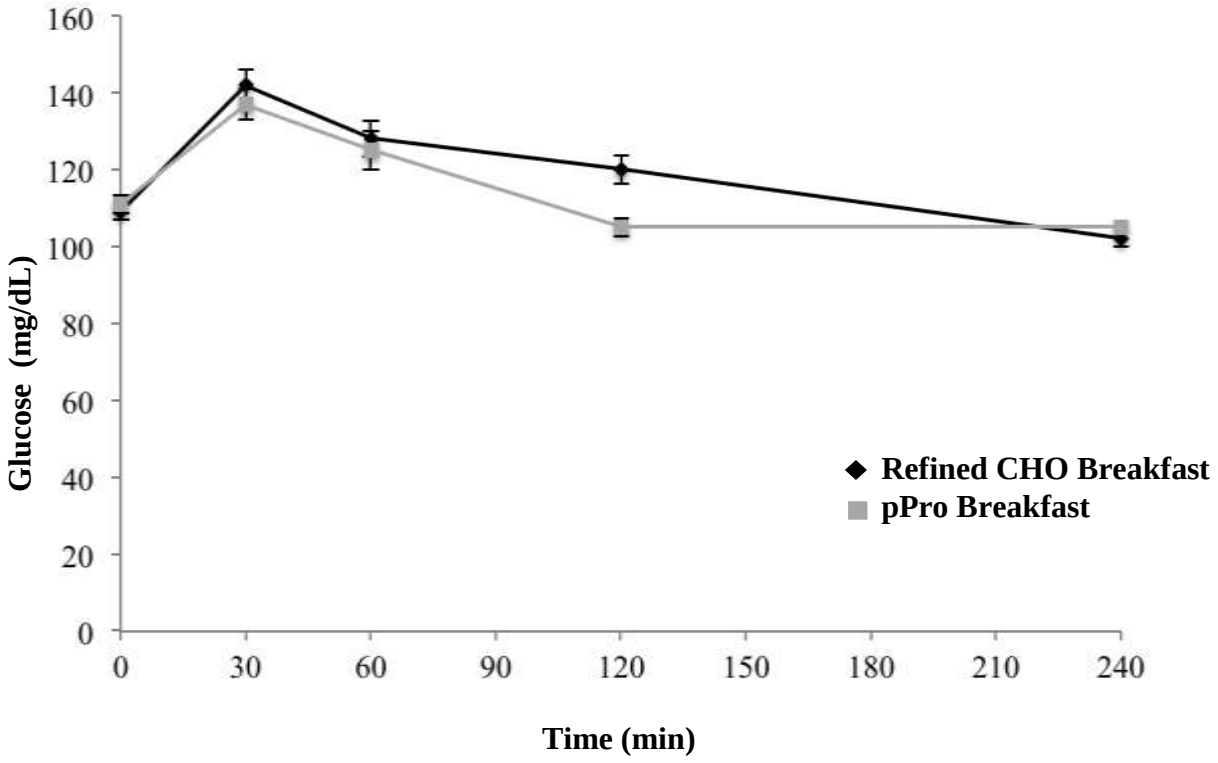
Figure 13. Mean (SEM) triglyceride levels (mg/dL) at pre-meal (t = -15 min) and 120 min and 240 min post-meal



Abbreviations: CHO, carbohydrate; pPro, lean pork, high-protein; SEM, standard error of the mean

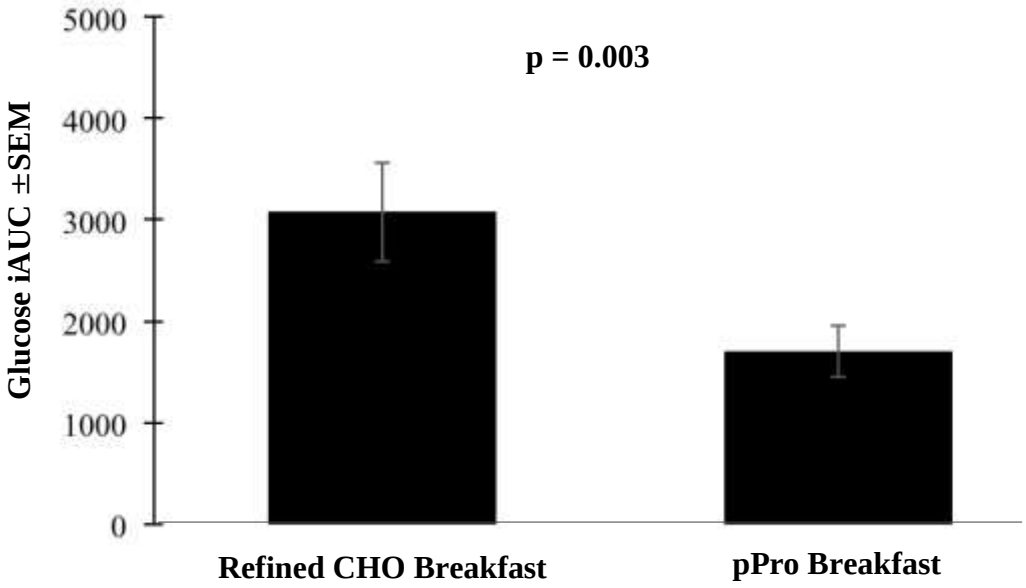
*p = 0.006 for percent change from baseline to 120min post-meal intake between pPro breakfast intake (10.0 ± 6.8% increase) and refined CHO breakfast intake (32.3 ± 7.7% increase)

Figure 14. Mean (SEM) glucose levels (mg/dL) from pre-meal (t = -15 min) to 240 min post-meal



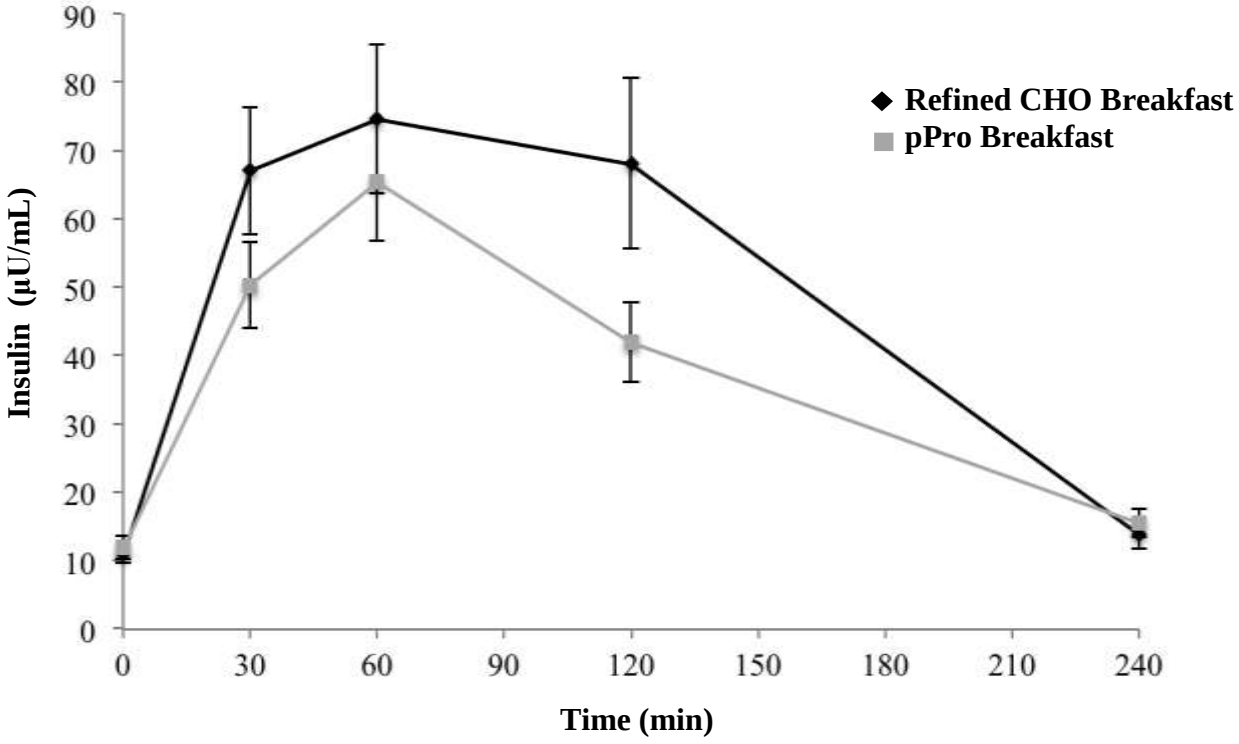
Abbreviations: CHO, carbohydrate; pPro, lean pork, high-protein; SEM, standard error of the mean

Figure 15. Mean (SEM) glucose iAUC from pre-meal (t = -15 min) to 240 min post-meal



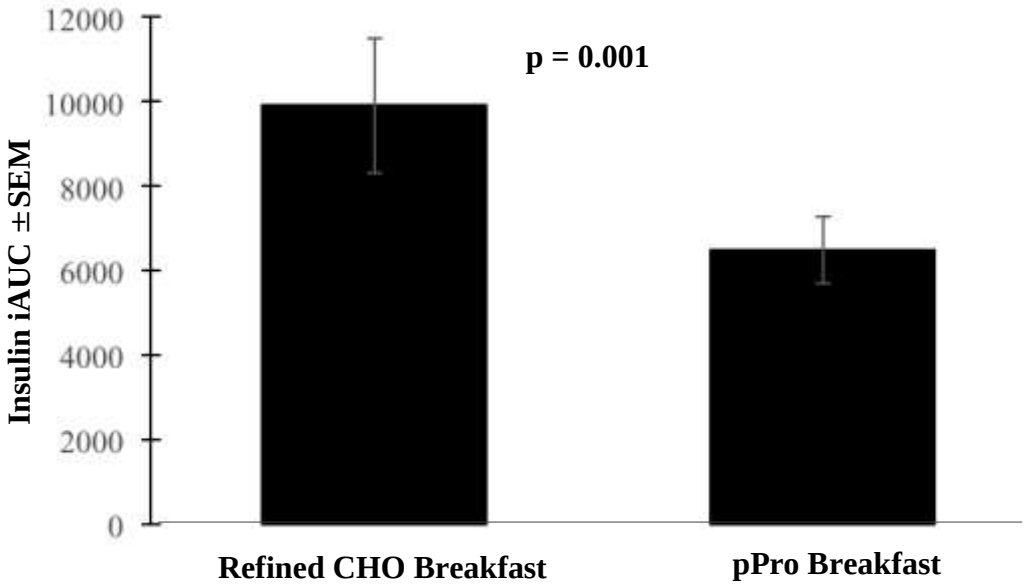
Abbreviations: CHO, carbohydrate; iAUC, incremental area under the curve; pPro, lean pork, high-protein; SEM, standard error of the mean

Figure 16. Mean (SEM) insulin levels (µU/mL) from pre-meal (t = -15 min) to 240 min post-meal



Abbreviations: CHO, carbohydrate; pPro, lean pork, high-protein; SEM, standard error of the mean

Figure 17. Mean (SEM) insulin iAUC from pre-meal (t = -15 min) to 240 min post-meal



Abbreviations: CHO, carbohydrate; iAUC, incremental area under the curve; pPro, lean pork, high-protein; SEM, standard error of the mean

Table 2. Fasting lipids at baseline and percent change from baseline

Abbreviations: %Δ, percent change; HDL-C, high-density lipoprotein cholesterol; IQL, interquartile limits; LDL-C, low-density lipoprotein cholesterol; Non-HDL-C, non-high-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; SEM, standard error of the mean

Lipid Parameter	Baseline (mg/dL)	Refined CHO Breakfast (%Δ)	pPro Breakfast (%Δ)
	Mean (SEM) or Median (IQL)[1]		
Fasting TC (mg/dL)	198 (6.5)	2.4 (2.7)	2.9 (2.0)
Fasting Non-HDL-C (mg/dL)	149 (5.8)	1.5 (3.0)	2.4 (2.4)
Fasting LDL-C (mg/dL)	127 (5.3)	-3.8 (2.9)	-1.9 (3.0)
Fasting HDL-C (mg/dL)	49 (3.4)	4.9 (3.1)	3.8 (3.1)
Fasting TG (mg/dL)	93 (69, 136)	-1.0 (-11, 15)	1.2 (-18, 17)

[1]No statistically significant differences were present at baseline or for percent change from baseline.

Variable	Refined CHO Breakfast	pPro Breakfast	Full Model			
			P-Trt	P-Seq	P-Trt*Seq	P-Covariate
	Mean ± SEM					
VAS						
Hunger niAUC	-6948.79 ± 1240.45	-8318.88 ± 1257.63	**0.041**	0.207	0.281	<**0.001**
BL value	64.52 ± 6.542	62.48 ± 5.340				
Fullness niAUC	9791.83 ± 1218.20	8684.31 ± 926.38	0.257	0.687	0.161	<**0.001**
BL value	17.43 ± 4.361	31.48 ± 5.408				
Desire niAUC	-5898.50 ± 1323.50	-7354.91 ± 1424.17	**0.040**	0.246	0.306	<**0.001**
BL value	61.71 ± 6.56	59.38 ± 5.232				
PrCons niAUC	-6413.38 ± 1108.04	-7861.357 ± 1260.25	0.061	0.496	0.398	<**0.001**
BL value	66.24 ± 4.95	63.90 ± 4.42				
Energy niAUC	3669.26 ± 99.31	3525.43 ± 866.97	0.139	0.304	0.679	<**0.001**
BL value	52.19 ± 6.272	59.48 ± 5.93				
Focus niAUC	804.55 ± 890.19	2980.88 ± 837.60	0.055	0.359	0.670	<**0.001***
BL value	69.81 ± 5.03	65.10 ± 5.40				
Daily VAS	**Refined CHO Breakfast**	**pPro Breakfast**				
Hunger (Week 1 average)	3.87 ± 0.40	4.18 ± 0.48	0.596	0.210	0.515	-
Hunger (Week 2 average)	3.96 ± 0.47	4.16 ± 0.49	0.649	0.329	0.963	-
Fullness (Week 1 average)	7.21 ± 0.25	7.11 ± 0.35	0.903	0.498	0.606	-
Fullness (Week 2 average)	7.56 ± 0.31	7.36 ± 0.33	0.454	0.732	0.087	-
Lunch Energy Intake	**Refined CHO Breakfast**	**pPro Breakfast**				
Grams consumed at lunch	503.00 ± 52.59	498.86 ± 47.51	0.912	0.785	0.543	-

Appendix

Glucose/Insulin	**Refined CHO Breakfast**	**pPro Breakfast**				
Glucose iAUC	3074.83 ± 487.58	1703.70 ± 254.22	**0.003**	0.970	0.516	0.094
BL value	108.86 ± 2.01	110.83 ± 2.37				
Insulin iAUC	9907.87 ± 1590.18	6470.93 ± 788.75	**0.001**	0.162	0.106	**0.016**
BL value	10.87 ± 1.26	11.96 ±1.70				
FPG	108.86 ± 2.01	110.83 ± 2.37	0.190	**0.022**	0.764	<**0.001**
BL value	109.16 ± 1.78	109.16 ± 1.78				
% change from BL	-0.21 ± 1.17	1.58 ± 1.57				
FPI	10.87 ± 1.26	11.96 ± 1.70	0.243	0.615	0.684	<**0.001**
BL value	12.62 ± 1.68	12.62 ± 1.68				
% change from BL	-9.54 ± 5.27	-1.94 ± 7.15				
HOMA-%S	85.11 ± 12.40	72.21 ± 10.61	0.346	-	-	<**0.001**
BL value	76.96 ± 8.23	76.96 ± 8.23				
Change from BL	8.16 ± 8.37	-4.75 ± 6.51				
HOMA-%B	107.96 ± 10.95	112.34 ± 12.20	0.828	-	-	<**0.001**
BL value	91.77 ± 9.00	91.77 ± 9.00				
Change from BL	16.19 ± 5.31	20.57 ± 6.73				

Fasting Lipids	**Refined CHO Breakfast**	**pPro Breakfast**				
TG (Fasting)	112.10 ± 11.49	106.47 ± 10.56	0.335	0.279	0.750	<**0.001**
BL value	109.03 ± 11.32	109.03 ± 11.32				
% change from BL	5.69 ± 5.93	1.59 ± 6.27				
TC (Fasting)	200.48 ± 6.46	202.36 ± 6.39	0.680	0.247	0.353	<**0.001**
BL value	197.97 ± 6.46	197.97 ± 6.46				
% change from BL	2.00 ± 2.58	2.74 ± 2.08				
LDL-C (Fasting)	127.95 ± 6.08	129.86 ± 5.71	0.555	0.228	0.342	<**0.001**
BL value	126.86 ± 5.26	126.86 ± 5.26				
% change from BL	1.15 ± 3.04	2.85 ± 2.93				
Non-HDL-C (Fasting)	150.38 ± 6.81	151.22 ± 6.00	0.800	0.457	0.234	<**0.001**

BL value	148.73 ± 5.81	148.73 ± 5.81				
% change from BL	1.21 ± 2.68	1.97 ± 2.19				
HDL-C (Fasting)	50.10 ± 2.81	51.14 ± 3.66	0.540	0.063	0.709	<**0.001**
BL value	49.24 ± 3.41	49.24 ± 3.41				
% change from BL	4.45 ± 3.64	4.70 ± 3.11				
EOT Lipids	**Refined CHO Breakfast**	**pPro Breakfast**				
TG (t=120 min)	140.73 ± 14.61	117.21 ± 12.33	**0.006**	0.260	0.726	<**0.001**
BL value	109.03 ± 11.32	109.03 ± 11.32				
% change from BL	32.30 ± 7.74	10.01 ± 6.84				
TG (t=240 min)	145.37 ± 17.53	139.65 ± 14.01	0.529	0.332	0.545	<**0.001**
BL value	109.03 ± 11.32	109.03 ± 11.32				
% change from BL	32.65 ± 8.72	32.08 ±7.91				
TC (t=240 min)	201.66 ±7.24	203.07 ± 6.96	0.767	0.412	0.164	<**0.001**
BL value	197.97 ± 6.46	197.97 ± 6.46				
% change from BL	2.43 ± 2.68	2.89 ± 1.99				
LDL-C (t=240 min)	122.10 ± 6.39	124.38 ± 6.21	0.439	0.258	0.291	<**0.001**
BL value	126.86 ± 5.26	126.86 ± 5.26				
% change from BL	-3.81 ± 2.90	-1.91 ± 2.99				
Non-HDL-C (t=240 min)	145.52 ± 10.43	152.30 ± 6.77	0.750	0.493	0.127	<**0.001**
BL value	148.73 ± 5.81	148.73 ± 5.81				
% change from BL	1.47 ± 2.96	2.37 ± 2.35				
HDL-C (t=240 min)	50.38 ± 2.83	50.76 ± 3.66	0.848	0.116	0.390	<**0.001**
BL value	49.24 ± 3.41	49.24 ± 3.41				
% change from BL	4.87 ± 3.14	3.83 ± 3.08				

NOTES:

- Means are raw means.
- Models: Fixed factors= Treatment, Sequence, Treatment*Sequence, Baseline value; Random factor= Screen; Covariance structure= VC; Estimation method= ML; No repeated factor
- *Model received following warning: The final Hessian matrix is not positive definite although all convergence criteria are satisfied. The MIXED procedure continues despite this warning. Validity of subsequent results cannot be ascertained.

Appendix

- Baseline values
    - niAUC VAS: Baseline values are the t= -15 min VAS ratings
    - iAUC Glucose/Insulin: Baseline values are the t= -15 min Glucose/Insulin values
    - Fasting Labs: Baseline values are the V2 fasting lab values
    - HOMA: Baseline values are the HOMA values at V2 (calculated using fasting glucose/insulin at V2)
    - EOT Lipids: Baseline values are the V2 fasting lab values
- iAUC FOR Glucose and Insulin were calculated as sum of iAUC values from t= -15 to t=240 mins.
- Fasting labs are lab values at t= -15 min.
- HOMA: Change from BL= HOMA value-BL value

Abbreviations: BL, baseline; BMI, body mass index; CHO, carbohydrate; EOT, end of treatment; FPG, fasting plasma glucose; FPI; fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; HOMA2-%B, homeostatic model assessment of beta-cell function; HOMA2-%S, homeostatic model assessment of insulin sensitivity iAUC, incremental area under the curve; IQL, interquartile limits; LDL-C, low-density lipoprotein cholesterol; niAUC, net incremental area under the curve; non-HDL-C, non-high-density lipoprotein cholesterol; pPRO, pork protein; TC, total cholesterol; TG, triglycerides; Trt, treatment; SEM, standard error of the mean; Seq, sequence; VAS, visual analog scale