The Effect of a Protein and Carbohydrate Mixture on Early Morning Aerobic Exercise Performance

THESIS

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By

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Abstract

Carbohydrates are used by the body as fuel during exercise; eating or drinking a small amount of carbohydrate before or during exercise is beneficial in some circumstances. The amount, type, and timing of carbohydrate have been researched in the past, but more data is needed to formulate an ideal plan for consumption around exercise. A product called ModCarb™ has been developed from grains thought to inhibit the blood glucose lowering reaction. The present study tested pre-exercise consumption of ModCarb™, with and without added protein, for effects on aerobic exercise performance in moderately trained male subjects age 18-30. Seventy-five subjects were randomly divided into five groups, including placebo, ModCarb™ (3grams), ModCarb™ plus whey protein (3 grams, 14 grams), whey protein (14 grams), and whey protein plus sucrose (14 grams, 20 grams) to provide additional Calories more similar to that found in commercial sports beverages. Subjects completed two exercise testing sessions separated by 10-21 days which assessed time to complete a three-mile run, distance covered in a 25 minute stationary bicycle activity and number of steps completed in a 90 second step test.

A one time consumption of the product(s) resulted in improved run time within all groups including placebo except the protein plus added sugar group. An improvement in the step test was seen for all groups (one-tailed paired t-test, p-value ≤ 0.05). There was also an improvement in bike distance seen for all groups except the Modcarb™ group (one-tailed paired t-test, $p \leq .05$). None of the supplement combinations resulted in improved aerobic exercise performance in any of the groups compared to placebo (unpaired t-test, p-value ≤ 0.05). No significant changes in plasma glucose levels were seen between baseline and treatment sessions within the groups (paired t-test, p-value ≤ 0.05), and there was no overall change in plasma glucose comparing each group to placebo (unpaired t-test, p-value $\leq .05$). Possible confounding factors such as hydration status, intrinsic motivation and familiarity with the testing protocol may have contributed to the improvement in aerobic exercise seen within the groups. The amount of ModCarb™ supplement provided to each group may also need to be increased to see more significant effects. In conclusion, a one-time consumption of ModCarb™ in the amount provided did not improve aerobic exercise performance or change plasma glucose responses following exercise.

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Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

The selection and timing of nutrients used by the body in aerobic exercise is a highly scrutinized topic. The performance of athletes at all levels can be greatly impacted by the fuel provided to them for energy. There are numerous recommendations on the distribution of macronutrients and the appropriate timing of their ingestion before, during, and after an aerobic training session or competition. A study done by Breen et. al did not show any improvement when adding protein to a carbohydrate beverage, but the supplement was only consumed during exercise (2). Because the majority of previous research has looked at the efficacy of carbohydrate prior to exercise or protein during or following exercise, a study is needed to test subjects using a combination of these macronutrients before completing a specific exercise protocol. The goal of the study is to provide a practical approach to fueling before exercise and provide a commercially available supplement to the general population.

A measure of performance (i.e. time to complete a race) is the most commonly used variable when examining the outcomes of this type of research. There are other markers that can also show an impact on subsequent performances and affect the performance of an individual during a specific time period. New research was done to test the acute effects of a ModCarb™ plus protein supplementation on the aerobic performance of moderately trained young men. Also examined was plasma glucose before and after both a baseline and treatment exercise session. The project tested the hypothesis that addition of a protein source to a carbohydrate supplement will improve the aerobic exercise performance.

The primary goal of this study will be to determine whether one-time consumption of ModCarb™ in combination with a protein product will improve aerobic exercise performance in moderately trained young men. A secondary objective will be to assess plasma blood measures of glucose before and after exercise in all subjects.

Chapter 2: Review of Literature

2.1 Pre-Exercise Nutrient Timing

Consuming carbohydrate prior to exercise has been studied extensively and has been thought to maximize endogenous glucose stores and prevent hypoglycemia during exercise (10). The body can only store enough glucose to last 90 minutes to 3 hours during moderate intensity exercise, so pre-exercise nutrient status is of utmost importance to performance. When examining exercise lasting less than 60 minutes, it is important to remember that endogenous glucose stores are most likely not going to be completely depleted as long as the individual has been consuming adequate amounts of carbohydrates for several days leading up to the exercise bout. As glucose stores decrease; however, exercise intensity and work output may decrease (10). Immune system suppression and higher amounts of muscle tissue breakdown may also occur with decreases in muscle glycogen. Appropriate nutrient timing in regards to exercise is focused on preventing these negative effects of diminished muscle glycogen.

There is some concern that consuming carbohydrate shortly before exercise could result in rebound hypoglycemia. This is due to a rapid increase in the uptake of glucose resulting in a decrease in serum blood glucose. Several studies by Jeukendrup and Miller (9) found that there was no decrease in performance even if this hypoglycemia was present. The studies used a testing protocol of 20 minutes of steady state exercise followed by a 40 minute time trial. The subjects received a 75 gram dose of carbohydrate 45 minutes prior to exercise (9). Similar responses in blood glucose have been seen when several different amounts of carbohydrate have been consumed within 60 minutes of exercise. No performance differences have been found with a smaller or larger amount of ingested carbohydrate (9). One of the mechanisms involved in maintaining blood glucose levels is liver glucose output. Marmy-Conus et. al (11) determined that pre-exercise ingestion of carbohydrate results in lower liver glucose output throughout exercise when compared to a placebo. The ingested carbohydrate appears to maintain plasma glucose levels throughout exercise to prevent hypoglycemia.

In a study that provided trained cyclists a 75 gram dose of carbohydrate at different times before exercise, no significant difference in performance was found among the test groups (13). The subjects received the carbohydrate beverage 15, 45, or 75 minutes pre-exercise, but the timing also had no effect on gastrointestinal discomfort or rate of perceived exertion. There were significant differences in the insulin and glucose levels among the subjects, but this leveled off as exercise proceeded. Moseley et. al (13) states that with performances lasting under one hour, the timing of carbohydrate ingestion prior to exercise has no significant effect on performance. In regards to consuming carbohydrate within 60 minutes of exercise; however, there have been conflicting results showing that more studies are needed in this area.

It has been hypothesized that consuming protein prior to exercise may give the body a longer period to digest the protein to make it available for muscle recovery postexercise (21). If protein is consumed farther in advance than immediately prior to exercise, there is more time for the digestion of protein to free amino acids which can be used by the body for recovery. More research is needed in this area to determine if this does occur in the body and the mechanism by which it occurs.

2.2 Sucrose Consumption

Although there lacks a lot of research regarding the consumption of sucrose prior to exercise, it has been suggested to be beneficial to performance because it has a moderate glycemic index. There is some fear that rebound hypoglycemia could occur if high glycemic index foods are consumed shortly before exercise. These effects; however, vary greatly by individual and there is no concrete evidence that a negative impact on performance or clinically diagnosed hypoglycemia occurs (9). Sucrose consumption prior to exercise has not been compared to other types of carbohydrate in healthy individuals and the metabolic response to sucrose needs further research to define any potential role in exercise performance (23).

2.3 Carbohydrate Plus Protein

It is important to determine whether any performance improvements found when adding protein to a carbohydrate supplement is not due to the added energy consumption that occurs when protein is added to the pre-exercise protocol (2). Saunders et. al (19) performed a study in which a carbohydrate plus protein energy gel was beneficial for endurance performance when compared to a carbohydrate gel. This study looked at both males and females and determined that cyclists performed longer at 75% VO_2 max when consuming the carbohydrate plus protein gel (19). The carbohydrate content of both gels used in this study was identical but the added protein did provide 25% more Calories in the carbohydrate plus protein gel. Another similar study showing increased performance following protein ingestion in addition to carbohydrate also used higher caloric intake from the added protein (18). This makes it difficult to distinguish whether the added benefits were from protein or additional Calories. Hall et. al (7) completed a trial of a carbohydrate plus protein beverage compared to a carbohydrate beverage in cyclists. Although the 1.8% improved performance was not statistically significant, the added protein did result in a reduced heart rate, lower rate of perceived exertion, and reduced muscle damage when compared to the carbohydrate alone. This supplement was also consumed during exercise, and the enhanced recovery due to protein intake may also be beneficial for athletes (7). Another study looking at carbohydrate and carbohydrate plus protein supplements consumed during exercise found an improved endurance performance with both of the mentioned supplements (8). This study examined trained cyclists over extended exercise duration while consuming a carbohydrate or carbohydrate plus protein beverage every 20 minutes. The exact mechanism for the increase in performance of both groups over the placebo groups could not be determined; however (8). There is a possibility that ergogenic effects seen were because the amount of carbohydrate administered to the test subjects was less than optimal amounts needed to complete the exercise testing protocol. Adding protein to a carbohydrate supplement that contains the recommended 6% carbohydrate has not been shown to be beneficial in a cycling time trial (22).

All of the previous studies used supplements that contained equal amounts of carbohydrates with the added protein in the test group also adding extra Calories. Romano-Ely et. al (16) looked at isocaloric beverages and found no improvement in cycling performance when comparing a carbohydrate beverage to a carbohydrate beverage with added protein. Coletta et. al (3) also found no improvement in performance following supplementation of either a carbohydrate or carbohydrate plus protein supplement. The supplement was administered in 120 ml servings five minutes before the start of a 19.2 km run and every 4 km throughout the run for a total of 600 ml. This study focused on supplementation during a run lasting longer than 60 minutes as opposed to the current study which looks at aerobic exercise lasting less than 60 minutes with supplementation occurring 30 minutes prior to exercise. These studies done by Romana-Ely et. al (16) and Coletta et. al (3) were both in contrast to the previously mentioned studies that show performance improvements following the addition of protein to a beverage during exercise. Thus, it still remains in question whether the benefit in these studies came from the protein or from the additional Calories. More research is needed in this area, specifically using different types of athletes (elite vs. recreational), different modes of exercise, and different lengths of performance.

Using a different form of exercise from the previous studies, one study conducted by Alghannam (1) determined that a carbohydrate beverage consumed prior to and during a simulated soccer match enhanced running capacity when compared to an isocaloric carbohydrate beverage. The exact mechanism by which running capacity and subsequent recovery are improved has yet to be determined and the possibility of an internal drive to perform better has not been ruled out (1).

In addition to the challenges faced when comparing non-isocaloric beverages, there remains the dilemma in choosing the type and amount of protein to provide to subjects. Saunders has stated that protein most likely interacts with various beverage compositions such as the carbohydrate content, total volume ingested, protein type, osmolality, and individual tolerance (17). He also explains that the protein content of supplements intended for use in endurance activities should be lower than the carbohydrate content since the latter is the major energy source. The threshold level of protein for improvement is still unknown and more research is needed to determine if higher protein intake will decrease performance in aerobic activities (17).

2.4 Plasma Blood Glucose

Exercising outside a normal blood glucose range could be dangerous for individuals. It is recommended that blood glucose be between 100 and 300 mg/dL before starting any workout regimen (12). Although more research needs to be done in this area, a study looking at the glycemic response to glucose intake compared to glucose and protein intake prior to exercise displayed that the area under the curve was significantly lower in the latter group (15). These young, healthy subjects received 50 grams of glucose powder with or without 20 grams of whey isolate protein powder and were asked to complete 45 minutes on a cycle ergometer at 60% of their age-predicted maximum heart rate. The effect of the protein seemed to cease at approximately one hour after ingestion. This effect on lowering the glycemic response by adding protein to carbohydrate beverages was demonstrated in the subjects both during exercise and on a separate trial in which the subjects were at rest. It is thought that protein consumption with carbohydrates prior to exercise can delay gastric emptying as well as stimulate secretion of glucagon-like peptide and gastric inhibitory protein in the stomach (15).

2.5 Hydration

Hydration status prior to exercise can impact performance for various reasons. Core body temperature may increase during reduced hydration states and psychological response to exercise can vary according to hydration status. Heart rate may increase to maintain cardiac output during times when athletes are hypovolemic due to dehydration and it is common for recreational athletes to be inadvertently hypohydrated prior to exercise. A study performed by Oliver J. Peacock et al (14), displayed that 37% of participants recruited at a gymnasium began to workout in a hypohydrated state and did not drink sufficient fluids to replace sweat loss. Those subjects who were at a hydrated state prior to exercise reported lower levels of fatigue during freely-chosen exercise routines. A urine osmolality of over 900 $mOsmol/kg^{-1}$ is associated with about a 2% body water deficit and one-third of the participants in this study came to the gym ready to exercise with a urine osmolality above this level (14).

There is not a large body of research examining the effect that protein ingestion prior to exercise has on hydration status. A study completed in 13 experienced endurance athletes who ranged in age from 20-28 years old reported that protein added to a sports beverage enhances fluid retention when compared to a carbohydrate only beverage. Subjects performed a cycling exercise session at 80% of maximal heart rate to achieve dehydration after which they consumed one of three test beverages. These beverages were consumed in an amount equal to the amount of body weight lost during the exercise. Subjects were monitored for a three hour recovery period in which it was reported that the carbohydrate/electrolyte/protein beverage significantly increased fluid retention when compared to a placebo and a carbohydrate/electrolyte beverage (20). This study examined consumption of protein with carbohydrate following exercise, so more research is needed to determine the effect that protein has on hydration status when consumed prior to exercise.

Chapter 3: Study Design and Methodology

3.1 Placebo and Treatments

The study was a double-blind, placebo-controlled, single dose clinical study with five test groups consisting of 15 subjects per group. These study groups included:

a. Placebo
b. ModCarb™
c. ModCarb™ + Protein Product
d. Protein Product
e. Protein product + sugar to provide additional Calories similar to that found in commercial sports beverages.

All of the products were dissolved in 16 ounces of water prior to consumption by the participants.

ModCarb Study Supplement Composition					
	Supplement Composition	**Calories**	**CHO**	**PRO**	**FAT**
Placebo	1 packet Crystal Light	10	0 g	0 g	0 g
ModCarb	1 packet Crystal Light + 3 g ModCarb	21.5	2.17 g	0.5 g	0.2 g
ModCarb + PRO	1 packet Crystal Light + 3 g ModCarb + 18.2 g PRO	94.5	3.27 g	14.5 g	1.04 g
PRO	1 packet Crystal Light + 18.2 g PRO	83	1.1 g	14 g	0.84 g
PRO + Sugar	1 packet Crystal Light + 18.2 g PRO + 20 g Sugar	158	21.1 g	14 g	0.84 g

Table 1. Composition of Supplements

3.2 Subjects

Since this study was a pilot study, it only used male test subjects in order to decrease costs associated with a higher number of subjects. Also, the background studies noted above have generally studied males. The inclusion criteria used were recreational, aerobically trained subjects age 18-30 and having a BMI of 20-27 kg/m². The included subjects participated in 120-180 minutes of aerobic exercise per week for at least six months, with at least 2 days per week consisting of runs of at least 2.5 miles. Individuals were excluded from the study based on the following criteria:

a. Cigarette smoking
b. Injuries that restrict the ability to do aerobic exercise
c. Acute major health problems or chronic conditions that affect the ability to exercise (i.e. muscular dystrophy, multiple sclerosis, heart conditions, cancer or arthritis)
d. Stationary or mobile bicycling as part of recent training (since the bicycle testing is supposed to test performance in an unfamiliar activity)
e. Currently being on an Ohio State athletics team (due to NCAA issues and variations on training schedules)
f. Already regularly using carbohydrate drinks just before or during exercise

3.3 Recruitment and Randomization

Recruitment for this study took place by two different methods. Initially, recruitment flyers were placed in various buildings on the campus of The Ohio State

University. Following completion of the study by the first few subjects, recruitment continued through word of mouth by these subjects. The response to the study by this latter method provided sufficient subjects to complete the study. All subjects were compensated for their participation.

Prior to subject recruitment, the numbers one through eighty were randomized into five groups representing the five different supplement groups. The extra subjects were recruited to account for potential drop-outs in the study. During the first testing session, subjects were allowed to draw a number from a bag containing numbers one through eighty and were placed in that numbers respective supplement group. Following completion of all participants in the study, the researchers became unblinded to the participants in each study group.

3.4 Exercise Testing

Subjects completed the exercise testing protocol two times. The first was without any supplementation of ModCarb™ or protein and the second was with a single intake of ModCarb™ plus protein or one of the comparison treatments. There was approximately 10-21 days between the two testing sessions. Each session consisted of the following exercise protocol:

a. 3 mile run which will be evaluated for time (used as a familiar activity)
b. 25 minute stationary bicycle evaluated for distance (used as an unfamiliar activity; started after 1 lap of walking following run)

c. A 90 second step test as an assessment of short term muscle recovery (started 1 minute after the bicycling).

The stationary bicycle activity was done at a predetermined resistance level. During the first session and prior to starting the run, all subjects chose an appropriate bicycle seat height as well as a resistance level that would be challenging for 25 minutes. Participants were instructed to choose a level that would not need to be changed during the course of testing and this level was recorded to be used in both sessions.

The subjects refrained from consuming all food and liquid except water for approximately ten hours prior to each testing session. All subjects received instructions to consume a small snack 10-12 hours prior to testing and were given a list of appropriate snack choices. Subjects were instructed to keep the snack the same or similar for each session. When completing this protocol for the second time, the intervention products or placebo were given 30 minutes prior to exercise. The dose of the ModCarb™ product was 3 grams and the dose of protein was 14 grams.

The study examined the following end points:

a. Time in a 3 mile run; third lane of a four lane indoor track (24 laps)
b. Distance covered in 25 minutes, stationary bicycle (done after walking 1 lap after the 3 mile run)
c. 90 second step test: total number of steps (done 60 seconds after the bicycling); a-c has been used in 3 unpublished studies and in a published study (5)
d. Saliva tests for lactate, IL-6, and IL-15 (analysis to be completed at a later date)
e. Blood tests to measure plasma glucose

3.5 Blood and Saliva Samples

For each testing session, baseline and post-exercise blood and saliva samples were taken 35 minutes before exercise and shortly after exercise by a licensed phlebotomist. Following the blood draws, each sample was spun in a centrifuge at 3000 repetitions per minute for 15 minutes. The plasma was then removed from each sample and stored at -80^{0}F until thawed for analysis. The saliva samples were transferred to microfuge tubes and also stored at -80^{0}F until needed for further analysis.

3.6 Plasma Glucose Test

Plasma glucose was measured with a glucose hexokinase reagent set (Pointe Scientific). Hexokinase catalyzes a reaction with glucose and adenine triphosphate (ATP) to form glucose-6-phosphate. Glucose-6-phosphate then reacts with nicotinamide adenine dinucleotide (NAD) and forms NADH. At an absorbance of 340 nanometers, NADH causes an increase that is proportional to the amount of glucose in the sample. One microliter of each sample was combined with 200 microliters of the glucose reagent in a test well. Using the absorbance of both a blank and an aqueous glucose standard, the absorbance of each sample was used to calculate the glucose in milligrams/deciliter for each sample.

3.7 Statistical analysis

For subject number per group, a power calculation was done partially based on placebo effects and variations in published and unpublished data from recent studies from the investigators. Based on this work, a 9 second improvement for a 3 mile run with a variation of 5 seconds ± 8 seconds was deemed reasonable for the placebo. A minimal desired change for any of the interventions was set at 17 ± 8 seconds. If 15 subjects are used per group, for a two- tailed t-test and a p-value of 0.05, a power value of 0.98 is given.

For statistical analysis of the results, significance was set at $p < 0.05$ for each comparison. Initially, for each treatment group, baseline and post-intervention measures were compared using one-tailed paired t-tests. The change in each parameter for the treatment groups was compared to the change in parameter for the placebo group using a two-tailed unpaired t-test at a significance level of $p < .05$. Using a Bonferroni correction for the four unpaired t-tests performed for each exercise parameter, significance would be set at the 0.0125 level. Change in parameters was also compared among groups using ANOVA but the results were not included because no significance was detected.

Chapter 4: Results

4.1 Subject Characteristics

The age and body mass index of each subject group were not significantly different from one another using a one-way ANOVA. The average and standard deviation of each group is displayed in Table 2.

Subject Group	Age (years)	BMI (kg/m^2)
Placebo	22.13 ± 2.36	24.21 ± 2.22
ModCarb	22.93 ±3.15	23.21 ± 1.30
ModCarb+PRO	22.06 ± 3.21	23.72 ± 2.01
PRO	21.40 ± 3.18	24.38 ± 1.46
PRO+Sugar	21.80 ± 1.32	23.45 ± 1.89

Table 2. Age and BMI of subjects in each group, reported as mean ± standard deviation. No significant differences were seen using a one-way ANOVA at p<.05.

4.2 Run Time

When comparing the run time for the placebo group as well as each treatment group using one-tailed paired t-tests, the protein plus added sugar group was the only group that did not see a statistically significant improvement (p<.05). Table 3 displays the mean and standard error for the pre and post treatment run times for each group as well as the significance level. The average run time for each group is shown in Figure 1.

	Placebo		ModCarb		ModCarb + PRO		PRO		PRO + Sugar	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Mean	24.46	23.67	23.63	22.52	23.85	23.16	24.25	23.56	23.37	23.05
Standard Error	0.54	0.47	1.16	0.94	0.64	0.47	0.40	0.38	0.61	0.57
P-Value	**0.0033**		**0.0041**		**0.0145**		**0.0055**		**0.15**	

Table 3. Run Time for each group in minutes, p-values are following a one-tailed paired t-test for each group.

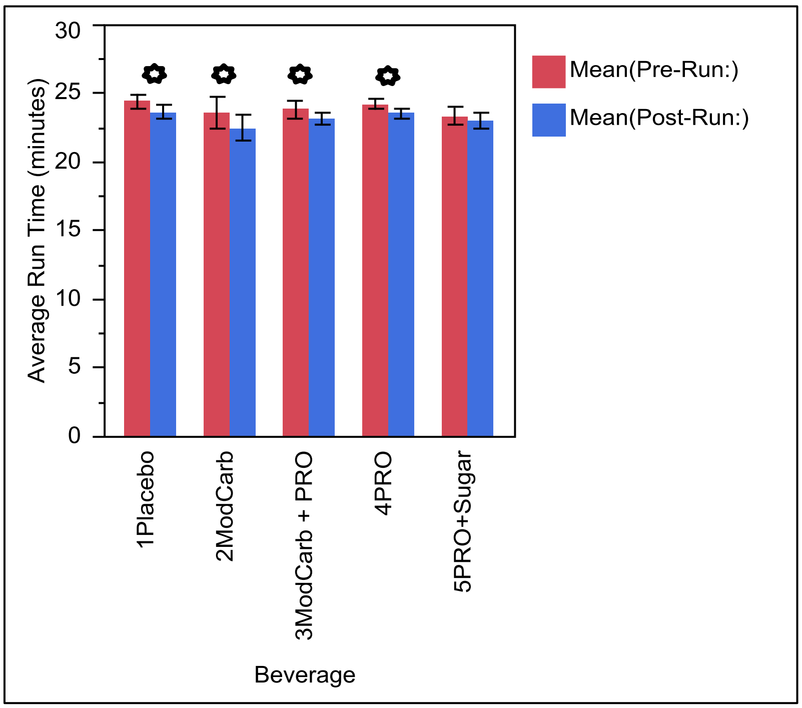

Figure 1. Average pre and post run times for each group, showing significance (p < 0.05). Each error bar is constructed using 1 standard error from the mean.

The average change in run time and the standard error for each group is shown in Table 4. After comparing each treatment group to the placebo, no statistically significant changes were seen (p <.05). The protein plus added sugar group was the most different from the placebo group; however, the difference was not great enough to be considered statistically significant. The average change in run time, displayed in minutes, can be seen in Figure 2.

	Placebo	ModCarb	ModCarb + PRO	PRO	PRO + Sugar
Mean	-0.782	-1.11	-0.69	-0.68	-0.32
Standard Error	0.24	0.36	0.28	0.23	0.30
P-Value	**NA**	**0.448**	**0.807**	**0.771**	**0.25**

Table 4. Δ Run Time in minutes, p-values are following an unpaired t-test with each group compared to placebo.

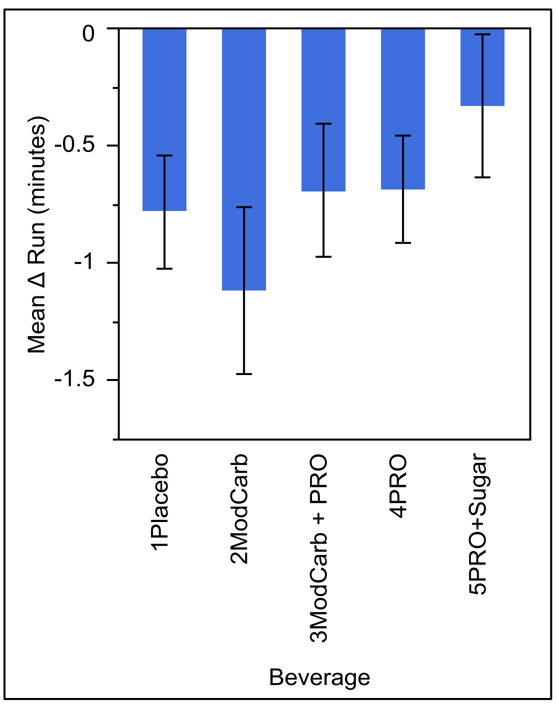

Figure 2. Average change in pre and post run time for each group. Each error bar is constructed using 1 standard error from the mean.

4.3 Bike Distance

The average miles biked for each group pre and post treatment along with the standard error can be seen in Table 5. The comparisons within each group were made using a paired t-test and the p-value for a one-tailed test is shown. Because the goal of supplementation is to see an improvement in bike distance, it can be argued that a one-tailed test would also be appropriate. As seen by the second p-value listed for each group in Table 5, all of the groups had a significant improvement except the ModCarb™ group.

	Placebo		**ModCarb**		**ModCarb + PRO**		**PRO**		**PRO + Sugar**	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Mean	5.88	6.39	6.61	6.84	6.36	6.71	5.98	6.30	6.01	6.54
Standard Error	0.38	0.40	0.31	0.40	0.34	0.31	0.37	0.34	0.28	0.30
P-Value	**0.032**		**0.26**		**0.02**		**0.038**		**0.039**	

Table 5. Bike Distance for each group in miles, p-values are following a one-tailed paired t-test for each group.

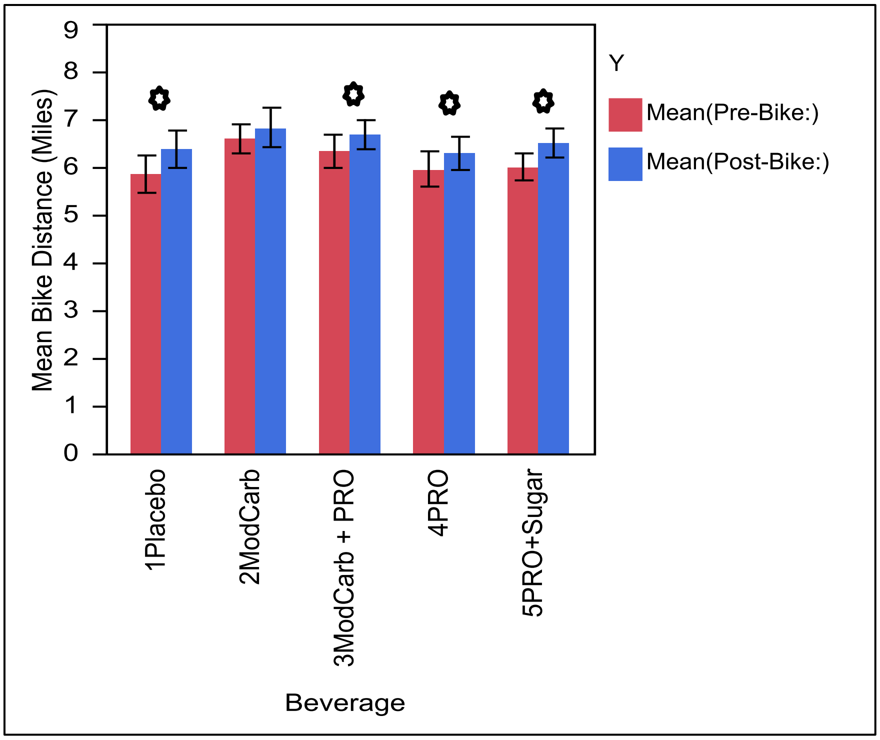

Figure 3. Average pre and post bike distance for each group, showing significance based on a one-tailed paired t-test. Each error bar is constructed using 1 standard error from the mean.

The average change in bike distance for each group is shown in Table 6. After comparing the treatment groups to the placebo, no statistically significant differences were seen. The protein plus added sugar group was nearly identical to the placebo group and the other three treatment groups were very similar to one another. Although Figure 4 seems to display a relatively large difference between the ModCarb™ group and the placebo group, this change was not large enough to be considered significant.

	Placebo	ModCarb	ModCarb + PRO	PRO	PRO + Sugar
Mean	0.516	0.236	0.35	0.32	0.53
Standard Error	0.25	0.36	0.16	0.17	0.28
P-Value	**NA**	**0.528**	**0.593**	**0.526**	**0.969**

Table 6. Δ Bike Distance in miles, p-values are following an unpaired t-test with each group compared to placebo.

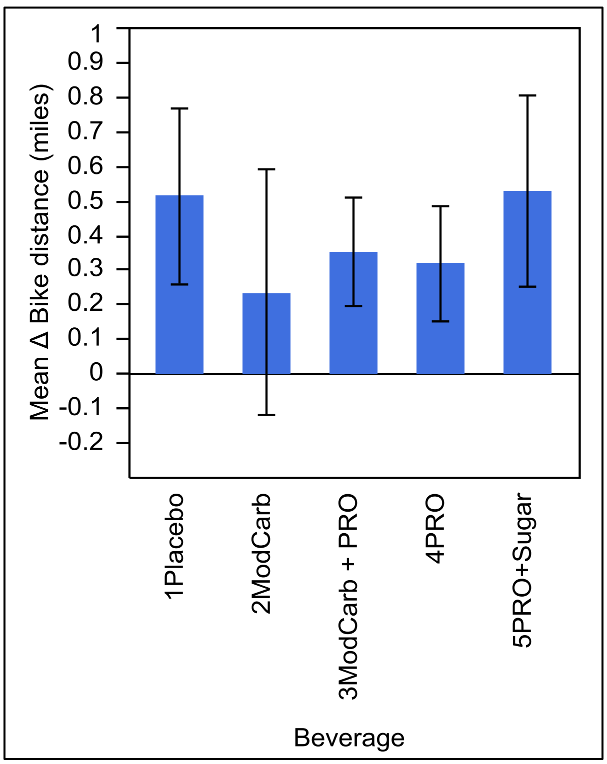

Figure 4. Average change in bike distance (miles) for each group. Each error bar is constructed using 1 standard error from the mean.

4.4 Steps Completed

The change in steps completed within each group was a statistically significant improvement for all groups. The average pre and post treatment steps as well as the standard error are shown in Table 7 with Figure 5 graphing these results. The p-values listed are following a paired t-test for each group.

	Placebo		**ModCarb**		**ModCarb + PRO**		**PRO**		**PRO + Sugar**	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Mean	43	47.4	42	45.13	40.47	44.8	41.8	48.93	41.67	48.2
Standard Error	1.65	2.40	2.00	1.89	2.29	2.09	2.60	2.98	1.36	1.99
P-Value	**0.00185**		**0.0032**		**0.00015**		**0.00015**		**4.2515e-5**	

Table 7. Steps completed for each group, p-values are following a one-tailed paired t-test for each group.

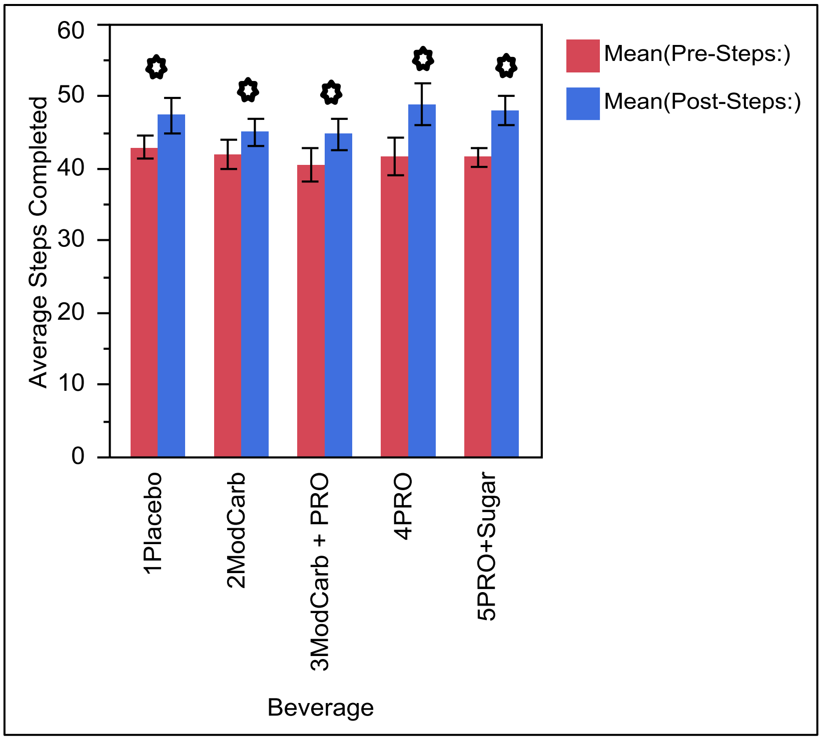

Figure 5. Average pre and post steps completed for each group, showing significance. Each error bar is constructed using 1 standard error from the mean.

The average change in number of steps completed for all groups can be seen in Table 8. When comparing the treatment groups to the placebo, there were no statistically significant differences; however, several of the groups did show a greater improvement than the others. Specifically, the protein and protein plus added sugar groups improved more than the ModCarb™ and ModCarb™ plus protein groups, but not with statistical significance.

	Placebo	ModCarb	ModCarb + PRO	PRO	PRO + Sugar
Mean	4.4	3.13	4.33	7.13	6.53
Standard Error	1.23	0.96	0.85	1.41	1.07
P-Value	**NA**	**0.425**	**0.965**	**0.156**	**0.202**

Table 8. Δ Steps Completed, p-values are following an unpaired t-test with each group compared to placebo.

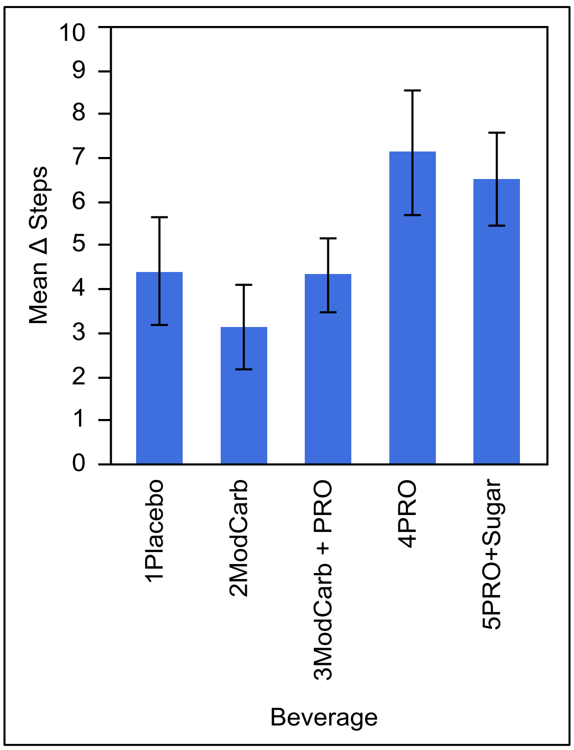

Figure 6. Average change in steps completed for each group. Each error bar is constructed using 1 standard error from the mean.

4.5 Plasma Glucose

As seen in Table 9, the change in plasma glucose levels during baseline and treatment exercise sessions was highly variable across the groups. All of the groups except the ModCarb™ plus protein group had relatively stable plasma glucose levels within the group. Using a paired t-test at the .05 level of significance, the figure shows that there were no significant differences between the change in plasma glucose levels during baseline when compared to the change in plasma glucose levels during treatment for each group. The ModCarb™ plus protein group was very close to being significantly different, most likely due to the large average drop in glucose levels during the baseline testing session. Figure 7 displays the average change in plasma glucose levels for each group during baseline and treatment sessions.

	Placebo		**ModCarb**		**ModCarb + PRO**		**PRO**		**PRO + Sugar**	
	Δ_B	Δ_{Tr}	Δ_B	Δ_{Tr}	Δ_B	Δ_{Tr}	Δ_B	Δ_{Tr}	Δ_B	Δ_{Tr}
Mean	0.32	2.29	-1.06	-7.22	-15.13	1.48	-0.44	2.36	-2.08	3.77
Standard Error	5.84	4.24	5.30	4.79	6.76	4.78	4.33	4.21	4.22	3.66
P-Value	**0.719**		**0.488**		**0.063**		**0.562**		**0.355**	

Table 9. Δ in Plasma Glucose (Δ_{Baseline} verse $\Delta_{\text{Treatment}}$) in mg/dL, p-values are following a paired t-test for each group

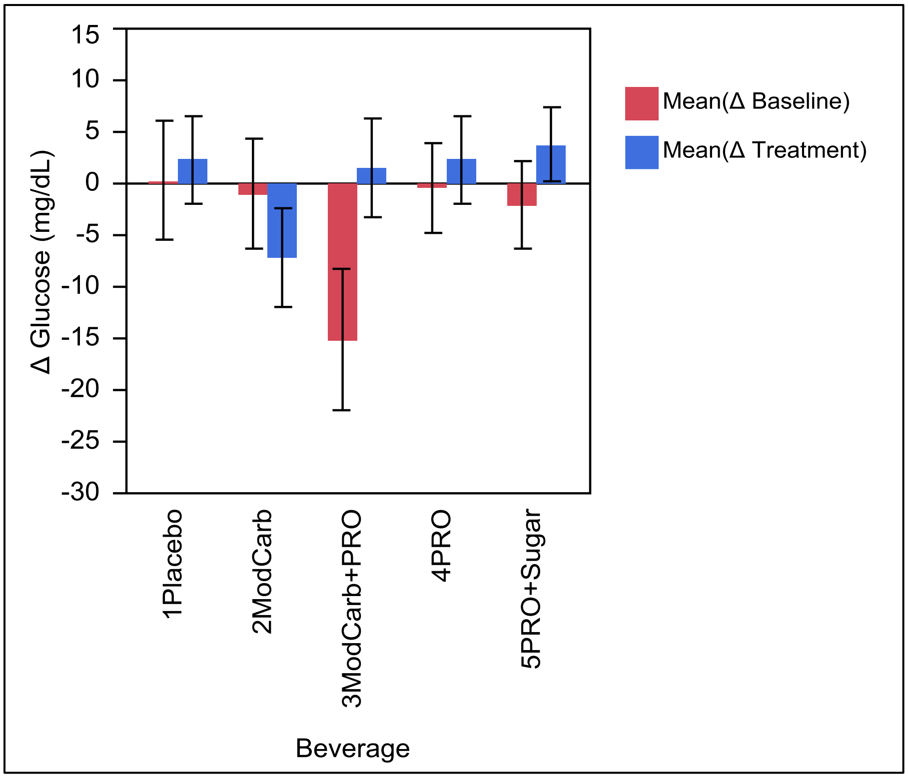

Figure 7. Average change in plasma glucose levels (mg/dL) during baseline and treatment sessions. Each error bar is constructed using 1 standard error from the mean.

Because the ModCarb™ plus protein groups would have displayed significance using a one-tailed paired-test, we then examined patterns in the plasma glucose changes. Using the subjects in all groups that had a decrease in plasma glucose levels during the baseline session, using a paired t-test, we found that the change from baseline to treatment sessions for these individuals was statistically significant. The mean plasma glucose levels and standard error for this group is displayed in Table 10.

	Δ Baseline	Δ Treatment
Mean	-16.57	0.143
Standard Error	2.72	2.55
p-value	**0.0001**	

Table 10. Paired t-test using all subjects displaying a decrease in plasma glucose levels at baseline, p<.05

After subtracting the change in plasma glucose level during the baseline testing session from the change in plasma glucose level during the treatment session, the overall change in plasma glucose levels for each individual was determined. Table 10 displays this average change and the standard error of the mean for each group. Using an unpaired t-test in which each group was compared to the placebo, it was determined that there were no significant differences. Similar to the paired t-test previously mentioned, the ModCarb™ plus protein group was close to showing significance. The plasma glucose levels in this group tended to remain more stable during the treatment session than during the baseline session, resulting in a large positive overall change of plasma glucose levels. Figure 8 displays the average change in plasma glucose levels for each group during the course of the study.

	Placebo	**ModCarb**	**ModCarb + PRO**	**PRO**	**PRO + Sugar**
Mean	1.96	-6.16	16.61	2.79	5.85
Standard Error	5.21	8.65	7.24	4.70	6.10
P-Value	**NA**	**0.431**	**0.145**	**0.908**	**0.636**

Table 11. Overall change in plasma glucose ($\Delta_{Treatment}$-$\Delta_{Baseline}$) in mg/dL, p-values are following an unpaired t-test with each group compared to placebo

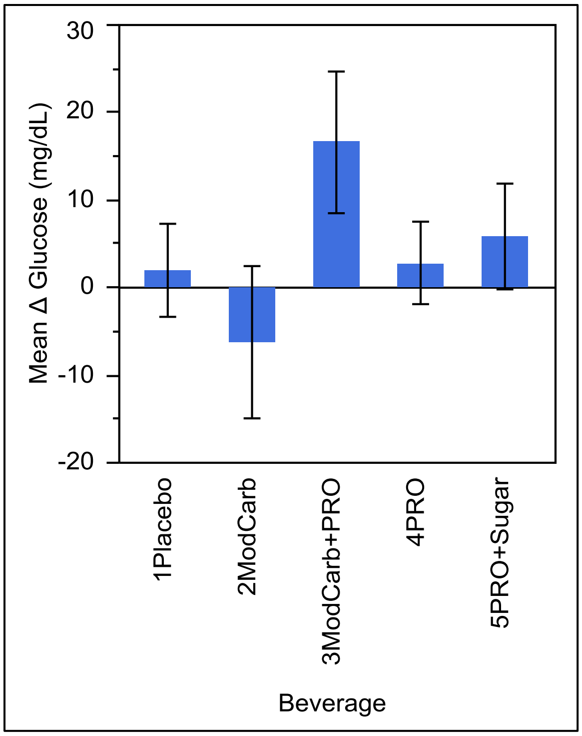

Figure 8. Overall average change in plasma glucose (mg/dL) levels from treatment to baseline. Each error bar is constructed using 1 standard error from the mean.

Chapter 5: Discussion

This study showed that an improvement in aerobic exercise performance completed in the morning following a fast occurred for a variety of carbohydrate and protein mixtures. There was a statistically significant improvement in run time for all of the groups except protein plus added sugar, bike distance for all groups except ModCarb™, and steps completed for all groups. It is important to note that in three previous studies from this laboratory, a repeat of this exercise testing in itself did not produce big improvements (4, 5, 6). However, exercise was not done in the early morning on an empty stomach in those studies. Thus the findings from the current study are important since there is limited research on carbohydrate consumption in the morning. The timing of supplement ingestion for the study, 30 minutes prior to exercise, is representative of a time frame in which recreationally trained athletes may consume nutrients in a real life situation.

Although we did not see significant differences in the change in run time, bike distance, or steps completed for any of the groups compared to the placebo, the amount of supplement the subjects received needs to be considered. When looking at the group receiving ModCarb™, the Calories consumed by each subject from the supplement were roughly ten Calories. The three grams of carbohydrates provided by this supplement may not be enough to show a significant improvement in any of the end points of the study. A much larger dose may be needed to have an impact on performance.

As for the protein only group, the Calories provided by the supplement was larger than the placebo or ModCarb™ groups, but 83 Calories may still not be enough to see an improvement. Along with the issue of the actual amount of Calories, this group only received 1.1 grams of carbohydrate to go along with the fourteen grams of protein. Because the exercise was completed roughly 30 minutes after ingestion of the supplement, it is likely that the protein was not used by the body as a source of fuel for exercise. The subjects were fasted prior to both testing sessions, so glycogen stores were most likely utilized during the testing sessions. The added Calories from the protein in the supplement would not have had enough time to be metabolized and incorporated into fuel for aerobic energy.

Similar to the protein group, the ModCarb™ plus protein group would not have ingested a high enough amount of carbohydrate to show a difference in performance. The Calorie level was greater than that of the previous two groups, but the composition of the supplement may not have been ideal to see an improvement in exercise performance. The final group, protein plus added sugar, was the only group that did not show a significant improvement in run time from baseline to post-intervention. The group did show a significant improvement in bike distance and steps completed. However, because the subjects were fasted, it was a concern that the pure sugar added to the supplement would cause a hypoglycemic effect during the course of the exercise. Adding the protein to the sugar was intended to slow down the rate of emptying from the gut in order to prevent this hypoglycemia and its effect on performance.

A part of the increase in number of steps completed by each group could be due to an increased familiarity with the testing protocol. Subjects were instructed on how to complete the step test prior to both sessions, but there is a degree of muscle memory that could contribute to the improvement from baseline to post-treatment. Again, this did not occur in three other studies of this same protocol, though testing did not occur on an empty stomach in the morning. Interestingly, in two of the studies, no improvement was seen in running time from the first trial to the second trial with either placebo, Gatorade or a substitute drink (5, 6). Effects were seen on the other two measures of performance for Gatorade or a substitute drink, but not for the placebo.

Because the 25 minutes of biking was intended to be an unfamiliar activity, it is somewhat surprising that we did not see as significant of an increase within each group as we did with the three mile run. This could be related to a lack of knowledge in each subject about what a satisfactory bike mileage for the given time would be. In contrast, if the subject was familiar with his typical time in a three mile run, there may be more intrinsic motivation to perform better during the second testing session. It is also possible; however, that the larger improvement seen in the run as compared to the bike could be related to the relatively small amount of Calories provided by the supplements. Because the run was performed first, the nutrients provided by the supplements could have aided the subject in the run, but may have been exhausted by the time the subjects were performing the stationary bike ride.

After analyzing the change in plasma glucose levels for each group, it was determined that no statistically significant differences were present within the groups as well as between the groups using an overall change in plasma glucose levels. Although this was the case, the relatively large difference in glucose levels between the two sessions in the ModCarb™ plus protein group should not be discounted. This group did show a drop in glucose levels during the baseline session which was stabilized during the treatment session. The amount of carbohydrate provided to this group was low, but the addition of protein may have had the expected effect of slowing down a hypoglycemic response during exercise. Plasma glucose levels vary greatly among individuals, as was seen in this study. It is difficult to determine the true effect of the addition of protein to the ModCarb™ supplement, so a larger dose of this supplement should be given in order to verify the effects of added protein on changes in plasma glucose levels.

A limitation of the study was the difference in amount of water consumed prior to the pre and post treatment exercise testing sessions. The subjects were instructed to fast all food and liquid except for water prior to each session; however, the amount of water that each subject ingested prior to exercise was not recorded. Regardless if the subject was in the placebo or a treatment group, each individual consumed approximately sixteen ounces of water prior to the second testing session. If this was significantly different than the amount consumed prior to the first session, the difference in hydration status could have an impact on performance. Because there was a significant improvement in all the groups from baseline to post-intervention but no difference among the groups, this fuels the belief that hydration status is an important factor in the study. The lack of effect on run time by the protein plus added sugar group may have been due to inferior hydration compared to the other treatments.

Intrinsic motivation can play a role in exercise performance and cannot be discounted in this study. Although subjects were not told their run time, bike distance or steps complete at baseline prior to the start of the post-treatment exercise session, it is possible that individuals remembered their previous performances. Because the subjects were recreationally trained, a performance improvement due to this motivation could have occurred. If using elite athletes already performing at a high level, this motivation is most likely still present but large changes in performance may be harder to achieve. With that being said, the target population of the ModCarb™ supplement is mainly the recreationally trained athletes so completing the study in this population is beneficial.

Chapter 6: Conclusion

All groups except the protein plus added sugar improved in three mile run time and all groups except the ModCarb™ group improved in distance biked from baseline to post intervention. All of the groups significantly improved from baseline to post-intervention for amount of steps completed. This study did not show any statistically significant improvement in three mile run time, distance biked in 25 minutes, or the number of steps complete in ninety seconds for any of the supplement groups when compared to placebo.

It is possible that hydration status played an important role in the improvement seen from baseline to post-intervention, so future studies should also provide the same amount of water prior to both testing sessions. Subjects should also be provided with an amount of ModCarb™ that is more similar to the suggested serving size and that usually ingested by recreational athletes prior to exercise. It is possible that this would result in an improvement in exercise performance and may provide more evidence that the addition of protein to the ModCarb™ supplement would have positive effects on changes in plasma glucose levels. Despite these limitations, a combination of carbohydrate and protein mixtures had a significant effect on early morning aerobic exercise performance.

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Appendix A: Screening Questionnaire

Eligibility Questionnaire for the Study Titled: Acute effects of ModCarb™ on Exercise Performance

Name_________________________________

Are you male or female? __________ What is your age? _______

What is your approximate height and weight? ______________________

Do you do aerobic exercise? ______   If yes:

How often? _____________________

What do you do? ____

About how long per workout (time or distance)? ___________________

And for how long have you been training? ____________________

Do you run 2.5 miles or more at least once a week? _______

Are you on any OSU sports teams? ________________________

Do you now have any injuries, diseases or health problems that limit your ability to exercise? Examples of relevant diseases are muscular dystrophy, multiple sclerosis, heart conditions, cancer or arthritis______

If yes to this question, please do not return this form because you are not eligible.

Do you take any nutritional supplements? If yes, what are they, how often and for how long have you been taking them?

____

____

Do you smoke cigarettes? ________

Do you drink any types of nutrition products just before or during exercise? _____

If yes, what product(s)?

____

Appendix B: Pre-Study Snack Guidelines

It is important to eat a snack about 10 hours before you are scheduled to complete this study. An ideal snack would contain about 250-350 Calories, 40-50 grams carbohydrate, 15-20 grams protein, and 5-10 grams fat. This will help fuel your workout in the morning, while providing a good balance of nutrients needed for exercise. There are many resources available on the internet to use as a guideline (ex. www.choosemyplate.gov, www.myfitnesspal.com, www.nutritiondata.com), but here are a few recommendations.

<u>How to Read a Nutrition Label</u>

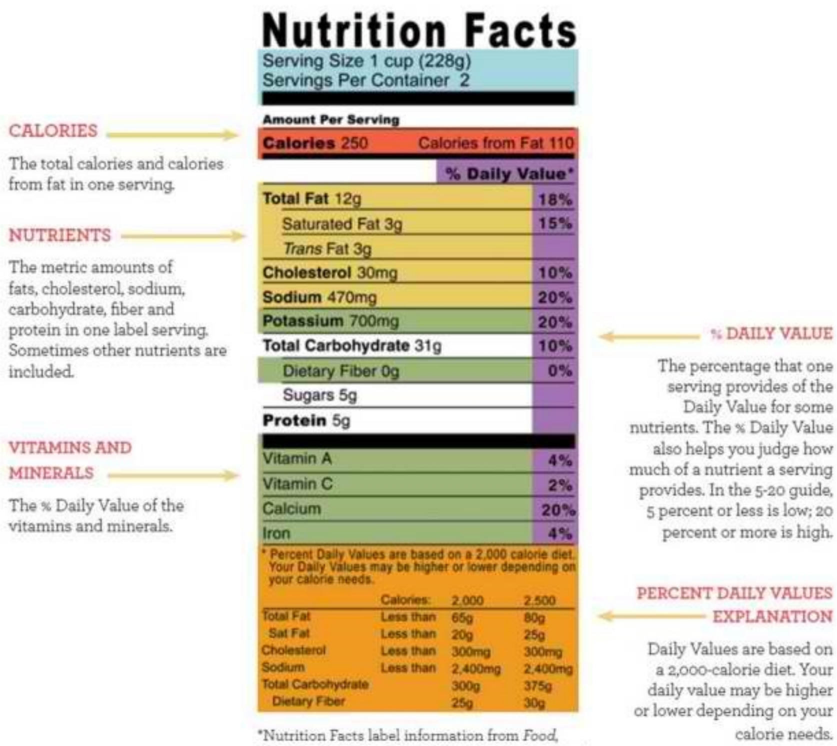
If there is a normal snack that you prefer to eat the night before a workout, this is completely fine. It is important to avoid trying new foods before this study because there is the potential for GI distress during workouts. Here are a few tips on how to read a nutrition label to ensure your snack is close to our nutrient guidelines.

1. Start your label reading by looking at the serving size printed right under nutrition facts. It is important to understand how much counts as one serving size so you can accurately estimate the nutrients you are consuming.
2. Next you'll see how many Calories are in a serving and how many of those Calories come from fat.
3. Look at the total grams of fat which includes fats that are good for you, such as monounsaturated, polyunsaturated and omega-3 fats (usually from liquid and

plant sources, such as canola oil and nuts) and fats that are not so good, such as saturated and trans fats (from animal or vegetable sources).

4. Identify the total carbohydrates. This number represents the total of all the different types of carbohydrates you consume from eating one serving of the food.
5. View the protein amount. This number tells you how many grams of protein you obtain from consuming one serving of the food.
6. Remember that %DV (daily value) is based on 2,000 Calories a day.

<u>Example of a Nutrition Facts Label</u>



*Nutrition Facts label information from *Food, Nutrition & Wellness* by Roberta Duyff, Woodland Hills, CA: Glencoe/McGraw-Hill, 2010

<u>Snack Options</u>

The following list of snacks falls close to the recommended range of nutrients:

1. 2 Eggs and 2 slices whole wheat toast
2. ¾ cup Greek yogurt, 1 cup berries, and ½ ounce almonds

3. 1 large apple, 1 tbsp peanut butter, and 1 cup milk (cow's milk or soy milk)
4. Energy bar such as ClifBar, Luna Bar, or Kind Bars (use the above guide to read the nutrition label)
5. 2 cups low-fat chocolate milk
6. ¾ cup cottage cheese, 1 tbsp honey, ¾ cup whole grain cereal
7. 1 small whole wheat pita with 2 tbsp hummus, 2 ounces beef jerky
8. 1 small whole wheat bagel, 2 ounces turkey, 1 slice cheese
9. 7-8 whole grain crackers, 1 ounce string cheese, 1 cup baby carrots
10. Fruit smoothie with ½ banana, ¾ cup low-fat greek yogurt, ½ cup orange juice, and 4-5 ice cubes

Appendix C: ModCarb™ Nutrition Label

Nutrition Facts

Serving Size 1 (30g)
Servings Per Container 15

Amount Per Serving
Calories 125 — Calories from Fat 18

	% Daily Value*
Total Fat 2g	3%
Saturated Fat 0.4g	2%
Cholesterol 0mg	0%
Sodium 7mg	0%
Total Carbohydrate 21.7g	7%
Dietary Fiber 3.4g	13%
Sugars 0.7g	
Protein 5g	10%

Vitamin A 0% • Vitamin C 0%
Calcium 3% • Iron 5%

* Percent Daily Values are based on a 2,000 calorie diet. Your Daily Values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Sat Fat	Less than	25g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

** Percent Daily Values not established.

The above statement has not been evaluated by the FDA. This product is not intended to diagnose or treat any disease.

Amino Acid Profile

Per 100 grams	
Alanine	0.78
Arginine	1.11
AsparticAcid	1.26
Cystine	0.37
GlutamicAcid	3.29
Glycine	0.78
Histidine	0.35
Isoleucine	0.71
Leucine	1.22
Lysine	0.53
Methionine	0.3
Phenylalanine	0.87
Proline	0.86
Serine	0.63
Threonine	0.5
Tryptophan	0.27
Tyrosine	0.62
Valine	0.89

The above statement has not been evaluated by the FDA. This product is not intended to diagnose or treat any disease.

Appendix D: Whey Protein Nutrition Label

NUTRITIONAL INFORMATION / 100G

Calories	400
Calories from Fat	40
Total Fat	4.6 g
Saturated Fat	2.4 g
Polyunsaturated Fat	0.7 g
Monounsaturated Fat	1.3 g
Trans Fatty Acid	0.2 g
Cholesterol	240 mg
Total Carbohydrate	6.0 g
Dietary Fiber	-
Sugars	5.0 g
Protein (as is)	77 g
Vitamin A	-
Vitamin C	-
Thiamin	-
Niacin	-
Riboflavin	-
Calcium	380 mg
Sodium	210 mg
Potassium	380 mg
Magnesium	60 mg
Iron	0.9 mg
Phosphorus	310 mg

MICROBIOLOGICAL ANALYSIS

Standard Plate Count	<30,000 cfu/g
Coliform	<10 cfu/g
Yeast and Mold	<50 cfu/g
Coag. Pos. Staph	<10 cfu/g
Salmonella	Negative/375g
Listeria	Negative/50g

AMINO ACID PROFILE / 100G POWDER

Aspartic Acid	9.0
Threonine	5.9
Serine	3.8
Glutamic Acid	13.3
Glycine	1.6
Alanine	4.4
Valine	4.3
Isoleucine	5.3
Leucine	8.4
Tyrosine	2.2
Phenylalanine	2.3
Histidine	1.2
Lysine	7.3
Arginine	1.7
Proline	5.3
Cystine	2.2
Methionine	1.7
Tryptophan	0.9

PACKAGING AND STORAGE

Multi-wall, Kraft paper sacks, having inner food grade polyethylene liner

Net weight 20 kg (44.092 lbs)

Store in a cool, dry, clean environment below 25° C (77° F) and at relative humidity below 65%. Keep away from strong odors and other contaminants.

Use stocks in rotation for up to two years

Appendix E: Recruitment Flyer

MEN'S NUTRITION-EXERCISE STUDY
SUBJECTS NEEDED

NEED: **MEN** AGE 18-30 WHO RUN 2.5 MILES OR MORE REGULARLY
FOR: NUTRITION-EXERCISE STUDY
PAY: Up to $220

STUDY REQUIREMENTS
2 EXERCISE TESTING SESSIONS-3 MILE RUN + OTHER AEROBIC EXERCISE (an hour or less each)
1 SHORT MEETING + PRELIMINARY EXERCISE TESTING (20 minutes)
4 SMALL BLOOD AND SALIVA DONATIONS
DRINK ONE BEVERAGE BEFORE THE SECOND EXERCISE TESTING

EMAIL modcarbstudy@gmail.com

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Modcarbstudy@gmail.com
Modcarbstudy@gmail.com
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Modcarbstudy@gmail.com

Appendix F: Initial Email Script

Thank you for contacting us about our study. We are studying whether intake of certain food derived products before exercise can improve aerobic exercise performance. I am attaching a questionnaire to see if you qualify for the study. The study has several qualification requirements, but it is especially important that you be able to run 3 miles.

I am also attaching a consent form that explains what may be required of you. If after reading the consent form, you want to be considered for the study, please return the questionnaire. Returning the questionnaire does not mean that you are agreeing to be in the study if selected. You still have a chance to decline after we look at your questionnaire and re-contact you. Do not sign or return the consent form. If you actually participate in the study, we will furnish another copy of the consent form at the research site for you to sign there. You can keep the copy we are sending now or ask at the research site for another one to keep. If you have any questions, please let me know and thank you again for your interest in our study.

Appendix G: Follow-up Email Script

Thank you again for your interest in our study, and you have qualified to participate. The next step is to sign the consent form, determine your stationary bike settings, and perform the baseline testing session. This includes the first two blood draws and saliva samples as well as completing the exercise protocol. The entire session should last around 1 hour and 30 minutes and it is very important that you are fasted (no food and drink **other than water** for 10 hours prior to testing). It is for this reason that the testing sessions take place in the morning between 7 am and 11 am. I have attached a handout of some snack options that should be consumed around 10 hours prior to testing. If you have a normal snack routine at that time; however, feel free to consume that instead. On the day of your testing, come to the basement/lower level of the PAES building. Follow the signs to the exercise science labs. Go through the double doors, and have a seat in the little seating area. The room is A25 which is right in that area. We currently have a testing session scheduled for _______so please let me know if this works. If not, we are more than willing to find a time that works in your schedule. Thank you again for your interest and I look forward to meeting you soon.

Appendix H: Raw Data for Exercise Tests

Group	Subject	Pre-Run	Post-Run	Pre-Bike	Post-Bike	Pre-Steps	Post-Steps
1	44	27.57	26.23	4.19	6.13	35	35
1	45	25.00	23.40	5.50	7.04	41	47
1	75	24.67	23.40	4.80	6.04	46	41
1	8	23.10	21.53	8.11	8.15	43	47
1	51	23.03	22.22	6.96	8.21	50	57
1	10	22.95	24.30	3.84	2.48	34	31
1	60	24.38	24.67	3.79	6.10	52	53
1	59	23.52	23.58	6.00	6.57	45	58
1	17	27.73	26.27	6.00	5.94	39	41
1	32	24.27	23.02	4.28	4.40	49	58
1	39	23.72	23.18	6.70	6.14	33	37
1	47	22.63	21.30	7.15	7.43	48	55
1	49	24.07	24.33	5.72	5.37	51	60
1	18	21.33	20.90	7.99	8.42	37	42
1	77	28.87	26.78	7.10	7.45	42	49
2	12	23.78	23.82	6.31	6.46	56	56
2	52	19.37	18.35	6.23	8.02	36	41
2	56	30.57	28.68	6.37	5.99	41	42
2	43	26.03	23.23	5.98	4.26	41	45
2	2	21.83	20.70	6.57	7.09	34	31
2	48	21.88	23.63	6.48	5.22	42	43
2	28	22.65	21.27	7.15	7.34	52	51
2	58	19.93	20.12	6.65	6.52	49	51
2	57	35.25	31.15	7.40	5.14	32	35
2	35	23.13	21.75	5.83	6.38	49	51
2	13	25.68	23.90	5.80	7.97	35	48
2	70	20.73	20.78	7.49	8.39	35	40
2	3	20.05	18.75	6.36	6.05	33	38
2	50	18.33	18.08	10.06	10.56	47	53
2	19	25.37	23.67	4.40	7.23	48	52

3	24	21.45	22.38	4.72	6.52	38	40
3	40	21.98	21.77	7.68	7.52	45	50
3	74	27.87	26.15	5.63	6.49	31	34
3	27	23.00	23.00	6.56	6.57	34	40
3	36	24.48	21.52	8.08	8.49	44	48
3	63	26.78	25.20	4.01	4.16	40	40
3	72	22.50	23.03	5.49	5.30	35	38
3	29	20.13	20.57	7.77	8.39	38	45
3	41	23.30	22.77	8.07	8.23	35	42
3	73	21.67	21.10	7.52	7.14	33	39
3	23	23.93	23.57	5.90	6.19	68	66
3	61	26.05	25.75	5.25	5.53	38	40
3	54	27.63	25.40	5.26	6.68	39	44
3	76	25.72	24.18	6.02	6.17	42	54
3	78	21.23	20.97	7.39	7.29	47	52
4	28	26.00	25.88	5.95	6.87	44	55
4	34	24.13	24.82	9.00	9.32	57	65
4	26	23.52	23.53	5.95	6.46	65	70
4	65	28.37	25.67	5.94	6.34	46	53
4	66	23.95	22.12	5.18	5.71	42	52
4	42	24.53	23.42	6.38	6.62	39	52
4	1	23.18	22.40	5.73	5.16	35	41
4	46	22.25	21.10	7.62	6.88	52	61
4	5	24.83	24.82	5.33	5.66	26	38
4	71	25.38	23.97	5.55	7.23	41	43
4	20	23.35	22.12	6.26	5.93	31	32
4	62	22.40	21.83	6.57	7.21	40	51
4	7	22.88	22.88	5.91	5.54	36	52
4	55	24.98	24.93	2.12	3.18	35	32
4	9	23.93	23.93	6.15	6.34	38	37
5	69	18.23	17.97	6.83	7.59	44	45
5	16	20.98	21.10	6.02	5.70	53	68
5	31	26.37	25.23	5.36	6.38	40	50
5	11	21.83	22.42	8.30	8.54	39	48
5	38	23.48	25.12	5.36	4.70	39	49
5	15	21.67	20.43	6.54	7.82	42	51
5	14	26.93	24.17	6.71	7.00	50	53
5	4	22.80	22.43	5.60	5.98	39	43
5	37	21.03	21.23	6.52	7.15	47	58

5	25	24.48	23.15	5.83	7.21	44	50
5	33	23.28	22.27	6.63	7.71	35	42
5	64	24.25	24.30	4.37	6.04	37	39
5	30	25.93	24.77	4.78	6.45	38	45
5	22	25.77	25.78	7.05	4.57	35	37
5	21	23.57	25.33	4.20	5.22	43	45

Appendix I: Raw Data for Plasma Glucose

Group	Subject	Pre-Baseline	Post-Baseline	Pre-Treatment	Post-Treatment
1	8	92.59	81.92	94.22	91.69
1	10	82.82	84.27	84.63	64.37
1	17	99.47	62.92	101.09	92.41
1	18	87.16	89.15	103.08	128.41
1	32	91.31	102.39	87.66	67.71
1	39	81.17	94.00	96.69	114.43
1	44	106.31	104.50	92.79	90.09
1	45	97.30	127.93	87.39	110.81
1	47	76.58	100.00	87.39	86.49
1	49	83.78	59.46	106.31	102.70
1	51	100.90	90.09	81.08	71.17
1	59	96.40	83.78	102.70	113.51
1	60	59.46	109.91	78.38	101.80
1	75	99.28	78.42	74.10	56.83
1	77	71.22	61.87	55.40	74.82
2	2	90.42	78.30	88.61	80.11
2	3	98.74	58.58	88.97	81.19
2	12	102.54	76.85	88.43	107.78
2	13	88.61	85.90	93.50	78.30
2	19	91.14	105.44	73.05	60.21
2	28	55.19	87.35	79.27	60.43
2	35	95.42	94.00	95.26	82.28
2	43	85.59	114.41	90.99	91.89
2	48	101.80	97.30	101.80	126.13
2	50	84.68	79.28	122.52	101.80
2	52	101.80	84.68	113.51	105.41
2	56	91.89	91.89	101.80	100.90
2	57	85.59	79.28	82.88	94.59
2	58	100.90	132.43	115.32	61.26
2	70	78.42	71.22	72.26	67.63

3	23	80.69	79.43	83.39	85.92
3	24	99.38	82.12	119.02	66.12
3	27	76.42	81.49	83.54	89.72
3	29	85.60	73.88	82.59	82.91
3	36	81.96	78.32	88.77	98.59
3	40	115.38	103.82	112.21	114.11
3	41	101.80	91.89	91.89	111.71
3	54	101.80	114.41	102.70	92.79
3	61	91.37	63.31	75.54	88.49
3	63	149.64	60.43	67.63	55.40
3	72	97.12	78.42	76.26	69.06
3	73	72.66	73.38	62.77	74.10
3	74	97.84	42.45	53.24	82.01
3	76	73.79	75.17	97.38	101.02
3	78	59.71	59.71	53.96	61.15
4	1	61.29	57.13	79.75	71.97
4	5	93.13	97.30	94.58	100.01
4	7	83.00	82.82	83.55	86.98
4	9	111.77	85.90	95.49	98.56
4	20	93.68	87.16	96.93	80.29
4	26	60.74	75.47	66.44	90.99
4	28	49.64	79.86	89.21	122.03
4	34	82.75	84.02	93.36	90.51
4	42	81.08	112.61	82.88	93.69
4	46	93.69	67.57	81.08	94.59
4	55	86.04	92.79	97.30	93.69
4	62	89.93	74.10	82.01	92.81
4	65	74.10	68.35	56.20	55.40
4	66	71.22	64.03	102.88	66.91
4	71	79.86	76.26	88.49	87.05
5	4	97.66	98.20	107.25	112.13
5	11	104.17	77.03	82.82	96.39
5	14	84.27	74.86	59.95	68.35
5	15	92.23	71.42	104.89	103.63
5	16	95.49	100.19	87.35	80.65
5	21	77.21	101.12	75.31	83.39
5	22	69.92	62.32	73.41	88.45
5	25	71.35	69.92	107.14	93.52
5	30	87.98	90.04	97.32	111.58

5	31	77.21	90.83	92.73	118.86
5	33	75.94	84.49	83.70	99.07
5	37	98.27	61.37	61.21	77.53
5	38	110.31	112.05	111.58	107.93
5	64	56.12	56.83	81.29	58.99
5	69	85.90	102.16	94.96	76.98