

Appendix A
Methodology

Methodology

Selection of Subjects

Twenty-two male experienced resistance trainers between the ages of 18 and 26 volunteered to participate in this study. They responded to flyers posted at The Weight Club, The Hokie Gym, and weight training classes. They met the following conditions: 1) they had participated in resistance training for at least 1 year, for a minimum of 3 times a week, and for an hour or longer each session and 2) each subject signed a statement saying he had not used anabolic steroids for at least the past year. Subjects were screened for contraindications to weight loss and resistance exercising including, but not limited to, diabetes, heart conditions, orthopedic limitations or injuries, and major organ malfunctions. They agreed to forgo any nutritional supplements 2 weeks before and during the study. Each subject was informed of the risks involved in participating in the study. All subjects signed an informed consent before participating (see Appendix C).

Sampling Procedures

It took approximately six weeks to recruit subjects. The subjects in the two experimental groups were randomly assigned to the placebo (P, n=8) or treatment (C, n=8) groups. The remaining six subjects were placed in the control group (N, n=6). One control subject was unable to complete the study because he suffered an orthopedic injury at home and another control subject failed to report for his final performance test. This left only six subjects in the control group.

General Methodology

After passing an initial screening (see Appendix C), each subject met with the investigator to discuss time requirements and details of the study. After this meeting, those subjects who remained interested read and signed informed consents.

Selection of Criterion Scores

The dependent measures investigated in this study were the total number of repetitions performed during the last sets of bench press and leg extension, plasma cortisol and creatine kinase levels, and RPE scores during the resistance performance test. Plasma cortisol and CK levels were analyzed from blood samples taken before, 10 minutes, six hours, and 24 hours after exercise on the days of the last two performance tests (Trials 2 and 3, Days 7 and 11).

Reliability and Validity Estimates

Reliability estimates were determined for the resistance exercise performance test during a pilot study. The test/retest score for 5 males was $p=0.99$ for leg extension. The reliability of the dependent performance measures was assessed for the actual study using the number of repetitions completed during Trials 1 and 2. The test/retest score for the 22 subjects was $p=0.696$ for the bench press and $p=0.847$ for the leg extension.

Experimental Procedures

Body fat was assessed using skin folds. The sites were chest, abdomen, and thigh. Measurements were done in triplicate and the mean of each site was used in the equation by Jackson and Pollack (18).

The week before beginning the study, 10 repetition maximum (10RM) was determined for parallel squats (free weights), bench press (free weights), leg press (Nautilus Inc), and leg extension (Nautilus Inc). This was the order the exercises were done for the strength test, workouts, and performance tests. The strength tests were performed in the same gym that was used for resistance performance tests. Subjects chose a light resistance for a warm up to each exercise. Then subjects tired to chose a resistance that would result in muscle failure after 10 repetitions. This usually took 2 or 3 times to achieve the correct resistance. Subjects rested for approximately 3 minutes in between sets. This strength test was done twice, 3 to 4 days apart. The first strength test lasted one hour and second test generally took a shorter amount of time. Percentages of the 10RM were then calculated to determine the exercise prescription, see Appendix B.

The entire study lasted 11 days. The control subjects only participated in the standardized workouts and performance test. The carbohydrate and placebo groups adhered to the following schedule.

Diet: Subjects were required to keep a diet record for 3 days (D1 - D3) and have body weight assessed several times during the week before energy restriction to insure that they were maintaining a stable body weight (D1, D3, D5, and D7). Subjects received oral and written instructions for keeping the diet records. Subjects recorded what they ate for dinner the night (D4) before Trial 1, this meal was duplicated the night (D6) before Trial 2. They arrived at each performance test after an overnight fast.

Subjects were asked to follow an exchange diet for the days of Trial 2 (D7) and Trial 3 (D11). This diet was based on the number of calories reported on the diet records. It contained the same or similar foods that were recorded on the diet records and consisted of 60% carbohydrate, 15% protein, and 25% fat. This was to ensure similar proportions of carbohydrate ingestion for the days involving blood draws.

For days 8 through 10, subjects were given a formula diet. The formula diet was $18 \text{ kcal kg}^{-1} \text{ day}^{-1}$ (54.7% carbohydrate, 21.3% protein, 24% fat, Ensure High Protein, Ross Laboratories). Subjects were given oral and written instructions regarding the formula diet (see Appendix B). They were told not consume any other food during this time, although noncaloric beverage could be consumed. Subjects were encouraged to drink plenty of water. Body weight was measured and formula diets were picked up daily during the hypoenergy period.

Standardized workouts: Subjects were instructed to do no other exercise than what they were prescribed during the 11 days of the study. Subjects performed a standardized workout on D1, D3, and D9. This workout was similar to the performance test. For the standardized workout, the experimenter doubled the 10RM for one-legged leg extensions and this weight was used to work both legs during leg extensions. Subjects did 5 sets of squats (free weights), bench press (free weights), leg press (machine), and leg extensions (machine) at 80%, 80%, 70%, 60% and 60% of 10RM. Each set consisted of 10 repetitions and there was 2 minutes of rest between set. These workouts were unsupervised and recorded by the subjects. Subjects were allowed to do the standardized workouts at times convenient for them on the assigned days. The standardized workouts lasts approximately one hour.

On Day 1, subjects did the workout under the experimenters supervision to ensure that they understood exactly what the workout entailed. During this workout, subjects did the 5th sets of bench press and leg extension to failure, so that they would have some experience with performing repetitions to failure before the resistance performance tests.

Exercise Testing: Subjects arrived at each test after an overnight fast. There were two performance tests [Trial 1 (D5) and 2 (D7)] before energy restriction to determine reliability of the dependent measures. For the test after energy restriction [Trial 3 (D11)], subjects were required to ingest their assigned beverage 30 minutes prior to beginning exercise.

Treatment: The treatment group consumed a beverage consisting of 1.0 g of carbohydrate per kilogram of body weight (Gatorade, glucose and fructose). The placebo group consumed a NutraSweet beverage of the same color and volume. Subjects were blind to which beverage they received. They were told all the drinks were the same, but they were not told what the drink was.

Performance test: Subjects arrived for all performance tests after an overnight fast. The performance test consisted of 5 sets of squats, bench press, leg press, and one-legged leg extensions. These exercises were always done in the same order. This was similar to the standardized workout except the 5th sets of bench press and one-legged leg extensions were done to exhaustion at the intensity of 80% of 10RM. This means for these sets subjects did as many repetitions as possible. For a repetition to be counted, full range of motion had to be executed. If the full range of motion was not attained the repetition was not counted and the test ended. No encouragement was given to subjects during these sets. All performance tests were supervised. The investigator and assistants recorded the number of repetitions, RPE, and timed the rest intervals.

During each performance test, subjects gave perceived rate of exertion (RPE) using the Borg 6-20 scale after the 5th set of every exercise and after the 4th set of bench press and leg extension.

Blood Draws: Subjects reported for morning blood draws in a fasted state. Subjects had blood samples taken before and after Trial 2 (D7) and Trial 3 (D11). One tube (either 7 or 10 ml) of plasma was drawn from a forearm vein 30 to 60 minutes before, 10 minutes post, 6 hours post, and 24 hours post exercise. On Day 11, subjects had blood taken when they arrived at the laboratory. This was at the same time as they arrived for Trial 2. Thirty minutes prior to the start of exercise, they consumed their beverage. Blood samples were placed on ice for 30 minutes. The serum was separated and frozen for later analysis of cortisol (radioimmuno assay, DRC, TKCO2), CK (colorimetric procedure, Sigma #47), and glucose (colorimetric procedure, Sigma #115). All samples were run in duplicate.

Research Design and Statistical Procedures

The performance data and body weight were analyzed using a repeated measures ANOVA. A Tukey post hoc procedure was conducted to make comparisons of means. Diet records were analyzed using a one-way ANOVA to determine if there were differences between groups. Blood

parameters were analyzed with a three-way ANOVA. A post hoc analysis of least square means was used to make comparisons of means. A p-value < 0.05 was considered significant.

Appendix B
Instructions to Subjects
Diet and Weight Lifting Protocols
Exercise Testing Forms

Each subject was given a schedule like this one. The exact dates were written in above the Days row.

Days	1	2	3	4	5	6	7	8	9	10	11
Standardized workout (WO)	WO		WO						WO		
matched dinners (MD)				MD		MD					
exchange diet (X)											X
hypoenergy diet (Ensure)								E	E	E	
performance test					T1		T2				T3
RPE (R)							R				R
Blood Samples (B)							B				B

Directions for the Diet Record Form

The Diet Record form was developed to help you keep an accurate record of what you eat and drink during days 1 through 3 of the study. Please record the name of the food (brand names if possible), the portion size, and the method of preparation. If you have any question or need additional forms, please call (552-1759) or email me (redalton@vt.edu).

Tips for Filling out the Diet Record Form

1. Record the food you eat as soon as possible after meals and snacks or while eating. It is easier to remember what you ate if you don't wait long to record it. This way you won't forget things like butter, salad dressing, mayonnaise, sugar, drinks, etc.
2. Beverages: Don't forget to include all drinks consumed. Record water and non-caloric beverages also.
3. Portion Size: Recording the amount of each food eaten is very important. Estimate portion sizes as closely as possible using cups (C), ounces (oz), teaspoons (tsp), slices (for bread or cheese), etc.
4. Preparation: How was the food cooked? Please list if it was fresh, canned, frozen, steamed, broiled, boiled, baked, or fried. If you prepare a mix (example macaroni and cheese), did you add butter, milk or water or some other ingredient? Also, record if you substituted an ingredient.
5. Break down the recipe into specific food items if the dish includes more than one ingredient. For example, a deli sub should be broken down into certain portion sizes and types of bread, meat, cheese, and condiments. The same is true for other combination foods like casseroles, salads, spaghetti, etc. Don't forget to the sauce and dressing!
6. Canned Food: Record all brand names. For fruits, was it packed in water, it's own juice, or syrup? Did you drain off the syrup, oil, or juice before eating it? For meat, was it in packed in water or oil?
7. Condiments: Please indicate the type and quantity if you added spices or condiments to your food. For example, mustard, mayonnaise, butter, salt, sugar, steak sauce, salad dressing, ketchup, etc.
9. Specialty Items: Record the brand names of any food item that has reduced fat, calorie, or sugar content. For example, Healthy Choice Microwave Popcorn, Kraft Free Ranch Salad Dressing, reduced fat cheeses, etc.

10. Supplements: If you take vitamin or mineral supplements, record the brand name and amount/dosages (i.e. mg) for each (or bring label). Remember that other nutritional supplements are prohibited during this study (e.g. creatine, amino acids).
11. Eat what you *normally* eat. Don't choose different foods because you have record your diet.

Directions for Exchange Diet

The exchange diet is prescribed to ensure similar rates of carbohydrate ingestion. It is based on foods that you consumed while keeping your diet record. Please eat these items or foods that are similar. Please record what you do eat while following the exchange diet. If you have any questions please contact me. 552-1759 redalton@vt.edu

Directions For Formula Diet

For 3 days, you will consume a low calorie formula diet. Every morning you will meet with me to be weighed and receive your formula diet. Below are instructions for following the formula diet. Failure to comply will result in dismissal from the study.

1. Keep the formula diet refrigerated.
2. Don't eat anything during this time. This includes candy and Certs. You can have sugarless gum (no more than 3 sticks per day).
3. Drink only non-caloric drinks. You can have diet sodas, coffee (no cream, artificial sweeteners only), and tea (artificial sweeteners only).
4. Drink plenty of water (at least 6 glasses a day).
5. Don't add any food to the formula diet, such as bananas.
6. You can put the formula diet in a blender with ice or you can heat in the microwave.
7. Consume all of the formula diet.

You may feel fatigued from the diet but let me know if you experience any unusual symptoms (552-1759 redalton@vt.edu).

The following is an example of a standardized workout.

Subject ID: _____

Standardized Workout

You will complete this workout on days 1, 3, and 9. This will be Tuesday March 18, Thursday March 20, and Wednesday March 25. Please complete this form during the standardized workout. Do 10 repetitions during each set. Remember to **rest 2 minutes** between each set. The weight in parenthesis () is the amount that goes on each side of the bar. This is given to ease the transition between sets and subjects.

Exercise	Weight (lb.)	Reps (10)
Example	150	<u>10</u> _____
Squats	180 (67.5)	_____
	180	_____
	155 (55)	_____
	135 (45)	_____
	135	_____
Bench Press	175 (47.5)	_____
	175	_____
	125 (40)	_____
	105 (30)	_____
	105	_____
Leg Press	290 (145)	_____
	290	_____
	250 (150)	_____
	220 (110)	_____
	220	_____
Two-legged Leg Extension	190	_____
	190	_____
	170	_____
	145	_____
	145	_____

The following is an example of a resistance exercise performance test.

Subject ID: _____

Performance Test

Someone will time your rest intervals (**2 minutes**) and record your repetitions to exhaustion (these sets are marked with ***). The weight in parenthesis () is the amount that goes on each side of the bar. This is given to ease the transition between sets and subjects.

Exercise	Weight (lb.)	Reps (10)	RPE
Example	150	<u>10</u>	<u>17</u>
Squats	180 (67.5)	_____	
	180	_____	
	155 (55)	_____	
	135 (45)	_____	
	135	_____	_____
Bench Press	175 (47.5)	_____	
	175	_____	
	125 (40)	_____	
	105 (30)	_____	_____
	175***	_____	_____
Leg Press	290 (145)	_____	
	290	_____	
	250 (150)	_____	
	220 (110)	_____	
	220	_____	_____
One-legged Leg Extension	95	_____	
	95	_____	
	85	_____	
	72.5	_____	_____
	95***	_____	_____

Appendix C

Informed Consent
Human Subject Forms
Medical History Form

VIRGINIA TECH

Informed Consent for Participants of Investigative Projects

Title of Project: The effects of a sports nutritional supplement on resistance exercise performed by males in negative energy balance.

Principle Investigators: Renee Dalton and Janet Walberg Rankin Ph.D., faculty advisor.

The Purpose of This Research Project:

You are invited to participate in a study about the effects of a sports nutritional supplement on resistance performance after weight loss through dieting. Most of the research on supplementation focuses on aerobic endurance performance. The purpose of this study is to determine if a sports nutritional supplement can improve resistance performance after energy restriction.

PROCEDURES

Five to seven days before the start of the study, 10 repetition maximums (10 RMs) will be determined for squats, bench press, leg press, and one-legged leg extensions. (A 10 RM means you can lift the weight 10 times but not 11.) This will take about an hour. Two to three days after the first 10 RM test, you will be required to repeat these lifts in order to ensure a reliable 10 RM.

Table 1 shows the time line for the study.

Table 1

Days	1	2	3	4	5	6	7	8	9	10	11	12
Diet record	DR	DR	DR									
Standardized workout	WO		WO						WO			
Matched dinners (md)				MD		MD						
Exchange diet							X				X	
Hypoenergy diet (Ensure)								E	E	E		
Performance test					T1		T2				T3	
Blood Draws (B)							B	24 h post			B	24 h post

Diet: You will be required to keep a diet record for 3 days (D1 - D3) and have your body weight assessed the week before energy restriction to insure that you are maintaining a stable body weight (D5 - D9). Keeping a diet record involves writing down everything you eat and drink on these days. You will record what you eat for dinner the night (D4) before Trial 1, this meal will be duplicated the night (D6) before Trial 2. You will arrive at each performance test after an overnight fast. It is very important that you eat and drink the same things in the same quantity.

You will be asked to follow an exchange diet (i.e. foods combined as groups such as “Dairy”, “Breads”, etc.) for the days of Trial 2 (D7) and Trial 3 (D11). This diet will be based on the number of calories you reported on your diet records. It will consist of 60% carbohydrate, 15% protein, and 25% fat. This is to ensure similar proportions of carbohydrate ingestion. You will be asked to keep a diet record on these days.

For days 8 through 10 you will be given a formula diet. The formula diet will be 18 kcal kg⁻¹ day⁻¹ (54.7% carbohydrate, 21.3% protein, 24% fat, Ensure High Protein, Ross Laboratories), about half of your energy requirement for the day. You should not consume any other food during this time, although noncaloric beverage may be consumed as you wish. Body weight will be measured daily during the hypoenergy period.

Standardized workouts: You will perform a standardized workout on D1, D3, and D9. This workout is similar to the performance test. It is important that you do no other exercise during the experiment than that prescribed. For the standardized workout, you will double your 10RM for one-legged extensions and this weight will be used to work both legs during leg extensions. You will do 5 sets of squats (free weights), bench press (free weights), leg press (machine), and leg extensions (machine) at 80%, 80%, 70%, 60% and 60% of 10RM. Each set will consist of 10 repetitions and there will be 2 minutes of rest between set. You will record these workouts. The standardized workout will last approximately one hour.

Exercise Testing: There will be two performance tests [Trial 1 (D5) and 2 (D7)] before energy restriction to determine reliability of the dependent measures. For the test after energy restriction [Trial 3 (D11)], you will be required to ingest a sports nutritional drink 30 minutes before the exercise testing begins. Each performance test will last approximately one hour.

The performance test will consist of 5 sets of squats, bench press, leg press, and one-legged leg extensions. This is similar to the standardized workout except the 5th sets of bench press and one-legged leg extensions will be done to exhaustion at the intensity of 80% of 10RM. This means for these sets you will do as many repetitions as you possible can. You will arrive for testing after an overnight fast.

During each performance test you will give information about your perceived rate of exertion (RPE) to let us know how hard you feel that you are working.

You will have blood samples taken before and after Trial 2 (D7) and Trial 3 (D11). One tube will be withdrawn from forearm vein just before, immediately post, 6 hours post, and 24 hours post exercise bout. We will measure levels of glucose as well as several indicators of muscle metabolism (cortisol and creatine kinase).

The time and conditions required for you to participate in this project are:

1. Initial meeting for orientation and health screening; lasting approximately 1 to 1.5 hours.
2. Time required to determine the maximum amount of weight you are able to lift for different resistance exercises (1 hour).
3. Time for 3 resistance performance tests (1 hour each).
4. You must do the standardized workouts (1 hour each), but it may be done at any time convenient for you.
5. You will have to undergo a low calorie formula diet for 3 days before the last performance test.
6. Your word that you have not used any form of steroids within the past year.
7. You must forgo any nutritional supplements 2 weeks before and during the study.

POSSIBLE RISKS OF PARTICIPATION AND EFFORTS TO MINIMIZE RISK:

Possible risks during the low calorie formula diet include hunger, fatigue, and irritability.

Possible risk during all of the resistance exercises include muscle soreness or fatigue. There is a small risk of strains, sprains, tendonitis, and bursitis from the resistance performance tests, strength test, and standardized workouts. These risks should be minimal because all subjects have at least two years experience with resistance training.

Possible risks during blood draws include infection and bruising. This will be minimized by having a an experienced phlebotomist conduct the entire blood drawing procedure. The phlebotomist will use universal precautions including the use of gloves when handling blood samples. Your blood may be screened for HIV if there is exposure.

APPROVAL OF RESEARCH

This project has been approved by the Institutional Review Board for projects involving human subjects at Virginia Tech.

SUBJECTS' RESPONSIBILITIES

I know of no reason why I cannot participate in this study. I have the following responsibilities:

1. To advise the researchers of any pre-existing medical problems that may affect my participation, such as, but not limited to, diabetes, heart conditions, muscle, bone, or joint problems, and major organ malfunctions. Should you have any questions regarding a particular condition, please ask an investigator.
2. To advise researchers of any medical problems that might arise in the course of this experiment, such as signs of strain, sprains, tendonitis, or bursitis; or any signs and or symptoms of illness.
3. To refrain from the consumption of alcoholic beverages throughout the entire duration of the study.
4. To refrain from the consumption of caffeine and use of tobacco at least 4 hours prior to each resistance performance test.
5. Do no other exercise than that we prescribe.
6. Eat no other foods than what you are prescribed.
7. Give maximum efforts on all strength and performance tests.

Safeguards that will be used to minimize your risk or discomfort include:

1. A telephone is available which would be used to call for emergency service.
2. The investigators are trained in basic first aid and CPR.

BENEFITS OF THIS PROJECT

Your participation in the project will provide the following information that may be helpful:

1. Knowledge of your strength on a variety of lifts.
2. Knowledge of your percent body fat.

Subjects in the experimental groups will be paid \$40, and control subjects will be paid \$10 at the completion of the study.

You may receive a synopsis or summary of this research when completed.

EXTENT OF ANONYMITY AND CONFIDENTIALITY

The results of this study will kept confidential. Without your written consent, at no time will the researcher release the results of your tests to anyone other than individuals working on the research project. The information you provide will have your name removed and only a

subject number will identify you during analyses and any written reports of the research. Only the researcher will know your identity.

FREEDOM TO WITHDRAWAL

You are free to withdrawal from this study at any time without penalty. There may the following circumstances under which the investigator may determine that you should not continue as a subject in this study:

1. Failure to comply with prescribed workout sessions and/or diet.
2. Illness.

SUBJECT’S PERMISSION

I, (print name) _____ have read and understand the informed consent and conditions of this projects. I have had all my questions answered. I thereby acknowledge the above and give my voluntary consent for participation in this project.

If I participate, I am free to withdraw at any time. I agree to abide by the rules of this project.

Signature of Participant:

_____ Date: _____

Signature of investigator:

_____ Date: _____

Should I have any questions about this procedure or its conduct, I can contact:

Janet Walberg Rankin, Ph.D.

Associate Professor

Department of Human Nutrition, Foods, and Exercise

231-6355

Renee Dalton

Master’s Candidate

Department of Human Nutrition, Foods, and Exercise

552-1759

Tom Hurd, Ph.D.

Chairmen of the IRB at Virginia Tech

231-5281

This was the informed consent form for control subjects.

VIRGINIA TECH

Informed Consent for Participants of Investigative Projects

Title of Project: The effects of a sports nutritional supplement on resistance exercise performed by males in negative energy balance.

Principle Investigators: Renee Dalton and Janet Walberg Rankin Ph.D., faculty advisor.

The Purpose of This Research Project:

You are invited to participate in a study about the effects of a sports nutritional supplement on resistance performance after weight loss through dieting. Most of the research on supplementation focuses on aerobic endurance performance. The purpose of this study is to determine if a sports nutritional supplement can improve resistance performance after energy restriction.

PROCEDURES

Five to seven days before the start of the study, 10 repetition maximums (10 RMs) will be determined for squats, bench press, leg press, and one-legged leg extensions. (A 10 RM means you can lift the weight 10 times but not 11.) This will take about an hour. Two to three days after the first 10 RM test, you will be required to repeat these lifts in order to ensure a reliable 10 RM.

Table 1 shows the time line for the study.

Table 1

Days	1	2	3	4	5	6	7	8	9	10	11
Standardized workout	WO		WO						WO		
Matched dinners (md)				MD		MD				MD	
Performance test					T1		T2				T3

Diet: On days 4,6 and 10 you will be asked to eat the same dinner. It doesn't matter what it is, just eat the same thing the 3 nights and no food after midnight. Arrive at the performance test fasted.

Standardized workouts: You will perform a standardized workout on D1, D3, and D9. This workout is similar to the performance test. It is important that you do no other exercise during the experiment than that prescribed. For the standardized workout, you will double your 10RM for one-legged extensions and this weight will be used to work both legs during leg extensions. You will do 5 sets of squats (free weights), bench press (free weights), leg press (machine), and leg extensions (machine) at 80%, 80%, 70%, 60% and 60% of 10RM. Each set will consist of 10 repetitions and there will be 2 minutes of rest between set. You will record these workouts. The standardized workout will last approximately one hour.

Exercise Testing: There will be three performance tests [Trial 1 (D5) ,2 (D7), and 3 (D10)]. Each performance test will last approximately one hour.

The performance test will consist of 5 sets of squats, bench press, leg press, and one-legged leg extensions. This is similar to the standardized workout except the 5th sets of bench press and one-legged leg extensions will be done to exhaustion at the intensity of 80% of 10RM. This means for these sets you will do as many repetitions as you possible can. You will arrive for testing after an overnight fast.

During each performance test you will give information about your perceived rate of exertion (RPE) to let us know how hard you feel that you are working.

The time and conditions required for you to participate in this project are:

1. Time required to determine the maximum amount of weight you are able to lift for different resistance exercises (1 hour).
2. Time for 3 resistance performance tests (1 hour each).
3. You must do the standardized workouts (1 hour each), but it may be done at any time convenient for you.
4. Your word that you have not used any form of steroids within the past year.
5. You must forgo any nutritional supplements 2 weeks before and during the study.

POSSIBLE RISKS OF PARTICIPATION AND EFFORTS TO MINIMIZE RISK:

Possible risk during all of the resistance exercises include muscle soreness or fatigue. There is a small risk of strains, sprains, tendonitis, and bursitis from the resistance performance tests, strength test, and standardized workouts. These risks should be minimal because all subjects have experience with resistance training.

APPROVAL OF RESEARCH

This project has been approved by the Institutional Review Board for projects involving human subjects at Virginia Tech.

SUBJECTS' RESPONSIBILITIES

I know of no reason why I cannot participate in this study. I have the following responsibilities:

1. To advise the researchers of any pre-existing medical problems that may affect my participation, such as, but not limited to, diabetes, heart conditions, muscle, bone, or joint problems, and major organ malfunctions. Should you have any questions regarding a particular condition, please ask an investigator.
2. To advise researchers of any medical problems that might arise in the course of this experiment, such as signs of strain, sprains, tendonitis, or bursitis; or any signs and or symptoms of illness.
3. To refrain from the consumption of alcoholic the night before performance tests.
4. To refrain from the consumption of caffeine and use of tobacco at least 4 hours prior to each resistance performance test.
5. Do no other exercise than that we prescribe.
6. Give maximum efforts on all strength and performance tests.

Safeguards that will be used to minimize your risk or discomfort include:

1. A telephone is available which would be used to call for emergency service.
2. The investigators are trained in basic first aid and CPR.

BENEFITS OF THIS PROJECT

Your participation in the project will provide the following information that may be helpful:

1. Knowledge of your strength on a variety of lifts.
2. Knowledge of your percent body fat.

Control subjects will be paid \$10 after the completion of the study.

You may receive a synopsis or summary of this research when completed.

EXTENT OF ANONYMITY AND CONFIDENTIALITY

The results of this study will kept confidential. Without your written consent, at no time will the researcher release the results of your tests to anyone other than individuals working on the research project. The information you provide will have your name removed and only a subject number will identify you during analyses and any written reports of the research. Only the researcher will know your identity.

FREEDOM TO WITHDRAWAL

You are free to withdrawal from this study at any time without penalty. There may the following circumstances under which the investigator may determine that you should not continue as a subject in this study:

1. Failure to comply with prescribed workout sessions and/or diet.
2. Illness.

SUBJECT’S PERMISSION

I, (print name) _____ have read and understand the informed consent and conditions of this projects. I have had all my questions answered. I thereby acknowledge the above and give my voluntary consent for participation in this project.

If I participate, I am free to withdraw at any time. I agree to abide by the rules of this project.

Signature of Participant:

_____ Date: _____

Signature of investigator:

_____ Date: _____

Should I have any questions about this procedure or its conduct, I can contact:

Janet Walberg Rankin, Ph.D.
Associate Professor
Department of Human Nutrition, Foods, and Exercise
231-6355

Tom Hurd, Ph.D.
Chairmen of the IRB at Virginia Tech
231-5281

Renee Dalton
Master’s Candidate
Department of Human Nutrition, Foods, and Exercise
552-1759

REQUEST FOR APPROVAL OF RESEARCH PROPOSAL IN THE DEPARTMENT OF
HUMAN NUTRITION, FOODS, AND EXERCISE

Investigators: Renee Dalton and Janet Walberg-Rankin, Ph.D., advisor

TITLE:

The effects of carbohydrate supplementation on resistance exercise performed by males in negative energy balance.

JUSTIFICATION OF PROJECT:

The propose of this investigation is to determine if consuming a carbohydrate beverage before resistance training will improve performance and reduce markers associated with protein catabolism and muscle damage in male subjects who are in a negative energy balance.

A variety of athletes attempt to reduce their body weight in order to achieve a weight class (e.g. wrestlers), to improve performance by decreasing energy cost of activity (e.g. football players), or to change appearance (e.g. bodybuilders). Ideally, this weight loss would occur using modest energy restriction over a long period of time. However it is not unusual for athletes to use more severe energy restriction to cause rapid weight loss.

Energy restriction has been associated with decreased resistance performance and decreased muscle glycogen. Because resistance exercise uses muscle glycogen as a fuel, a carbohydrate supplement may allow some glycogen synthesis prior to exercise and have an overall glycogen sparing effect. Carbohydrate supplementation has been shown to improve aerobic performance in numerous studies, but few studies have looked at resistance performance.

Another potential benefit of carbohydrate consumption before resistance exercise may relate to the muscle damage and protein breakdown as observed following this activity as reflected by increases in plasma creatine kinase (CK) and cortisol. Decreased post exercise cortisol and CK levels have been reported when carbohydrate was consumed prior to aerobic exercise. If carbohydrate ingestion before resistance exercise decreases cortisol and CK responses, there may be a more favorable physiological environment for an increase in muscular mass and strength.

The purpose of this study is to determine if a carbohydrate drink (1g/kg) consumed by subjects in negative energy balance 45 minutes prior to resistance exercise would improve performance and decrease markers associated with protein catabolism and muscle damage in male subjects who are in a negative energy balance. This will have application to athletes who try to reduce their body weight but want to maintain high quality workouts and performance.

PROCEDURE:

Twenty-four male subjects will be selected. Male subjects will be tested because previous studies and data from our laboratory using similar diets involved male subjects. The genders cannot be combined for this study because of large differences expected in muscle strength and body composition. They have to meet the following requirements: 1) they must have done resistance training for at least 2 year, for a minimum of 3 times a week, and for an hour or longer each session and 2) each subjects has to sign a statement saying they have not used anabolic steroids for at least the past year. Subjects will be screened for contraindications to weight loss and resistance exercising including, but not limited to, diabetes, heart conditions, orthopedic

limitations or injuries, and major organ malfunctions (see screening form attached). Each subject will sign an informed consent.

The subjects will be randomly assigned to one of three groups: a control, a carbohydrate supplement, or a placebo. The control group will do the resistance performance tests, but will not undergo energy restriction or blood testing. The treatment and placebo groups will be given the same hypoenergy diet for three days. A resistance performance test will be done before and after the energy restriction period. The treatment group will consume a carbohydrate supplement and the placebo group will consume a placebo 45 minutes before the resistance exercise performance test. Blood samples will be taken before and after the performance test to determine effects on indicators of catabolic state (cortisol) and muscle damage (CK).

Percent body fat will be calculated on day 1 for each subject by using calipers that measure the width of a fold of skin on the chest, thigh, and abdomen. The measurement numbers are put into an equation that determines the percentage. Weight will be measured using a standard scale on day 1 and days 6 through 13 of the study.

The subjects will be required to undergo a strength test on day 1. During this test their 1 RM (repetition maximum) will be determined for different resistance exercises. 1 RM means that the subject can lift the weight one time, but cannot lift it twice. The resistance exercise will be typical of those performed by resistance trainers. They include bench press, rows, lat pulldowns, curls, squats, leg extensions, and other similar exercises. The subjects will warm up with one set of 5 - 10 reps using 40 - 60 % of perceived maximum. After approximately a minute of rest and some light stretching, subjects will perform one set of 3 to 5 reps with 60 - 80% of their perceived maximum. After another minute of rest and some easy stretching, subjects will perform 3 - 4 1 rep attempts with increments of increasing weight. Subjects will rest 30 seconds between these attempts. The last successfully executed lift will be recorded as their 1 RM.

Day 2 will be a day of rest for the subjects. There will two tests (Trial 1 and 2, on days 4 and 6) before energy restriction to determine reliability of the dependent measures. The subjects will arrive after an overnight fast. The exercise test consists of bouts of knee extensions done at 60% of 1 RM with 2 minute rest between sets. Set 1 will consist of 18 reps, set 2: 15 reps, set 3: 12 reps, set 4: 10 reps, set 5: 8 reps, and set 6: 6 reps. For set 7 (the final set) subjects will do as many reps as possible before muscle failure. The number of reps performed in the 7th set is the dependent measure.

These first two trials will be on days 4 and 6. Subjects will perform a standardized workout on days 3 and 5. Each lift will be 75-85% of 1RM. The subjects will perform three sets of 8 to 10 repetitions. Days 3 and 5 will be an upper body workout. On day 4 the subjects will do a lower body workout a few hours after doing the performance test. On day 6 they will only do the performance test.

They will record everything they eat for dinner on day 3. This meal will be duplicated for dinner on day 5.

The subjects will keep diet records on days 7 through 10 and have body weight assessed these days to insure that they are maintaining stable body weights. They will be asked to follow a prescribed exchange diet (i.e. foods combined as groups such as "Dairy", "Breads", etc.) for the week before the experimental period to insure similar amounts of carbohydrate among the subjects. The hypoenergy formula diet will be 18 kcal kg⁻¹ day⁻¹ (60% carbohydrate, 20% protein, 20% fat, Ensure, Ross Laboratories) for 3 days (days 11 through 13). This is approximately 50%

of the calories required to maintain energy balance. Body weight will be measured daily during the hypoenergy period.

Subjects will also perform a standardized resistance workout on days 7 through 13. Each lift will be 75-85% of 1RM. The subjects will perform three sets of 8 to 10 repetitions. On day 7 subjects will upper body exercises, on day 8 they perform lower body exercises, and on day 9 they will perform upper body exercises. This will be followed by one of no resistance training on day 10 and then the 3 days of training will be repeated on days 11 through 13.

For the test after energy restriction (Trial 3, day 14), the experimental subjects will be randomly assigned to either a carbohydrate drink, Gatorade, (1 gram of carbohydrate per kg) or a placebo drink (artificially sweetened). The drinks will be ingested 45 minutes before exercise testing begins. The exercise test is same as on days 4 and 6.

Blood samples will be taken before and after trial 3. Specifically, seven ml will be withdrawn from forearm vein just before, immediately post, 6 hours post, and 24 hours post exercise bout.

RISK AND BENEFITS

Risks to human subjects include hunger, irritability, and fatigue while on the low calorie diet. As a result of the performance test, subjects may experience some muscle soreness. There is a risk for bursitis, tendonitis, strains, and sprains while performing the resistance exercises. This is probably minimal since all subjects will have regular experience with resistance training. The primary investigator is CPR certified and has experience with resistance training.

Risk of blood collection include slight discomfort associated with placement of a needle. These risks will be minimized because an experienced phlebotomist will conduct the entire blood drawing procedure. The phlebotomist will use universal precautions including the use of gloves when handling blood samples. Blood may be screened for HIV if there is contamination.

Benefits to the human subjects include having first hand experience with a research study that may have results that may prove beneficial to athletes in the future. They will be given the results from the calculation of the percentage of body fat and muscle strength tests. Subjects in the experimental groups will given \$40 after they complete the study and those in the control group will be given \$10.

CONFIDENTIALITY/ANONYMITY

Results of this study will remain confidential. Unless there is written consent given by each subject the results of individual subjects in this study will remain unreleased to anyone except the principle investigator and her advisor. Names will be removed from any information and only a research number will remain. Only the researcher will know the subject's identity.

CONSENT:

An informed consent must be signed before any subject will be allowed to participate in the study. The informed consent will explain testing procedures and subject responsibility. Subjects will be told that they can withdraw from the study at anytime. Their payment will be prorated depending on the extent of their participation.

BIOGRAPHICAL SKETCH:

Renee Dalton, MS candidate: Graduate student in Muscle Function option in the Department of Human Nutrition, Foods, and Exercise. She received her bachelor's degree in Biology from Virginia Tech. She is currently CPR certified and experienced with resistance training. She has vast experience working with people and has helped with another research study involving human

subjects and the collection of blood samples. She is currently doing an internship in a hospital physical therapy department.

Janet Walberg Rankin, Ph.D., Faculty Advisor: She received a bachelor's degree in zoology from Duke University and doctorate in Nutrition with a minor in Exercise Physiology from the University of California at Davis. She has been on the faculty at Virginia Tech since 1982 and is currently an Associate Professor in the Department of Human Nutrition, Foods, and Exercise. She teaches undergraduate classes in "Exercise Physiology" and "Nutrition and Physical Performance" as well as a graduate class in "Metabolic Aspects of Exercise". Her primary research areas are nutritional manipulations in athletes and weight control. Her research has been published in journals such as: International Journal of Sports Nutrition, Medicine and Science in Sports and Exercise, and International Journal of Sports Medicine.

**VIRGINIA TECH LABORATORY FOR HEALTH AND EXERCISE SCIENCE
DEPARTMENT OF HUMAN NUTRITION, FOODS, AND EXERCISE
MEDICAL AND HEALTH HISTORY**

Name: _____ Age: _____ Birth Date: _____

Student ID: _____ Email: _____

Local Address: _____

City, State, ZIP: _____

Local Phone Number: _____ (home) _____ (office/other--specify)

Permanent Address: _____

City, State, ZIP: _____

Person to contact in case of emergency: _____

Relationship: _____ Phone: _____

Primary Care Physician: _____ Phone: _____

Medical History

Please indicate any current or previous conditions or problems you have experienced or have been told by a physician that you had:

	Yes	No
Heart disease or any heart problem	_____	_____
Rheumatic fever	_____	_____
Respiratory disease or breathing problems	_____	_____
Circulation problems	_____	_____
Kidney disease or problems	_____	_____
Urinary problems	_____	_____
Reproductive problems	_____	_____
Musculoskeletal problems	_____	_____
Fainting or Dizziness	_____	_____
High Cholesterol	_____	_____
Diabetes	_____	_____

Thyroid problems _____

High blood pressure _____

Lactose Intolerance (milk allergy) _____

Other Allergies _____

If "yes" to any of the above, please indicate the date, explain, and describe:

Please list any hospitalizations/operations/recent illnesses (date/type):

Please list all medications (prescription and over-the-counter) you are currently taking or have taken within the past week:

Please list any vitamins or other nutritional supplements (creatine, amino acids) you are currently taking or take on a regular basis:

Exercise Habits

Please describe the activities you currently participate in.

	<u>Curr.</u> (✓)	<u>How Often?</u> (minutes/wk)	<u>Date Last Participated</u>
Running	_____	_____	_____
Weight Training	_____	_____	_____
Road Cycling	_____	_____	_____
Mountain Biking	_____	_____	_____
Rowing	_____	_____	_____
Swimming	_____	_____	_____
Racquet Sports	_____	_____	_____
Hiking	_____	_____	_____
Volleyball	_____	_____	_____
Basketball	_____	_____	_____
Team Sport: _____	_____	_____	_____
Other: _____	_____	_____	_____
Other: _____	_____	_____	_____

Do you ever feel faint, short of breath, or chest discomfort with exercise?
Yes____ No____ If "yes", please describe:_____

Are there any orthopedic limitations you have which may restrict your ability to perform
exercise of moderate to high intensity? Yes____No____

If "yes", please describe: _____

Weightlifting Experience

Are you currently weightlifting? **Y or N**

How many years have you been weightlifting? _____

How many days per week? _____

How many minutes does each session typically last? _____

Where do you workout? _____

Do you typically use free weights? _____

Do you typically use weight machines? _____

Have you ever participated in a bodybuilding competition? **Y or N**

If yes, please list them below:

<u>Name</u>	<u>Date</u>	<u>Finishing Place</u>
-------------	-------------	------------------------

Please list your normal workout below. Include specific exercise, weight, reps, and number of sets.

Family History

Has anyone in your family been diagnosed or treated for any of the following:

	Yes	No	Relationship	Age (at occurrence)
Heart attack	___	___	_____	_____
Heart disease	___	___	_____	_____
High blood pressure	___	___	_____	_____
Stroke	___	___	_____	_____
Kidney disease	___	___	_____	_____
Diabetes	___	___	_____	_____

Dietary Habits

Please list your current height: _____(feet and inches)

Please list your current weight: _____(lbs.)

What would you like to weigh? _____(lbs.)

What is the most you have weighed since age 18: _____(lbs.)

What is the least you have weighed since age 18: _____(lbs.)

Has your weight fluctuated more than 5 lbs. in the past year? **Y or N**

If yes, how much? _____(lbs)

If yes, was this weight loss/gain on purpose? **Y or N**

How much coffee/tea do you drink per day? _____cups

What do you add to your coffee/tea and how much? _____

How many soft drinks (cans) do you consume a day? _____cans

What type and brands of soft drinks do you consume (indicate diet/non-diet): _____

Do you ever feel weak or faint when you haven't consumed an adequate amount of food?

Y or N

Do you feel you can complete this study knowing that you will be consuming a low calorie formula diet for 3 days?

Y or N

Do you think that you will have problems complying with the dietary regimen prescribed to you? **Y or N**

Do you have any specific food allergies? **Y or N**

If yes, please describe: _____

Drinking Habits

During the past month, how many days did you drink alcoholic beverages? _____days

During the past month, how many times did you have 5 or more drinks? _____times

On average, how many glasses of the following beverages do you consume in a week?

Beer _____ glasses or cans

Wine _____glasses

Mixed Drinks _____glasses

Shots _____number

Other (specify): _____number

Blood Draws

Do you feel faint when you have your blood drawn? _____ **Yes** **No**
Do you feel faint at the sight of other people's blood? _____

If "yes" to any of above, please describe: _____

Schedule

Please list your class schedule (class, room, and times)

Mon Tues Wed Thurs Fri

Please list your work schedule:

Mon Tues Wed Thurs Fri Sat Sun

Will you be here over spring break? **Y or N**

Please describe which part of the study you feel will be the most difficult for you to comply with.

Please sign below to indicate the above information is correct.

_____ _____ _____
Print Name **Signature** **Date**

All information is confidential.

Appendix D
Raw Data
Statistical Analyses

Results of the pilot study:

Subject	80% of 10RM	Trial 1 (number of repetitions for 6 th set of one-legged leg extensions)	Trial 2 (number of repetitions for 6 th set of one-legged leg extensions)	Days between Trial 1 and Trial 2
1	90 lb.	15	16	4
2	80 lb.	14	15	2
3	80 lb.	7	8	3
4	80 lb.	10	11	5
5	65 lb.	7	8	5

Physical characteristics

This data was analyzed to determine if there were differences between groups at baseline.

Summary ANOVA for age.

Source	SS	df	MS	F	P
Group	10.98	2	5.49	1.37	0.279
Error	76.33	19	4.02		
Total	87.32	21			

Group-C,P,N

Summary ANOVA for height.

Source	SS	df	MS	F	P
Group	20.6	2	10.3	0.19	0.827
Error	1019.9	19	53.7		
Total	1040.5	21			

Group-C,P,N

Summary ANOVA for weight.

Source	SS	df	MS	F	P
Group	126.7	2	63.4	0.65	0.531
Error	1840.8	19	96.9		
Total	1967.6	21			

Group-C,P,N

Summary ANOVA for body fat.

Source	SS	df	MS	F	P
Group	52.2	1	52.2	5.06	0.041
Error	144.3	14	10.3		
Total	196.5	15			

Group-C,P

Summary ANOVA for lean body mass.

Source	SS	df	MS	F	P
Group	33.9	1	33.9	0.83	0.377
Error	571.4	14	40.8		
Total	605.4	15			

Group-C,P

Raw data for physical characteristics

Subject	Group	Age	Height	Weight	Body fat	Lean Body Mass
1	C	24	175.26	87.9	8.0	80.868
2	C	22	180.34	77.5	7.0	72.075
3	C	20	172.72	78.9	8.9	71.878
4	C	20	177.80	77.0	13.6	66.528
5	C	20	175.26	81.5	12.0	71.720
6	C	26	193.04	94.7	11.3	83.999
7	C	20	182.88	77.5	7.0	72.075
8	C	22	187.96	98.7	17.0	81.921
9	P	23	187.96	94.0	17.0	78.020
10	P	18	172.72	76.4	8.9	69.600
11	P	19	193.04	91.5	16.0	76.860
12	P	22	185.42	82.1	13.4	71.099
13	P	21	170.18	71.6	12.0	63.008
14	P	19	177.80	74.4	13.4	64.430
15	P	20	177.80	87.0	16.0	73.080
16	P	22	190.50	98.4	17.0	81.670
17	N	21	185.42	75.0		
18	N	22	172.72	83.2		
18	N	26	187.96	100.0		
20	N	21	177.80	72.7		
21	N	21	177.80	68.2		
22	N	22	175.26	74.5		

Subject	Group	Squats (lb.)	Bench Press (lb.)	Leg Press (lb.)	One-legged leg extension (lb)
1	C	355	260	660	130
2	C	245	170	450	130
3	C	225	170	175	100
4	C	195	170	300	90
5	C	250	215	475	150
6	C	175	155	285	110
7	C	205	160	325	80
8	C	205	240	380	90
9	P	275	170	500	160
10	P	225	175	415	140
11	P	165	155	290	60
12	P	215	180	380	110
13	P	185	165	365	90
14	P	145	155	290	80
15	P	225	150	360	80
16	P	225	175	365	120
17	N	195	165	325	120
18	N	205	140	275	90
19	N	140	125	180	70
20	N	165	120	230	70
21	N	105	95	120	60
22	N	205	190	325	80

Dietary Statistics

This data was analyzed to determine if there were any significant differences between experimental groups for the diet records recorded during the first three days of the study.

Summary ANOVA for total calories reported on three day diet records.

Source	SS	df	MS	F	P
Group	813604	1	813604	2.1	0.169
Error	5417915	14	386994		
Total	6231519	15			

Group-C,P

Summary ANOVA for carbohydrate intake reported on three day diet records.

Source	SS	df	MS	F	P
Group	0	1	0	0.0	0.984
Error	2002	14	143		
Total	2002	15			

Group-C,P

Summary ANOVA for protein intake reported on three day diet records.

Source	SS	df	MS	F	P
Group	64.0	1	64.0	2.31	0.151
Error	387.8	14	27.7		
Total	451.7	15			

Group-C,P

Summary ANOVA for fat intake reported on three day diet records.

Source	SS	df	MS	F	P
Group	60.1	1	60.1	0.67	0.426
Error	1252.9	14	89.5		
Total	1312.9	15			

Group-C,P

Analysis of 3 day diet records

Subject	Group	Total Calories	% CHO	% Protein	% Fat
1	C	3463	62	18	21
2	C	2941	45	19	36
3	C	2606	53	20	27
4	C	3593	80	8	12
5	C	2965	48	29	22
6	C	3365	42	28	30
7	C	3320	54	16	30
8	C	2317	71	15	14
9	P	3013	56	15	29
10	P	2008	77	16	7
11	P	2859	52	14	33
12	P	2038	46	19	35
13	P	3530	48	11	41
14	P	1374	55	15	30
15	P	2670	68	12	19
16	P	3470	52	19	29

Performance Data

Correlation of performance for Trial 1 and Trial 2 as measured by the number of repetitions performed to failure during the final set of bench press.

Pearson's Product Moment Correlation R=0.696

Correlation of performance for Trial 1 and Trial 2 as measured by the number of repetitions performed to failure during the final set of one-legged leg extension.

Pearson's Product Moment Correlation R=0.847

Summary ANOVA for repetitions performed to failure during the final set of bench press.

Source	SS	df	MS	F	P
Group	21.623	2	10.812	0.317	0.695
Group(subject)	553.854	19	29.150		
Trial	15.052	1	15.052	10.086	0.005
Interaction (Group X Trial)	14.941	2	7.471	5.006	0.018
Residual	28.354	19	1.492		
Total	632.977	43	14.720		

Group-C,P,N

Trial- Pre-test, Post-test

Summary ANOVA for repetitions performed to failure during the final set of one-legged leg extension.

Source	SS	df	MS	F	P
Group	4.504	2	2.52	0.0711	0.932
Group(subject)	601.792	19	31.673		
Trial	1.408	1	1.408	0.578	0.456
Interaction (Group X Trial)	1.095	2	0.547	2.436	0.801
Residual	46.292	19	2.436		
Total	654.795	43	15.228		

Group-C,P,N

Trial- Pre-test, Post-test

Raw data for bench press (repetitions performed to failure during the 5th set)

Subject	Group	Trial1	Trial2	Trial3
1	Carb	16	18	16
2	Carb	15	17	15
3	Carb	16	17	16
4	Carb	13	19	20
5	Carb	20	19	21
6	Carb	13	14	13
7	Carb	17	20	19
8	Carb	16	17	18
9	Placebo	10	10	14
10	Placebo	17	17	19
11	Placebo	11	12	16
12	Placebo	21	21	21
13	Placebo	19	18	18
14	Placebo	18	17	18
15	Placebo	15	14	15
16	Placebo	13	11	17
17	Control	9	9	12
18	Control	10	9	12
19	Control	9	10	12
20	Control	20	24	25
21	Control	22	20	21
22	Control	13	18	18

Raw data for leg extensions (repetitions performed to failure during the 5th set)

Subject	Group	Trial1	Trial2	Trial3
1	Carb	12	13	11
2	Carb	11	11	13
3	Carb	21	21	21
4	Carb	15	17	18
5	Carb	15	16	16
6	Carb	13	19	19
7	Carb	20	20	21
8	Carb	15	14	13
9	Placebo	12	13	12
10	Placebo	14	14	13
11	Placebo	15	16	18
12	Placebo	16	17	15
13	Placebo	14	12	16
14	Placebo	20	20	20
15	Placebo	14	11	13
16	Placebo	22	27	24
17	Control	15	16	18
18	Control	15	18	19
19	Control	12	16	20
20	Control	19	21	24
21	Control	15	13	12
22	Control	16	16	12

Summary ANOVA for RPE after the 5th set of parallel squats.

Source	SS	df	MS	F	P
Group	32.583	2	16.292	2.058	0.155
Group(subject)	150.417	19	7.917		
Trial	20.833	1	20.833	5.447	0.031
Interaction (Group X Trial)	0.879	2	0.439	0.155	0.892
Residual	72.667	19	3.825		

Group-C,P,N
Trial- Trial 2, Trial 3

Summary ANOVA for RPE after the 4th set of bench press.

Source	SS	df	MS	F	P
Group	3.432	2	1.716	0.239	0.790
Group(subject)	136.50	19	7.184		
Trial	1.408	1	1.408	0.503	0.487
Interaction (Group X Trial)	2.220	2	1.110	0.397	0.678
Residual	53.167	19	2.798		

Group-C,P,N
Trial- Trial 2, Trial 3

Summary ANOVA for RPE after the 5th set of bench press.

Source	SS	df	MS	F	P
Group	10.328	2	5.164	1.057	0.367
Group(subject)	92.854	19	4.887		
Trial	4.219	1	4.219	0.944	0.344
Interaction (Group X Trial)	1.608	2	0.804	0.180	0.837
Residual	84.937	19	4.470		

Group-C,P,N
Trial- Trial 2, Trial 3

Summary ANOVA for RPE after the 5th set of leg press.

Source	SS	df	MS	F	P
Group	14.608	2	7.304	0.907	0.420
Group(subject)	152.938	19	8.049		
Trial	14.352	1	14.352	6.800	0.017
Interaction (Group X Trial)	10.078	2	5.039	2.387	0.119
Residual	40.104	19	2.111		

Group-C,P,N
Trial- Trial 2, Trial 3

Summary ANOVA for RPE after the 4th set of one-legged leg extension.

Source	SS	df	MS	F	P
Group	34.311	2	17.155	1.890	0.178
Group(subject)	172.417	19	9.075		
Trial	4.408	1	4.408	1.886	0.186
Interaction (Group X Trial)	6.311	2	3.155	1.350	0.283
Residual	44.417	19	2.338		

Group-C,P,N
Trial- Trial 2, Trial 3

Summary ANOVA for RPE after the 5th set of one-legged leg extension.

Source	SS	df	MS	F	P
Group	5.409	2	2.705	0.551	0.585
Group(subject)	93.250	19	4.908		
Trial	3.675	1	3.675	4.816	0.041
Interaction (Group X Trial)	0.159	2	0.0795	0.104	0.902
Residual	14.500	19	0.763		

Group-C,P,N
Trial- Trial 2, Trial 3

RPE for 5th set of squats

Subject	Group	T2	T3
1	C	13	12
2	C	7	7
3	C	14	16
4	C	11	11
5	C	12	12
6	C	13	11
7	C	13	10
8	C	15	7
9	P	11	12
10	P	10	10
11	P	11	10
12	P	15	11
13	P	11	10
14	P	9	10
15	P	12	9
16	P	8	7
17	N	6	6
18	N	13	9
19	N	13	8
20	N	12	7
21	N	10	13
22	N	7	8

RPE for 4th set of bench press

Subject	Group	T2	T3
1	C	10	10
2	C	6	6
3	C	9	11
4	C	10	8
5	C	8	8
6	C	13	11
7	C	7	9
8	C	7	9
9	P	10	8
10	P	7	7
11	P	11	10
12	P	8	6
13	P	2	8
14	P	11	12
15	P	9	7
16	P	10	6
17	N	7	8
18	N	9	9
19	N	11	9
20	N	9	6
21	N	13	10
22	N	6	8

RPE for 5th set of bench press

Subject	Group	Trial 2	Trial 3
1	C	17	18
2	C	17	18
3	C	15	20
4	C	15	15
5	C	16	17
6	C	15	13
7	C	20	17
8	C	13	19
9	P	15	17
10	P	17	16
11	P	15	16
12	P	17	13
13	P	16	17
14	P	19	19
15	P	19	17
16	P	15	20
17	N	13	12
18	N	17	12
19	N	14	18
20	N	15	16
21	N	17	18
22	N	16	19

RPE for 5th set of leg press

Subject	Group	Trial 1	Trial 2
1	C	10	13
2	C	7	10
3	C	11	16
4	C	12	13
5	C	9	9
6	C	13	15
7	C	13	13
8	C	13	17
9	P	10	17
10	P	13	14
11	P	13	13
12	P	12	14
13	P	13	11
14	P	10	11
15	P	10	10
16	P	9	11
17	N	9	11
18	N	12	11
19	N	14	13
20	N	9	8
21	N	12	13
22	N	9	8

RPE for 4th set of leg extension

Subject	Group	T2	T3
1	C	12	11
2	C	9	9
3	C	12	11
4	C	11	12
5	C	11	10
6	C	13	11
7	C	10	8
8	C	11	13
9	P	10	14
10	P	11	13
11	P	12	11
12	P	10	13
13	P	15	12
14	P	8	8
15	P	13	10
16	P	6	6
17	N	8	7
18	N	13	11
19	N	15	9
20	N	7	6
21	N	8	9
22	N	7	6

RPE for 5th set of leg extension

Subject	Group	T2	T3
1	C	16	18
2	C	16	17
3	C	15	17
4	C	15	15
5	C	17	17
6	C	15	15
7	C	19	18
8	C	17	19
9	P	17	17
10	P	14	15
11	P	15	16
12	P	16	15
13	P	17	18
14	P	19	19
15	P	19	19
16	P	18	20
17	N	14	17
18	N	17	17
19	N	17	16
20	N	14	13
21	N	17	17
22	N	17	19

Body Weight Analysis

Summary ANOVA for body weight for experimental group pre and post energy restriction.

Source	SS	df	MS	F	P
Group	1.163	1	1.163	.000703	0.934
Group(subject)	2317.242	14	165.517		
Trial	40.275	1	40.275	93.829	<0.001
Interaction (Group X Trial)	0.340	1	0.340	0.793	0.388
Residual	2365.030	14	0.429		

Group-C,P

Trial- Pre-test, Post-test

Body Weight

Subject	Group	<u>Energy Restriction</u>						
		D1	D5	D7	D8	D9	D10	D11
1	Carbohydrate	87.9	87.9	88.2	88.0	86.2	86.0	85.7
2	Carbohydrate	77.5	77.7	77.2	77.2	76.0	75.8	75.7
3	Carbohydrate	78.9	78.8	79.2	80.0	77.5	76.4	76.2
4	Carbohydrate	77.0	77.1	77.2	77.0	76.5	76.1	75.6
5	Carbohydrate	81.5	81.2	81.3	81.5	81.4	81.0	80.6
6	Carbohydrate	94.7	94.5	94.2	94.9	92.7	91.6	91.0
7	Carbohydrate	77.5	77.5	77.3	77.5	75.4	75.5	74.5
8	Carbohydrate	98.7	98.6	98.8	98.6	97.5	97.2	95.8
9	Placebo	94.0	93.5	94.0	94.5	92.7	91.8	91.2
10	Placebo	76.4	76.2	76.0	76.0	75.4	74.6	74.6
11	Placebo	91.5	91.6	91.7	91.7	90.6	90.0	90.2
12	Placebo	82.1	82.0	82.0	82.1	80.3	80.0	80.1
13	Placebo	71.6	71.7	72.0	71.7	71.5	70.0	69.4
14	Placebo	74.4	74.5	74.2	74.6	74.2	72.7	72.7
15	Placebo	87.0	87.0	87.1	86.9	86.4	85.9	85.6
16	Placebo	98.4	98.3	98.5	98.6	97.5	97.2	96.0

Summary ANOVA Tables for changes in blood parameters

Cortisol					
Source	NDF	DDF	TYPE III F	Pr>F	
Group	1	14	5.53	0.0325	
Trial	1	98	1.32	0.2527	
Draw	1	98	48.59	0.0001	
Interaction (Group*Trial)	1	98	0.10	0.7472	
Interaction (Trail*Draw)	3	98	3.36	0.0219	
Interaction (Group*Trial*Draw)	6	98	0.72	0.6313	

CK					
Source	NDF	DDF	TYPE III F	Pr>F	
Group	1	14	2.07	0.1726	
Trial	1	98	0.43	0.5123	
Draw	1	98	5.70	0.0012	
Interaction (Group*Trial)	1	98	0.83	0.648	
Interaction (Trail*Draw)	3	98	0.41	0.7467	
Interaction (Group*Trial*Draw)	6	98	1.03	0.4081	

Glucose					
Source	NDF	DDF	TYPE III F	Pr>F	
Group	1	14	1.13	0.3061	
Trial	1	98	0.22	0.6428	
Draw	1	98	1.53	0.2230	
Interaction (Group*Trial)	1	98	0.22	0.8016	
Interaction (Trail*Draw)	3	98	2.86	0.0639	
Interaction (Group*Trial*Draw)	6	98	0.93	0.4318	

		Cortisol T2			
Subject	Group	Pre Exercise	10 Minutes Post	6 Hours Post	24 Hours Post
1	C	271.7	190.8	120.9	223.6
2	C	230.2	271.7	89.6	162.8
3	C	255.6	157.0	90.7	218.9
4	C	301.4	241.5	159.8	247.5
5	C	203.7	75.0	237.9	240.9
6	C	203.6	236.0	133.3	238.9
7	C	216.3	363.8	74.0	261.2
8	C	344.0	344.3	210.0	332.7
9	P	198.7	163.8	62.3	202.5
10	P	264.8	250.3	90.5	228.9
11	P	229.9	188.5	82.1	186.2
12	P	268.4	200.5	90.1	237.6
13	P	298.7	132.3	89.1	191.0
14	P	317.8	153.5	12.9	265.0
15	P	247.7	226.8	102.9	180.6
16	P	181.0	192.3	84.8	232.2

		Cortisol T3			
Subject	Group	Pre Exercise	10 Minutes Post	6 Hours Post	24 Hours Post
1	C	428.1	186.6	310.2	230.2
2	C	347.1	178.9	141.0	136.0
3	C	346.1	268.7	90.8	226.0
4	C	389.3	204.8	210.5	318.8
5	C	221.6	119.9	171.6	254.2
6	C	237.5	199.3	72.6	222.7
7	C	248.2	231.0	102.5	223.3
8	C	357.2	184.0	195.6	126.1
9	P	246.8	280.8	202.9	133.3
10	P	247.6	256.1	90.3	187.9
11	P	269.2	156.5	90.7	223.6
12	P	366.9	259.4	129.4	198.4
13	P	319.3	171.7	114.2	184.5
14	P	280.5	165.8	101.0	184.4
15	P	226.0	241.5	82.7	196.6
16	P	372.7	155.0	128.5	138.9

Subject	Group	Pre Exercise	CK T2		
			10 Minutes Post	6 Hours Post	24 Hours Post
1	C	98.4	123.0	155.6	106.6
2	C	65.6	180.4	137.4	180.4
3	C	86.1	116.9	137.4	188.6
4	C	114.8	149.7	176.8	268.6
5	C	123.0	118.9	121.0	96.4
6	C	75.9	92.3	98.4	61.5
7	C	121.0	186.5	166.1	172.1
8	C	157.9	176.3	178.4	149.6
9	P	118.9	75.9	244.0	205.0
10	P	127.1	194.8	254.2	311.6
11	P	121.0	125.1	164.0	162.0
12	P	104.6	116.9	147.6	235.8
13	P	77.9	104.6	186.6	153.8
14	P	49.2	71.8	61.5	86.1
15	P	114.8	125.1	145.6	102.5
16	P	254.2	176.3	237.8	135.3

Subject	Group	Pre Exercise	CK T3		
			6 Minutes Post	6 Hours Post	24 Hours Post
1	C	49.2	57.4	75.9	80.0
2	C	84.1	106.6	110.7	123.0
3	C	75.9	92.3	108.7	114.8
4	C	149.7	155.8	239.9	135.3
5		75.9	123.0	118.9	129.2
6	C	108.7	151.7	196.8	282.9
7	C	160.0	69.7	61.5	127.6
8	C	53.3	73.8	96.4	88.2
9	P	123.0	155.8	190.7	149.7
10	P	82.0	135.3	174.3	184.5
11	P	82.0	96.4	98.4	147.6
12	P	108.7	153.8	110.7	190.7
13	P	75.9	98.6	112.8	88.2
14	P	104.6	110.7	135.3	37.7
15	P	188.6	371.1	235.8	149.6
16	P	155.8	209.1	217.3	430.5

Subject	Group	Pre Exercise	Glucose T2	
			10 Minutes Post	6 Hours Post
1	C	6	5.68	4.84
2	C	5.2	3.6	4.48
3	C	2.14	2.71	3.46
4	C	4.3	4.87	6.64
5	C	5.14	8.17	10.44
6	C	5.12	5.31	4.75
7	C	4.37	6.37	5.75
8	C	5.98	4.15	8.84
9	P	6	4.92	7.08
10	P	4.33	4.22	3.9
11	P	4.74	5.63	5.9
12	P	3.04	6.2	4.8
13	P	4.88	4.56	4.56
14	P	4.99	6.62	8.09
15	P	5.53	4.63	5.05
16	P	3.49	5.04	3.69

Subject	Group	Pre Exercise	Glucose T3	
			10 Minutes Post	6 Hours Post
1	C	5.56	7.44	3.68
2	C	3.92	2.88	4.56
3	C	4.24	3.92	4.24
4	C	4.15	4.29	5.34
5	C	6.69	6.88	6.62
6	C	7.65	6.45	5.12
7	C	6.7	5.56	6.61
8	C	6.07	6.49	7.12
9	P	4.52	3.76	5.64
10	P	4.77	3.79	3.75
11	P	4.52	5.82	5.91
12	P	5.12	5.84	5.56
13	P	4.03	3.42	3.56
14	P	5.9	4.84	4.84
15	P	4.39	4.63	5.56
16	P	5.6	4.43	3.88

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