

APPENDICES

APPENDIX A: DETAILED DESCRIPTION OF TECHNICAL PROCEDURES AND RESEARCH METHODS

Subject Selection and Screening

Fourteen sedentary overweight males ages 18-40 years old were selected as subjects. Subjects were recruited from students, faculty, and staff at Virginia Polytechnic Institute and State University through flyers, newspaper advertisements, and email announcements. The potential subjects were screened for previously diagnosed health risks such as hypertension, diabetes, and heart disease. The subjects' BMI was calculated to determine if they were considered overweight ($BMI \geq 25 \text{ kg/m}^2$) but not morbidly obese ($BMI \leq 45 \text{ kg/m}^2$). The subjects could not be participating in any type of regular physical activity to be considered for the study.

Preliminary Testing Procedures

All potential subjects were provided with detailed written and oral descriptions of the study procedures. The subjects were familiarized with the facilities and equipment to be used. The subjects were shown pictures of the muscle biopsy procedure and the resulting scar.

Those interested in participating were required to receive a doctor's approval prior to beginning the study. The subjects completed an informed consent form prior to initiation of the study. The study was approved by the Virginia Polytechnic Institute and State University Institution Review Board.

Experimental Conditions and Testing Protocol

Overall Study Design

The subjects were pair matched according to their initial %BF and divided into two groups of seven. The subjects participated in a nine week exercise training program consisting

of either a MIT or HIIT on a cycle ergometer three times per week. The week prior to beginning and the week following the treatment period the subjects had their body composition determined through the hydrostatic weighing technique. Body fat distribution was determined through WHR, WTR, and waist circumference. A resting muscle biopsy was used to determine the activity of HADH. The subjects in both groups attended weekly nutrition education sessions and followed a modest energy restricted diet.

Nutrition Education

Weekly sessions were held to educate the participants in various aspects of nutrition and health. The sessions were discussion based and the subjects were educated through demonstrations and activities. The weekly sessions were designed as follows.

Week 1: Introduction to the program and how to record food intake accurately

Week 2: Food Guide Pyramid and reading food labels, distribution of diet plans

Week 3: Fat Facts

Week 4: Fast food eating, eating on the go, travelling and vacationing, and packing a healthy meal

Week 5: Recipe modification, bring a friend/spouse

Week 6: What happens to the foods I eat? (A look at basics of digestion and metabolism)

Week 7: Exercise and Weight Loss

Week 8: Weight Maintenance

Week 9: Popular diets, wrap up, and evaluation

Diet Modification

Based on the initial age, height, and weight of the subjects, individual basal energy expenditure (BEE) was determined by the Harris-Benedict Equation. The estimated total energy expenditure (TEE) was determined by multiplying the BEE by an activity factor of 1.5 for lightly active individuals (53). Lightly active consisted of performing everyday activities with the exclusion of physical activity. The subjects were then provided with either an 1800, 2000, 2200, 2500, or 2800 calorie diet based on the estimated TEE to provide a deficit of 500 calories per day.

Dietary treatment consisted of limiting fat intake to 25% of total calories, protein to 15-20% of total calories, and carbohydrates (CHO) to 55-60% of total calories. The subjects were provided with the number of recommended servings from each of the food groups included in the Food Guide Pyramid for their individual diet. The estimated serving sizes were based on the food exchange system.

The subjects kept three-day diet records during weeks 1, 4, 7, and 9 of the study. The diets were analyzed using the Nutritionist III program (First Data Bank, Inc.). Upon completion of the nutrient analysis of each food record the subjects were provided a written evaluation of their diet. The macronutrients, micronutrients, sodium, fiber, and cholesterol were included in the diet evaluation. Also included were recommendations for change to meet the dietary requirements. Individual and informal nutrition counseling sessions were provided at the subject's request. Reasons for individual counseling were to clarify the diet evaluation or to learn more specific ways to improve their diets. Three subjects, two from the HIIT group and one from the MIT group consistently sought additional nutrition information.

Moderate Intensity Exercise Protocol

The subjects cycled on a Monark cycle ergometer in our laboratory under supervision beginning at 20 minutes per session during week one and two and increasing to 30 minutes during week two. The exercise duration for the subsequent weeks was 30-40 minutes to allow for energy expenditure equal to that of the HIIT group. The intensity began at 45% VO_2max for week one, increased to 50% VO_2max during week two, 55% VO_2max during weeks three and four, to 60% VO_2max for weeks five, six, and seven, and to 65% VO_2max that was maintained for weeks eight and nine. The subjects cycled at 60 revolutions per minute (rpm). There was a five minute warm-up and warm-down at 50% VO_2max before and after each exercise session. Heart rate was monitored during the sessions to validate exercise intensity.

High Intensity Exercise Protocol

The first two weeks of training of the HIIT group were the same as the first two weeks of the MIT group. The HIIT began during the third week with each of the three days of exercise during the week denoted as a short, medium, or long interval. The initial week of the interval session consisted of exercising for 30 seconds at 80% VO_2max followed by a recovery period of 60 seconds at 40% VO_2max for the short interval. The high intensity interval was repeated 16 times. The high intensity interval increased to 90% VO_2max during week four, and by 5% each week to reach a maximum of 110% VO_2max by week nine. The medium interval began at 70% VO_2max for 90 seconds increasing to 100% VO_2max by week nine. There were two minutes of cycling at 40% VO_2max between each interval, and a total of eight intervals in the exercise session. The long interval consisted of cycling at 65% VO_2max for three minutes increasing to 95% VO_2max with three minutes of recovery at 40% VO_2max . The long interval cycle was repeated four times within the session. There was a five minute warm-up and warm-down at

50% VO_2max before and after each exercise session. The subjects' heart rate was monitored to validate exercise intensity. The estimated exercise energy expenditure for the two exercise protocols ranged from 250 and 350 kilocalories per session.

Muscle Biopsy Technique

A muscle biopsy was obtained from the middle of the vastus lateralis of the left leg using the percutaneous needle biopsy technique. Two experienced technicians under the supervision of a physician performed the procedure. A small area of the leg was cleansed and shaved. The shaven area was then treated with iodine. A sterile drape with an opening was then placed over the leg, exposing only the area from which the biopsy was to be obtained. The subject was then administered lidocaine, a local anesthetic in multiple injections. Upon anesthetization, a 1 cm wide and deep incision was made. The biopsy needle was then inserted. Application of suction allowed for the sample to be obtained. The muscle was immediately frozen in liquid nitrogen and stored at -80°C for further analysis. The incision was closed with adhesive bandage strips and the subjects were instructed on proper care for the incision.

Measurement Procedures for Dependent Variables

Body Weight

Body weight was measured to the nearest 0.1 kg on a balance beam scale.

Residual Lung Volume Technique

The residual volume of the lungs was measured using the oxygen dilution technique as described by Wilmore et al. (69). A rebreathing bag was filled with 5 liters (L) of oxygen. The bag was attached to a 3-way valve with a mouthpiece connected at one side. The mouthpiece

was placed in the subject's mouth, and the subject's nose was clipped. The subject performed a maximal exhalation into room air. Upon completion of the exhalation the valve was turned and the subject breathed 5 deep breaths from the oxygen bag and following the fifth inhalation, a maximal exhalation was performed into the rebreathing bag. The bag was sealed and the air in the bag was analyzed for oxygen and carbon dioxide content. This procedure was done in duplicate to ensure repeatability. The residual volume was calculated and used in determining %BF.

Hydrostatic Weighing Technique

Body composition was determined through hydrostatic weighing technique. The subjects were seated on a chair suspended within a water tank (Novel Products, Rockton, IL). The subjects were trained on the procedure for obtaining underwater weight and were allowed to practice several times prior to collecting the underwater weight. The chair was attached to a load cell connected to a computer used to calculate underwater weight based on output from the load cell. The subjects completely submerged themselves while giving a maximal exhalation. The subjects were submerged for approximately 3-5 seconds once the maximal exhalation was complete. At this time the weight in pounds was obtained. Three highest values from 8 submersions were used to determine %BF according to Siri et al. (60).

Body Fat Distribution

Waist circumference, WHR, and WTR were determined to estimate the distribution of body fat. The waist measurement was taken at the narrowest part of the torso below the xiphoid process and above the umbilicus. The hip circumference was measured around the buttocks at the point where the buttocks are at maximal extension above the gluteal fold. The maximal

circumference of the thigh below the gluteal fold was measured (1). A nonelastic measuring tape was used.

Tissue Homogenization

The muscle biopsy was homogenized according to the procedures outlined by Passonneau and Lowry (42). A 10-20 mg sample of muscle was homogenized manually in a glass homogenizer on ice. The muscle sample was homogenized in 50 volumes of a phosphate-based buffer. The buffer consisted of 16 mM sodium phosphate (Na_2HPO_4), 4 mM potassium phosphate (KH_2PO_4), 0.02% bovine serum albumin (BSA), 5 mM mercaptoethanol, 0.5 mM EDTA, and 100 ml of water brought to a pH of 7.4. Once the proper pH was achieved, 100 ml of glycerol was added, and the solution was mixed well. The buffer was stored at -80°C for up to three weeks.

The homogenate was removed and placed in a 3.0-ml tube and stored at -80°C for further analysis. Prior to analysis the sample was thawed and diluted to a total of 100 volumes by adding 50 volumes of Lowry diluting media (42). The diluting media consisted of 2 ml 1M imidazole, 200 μ l 10% BSA, and 100 ml water. The solution was brought to a pH of 7.0, and was stored at -80°C .

Enzyme Analysis

The procedure for analysis of the enzyme activity was modified from that previously described by Passonneau and Lowry (1992). The activity of the enzyme was determined spectrophotometrically at a wavelength of 340 nm. The assay reagent consisted of 2.5 ml 1 M tris-HCl at a pH of 7.0, 0.5 ml 200mM EDTA, and water brought to volume of 50 ml. Seven and a half milliliters of 5 mM NADH was then added to the assay mixture to allow

the initial absorbance to be 1.0-1.5. The cuvette contained 200 μ l of the assay mixture including NADH, 40 μ l homogenate (100 times dilution factor), and 10 μ l 10% triton. The mixture was stirred and allowed to sit for two minutes. The basal activity was then recorded every 30 seconds for two minutes. After the basal activity was recorded, 10 μ l 5mM acetoacetyl CoA was added to the cuvette to promote the reaction. The absorbance was recorded every 30 seconds for five minutes. Each sample was analyzed in triplicate. The coefficient of variation (CV) of the assay was 25% which is in agreement with that of Simoneau et al. (37).

Statistical Analyses for Dependent Measures

Data was statistically analyzed by using the Sigma Stat program (Version 2.03, SPSS software ? 1992-1997). A two-way analysis of variance with repeated measures and a significance level of 0.05 was used to determine a difference between groups over time in the dependent measures.