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Stewart G. Trost for the degree of Master of Science in Human Performance presented on January 6, 1994.

Title: The Effect of Substrate Utilization, Manipulated by Nicotinic Acid, on Excess Postexercise Oxygen Consumption.

Abstract Approved: _____

Anthony R. Wilcox Ph.D.

Increased fat oxidation during the recovery period from exercise is thought to be a contributing factor for the EPOC. In an attempt to study the effect of serum free fatty acid (FFA) availability during exercise and recovery on EPOC, nicotinic acid (NA), a potent inhibitor of FFA mobilization from adipose tissue, was administered to six trained male cyclists (VO_2 max 65 ± 8.5; age 25 ± 4.6 y) prior to, during, and after a bout of cycling at 65% VO_2 max. In the NA trial, a 500 mg dose of NA was ingested prior to exercise, and 100 mg doses were ingested at 15, 30, and 45 min exercise, and 30 min recovery. The cyclists also completed a trial under control (C) conditions. Serum FFA, serum glycerol, and VO_2 were monitored during rest, exercise, and recovery, each of which was 1h in duration. NA ingestion prevented the increase in serum FFA that occurred during exercise in the C trial; FFA levels were significantly lower than C values ($p < .05$) during both exercise and recovery in the NA trial. Serum glycerol levels were significantly lower ($p < .05$) during exercise in the NA trial. The respiratory exchange ratio (R) was not significantly lower during exercise. However, R was significantly lower, indicative of greater fat utilization, during recovery in the C trial (0.77 C, 0.83 NA) ($p < .05$). There was a tendency for VO_2 values to be

greater in the C condition, but the difference in the 1h EPOC was not statistically significant (5.71 ± 1.49 L C;4.46 ± 2.5 L NA)(P=.23). While the expected shifts in substrate metabolism occurred following NA ingestion, the reductions in fat utilization during recovery did not significantly alter the EPOC.

The Effect of Substrate Utilization, Manipulated by Nicotinic Acid, on Excess Postexercise Oxygen Consumption.

by

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Associate Professor of Exercise and Sports Science in charge of major

Redacted for Privacy

Chair of Department of Exercise and Sports Science

Redacted for Privacy

Dean of College of Health and Human Performance

Redacted for Privacy

Dean of Graduate School

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Introduction

It is well-established that upon cessation of exercise, oxygen consumption (VO_2) remains elevated above preexercise resting levels for a transient period of time (Figure 1). This phenomenon, which has been documented since the early 1900's, is commonly referred to as excess postexercise oxygen consumption (EPOC).

Currently, the underlying mechanisms responsible for the EPOC are not fully understood. The classical O_2 debt hypothesis of Hill and Lupton (1923) proposed that elevated VO_2 after exercise was necessary for the repayment of an O_2 deficit which was incurred during exercise. This elevation in postexercise VO_2 consumption represented the oxidation of a portion (20%) of the lactate formed during exercise which provided the necessary energy for the reconversion of the remaining portion (80%) of lactate to glycogen.

The original O_2 debt hypothesis was modified by Margaria, Edwards, and Dill (1933), who distinguished the fast and slow components of postexercise VO_2 as the "alactacid debt" and the "lactacid debt", respectively. Margaria and his colleagues concluded that the rapid decline in VO_2 after exercise was not related to the oxidation of lactate, but rather, represented the replenishment of creatine phosphate stores. Consequently, only the slow phase of postexercise VO_2 represented the oxidative removal of a portion of the lactate formed during exercise.

In conflict with the classical O_2 debt explanation of elevated postexercise VO_2, numerous investigators using isotopic tracers have demonstrated the primary fate of lactate to be oxidation and not glycogen synthesis (Gladden, 1989).

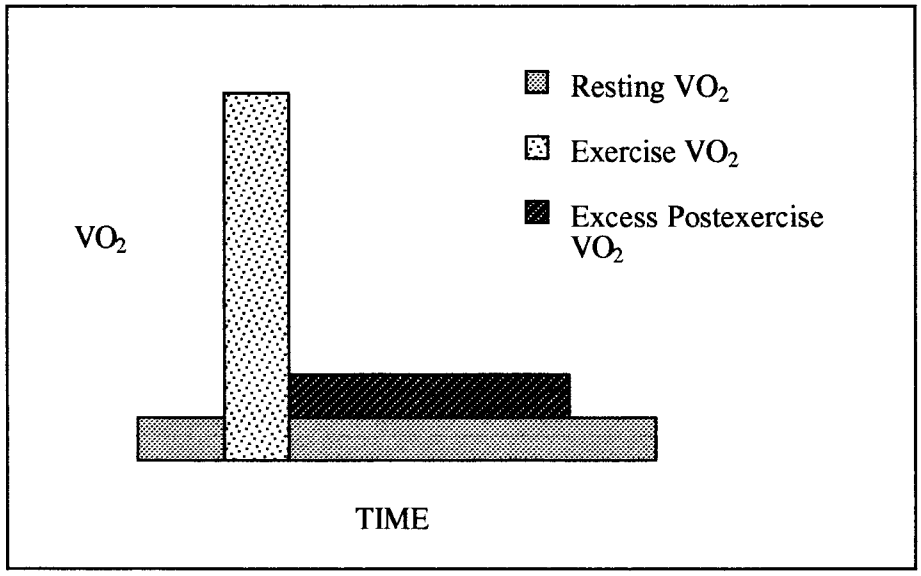


Figure 1. Schematic illustration of excess postexercise oxygen consumption (EPOC)

Furthermore, researchers have failed to observe any association between the kinetics of lactate removal, glycogen repletion, and postexercise VO_2 (Gaesser & Brooks, 1984; Harris, 1969). As a result, contemporary explanations of the EPOC have focused on the physiological and biochemical factors that directly or indirectly stimulate cellular respiration. These factors include adenosine triphosphate (ATP), adenosine diphosphate (ADP), inorganic phosphate (Pi) and creatine phosphate (CP) concentrations, catecholamines, thyroxine, glucocorticoids, fatty acids, calcium ions, and temperature (Gaesser & Brooks, 1984). Presently there exists no complete or universally accepted explanation of increased postexercise metabolism.

There is some evidence to suggest that increased utilization of fat during and after exercise may play a role in the elevation of metabolic rate after exercise. Chad and Quigley (1991) measured postexercise VO_2 in trained and untrained females following 30 minutes of cycle ergometry at 50% and 70% VO_2 max. For both the trained and untrained groups, postexercise VO_2 was significantly greater after exercise at 50% VO_2 max than after 70% VO_2 max. The significantly lower respiratory exchange ratio (RER) values observed during recovery after the 50% VO_2 max trial in both the trained and untrained groups suggested that increased fat mobilization and utilization during exercise may be involved in the long-term elevation of postexercise metabolism.

Bahr, Ingnes, Vaage, Sejersted, and Newsholme (1987) and Maehlum, Grandmontagne, Newsholme, and Sejersted (1986) reported significantly lower RER values in the postexercise state compared to rest and exercise, indicating a greater reliance on fat metabolism during exercise recovery. The result prompted

the investigators to suggest that part of the observed EPOC may be accounted for by an increased rate of triglyceride-fatty acid (TG-FA) cycling, in which FFAs released from TG are not oxidized but reesterified to reform TG.

A substrate cycle is said to exist when two opposing, non-equilibrium reactions catalyzed by different enzymes are operating simultaneously. At least one of the reactions must involve the hydrolysis of ATP. Thus, the result of the substrate cycle is the liberation of heat and consumption of energy with no net conversion of substrate to product. According to Newsholme (1978), substrate cycles form part of a logical series of biochemical mechanisms that exist to increase the sensitivity of non-equilibrium reactions to changes in concentrations of metabolic regulators.

Recently, several investigations have shown the rate of the TG-FA cycle to be increased after prolonged exercise at moderate intensity, and that the energy cost of this increase may account for a significant portion of the EPOC. Bahr, Hansson, and Sejersted (1990) measured the rate of TG-FA cycling after exercise and assessed its relative contribution to the EPOC. After two hours of exercise at 51% of VO_2 max, TG-FA cycling was found to increase from 414 ± 90 μmol FA/min (control) to 1473 ± 435 μmol FA/min (3 hours postexercise). When converted to energy cost, the increased rate of cycling accounted for as much as 50% of the prolonged component of EPOC.

Wolfe, Klein, Carraro, and Webber (1990), using an isotopic infusion technique and indirect calorimetry, assessed the importance and the energy cost of total TG-FA cycling in coordinating the availability of fatty acids with energy requirements during exercise and recovery. The observed rapid changes in the percentage of FFAs mobilized and reesterified at the beginning of exercise and recovery demonstrated the importance of the TG-FA substrate cycle in amplifying the ability of stored triglyceride to respond rapidly to major changes in energy

requirements. At rest and during exercise, the energy cost of TG-FA cycling was less than 2% and 0.5% of total energy expenditure, respectively. However, during recovery from exercise, the high rate of cycling accounted for a considerable percentage (14%) of the increase in energy expenditure above the resting value before exercise. Importantly, the increased rate of TG-FA cycling persisted for two hours after exercise, prompting the authors to conclude that TG-FA cycling was important not only in the control of substrate flux, but also in elevation of energy expenditure after exercise.

Few investigators have directly examined the effect of substrate utilization on the magnitude and duration of the EPOC. Chad and Quigley (1989) studied the effect of substrate utilization on postexercise VO_2 in five untrained women. When fatty acid mobilization and oxidation were stimulated by caffeine ingestion prior to exercise, postexercise VO_2 over the one-hour recovery period was significantly higher in the caffeine trial compared to the non-caffeine control trial. Although the finding provided support for the hypothesis that increased fat metabolism may account for a significant portion of the EPOC, it is possible that the greater postexercise VO_2 may have been a function of the stimulatory effect of caffeine on metabolic rate. This was evidenced by the significantly higher resting and exercise VO_2 values recorded in the caffeine trial compared to the control trial.

The antilipolytic effect of nicotinic acid (NA) on adipose tissue has been well-documented by a number of investigators (Carlson & Oro, 1962; Carlson, 1963; Carlson, Havel, Ekeland, & Holmgren, 1963; Carlson, Oro, & Ostman, 1968; Madson & Malchow-Moller, 1983). The antilipolytic properties of NA have been attributed to its inhibiting effect on adenyl cyclase, resulting in decreased levels of cyclic adenosine monophosphate (c-AMP) in the adipose tissue cell (Butcher,

Baird, & Sutherland, 1968). Several studies have examined the acute effects of nicotinic acid during exercise. Carlson et al. (1963) reported an inhibition of FFA mobilization and a shift towards carbohydrate metabolism during two hours of cycle ergometry following infusion of nicotinic acid. Jenkins (1965) reported decreased fat utilization during treadmill running after administration of nicotinic acid. Bergstrom, Hultman, Jorfeldt, Pernow, and Wahren (1969) reported significantly elevated RER values and increased glycogen utilization with respect to control bouts, indicating a greater dependence on carbohydrate as substrate following nicotinic acid administration.

To date, no investigation has examined the effect of substrate utilization, manipulated by nicotinic acid ingestion, on postexercise VO_2. If enhanced fat metabolism is responsible for a significant portion of the EPOC, then it is reasonable to theorize that inhibition of fatty acid metabolism by nicotinic acid should reduce the magnitude of the EPOC. The results of such an investigation would provide evidence to support or reject the hypothesis that increased fat metabolism may be related to elevated metabolism after exercise. Thus, the purpose of this study is to examine the effect of substrate utilization, manipulated by ingestion of 900 mg of nicotinic acid, on excess postexercise oxygen consumption in trained male cyclists, 21-35 years of age, after 60 minutes of cycle ergometry at 65% of VO_2 max.

Methods

Subjects

Six well-trained male cyclists between the ages of 21 and 36 were solicited to serve as subjects. This group was selected in order to maximize subject homogeneity with respect to body composition, fitness level, and training experience. Well-trained subjects also utilize a greater percentage of energy metabolism from fat oxidation during submaximal exercise. An informed consent document (Appendix III) and a medical questionnaire (Appendix IV) were read, completed, and signed by each subject prior to participation. The study was approved by the Oregon State University Institutional Review Board for use of Human Subjects.

Protocol

Subjects reported to the Human Performance Laboratory on four occasions. On the first visit, each subject read and signed an informed consent form and completed a medical questionnaire. The experimental procedures were fully explained both verbally and in writing. Subjects then performed a ramped maximal exercise test on a bicycle ergometer for the determination of maximal oxygen consumption (VO_2 max). For each subject, a plot of VO_2 versus power output was used to calculate the workload corresponding to 65% VO_2 max. On the second visit, subjects performed a 15-min pretest to confirm the accuracy of the calculated workload. Following this procedure, percent body fat was determined by hydrostatic weighing. On the third and fourth visits, the submaximal experimental trials were completed, during which subjects rested 60 min, exercised 60 min at 65% VO_2 max, and rested for another 60 min. In the nicotinic acid trial (NA), fat metabolism was inhibited by ingestion of 900 mg of

nicotinic acid. Results were compared to an identical control trial (C) during which nicotinic acid was not ingested. Trials were counterbalanced in order to prevent the possibility of an order effect.

Maximal Exercise Test

In order to determine the workload for the submaximal experimental trials, each subject performed a maximal incremental exercise test for determination of VO_2 max. Prior to the start of each test, each subject was fully briefed on the procedures involved in the test. The subjects peddled at a self-selected rpm on a Sensormedics electrically-braked cycle ergometer. After a 5-min warm-up at 50 watts, power output was increased by 35 watts every minute until volitional termination due to fatigue. Criteria for VO_2 max was attainment of any two of the following: (a) an increase in work load with little or no change in VO_2 (plateau), (b) a respiratory exchange ratio above 1.1, and (c) a heart rate approximating the age-predicted maximum (220 - age). VO_2 max was recorded as the mean of the largest three consecutive 20 s measurements of VO_2 during the test.

An open-circuit, indirect calorimetry technique was used, in which the subject, wearing a nose clip, breathed through a mouthpiece into a 2700 Hans-Rudolf nonrebreathing two-way valve connected by a hose to a mixing chamber on the exhalation side. Expired air for the determination of VO_2, carbon dioxide production (VCO_2), ventilation (Ve), and respiratory exchange ratio (RER) was collected and analyzed in 20 s intervals by the Sensormedics 2900 metabolic cart. Prior to each test, the metabolic cart was calibrated using gas samples with known concentrations of O_2 and CO_2. At the start of each testing day, the flow meter was calibrated with a 3 L calibration syringe. Heart rate and ECG for each subject was monitored continuously with a Marquette electrocardiograph using a V5 electrode lead configuration. A hard copy of the ECG was made at rest and

during the last 10 s of each minute of exercise. Heart rate was also monitored by a Polar Trainer heart rate monitor and was recorded during the last 10 s of each stage. The subjects's rate of perceived exertion (RPE) was recorded at 2-min intervals.

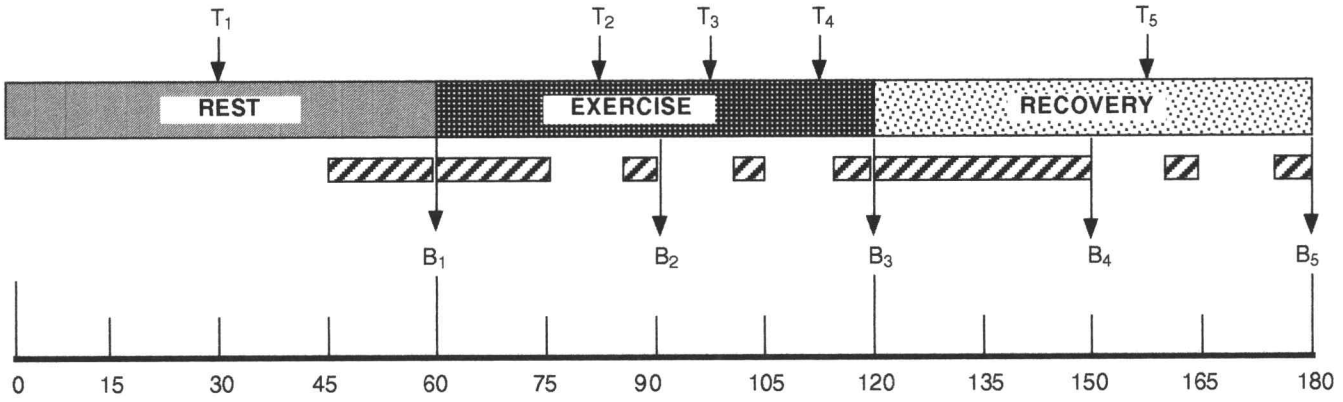
Hydrostatic Weighing

Following the work load confirmation test, residual lung volume (RV) was measured with the GO-MI LFT 3000 pulmonary function testing equipment, using a modification of the helium dilution technique, where the tracer gas was neon. Body density was then assessed by hydrostatic weighing. Underwater weight was determined from a digital display connected to a load cell attached to a PVC pipe platform. Subjects were completely submerged while sitting in the platform and were instructed to exhale maximally. The number of trials and mean underwater weight was determined according the criteria of Bonge and Donnelly (1989). Body density was calculated from mean underwater weight using the equations outlined by Brozek and Henschel (1959) and converted to percent body fat using the Siri equation (1956).

Submaximal Experimental Trials

Rest Period. The submaximal experimental protocol is outlined in Figure 2. Subjects reported to the laboratory at approximately 7:00 a.m. having refrained from hard physical activity for a minimum of 24 h. Subjects were requested to fast from 7:00 p.m. the night before. Upon entry to the laboratory, a heart rate monitor was fitted via an elastic strap below the chest area. A teflon catheter was then inserted into a forearm vein and kept patent with an infusion of heparin-free isotonic saline. Subjects then rested quietly in a semi-recumbent position for a total of 60 min. After 30 min, subjects ingested either 500 mg of nicotinic acid (NA) with water, or water alone (C). A valve and mouthpiece with supporting headgear was then fitted; however, the mouthpiece was not inserted.

EXPERIMENTAL TRIALS



T_1 = 500 mg NA or Water
T_2 - T_5 = 100 mg NA or Water
B_1 - B_6 = Blood Sample Collection (10 ml)

Expired air collections for determinations of VO2, VCO2, Ve, RER

Figure 2. Protocol for submaximal experimental trials

During the last 15 min of the rest period, the mouthpiece was inserted, the nose was clipped, and expired air was collected and analyzed for O_2 and CO_2 concentration as described in the section above. At the conclusion of the 60-min rest period, a 10-15 ml blood sample was taken. Resting baseline VO_2 and RER values were determined by averaging the values recorded during the last 10 min of the 60-min rest period.

Exercise Period. After resting baseline measurements were completed, subjects cycled for 60 min on a Sensormedics bicycle ergometer at an intensity corresponding to 65% of VO_2 max. Subjects consumed water *ad libitum.* Expired air for determination of VO_2 and RER was collected and analyzed continuously for the first 15 minutes of exercise, and between 25 and 30 min, 40 and 45 min, and 55 and 60 min of exercise. In between each sampling period, subjects ingested 100 mg NA with water or plain water alone (C). Thus, subjects ingested an additional 300 mg NA during the exercise portion of the NA trial. Blood samples for the determination of serum FFA and glycerol concentrations were taken after 30 and 60 min of exercise. VO_2 and RER values at 15, 30, 45, and 60 min of exercise were determined by averaging the values recorded during the final three minutes of each sampling period.

Postexercise Period. On completion of the 60 min exercise bout, subjects immediately resumed their seated resting positions with the mouthpiece and breathing valve left in place. Expired air for determination of VO_2 and RER was collected and analyzed continuously for the first 30 min of recovery, and between 40-45 and 55-60 min of recovery. After 30 min of recovery, subjects ingested 100 mg NA with water or plain water alone (C). Blood samples for determination of postexercise serum FFA and glycerol concentrations were taken after 30 and 60 min of recovery. Postexercise VO_2 and RER values 15 min into

recovery were determined by averaging values recorded between the 12th and 15th min of the recovery period. Postexercise VO_2 and RER values 30, 45, and 60 min after exercise were determined by averaging the values recorded during the last three minutes of each respective sampling period.

The 1-h EPOC was calculated as the time integral of the difference between postexercise VO_2 and preexercise resting VO_2 over the one hour recovery period. Postexercise VO_2 was judged to have returned to baseline when VO_2, averaged over three consecutive minutes, was equal to or less than the preexercise resting VO_2 value. Because of the extended duration of the experimental trials, the metabolic cart was recalibrated after 30 and 50 min of exercise, and after 30 min of recovery.

Dietary Record

Each subject completed a 24-h dietary record prior to each experimental trial. For both trials, subjects were given instructions and a sample dietary record to assist them and were requested to be as explicit as possible with respect to the quantity of food eaten, food preparation, and brand names. The purpose of the dietary record was to establish if the subjects had maintained their regular mixed diet prior to each experimental trial.

Analysis of Blood Parameters

Assays for the determination of serum FFA and glycerol concentration were performed on a Milton Roy Spectronic 401 spectrophotometer using the Wako kit NEFA C and Sigma kit number 337 for FFA and glycerol, respectively.

Experimental Design

The study employed a repeated measures experimental design in which subjects served as their own controls. For each treatment, the dependent variables were VO_2, RER, serum free fatty acid concentration, and serum glycerol

concentration. In order to counteract the possibility of any carry-over effects from previous exercise or administrations of nicotinic acid, each trial was separated by a minimum of one week.

Statistical Analysis

All data were analyzed using the SAS statistical software package (Release 6.03). Means and standard deviations were used to describe subjects' age, height, weight, VO_2 max, and percentage body fat. The effects of NA ingestion on VO_2, RER, serum FFA concentrations, and serum glycerol concentrations were analyzed using a two-way analysis of variance (ANOVA) with repeated measures across both factors of treatment (Control, Nicotinic Acid) and time. Checks were undertaken for violations of the assumptions underlying all analyses by Mauchly's test of sphericity. When the sphericity assumption was violated, the Huynh-Feldt Epsilon was used to adjust the respective degrees of freedom. Upon a significant *F*-ratio, *post hoc* pairwise comparisons between treatments at each time interval were performed using Tukey's HSD test. The treatment differences with respect to mean exercise VO_2 and the 1-h EPOC were tested by Student's *t*-test for paired samples. An alpha of 0.05 was used as the level of significance. All data are given as the mean and standard deviation, unless otherwise stated.

Results

Physical characteristics of subjects

The physical characteristics of the subjects are shown in Table 1. The high mean VO_2 max (65 ± 8.5 $ml.kg^{-1}.min^{-1}$) and the low percentage body fat (8.9% ± 3.6%) are indicative of the high fitness levels within the study group. Dietary analysis using the Nutritionist III (version 4.5, N2 Computing Salem, OR), software package confirmed that the subjects had maintained their normal mixed diets prior to both submaximal experimental trials (Figure 3).

Oxygen Consumption

VO_2 during the rest and postexercise period for both treatment and control trials are shown in Figure 4. Under both treatment conditions, resting metabolic rate (RMR) was almost identical, at 0.26 ± 0.05 $l.min^{-1}$ and 0.26 ± 0.03 $l.min^{-1}$ for the C and NA trials, respectively. The average workload for the 60 minutes of cycle ergometry was 249 ± 23 watts. VO_2 during exercise was not significantly different between trials (t [5] = 0.14, p = .17), averaging 3.19 ± 0.13 $l.min^{-1}$ (67% ± 2% VO_2 max) and 3.13 ± 0.37 $l.min^{-1}$ (66% ± 2% VO_2 max) for the C and NA trials, respectively. As shown in Figure 5, exercise remained at a steady-state under both experimental conditions. In both trials, postexercise VO_2 declined rapidly, reaching a steady state approximately 15 min into the recovery period. One hour after exercise, VO_2 remained significantly elevated above preexercise RMR($p < .01$) at 0.30 ± 0.01 $l.min^{-1}$ (115%) and 0.29 ± 0.02 $l.min^{-1}$ (110%) for the C and NA trials, respectively. Although postexercise VO_2 was consistently higher in the C trial, the non-significant treatment main effect (F = 2.59, 1,5 df, p =.17)

Subject	Age (year)	Height (m)	Weight (kg)	Body fat (%)	VO_2 max ($ml.kg^{-1}.min^{-1}$)
1	26	1.80	75	6.3	47.6
2	35	1.83	81	16.4	59.6
3	22	1.78	69	9.6	68.8
4	24	1.83	71	7.5	73.6
5	21	1.73	64	6.3	69.1
6	26	1.91	83	7.1	65.3
Mean	25.7	1.81	74.8	8.9	64.0
SD	4.6	0.06	6.3	3.6	8.5

Table 1. Physical characteristics of trained male cyclists (n = 6)

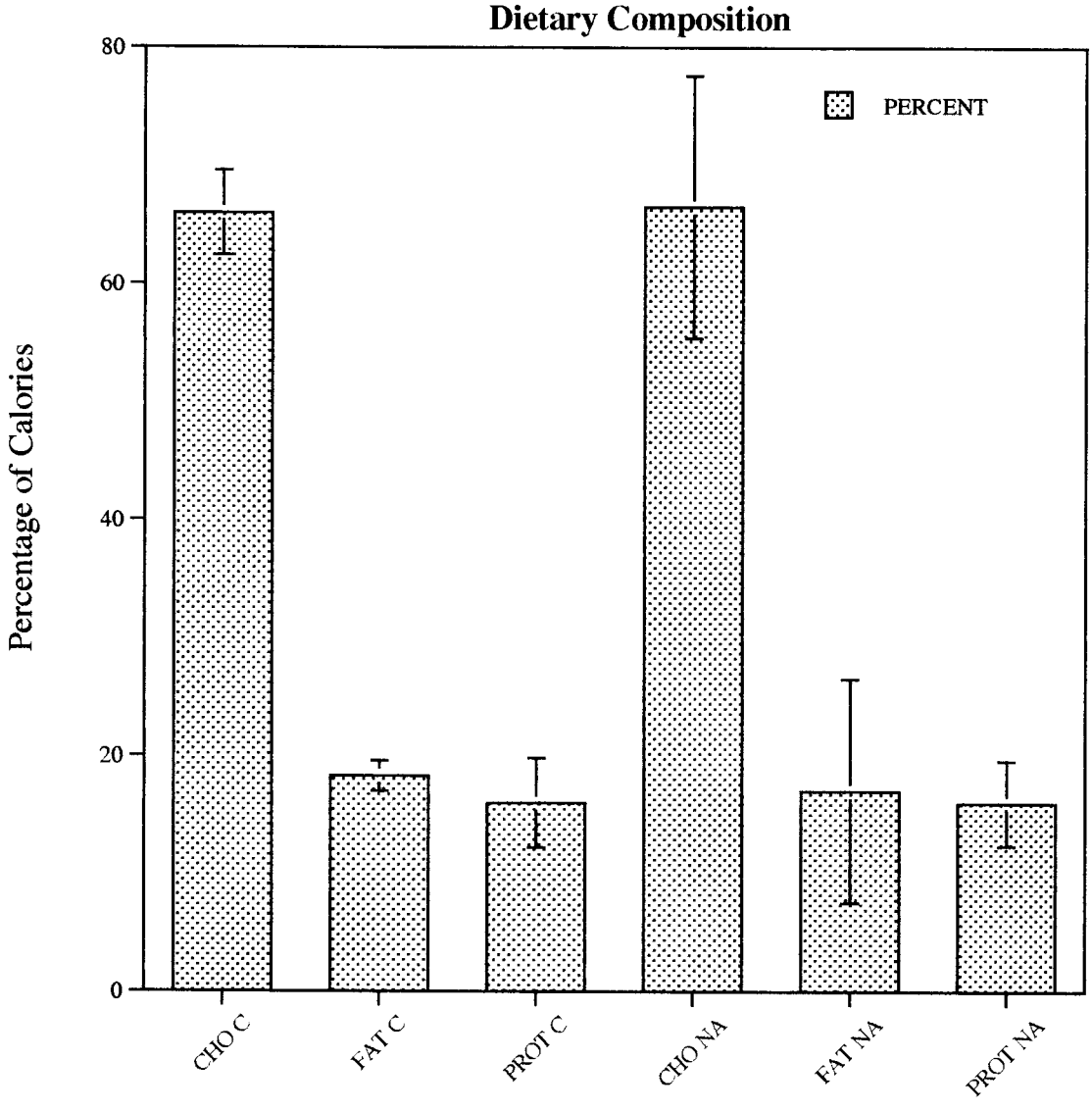


Figure 3. Dietary composition 24-h prior to the C and NA experimental trials

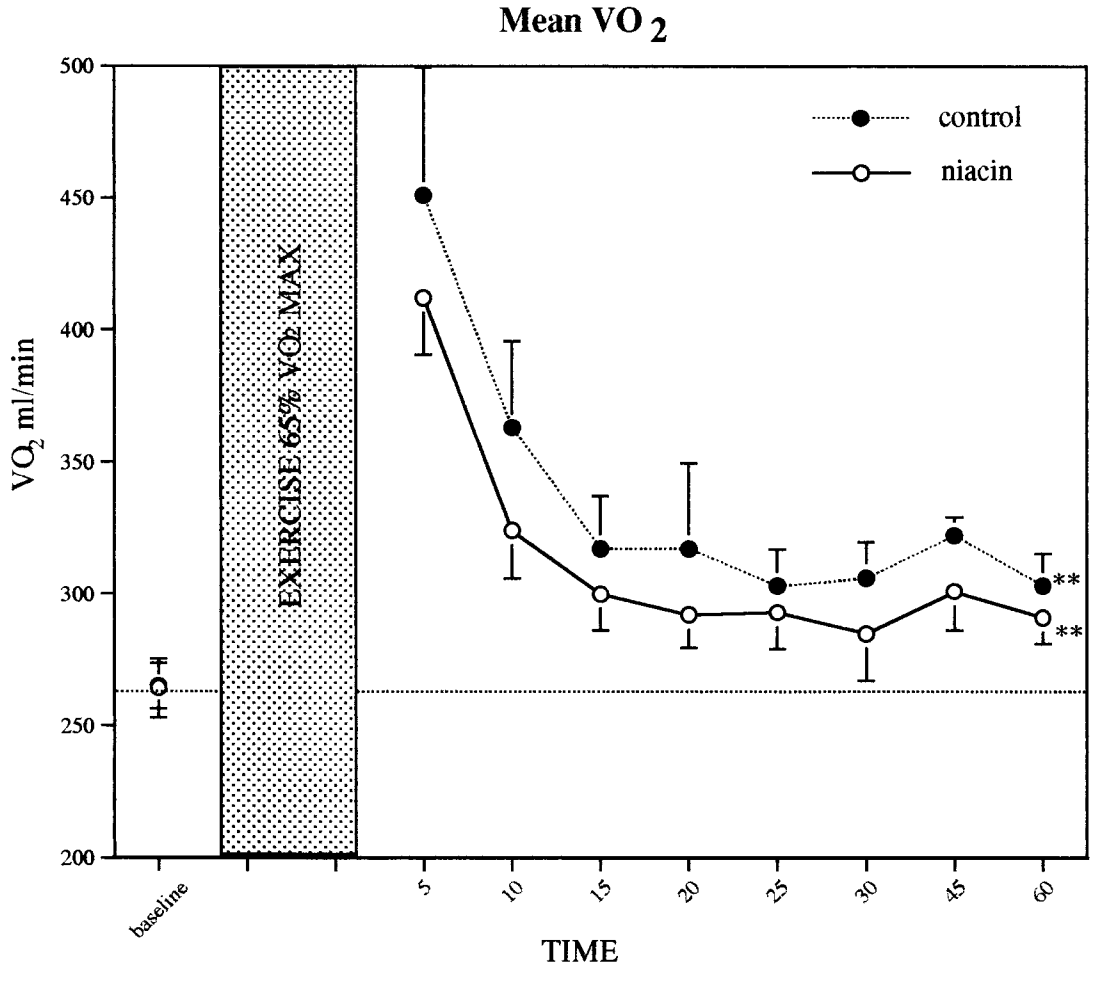


Figure 4. Mean VO_2 for both treatments at all time intervals. Values are means with SE (n=6). ** Greater than RMR $P < .05$.

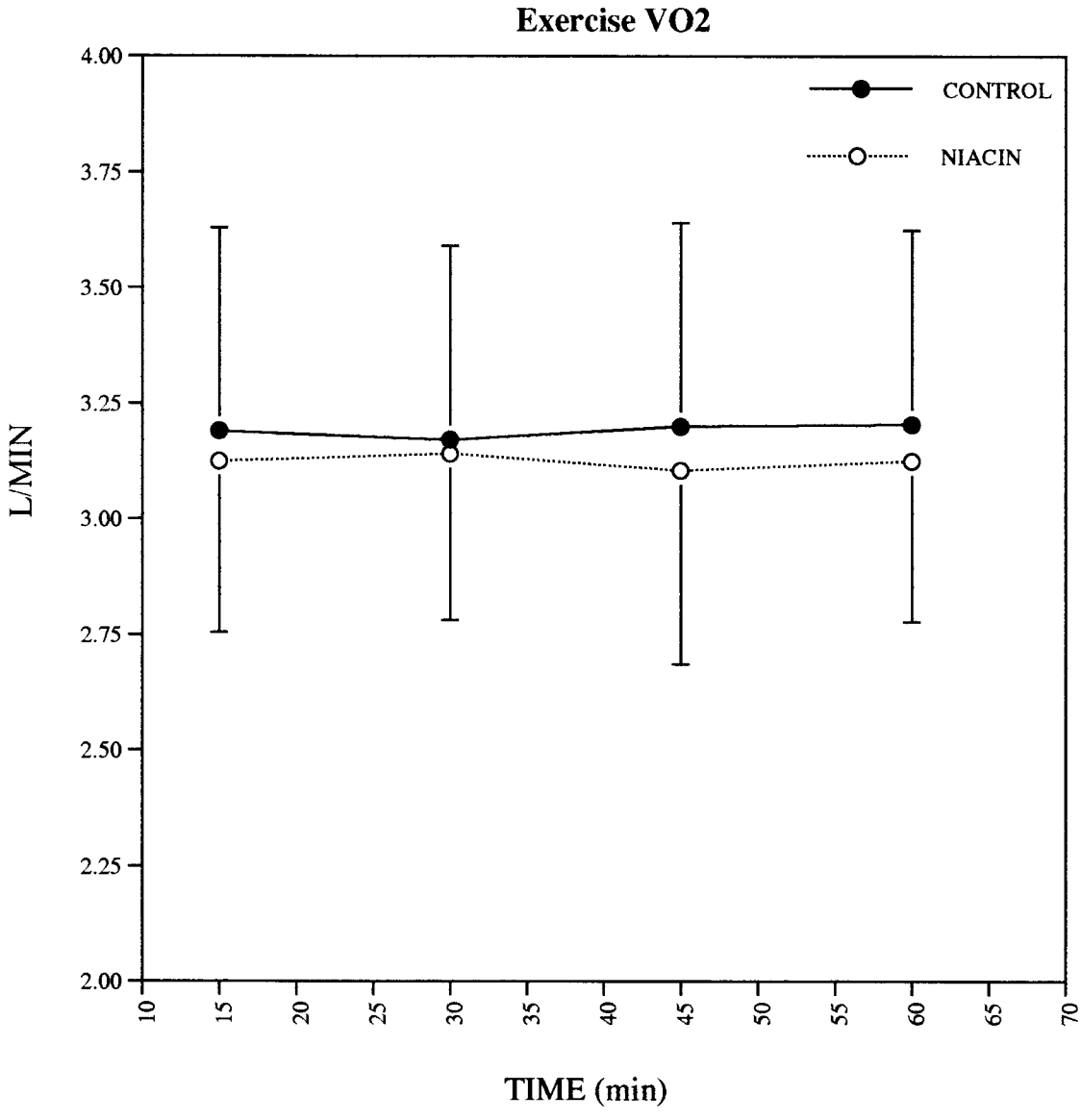


Figure 5. Mean VO_2 during 1-h cycle ergometry.

and treatment by time interaction ($F = 1.46$, 8,40 df, $p = .20$) indicated that therewere no statistically significant differences in VO_2 between the C and NA trials over the 1-h postexercise period.

The 1-h EPOC represented net oxygen consumption above RMR over the 1-hour postexercise examination period (Figure 6). Under both experimental conditions, the 1-h EPOC was highly variable, with values ranging from 3.69 L to 7.64 L and 1.11 L to 8.54 L for the C and NA trials, respectively In the C trial, the 1-h EPOC tended to be greater than that observed in the NA trial (5.71 ± 1.49 L vs 4.46 ± 2.51 L), however, this difference failed to reach statistical significance ($t[5] = 1.35$, $p = .23$). The 1-h EPOC for the C and NA trials amounted to only 3% and 1.7%, respectively, of the total O_2 consumption during exercise.

Respiratory Exchange Ratio

RER values for both experimental trials are shown in Figure 7. Mean RER values at rest and during exercise were consistently lower in the C trial, however, between treatment differences failed to reach statistical significance ($.05 < p < .10$). Mean RER values during exercise were 0.88 ± 0.05 and 0.91 ± 0.04 for the C and NA trials, respectively, indicating a marginally greater utilization of fat during exercise in the C trial (61% Carbohydrate, 39% Fat vs 71% Carbohydrate, 29% Fat). For both trials, the primary source of energy was carbohydrate metabolism. Over the postexercise period, RER values declined significantly ($p < .01$), falling to 0.74 ± 0.06 and 0.79 ± 0.05 for the C and NA trial, respectively. This decline was indicative of a pronounced shift from carbohydrate to fat metabolism over the 1-h recovery period. Between treatment comparisons showed C trial RER values to be significantly lower ($p < .05$) at 15 and 45 min of recovery, indicating that the postexercise shift towards fat metabolism was generally more pronounced in the control condition.

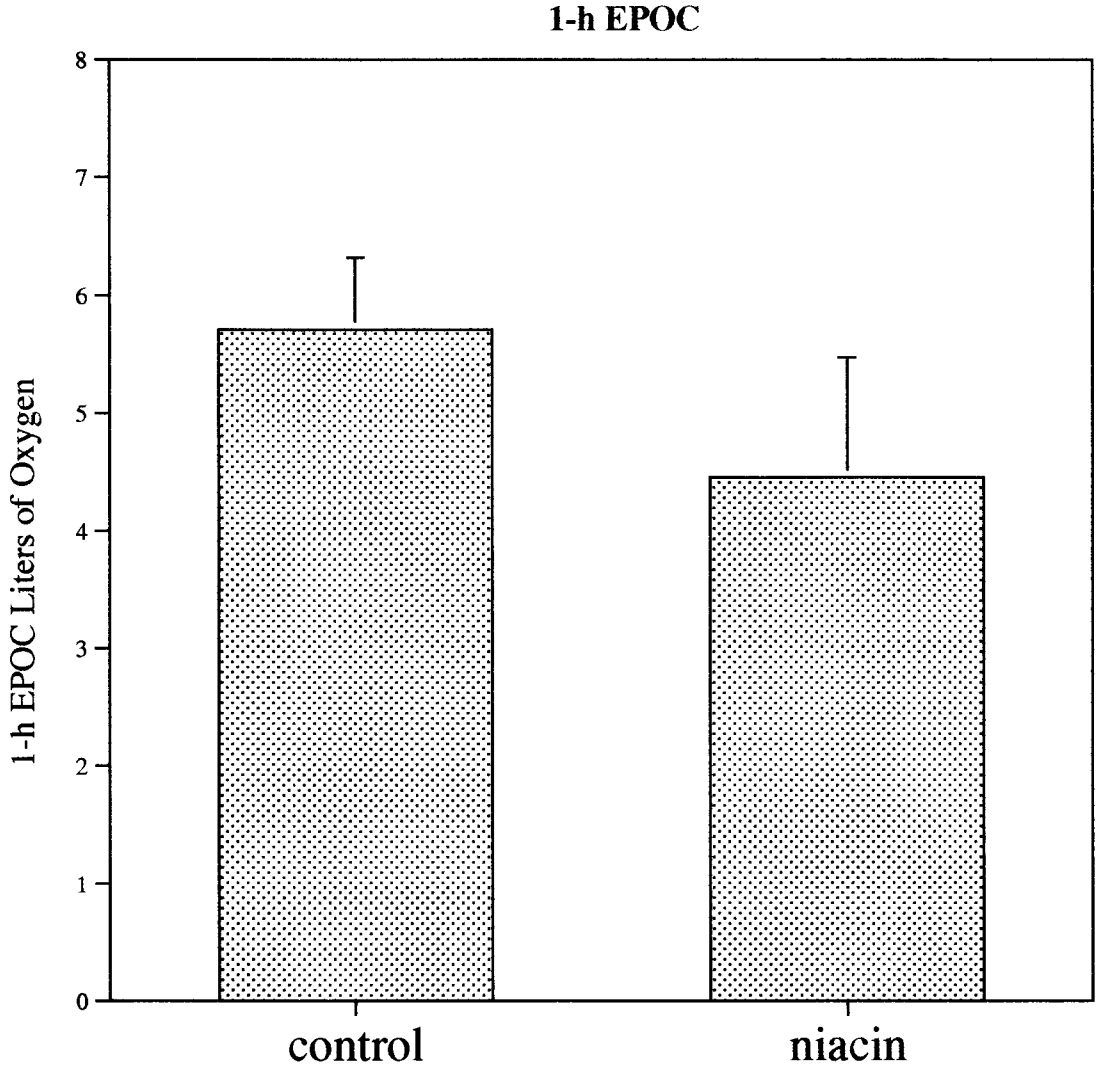


Figure 6. 1-h EPOC for C and NA trials. Values are means with SE (n=6). Difference is NS.

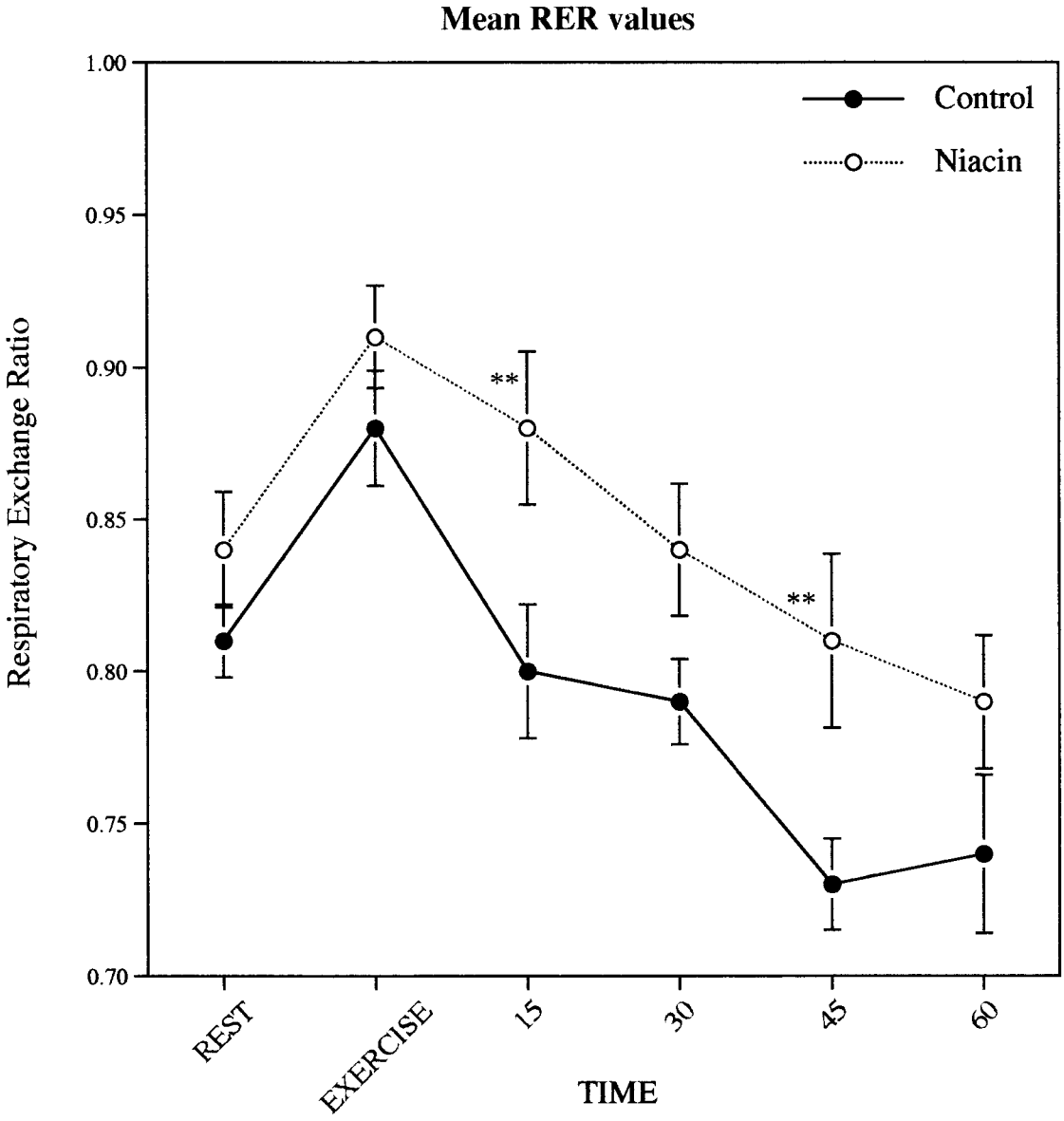


Figure 7. RER values for both treatments at all time intervals. Values are means with SE (n=6) ** Greater than C trial, $P < 0.05$

Free Fatty Acid Concentration

Serum FFA concentrations for both experimental trials are shown in Figure 8. Although higher in the C trial, preexercise FFA concentration under both experimental conditions were not significantly different ($p > .05$) serum. In the C trial, serum FFA concentration increased significantly ($p < .01$) during cycle ergometry, rising from 0.69 ± 0.30 mmol.l^{-1} at 30 min of exercise, to 1.42 ± 0.49 mmol.l^{-1} at the end of the exercise period. During recovery, C trial FFA levels remained significantly elevated ($p < .01$) above resting level, declining only marginally to 1.15 ± 0.44 mmol.l^{-1} and 1.07 ± 0.40 mmol.l^{-1} at 30 and 60 min of recovery, respectively. In contrast, ingestion of NA completely abolished the expected increase in serum FFA concentration during prolonged exercise, with FFA levels remaining at or around preexercise values over the entire experimental period. Between-treatment comparisons at each time interval during exercise and recovery showed FFA levels to be significantly higher ($p < .05$) in the C trial, indicating that NA was successful in inhibiting the mobilization of FFA from adipose tissue.

Serum Glycerol Concentration

Serum glycerol concentrations for both experimental trials are shown in Figure 9. Serum glycerol concentrations prior to exercise did not differ significantly between treatments ($p > .05$). In the C trial, serum glycerol concentration increased significantly ($p < .05$) to 0.26 ± 0.09 mmol.l^{-1} and 0.32 ± 0.09 mmol.l^{-1} at 30 and 60 min, respectively. Serum glycerol concentrations in the postexercise period fell rapidly towards resting level, declining to 0.15 ± 0.07 mmol.l^{-1} by the end of recovery. In contrast, serum glycerol concentration in the NA trial failed to rise appreciably during exercise, increasing only marginally to 0.17 ± 0.06 mmol.l^{-1} and 0.18 ± 0.10 mmol.l^{-1} at 30 and 60 min, respectively.

During recovery, serum glycerol concentration declined towards resting level, falling to 0.11 ± 0.02 mmol.l^{-1} by the end of the postexercise period. Between-treatment comparisons showed C trial glycerol levels to be significantly higher ($p < .05$) at 30 min and the completion of exercise, indicating that NA ingestion had inhibited lipolysis during the 60 min of cycle ergometry. Between-treatment differences in glycerol levels during recovery were not significantly different ($p > .05$).

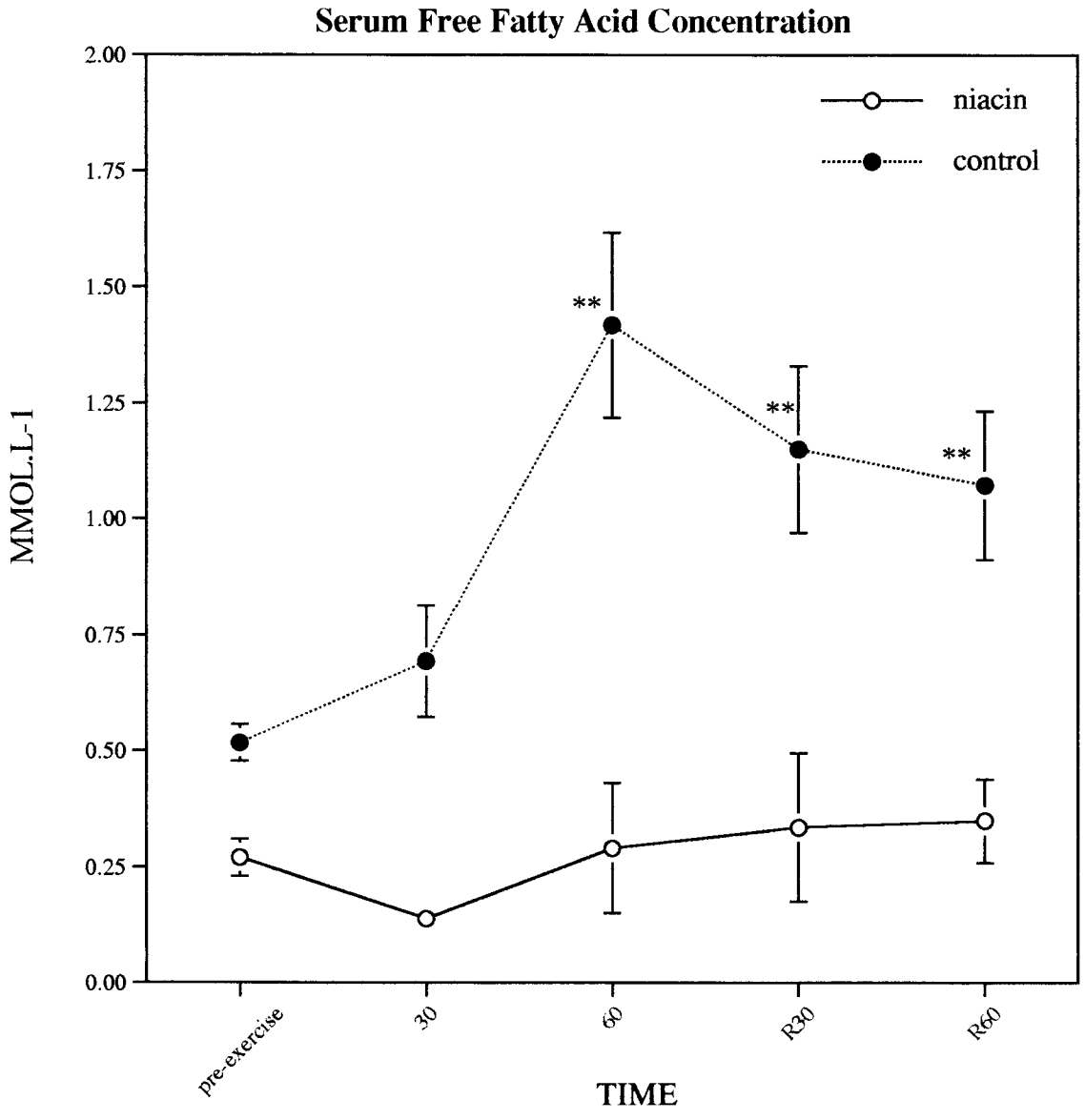


Figure 8. Serum free fatty acid concentration for both treatments at all time intervals. Values are means with SE (n=6). ** Greater than NA trial, $P < 0.05$.

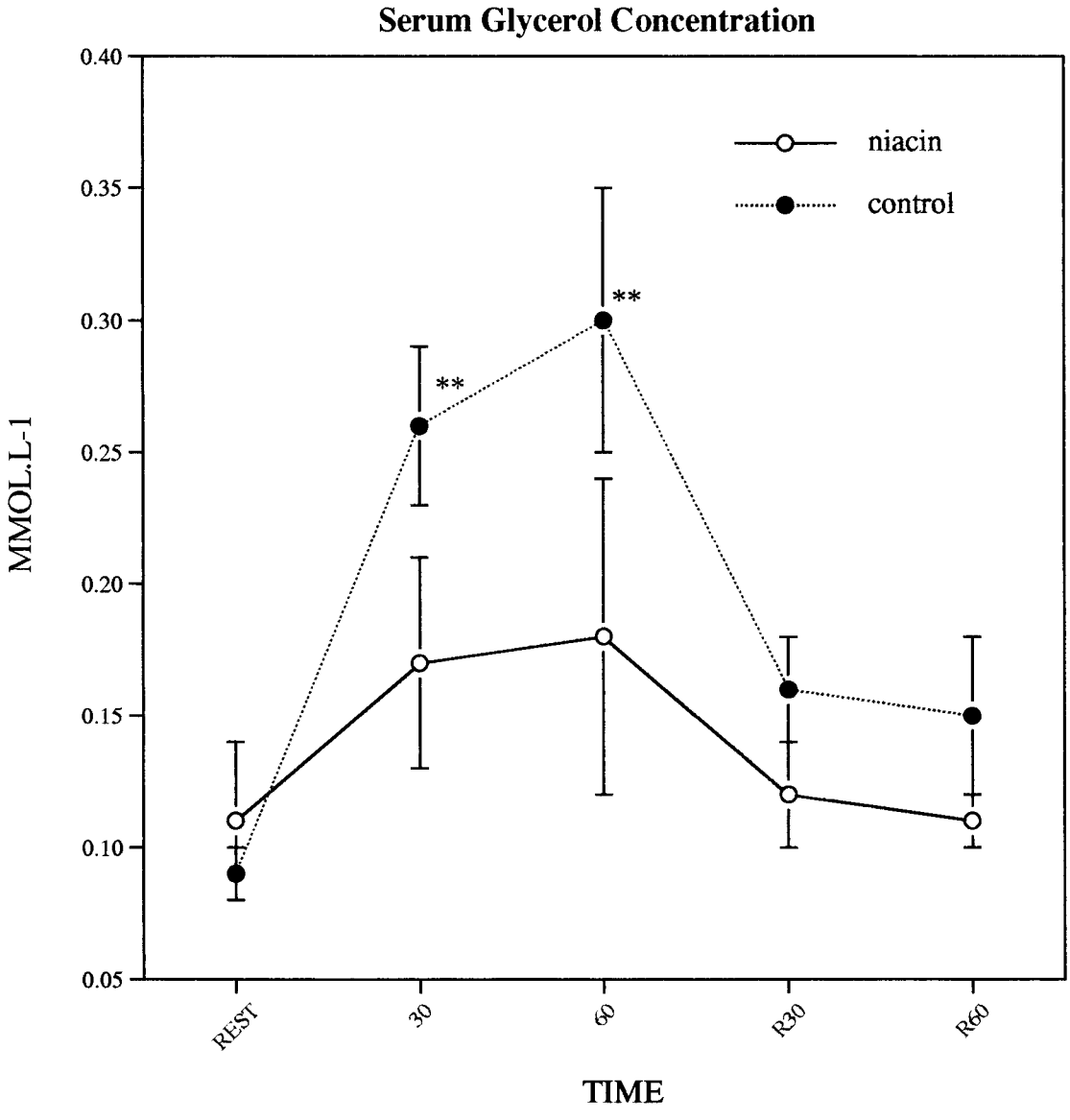


Figure 9. Serum glycerol concentration for both treatments at all time intervals. Values are means with SE (n=6). ** Greater than NA trial, $P < 0.05$

Discussion

Little is known regarding the role of fat metabolism in elevating postexercise energy expenditure. Previous investigations have reported significantly greater elevations in postexercise RMR after increasing the mobilization and utilization of FFA during exercise (Chad & Quigley, 1989, 1991). These findings are consistent with the theory that an increased rate of TG-FA cycling is responsible for a significant portion of the EPOC (Bahr et al., 1990; Newsholme, 1978; Wolfe et al., 1990). In the present study, ingestion of NA completely abolished the increase in serum FFA observed during exercise in the C trial. During recovery, C trial RER values were significantly lower at 15 and 45 min of recovery, indicating a greater utilization of FFA for energy metabolism. Yet, despite these shifts in metabolism, postexercise VO_2 was not significantly different between trials, although VO_2 tended to be marginally lower in the NA condition. Furthermore, the 1-h EPOC, a measure of the total oxygen consumed above preexercise RMR, was not significantly different between trials. These findings do not support the hypothesis that increased fat metabolism during exercise and recovery is a contributing factor for the EPOC.

The results of this study are in conflict with those of Chad and Quigley (1989), who reported postexercise VO_2 to be significantly greater after increasing the mobilization and utilization of FFA with caffeine ingestion prior to exercise. In their investigation, five untrained females completed 90 min of treadmill running at 55-74% VO_2 max. Importantly, compared to the present study, the elevations in RMR 1-h after exercise were considerably larger, averaging 104% and 54% for the caffeine and control trials, respectively. In addition, absolute FFA levels in the caffeine condition were noticeably higher, averaging 1.89 ±

0.20 mmol.l^{-1} at the end of the recovery period. Therefore, in the present study it is possible that the relatively minor elevations in postexercise VO_2 (15% C, 10% NA) may have prevented the detection of any treatment effect, and/or that the intensity and duration of exercise (65% VO_2 max, 60 min) may not have stimulated lipolysis sufficiently to have a significant effect on postexercise energy expenditure. In opposition to the later, Chad and Quigley (1991) reported RMR to be significantly elevated for three hours after just 30 min of cycle ergometry at 50 and 70% VO_2 max. Unfortunately, FFA metabolism was not directly assessed. Future studies should attempt to assess the role of fat metabolism in elevating postexercise energy expenditure over a range of exercise intensities and durations.

In the present study, NA ingestion had no significant effect on VO_2 at rest or during exercise. This finding is consistent with previous investigators who have reported no significant changes in VO_2 at rest or during exercise following NA ingestion (Bergstrom et al.,1969; Carlson et al.,1963; Wilcox, Gillis, Wegner, Harris, & Wander, 1993). Under both experimental conditions, VO_2 remained significantly elevated above RMR at the end of the 1-h recovery period. However, as previously mentioned, the magnitude of these elevations were quite small, representing an additional energy expenditure of 27.2 kcal and 21.5 kcal for the C and NA trials, respectively. This finding is consistent with previous investigators who have reported the EPOC to be small in magnitude following exercise of moderate intensity and duration ($\leq$ 70% VO_2 max, $\leq$ 60 min) (Brehm & Gutin, 1986; Freedman-Akabas, Colt, Kissileff, & Pi-Sunyer, 1985; Kaminsky, Padjen, & Laham-Saeger, 1990; Kaminsky, Kanter, Lesmes, & Laham-Saeger, 1987; Pacy, Barton, Webster, & Garrow, 1985, Sedlock, Fissinger, & Melby, 1989; Sedlock, 1991, Sedlock, 1992). In contrast, other investigators

have reported the EPOC to be substantial in magnitude (Bahr et al., 1987; Bielinski, Schutz, & Jequier, 1985; Hermansen, Grandmontagne, Maehlum, & Ignes, 1984; Maehlum, Grandmontagne, Newsholme, & Sejersted, 1986; Passmore & Johnson, 1960). However, in most cases, these studies used exercise bouts of moderately high intensity and duration ($\geq$ 70% VO_2 max, $\geq$ 60 min) and, moreover, incorporated meals into the experimental protocol, thus confounding their results with a possible interaction with diet-induced thermogenesis. In a review of the EPOC literature, Brehm (1988) concluded that an exercise intensity threshold exists at around 70% VO_2 max, under which exercise has little effect on postexercise metabolism, unless it is of substantial duration. The relatively minor EPOC observed in the present study after 60 min of cycle ergometry at approximately 65% VO_2 max lends support to this contention.

Ingestion of NA completely blocked the increase in FFA concentration normally associated with prolonged exercise of moderate intensity. This finding is consistent with previous reports documenting the antilipolytic properties of NA during exercise (Bergstrom et al, 1969; Carlson et al., 1963; Heath, Wilcox, & Quinn, 1993; Jenkins, 1965). Interestingly, postexercise FFA levels in the NA trial increased slightly but not significantly. This slight increase was primarily attributable to the results two subjects in which FFA levels increased somewhat abruptly at the end of the recovery period. Such an overshoot effect was noted by Carlson, Oro, and Ostman (1968) who reported a dramatic increase in FFA concentration 4 to 6 h after ingestion of 1 g of NA. From their data it was concluded that a plasma concentration of above 1 $\mu g.ml^{-1}$ was required to maintain low FFA levels. In the present study, subjects were given an initial dose of 500 mg at rest and four additional doses of 100 mg, three during exercise and one during recovery. It is probable that in these two subjects, the follow-up

doses were insufficient to maintain plasma levels of NA at an effective level. Future investigations should take into account the possibility of between-subject variability with respect to NA metabolism.

In contrast to the NA trial, serum FFA concentration in the C condition increased after 30 min of exercise and remained elevated above baseline over the 1-h recovery period. This finding is in agreement with other investigators who observed significant elevations in postexercise FFA levels relative to baseline values (Bahr et al., 1990; Chad & Quigley, 1989; Walker, Cooper, Elliot, Reed, Orskov, & Alberti, 1991, Wolfe et al., 1990).

Serum glycerol levels at the completion of exercise were significantly higher in the C trial, indicating that NA ingestion had significantly inhibited lipolysis during the 60 min of cycle ergometry. There were no significant differences in serum glycerol concentration in the postexercise period. Walker et al. (1991), using the NA analog acipimox to inhibit lipolysis, reported similar results, observing glycerol concentrations to be significantly different during the exercise period only. In the C trial, serum glycerol concentrations declined dramatically during recovery. Such a decline may have represented an increased uptake of glycerol by the liver, a reduction in the rate of lipolysis, or a combination of both processes.

Despite its inhibitory effect on adipose tissue lipolysis, NA ingestion had no significant effect on the RER during exercise. This indicated that FFA utilization during exercise was not significantly different between trials. Therefore, an alternative source of FFA must have contributed to energy metabolism during exercise. Given the fasted state of the subjects, the most likely alternative source of FFA available for oxidation would have been intramuscular triglyceride (TG) stores. Importantly, NA has been shown to have little effect on the lipolysis of intramuscular TG (Carlson and Hanngren, 1945). Several studies

have shown intramuscular TG to be a major fuel source during exercise. Havel et al. (1967) demonstrated that as much 50 percent of the lipid oxidized during exercise was supplied by intra-muscular TG stores. Walker et al. (1991) reported lipid oxidation to be decreased by only 50 percent after complete inhibition of lipolysis by the NA analog, acipimox. Recently, Romijn, Coyle, Sidossis, Horwitz, and Wolfe (1992) demonstrated significant utilization of intramuscular TG stores during cycle ergometry at 25 and 65% VO_2 max. Thus, the failure of NA to significantly alter lipid oxidation levels during exercise may have been a result of its inability to effectively block the mobilization and utilization of FFA from intramuscular TG stores. Also, given the fact that carbohydrate oxidation was the primary contributor to energy metabolism during exercise, it is possible that the intensity of exercise (65% VO_2 max) may have been too high to effectively examine any changes in FFA utilization. In support of this notion, Bergstrom et al. (1969) reported the RQ to be significantly higher after NA infusion at lower workloads, but not significantly different at workloads of higher intensity.

The use of the RER as an estimate of substrate utilization is not without risk, however, as changes in the acidity of the blood, hyperventilation, and changes in dissolved CO_2 levels may cause the RER to be different from the true respiratory quotient (RQ), thus invalidating its use as a measure of substrate utilization. In the present study, care was taken to select an exercise intensity that would enable the trained cyclists to maintain steady-state conditions. For each trial VO_2 and VCO_2 remained constant, with no increase in the ventilatory equivalent for O_2. Furthermore, the duration of the exercise task was such that a sustained hyperventilation was unlikely.

During recovery, RER values declined significantly in both experimental trials, indicating a shift towards fat metabolism during the postexercise period. Significant decreases in postexercise RER values have been noted by several investigators (Bahr et al., 1987; Maehlum et al., 1986). The decrease, however, was significantly greater in the C trial, indicating a greater utilization of FFA for energy metabolism relative to the NA trial. This result is consistent with Walker et al. (1991), who reported significantly higher postexercise RER values after ingestion of the NA analog, acipimox.

A limitation of the present study was the lack of statistical power due to the small sample size (n=6). According to Barcikowski & Robey (1985), a sample size of 15 was required to achieve a statistical power of 0.80 using a large effect size (Cohen, 1973), and a level of significance of 0.05. Thus, it is possible that in several instances, non-significant differences between treatments may have represented type II errors, however, in most cases the differences were small in magnitude and unlikely to be of physiological significance.

In conclusion, inhibiting lipolysis with NA had no significant effect on the magnitude of the EPOC. This result is not consistent with the theory that an exercise-induced increase in the rate of TG-FA cycling is responsible for a significant portion of the increased energy expenditure postexercise. For both trials, the magnitude of the 1-h EPOC was small and of little consequence to weight control. NA administration had a non-significant effect on exercise RER values, suggesting that during exercise, an alternative lipid source such as intramuscular TG was utilized for energy metabolism. Consequently, the EPOC may be more related to FFA utilization rather than the level of substrate availability. Clearly, more studies using greater numbers of subjects and more sophisticated measures of substrate turnover are required in order to definitively elucidate the role of FFA in elevating postexercise metabolic rate.

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APPENDICES

Appendix I

Statement of the Problem

The purpose of this study is to examine the effect of substrate utilization, manipulated by ingestion of 900 mg of nicotinic acid (NA), on excess postexercise oxygen consumption (EPOC) in trained male cyclists, 21-35 years of age, after 60 minutes of cycle ergometry at 65% of VO_2 max.

Research Hypotheses

The following research hypotheses were tested in this study.

1. Resting baseline VO_2 in the NA trial and the control trial will not be significantly different.

2. VO_2 at 60 minutes of exercise in the control and the NA trial will not be significantly different.

3. VO_2 at 15, 30, 45, and 60 minutes after exercise will be significantly greater in the control trial compared to the NA trial.

4. RER values at 60 minutes of exercise and 15, 30, 45, and 60 minutes of recovery will be significantly lower in the control trial compared to the NA trial.

5. Serum glycerol concentrations at 30 and 60 minutes of exercise and 30 and 60 minutes of recovery will be significantly greater in the control trial compared to the NA trial.

6. Serum fatty acid concentrations at 30 and 60 minutes of exercise and 30 and 60 minutes of recovery will be significantly greater in the control trial compared to the NA trial.

7. EPOC magnitude will be significantly greater in the control trial compared to the NA trial.

Statistical Hypotheses

The following statistical hypotheses were tested in this study.

1. Resting baseline VO_2, where 1 = control trial and 2 = NA trial:

$$H_0: \mu_1 \neq \mu_2 \qquad H_A: \mu_1 = \mu_2$$

2. Exercise VO_2, where 1 = control trial and 2 = NA trial:

$$H_0: \mu_1 \neq \mu_2 \qquad H_A: \mu_1 = \mu_2$$

3. Postexercise VO_2 at 15, 30, 45, and 60 minutes, where 1 = control trial and 2 = NA trial:

15 minutes: $H_0: \mu_1 \leq \mu_2 \qquad H_A: \mu_1 > \mu_2$

30 minutes: $H_0: \mu_1 \leq \mu_2 \qquad H_A: \mu_1 > \mu_2$

45 minutes: $H_0: \mu_1 \leq \mu_2 \qquad H_A: \mu_1 > \mu_2$

60 minutes: $H_0: \mu_1 \leq \mu_2 \qquad H_A: \mu_1 > \mu_2$

4. RER values at 60 minutes exercise and at 15, 30, 45 and 60 minutes of recovery, where 1 = control trial and 2 = NA trial:

exercise (60 minutes): $H_0: \mu_1 \geq \mu_2 \qquad H_A: \mu_1 < \mu_2$

recovery (15 minutes): $H_0: \mu_1 \geq \mu_2 \qquad H_A: \mu_1 < \mu_2$

recovery (30 minutes): $H_0: \mu_1 \geq \mu_2 \qquad H_A: \mu_1 < \mu_2$

recovery (45 minutes): $H_0: \mu_1 \geq \mu_2 \qquad H_A: \mu_1 < \mu_2$

recovery (60 minutes): $H_0: \mu_1 \geq \mu_2 \qquad H_A: \mu_1 < \mu_2$

5. Serum glycerol concentrations at rest, during exercise at 30 and 60 minutes, and 30 and 60 minutes of recovery, where 1 = control trial and 2 = NA trial:

rest : $H_0: \mu_1 \leq \mu_2$ $H_A: \mu_1 > \mu_2$

exercise (30 minutes): $H_0: \mu_1 \leq \mu_2$ $H_A: \mu_1 > \mu_2$

exercise (60 minutes): $H_0: \mu_1 \leq \mu_2$ $H_A: \mu_1 > \mu_2$

recovery (30 minutes): $H_0: \mu_1 \leq \mu_2$ $H_A: \mu_1 > \mu_2$

recovery (60 minutes): $H_0: \mu_1 \leq \mu_2$ $H_A: \mu_1 > \mu_2$

6. Serum free fatty acid concentrations during rest, during exercise at 30 and 60 minutes, and 30 and 60 minutes of recovery, where 1 = control trial and 2 = NA trial:

rest : $H_0: \mu_1 \leq \mu_2$ $H_A: \mu_1 > \mu_2$

exercise (30 minutes): $H_0: \mu_1 \leq \mu_2$ $H_A: \mu_1 > \mu_2$

exercise (60 minutes): $H_0: \mu_1 \leq \mu_2$ $H_A: \mu_1 > \mu_2$

recovery (30 minutes): $H_0: \mu_1 \leq \mu_2$ $H_A: \mu_1 > \mu_2$

recovery (60 minutes): $H_0: \mu_1 \leq \mu_2$ $H_A: \mu_1 > \mu_2$

7. EPOC magnitude (1-h EPOC), where 1= control trial and 2 = NA trial

$H_0: \mu_1 \leq \mu_2$ $H_A: \mu_1 > \mu_2$

Operational Definitions

Substrate Utilization: For the purpose of this study, substrate utilization will be determined by indirect calorimetry using the nonprotein respiratory exchange ratio in which R equals 0.70 and 1.00 for fatty acids and

carbohydrates, respectively. Percentage fatty acid = (1 - R)/ 0.3 X 100% and percentage carbohydrate = (R - 0.7)/ 0.3 X 100%. Plasma FFA and glycerol concentrations will be used for estimations of fatty acid flux.

Trained Cyclists: Those individuals who cycle a minimum of four times per week for a duration of one hour or more and can maintain a workload corresponding to 65% VO_2 max for one hour in an aerobic steady state.

EPOC Magnitude: The EPOC magnitude will be calculated as the time integral of the difference between postexercise VO_2 and resting baseline VO_2.

Aerobic Steady State: For the purpose of this study, an aerobic steady state is defined as a condition in which VO_2 and ventilation (V_E) are kept constant throughout the exercise period, and respiratory exchange ratios are less than 1.0.

Assumptions

In this study the following assumptions were made.

1. Subjects will maintain their normal training and dietary regimens throughout the duration of the study.
2. Subjects will refrain from hard physical activity 24 hours prior to each experimental trial.
3. Subjects will perform each experimental trial in a 12 hour fasted state.

Delimitations

The following were the delimitations of this study.

1. In order to maintain a high level of homogeneity with respect to fitness level and body composition, the study will be delimited to well-trained male subjects 18 to 40 years of age. Female subjects will not be used in this study in light of reported differences in fat utilization during the follicular phase of the menstrual

cycle (Tarnopolsky, MacDougall, Atkinson, Tarnopolsky and Sutton, 1990), and significant variations in basal metabolic rate with phases of the menstrual cycle (Soloman, Kurzer and Calloway, 1982).

2. The experimental trials in this study will be delimited to 60 minutes of cycle ergometry at 65% of maximal oxygen consumption. This duration and intensity was selected to ensure that a significant EPOC occurred. Cycle ergometry was chosen for ease of blood sample collection.

3. The study will be delimited to trained cyclists able to maintain an aerobic steady state at 65% of maximal oxygen consumption for a duration of 60 minutes.

4. Due to the length of the experimental protocol and the schedules of the subjects, the examination of postexercise metabolism will be delimited to 60 minutes.

Limitations

The following are the limitations of the study.

1. The findings of this study were limited by use of indirect measures such as respiratory exchange ratios and serum free fatty acid and glycerol concentrations rather than isotopic and non-isotopic infusion techniques to determine substrate utilization and FFA flux.

2. Nicotinic acid inhibits FFA mobilization from adipose tissue, but necessarily from intramuscular triglyceride stores.

3. The findings of this study were limited by a small sample size, and hence a loss statistical power.

Definitions

The following are general definitions of some of the dependent variables in this study.

Free Fatty Acids (FFA): The acid portion of triglycerides (fat) that is oxidized by the process of beta oxidation to provide energy.

Glycerol: The alcohol portion of triglycerides which is converted to 3-phosphoglyceraldehyde which is one of the substances in the glycolytic pathway.

Respiratory Exchange Ratio (RER): The quantity of carbon dioxide produced in relation to oxygen consumed. Varies according to the substrate metabolized. RER = CO_2 produced/O_2 consumed.

Appendix II

Review of Literature

The following chapter is a review of the literature pertaining to excess postexercise oxygen consumption (EPOC). Part I will focus on the early and recent studies documenting the magnitude and duration of the EPOC. Following this, the effect of exercise intensity, duration, mode, and the thermogenic effect of food on the EPOC will be examined. Part II will focus on the physiological and biochemical mechanisms responsible for the EPOC. Here, the classical debt theory will be discussed and evaluated, along with the contemporary metabolic explanations of increased postexercise oxygen consumption. Finally in Part III, studies identifying the antilipolytic effect of nicotinic acid during rest and exercise will be discussed followed by chapter summary.

Part I

Excess postexercise oxygen consumption - early studies

Elevated resting metabolism after exercise was first documented by Benedict and Cathcart (1913). Using only one subject, they reported resting oxygen consumption (VO_2) during sleep after very severe and severe work to be increased by 25 and 8%, respectively. It should be noted, however, that after the very severe work the subject slept for only half of the measurement period and this could have accounted for the large increase in metabolic rate. The authors concluded that the increase in resting VO_2 was a result of a stimulated metabolism.

Several German studies conducted during the late 1920's supported the notion of increased energy expenditure after exercise. Herxheimer, Wissing, and

Wolff (1926) investigated basal metabolic rate (BMR) in nine students training for the marathon. At the end of a training day, their BMR was elevated 14.7 ± 8.6%, while after a rest day, BMR was elevated 5.0 ± 6.6%. Radtke (1927) observed an average increase in VO_2 of 15% in nine athletes 24 hours after completion of an 800 meter race. Schenk and Stahler (1929) used three experienced athletes as subjects for five experimental trials in which two subjects ran 200 meters, two ran 800 meters, and one ran 3000 meters. VO_2 returned to resting values in 30-60 minutes. A seven-month training study on one of the three subjects revealed a modest increase in morning resting VO_2, although this result could have been explained by normal within-subject variability in relation to metabolic measurements.

Hill, Long, and Lupton (1924) reported a seven percent rise in basal metabolism after severe exercise, however, they concluded that the rise was not part of the recovery process, but merely an effect of the general circulatory and metabolic disturbance produced by exercise. Margaria, Edwards, and Dill (1933) observed a prolonged elevation in resting VO_2 after work. In similar fashion, they interpreted the observation as a general metabolic disturbance unrelated to the recovery process. Edwards, Thorndike, and Hill (1935) reported an increase in VO_2 of 25% for 15 hours after two hours of American football. On the basis of their results the authors concluded that 50% of the energy requirement of football participation was accounted for after the game.

In summary, these studies were consistent in observing prolonged elevations in resting metabolic rate after exercise. However, a number of key elements should be considered when evaluating the validity of these studies. First, there is considerable variation in the types of activities examined, and in most cases, exercise duration and intensity are either poorly reported or omitted

altogether. Consequently, direct comparisons between studies are impossible. Second, the determination of resting VO_2 is usually taken from a single preexercise measurement. Additionally, activity levels prior to measurement of resting values are not reported. Third, since there is usually no report of food intake, one must assume that the thermogenic effect of food is not controlled for. Fourth, resting conditions are not clearly defined so that variations in the activity level after exercise may be responsible for the increases in energy expenditure (Bahr & Maehlum, 1986).

Excess postexercise oxygen consumption - recent studies

The results of recent studies examining the magnitude and duration of the EPOC are equivocal. While some studies have reported prolonged elevations in the metabolic rate after exercise, others have found the EPOC to be small and physiologically insignificant. Studies reporting a prolonged EPOC will be discussed first.

Passmore and Johnson (1960) measured VO_2 in 10 men after a 10 mile walk on a treadmill at four mph. Postexercise VO_2 did not return to baseline values for at least seven hours after completion of exercise, although no control study was performed, and no attempt was made to quantify the magnitude of EPOC observed. DeVries and Gray (1963) examined postexercise resting metabolism after a mixed exercise bout consisting of cycle ergometry, bench stepping, and running/walking for a total of 45 minutes. They reported an increase in resting VO_2 for six to eight hours relative to control experiments in which no exercise was performed. The investigators estimated the mean total increase in metabolism in the postexercise state to be 53 kcal. Although the results were taken as

evidence in support of the use of exercise as a means of weight control, the variation with respect to relative exercise intensity and the small sample size (N=3) limited the overall usefulness of the study.

Hermansen, Grandmontagne, Maehlum, and Ignes (1984) examined postexercise VO_2 in one subject following exercise for 80 minutes at 75% of VO_2 max. VO_2 was measured hourly in the 12 hours that followed exercise and again at 24 hours postexercise. Using the same subject, the protocol was repeated several weeks later without exercise. VO_2 during the 12 hours after exercise was 19.3% higher than that observed during the corresponding 12 hours in the control experiment. There was no significant difference in postexercise VO_2 24 hours after exercise. Rectal temperatures, heart rate, and ventilation were also increased above control values 12 hours postexercise. It was concluded that metabolic rate was increased after prolonged severe work, and that this prolonged elevation in metabolic rate may effectively double the energy cost of exercise. As in all single subject studies, such findings should be viewed with caution.

Bielinski, Schutz, and Jequier (1985) explored the effect of exercise on resting energy expenditure in 10 healthy males living in a respiratory chamber. The investigators used a two-day protocol in which the subjects rested on day one, and exercised for three hours at 50% of VO_2 max on day two. Food intake was identical on both days, with the exercise session being performed immediately after lunch on the second day. Postabsorptive resting metabolic rate was elevated above control values for nine hours after cessation of exercise, but was not significantly elevated during the sleeping period (9-17 hours postexercise). Importantly, resting metabolic rate the next morning was significantly higher (4.7%) than that measured after the non-exercise day.

Furthermore, in the exercise condition, the fuel mix after the meal, as determined by indirect calorimetry, was characterized by a greater contribution by fat oxidation to total energy expenditure. It was concluded that intense exercise stimulates both energy expenditure and fat metabolism for a prolonged period.

Maehlum, Grandmontagne, Newsholme, and Sejersted (1986) investigated postexercise VO_2 in eight healthy males. The subjects performed exercise for approximately 80 minutes at 70% of their maximum VO_2 and then rested in bed for 24 hours. VO_2 was measured continuously during the first hour of recovery, hourly for the next 11 hours, and finally 24 hours after exercise. Comparing the results to an identical control experiment during which no exercise was performed, mean VO_2 12 hours after exercise was 211 ± 13 ml/min compared to 185 ± 13 ml/min. VO_2 24 hours postexercise was significantly higher than that recorded 24 hours after the start of the control experiment. Additionally, the first meal taken after the cessation of exercise increased VO_2 markedly in comparison to the increase observed following the same meal in the control experiment. The fact that EPOC was observed for at least 12 hours, coupled with the finding that exercise significantly enhanced the thermogenic effect of food, led the authors to conclude that exercise may be important in weight control by not only increasing caloric expenditure during exercise, but also through its stimulatory effect on basal metabolism.

In contrast to the above, numerous studies have failed to observe a significant prolonged elevation in resting metabolic rate after exercise. Freedman-Akabas, Colt, Kissileff, and Pi-Sunyer (1985) measured postexercise VO_2 in 23 subjects (males and females) classified as having low, medium, and high fitness levels. The subjects walked on a treadmill for 20 minutes at approximately the anaerobic threshold. Resting metabolic measures were compared to an identical

non-exercise control experiment. Resting values 40 minutes to three hours after exercise were not significantly different to those observed in the non-exercise control experiment. Importantly, the results were consistent across all three fitness categories. In an additional experiment, seven subjects exercised for either a longer duration or at a greater intensity. Again, there was no significant difference in VO_2 from 40 to 220 minutes after exercise between the exercise and non-exercise experiment. It was concluded that the caloric expenditure beyond that generated by the exercise period itself was negligible, and that elevated energy expenditure after exercise should not be considered an important contributor to weight loss.

Pacy, Barton, Webster, and Garrow (1985) examined the thermogenic effect of moderate aerobic exercise in the fasted and fed state in four non-obese subjects. Exercise was performed at a constant rate on a cycle ergometer (33-55% of VO_2 max) during the first 20 minutes of four consecutive hours. The first two exercise periods were performed in a fasted state while the last two followed an 800 kcal mixed meal. Analysis of variance revealed a significant but similar (13.6%) elevation in mean VO_2 following exercise in either the fasted or fed state. There was no interaction, either additive or synergistic, between exercise and diet-induced thermogenesis. Sixty minutes after cessation of exercise, postexercise VO_2 was not significantly different from preexercise level.

Brehm and Gutin (1986) examined the effects of intensity, mode of exercise, and aerobic fitness on postexercise energy expenditure. Eight runners (4 males, 4 females) completed 3.2 km of walking and running at rates of 3.2 and 6.4 km/h (walking) and 8.1 and 11.3 km/h (running), respectively. Additionally, eight sedentary subjects completed the 6.4 km/h trial. The EPOC durations for the four trials were 18.75 ± 5.87, 42.25 ± 7.28, 31.13 ± 6.35, and 48.00 ± 6.93

minutes, respectively. This corresponded to mean caloric expenditures of 4.52 ± 0.97, 9.19 ± 1.88, 9.99 ± 1.17, and 15.33 ± 1.94 kcal, respectively. In support of Freedman-Akabas et al. (1985), there were no significant differences in postexercise VO_2 between the runners and the sedentary group. The finding that recovery energy expenditure amounted to only 3-17 kcal prompted the authors to describe the notion of prolonged elevated postexercise energy expenditure as "misleading" to those exercising at low to moderate intensities (18-68% of peak VO_2).

Sedlock, Fissinger, and Melby (1989), and Sedlock (1991, 1992), reported an absence of a prolonged EPOC following (a) cycle ergometry at high intensity-short duration (75% VO_2 max, 20 minutes), low intensity-short duration (50% VO_2 max, 30 minutes), and low intensity-long duration (50% VO_2 max, 60 minutes), (b) arm cranking and cycle ergometry for 20 minutes at 60% of mode-specific peak VO_2, and (c) cycle ergometry and treadmill running for 30 minutes at 60-65% of mode-specific peak VO_2.

Kaminsky, Kanter, Lesmes, and Laham-Saeger (1987) failed to observe a prolonged EPOC after either walking at 35% peak VO_2 for 60 minutes, or running at 70% peak VO_2 for 30 minutes. Interestingly, there were no significant differences in recovery VO_2, which remained significantly elevated for 15 minutes and 10 minutes for the walking and running group, respectively. In another study, Kaminsky, Padjen, and Laham-Saeger (1990) investigated the effect of split exercise sessions on EPOC. Six female subjects completed one 50 minute run and two 25 minute runs at 70% of peak VO_2. After all three trials, postexercise VO_2 returned to baseline within 30 minutes. The combined EPOC of the two 25

minute runs was significantly greater than the single 50 minute run, however, the overall magnitude of the EPOC was small, averaging 1.3% and 2.8% of exercise VO_2 for the continuous and split exercise sessions, respectively.

From the above discussion it is clear that there is considerable controversy concerning the magnitude and duration of the EPOC. The situation is complicated by methodological differences with respect to the mode, intensity and duration of exercise, as well as food intake during the recovery period. The next section will examine the effect of exercise mode, intensity, and duration on EPOC. In addition, studies investigating the possible interaction of diet-induced thermogenesis and exercise-induced thermogenesis will be examined.

Exercise intensity, duration, mode, and food intake and excess postexercise oxygen consumption.

Early Studies. During the 1960's and 70's, investigations into EPOC, or O_2 debt as it was still referred to, centered on lactate kinetics and its relationship to the magnitude and duration of post-exercise VO_2. As a result, a number of meaningful observations were made concerning the magnitude of the O_2 debt and the intensity and duration of exercise.

Knuttgen (1972) investigated O_2 debt after exercise at intensities ranging from 48% to 98% of VO_2 max. After 15 minutes of work, the O_2 debt increased only marginally throughout the lower work ranges (45-65%), but increased exponentially at higher work loads. At 60% of VO_2 max, increasing the duration of exercise to 30 and 55 minutes, respectively, resulted in a greater O_2 debt, principally in the slow phase. In a later study, Knuttgen & Saltin (1972) investigated the O_2 debt in young males following four minutes of cycle ergometry at 15, 30, 45, 60, 75, and 95% of VO_2 max. Interestingly, the magnitude

of the O_2 debt appeared to increase exponentially at 60% of VO_2 max. The magnitude of the fast component of O_2 debt was found to be proportional to exercise intensity and was always completed within a six-minute period. The "repayment" of the slow period was also found to be proportional to exercise intensity, particularly at workloads greater than 60% of VO_2 max, and was completed within a 45 minute period in all cases.

Schneider, Robinson, and Newton (1968) examined recovery VO_2 following treadmill exercise at 30 and 50% of VO_2 max for periods of 3, 8, 14, and 25 minutes, respectively. In contrast with Knuttgen, O_2 debts did not vary with the duration of work performed. In all instances, VO_2 declined rapidly, reaching baseline asymptote within a 10 minute period.

Exercise duration and excess postexercise oxygen consumption. A number of studies have investigated the effect of exercise duration on the magnitude of EPOC. Bahr, Ingnes, Vaage, Sejersted, and Newsholme (1987) measured the 12-h EPOC in males following cycle ergometry at 70% VO_2 max for durations of 80, 40, and 20 minutes. The magnitude of 12-h EPOC was found to be proportional to exercise duration. On average, 12-h EPOC equaled 15.2 ± 2.0% of total exercise oxygen consumption. It should be noted, however, that food was taken at 2, 7, and 12 hours postexercise. Thus, the calculation of EPOC was made with reference to a control trial in which food was taken, but no exercise was performed.

Chad and Wenger (1988) measured the EPOC in males and females (N=5) following cycle ergometry at 70% VO_2 max for durations of 30, 45, and 60 minutes. EPOC increased 2.35- and 5.3-fold when exercise duration was increased from 30 to 45 minutes and from 30 to 60 minutes, respectively. The time for VO_2 to return to resting baseline was 128 ± 4.4 minutes, 204 ± 15.9

minutes, and 455 ± 30.0 minutes for 30, 45, and 60 minutes exercise bouts, respectively. No food was eaten during the recovery period.

In contrast, Kaminsky, Padjen, and Laham-Saeger (1990) reported no significant differences in the magnitude of EPOC in females following treadmill running at 70% of VO_2 max for durations of 25 and 50 minutes, respectively. No food was taken in during the recovery period.

Exercise intensity and excess postexercise oxygen consumption. A number of studies have examined the independent effect of exercise intensity on EPOC. Bahr and Sejersted (1991) tested the hypothesis that exercise intensity must exceed a certain level in order to trigger an extended EPOC. Six healthy males cycled for 80 minutes at 29, 50 and 75% of VO_2 max. Compared to non-exercise control values, recovery remained significantly elevated for 0.3 ± 0.1 hours (29%), 3.3 ± 0.7 hours (50%), and 10.5 ± 1.6 hours (75%). Regression analysis revealed an exponential relationship between exercise intensity and total EPOC, with a intensity threshold at approximately 50% of VO_2 max. It was concluded that prolonged exercise at intensities above 40 to 50% of VO_2 max are required to elicit a prolonged EPOC component of approximately two hours.

The effect of exercise intensity on the EPOC was further examined by Bahr, Gronnerod, and Sejersted (1992) who measured the time course and magnitude of EPOC after supramaximal exercise. Six male subjects performed three intermittent two-minute (3x2) exercise bouts on a cycle ergometer at 108% of VO_2 max with a three minute recovery period. On separate days subjects performed a two-by-two minute trial (2x2) and a one-by-two minute trial (1x2). Following the 3x2 trial, 2x2 trial, and 1x2 trial, recovery VO_2 remained significantly elevated for four hours, 50 minutes, and 30 minutes, respectively. The study demonstrated the existence of a prolonged EPOC after exhaustive,

supramaximal exercise of extremely short duration. However, the extra energy expenditure after the 3x2 trial amounted to only 80 kcal and was considered insignificant in relation to overall energy balance.

There is some evidence to suggest that low-intensity exercise performed for extremely long durations results in significantly elevated postexercise VO_2. Bielinski, Schutz, and Jequier (1985) found postexercise energy expenditure to be significantly elevated for 4.5 hours following three hours of treadmill exercise at 50% VO_2 max. Wolfe, Klien, Carraro and Weber (1990) found recovery VO_2 to be significantly elevated two hours postexercise following four hours of treadmill walking at 40% of VO_2 max.

Chad and Quigley (1991) compared three-hour EPOC measurements following 30 minutes of cycling at 50 and 70% of VO_2 max in trained and untrained females. In conflict with other studies, exercise at 50% of VO_2 max produced a greater three hour EPOC than exercise at 70% VO_2 max in both trained and untrained females. The authors suggested that enhanced fat metabolism during the lower intensity trial may have been responsible for the larger EPOC.

Exercise intensity and duration on excess postexercise oxygen consumption. Energy expenditure during exercise is a function of both intensity and duration. Therefore, studies in which exercise intensity and duration are manipulated as independent variables are more powerful in determining the effect of total work output or caloric expenditure during exercise on the magnitude and duration of EPOC.

Hadberg, Mullin, and Nagle (1980) exercised 18 men at 50, 65, and 80% of VO_2 max for durations of five and 20 minutes. The slow component of recovery VO_2 was not significantly altered by exercise intensity or duration at 50 and 65%

of VO_2 max. However, after 20 minutes of exercise at 80% of VO_2 max, the slow component of recovery VO_2 was five times larger than that recorded for the five minute trial at 80% VO_2 max. It was concluded that the slow component of postexercise VO_2 was independent of work load and duration unless exercise is of a high intensity (> 65% VO2 max) and of a duration longer than 5 minutes

Chad and Wenger (1985) concluded that exercise duration was more important than intensity in determining the size of EPOC. After total work at two different intensities was equated, the EPOC after 38 minutes at 50% VO_2 max was significantly larger than 30 minutes at 70% VO_2 max. In contrast, Kaminsky et al. (1987) found no significant difference in the EPOC following walking at 35% peak VO_2 for 60 minutes and running at 70% peak VO_2 for 30 minutes. Importantly, the group failed to observe a prolonged EPOC with postexercise VO_2 returning to resting baseline with 15 minutes.

Sedlock, Fissinger, and Melby (1989) also failed to observe a prolonged EPOC following cycle ergometry at high intensity-short duration (HS) (75% VO_2 max, 20 minutes), low intensity-short duration (LS) (50% VO_2 max, 30 minutes), and low intensity-long duration (LL) (50% VO_2 max, 60 minutes). The durations of the EPOC were similar for both the HS trial (33 ± 10 minutes) and the LL (28 ± 14 minutes). Both, however, were significantly longer than the EPOC observed in the LS trial (20 ± 5 minutes). Despite having a similar duration to LL trial, total net caloric expenditure was significantly greater after the HS trial (29 ± 9 kcal) than either the LS trial(14 ± 6 kcal) or the LL trial (14 ± 7 kcal). It was concluded that exercise intensity affects both the magnitude and length of the EPOC, whereas exercise duration affects only the length of the EPOC.

In a well-designed experiment, Gore and Withers (1990) investigated the critical threshold hypothesis with respect to both exercise intensity and duration. EPOC values for nine male subjects were examined in a three-by-three repeated measures design. Each subject walked or ran on a treadmill for durations of 20, 50, and 80 minutes at intensities of 30, 50 and 70% VO_2 max. Post hoc analysis of the significant intensity-by-duration interaction revealed no significant differences between the EPOCs for the 20, 50 and 80 minute trials at 30% VO_2 max. However, above this intensity, the EPOC increased significantly with duration. The increase in EPOC after 80 minutes of running at 50% VO_2 max compared with 20 minutes at 50% VO_2 max was much smaller than the increase observed when duration was increased from 20 to 80 minutes at 70% VO_2 max. Importantly, intensity was identified as the major determinant of EPOC, with the intensity effect accounting for 45.5% of the systematic variance of the EPOC, while the duration effect and the interaction between intensity and duration accounted for only 8.9% and 7.7%, respectively. The mean EPOC was calculated to be 4.8% of net total oxygen consumption. Consequently, it was concluded that elevated postexercise metabolism in itself was of little physiological value, unless exercise was undertaken on a regular basis.

Exercise mode and excess postexercise oxygen consumption. There is a paucity of information regarding the effect of different exercise modes on EPOC. Sedlock (1991) compared the magnitude and duration of the EPOC following upper body (arm cranking) and lower body (cycle ergometry) exercise. Eight untrained subjects exercised for 20 minutes at 60% of mode-specific peak VO_2. The results indicated that the EPOC response following moderate intensity upper body exercise was similar to that of lower body exercise when performed at equivalent metabolic rates. It was concluded that postexercise energy

expenditure following moderate intensity exercise may be related to the relative metabolic rate of the active musculature, as opposed to the absolute rate of exercise energy expenditure or the quantity of active muscle mass involved in the mode of exercise.

In a further experiment, Sedlock (1992) found no significant difference in the magnitude and duration of the EPOC following 30 minutes of cycle ergometry and treadmill running at 60-65% of mode-specific peak VO_2. Again, it was concluded that moderate intensity exercise at an equivalent metabolic rate elicits a similar postexercise response, and that the use of different modes of exercise is not a contributing factor to the discrepancy in the literature regarding the magnitude and duration of the EPOC.

Food intake and excess postexercise oxygen consumption. In determining the possible contribution of food intake on the magnitude and duration of the EPOC, one must consider if the thermogenic effect of food is enhanced after exercise. Numerous studies have demonstrated that exercising after a meal significantly enhances the thermogenic effect of food (Bray, Whipp, & Koyal, 1974; McDonald, Wickler, Horwitz, & Stern, 1988; Miller, Munford, & Stock, 1967; Segal & Gutin, 1983; Segal, Presta, & Gutin, 1984; Zahanska-Markiewicz, 1980). Few, however, have examined the extent of diet-induced thermogenesis after an exercise bout.

Bahr et al. (1987) noted that 42% of the EPOC occurred after the first meal (two to seven hours post exercise) and that 22% occurred after the second meal (more than seven hours postexercise). Maehlum et al. (1986) observed that the food given two hours postexercise significantly increased VO_2 compared to the control study when food was given without exercise.

Davis, Sadri, Sargent, and Ward (1989) examined the thermogenic effects of pre- and postprandial exercise in seven lean, active females. The subjects

completed four experimental treatments: Exercise Only (25 minutes treadmill running at 60% VO_2 max), Meal Only (910 kcal mixed meal), Exercise-Meal, and Meal-Exercise. All treatments resulted in significantly elevated energy expenditures over the three hour post treatment period. Importantly, there was no significant difference in postexercise energy expenditure between the Meal Only (1.30 ± 0.03 kcal/min) and the Exercise-Meal (1.3 ± 0.03 kcal/min) treatments. Energy expenditure after the Meal-Exercise treatment was significantly greater than both Meal Only and Exercise-Meal treatments. The Exercise Only treatment showed the lowest thermogenic response. It was concluded that exercise following a meal would be more beneficial than exercise before a meal in increasing and maintaining an elevated energy expenditure.

Davis, Sargent, Brayboy, and Bartoli (1992) repeated the experiment with six obese females with an additional resting control trial. Energy expenditure after the Meal Only, Exercise-Meal, and Meal-Exercise was significantly higher than that recorded in the Rest Control and Exercise Only treatments. Again, the Meal-Exercise treatment elicited the greatest energy expenditure. Although not tested statistically, postexercise energy expenditure following the Exercise-Meal treatment (1.22 ± 0.01 kcal/min) was greater than that observed for the Meal Only trial (1.13 ± 0.01 kcal/min). Given the fact that no significant elevation in energy expenditure was observed during the Exercise Only trial, such a result may be viewed as a potentiation of diet-induced thermogenesis through exercise performed before a meal. However, the fact that this difference was observed in obese subjects and not in lean subjects makes interpretation of this result difficult.

Young, Treadway, and Ruderman (1985) examined the effect of prior exercise on diet-induced thermogenesis. Seven subjects (male and female) exercised 45 minutes at 70% of VO_2 max. Two hours after exercise, the subjects ingested a 100 gram oral glucose load. Results were compared to an identical trial

during which no exercise was performed. In the control condition, ingestion of the glucose load increased VO_2 by 9.1% over resting baseline. In contrast, the glucose load after exercise increased VO_2 by 20.5% over resting baseline. The results indicated that the thermic effect of food is potentiated by prior exercise.

Several investigators have proposed that the discrepancy in the literature regarding the magnitude and duration of the EPOC may be attributable to the potential interaction of postexercise VO_2 and diet-induced thermogenesis (Kaminsky, Padjen, & LaHam-Saeger, 1990; Sedlock, Fissenger, & Melby, 1989). Interestingly, of the studies reporting prolonged EPOCs of greater than 10 hours (Bahr et al., 1987; Bahr & Sejersted, 1991; Bielinski, Schutz, & Jequier, 1985; Hermansen et al., 1984; Maehlum et al., 1986), all incorporated food intake into the experimental design, comparing postexercise VO_2 to corresponding measures in a control experiment during which food was eaten but no exercise was performed.

More studies are needed to fully evaluate the effect of food ingestion on postexercise metabolism. Clearly, one needs to perform a study in which postexercise VO_2 following high intensity work is compared under both fasting and fed conditions. Additionally, these treatments should be tested over a wide range of exercise intensities and durations. In this way, the possible exercise by food interaction can be fully evaluated.

Part II

Explanation of excess postexercise oxygen consumption

In this section the proposed underlying mechanisms responsible for the EPOC will be examined. The classical O_2 debt theory will be discussed first, followed by an evaluation of this theory. Next, the metabolic basis of elevated

energy expenditure will be discussed. Here the roles of temperature, calcium ions, catecholamines, fatty acids and substrate cycling in elevating postexercise metabolism will be examined.

The classical oxygen debt theory.

The classical O_2 debt theory of Hill and Lupton (1923) proposed that elevated VO_2 after exercise was necessary for the repayment of an O_2 deficit which was incurred during exercise. This elevation in VO_2 could not be attributed to the resaturation of hemoglobin and myoglobin stores, as the volume of O_2 associated with these processes represented only a small fraction of the debt. Rather, the increased VO_2 after exercise represented the oxidation of a portion (20%) of the lactate formed during exercise which provided the necessary energy for the reconversion of the remaining portion (80%) of lactate to glycogen. The initial rapid phase was attributed to the oxidative removal of lactic acid in the muscle where it was formed. The second and prolonged phase was attributed to the oxidative removal of the lactic acid which had escaped by diffusion from muscle into the bloodstream.

The O_2 debt theory was based on a number of earlier studies on stimulated amphibian muscle. In 1907, Fletcher and Hopkins found small quantities of lactate in freshly exercised frog muscle. When the muscle was placed in an anaerobic environment there was a consistent increase in the concentration of lactate until the tissue was unable to contract. Conversely, in the presence of oxygen, the lactate disappeared. The investigators concluded that the production of lactate was a necessary stimulus for muscle contraction and that oxygen was necessary for recovery.

In 1910, A.V. Hill published the first of a series of papers on the production of heat during muscle contraction. Using the amphibian model, Hill reported the the quantity of heat produced during contraction to be the same in the presence or absence of oxygen. The result was taken as confirmation that the chemical processes underlying muscle contraction did not require oxygen. In 1913, Hill reported evidence of important chemical activity requiring oxygen in the period of recovery following muscular contraction. It was noted that in the presence of oxygen, muscles failed to cool as rapidly as would be expected by "simple physical processes." According to Hill, this was due to the liberation of "recovery heat" which, in the presence of oxygen, was greater than or equal to the heat liberated during the contraction itself. In the absence of oxygen, no recovery heat was recorded.

Based upon his own findings, and those of Fletcher and Hopkins (1907), Hill concluded that the energy for contraction was derived from the breakdown of some precursor to lactate and that this process was accompanied by the production of heat and did not require oxygen. Furthermore, the recovery period immediately after contraction was associated with the oxidation of lactate, a process accompanied by the production of heat, but dependent on the presence of oxygen (Harris, 1968).

In 1920, Meyerhof identified the precursor of lactic acid as glycogen. During recovery, when lactate disappeared, glycogen was found to reappear in a corresponding amount less a quantity which had been lost by oxidation. Meyerhof observed that the oxygen absorbed during recovery was equivalent to only one third of the lactate which disappeared and the heat given off during recovery was also approximately equal to the heat of combustion of one third of the lactate disappearing. It was concluded that during recovery, one third of the

lactate was oxidized, thus providing the energy for the resynthesis of the remaining lactate into glycogen (Gaesser & Brooks, 1984; Harris, 1968).

In their own experiments, Hill (1910, 1913, 1914) and Hartree and Hill (1922) also noted that only part of the lactate was oxidized during recovery, the rest being resynthesized into its precursor. They reported that the amount of heat liberated during recovery could account for only one sixth to one fifth of the lactate removed during recovery. Thus, while the results were quantitatively different to those of Meyerhof, the conclusion was essentially the same: That the majority of lactate formed during contraction was resynthesized back to glycogen (Gaesser & Brooks, 1984).

The original O_2 debt hypothesis of Hill and Lupton (1923) was modified by Margaria, Edwards, and Dill (1933), who distinguished the fast and slow components of the O_2 debt as the "alactacid debt" and the "lactacid debt", respectively. Using running protocols of three to ten minutes duration, Margaria, Edwards, and Dill (1933) observed that the decline in blood lactate did not occur until after the fast phase of postexercise VO_2 was completed. With knowledge of the recent discovery of phosphagens and their role in muscle contraction, Margaria and his colleagues hypothesized that the rapid decline in VO_2 after exercise, the so called "lactacid debt", was not related to the oxidation of lactic acid, but rather, represented the replenishment of creatine phosphate stores. Thus, the slow phase or "lactacid debt" was attributed to the oxidation of lactic acid as described by Hill and Lupton (1923). Consequently, after mild exercise, when lactate did not accumulate in muscle and blood, the O_2 debt was solely comprised of the "alactacid portion". With more severe forms of exercise, during which lactate accumulated in muscle and blood, the O_2 debt was comprised of both the "alactacid" and "lactacid" portions.

Evaluation of the classical oxygen debt theory.

The classical O_2 debt theory, was based on the assumption that (a) a temporal relationship existed between the decline in blood lactate concentration after exercise and the slow component of postexercise VO_2, and (b) the slow component of postexercise VO_2 represented the oxidative removal of a portion of the lactate formed during exercise to supply energy for reconverting the remaining portion to glycogen. Experimentally, however, it appears that the kinetics of postexercise VO_2 and lactate disappearance are not tightly coupled. Furthermore, investigations into lactate metabolism and glycogen synthesis after exercise have generally failed to identify lactic acid as a significant glyconeogenic precursor.

Lactate removal and postexercise VO_2. A number of studies on a variety of species has provided substantial evidence of a dissociation between postexercise VO_2 and changes in blood and muscle lactate concentration. Abramson, Eggleton, and Eggleton (1927) evaluated the relationship between lactic acid metabolism and postexercise VO_2 by infusing sodium lactate into anesthetized dogs. Although the infusions produced blood lactate levels as high as those observed during heavy exercise, the mean increase in VO_2 of approximately 23% was not significantly different from when sodium bicarbonate alone was infused. Moreover, the lactate infusion resulted in no significant increase in muscle or liver glycogen synthesis.

Alpert and Root (1954) reported no consistent relationship between the amount of lactate infused or utilized and excess postexercise VO_2 in dogs.

Reductions in cardiac output through tamponade or inhalation of hypoxic gas mixtures resulted in O_2 debts completely unrelated to the magnitude of the induced O_2 deficit.

Kayne and Alpert (1964) measured VO_2 and plasma lactate and pyruvate concentrations in normal, eviscerated, eviscerated-hepatechomized, and eviscerated-hepatechomized-insulin treated dogs before and after five minutes of mild exercise. Elevated VO_2 after exercise was not significantly different in all four groups despite the fact that lactate and pyruvate uptake was severely impaired in the operated animals. The finding of a complete dissociation between postexercise VO_2 and metabolic removal of lactate prompted the authors to describe the relationship as "coincident not causal" (Kayne & Alpert, 1964, p. 372).

Studies involving lower vertebrates have also yielded little evidence in support of the classical O_2 debt hypothesis. Bennett and Licht (1973), in their study of the amphibian (*Batrachoseps attenuatus*), observed postexercise VO_2 to return to preexercise levels before any net lactate was catabolized. Gleeson (1980), studying the lizard (*Amblyrhynchus Cristatus*), reported blood lactate concentrations to remain elevated for several hours after VO_2 had returned to resting baseline levels.

Studies involving human subjects in which blood lactate concentration was manipulated have also shown a dissociation between lactate removal and postexercise VO_2. Segal and Brooks (1979) exercised 11 male subjects on a bicycle ergometer at moderate (55-60% VO_2 max) and heavy (90-95% VO_2 max) workloads in both normal and glycogen-depleted states. For both moderate and heavy exercise, there was no significant difference in postexercise VO_2 between

the normal glycogen and the glycogen depleted state, despite the fact that blood lactate concentration was significantly higher in the normal glycogen state. At the end of the recovery period following heavy exercise in either glycogen state, blood lactate concentration remained significantly elevated even though VO_2 had fallen to or below initial resting values by this time. The result was clearly inconsistent with the traditional lactic acid explanation of increased postexercise VO_2.

Roth, Stanley, and Brooks (1988) examined the relationship between blood lactate and the EPOC in nine male subjects by observing the effects of induced lactacidemia on postexercise VO_2. The subjects performed two 12 minute experimental trials, each performed at 40% VO_2 max. During one trial, leg blood flow was occluded with surgical thigh cuffs placed below the buttocks and inflated to 200 mm Hg. Occlusion spanned minutes six through eight of the 12 minute exercise trial, and elicited a mean blood lactate concentration 380% greater than that measured in the control trial. The induced lactacidemia and the subsequent fall in lactate concentration during the postexercise period had no significant effect on recovery VO_2. Neither gross VO_2 or net VO_2 (amount of O_2 consumed above the asymptotic recovery baseline) were significantly different between the control and occluded conditions. It was concluded that the time course and magnitude of oxidative recovery after exercise was not governed by blood lactate concentration.

Lactate as a glyconeogenic substrate. Attempts to establish lactic acid as a major glyconeogenic substrate has yielded mixed results. While the results of some studies clearly support the notion that a large portion of the lactate formed during exercise is converted to glycogen within recovering muscle, others have found the amount of lactate converted to glycogen to be small. Furthermore, if

lactic acid is indeed a major glyconeogenic substrate during recovery, skeletal muscle should possess the necessary biochemical machinery for the synthesis of glycogen from lactate. However, experimental evidence, particularly with respect to humans, is severely lacking.

Early attempts to identify the necessary glyconeogenic machinery in skeletal muscle were unsuccessful. Krebs and Woodford (1965) investigated the occurrence of the enzymes fructose-1,6-bisphosphatase (FBP), pyruvate carboxylase, and phosphoenolpyruvate carboxykinase with reference to the question of whether lactate can be converted into glycogen in muscle. Although the presence of FBP was detected in the skeletal muscle of a variety of species, including man, the virtual absence of the enzymes pyruvate carboxylase and phosphoenolpyruvate carboxykinase, led the authors to conclude that lactate and pyruvate could not be converted into glycogen in skeletal muscle.

Moorthy and Gould (1969) examined glycogen synthesis from glucose and lactate in the isolated rat soleus. The results showed that lactate stimulated the formation of glycogen in the presence of glucose, but was itself ineffective as a precursor for glycogen synthesis. This was evidenced by the fact that glycogen was synthesized from glucose approximately 10 times faster than [^{14} C] lactate was incorporated into the muscle. Pearce and Connett (1980) also reported an absence of any significant glycogen synthesis from lactate in isolated rat soleus muscle.

The metabolic fate of lactate after exercise may in fact depend upon the biochemical profiles of the muscle fibers involved. Opie and Newsholme (1967) examined the activities of FBP, phosphofructokinase (PFK), and phosphoenolpyruvate carboxykinase in both white and red rat muscle.

Results indicated that the activity of FBP, the enzyme necessary for the synthesis of glycogen from lactate, was very low in red (slow-twitch) muscle compared to that observed in white (fast-twitch) muscle.

McLane and Holloszy (1979) examined glycogen synthesis from lactate in three types of skeletal muscle. When rat hind limb was perfused with a medium containing 12 mmol lactate as the only substrate, a rapid increase in glycogen was observed in the fast-twitch red and fast-twitch white muscle types. In contrast, glyconeogenesis in slow-twitch red muscle was physiologically insignificant. When the hind limb was perfused with [^{14}C] lactate, the label was rapidly incorporated into glycogen in the plantaris muscle (94% fast-twitch fibers) while there was little incorporation of the label into glycogen in the soleus muscle (predominantly slow-twitch fibers). Determinations of FBP activity provided an explanation for the insignificant conversion of lactate to glycogen in slow-twitch red muscle, in that this muscle type exhibited an extremely low level of FBP activity. The results supported the notion that (a) fast-twitch fibers possess the necessary biochemical machinery to convert lactate to glycogen, and (b) FBP activity is the rate limiting step in the conversion of lactate to glycogen in skeletal muscle.

Experimental evidence regarding glyconeogenic enzyme activities in human skeletal muscle is scant, to say the least. Hintz, Lowry, Kaiser, McKee, and Lowry (1980) reported the slow- and fast-twitch muscle fibers of the biceps brachi muscle in one male subject to have considerable FBP activity. Despite the lack of information regarding glyconeogenic enzyme activities in human skeletal muscle, several investigators have reported significant glycogen synthesis from lactate in skeletal muscle following exercise.

Hermansen and Vaage (1977) examined lactate disappearance and glycogen synthesis in 24 men following intermittent maximal exercise. Based on determinations of muscle biopsies, leg blood flow, muscle water content, arteriovenous differences for lactate, glucose, and alanine, it was calculated that 90% of the lactate formed during intense exercise remained within the muscle, and that 75% of this amount was converted to glycogen. Importantly, lactate disappearance and glycogen synthesis were observed to coincide in both time and magnitude, with the average rates of lactate disappearance and glycogen synthesis, corrected for changes in water content during recovery, being 0.74 mmol min^{-1} kg^{-1} wet wt and 0.51 mmol glucosyl U min^{-1} kg^{-1} wet wt, respectively. The small efflux of lactate from muscle into blood (less than 10%), the marked increase in intramuscular glycogen synthesis, and the low uptake of glucose from circulation, was taken as evidence in support of the traditional O_2 debt concept that a significant portion of the lactate formed during exercise was converted into glycogen during recovery.

Astrand, Hultman, Juhlin-Dannfelt, and Reynolds (1986) examined lactate disappearance after exhaustive arm and leg ergometry in seven healthy males. Based on estimates of anaerobic energy production, mean lactate production was calculated to be 830 mmol. Of this amount, splanchic uptake was calculated to be 80 mmol, and assuming that only lactate was metabolized during the recovery period, the maximum amount that could be oxidized was 330 mmol. At the end of the resting period, approximately 60 mmol was accounted for in body water, leaving the remaining 360 mmol of lactate in the muscle where, according to the authors, it was synthesized into glycogen. It was concluded that approximately 50% of the lactate formed during heavy exercise was converted to muscle glycogen during recovery and that lactate uptake by the liver could account for only 10% of postexercise lactate metabolism.

The conclusions of Hermansen and Vaage (1977) and Astrand et al. (1986) have been challenged on several fronts. With respect to Hermansen and Vaage (1977), determinations of splanchic uptake of lactate were not made, thus ignoring a potentially large contribution to postexercise lactate metabolism. Furthermore, it is likely that the use of plethysmography to determine blood flow resulted in serious underestimation. Hence, calculations of lactate release and glucose uptake may have been artificially low and the role of lactate in muscle glyconeogenesis may have been overestimated. Gaesser and Brooks (1984) have highlighted the fact that different subject groups were used for obtaining muscle biopsies, arteriovenous substrate concentrations, and estimates of blood flow. Consequently, in their opinion, Hermansen and Vaage (1977) failed to adequately control for intersubject variability associated with muscle/blood lactate gradients at given blood lactate concentrations.

With respect to Astrand et al. (1986), lactate release from the quadriceps muscle was based on whole-body estimates of lactate clearance from the circulation. This is problematic in that the dilutional space for lactate has not been well-defined and the turnover rate for lactate has been shown to be high (Brooks, 1985). Consequently, the rate of lactate clearance could have been underestimated, resulting in overestimations of intramuscular glyconeogenesis.

A number of investigators have failed to identify lactate as a significant glyconeogenic substrate. Brooks, Brauner, and Cassens (1973), using a rat model, directly tested the classical O_2 debt assumption that the elevation in postexercise VO_2 was attributable to the reconversion of lactate to glycogen. Blood, muscle, and liver lactate concentrations, muscle and liver glycogen content, and blood glucose levels were monitored 24 and 36 hours after exhaustive exercise. Exhaustive exercise resulted in severe depletion of liver and muscle glycogen

stores. Lactate and blood glucose concentrations were found to return to non-exercised levels within 15 to 30 minutes. After 24 hours without food, liver glycogen levels in the exercised rats were not significantly different from immediate postexercise values, indicating an absence of glycogen synthesis. Compared to the 24-hour group, liver glycogen 36 hours after exhaustive exercise was slightly greater, however, when compared to the non-exercise 12-hour controls, the extent of liver glycogen synthesis appeared negligible. Moreover, there was no evidence of muscle glycogen synthesis during the postexercise examination period. The failure to observe significant glycogen synthesis in either muscle or liver 24 and 36 hours after exhaustive exercise, coupled with the finding that blood lactate concentrations had returned to resting levels within 15 minutes of recovery, led to the conclusion that lactate was not a significant substrate in the replenishment of glycogen stores after exhaustive exercise.

Noting reports of rapid glycogen supercompensation in rat cardiac muscle, Gaesser and Brooks (1980) conducted a further investigation into postexercise lactate metabolism. On this occasion, the postexercise examination period was reduced to four hours, and in order to assess possible differences with respect to aerobic and anaerobic metabolism, both continuous (CE) and intermittent (IE) exhaustive exercise protocols were employed. Both exercise protocols resulted in substantial glycogen depletion in all tissues. In the immediate postexercise period, lactate concentrations in the blood, skeletal muscle, and kidney declined rapidly, reaching levels equal to or less than preexercise values within 15 minutes. At the end of the four hour recovery period, blood glucose was restored to 83.9% and 76.9% of preexercise levels following CE and IE, respectively. During this time, cardiac muscle was the only tissue capable of complete glycogen restoration. Muscle glycogen concentration increased 51.1% and 51.6% of

preexercise levels following CE and IE, respectively, while liver and kidney showed no indications of significant glycogen repletion. Similar to the earlier study, results indicated a marked difference in the kinetics of lactate removal and glycogen synthesis after exercise. Assuming a 100% conversion to glycogen, the authors calculated that lactate removal could only account for approximately 40% of all glycogen synthesis in the first 15 minutes of recovery. The results supported the concept that lactate removal after exercise contributed only minimally to the repletion of glycogen.

Bangsbo, Gollnick, Graham, and Saltin (1990) examined intramuscular glyconeogenesis in human subjects following exhaustive supine leg ergometry. Importantly, calculations of lactate clearance were restricted to the quadricep muscles, employing a direct Fick calculation to determine lactate release. The group reported that approximately two thirds of the lactate accumulated in muscle during intense exercise was released into the blood. Based on estimates of carbohydrate oxidation in the first 10 minutes and last 50 minutes of recovery, it was calculated that between 13 and 27% of the lactate formed could have been converted to glycogen. In opposition to Hermansen and Vaage (1977) and Astrand et al. (1986), it was concluded that intramuscular glycogen synthesis from lactate was not a dominant metabolic pathway during recovery from exercise.

Isotopic tracer studies. Several studies using isotopically labeled lactate have demonstrated the major fate of lactate during and after exercise to be oxidation. Brooks, Brauner, and Cassens (1973) infused exercise-exhausted and pair fasted control rats with 1-^{14}C labeled lactate. Within two hours of infusion, 75% of the isotope was collected as $^{14}CO_2$ in the exercised animals, indicating that the primary fate of lactate after exercise was oxidation.

Brooks and Gaesser (1980) measured postexercise VO_2 while quantitatively assessing the metabolic endpoints of 1-^{14}C-lactate and 1-^{14}C-glucose injected into rats at exhaustion. After exercise, VO_2 declined rapidly for the first 15 minutes, thereafter, declining slowly and remaining elevated above resting levels for 120 minutes. Importantly, this slow phase did not coincide with lactate removal, which occurred within 15 minutes of recovery. Two dimensional radiochromatograms produced from blood, kidney, liver, and cardiac and skeletal muscle indicated a rapid and unexpected incorporation of the ^{14}C label into several amino acid pools. Analysis of all major glycogen containing tissues indicated that less than 20% of the lactate present at the end of exercise was reconverted to glycogen during the first four hours of recovery. In the same period, 55 to 70% of lactate could be accounted for as $^{14}CO_2$ and $H^{14}CO_3^-$ indicating that the primary postexercise metabolic pathway for lactate was oxidation.

Depocas, Minaire, and Chatonnet (1969) measured the rates of formation and oxidation of plasma lactic acid in dogs either resting quietly or running at six km/h on the level. Dogs were infused with uniformly labeled [^{14}C] lactate. In running dogs, 74% of the lactate turnover was converted to $^{14}CO_2$, with approximately 12% of the respiratory carbon being derived from lactate acid. Issekutzz, Shaw, and Issekutzz (1976) examined the effect of treadmill running on the rates of oxidation of [^{14}C] lactate and the conversion of [^{14}C] lactate to glucose in dogs. At rest, approximately 50% of the lactate was oxidized to $^{14}CO_2$ and 18-19% was used to form glucose. During exercise, the corresponding values

were 55-56% oxidized, with about 25% converted to glucose. It was concluded that working muscle produces and utilizes lactate simultaneously and that the major factor controlling both oxidation and gluconeogenesis is substrate supply.

Jorfeldt (1970) injected [U-^{14}C] lactate into the brachial artery of human subjects during forearm exercise and demonstrated that working muscle was capable of taking up and oxidizing lactate. Hubbard (1973) reported that during 30 minutes of exercise, 35-68% of the administered [U-^{14}C] lactate was recovered in the expired gas as $^{14}CO_2$, whereas at rest, only 3-7% was recovered in the same period. Mazzeo, Brooks, Schoeller, and Budinger (1986) examined the disposal of blood [1-^{13}C] lactate in six male subjects during rest, easy exercise (cycling 140 minutes at 50% VO_2 max), and hard exercise (cycling 65 minutes at 75% VO_2 max). The lactate oxidation rate was linearly related to VO_2 ($r = 0.81$), while a curvilinear relationship was found between lactate oxidation and blood lactate concentration. Importantly, the percentage of lactate turnover oxidized increased from 49.3% at rest to 87% during exercise. It was concluded that in humans, (a) lactate turnover is directly related to the metabolic rate, (b) oxidation is the major fate of lactate removed during exercise, and (c) blood lactate concentration is not an accurate indicator of lactate disposal and oxidation.

Collectively, these studies provide support for the notion that most of the lactate removal during exercise and rest is by way of oxidation, and that after exercise, lactate contributes minimally to the repletion of glycogen stores.

Summary. Many studies have investigated the metabolic fate of lactate during exercise recovery and the temporal relationship between lactate removal and postexercise VO_2. The experimental evidence strongly suggests that the primary fate of lactate following exercise is oxidation and that intramuscular glycogen synthesis from lactate is not a dominant metabolic pathway during

recovery from exercise. Furthermore, the kinetics of postexercise VO_2 and lactate disappearance are not tightly coupled. Consequently, the classical O_2 debt theory of Hill and Lupton (1923) cannot be supported as a valid explanation of the physiological events occurring after exercise.

Metabolic basis of the excess postexercise oxygen consumption

Presently, there exists no complete or universally accepted explanation of increased postexercise metabolism. Contemporary explanations of the EPOC have focused on the biochemical and physiological factors that directly or indirectly influence mitochondrial O_2 consumption. In this section, the contributing roles of temperature (Q_{10} effect), catecholamines, calcium ions, fatty acids, and substrate cycling in elevating postexercise VO_2 will be examined.

Temperature. According to Gaesser and Brooks (1984), elevated core temperature is perhaps the most important factor contributing to elevated postexercise VO_2. Brooks, Hittelman, Faulkner, and Beyer (1971a) monitored rat muscle and tissue temperatures after moderate and exhaustive exercise. During exercise, rectal and muscle temperatures increased 5.1°C and 8.1°C, respectively. After exercise, muscle temperature fell exponentially but did not return to control values in one hour. Rectal temperature fell rapidly for the first 20 minutes, thereafter declining very slowly, remaining 1.6°C above the control value at the end of the one hour postexercise period. Postexercise VO_2 immediately after exercise was high, but declined rapidly during the first 20 minutes of the recovery period, reaching asymptote at a level significantly above resting. From examination of the temporal relationship between rectal and muscle temperatures and postexercise VO_2, it was concluded that in the first 20 minutes of recovery, both elevated muscle and internal temperatures contribute significantly to

elevated postexercise VO_2. During the second or slow phase of recovery, elevated postexercise VO_2 was associated primarily with elevated internal temperatures.

In another experiment, Brooks, Hittelman, Faulkner, and Beyer (1971b) investigated the relationship between temperature, respiration, and the VO_2 of isolated rat muscle at temperatures between 25°C and 45°C. Increasing temperature had a striking effect on mitochondrial functions. Within the physiological range of 37-45°C, the rate of state four respiration (the lowest possible level of resting metabolism in skeletal muscle) increased over 200%, with mitochondrial phosphorylative coupling efficiency (ADP:O_2 ratio) decreasing 18%. The results supported the notion that elevated temperature (Q_{10} effect) must at least be partially responsible for elevated postexercise VO_2.

In human subjects, Hadberg, Mullin, and Nagle (1980) calculated that the Q_{10} effect of temperature on metabolism could account for 60-70% of the slow component of recovery VO_2 after exercise eliciting 50-80% of VO_2 max. Claremont, Nagle, Reddan, and Brooks (1975) compared metabolic, temperature, heart rate and ventilatory responses to exercise at 0°C and 35°C. Despite an elevated VO_2 response in the cold condition, the higher body temperatures recorded during the heat condition were associated with a significantly higher postexercise VO_2.

In contrast, several studies have demonstrated the contribution of elevated body temperature to elevated postexercise VO_2 to be minor. Maehlum et al. (1986), Bahr et al. (1987), and Bahr and Sejersted (1991) observed rectal temperatures to decline rapidly to control values in less than one hour, despite the fact that in all cases, VO_2 was significantly elevated for more than 10 hours after

exercise. Gore and Withers (1990), using the van't Hoff-Arrhenius equation, computed the excess VO_2 caused by temperature alone. The results indicated that only 2-24% and 11-36% of the eight hour EPOC could be attributed to the rectal and body temperature increases, respectively.

Catecholamines. There is considerable evidence to suggest that a significant portion of the EPOC is attributable to the effect of elevated catecholamine concentrations on metabolism. Chapler, Stainsby, and Gladden (1980) examined the effect on changes in blood flow, norepinephrine, and pH on VO_2 in the dog gastrochnemius-plantaris muscle group. Norepinephrine infusion caused an average increase of about 30% in resting muscle VO_2 which was sustained over the 15 minute sampling period. In support of this finding, Gladden, Stainsby, and Macintosh (1982) reported that norepinephrine infusion increased skeletal muscle postexercise VO_2 in dogs by 40%. In humans, Bahr et al. (1992) reported a significant correlation ($r = 0.70$, $p < 0.005$) between the mean increase in plasma norepinephrine concentration over the first hour of exercise and the magnitude of the one hour EPOC.

Bernard and Foss (1969) used the beta-adrenergic blocking agent, propranolol, to investigate the involvement of catecholamines in elevating postexercise VO_2. Dogs were run on a treadmill at four mph with a 20% grade for 19 minutes. In the control condition, the mean "O_2 debt" was 23.66 ± 2.78 L. When propranolol was administered, the "O_2 debt" was reduced to 14 ± 1.62 L, which represented a mean reduction of 35.4%. It was concluded that catecholamines, acting through beta-adrenergic receptors, have an effect on the magnitude of the "O_2 debt". In similar fashion, Cain (1971) examined postexercise VO_2 in eight dogs with and without propranolol administration.

Beta-block reduced exercise VO_2 by approximately 20% and reduced the "O_2 debt" to approximately 50% of that observed without beta block.

Although experimental evidence in humans is lacking, these studies support the notion that at least some portion of elevated postexercise VO_2 is attributable to the stimulatory effects of circulating catecholamines.

Calcium ions. According to Gaesser and Brooks (1984), calcium ions may influence postexercise VO_2 by stimulating mitochondrial respiration. In this process, mitochondria sequester calcium ions, which results in O_2 consumption but not ATP production. Additionally, increased calcium ion concentration within the mitochondria results in impaired oxidative phosphorylation, a process which would result in increased VO_2. Thus, calcium uptake by cardiac and skeletal muscle mitochondria during exercise may result in elevated mitochondrial respiration after exercise.

Fatty acids and substrate cycling. Fatty acids may play a significant role in the elevation of postexercise VO_2. Bielinski, Schutz, and Jequier (1985) observed significantly lower respiratory quotients after exercise compared with control conditions, indicating a greater mobilization and utilization of fat during recovery. Similarly, Maehlum et al. (1986) and Bahr et al. (1987) reported significantly lower respiratory exchange ratios during recovery from exercise compared to those obtained during resting control experiments. Bangsbo et al. (1990) reported intramuscular triglyceride to be a major source of fat oxidation during recovery from exhaustive leg exercise.

Chad and Quigley (1991) reported postexercise VO_2 to be greater in trained and untrained females following cycle ergometry at 50% VO_2 max compared to exercise at 70% VO_2 max. The significantly lower respiratory exchange ratios observed during recovery after the 50% trial in both trained and

untrained groups suggested that increased fat mobilization and utilization during physical activity may be involved in the long-term elevation of postexercise metabolism.

Recently, several investigations have shown the rate of the triglyceride-fatty acid substrate cycle (TG-FA) to be increased after prolonged exercise at moderate intensity, and that the energy cost of this increase may account for a significant portion of the EPOC. In this substrate cycle, the fatty acids released during the process of lipolysis are not oxidized, but are reesterified back into triglyceride. According to Newsholme (1978), substrate cycles form part of a logical series of biochemical mechanisms that exist to increase the sensitivity of non-equilibrium reactions to changes in concentration of metabolic regulators such as hormones. At least one of the reactions must involve the hydrolysis of ATP. Therefore, the result is the liberation of heat and consumption of energy, with no net conversion of substrate to product.

Bahr, Hansson, and Sejersted (1990) measured the rate of TG-FA cycling after exercise and assessed its relative contribution to the EPOC. After two hours of exercise at 51% of VO_2 max, TG-FA cycling was found to increase from 414 ± 90 μmol FA/min (control) to 1473 ± 435 μmol FA/min (three hours postexercise). When converted to energy cost, the increased rate of cycling accounted for as much as 50% of the prolonged component of EPOC.

Wolfe, Klein, Carraro, and Webber (1990), using an isotopic infusion technique and indirect calorimetry, assessed the importance and the energy cost of total TG-FA cycling in coordinating the availability of fatty acids with energy requirements during exercise and recovery. At rest, approximately 70% of all fatty acids released during lipolysis were reesterified. During the first 30 minutes of exercise, reesterification dropped to 25%, whereas total fatty acid release from triglyceride hydrolysis tripled. This coordinated response allowed a six-fold

increase in free fatty acid (FFA) availability for oxidation. Immediately at the cessation of exercise, almost 90% of fatty acids released from lipolysis were reesterified. This dramatic increase in the percentage of fatty acids reesterified was responsible for the rapid fall in FFA concentration after exercise. According to the authors, had the percentage of FFA reesterified in the recovery period stayed the same value during exercise (25-30%), plasma FFA concentration would have risen to a level beyond the binding capacity of albumin. The rapid changes in the percentage of FFA that were reesterified at the beginning of both exercise and recovery, demonstrated the importance of the TG-FA substrate cycle in amplifying the ability of stored triglyceride to respond rapidly to major changes in energy requirements caused by starting, maintaining, and stopping exercise.

At rest and during exercise, the energy cost of TG-FA cycling required less than two percent and 0.5% of total energy expenditure, respectively. In contrast, during recovery from exercise, the high rate of cycling accounted for a considerable percentage (14%) of the increase in energy expenditure above the resting value before exercise. Importantly, the increased rate of TG-FA cycling persisted for two hours after exercise, prompting the authors to conclude that TG-FA cycling was important not only in the control of substrate flux, but also in elevation of energy expenditure after exercise.

Few investigators have directly examined the effect of fat mobilization and utilization on the magnitude and duration of the EPOC. By manipulating postexercise substrate utilization with factors known to influence fat metabolism, the role of fatty acids in elevating VO_2 after exercise can be further evaluated.

Chad and Quigley (1989) studied the effect of substrate utilization on postexercise VO_2 in five untrained women. When fatty acid mobilization and oxidation was stimulated by caffeine ingestion prior to exercise, postexercise VO_2

over the one hour recovery period was significantly higher in the caffeine trial than in the non-caffeine trial. Although the finding provided support for the hypothesis that increased fat metabolism may account for a significant portion of the EPOC, it is possible that the greater postexercise VO_2 may have been a function of the stimulatory effect of caffeine on metabolic rate. This was evidenced by the significantly higher resting and exercise VO_2 values recorded in the caffeine trial compared to the control trial.

Other chemical agents are known to have an effect on fatty acid metabolism. The antilipolytic effect of nicotinic acid on adipose tissue has been well-documented by a number of investigators (Carlson & Oro, 1962; Carlson, 1963; Carlson, Havel, Ekeland, & Holmgren, 1963; Carlson, Oro, & Ostman, 1968; Madson & Malchow-Moller, 1983). To date, no investigation has examined the effect of substrate utilization, manipulated by nicotinic acid ingestion, on postexercise VO_2. If enhanced fat metabolism is responsible for a significant portion of the EPOC, then it is reasonable to conclude that inhibition of fatty acid metabolism by nicotinic acid should reduce the magnitude and duration of the EPOC. The results of such an investigation would provide support for or against the hypothesis that increased fat metabolism is responsible for a significant portion of the elevation in metabolic rate after exercise.

Part III

Nicotinic Acid

Nicotinic acid is one form of the B-group vitamin, niacin. The purpose of this section is to (a) examine the effect of nicotinic acid ingestion on fat metabolism, (b) identify the underlying biochemical mechanisms responsible for its action, and (c) examine the effect of nicotinic acid ingestion on fat metabolism during exercise.

The effect of nicotinic acid on fat metabolism. In large doses (3-6 g/day), nicotinic acid has the pharmacological effect of producing peripheral vasodilation, flushing and reducing serum cholesterol levels. The cholesterol-lowering effect of nicotinic acid was first documented by Altshul, Hoffer, and Stephen (1955). In this study, nicotinic acid was administered to 11 medical students in tablet form, one gram at 9:30 am, one at 2:30 pm, another at bedtime, and a fourth gram on rising. In all cases nicotinic acid decreased serum cholesterol levels.

Carlson and Oro (1962) investigated the acute effects of nicotinic acid on plasma FFA fraction. Five dogs were infused intravenously with norepinephrine at a constant rate for two 20-minute periods. In between periods, three dogs were given nicotinic acid (100 mg/kg body weight), divided into 10 equal doses. In the experimental dogs, administration of nicotinic acid almost completely abolished the increase in plasma FFA induced by norepinephrine, with no effect on blood pressure. The findings prompted the authors to conclude that nicotinic acid had an effect in which the pressor response of infused epinephrine is unaffected but the metabolic effect is blocked.

Carlson (1963) examined the effect of nicotinic acid, nicotinamide, and nicotinuric acid on basal release and norepinephrine-stimulated release of glycerol and FFA from adipose tissue *in vitro*. None of the substances significantly affected the basal release of FFA or glycerol from adipose tissue. However, nicotinic acid and nicotinuric acid significantly reduced the norepinephrine-stimulated release of glycerol and FFA. The results supported the hypothesis that nicotinic acid blocked the direct stimulating effect of norepinephrine on the release of FFA from adipose tissue.

Havel, Carlson, Ekelund, and Holmgren (1964) investigated the effects of norepinephrine and nicotinic acid injection on energy metabolism in six male subjects. The subjects were injected with either 100 mg or 200 mg doses of nicotinic acid between two 19-minute infusions of norepinephrine through a venous catheter at a rate of 25 to 28 μg per minute. Initial infusion with norepinephrine resulted in rapid increases in FFA, glycerol, glucose, and protein concentrations. After nicotinic acid injection, plasma FFA, glycerol, and glucose concentrations fell and then stabilized after 30 minutes. Respiratory exchange ratios significantly increased after administration of nicotinic acid, indicating a greater energy contribution from carbohydrate metabolism. Importantly, the effect of the second infusion of norepinephrine on FFA and glycerol levels was completely blocked by the nicotinic acid.

Carlson, Oro, and Ostman (1968) investigated the metabolic and clinical effects of nicotinic acid administration on patients with ischemic cardiovascular disease and hyperlipidemia. The group hypothesized that the inhibition of FFA mobilization from adipose tissue was a possible mechanism by which nicotinic acid reduced serum lipoprotein levels. To test this hypothesis, eleven patients with uncomplicated ischemic cardiovascular disease and moderate hyperlipidemia were studied for six hours after the administration of one gram of nicotinic acid. In the first three hours after administration, plasma FFA concentrations were significantly depressed by nicotinic acid compared to the control study. Following this period a pronounced rebound of FFA occurred. Glycerol concentrations in plasma followed the same pattern as FFA. Cholesterol values remained unchanged during the six hour study period, while triglyceride levels decreased significantly four to six hours after nicotinic acid administration. The authors concluded that the results were compatible with the hypothesis that acute inhibition of FFA mobilization reduces the concentration of plasma

triglycerides, but not plasma cholesterol. It was hypothesized that reductions in plasma cholesterol were secondary to the reduction of circulating FFA and triglycerides.

Grundy, Mok, Zech, and Berman (1981) assessed the metabolism of triglyceride and cholesterol in hyperlipidemic patients before treatment and during treatment with nicotinic acid. During the treatment phase, plasma triglycerides and very low density lipoproteins (VLDL) decreased by an average of 52% and 36%, respectively. Furthermore, the investigators noted a 21% reduction in VLDL synthesis. The reduced synthesis was attributed to a reduction in particle size, with less triglyceride per particle, rather than a decrease in particle number. The results suggested that the predominant mechanism for cholesterol and triglyceride lowering by nicotinic acid was the reduction in hepatic production of VLDL, which in turn, was attributed to the inhibition of FFA release from adipose tissue.

Biochemical mechanism. Adipose tissue lipolysis is regulated by hormones and paraendocrine agents through the cyclic adenosine monophosphate (c-AMP) system (Dipalma & Thayer, 1991). Elevated levels of c-AMP activates a protein kinase that phosphorylates a hormone-sensitive lipase, thus activating the lipase to catalyze triglyceride hydrolysis (Butcher, Baird, & Sutherland, 1968). Hormonal signals are transduced through guanine nucleotide-linked G-protein system, which interacts with adenyl cyclase to modulate c-AMP levels. Both stimulatory (Gs) and inhibitory (Gi) pathways have been identified. Lipolytic agents such as catecholamines, adrenocorticotrophic hormone, and glucagon bind to distinct stimulatory receptors. The ligand-receptor complexes then interact with a common Gs, leading to a stimulation of adenyl cyclase activity, which results ultimately in fat breakdown. Antilipolytic agents such as

nicotinic acid bind to inhibitory receptor sites, which in turn interact with Gi proteins. These interactions lead to inhibition of adenyl cyclase and, subsequently, decreased mobilization of fat from adipose tissue (Dipalma & Thayer, 1991).

Effect of nicotinic acid on fat metabolism during exercise. Relatively few studies have examined the effect of nicotinic acid on exercise metabolism. In all cases, the administration of nicotinic acid inhibited the increased mobilization of FFAs from adipose tissue normally seen during endurance exercise.

Carlson, Havel, Ekelund, and Holmgren (1963) investigated the effect of nicotinic acid administration on the turnover rate and oxidation of FFA during rest and exercise in four male subjects. Catheters were placed in a brachial artery and two brachial veins in the opposite arm. The subjects rested in a chair as a constant infusion of labeled palmitate was given through one brachial vein. After a one hour rest, the subjects performed work on a bicycle ergometer for two hours at an intensity sufficient to maintain a heart rate of 130 bpm. Two of the subjects received a constant intravenous infusion of 20% glucose solution at a rate of one g/min, while the other two were infused with 200 mg of nicotinic acid at the midpoint of the first rest period, followed by 100 mg doses every 15 minutes during exercise and recovery. Both nicotinic acid and glucose reduced the concentration and turnover rate of FFA and glycerol at rest and inhibited the expected increases in concentration and turnover during exercise. It was concluded that nicotinic acid prevented the FFA mobilizing action of catecholamines at receptor sites in adipose tissue.

Jenkins (1965) also reported decreased fat utilization during treadmill running following administration of nicotinic acid. Three healthy male subjects performed two treadmill runs to exhaustion. In the second trial, each subject took

an initial dose of 200 mg nicotinic acid followed by a 100 mg dose an hour later. Nicotinic acid ingestion resulted in a significant depression in plasma FFA levels and an increase in carbohydrate oxidation.

Bergstrom, Hultman, Jorfeldt, Pernow, and Wahren (1969) examined the effect of nicotinic acid on physical work capacity. Results indicated that the ability to perform either short duration, high intensity work or prolonged submaximal work was neither enhanced nor reduced by the administration of nicotinic acid. Muscle biopsies performed on the quadriceps femoris muscle revealed significantly greater decreases in glycogen content compared to controls following nicotinic acid administration. In addition, respiratory exchange ratios were significantly higher during prolonged exercise after administration of nicotinic acid, indicating a greater reliance upon carbohydrate oxidation for energy during exercise. It was concluded that the reduced delivery of FFA to the muscles after administration of nicotinic acid was compensated by an increased metabolism of muscle glycogen in a manner that did not diminish the ability to perform physical work.

Pernow and Saltin (1971) utilized nicotinic acid to examine the question of whether reduced availability of FFA diminished physical work capacity. After exhaustive exercise, glycogen content in the quadriceps femoris muscle, determined from needle biopsy samples, decreased from 11.7 to 0.3 g/kg wet wt. On the following day, subjects were given a no-carbohydrate diet and the experimental procedure was repeated. The decreased glycogen levels resulted in markedly reduced work output and exercise duration. FFA blockade with nicotinic acid resulted in further decreases in work output and exercise duration. The mean respiratory exchange ratio during exercise decreased from 0.96 on day one to 0.77 on day two. After administration of nicotinic acid, the mean respiratory exchange ratio increased to 0.84, indicating a greater energy

contribution from carbohydrate metabolism. It was concluded that when glycogen stores are reduced, prolonged work can still be performed at levels less than 60-70% of VO_2 max provided that the supply of FFAs is adequate. Restriction of both muscle glycogen and FFA supply seriously impairs the ability to perform prolonged exercise.

Heath, Wilcox, and Quinn (1993) examined the changes in fuel utilization in trained runners during submaximal treadmill running over a three week period during which pharmacological doses of nicotinic acid were ingested. Nicotinic acid treatment resulted in significant decreases in fat utilization during exercise. Over the three week period, respiratory exchange ratios exhibited a declining trend towards greater fat utilization, which was interpreted as evidence of an incomplete adaption to chronic nicotinic acid ingestion. However, serum FFA, glycerol, and glucose concentrations remained unchanged. Interestingly, the three week treatment resulted in significant changes in total and HDL cholesterol levels.

Summary

It is well-known that physical activity results in an elevation in metabolic rate which persists for some time after cessation of the activity. This elevation is commonly referred to as excess postexercise oxygen consumption or EPOC. The magnitude and duration of the EPOC, and hence, its physiological importance, remains controversial. Reports of EPOC durations vary from less than 30 minutes to more than 24 hours. Much of this controversy can be attributed to differences in exercise intensity and duration, and food intake during the recovery period. It appears that exercise must be performed at intensities of greater than 50 to 60% of VO_2 max for durations of equal to or greater than one hour to elicit a prolonged EPOC. The possible confounding interaction of exercise-induced

thermogenesis and diet-induced thermogenesis remains unresolved. However, it seems likely that this interaction may explain the discrepancy in the literature regarding the size of the EPOC.

The classical O_2 debt explanation of elevated postexercise VO_2 has been rejected on the basis that (a) lactate disappearance and the EPOC are not tightly coupled, and (b) during recovery, the primary fate of lactate formed during exercise is oxidation and not conversion to glycogen. Contemporary explanations of EPOC have focused on the physiological and biochemical factors that directly or indirectly stimulate mitochondrial respiration. Increased core temperature (Q10 effect), elevated levels of catecholamines, and calcium ions have been theorized to account for a significant portion of the EPOC. A number of studies have reported an association between increased fat metabolism during exercise and the magnitude and duration of the EPOC. In addition, an increased dependence on fatty acids as a fuel substrate during recovery has been shown to stimulate the triglyceride-fatty acid cycle, which has been demonstrated to account for a significant portion of the EPOC.

The role of fat metabolism in elevating metabolic rate after exercise can be further evaluated by manipulating fatty acid mobilization and utilization during exercise and recovery. The B-group vitamin niacin, in the form of nicotinic acid, has been shown to inhibit the mobilization of free fatty acids from adipose cells. To date, no investigation has examined the effect of nicotinic acid ingestion on postexercise VO_2. The results of such an investigation would provide evidence for or against the theory that enhanced fat metabolism is responsible for a significant portion of the elevation in metabolic rate after exercise.

Appendix III

Consent Form

Title: The effects of substrate utilization, manipulated by nicotinic acid, on postexercise oxygen consumption.

Investigators: Stewart G. Trost, Anthony R. Wilcox Ph.D.

Purpose: It is well-known that physical activity results in an elevation in metabolic rate which returns to pre-exercise levels some time after cessation of the activity. This elevation is commonly referred to as excess postexercise oxygen consumption or EPOC. Several studies have shown that burning more fat during physical activity will result in a greater elevation in metabolic rate after exercise. Therefore, by manipulating the amount of fat used for a given intensity and duration of exercise, the role of fat metabolism in elevating metabolic rate after exercise can be evaluated. To date, no investigation has examined the effect of niacin ingestion on post-exercise metabolic rate. Niacin, in the form of nicotinic acid is a B-group vitamin known to decrease the amount of fat used for energy during exercise. The results of such an investigation would provide evidence for or against the theory that enhanced fat metabolism is responsible for a significant portion of the elevation in metabolic rate after exercise.

I have received and oral explanation of the study procedures and understand that they entail:

1. **A test of maximal oxygen consumption on a cycle ergometer.** On the first visit to the laboratory, I will undergo an incremental exercise test to determine maximal oxygen consumption. The test will begin at an easy workload of 50 watts. After five minutes, the workload will be increased by 30 watts every minute until termination of the test. The test will be terminated when I feel too fatigued to continue pedaling. It is anticipated that the test will take 10-15 minutes, with only the last few minutes at a high intensity. I will be breathing through a mouthpiece and valve with my nose clipped so that the amount of oxygen my body is using during the

test can be determined. My heart rate, electrocardiogram (ECG), and rate of perceived exertion (RPE) will be monitored continuously by a certified exercise test technologist.

2. **A test of body composition using hydrostatic weighing.**
On the second visit to the laboratory, my body composition (percent body fat) will be determined using an underwater weighing procedure in a specially designed indoor tank. Water in the tank will be near body temperature (35-37° C). Sitting on a chair that is suspended from a scale, I will submerge myself following a maximal exhalation and remain underwater for 2-3 seconds while the scale is read. This procedure is repeated 3-6 times. I will also perform a test to determine my residual volume (volume of air left in the lungs following a maximal exhalation), which entails breathing into a spirometer filled with pure oxygen for a period of 30-60 seconds. Two or three trials will be performed.

3. **A pretest trial to confirm calculated submaximal workload.**
In addition to the hydrostatic weighing, I will perform a 15-20 minute pretest exercise trial on a cycle ergometer. After a five minute warm-up at 50 watts, the workload will be increased to the value calculated to be 65% of maximal oxygen consumption. The workload will then be adjusted up or down in 5 watt increments until my oxygen consumption is as close as possible to 65% of my previously measured maximal oxygen consumption. I will be breathing through a mouthpiece and valve with my nose clipped in a manner identical to the maximal exercise test.

4. **Two tests to investigate fat utilization and its relationship to increased metabolic rate after exercise.**
On the the third and fourth visits to the laboratory, I will participate in a three-hour experimental trial in which I will rest one hour, exercise for one hour at 65% of max, and rest for another hour. For each test I will arrive by car or similar means at approximately 7:00 am. I will be required to fast from 7:00 pm the night before. I will also refrain from hard exercise for a minimum of 48 hours before each trial.

Resting Period

Upon arrival I will be fitted with a heart rate monitor. A sterile teflon intravenous catheter will be inserted into a forearm vein by a certified medical technologist/ phlebotomist and held in place with surgical tape. At the beginning of each sampling period, a 3 ml blood sample (less than one teaspoon) will be drawn and discarded, after which, the catheter will be flushed with saline solution. This will be done to prevent blood clotting within the catheter. A 10-15 ml (2-3 teaspoons) blood sample will be then taken immediately. The subject will then sit quietly in a reclining chair with their feet

elevated for a total of 60 minutes. After 30 minutes, I will ingest either 500 mg (1 capsule) of niacin with water or water alone. A valve and mouthpiece with supporting headgear will then be fitted but the mouthpiece will not be inserted. During the last 15 minutes of the resting period, the mouthpiece will be inserted, the nose clipped, and expired air will be collected and analyzed for oxygen and carbon dioxide concentration. During the last minute of rest, a further 10-15 ml (2-3 teaspoons) blood sample will be taken.

Exercise Period.
After resting baseline measurements are completed, I will cycle for one hour at an intensity corresponding to 65% of maximal oxygen consumption. I will breathe through the mouthpiece and valve for the first 15 minutes of exercise, and between 25 and 30 minutes, 40 and 45 minutes, and 55 and 60 minutes of exercise. A blood sample will be drawn from the catheter after 31 and 60 minutes of exercise.

Recovery Period.
On completion of the one hour exercise bout, I will immediately resume the seated resting position with the mouthpiece and valve left in place. I will breathe through the mouthpiece and valve for the first 30 minutes of the recovery period, and between 40 and 45 minutes, and 55 and 60 minutes of recovery. Blood samples will be drawn from the catheter 31 and 60 minutes into the recovery period.

Risks
I understand that the test of maximal oxygen consumption has a very remote chance of precipitating a cardiac event (such as abnormal heart rhythms) or even death. However, the possibility of such an occurrence is very slight (less than 1 in 10,000), since I am in good physical condition with no known symptoms of heart disease. Furthermore, the test will be administered by trained personnel who will be monitoring for signs of exercise intolerance.

I am aware that there is a chance I may experience short lasting uncomfortable side effects from niacin ingestion. The most common side effect is a flushing, prickly-heat sensation in the face, neck, hands, and arms. The effect may begin within minutes of ingestion and last for 30-45 minutes. Other possible side effects include dizziness and nausea. Since I am from a healthy population, and since I will be taking a single dose, there is little chance of experiencing elevated liver enzyme levels, a side effect associated with chronic niacin ingestion. Niacin is a water-soluble B vitamin that is not stored in the body.

I am aware that if for any reason the catheter fails to function properly, the remaining blood samples will be taken by venapuncture.

Benefits

The benefits of my participation in the study include contributing to the understanding of substrate utilization and its effect on excess postexercise oxygen consumption. I will also gain knowledge regarding my aerobic capacity and body composition, a service that usually entails a fee of $90.0. Such information may be useful to me in making decisions relating to my lifestyle and health habits.

I understand that my participation in the study will entail four laboratory sessions, requiring a total time commitment of approximately eight hours over a two to three week period.

I understand that confidentiality will be maintained by codifying my data files. Upon entry into the study, I shall receive a code number to identify my data and all records shall be kept using the code number. The list containing the names of the subjects and their appropriate code numbers will only be available to the researchers in this study. I will not be identified in any way in the presentation or publication of the results of the study.

Persons who have ever had hepatitis B or C, who have tested positive for HIV or any AIDS virus, or persons who have AIDS should not donate body fluids or tissues. Persons at risk for getting and spreading AIDS virus also should not donate body fluids or tissue and should not participate in this investigation. You are at risk if:

- you are a man who has had sex with another man since 1977, even one time.
- you have shared a needle, even one time, to inject drugs or medication.
- you have taken clotting factor concentrates for a bleeding disorder such as hemophilia.
- you have ever had a positive test for any AIDS virus or hepatitis B or C or any AIDS antibody.
- you have had sex with any person described above.
- you have had sex with a male or female prostitute since 1977.

Questions about the research, my rights, or research-related injuries should be directed to Stewart Trost (737-6795) or Anthony Wilcox (737-5922). I understand that the University does not provide a research subject with compensation or medical treatment in the event the subject is injured as a result of participation in the research project.

I have been completely informed and understand the nature and purpose of this research. The researchers have offered to answer any further questions that I may have. I understand that my participation in this study is completely voluntary and I may withdraw from the study at any time without prejudice or loss of the benefits to which my participation entitles me.

I have read the foregoing and agree to participate in this study.

Subject's Signature	Date
Subject's Address	
Investigator's Signature	Date

Appendix IV

Medical History Form

Name_____D.O.B._____

Address_____

Age_____ Phone_____

Height_____ Weight _____ Gender _____

Which best describes your ethnic identity? (optional)

_____ Caucasian
_____ African American
_____ Hispanic American
_____ Asian American
_____ American Indian/Alaskan Native
_____ Other (please specify)_____

PAST HISTORY

Do you now have or have you ever had any of the following conditions? If so, please check the appropriate line, give explanations and dates.

___ Heart Murmur
___ High Blood Pressure
___ Serum Cholesterol ≥ 240 mg/dl
___ Disease of Arteries
___ Surgery (eg. Heart, Back, or Knee)
___ Fainting Spells, Dizziness or Weakness
___ Epilepsy or Convulsions
___ Numbness or Tingling
___ Arthritis
___ Diabetes
___ Anemia
___ Thyroid Disorders
___ Allergies
    ___ Food
    ___ Medication
    ___ Skin
    ___ Asthma
___ Frequent Indigestion or Heartburn
___ Stomach Ulcer
___ Hernia

__ Other

Family History

Have any close relatives experienced:

__Heart Attack
__High Blood Pressure
__High Cholesterol
__Diabetes
__Congenital Heart Disease

Present Symptoms

Have you recently experienced:

__Chest Pain
__Chest Pain with Exertion
__Shortness of Breath
__Heart Palpitations
__Any Joint or Musculoskeletal Problems
__Other

Are you presently taking medication? Yes No

If yes, please explain

Do you smoke? Yes No

Have you recently quit smoking? Yes No If yes, how long ago?

What would you estimate your daily alcohol consumption to be? (circle one)
(1 Drink is equivalent to 12 oz beer, 3 oz of wine, or 1.5 oz of distilled spirits.)

none - less than 1 - less than 3 - less than 6 more than 6

Physical Activity Information

1. Please indicate how much physical activity you perform at work/school.

   very little - little - moderate - active - very active

2. Please indicate your physical activity level during your leisure hours.

   very little - little - moderate - active - very active

3. Briefly describe any activity you have been doing in the past few months.