Protein Metabolism and Energy Utilization in Females Participating in Endurance Activity: The Effect of Nutritional Supplement Timing.

by

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A Thesis

Submitted to the School of Graduate Studies in Partial Fulfillment of the

Requirements for the Degree

Master of Science.

McMaster University

October 30, 1997.

MASTER OF SCIENCE
(Human Biodynamics)

McMASTER UNIVERSITY
Hamilton, Ontario

Title: **Protein Metabolism and Energy Utilization in Females Participating in Endurance Activity: The Effect of Nutritional Supplement Timing.**

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NUMBER OF PAGES: 198

This thesis is dedicated to my parents,

James Henry Bosman and Heather Barbara Bosman,

And to my Friends.

With whom my McMaster experience,
both undergraduate and graduate,
was bearable, joyous, and truly rewarding.

ABSTRACT.

Ten healthy young females (age 22.3 $\pm$ 0.2 y, VO_{2max} > 40 $ml \cdot kg^{-1} \cdot min^{-1}$) volunteered to participate in a randomized, double-blinded study that examined the effect of both (A) the timing of PRO/CHO/FAT supplementation, pre- and post-endurance exercise, and (B), extra-energy supplementation (~ 400 kcal) on indices of protein metabolism. Each subject completed each of three, 7-day supplementation trials conducted at least 1 week apart: a pre-exercise trial (PRE), a post-exercise (PO) trial, and a post-exercise with extra energy (POE) trial. All females were eumenorheic and tested during the early (days 4-7), middle (days 8-11), and late (days 12-14) periods of the follicular phase of their menstrual cycle. Subjects were weighed prior to and following each trial to determine weight loss. During each trial, subjects would consume a checklist diet (days 1-3) and a prepackaged diet (days 4-6) that were isoenergetic, isonitrogenous, and matched for diet composition (carbohydrates, protein, and fat) to the individual's habitual dietary intake.

Subjects cycled at 65% VO_2max for 1 hr, according to their target heart rate on Days 1,3, and 4 and for 1.5 hrs on Day 6 with Days 2 and 5 being rest days. Expired gas and blood samples were collected on Day 6 during exercise (T=0,30,60,90 min) and post exercise for 2 hrs (Blood t=30,60,90,120, Gas t=10,20,30,45,60,90,120 min). On Day 5, expired gas samples were collected post- supplement consumption at the same timepoints as Day 6. 24 h-urine collections were performed on Days 5 and 6. A 75% VO_2max performance ride to exhaustion was performed in a fasted state on the morning of Day 7.

During each trial, subjects had consumed their daily meals at regular intervals by 1500 h prior to the exercise bout (1600 h). In the PRE trial, a PL can + PRO/CHO/FAT Supplement (Results® + 0.5 g·kg CHO powder[Gatorade®]) was consumed with breakfast (BKFT), and a PL Supplement (1 can + powder) was consumed immediately following the cessation of exercise. In the PO trial, a PL Supplement (2 cans + powder) was taken at BKFT and the PRO/CHO/FAT Supplement was consumed post-exercise. The POE trial was identical to the PO trial with the exception that subjects received an extra 400 kcal of energy·d^{-1} at BKFT (Boost®~250 kcal and an extra 150 kcal CHO powder). During the POE checklist diet (Days 1-3), the extra energy was given by the addition of extra items to their daily food consumption.

Although plasma estrogen levels were significantly higher during the PO trial ($P < 0.05$), plasma progesterone levels were not significantly different between trials, and both hormones indicated that subjects were in the follicular phase of their menstrual cycle. Significant main effects for time occured in which hematocrit, sodium, potassium, and lactate were observed to increase during exercise and decrease post-exercise. Glucose concentration increase was signiciant for time ($P < 0.01$) increasing post-exercise after consumption of the supplement. Significant main effects for insulin were observed across time ($P < 0.000001$) and for trial ($P < 0.01$) with the POE trial having the largest post-exercise insulin response. No significant differences were observed between the three trials for urinary creatinine (g·24 h^{-1}) and urea (g·24 h^{-1} and g·g Cr^{-1}). Day 6 urinary 3-methylhistidine (μmol·g Cr^{-1}) was significantly different between trials (POE>PO>PRE)($P < 0.01$). Similarly, no significant differences were observed between

trials for resting metabolic rate, thermic effect of food, thermic effect of exercise, and excess post-exercise oxygen consumption. However, on Day 5, Apparent Nitrogen Balance trended toward signifcance for trial ($P=0.086$) becoming significant on Day 6 ($P<0.005$) wherein POE and PO trials were positive and PRE trial was negative. There was a trend towards increased performance times when comparing the POE and PO trials versus the PRE trial ($P=0.074$). Furthermore, weight loss was significantly lower with post-exercise supplementation (POE<PO<PRE)($P < 0.01$). This study suggests that the protein balance of females regularly engaging in endurance activity may benefit from immediate nutritional supplementation following exercise.

PREFACE.

The following is a list of the abbreviations and operational definitions used throughout this manuscript.

3-MH- 3-methylhistidine
AA - amino acid
ADP - adenosine diphosphate
ATP - adenosine triphosphate
Ala - alanine
BCAA - branched- chain amino acid
BCAAT - branched-chain amino acid transferase
BCGND - background
BCKAD - branched-chain keto acid dehydrogenase
BCOAD - branched-chain 2-oxo adid dehydrogenase
Ca^{2+} - calcium cation
CHO - carbohydrate
CO_2 - carbon dioxide
E_{BAL} - energy balance
E_{IN} - energy intake
E_{OUT} - energy expenditure
EE_{ACT} - energy cost of physical activity
FFA - free fatty acid
FFM - fat free mass
FSR - muscle protein fractional synthetic rate
IGF-I - insulin-like growth factor
IGFBP - insulin-like growth factor binding protein
Ile - isoleucine
INS - insulin
K^+ - potassium
Leu - leucine
MPB - muscle protein breakdown
MPS - muscle protein synthesis
Na^+ - sodium cation
N_{BAL} - nitrogen balance
NH_3 - ammonia
NH_4^+ - ammonium
O_2 - oxygen
Phe - phenylalanine
P_i - inorganic phosphate
PRO - protein
PRO_{BAL} - protein balance
PRO_{IN} - protein intake
RDI - recommended daily intake
SPA - spontaneous physical activity
Tyr - tyrosine
tRNA - transfer ribonucleic acid
Val - valine
VCO_2 - carbon dioxide consumption
VO_2 - oxygen consumption
VO_2max - maximum oxygen consumption
WBPD - whole-body PRO degradation
WBPS - whole-body protein synthesis

ACKNOWLEDGMENTS.

I would like to extend my deepest gratitude to a number of individuals who helped me make this thesis a reality. First, I am indebted to my advisor, Dr. M.A. Tarnopolsky, for his guidance, genius, and choice in me. I must also thank Dr. J.D. MacDougall- whose personal council and influence I am forever indebted to. I am grateful for the guidance of my other committee members; Dr. S. Atkinson, Dr. Deion Neame, and Dr. Audrey Hicks. I wish to thank John Moroz, Zoran, and Dr. J. Rosenfeld for their technical expertise. My regards to the talented members of "The Lab"; Joan Martin, Scott McKenzie, Gianni Parise, Stuart Lowther, Dan MacLennan, and Sasa Mihic, for their great friendship, courtesy, deviance, and ability to laugh. Furthermore, thanks to all the subjects who made this research project possible and enjoyable.

My parents deserve a special thanks for covering countless miles, supplying my shelves, and providing stability in an unstable environment. Finally, I must thank those that may be quite distant now- former classmates, teammates, bandmates, and roommates.

"We see so far because we stand on the shoulders of giants." - Sir Isaac Newton.

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FOREWORD.

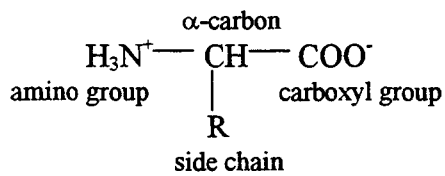
This thesis is presented in three separate chapters. The first chapter discusses protein and related metabolic pathways, metabolites, synthesis, degradation, sex differences, and the effect of exercise. A brief review of post-exercise nutritional supplementation is also be included in this chapter. Chapter II is a review of the relationship between nutritional intake and energy expenditure, and the contributing factors that influence whole-body energy balance. The final chapter is a research paper presented in publication format. Following chapter III are all references, tables, figures, statistics, and appendices relevant to the entire manuscript.

Chapter I. Protein Metabolism.

Proteins are the most abundant macromolecules in living cells, occuring in great variety and exhibiting a diversity of biological functions. These functions include enzymatic catalysis and cellular transportation, motility, contraction, defense, regulation, and the provision of structure.

1.1. Amino Acids/Protein.

Proteins, (the name being derived from the Greek *protos*, meaning "foremost"), are constructed from the same set of 20 AAs. Each AA monomer has a side chain, an amino group (or N-terminal), and a carboxyl group (or C-terminal):



Each side chain, or R group, differs from the other in structure, size, and polarity and, therefore, serves as a basis with which to classify AAs (Table I).

Polymers of AAs are called peptides and range in size from 2-3 AAs to macromolecules containing thousands of AAs. A dipeptide is formed when 2 AAs are covalently joined by a peptide bond in a condensation reaction. In such a reaction, the α-amino group of one AA acts as a nucleophile to displace the hydroxyl group of an adjacent AA yielding water. Oligopeptides are formed when a few AAs are linked. Polypeptides have thousands of AAs and are often used interchangeably with the term "protein". However, the term "protein" is generally referred to as having a molecular weight of < 10000 (Lehninger *et al.*, 1995).

1.2. Biological Pathways.

Amino acids exist principally in the ionized state as anions and the plasma concentration of each AA is maintained at a reasonably constant value. Tracer studies have estimated that about 400 grams of body PRO are synthesized and degraded each day (Brooks *et al.*, 1995).

In the small intestine, pancreatic enzymes hydrolyse most dietary PRO to AAs and most of the remaining peptide bonds are cleaved by additional amino polypeptidase and dipeptidase enzymes in the epithelial cells of the small intestine.

At the brush border membrane of intestinal villi, AAs are cotransported with sodium ions in four carrier systems, one each for neutral, basic, and acidic AA as well as one for proline and hydroxyproline (Brooks *et al.*, 1995). After leaving the mucosal cells, AAs diffuse into the portal circulation. Amino acid concentration in the blood rises very slowly after a meal because PRO digestion and absorption is extended over 2 to 3 hours. Once in the blood, AAs are absorbed via facilitated and active transport within 5 to 10 minutes by cells throughout the entire body, especially by the liver (Guyton, 1995).

1.2.1. Synthesis and Storage of Cellular Protein.

Protein synthesis requires the participation of over 70 different ribosomal PROs; 20 or more enzymes to activate the AA precursors; a dozen or more auxilliary enzymes and other PRO factors for the initiation, elongation, and termination of polypeptides; about 100 additional enzymes for the final processing of different kinds of PRO; and at least 40 kinds of tranfer and ribosomal RNAs (Lehninger *et al.*, 1995). Upon entering the cell, AAs are combined via peptide linkages, under the direction of the messenger RNA

and ribosomal system to form cellular PRO. Growth hormone and INS increase the formation of tissue PRO. The liver, kidney, and intestinal mucosa participate to a greater extent in PRO storage.

Essential AAs (those with carbon skeletons that cannot be synthesized by animals and must be obtained in the diet) can be synthesized from appropriate α-keto acid precursors by transamination. For example, pyruvate is the α-keto acid precursor for Ala. During the transamination reaction, an aminotransferase promotes the transfer of the amine group from glutamate to pyruvate, which thus becomes Ala. Concommitantly, the keto-oxygen is transfered from pyruvate to glutamate, which thus becomes α-ketoglutarate.

1.2.2. Degradation of Cellular Protein.

When the plasma is depleted of PRO during starvation and prolonged periods of exercise, tissue PRO can be hydrolysed by tissue proteases to constituent AAs and transported back into the plasma. In this way, the ratio of total tissue PRO to total plasma PRO remains at approximately 33:1.

The liver, being the organ responsible for oxidative deamination and the urea cycle, is the major site of AA degradation. Ten-30% of total liver PRO can be mobilized for metabolism (Dohm *et al.*, 1987). Proteolysis in the liver occurs because of an increase in non-sedimentable (free) lysosomal enzyme activities (Kasperek *et al.*, 1985b). Although skeletal muscle is capable of BCAA oxidation, the liver catabolizes large amounts of PRO into AAs and change the types of AAs present because of a high activity of transaminases.

1.2.3. Oxidative Deamination and Transamination.

Tissue PRO degradation begins with the removal of the amine group from AAs by two mechanisms: oxidative deamination or transamination (Figure 1). The basic strategy for nitrogen removal in both of these processes is to convert AAs to glutamate. When there is a shortage of substrates for the TCA cycle, glutamate is deaminated by glutamate dehydrogenase to yield α-ketoglutarate through the use of NAD^+ as the oxidizing agent (Figure 1). Alpha-ketoglutarate can then be used as a TCA cycle intermediate and NADH can yield several ATP molecules through mitochondrial oxidative phosphorylation. The reaction is reversed when sufficient quantities of other substrates are available to provide material for the TCA cycle.

Transamination is by far the most common route for amino group exchange in most tissues, including muscle. In these transamination reactions, the α-amino group is transferred to the α-carbon atom of α-ketoglutarate, leaving behind the corresponding α-keto acid analogue of the AA. There is no net deamination in such reactions because the α-ketoglutarate becomes aminated as the α-AA is deaminated (Lehninger *et al.*, 1995). Thus, α-ketoglutarate becomes glutamate upon accepting an amino group from Ala. Likewise, Ala becomes pyruvate upon accepting a keto group from α-ketoglutarate. The effect of transamination reactions is to collect the amino groups from many different AAs in the form of only one, namely, L-glutamate. Glutamate can transfer the amino group to other substances or can release it in the form of NH_4^+. Mole and colleagues (1971) have demonstrated that endurance training can double the levels of important transaminases such as glutamate-pyruvate transaminase.

1.2.4. Glucose-Alanine Cycle.

During periods of fasting and prolonged exercise, AAs from degraded muscle PRO circulate to the liver, where deamination, transamination, and gluconeogenesis take place. Due to the aforementionned *de novo* alanine synthesis in muscle, half or more of the AAs taken up by liver for these processes are in the form of Ala (Goldberg and Odessey, 1972). In a reversal of the Ala aminotransferase reaction, Ala transfers its amino group to α-ketoglutarate, forming glutamate in the cytosol. Some of this glutamate is transported into the mitochondria and deaminated by glutamate dehydrogenase, releasing NH_4^+. In the liver, Ala yields pyruvate, the starting material for gluconeogenesis, thus relieving the muscle of the energy burden of gluconeogenesis so that the available ATP in muscle can be devoted to muscle contraction (Lehninger *et al.*, 1995).

1.3. Relevant Indices of Protein Metabolism.

1.3.1. Nitrogen Balance Studies

N excretion is considered to be a quantitative index of net PRO degradation since PRO is ~16% N by weight. When the dietary input (g $N \cdot d^{-1}$) is equal to that excreted, the individual is in zero N_{BAL}. When an individual catabolizes more PRO than can be replenished with food consumption, a negative N_{BAL} results. Similarly, if more nitrogen is consumed in the diet than that excreted, a positive N_{BAL} results.

Tarnopolsky *et al.* (1988) demonstrated, using the N_{BAL} technique, that endurance athletes required about 67% more dietary PRO than sedentary individuals to maintain N_{BAL}. This amount of PRO represents approximately twice the Canadian RDI (0.86 $g \cdot kg^{-1} \cdot d^{-1}$).

Meredith *et al.* (1989) studied 6 young (26.8 ± 1.2 yr) and 6 middle-aged (52 ± 1.9 yr) endurance-trained men consuming 0.6, 0.9, or 1.2 g $N \cdot kg^{-1} \cdot d^{-1}$ over three separate 10 day periods, while maintaining endurance training and constant body weight. All subjects were in negative N_{BAL} at the lowest PRO_{IN}. The zero intercept was 0.94 ± 0.05 g $N \cdot kg^{-1} \cdot d^{-1}$ and the protein requirement was about 25% higher still for the 12 men with no effect for age.

Later work by Tarnopolsky *et al.* (1992) determined that the dietary PRO requirement of strength athletes FOR zero N_{BAL} was 1.41 $g \cdot kg^{-1} \cdot d^{-1}$. A low PRO (0.86 $g \cdot kg^{-1} \cdot d^{-1}$) diet did not provide adequate PRO and resulted in an accommodated state (decreased WBPS). The medium PRO (1.40 $g \cdot kg^{-1} \cdot d^{-1}$) diet resulted in a state of adaptation (↑ WBPS but no change in Leu oxidation). The high PRO (2.40 $g \cdot kg^{-1} \cdot d^{-1}$) diet did not further increase WBPS, but resulted in an increase in Leu oxidation, indicating nutrient excess.

Lemon *et al.* (1992) randomized 12 men to a weight-training group receiving either an isoenergetic PRO (total PRO_{IN} 2.62 $g \cdot kg^{-1} \cdot d^{-1}$) or CHO (total PRO_{IN} 1.35 $g \cdot kg^{-1} \cdot d^{-1}$) supplement for a 1 month each. The PRO_{IN} necessary to maintain zero N_{BAL} was 1.4-1.5 $g \cdot kg^{-1} \cdot d^{-1}$ at 3.5 weeks. However, the investigators suggest that a PRO_{IN} of 1.6-1.7 $g \cdot kg^{-1} \cdot d^{-1}$ be employed to compensate for larger PRO demands early in the training program.

1.3.2. Urinary Urea.

The ammonia released during deamination is almost entirely removed from the blood by conversion into urea by the liver. This occurs by the combination of two

molecules of NH_3 and one molecule of CO_2 in the urea cycle (Figure I). It is the function of the kidneys to remove the circulating urea and to excrete it in urine. Urea excretion via sweat is also increased during exercise (Tarnopolsky *et al.*, 1988).

Urea N atoms can be re-cycled because the bacteria in the gut contain urease; thus, urea diffusing into the gut can be hydrolyzed to CO_2 and NH_3. The NH_3 can diffuse back into the plasma, to be re-incorporated into urea or AAs (Wolfe, 1987).

Urea production is elevated during exercise in a controlled laboratory setting (Calles *et al.*, 1984) and after dietary manipulation such as CHO restriction (Freund *et al.*, 1979) or excess caloric and PRO_{IN} (Mole & Johnson, 1971). Estimations of nitrogen loss from urea production during exercise may underestimate PRO degradation since kidney blood flow is reduced during exercise (Castenfors, 1967). Moreover, using tracer methodology, Wolfe (1987) found no increase in the R_a of urea after infusing [$^{15}N_2$]urea into subjects that cycled at 30% VO_2max for 105 min of exercise. Likewise, at a slightly higher intensity (53% VO_2max), Stein *et al.* (1987) found no increase in urea production during a simulated triathalon in which the subjects cycled for 5 h and then ran on a treadmill for 3 h. The reasons for this discrepancy may be due to the low intensities of exercise which subjects performed and this would have shifted substrate utilization towards a higher proportion of lipid.

1.3.3. Urinary Creatinine.

Burger (1919) was the first to propose that urinary creatinine was proportional to muscle mass, assuming that: nearly all body creatine is within skeletal and smooth muscle; on a creatine-free diet, the total creatine pool remains constant; creatine is converted

nonenzymatically and irreversibly to creatinine at a constant daily rate; and creatinine, once formed, undergoes diffusion from the cell to appear in the urine after glomerular filtration.

Virgili *et al.* (1994) determined that urinary creatinine excretion can be an indirect method for evaluating body composition in healthy adult subjects. Urinary creatinine was analysized in 20 subjects over a meat-free 2 day period following 4 days of a meat-free diet. Although men had significantly higher urinary creatinine concentrations than women, creatinine excretion was found to be well associated with FFM for both sexes (R^2 =0.89).

1.3.4. Urinary 3-Methyl Histidine

Since 3-MH is found exclusively in contractile protein its release provides an index of myofibrillar protein degradation (Balon *et al.*, 1990). It is formed by the post-translational modification of histidine residues. The methyl group is attached to free histidine after the formation of the aminoacyl-tRNA, since 3-MH has no specific histidyl-tRNA and therefore cannot be resynthesized (Young *et al.*, 1972). When contractile PRO is degraded, 3-MH is released, but enzymatic degradation of the methylated histidine ring does not occur. The 3-MH can not be reincorporated into PRO and its only fate is to be excreted in the urine (Young & Munro, 1978). Long and colleagues (1975) administered labelled radioactive 3-[Me-^{14}C] MH to rats and found that 99% of the dose was recovered in urine with neglibible amounts excreted in feces or expired CO_2.

Analysis of the major tissues of the rat suggests that more than 90% of the whole body protein bound 3-MH is present in actin and myosin of skeletal muscle, 8.4% in skin and hair, and 1.0% in the gastrointestinal tract with rates of myofibrillar PRO turnover of

approximately 7.3%·d^{-1} in skeletal muscle and 5%·d^{-1} in the gut and skin (Nishizawa *et al.*, 1977). Other investigators have reported the rate of myofibrillar PRO turnover to be as low as 1.7%·d^{-1} (Rennie & Millward, 1983; Ballard & Tomas, 1983). Doubts as to whether 3-MH is a reliable indicator of skeletal muscle PRO breakdown have been expressed (Wassner & Li, 1982; Rennie & Millward, 1983). Wassner & Li (1982) used fed rats to report that the gastrointestinal tract contributed to 40% of the total 3-MH excretion with a fractional myofibrillar PRO catabolic rate of 24%·d^{-1} vs.1.4%·d^{-1} for skeletal muscle. In another study using the rat model, Rennie & Millward (1983) reported an intestinal 3-MH turnover of 29%·d^{-1}, almost 20 times as fast as that of skeletal muscle, so that the gut and skeletal muscle accounted for ~20% and 25% respectively of total 3-MH excretion.

Long *et al.* (1981) did not find any significant differences in splanchnic PRO metabolism and 3-MH excretion between patients with short bowel syndrome and controls. Perhaps the most elucidating study was a single-case study (Afting *et al.*, 1981) of a completely paralyzed patient with neither macroscopically nor microscopically detectable skeletal muscle that was fully turned into connective tissue. Excretion rates of 3-MH in this patient were about 28% of the control subjects' rates, showing that 72% of the total 3-MH production was accounted for by skeletal muscle. Taken together with the aforementionned studies (Ballard & Tomas, 1983; Long *et al.*, 1981; Nishizawa *et al.*, 1977), it is reasonable to assume that skeletal muscle is the primary contributor to 3-MH appearance in urine, and that 3-MH excretion is representative of directional changes in myofibrillar PRO breakdown.

3-Methylhistidine excretion is normally expressed as a ratio of creatinine excretion to correct for differences in muscle mass and for changes in the rates of kidney clearance. Lukaski *et al.* (1981) determined that for the quantification of 3-MH in urine to be valid, a flesh-free diet must be consumed for at least 3 days prior to urine sampling. In addition, 3-MH excretion may be limited by renal failure and completeness of urine collection (Young & Munro, 1978).

1.3.5. Amino Acid Metabolism In Skeletal Muscle.

The preferred AA fuels for exercise by skeletal muscle are the essential BCAAs (Leu, Ile, and Val). In contrast, Tyr and Phe are neither catabolized nor synthesized by muscle; therefore, these AAs reflect the average rate of degradation of both myofibrillar and nonmyofibrillar PRO pools and their release from tissues may provide a measure of the balance between PRO synthesis and degradation (Goldberg & Chang, 1978). It has been shown that the release of Phe and Tyr from muscle is comparable (Davis & Karl, 1986).

Branched chain amino acids can be oxidized for energy or be used in ketogenic pathways (the latter not in muscle)(Waterlow, 1985). The metabolism of BCAAs begins with a transamination reaction via BCAAT to yield glutamate and a keto acid (Figure 1)(Felig & Wharen, 1971). The glutamate can then, via the alanine aminotransferase reaction, donate a nitrogen to pyruvate to form Ala. The ketoacids can be dehydrogenated by the rate limiting enzyme in BCAA catabolism, BCKAD, to yield acetyl-CoA or to acetoacetate which can then undergo oxidation.

1.4. Protein Metabolism Following Endurance Exercise.

1.4.1. Decreased Proteolysis, Increased Protein Synthesis.

An elevation of PRO synthesis has been reported in the recovery period after exercise. This is evidenced by nitrogen retention, and an elevated rate of AA incorporation into various fractions of skeletal muscle PRO (Seene *et al.*, 1986; Wenger *et al.*, 1981). In addition to synthesis, muscle PRO turnover post-exercise is thought to renew the damaged actin-myosin complex to ensure improvement in contractile function of muscles in training (Viru, 1987). Muscle hypertrophy is accompanied by an increased rate of PRO turnover (Waterlow, 1984).

After moderate-intensity exercise (~50% VO_2max), rates of PRO synthesis increase and proteolysis decrease so that net PRO_{BAL} is positive (Rennie *et al.*, 1981). However, this study estimated proteolysis by the Ra of L-[1-^{13}C]-Leu in plasma and the authors have advocated using enrichments of α-[1-^{13}C] KIC as an indicator of intracellular Leu enrichment to assess rates of WBPB (Rennie *et al.*, 1982).

The time course for the postexercise increase in PRO synthesis and decreased PRO degradation is unknown. In animals, MPS is reduced during exercise and is increased at about 1-2 h after exhaustive exercise (Rogers *et al.*, 1979). Devlin *et al.* (1990) demonstrated a decrease in Leu oxidation between 2-3 h after endurance exercise (75%VO_{2max} to exhaustion), estimated from KIC Ra, while NOLD, an indicator of WBPS, was increased. Devlin *et al.* (1990) postulated that fat oxidation may have decreased Leu oxidation after exercise. Decreased levels of Leu oxidation have been

found with physiological levels of FFAs (0.5 to 1.2 mM) (Tessari *et al.*, 1986) and/or β-hydroxybutyrate (Nair *et al.*, 1988).

Using the perfused hindquarter preparation in rats, the post-exercise Ra of Tyr from muscle was compared between sedentary controls and rats that swam for 1 h (Dohm *et al.*, 1980). In another experiment that utilized the same tracer (Kasperek & Snyder, 1985a), small rats (60 to 80 g) ran at 16 $m \cdot min^{-1}$ down a 16% grade for 120 min, and then the soleus and extensor digitorum longus muscles were removed and incubated *in vitro* to determine the rates of Tyr release. Both experiments showed Tyr release, and thus MPD, was increased by prior exercise. Although post-exercise MPD was increased compared to rest, the amount of MPD observed was thought to be less than that observed during the exercise bout.

Tipton *et al.* (1996) infused ring-[$^{13}C_6$] Phe over a 5 h period into 7 early-follicular (days 1-8) collegiate female swimmers after 3 randomly ordered workouts: (1) 4,600 m of intense swimming (~1.5 h); (2) resistance training (~1 h); and (3) both workouts combined. Surprisingly, WBPB values, as determined from the Ra of Phe, were similar to rest values after each of the workouts. The FSR in the posterior deltoid was increased 81% over resting levels with the combined resistance and endurance exercise. Even though the posterior deltoid was only used in 9 of 33 sets, it is possible that the stimulatory effect of the combination workout on the FSR was primarily due to the increased work performed by this muscle group.

1.4.2. Tissue Specificity.

Interestingly, Devlin *et al.* (1990) found that net forearm [^{15}N] Lys and [^{13}C]Leu balance was unchanged after exercise, but there were decreases in forearm MPS and MPD. This suggested that increases in post-exercise PRO synthetic rates may be confined to the previously exercised muscles and/or the increased WBPS during recovery may occur in the liver or other splanchnic tissues (Dohm *et al.*, 1985; Rennie *et al.*, 1981).

1.4.3. Studies Documenting Long-Term Protein Breakdown in Myofibrillar Protein.

Post-exercise contractile PRO breakdown, as indicated by most studies utilizing 3-MH, is elevated for periods greater than one day. Although Dohm and colleagues (1985) found a decrease in the 3-MH/creatinine ratio during a 90 min run at 70% VO_{2max} which is in agreement with the earlier findings of others (Rennie *et al.*, 1981; Calles-Escandon *et al.*, 1984), subjects had an unchanged 3-MH excretion 1 day after the exercise bout. However, there was a significant increase in the 3-MH/creatinine ratio on the second day (Dohm *et al.*, 1985). Similar results were found in 10 soldiers who ran as far as possible during a 2 h period each day for seven consecutive days (Dohm *et al.*, 1985). There was no change in 3-MH excretion on the first day of exercise, but on successive days the excretion was elevated ~20% above resting levels. Later work by the same group (Dohm *et al.*, 1987) found 24-h post-exercise 3-MH excretion increased in proportion to exercise intensity using exercised rats 2 $h \cdot d^{-1}$ for 5 consecutive days at various intensities.

It may be that contractile PRO is preferentially degraded post-exercise (Dohm *et al.*, 1985). Bylund-Fellenius *et al.* (1984) using a perfused rat hindquarter preparation, found the release of 3-MH during muscle contraction decreased dramatically. It is

possible that the biphasic response of contractile PRO breakdown follows the time course of lysosomal enzyme changes observed by Salminen *et al.* (1984).

Viru *et al.* (1984) found that untrained rats showed an increased PRO and free Tyr concentration in the quadriceps muscle 2 to 24 h after a 10 hour swimming regimen in addition to a significant increase in 24 h 3-MH excretion. These findings suggest a long-term simultaneous contractile and non-contractile degradation during recovery that may be related to the exercise duration.

1.5. Signalling Mechanisms Regulating Protein Metabolism.

1.5.1. The Presence of Amino Acids Alone.

Svanberg *et al.* (1996a) have proposed that the anabolic effects of AAs can occur independently of INS and INS-like growth factor (IGF-I). These investigators found an increased AA uptake across the non-exercising arm and leg (but not Tyr, Trp, or Cys), during an 8 h perfusion protocol during which plasma AA concentration progressively increased from 2.6 to 5.7 mM. The net release of AAs, representing globular-PRO breakdown in the fasted state, was changed to an uptake over this time period. However, myofibrillar (3-MH) PRO breakdown was unaffected. The aforementionned findings occured in response to progressively increased AA concentrations despite unchanged blood flow and circulating plasma levels of glucose, FFA, lactate, INS, IGF-I, and binding proteins IGFBP-I and -3. Svanberg and colleagues (1996a) postulate that skeletal muscle AAs are dependent upon a functional threshold for intracellular transport. This threshold would drain AAs from the plasma compartment only when AAs are present in excess. i.e., after feeding. Pool changes of AA concentration within the cell would then elicit a cellular

activation perhaps by phosphorylation/dephosphorylation of initiation factors, in concert with tissue IGF-I (paracrine function) (Svanberg *et al.*, 1996a). Interestingly, in accordance with these findings, Moller-Loswick *et al.* (1991) found that in the presence of subnormal and decreasing plasma AA concentrations, Tyr Ra, and thus PRO breakdown, was decreased.

In an earlier study by Fryburg *et al.* (1995), hyperaminoacidemia was observed to reverse postabsorptive Phe release by forearm muscle to a net uptake. Specifically, Phe Rd increased with no significant change in Phe Ra. Between 3 and 6 h of AA infusion, arterial Phe concentration rose very little (~10 $mol \cdot L^{-1}$). During this period the persistently positive Phe_{BAL} could not be attributed to raising the cell and interstitial water Phe concentration and suggests a true anabolic shift in PHE_{BAL}.

Following resistance exercise, Roy and colleagues (1997) found that FSR of the vastus lateralis was attenuated when a glucose supplement (1 $g \cdot kg^{-1}$) was given immediately post-exercise. The authors attributed the attenuation of FSR to a decrease in plasma AA availability which was likely caused by the increase in post-exercise INS response observed during the supplementation trial. In human and mice studies, in which either glucose, fat, or AAs were provided alone or in combinations, only AAs activated skeletal MPS independently of INS (Svanberg *et al.*, 1996a unpublished observations).

1.5.2. Insulin.

It is well-established that INS independently stimulates PRO anabolism and diminishes PRO catabolism (Kimball & Jefferson, 1988; Bennet *et al.*, 1990; Moller-Lowsick *et al.*, 1994). In addition, INS may act *in vivo* synergistically with nucleotides,

initiation factors, and hormones to regulate PRO synthesis (Garlick *et al.*, 1983). Glucocorticoids, glucagon, Ca^{2+} and prostaglandins are known to decrease PRO synthesis and/or increase PRO degradation in muscle (Fulks *et al.*, 1975; Walton & Gill, 1976; Preedy & Garlick, 1985; Simmons *et al.*, 1984) and, therefore, may be counter-regulatory to INS action post-exercise.

The isotopic study of INS on human muscle PRO metabolism *in vivo* has been difficult for both physiological and technical reasons. The availability of plasma AAs decreases due to INS action on extramuscular tissues, with the resultant hypoaminoacidemia counteracting the anabolic INS effect on muscle (Tessari *et al.*, 1991). Initial attempts to study the effect of INS on PRO_{BAL} by systemic infusion of INS, without concommitant AA infusion, such as that performed by Arfvidsson and colleagues (1991), caused hypoaminoacidemia. The authors investigated the effect hyperinsulinemia (~110 mU L-1) on leg tissue PRO_{BAL} and observed that balance of the BCAAs and methionine were switched from efflux toward influx and muscle tissue concentrations of six AAs, particularly the BCAAs, decreased significantly. Thus, it has been suggested that to best approximate the *in vivo* effects of INS on PRO_{BAL}, simultaneous AA and INS infusions must be performed. Furthermore, rates of MPS and MPB have been measured in human limbs as Ra and Rd from plasma that underestimate actual MPS and MPB because up to 50% of AAs resulting from the latter can be reincorporated into PRO (Biolo *et al.*, 1995).

Maintaining blood AA concentrations at levels higher than normal during systemic INS infusion has been shown by some to increase PRO synthesis (Bennett *et al.*, 1990;

Biolo *et al.*, 1995). Intravenous infusion of labeled AAs across the leg was observed to increase labeled Phe uptake by 50-60% with simultaneous INS infusion, whereas the uptake of labeled Leu rose by only ~10% (Bennett *et al.*, 1990). Although Phe and Leu share the same transporter, the disparity of results with the two labeled tracers suggests some caution should be exercised in the interpretation from these data.

Biolo and colleagues (1995) combined leg arteriovenous catheterization and muscle biopsy data and found PRO synthesis in 6 males, measured either as the Rd of AA (Phe and Lys) from the intracellular pool or as the FSR of muscle PRO increased by 40-65% after INS infusion. Although non-significant, MPB tended to decrease after INS infusion for Phe and Leu. For Lys, however, there was no change. Despite increased Ala, Lys, and Leu transport (+48%, +75%, and +22%, respectively), no change in transmembrane transport was observed for Phe. An observed increase in Ala synthesis after INS infusion was attributed to the INS-mediated increase in glucose uptake and subsequent *de novo* synthesis of intracellular pyruvate in muscle.

Other investigators have found that the mechanism of INS action is to selectively attenuate breakdown of nonmyofibrillar protein (Moller-Loswick *et al.*, 1994; Fryburg *et al.*, 1995) . Using the euglycemic clamp model, Moller-Loswick *et al.* (1994) infused 8 males with L-[U-^{14}C] Tyr, L-[$^{2}H_5$] Phe, and a balanced AA solution, during hyperinsulinemia. Net AA uptake was significantly increased across the arm and leg whereas disposal (PRO synthesis) of Tyr and Phe was unchanged. Tyrosine and Phe Ra (PRO degradation) significantly decreased and 3-MH release from skeletal muscles was totally unaffected in response to hyperinsulinemia.

Support for the findings demonstrated by Moller-Loswick *et al.* (1994) come from work done by Fryburg *et al.* (1995) who infused INS locally (0.035 $mU \cdot min^{-1} \cdot kg^{-1}$) into the forearm of 10 subjects over a period of 6 h via the brachial artery and found that although INS promoted a greater positive shift in Phe_{BAL} than the AA arm alone, INS did not stimulate PRO synthesis above the effect of AAs alone.

1.5.3. Protein Metabolism Sensitive to Insulin and/or Prior Exercise.

Balon and colleagues (1990), using exercised and control Sprague-Dawley rats, generated a model in which hindlimbs were perfused *in situ* with varying concentrations of INS and examined for PRO synthesis and degradation. The authors found that Phe incorporation into PRO was enhanced in the presence of INS (200 $\mu U \cdot ml^{-1}$) yet the increase was almost identical between exercised and unexercised rats 30 and 150 min after the high-intensity bout of exercise. An elevated PRO catabolism (tyrosine release) at 30 min post-exercise was diminished only at supraphysiological concentrations of INS (20000 $\mu U \cdot ml^{-1}$) and further elevated with high versus low intensity exercise. However, the catabolism was transient and Tyr levels returned to baseline values at 150 minutes post-exercise.

The authors suggest that although previous exercise enhances the ability of INS to stimulate glucose and AA transport it does not further enhance the ability of INS to stimulate PRO synthesis or inhibit PRO degradation. The authors further suggest that prior exercise may selectively enhance specific signals generated by INS in skeletal muscle but the site and mechanism of this enhancement must be distal to the INS receptor because

prior exercise does not alter INS binding (Zorzano *et al.*, 1986) or activation of the intrinsic PRO Tyr kinase of the β-subunit of the INS receptor (Treadway *et al.*, 1989).

It is possible that a local serum factor is responsible for an increase in the susceptibility of the AA transport process in muscle to stimulation by INS. Evidence supporting this possibility is provided by *in vitro* and *in situ* glucose transport experimentation utilizing rat epitrochlearis muscles (Gao *et al.*, 1994). The isolated epitrochlearis muscle preparation is useful for metabolic study, since it eliminates the influence of non-muscle tissues, innervation, hormones, and other humoral factors, particularly during prolonged incubation (Harter *et al.*, 1984).

Gao and colleagues (1994) stimulated rat epitrochlearis muscles to contract *in vitro* after 15 min of incubation in Krebs-Henseleit buffer (KHB) and did not observe an increase in INS sensitivity (30 $\mu U \cdot ml^{-1}$) determined by enhancement of 3-*O*-methyl-D-glucose transport 3.5 h later. However, muscles stimulated to contract immediately after being dissected showed a small but significant enhancement by INS. Furthermore, forelimb muscles stimulated to contract *in situ* via the ulnar nerve and then allowed to recover *in vitro* showed as large an increase in INS sensitivity as that which occured after prolonged swimming (4 x 30 min bouts). Taken together, these findings suggests that a local factor, necessary for induction of an increase in muscle INS sensitivity by muscle contractions, is rapidly lost when muscles are incubated in KHB *in vitro*. Such a postulate was suggested by Richter *et al.* (1984) who stimulated rat hindlimb muscles to contract *in situ* via the sciatic nerve and found a relatively brief stimulation of INS-independent glucose transport and a more prolonged increase in INS sensitivity.

The local serum factor responsible for INS sensitivity is likely a PRO of molecular mass >10000 as (1) charcoal treatment of serum did not decrease serum effectiveness, (2) serum protein digestion by trypsin removed serum factor activity, and (3) serum ultrafiltration found that the retained filtrate (molecular weight >10000) maintained INS sensitivity (Gao *et al.*, 1994). Further investigation determined that the local factor is not INS, IGF-I, albumin, or serum α-, β-, γ-globulin.

Davis & Karl (1986), using a incubated epitrochlearis muscle preparations of female rats accustomed to swimming (2 h, 5 days a week), found net PRO breakdown (Tyr release) increased and PRO synthesis ([^{14}C] Phe radioactivity) decreased immediately after single exercise bout. The authors contend the latter to be the primary change in muscle PRO turnover following endurance exercise (1990). Furthermore, the authors found that incubated muscles having experienced a prior acute bout of exercise were, *in vitro*, more sensitive to INS (0.1-100 μU·ml-1) as shown by increased levels of PRO synthesis and diminished degradation thus contradicting the findings of Balon *et al* (1990). Training was also shown to increase *in vitro* INS-sensitivity. An increase in INS-stimulated AA transport in muscle after exercise has been reported (Zorzano *et al.*, 1985).

Davis *et al.* (1987) again used the epitrochlearis muscle preparations of female rats that were made azotemic by a 60% left renal infarction plus a right nephrectomy. Seven days after surgery, azotemic and control rats were exercised for 4 weeks (swimming 2 h·d^{-1}, 5 d·wk^{-1}) or remained sedentary prior to a 2 hr swimming bout. Although INS (0.5 mU·ml^{-1}) increased MPS in all groups, and an increased INS sensitivity was observed in trained rats, only acute exercise in untrained animals (both control and azotemic) further

enhanced the sensitivity of MPS. Nonetheless, previous work by this same group (Davis & Karl, 1986) was largely supported by the present finding that exercise training attenuated the elevated MPB due to azotemia and/or acute exercise and enhanced INS action on muscle.

Further support for the findings of Davis and colleagues (1986; 1987) is provided by Rodnick *et al.* (1990) who trained rats for 6 wks before perfusing their hindquarters with 0, 60, 250 or 6000 $\mu U \cdot ml^{-1}$ INS after a 24 h rest period. Tyrosine release was suppressed by INS in all groups, but this effect was significantly greater in trained rats (T) compared with sedentary(SC) and diet(DC) controls at INS levels of 60 $\mu U \cdot ml^{-1}$ and greater. It also appears that the effect of INS on suppressing net proteolysis in ET rats was more sensitive than its effect on enhancing glucose uptake; at the INS concentration of 60 $\mu U \cdot ml^{-1}$, tyrosine release was suppressed to 50% of maximum wherease glucose uptake was stimulated to only 12% of maximum. Neither INS nor training decreased 3-MH release which confirms previous studies that INS does not modify myofibrillar protein turnover (Goodman *et al.*, 1987a; 1987 b).

1.5.4. Summary of Possible Signalling Mechanisms.

Thus, following exercise, evidence exists for (1) prior exercise independently increasing PRO synthesis and breakdown and (2) INS alone increasing PRO anabolism either by increasing PRO synthesis and attenuating the non-myofibrillar PRO breakdown (Davis *et al.*, 1986; 1987; Rodnick *et al.*, 1990; Balon *et al.*, 1990; Biolo *et al.*, 1995) or by reducing PRO breakdown alone (Moller-Loswick *et al.*, 1994; Fryburg *et al.*, 1995). Although most studies have found the effect to be additive (Davis *et al.*, 1986; 1987;

Rodnick *et al.*, 1990), some research contradicts this (Balon *et al.*, 1990). Gao and colleagues (1994) suggest that other PROs are capable of modulating contraction-induced increases in substrate uptake. In fact, some investigators (Fryburg *et al.*, 1995; Svanberg *et al.*, 1996b) attribute enhanced PRO anabolism to IGF-1to a greater extent than INS. Nonetheless, the increased PRO anabolism appears to be restricted to globular PRO (Svanberg *et al.*, 1996a; Goodman *et al.*, 1987a; 1987b) and may be limited by the availability of AAs (Fryburg, 1994; Fryburg *et al.*, 1995; Svanberg *et al.*, 1996a).

1.6. Sex Differences in Protein Metabolism.

At present, there is a paucity of research available on sex differences in PRO metabolism. Tarnopolsky *et al.* (1990) showed that males may catabolize more PRO than females consequent to endurance exercise. During a 24 hr period of urine collection on the day of a 65% VO_{2max} bout of exercise, males had a 32% greater urea N excretion than females. The investigator suggested that the greater glycogen depletion observed in males could have induced the greater PRO catabolism. In addition, males had lower INS concentrations, possibly related to the inhibitory effects of larger epinephrine concentrations (Tarnopolsky *et al.*, 1990), which may have accounted for an increased PRO catabolism. Data to support these findings can be drawn from those of Dohm & Louis (1978) wherein male rats have been demonstrated to excrete more urinary urea N after exhaustive exercise than female rats..

Phillips *et al.* (1993) adapted male and female subjects to a diet isoenergetic with their habitual intake for 10 days prior to completion of a 3 day N balance measurement period during which subjects ran at 65% VO_{2max} for 90 minutes on days 1 and 3.

Adaptation to the new nitrogen intake (Canadian RNI 0.86 $g \cdot kg^{-1} \cdot d^{-1}$) during this period was confirmed by no significant day-to-day variation in urea nitrogen excretion. N balance showed that the RNI was inadequate for females and males (-15.9 $\pm$ 6.0 and -26.3 $\pm$ 11.0 $mg \cdot kg^{-1} \cdot d^{-1}$ respectively). On day 3, the nonprotein RER data showed that males oxidized a greater amount of CHO during exercise. Leucine oxidation during endurance exercise increased 84% and 95% in trained men and women respectively but in absolute terms was 70% higher in men during exercise. No difference in NOLD was seen. Thus, higher Leu oxidation in male athletes, at rest and during exercise, could result in their having an even higher PRO requirement than female athletes as evidenced by the more negative N_{BAL}.

The greater lipid oxidation seen in females could be a result of increased oxidation of extracellular or intracellular FFAs, a process that may serve to "spare" muscle glycogen (Phillips *et al.*, 1993). Limiting access to this glycogen pool may result in a reduced usage of PRO for gluconeogenesis (Lamont *et al.*, 1990).

1.6.1. Menstrual Cycle Effects on Protein Metabolism.

Daily urinary urea nitrogen excretion resembled a bi-phasic sine wave function when studied across the menstrual cycle in a well-controlled nitrogen balance study (Calloway & Kurzer, 1982). Although this study failed to determine cycle phase by not using ovarian hormone concentrations, maximum urinary urea nitrogen excretion was observed during the mid-follicular and mid-luteal phase, while minimum excretion occured with the onset of menses and about the time of ovulation.

Lamont and colleagues (1987) had 9 females cycle for 60 min at 70% VO_{2max} when serum estradiol and progesterone were low (menses; 2-4 days after onset of menstural flow) and when both were elevated (mid-luteal; days 20-22 of menstrual cycle). No significant differences in serum or sweat urea nitrogen due to exercise or time of the menstrual cycle were found. However, mid-luteal exercise day urinary urea nitrogen excretion was significantly greater than menses (8.5 $\pm$ 0.96 vs 5.5 $\pm$ 0.81 g). In addition, total urea nitrogen excretion (sweat and urine for experimental days) was significantly greater in the mid-luteal phase compared to menses (24.8 $\pm$ 2.38 vs 19.3 $\pm$ 1.38 g). These data, when converted to protein equivalents, indicate that more protein was catabolized during the mid-luteal phase than during menses (235.7 $\pm$ 22.54 vs 182.7 $\pm$ 13.03 g). In other human studies, progesterone has been shown to have catabolic properties and estrogens to alter enzymes responsible for cortisol metabolism (Lamont *et al.*, 1987).

1.7. Post- Exercise CHO/PRO Supplementation.

Due to the paucity of research in post-exercise supplementation, especially on indices of protein metabolism, results from endurance (Blom *et al.*, 1987; Burke *et al.*, 1995; Ivy *et al.*,1988; Reed *et al.*, 1989; Zawadzki *et al.*, 1992; Parkin *et al.*, 1997), and resistance (Chandler *et al.*, 1994; Roy *et al.*, 1997) activity will be considered in the following review.

1.7.1. Post-Exercise CHO Supplementation.

Contraction-stimulated glucose transport is mediated by Glut-4 probably by a nitric-oxide dependent pathway (Roberts *et al.*, 1997). If glucose feeding is maintained post-exercise at 2-hr intervals, increased rates of glycogen storage are

demonstrated for up to 6-8 h after exercise (Blom *et al.*, 1987; Reed *et al.*, 1989). Although the most rapid rate of glycogen resynthesis has been shown to occur within the first 4 hours (Ivy *et al.*, 1988, Burke *et al.*, 1995; Roy *et al.*, 1996b), complete recovery to pre-exercise glycogen values may be accomplished within 24 h (MacDougall *et al.*, 1977). Consumption of a supplement containing CHO immediately post-exercise, results in the quickest replenishment of body glycogen stores.

Roy and colleagues (1997) investigated the effect of glucose supplementation (1 $g \cdot kg^{-1}$), given immediately and +1 h following post-resistance exercise, upon skeletal muscle FSR, urinary urea excretion, WBPD, and MPD in 8 healthy young males. FSR was ~30% greater in the CHO than observed in the PL condition. Plasma INS concentration was greater in the CHO condition. 3-MH excretion was lower in the than PL condition (CHO, 110.43 $\pm$ 3.62 vs. PL, 120.14 $\pm$ 5.82 $\mu mol \cdot g$ creatinine^{-1}) as was urinary urea nitrogen (CHO, 8.60 $\pm$ 0.66 vs PL, 12.28 $\pm$ 1.84 $g \cdot g$ creatinine^{-1}) suggesting that post-exercise CHO supplementation may attenuate PRO breakdown resulting in a more positive muscle and whole body N_{BAL}.

1.7.2. Addition of Protein to Post-exercise CHO Supplements.

Insulin release is stimulated primarily by CHO, but PRO has also been demonstrated to stimulate its release (Nuttal *et al.*, 1984). Bennet *et al.* (1990) have suggested that hyperinsulinemia appears to be most effective in promoting MPS when hyperaminoacidemia is present. Evidence has suggested that consumption of a combined CHO + PRO supplement may result in a greater plasma INS response (Zawadzki *et al.*, 1992; Chandler *et al.*, 1994).

Zawadzki *et al.* (1992) found combined PRO + CHO supplementation (112g CHO, 47 g PRO) was more effective in promoting glycogen resynthesis than either CHO (112 g) or PRO (47 g) supplementation alone because of the former's larger INS response. However, the author's interpretation of these findings is complicated by the fact that the nutritional interventions were non-isoenergetic. The combined PRO + CHO supplement was the individual CHO supplement added to the PRO supplement, thus resulting in a supplement with a 42% greater energy content. Furthermore, the increased glucose response opposes previous findings (Nuttal *et al.*, 1984; Spiller *et al.*, 1987) wherein increased INS concentrations were accompanied by decreased plasma glucose levels. Zawadski (1994) attributed this to a greater suppression of hepatic glucose production with CHO + PRO supplementation versus CHO supplementation alone.

Chandler *et al.*(1994) found larger post-resistance exercise INS and growth hormone concentrations using combined CHO/PRO (1.06 g CHO + 0.41 $g \cdot kg^{-1} \cdot$body $mass^{-1}$) supplementation compared to isoenergetic drinks of either alone (CHO, 1.5 vs PRO, 1.38 $g \cdot kg^{-1} \cdot$body $mass^{-1}$) in a group of weight-trainers. Supplements had no effect on IGF-I. The larger induced growth hormone concentrations observed in this study at 6 h post-exercise would, in addition to INS, promote MPS.

Burke *et al.* (1995) studied the effect of coingestion of fat and PRO with CHO feedings on muscle glycogen storage. Eight well-trained triathletes (VO_{2max} = 4.7 $\pm$ 0.4 $L \cdot min^{-1}$) cycled for 2 h at 75%VO_{2max}, followed by 4 X 30s Wingate tests. Subjects were then fed either a CHO diet (7 $g \cdot kg^{-1} \cdot d^{-1}$), a CHO/PRO/FAT diet (7 $g \cdot kg^{-1} \cdot d^{-1}$ CHO +1.6 $g \cdot kg^{-1} \cdot d^{-1}$ FAT + 1.2 $g \cdot kg^{-1} \cdot d^{-1}$ PRO), or a matched-energy diet (7 $g \cdot kg^{-1} \cdot d^{-1}$ CHO + 4.8

$g \cdot kg^{-1} \cdot d^{-1}$ CHO). Meals were eaten at t = 0,4,8,and 21 h of recovery. The CHO/PRO/FAT diet resulted in both a significantly elevated plasma triglyceride and serum FFA response, and a reduced plasma glucose area when compared to the other diets. No differences between trials in glycogen storage over 24 h was observed.

Roy and colleagues (1996b) have studied the effect of isoenergetic CHO and CHO/PRO/FAT (68% CHO, 22% PRO, 10% FAT) supplements given immediately (t=0 h) and 1 h (t=+1h) post-resistance exercise in ten healthy young males. Interestingly, no significant difference in INS response was observed between the isoenergetic CHO/PRO/FAT and CHO only supplements when taken post-resistance exercise. Muscle glycogen was significantly lower for the EX vs. CON leg immediately post-exercise for all three conditions. Furthermore, an increased rate of glycogen resynthesis was observed for both CHO and CHO/PRO/FAT (23.0 ± 4.5, 24.9 ± 5.1 $mmol \cdot kg^{-1}$ $dm \cdot h^{-1}$) vs. PL (EX=2.0 ± 2.3, CON= 3.4 ± 4.5 $mmol \cdot kg^{-1}$ $dm \cdot h^{-1}$). The rates of resynthesis were similar to those observed following endurance exercise (Zawadzki *et al.*, 1992).

Parkin and colleagues (1997) examined the effect of delaying the ingestion of PRO/CHO/FAT meals on muscle glycogen storage following prolonged exhaustive exercise. Following the exercise bout (70% VO_2max, 2 h, + four, 30 s sprints), subjects were fed 5 high glycemic index PRO/CHO/FAT meals (2.5 g $CHO \cdot kg^{-1}$) over a 24 h period, with the first 3 being fed either at 0-4 h or 2-6 h at 2 h intervals. The remaining meals were consumed at 8 and 22 h post-exercise. No significant difference between trials was observed for glycogen concentration at all muscle biopsy time points (0, 8, and 24 h). Furthermore, no differences were observed in the incremental glucose and INS areas after

each meal when trials were compared. This study indicated that, providing that sufficient CHO is ingested during the recovery period, a delay of a meal by 2 h has no effect on the rate of muscle glycogen resynthesis at 8 and 24 h post exercise.

1.8. Summary.

Protein requirements have been shown to be greater with exercise (Tarnopolsky *et al.*, 1988; 1992; Meredith *et al.*, 1989). Post-exercise nutritional supplements may improve PRO_{BAL} with no change in energy intake. Firstly, this is based upon a more rapid rate of recovery of glycogen stores with immediate post-exercise supplementation (Ivy *et al.*, 1988; Roy *et al.*, 1996b). This is important considering that AA oxidation has been shown to be directly related to decreasing availability of glycogen storage (Lemon & Mullin, 1980). Secondly, as Roy and colleagues (1997) have suggested, the corresponding INS response and contraction-stimulated Glut-4 migration to the sarcolemma may further improve glycogen stores. Finally, protein anabolism has been shown to improve in the presence of AAs alone (Svanberg *et al.*, 1996a) and intracellular transport of AAs has been shown to be enhanced in the presence of INS (Moller-Loswick *et al.*, 1994; Biolo *et al.*, 1995).

Chapter II. Energy Balance.

2.1. Introduction.

An individual in a steady state over a period of time may be expressed by the following equation:

$$E_{IN} = E_{OUT}$$

in which the amount and composition of energy consumed equals the amount and composition of fuel burned, the energy required to maintain that state can be determined by measuring either E_{IN} or E_{OUT} (Hill *et al.*, 1995).

Total E_{OUT} consists of three components:

$$E_{OUT} = RMR + TEF + EE_{ACT}$$

RMR is the energy expenditure measured in a resting subject after an overnight fast, and approximates internal work or the minimum energy expenditure necessary for maintenance of critical body functions. TEF is the increase in energy expenditure that occurs after ingestion of food and represents the cost of absorption, digestion, and metabolism of ingested nutrients (Flatt, 1978). EE_{ACT} is the increase in energy expenditure associated with performing external work (Hill *et al.*, 1995).

2.2. Resting Metabolic Rate (RMR)/ Internal Work.

RMR accounts for more than 50% of total daily energy expenditure in sedentary persons (Ravussin et al., 1986). A strong positive correlation exists between fat-free mass (FFM) and RMR (Ravussin *et al.*, 1986). FFM consists of all nonfat mass (eg. amount of skeletal muuscle, liver mass, etc.) and accounts for 60-80% of the between-subject variation in RMR (Ravussin *et al.*, 1986). Adipose tissue, being less metabolically active

than FFM and generally comprising a lower proportion of body mass, contributes less to RMR (Armellinin *et al.*, 1992).

2.2.1. The Effect of Diet Composition on RMR.

Hill and colleagues (1993) have concluded that diet composition may have secondary effects on RMR through long-term effects on body composition. Studies in humans have shown that voluntary E_{IN} tends to increase, with increases in the proportion of dietary energy supplied by fat (Tremblay *et al.*, 1989; Thomas *et al.*, 1992). Although some reports have indicated that diets high in sweet CHOs elevate long-term E_{IN} in animals (Ramirez & Friedman, 1990), a similar long-term effect has not been observed in humans. High PRO loads in humans acutely reduce subsequent E_{IN} relative to low PRO foods (Booth *et al.*, 1970). The effects of PRO are probably of limited importance in humans because PRO makes up a small and relatively consant proportion (10-15%) of calories (Hill *et al.*, 1993).

2.2.2. The Effect of Physical Activity on RMR.

Although a single bout of exercise will probably not change the active metabolic tissue mass or produce a new metabolic state (Hill *et al.*, 1995), chronic exercise may challenge the steady state system to adapt and acquire a new steady state.

The acute effect of exercise on RMR has been discounted in studies allowing 24 hours of rest post-exercise before RMR measurements were taken (Hill *et al.*, 1995). Such studies identified higher RMR in trained athletes to which Poehlman and colleagues (1988) attribute to adaptations to chronic exercise. Studies using longer intervals (48h-56h) between previous exercise and RMR measurement failed to find elevations of RMR

in trained subjects (Herring *et al.*, 1992; Schultz *et al.*, 1991). In cross-sectional studies both an elevated RMR (Poehlman, 1989; Poehlman *et al.*, 1990) and an unchanged RMR (Broeder *et al.*, 1992) have been seen in endurance-trained individuals compared with sedentary, untrained subjects, independent of differences in body composition. A recent study of more than 500 men and women reported that VO_{2max} was a significant predictor of RMR independent of body weight and composition (Anciero *et al.*, 1993).

Using a room calorimeter, Sharp and colleagues (1992) found that VO_{2max} was unrelated to RMR, TEF, EE_{ACT}, or total daily E_{OUT} independently of body composition. The discrepancy in results may be due in part to the time interval between the last bout of exercise and RMR measurement and to E_{IN} during the days prior to testing.

In trained women who had abstained from exercise for ≥ 36 h, Ballor and Poehlman (1992) observed an elevated RMR compared to untrained women. However, because E_{IN} was not controlled for post-exercise, a rapid increase in E_{OUT} may have been due to overfeeding (Horton *et al.*, 1995).

2.2.3. The Effect of Energy Intake and/or Physical Activity on RMR.

It has been observed that RMR decreases in exercising athletes when they move to an energy-deficient state (Melby *et al.*, 1990). Combining an increased EE_{ACT} with caloric restriction may preserve FFM, but the effect is small and is unlikely to have a measurable effect on the decrease in RMR that accompanies weight reduction (Hill *et al.*, 1987).

Poehlman and colleagues (1989) suggested that endurance-trained athletes, even when they are in E_{BAL}, could elevate their RMR with a high energy turnover (high E_{IN} and E_{OUT}). Bullough *et al.* (1995) found that, on a high E_{IN} diet after an acute bout of high-

intensity exercise, the RMR of trained subjects was elevated compared to untrained subjects but that the elevation was attenuated in the trained subjects as the time interval increased from the last exercise bout to the measurement of RMR. These investigators postulate that the RMR is elevated in individuals who engage in daily, high-intensity, prolonged exercise, as the result of an acute exercise rather than an adaptation to chronic exercise.

2.3. Thermic Effect of Food.

Using 24 h room calorimetry, Ravussin and colleagues (1986) determined that the TEF accounted for ~7% (165 kcal·d^{-1}) of daily E_{IN} (177 volunteers/12 females, age 27, body fat 32%, E_{IN} 2360). The inaccuracy of 24 h room calorimetry in measuring the TEF makes comparison with results of Bessard *et al.*(1983) and Segal *et al.* (1985) difficult. These investigators showed an inverse relationship between body fat and the TEF when measured over shorter periods of time.

2.3.1. The Effect of Diet Composition on TEF.

Flatt (1985) found that at the same E_{IN}, differences in both the amount of food consumed and the composition of the diet had a small effect on TEF, because of the differing costs of metabolizing PRO, CHO, and fat (Flatt, 1985). However, Hill *et al.*(1984) found no measurable difference in E_{OUT} during a 24-hour period when the proportion of dietary fat was increased from 20% to 60% of total calories and the proportion of PRO was kept constant. Furthermore, Kinabo and Durnin (1990) measured the TEF of non-obese females (n=18) for 6 hr postprandially and found TEF was not influenced by either meal frequency (1 meal: 90 $\pm$ 7.2 kcal vs. 2 meals: 91.0 + 6.3 kcal) or

differences in fat or CHO content ([70% CHO, 19% Fat, 11% Pro] 85.0 $\pm$ 5.5 kcal vs. [24% CHO, 65% Fat, 11% Pro] 81.0 $\pm$ 3.8 kcal).

2.3.2. The Effect of Caloric Restriction on TEF.

For a person consuming 2500 kcal•d^{-1}, the TEF of a diet high in fat content (40% of calories) is approximately 30 kcal•d^{-1} higher than the TEF of a diet low in fat content (20% of calories). Reducing caloric intake from 2500 kcal•d^{-1} to 1200 kcal•d^{-1} results in a 50% reduction in TEF. At this caloric intake, the absolute difference in TEF between the high (40% of calories) and low fat (20% of calories) diets was only 15 kcal•d^{-1}. Thus, differences in diet composition has a small overall effect on E_{OUT} and an even smaller effect during caloric restriction (Hill *et al.*, 1993).

When the body is in negative E_{BAL}, body energy stores are consumed to sustain metabolic processes, and weight loss ensues. When a negative E_{BAL} is achieved through reduced E_{IN}, the primary and short-term effect on E_{OUT} is a reduction in the TEF proportional to the reduction in E_{IN} (Hill *et al.*, 1993). With further caloric restriction, the decrease in body mass is concommitant with a reduced RMR and EE_{ACT}, the latter being due to a lower energy cost of movement (Weigle, 1988).

2.3.3. The Effect of Physical Activity on TEF.

Whether or not physical activity influences TEF remains to be determined. An increase in TEF has been correlated with increased physical fitness level by some (Hill *et al.*, 1984), but not all (Gilbert *et al.*, 1991; LeBlanc *et al.*, 1984; Owen *et al.*, 1986; Dauncy, 1990) investigators. Witt *et al.* (1993) proposed that the magnitude of TEF is

dependent upon the time interval between the last exercise bout and the TEF measurement.

2.3.4. The Effect of Physical Activity and Food Intake on TEF.

It has been shown that subjects eating prior to exercise or while exercising show a greater TEF (Gleeson *et al.*, 1979a,b; Segal & Bernard, 1993). It is thought that increased activation of the sympathetic nervous system during exercise is responsible for the observed increase in TEF. Epinephrine levels have been found to be positively correlated with the TEF in both males and females (Frey *et al.*, 1993; Jensen *et al.*, 1995). Segal (1989) proposed a second mechanism that would contribute to a greater TEF with exercise (↑ norepinephrine = ↑ Na^+/K^+ pump + oxidative phosphorylation uncoupling) (Frey *et al.*, 1993)), wherein exercise and food produce conflicting hormonal signals that would result in increased rates of glucose, FFA, and AA cycling.

2.3.5. Other Correlates of TEF.

Tataranne and colleagues (1995) used a respiratory chamber to examine the determinants of TEF in 471 subjects and found the TEF of females (n=180) correlated negatively with fasting plasma glucose and INS concentrations ($p<0.01$) while fasting INS levels were not significantly correlated with the TEF of males (n=291). Brundin *et al.*, (1992) showed that the TEF is a function of the heat leakage across the abdominal wall, which is inversely related to the thickness of the abdominal adipose tissue layer. Therefore, since females have a greater body fat percentage (Tarnopolsky *et al.*, 1990), they may exhibit a smaller TEF when compared to males.

2.4. Physical Activity/External Work (EE_{ACT}).

Physical activity varies greatly between individuals and within the same individual. Unlike RMR, the factors that can explain the intersubject variation in amount and energy cost of physical activity are not well understood. Some variation between individuals in EE_{ACT} can be attributed to differences in body weight and fitness level (Hanson, 1973) yet physical activity remains the largest source of individual differences in E_{OUT}.

EE_{ACT} varies with characteristics of both the exercise (ie. mode of exercise frequency, intensity, and duration) and the subject (ie. body weight and exercise economy). Whole room calorimetry has found EE_{ACT} to range from 830-4180 $kJ \cdot d^{-1}$ with non-purposeful SPA, i.e., fidgeting, accounting for 100-800 $kcal \cdot d^{-1}$ (Ravussin *et al*, 1986; Hill *et al.*, unpublished observations, 1995).

2.4.1. Excess Post-Exercise Oxygen Consumption (EPOC).

E_{OUT} does not return immediately to normal upon cessation of exercise and the magnitude and duration of this effect is controversial (Bahr & Maehlum, 1986). Frey and colleagues (1993) have reported an EPOC threshold of intensity and duration which produced a significantly longer EPOC of several hours. Maehlum and colleagues (1986) found EPOC was elevated for 12 hours in athletes (4 females, 4 males) that had exercised for 90 minutes on a cycle ergometer at 70% VO_{2max}. Mean total O_2 consumption after exercise was 211 ± 16 $L \cdot 12\ h^{-1}$ versus 185 ± 13 $L \cdot 12h^{-1}$ in the control trial, an increase of approximately 12% above resting VO_2 values.

Brehm and Gutin (1986) examined the effect of exercise intensity and aerobic fitness on the energy expended during recovery from walking and running exercise. Post-

exercise O_2 consumption was observed to increase with exercise intensity in both groups. However, no significant difference between runners and nonexercisers in recovery VO_2 response to steady state walking was found in this study.

Sedlock and colleagues (1989) found exercise intensity to have a greater influence on the magnitude of post-exercise metabolic rate to a greater extent than exercise duration. It appears that the magnitude of this post-exercise elevation of metabolic rate is dependent on fitness level. E_{OUT} returns to baseline within 5-40 min post-exercise (40-70% VO_{2max}, 15-40 min) and accounts for an additional 21-125 kJ expended beyond the exercise bout itself (Freedman-Akabas *et al.*, 1985). EPOC may be a larger contributor to total daily E_{OUT} in individuals capable of performing high-intensity, long-duration exercise (Bahr & Sejersted, 1991).

Chad & Quigley (1991) reported that well-trained women maintained higher VO_2 than an untrained group during 3 h of recovery. Exercise consisted of 30 min of cycling at 50 or 70% VO_2max. Trained subjects had a significantly higher rate of postexercise fat utilization that may have contributed to EPOC, but a suitable explanation for why this effect was more apparent in trained than untrained individuals was not provided.

Short & Sedlock (1997) had trained and untrained subjects complete two 30-min cycle ergometer tests (70% VO_2max) on separate days in the morning, after a 12 h fast. Trained subjects had a similar EPOC duration (Trained 40 $\pm$ 15 min, Untrained 39 $\pm$ 14 min) and EPOC magnitude was not different between groups. However, when a correction for the higher exercise VO_2 was made, trained subjects had a faster relative decline during the fast-recovery phase.

Conversely, *Frey et al.* (1993) have demonstrated that trained women who cycled at either 80% (24 min) or 65% VO_2max (45 min) had shorter EPOC duration than their untrained counterparts. Post-exercise O_2 consumption duration was considered longer in the untrained group due to a mean elevation of 30 ml min at 60 min of recovery (statistically different from basline). Total EPOC magnitude was not different between groups but EPOC accumulated during the fast portion of recovery (10 min) was larger for trained subjects. Approximately one-half of the total EPOC was accumulated during the fast phase so, in effect, the VO_2 at the conclusion of exercise can have a sizable impact on EPOC.

2.4.2. Proposed Mechanism of Excess Post-Exercise Oxygen Consumption (EPOC).

It has been suggested that an increase in lipid metabolism and substrate cycling post-exercise may contribute to a prolonged EPOC (Frey *et al.*, 1993; Maehlum *et al.*, 1986). In addition, high plasma lactate and plasma norepinephrine levels have been positively correlated with initial increases in EPOC (Frey *et al.*, 1993; Barnard *et al.*, 1969; Cain *et al.*, 1981). Trained subjects are known to have higher oxidative capacities in trained muscle groups (Saltin & Gollnick, 1983), which might be expected to result in greater fat utilization and lower RER after exercise (Short & Sedlock, 1997). Some studies have reported a significant correlation between EPOC and rectal temperature (Brehm & Gutin, 1986) whereas others have not (Maehlum *et al.*, 1986). Brehm & Gutin (1986) found post-exercise rectal temperature accounted for ~28% of elevations in O_2 consumption.

Sloman (1990) noted that an elevated plasma lactate concentration is likely a poor contributor to prolonged EPOC since the primary fate of post-exercise lactate is oxidation. Norepinephrine has been positively correlated with EPOC in animal studies that have directly infused antagonists of α- and β-receptors (Barnard *et al.*, 1969; Cain *et al.*, 1981).

2.5. Possible Sex Differences in Energy Balance.

Males have higher metabolic rates than females on the basis of body size alone (Ferraro *et al.*, 1992). When BMR is corrected for individual differences in FFM, similar metabolic rates are observed between the sexes (Cunningham, 1980). In a 24 h comparison of metabolic rate between males (n=7) and females (n=8), Webb (1981) reported that 24 E_{OUT} corresponded with FFM and was not influenced by sex. Ravussin *et al.* (1986) confirmed that FFM was the best determinant of 24 E_{OUT} in a multiracial population and sex was not a contributing factor. However, 27 subjects, including 15 females, had non-INS dependent diabetes mellitus, a condition in which metabolic rate is increased (Bogardus *et al.*, 1986) and thus may have been a confounding factor.

Women (n=121) may indeed have a 5-10% lower (124 + 38 kcal d) 24 h sedentary E_{OUT} than men (n=114) (Ferraro *et al.*, 1992). BMR, measured using a ventilated plastic hood placed over the subject's head, and SMR, measured between 2300 and 0500 hours were similar between sexes with a tendency toward higher values in males. Since 24 h SPA, expressed as the percent of time during which a subject was moving as measured continuously by two microwave radars, was not significantly different between males and females, none of the measured components of E_{OUT} could account for the difference.

Sex differences in EE_{OUT} have been explained by differing androgen levels in animal studies. Basal EE_{OUT} has been reported to be 18% higher in postpubertal bulls (Webster *et al.*, 1977) and 13-17% higher in rams compared with their castrated male counterparts (Graham, 1967).

In human studies, basal O_2 consumption, adjusted for body composition, correlated with urinary 17-ketosteroid excretion in maturing boys (r=0.66) but not with girls aged 8-18 yrs and the agent attributed to this difference was testosterone (Clark & Garn, 1954). Bruemann *et al.*(1983) determined that 24 h EE_{OUT} adjusted for FFM, FM, and age was higher in obese women with an android fat distribution compared to those with a gynoid distribution using whole body direct calorimetry. Free testosterone levels have been found to be higher in women with an android pattern of fat distribution compared with women with gyecoid distribution (Kirchner *et al.*, 1990).

2.5.1. Contribution of Menstrual Cycle Phase to Energy Balance.

Rogol *et al.* (1992) reported no alteration in menstrual cycle or reproductive hormone concentrations in older women who had normal function before initiating a progressive exercise program of moderate distance and intensity. In the women who trained at a higher intensity, a decrease in luteal phase length only was observed.

It has been reported that 24 h E_{OUT} is 9% higher in females during the luteal phase of the ovulatory cycle (Webb, 1986). Ferraro *et al* (1992) found that 24 h E_{OUT} (106 $\pm$ 39 $kcal \cdot d^{-1}$), BMR (199 $\pm$ 53 $kcal \cdot d^{-1}$), and SMR (108 $\pm$ 42 $kcal \cdot d^{-1}$) adjusted for differences in FFM, age, and SPA, were higher for females during the luteal a s compared to follicular phase. Furthermore, the authors found no significant differences in E_{OUT},

adjusted for body composition, age, and SPA between post-menopausal (n=28) and pre-menopausal (n=42; 30 follicular, 12 luteal) females.

Mulligan & Butterfield (1990) and Wilmore *et al.* (1992) report equivalent RMR levels among amenorrheic runners, eumenorrheic runners, and sedentary controls despite reports of the amenorrheic runners being in negative E_{BAL}. Baer (1993) reported that female runners with a consistent daily E_{IN} (Eumennorheic 1944 $\pm$ 45.0 kcal•d^{-1}; Amennorheic 1627 $\pm$ 75 kcal•d^{-1}) maintained their body weight on such an intake despite training a minimum of 4-8 miles·d^{-1}. To reconcile the disparity between reported low E_{IN} and higher E_{OUT} in amennorheic females, it has been suggested that amennorheic females become more metabolically efficient in an attempt to conserve energy stores and follicular development (Warren, 1983; Brownell *et al.*, 1987; Mulligan & Butterfield; 1990). It has also been suggested that amennorheic subjects underreport actual E_{IN} (Wilmore *et al.*, 1990) and, furthemore, that problems exist with the data collection and methodology used to estimate E_{OUT} (Mulligan & Butterfield; 1990). Interestingly, both Myerson and colleagues (1991) and Baer (1993) reported total T3 levels as well as RMR levels to be significantly lower in amennorheic vs eumennorheic female runners.

2.6. Summary of Energy Balance.

When BMR is corrected for individual differences in FFM, similar metabolic rates are observed between the sexes (Cunningham, 1980). Sex differences in EE_{OUT} have been explained by differing androgen levels in both animal (Graham, 1967; Webster *et al.*, 1977) and human (Clark & Garn, 1954; Kirchner *et al.*, 1990) studies.

Janssen and colleagues (1989) studied males and females during 18 months of training for a marathon. Despite a significant increase in E_{IN} and altered body composition for the males, no changes were observed for either E_{IN} or body composition in the females despite an increased energy requirement for the completion of the marathon. Similarly, Deuster and colleagues (1986) reported low caloric intakes for females running 10 miles·d^{-1}. Although females tend to underreport their dietary intakes (Deuster *et al.*, 1986), it has been suggested that females increase metabollic efficiency in case of energy restriction, as the body seeks to protect and replenish its energy stores (Warren, 1983; Brownell *et al.*, 1987; Mulligan & Butterfield; 1990). Thus, post-exercise nutritional supplementation may serve as a vehicle to increase the efficiency of energy storage when the body is in negative E_{BAL}.

Chapter III. The Research Paper.

Protein Metabolism and Energy Utilization in Females Participating in Endurance Activity: The Effect of Nutritional Supplement Timing.

INTRODUCTION.

To date a paucity of research exists on the effects of nutritional supplementation in the post-exercise period upon protein (PRO) balance. This is surprising considering amino acids (AA) and PRO are significantly important to exercise metabolism in at least three important ways: During prolonged exercise, AAs are essential to replete metabolic intermediates of the Krebs (TCA) cycle. Secondly, the AA formation of alanine by skeletal muscle is an important gluconeogenic precursor to maintain blood glucose homeostasis during exercise. Finally, although the oxidation of branched-chain amino acids (BCAAs) to substrate fuel is relatively small (5-10%), because of their large tissue masses, AAs and PRO represent a significant fuel supply to support prolonged exercise (Brooks *et al.*, 1996). Tarnopolsky *et al.* (1988) and Phillips *et al.* (1993) have demonstrated that the current Canadian RNI for protein is inadequate for well-trained endurance athletes.

Although female athletes do not catabolize protein to the same extent during exercise as their male counterparts (Tarnopolsky *et al.*, 1990; Phillips *et al.*, 1993), this population may be particularly at risk for protein deficiency since they characteristically have low protein intakes (Mulligan & Butterfield, 1990) and ususally do not increase their energy intakes to compensate for the increased energy expenditure which occurs with

regular endurance exercise (Janssen *et al.* (1989). When combined with the stress of competition, reduced body protein and energy stores may also contribute to the bone and menstrual irregularities frequently documented in such athletes (Bonen, 1994; Drinkwater, 1996; Dueck *et al.*, 1996). It was thus considered of primary importance to assess different strategies for maintaining a positive protein balance in such athletes.

Several methods for promoting a more positive nitrogen balance have been elucidated for females undergoing endurance training. An improved protein status has been shown with the consumption of more dietary protein per day (Tarnopolsky *et al.*, 1988; Meredith *et al.*, 1989; Phillips *et al.*, 1993). Furthermore, protein oxidation is decreased with carbohydrate loading prior to an exercise bout (Lemon & Mullin, 1980). However, since females may be limited in their ability to glycogen supercompensate by being unable to carbohydrate load (Tarnopolsky *et al.*, 1995), the presentation of carbohydrate immediately following exercise may attenuate PRO breakdown resulting in a more positive muscle and whole body N_{BAL} (Roy *et al.*, 1997).

Roy and colleagues (1997) investigated the effect of glucose supplementation (1 $g \cdot kg^{-1}$) given immediately post-resistance exercise and found a ~30% greater increase skeletal muscle FSR in the CHO vs. Placebo condition. Furthermore, urinary urea nitrogen and 3-methylhistidine (3-MH) excretion was lower in the PL condition suggesting that the post-exercise CHO supplementation improved whole-body protein balance.

It is known that, post-exercise uptake of amino acids is somewhat similar to that of glucose. Just as rates of glucose uptake are also enhanced post-exercise (Blom *et al.*,

1987; Reed *et al.*, 1989; Ivy *et al.*, 1988a; Roy *et al.*, 1996b), studies of protein metabolism have also demonstrated that (1) prior exercise independently increased PRO synthesis and breakdown and (2) insulin (INS) alone increased PRO anabolism either by increasing PRO synthesis and attenuating the non-myofibrillar PRO breakdown (Davis *et al.*, 1986; 1987; Rodnick *et al.*, 1990; Balon *et al.*, 1990; Biolo *et al.*, 1995) or by reducing PRO breakdown alone (Moller-Loswick *et al.*, 1994; Fryburg *et al.*, 1995).

Although most studies have found the effect of exercise and INS to be additive (Davis *et al.*, 1986; 1987; Rodnick *et al.*, 1990), others have not (Balon *et al.*, 1990). The increased PRO anabolism may be limited by the availability of AAs (Fryburg 1994; 1995; Svanberg *et al.*, 1996a). Thus, the consumption of a CHO/PRO/FAT nutrional supplement post-exercise may make use of an increased rate of amino acid uptake and/or insulin response and promote a more positive protein balance.

The purpose of this study was to examine the effect of both the timing of PRO/CHO/FAT supplementation and extra-energy supplementation (~ 400 kcal) on indices of protein metabolism and E_{BAL} in females engaging in habitual endurance activity. The implications were considered to be not only important to the competitive female athlete but also to the larger population of recreational fitness ethusiasts who represent a highly body image-conscious group in a highly diet-conscious society (Deuster *et al.*, 1986).

It was hypothesized that post-endurance exercise CHO/PRO/FAT supplementation during a period of relative energy deficiency in females would (1) decrease urinary 3-MH excretion, (2) promote a more positive 24 h N_{BAL}, (3) decrease weight loss, and (4)

improve performance times when compared to pre-exercise supplementation. The addition of extra-energy was expected to improve upon the aforementionned hypothesis and to increase TEF.

METHODS.

Subjects.

Ten young females (age 22.3 $\pm$ 0.2 y) volunteered to participate in the study. All gave informed written consent and the study was approved by the McMaster University Human Ethics Committee. All subjects were healthy (by medical history), taking no prescribed medications, participated regularly in some form of endurance activity (3 d·week, 1 $hr \cdot d^{-1}$), and met the inclusion criteria for the study by having a VO_{2max} of at least 40 $ml \cdot kg^{-1} \cdot min^{-1}$. They recorded the timing of their menstrual cycle and maintained their current training regimen prior to the beginning of the study. All were eumenorrheic, with a normal cycle length of 27-33 days, and 4 of the 10 females were taking oral contraceptives. The descriptive characteristics of the subjects are presented in Table II.

Study Design.

VO_{2max} was determined < 2 wk before the beginning of the study according to the protocol described previously for cycle ergometry (Tarnopolsky *et al.*, 1995). Based on the VO_{2max} test data, a power output which would elicit 65%VO_{2max} was estimated and then adjusted where necessary by having each subject cycle for 10 min after having recovered from the maximum test. This procedure was performed on a constant-load, electrically-braked cycle ergometer (Erich Jaeger Gm & Co., Hoechberg, Germany) and on a mechanically-braked cycle ergometer (Monarch®, Varberg, Sweden). To further

familizarize subjects with the testing protocol, a 75% $\dot{V}O_{2max}$ ride to exhaustion was performed the following morning on an electrically-braked ergometer. Subjects used a heart rate monitor (Polaris Vantage XL®, Stamford, CT) during these rides to determine their target heart rate for subsequent performance rides on the mechanically-braked ergometer. Body composition was estimated from measurements of body density, as determined by hydrostatic weighing.

Diet composition and habitual energy intake were determined from prospective 5-day food records (4 weekdays, 1 weekend day) collected immediately before the study, and these results were used to prepare both a 3-day checklist and a 3-day prepackaged diet that were isoenergetic, isonitrogenous, and matched for diet composition (carbohydrates, protein, and fat) to the individual's habitual dietary intake. Subjects abstained from caffeine and alcohol consumption during their participation in the study.

Each subject completed each of three 7-day supplementation trials: a pre-exercise trial (PRE), a post-exercise (PO) trial, and a post-exercise with extra energy (POE) trial. Females were tested during the early (days 4-7), middle (days 8-11), and late (days 12-14) periods of the follicular phase of their menstrual cycle. The trials were conducted at least 1 week apart. Subjects were asked not to comment upon or discuss with other subjects which trial they perceived themselves to be participating in. The administration of PRE, PO, or POE occurred in a random, double-blind fashion.

A) The Pre-Exercise (PRE) Supplementation Trial (Figure 3).

Days 1-3: The Checklist Diet.

Subjects arrived at the lab < 1 wk prior to the trial, to obtain their checklist diet. This 3-day diet was identical in energy and nitrogenous intake, and composition to their habitual diet and was composed of foods reported in subject's diet records. On **Day 1**, subjects were weighed in the morning before consuming their first checklist meal and, at 1600 h, asked to exercise on a cycle ergometer (Monarch®) for 1 h at 65% VO_{2max} as determined by their target heart rate. During exercise, subjects were allowed to consume water ad libutum and the volume of water ingested during this exercise bout was recorded and the same amount consumed during the subsequent performance rides (Days 3,4,6). Subjects were asked to consume the checklist requirements of each day by 1800 h in order to accomodate them to the strict timing of the prepackaged diet period (Days 4-6). On **Day 2** subjects were asked to perform no exercise, and, on **Day 3**, subjects repeated the sequence of events of Day 1. After their performance ride, subjects received their 3-day prepackaged diet.

Days 4-6: The Prepackaged Diet.

The 3-day prepackaged diet consisted of 10 possible foods (Appendix III). On **Day 4**, breakfast, a **Placebo can (PL can)**, and the **Supplement (Results® [Mead-Johnson, Ottawa, ON] + 0.5 g·kg CHO powder [Gatorade®, Peterborough, ON])** were consumed at 0800 h followed by lunch at 1100 h. A snack was administered at 1500 h before cycling at 1600 h for 1 hr at 65% VO_{2max} as performed during Days 1 and 3. Following exercise, subjects received a **Placebo Supplement (PL can + PL powder[Crystal Light®, Kraft, Don Mills, ON]).** Subjects were instructed to consume

all powdered supplements after dissolving them in water. Placebo cans were identical to supplement cans in visual appearance and gustation.

On **Day 5**, subjects began collecting urine and continued for 2 days (Days 5,6). The first urination of Day 5 was not collected but every urination thereafter up to and including the first urination of Day 6 was. Day 6 urinary collection included every urination thereafter up to and including the first urination of Day 7. Urine samples were collected via 1 L Nalgene containers and were immediately emptied into 4 L plastic containers that contained 5 ml of glacial acetic acid and were stored at -2 to -10°C. Subjects were informed to return all urine samples to the laboratory within 24 h of collection.

Subjects did not exercise on Day 5. They arrived at the laboratory at 1730 h to rest 10 minutes before having a baseline (T=B) expired gas determination of VO_2, VCO_2, and RER. In addition, HR was recorded via a 3-lead HR monitor (Respironics Inc., Monroeville, PA). After this, subjects were allowed 5 minutes to consume the **PL Supplement**. Immediately after completion (T=0), oxygen consumption was again determined by gas collections at T=0,10,20,30,45,60,90,120 min. Subjects sat in a chair and were permitted to read or watch movies. Room temperature remained constant throughout all trials at 22°C.

On **Day 6**, subjects repeated the events of Day 4 prior to testing. Subjects arrived at the laboratory to rest 10 min before a baseline (T=B) expired gas was collected and resting HR was determined. Following this, a 22-gauge catheter was inserted into a suitable median cubital vein and kept patent with a heparinized isotonic saline infusion

(total = 20 ml). A blood sample (T=0) was collected and stored at -50°C for subsequent analysis.

Further expired gas, HR, and blood collections were taken during the 90 min 65%VO_{2max} ride on the electrically-braked cycle ergometer at T=0,30,60,90 min. In addition, the rating of perceived exertion (Borg) was determined at T=30,60,90 min during exercise.

After the cessation of exercise, the **PL Supplement** was consumed as previously described and post-exercise expired gas collections were made at timepoints T=0,10,20,30,45,60,90,120 min. These timepoints were the same as those of Day 5 to determine EPOC (post-exercise oxidative consumption). Blood samples were taken during this period at T=30,60,90,120 min.

Day 7: The Performance Ride to Exhaustion.

On **Day** 7, subjects arrived at the laboratory at 0800 h after an overnight fast to have their body weight recorded. Subjects then cycled to exhaustion at a power output which elicited a VO_2 which was 75% of VO_{2max}. They were not given any temporal cues during this ride. Once subjects were no longer able to maintain a pedal frequency of 50 rpm in spite of vigorous verbal encouragement, the test was terminated.

B) The Post-Exercise (PO) Supplementation Trial (Figure 4).

The PO trial was similar to the PRE trial as seen in Figure 4. However, the **PL can + PL Supplement** was taken with breakfast and the **Supplement** was given immediately post-exercise on Days 4 and 6, and before the expired gas collection on Day 5. Thus, Day 5 expired gas collection was used to determine the Thermic Effect of Food.

C) The Post-Exercise + Extra Energy (POE) Supplementation Trial (Figure 5).

The POE trial was identical to the PRE trial as seen in Figure 5 with the exception that subjects received an extra 400 kcal of energy·d^{-1}. During the checklist diet (Days 1-3), the extra energy was given by the addition of extra items to their daily food consumption. This addition served to accomodate them to the extra energy they received in their prepackaged diet (Days 4-6). During the prepackaged diet period, the extra 400 kcal of energy·d^{-1} was administered at breakfast via both a **Supplement can (~250 kcal)[Boost®, Mead-Johnson, Ottawa, ON]** and an extra **150 kcal CHO [Gatorade®]** added to the PL powder. Thus, Day 5 expired gas collection was used to determine the Thermic Effect of Food.

Measurements. Diet composition and habitual energy intake were analyzed using a computer-based nutrient analysis program (Nutritionist IV, First Data Bank, San Bruno, CA).

Blood was analyzed at all time-points for hematocrit, lactate (YSI-23L), glucose (Sigma Diagnostics® Glucose, Kit #315-100, St. Louis, MO), and sodium (Na^+)/potassium(K+)(KNA2 Analyzer, Radiometer/Copenhagen) concentration. Insulin concentrations were determined for T=0 and 90 during exercise and at all post-exercise timepoints (Diagnostic Products Corporation/Coat-A-Count® RIA Kit #TKIN5, Los Angeles, CA). Estrogen (Diagnostic Products Corporation/Coat-A-Count® RIA Kit #TKE21, Los Angeles, CA) and progesterone (Diagnostic Products Corporation/Coat-A-Count® RIA Kit #TKPG1, Los Angeles, CA) concentrations were determined at T=B.

Pooled 24 h urinary volume was recorded and several proportional aliquots of each sample were stored at -50°C for later determination of 24 h urinary urea (Sigma Diagnostics, Kit# 640-A, St. Louis, MO), creatinine (Sigma Diagnostics®, Kit # 555-A, St. Louis, MO), and 3-methylhistidine concentration by high-performance liquid chromatography (HPLC) as previously described (Wassner *et al.*, 1985).

Respiratory measurements were made using two Turbofit software programs and two open-circuit gas collection systems ([Analyzer #1]: Applied Electrochemistry s-3a/I O_2 analyzer, Hewlett Packard 78358A CO_2 analyzer, Vacuumed Flow Turbine SC-521; [Analyzer #2]: Beckman Instrument OM11 O_2 analyzer, Hewlett Packard 47210A CO_2 analyzer, Vacuumed Flow Turbine SC-521). Each subject used the same open circuit system for all three trials.

Experimental Calculations

Oxygen consumption and RER for each timepoint was calculated as the mean of the most stable 3-5 values across a 5 min sampling period during which VO_2 was recorded every 30 seconds. Resting metabolic rate (RMR) for the PRE trial was determined as the mean VO_2 ($L \cdot min^{-1}$) at t = 120 on Day 5. To account for the downward shift in baseline observed on Day 5 between t=b and t=120, this difference was added to the mean resting (t = b)VO_2 of Day 5 and 6 of the remaining trials to determine their respective RMRs.

Excess Post-Exercise Oxygen Consumption (EPOC) for the PRE trial was determined by subtracting the area under the oxygen consumption curve (AUC) of Day 5 from that of Day 6. Furthermore, the Thermic Effect of Food (TEF) was calculated for the POE and PO trials as the difference between the AUC of POE and PRE and of PO and

PRE trials respectively. Thus, EPOC for the POE and PO trials was calculated as the difference between the AUC of Day 5 and 6 minus the TEF observed on Day 5. Energy expenditure (kcal) was determined using Zuntz's Table (Appendix V) using both the mean RER of the measured time points and the total oxygen consumed (L). It was assumed that the contribution of protein metabolism to energy expenditure would be insignificant since previous research has shown that females use mainly fats and carbohydrates during endurance exercise and at rest (Tarnopolsky *et al.*, 1995). The Thermic Effect of Exercise (TEE) was calculated according to the equation given by Frayn (1983) using the mean exercise values (t=30,60,90 min) of both VO_2 and RER .

Apparent nitrogen balances of both Day 5 and 6 were calculated according to the following equation:

$$N_{BAL} = N_{IN}\ (g \cdot d^{-1}) - [\text{Urea} + \text{Ammonia}\ (g \cdot d^{-1}) - \text{Creatinine}\ (g \cdot d^{-1})$$

$$- \text{Feces}\ (1.394\ g \cdot d^{-1}) - \text{Sweat}\ (0.581\ g \cdot d^{-1}) - \text{Miscellaneous}\ (0.41\ g \cdot d^{-1})].$$

Resting fecal and sweat N losses were estimated from the results of a recent study on endurance athletes with similar PRO_{IN} (Phillips *et al.*, 1993). Miscellaneous N losses (toothbrush, toilet paper, plate, hair, N_2 gas) were estimated at 0.14 g $N \cdot d^{-1}$ for all subjects (Calloway *et al.*, 1971).

Statistics. Statistical significance was determined by analysis of variance with a repeated-measures design. A paired T-test was used to determine significance for the Thermic Effect of Food. When a significant main effect and/or interaction occurred, the location of pairwise differences was determined using a Tukey *post hoc* analysis. Area under the curve (insulin, glucose) was calculated with a custom made software package (Fig P). The

level taken to indicate significance was $P < 0.05$. All data in text, figures, and tables are presented as mean $\pm$ SEM.

RESULTS.

Blood Chemistry.

Hematocrit (%). A significant main effect for time was observed for hematocrit measures ($P < 0.000001$). There was a trend toward increased hematocrit for the PRE trial when compared to the POE and PO trials. However, it was not statistically significant ($P = 0.052$)(Figure 6).

Sodium (Na^+)($mmol \cdot L^{-1}$). A significant main effect for time occured in which Na^+ values were observed to increase during exercise and then decrease post-exercise ($P < 0.0005$)(Figure 7).

Potassium (K^+)($mmol \cdot L^{-1}$). Plasma K^+ values were significantly different between PRE and PO trials (POE 4.55 ± 0.42, PRE 4.70 ± 0.45, PO 4.42 ± 0.32 $mmol \cdot L^{-1}$)($P < 0.001$) and across time ($P < 0.000001$). Potassium concentrations were observed to increase during exercise (T=0 min, 4.00 ± 0.22 to 90 min, 5.01 ± 0.44 $mmol \cdot L^{-1}$) and decrease post-exercise (T=30 min, 4.51 ± 0.52 to 120 min, 4.35 ± 0.52 $mmol \cdot L^{-1}$). A trial by time interaction was observed wherein potassium returned to basline much quicker with supplement consumption post-exercise ($P < 0.01$)(Figure 8).

Lactate ($mmol \cdot L^{-1}$). A signficant main effect for time occured for lactate ($P < 0.000001$)(Figure 9). Lactate values increased during exercise (T=0 min, 1.50 ± 0.49 to 90 min, 2.47 ± 0.71 $mmol \cdot L^{-1}$) and decreased post-exercise (T=30 min, 2.17 ± 1.02 to 120 min, 1.57 ± 0.87 $mmol \cdot L^{-1}$).

Glucose (mmol·L^{-1}). Although no significant main effect was observed for trial, glucose concentration increase was significant for time ($P < 0.01$)(Figure 10). As expected, plasma glucose was increased post-exercise after consumption of the supplement (T=30 min, POE 5.74 ± 1.36, PRE 4.58 ± 1.14, PO 5.23 ± 2.14 mmol·L^{-1}).

Hormones.

Estrogen (nmol·L^{-1}). A significant main effect for trial was observed wherein resting serum estrogen concentration during the PO trial was significantly higher than the POE or PRE trials (POE 0.046 ± 0.045, PRE 0.079 ± 0.051, PO 0.184 ± 0.215 nmol·L^{-1})($P < 0.05$)(Figure 11).

Progesterone (nmol·L^{-1}). No significant main effect was observed for trial (POE 1.51 ± 0.42, PRE 1.60 ± 0.39, PO 1.76 ± 0.55 nmol·L^{-1})($P = 0.192$) indicating that subjects were in the follicular phase of the menstrual cycle (Figure 12).

Insulin (μIU·L^{-1}). Significant main effects for trial occured for insulin concentration. The post-exercise trials had significantly higher average plasma insulin concentrations than the PRE trial (POE 34.92 ± 29.09, PRE 11.80 ± 11.44, PO 25.13 ± 12.46 μIU·L^{-1})($P < 0.01$). A main effect was observed for time ($P < 0.000001$), wherein insulin concentrations decreased with exercise (T=0 min, 31.29 ± 20.85 to 5.65 ± 3.62 mmol·L^{-1}) and increased with consumption of the supplement (Figure 13).

Urinary Metabolites.

Creatinine (g·24 h^{-1}). Urinary creatinine values (g·24 h^{-1}) were not significantly different between trials (POE 1.08 ± 0.40, PRE 1.20 ± 0.47, PO 1.17 ± 0.59 g·24 h^{-1})($P = 0.38$) or across Days 5 and 6 ($P = 0.48$)(Figure 14).

Urea (g·24 h^{-1}). Urinary urea values (g·24 h^{-1}) were not significantly different between trials (POE 10.17 ± 3.59, PRE 11.38 ± 2.74, PO 9.80 ± 3.91 g·24 h^{-1})(P = 0.10) or across Days 5 and 6 (P = 0.76)(Figure 15).

Urea (g·g $Creatinine^{-1}$). Urinary urea values expressed relative to creatinine excretion (g·g Cr^{-1}) were not significantly different between trials (POE 10.37 ± 4.37, PRE 11.78 ± 5.62, PO 9.61 ± 4.39 g·g Cr^{-1})(P =0.15) or across Days 5 and 6 (P = 0.75)(Figure 16).

3-Methylhistidine (µmol·24 h^{-1}). A significant main effect for trial was observed for urinary 3-methylhistidine (3-MH)(µmol·24 h^{-1}) wherein urinary 3-MH observed during the POE trial was significantly higher than that observed during PRE trial (POE 299.37 ± 70.58, PRE 231.73 ± 92.34, PO 269.77 ± 54.80 µmol·24 h^{-1})(P<0.050). No significant main effect was observed for day (P=0.943)(Figure 17).

3-Methylhistidine (µmol·g $Creatinine^{-1}$). A significant main effect for trial was observed for urinary 3-methylhistidine values expressed relative to creatinine excretion (µmol·Cr^{-1})(P<0.01). *Post-hoc* analysis revealed urinary 3-MH during the PRE trial was significantly lower than that observed for the post-exercise supplementation trials (POE 165.01 ± 79.75, PRE 97.37 ± 53.68, PO 149.72 ± 67.67 µmol·Cr^{-1}). No significant main effect was observed for day (P=0.865)(Figure 18).

Apparent Nitrogen Balance. A significant main effect for trial was observed for Apparent N_{BAL} (P<0.0005). However, no main effect was observed for day (P=0.837). *Post-hoc* analysis revealed Day 5 Apparent N_{BAL} to be significantly different between the POE and PRE trials (POE 2.99 ± 5.25, PRE -0.29 ± 3.74, PO 0.95 ± 4.13)(P <

0.01)(Figure 19). In addition, Day 6 Apparent N_{BAL} was significantly different between the POE and PRE trials (POE 3.91 ± 3.05, PRE -1.26 ± 2.73, PO 0.96 ± 4.81)($P < 0.005$)(Figure 20).

Gas Collection.

Day 5 (Rest).

Respiratory Exchange Ratio (RER). RER was observed to be significantly different for trial (POE 0.93 ± 0.04, PRE 0.88 ± 0.05, PO 0.91 ± 0.04)($P < 0.00005$) and to increase across time ($P < 0.00005$)(Figure 26). A trial by time interaction was also observed ($P < 0.001$) wherein RER remained elevated across time for the post-exercise supplementation trials.

Oxygen Consumption (VO_2)($L \cdot min^{-1}$). A significant main effect for trial (POE 0.28 ± 0.05, PRE 0.24 ± 0.04, PO 0.32 ± 0.08 $L \cdot min^{-1}$)($P < 0.05$) and time ($P < 0.0005$) was observed for VO_2 after supplement consumption (Figure 21). A time by trial interaction was also observed ($P < 0.005$) wherein VO_2 remained elevated across time with post-exercise supplementation.

Day 6 (During Exercise).

Respiratory Exchange Ratio (RER). RER was significantly different between trials (POE 0.93 ± 0.04, PRE 0.93 ± 0.03, PO 0.91 ± 0.04)($P < 0.05$) and was observed to rise significantly across time ($P < 0.000001$)(Figure 27). Resting RER was higher in the PRE trial (0.91 ± 0.05) than in the POE and PO trials (0.89 ± 0.03 and 0.86 ± 0.04 respectively). (EXPLAIN THIS - DURING VS POST-EX VALUES - NOT CLEAR)

Oxygen Consumption (VO_2)($L \cdot min^{-1}$). Oxygen consumption was not significantly different between trials (P =0.32) but was observed to significantly increase across time (P < 0.000001)(Figure 23).

Day 6 Post-Exercise.

Respiratory Exchange Ratio (RER) (Day 6). RER was observed to be significant for trial with the post-exercise trials being significantly different than the PRE trial (POE 0.90 ± 0.06, PRE 0.85 ± 0.06, PO 0.87 ± 0.07)(P < 0.05). In addition, RER was also observed to significant increase across time (P < 0.00005)(Figure 28). A trial by time interaction was also observed (P < 0.000001) wherein RER of the post-exercise trials remained elevated across time compared to the PRE trial.

Oxygen Consumption (VO_2)($L \cdot min^{-1}$)(Day 6). A significant main effect for trial was determined for VO_2. Oxygen consumption was significantly different between between the POE and PRE trials (POE 0.29 ± 0.04, PRE 0.27 ± 0.06, PO 0.28 ± 0.04 $L \cdot min^{-1}$)(P < 0.05) . A significant main effect was observed for time (P < 0.00005)(Figure 24).

Energy Expenditure.

Resting Metabolic Rate (RMR)(L $O_2 \cdot min^{-1}$). No significant difference was observed between trials for RMR (POE 0.234 ± 0.043, PRE 0.226 ± 0.037, PO 0.237 ± 0.035 $L \cdot min^{-1}$)(Table IV).

Thermic Effect of Food (TEF)(L O_2). No significant difference in TEF existed between trials [POE 5.73 ± 5.53 L O_2 (28.50 ± 27.5 kcal), PO 4.40 ± 8.08 L O_2 (21.28 ± 39.98 kcal)](P = 0.61)(Table IV).

Excess Post-Exercise Oxygen Consumption (EPOC)(L O_2). No significant difference was observed between trials for EPOC [POE -0.09 ± 5.56 L O_2 (-0.438 ± 25.90 kcal), PRE 2.40 ± 5.26 L O_2 (11.67 ± 25.57 kcal), PO -0.20 ± 8.18 L O_2 (-0.97 ± 39.98 kcal)](P = 0.45)(Table IV).

Thermic Effect of Exercise (TEE). No significant difference was observed between trials for TEE over 90 minutes (POE 904.90 ± 322.30, PRE 900.38 ± 185.76, PO 910.55 ± 321.22 kcal)(Table V).

Descriptive Results.

Time to Exhaustion (s). Although non-significant (P = 0.074), there was a trend towards increased performance times when comparing the POE and PO trials versus the PRE trial (POE 529.40 ± 282.18, PRE 346 ± 61.60, PO 509.50 + 282.55 s)(Figure 29).

Rating of Perceived Exertion (RPE). There was no significant difference between trials in RPE (P = 0.34)(Figure 30). However, RPE was observed to increase signficantly (P < 0.001) during exercise from 3.32 ± 0.75 at 30 min to 4.38 ± 0.90 at 90 min.

Weight Loss ($kg \cdot d^{-1}$). Weight loss was significantly different between trials (POE -0.46 ± 0.65, PRE -1.37 ± 0.56, PO -0.65 ± 0.75 kg)(P < 0.01)(Figure 31).

DISCUSSION.

The purpose of this investigation was to examine the effect of both (A) the timing of a PRO/CHO/FAT supplement, pre- and post-endurance exercise, and (B), extra-energy supplementation (~ 400 kcal) on indices of protein metabolism in females engaging in

regular endurance activity while consuming an energy deficient diet (- 400 kcal). In addition, (C) both the interaction between post-exercise oxidative consumption (EPOC) and the thermic effect of food (TEF), and performance on the morning after the supplement was measured.

The major finding of this paper was that both post-endurance exercise supplementation trials resulted in a positive apparent 24 h N_{BAL} compared to the negative N_{BAL} of the pre-exercise supplementation trial. It is interesting that on Day 5, N_{BAL} was already negative for the PRE trial and positive for the POE and PO trials. On Day 6, N_{BAL} became more negative for the PRE trial and more positive for the POE trial. Had whole-body N_{BAL} been measured during trials of longer duration (>6 days), perhaps these differences in N_{BAL} would have been exacerbated.

Assuming that sweat, fecal, and miscellaneous losses did not differ between the three trials (Tarnopolsky *et al.*, 1988; Phillips *et al.*, 1993), the observation of positive N_{BAL} for post-exercise supplementation trials (POE > PO), was likely the result of a higher N intake (POE), and slightly larger, but non-significant elevations in urea and creatinine excretion observed during the PRE trial. Comparison of the negative N_{BAL} of the PRE trial to the positive N_{BAL} of the PO trial indicated that timing of nutrient delivery was important.

The post-exercise supplementation trials may have replenished the nitrogen stores utilized during the exercise bout by increasing rae of protein synthesis and decreasing the degradation during exercise recovery (Viru, 1987). Support after exercise has been given for (1) prior exercise independently increasing protein synthesis and breakdown and (2)

INS alone increasing protein anabolism· either by increasing protein synthesis and attenuating the non-myofibrillar protein breakdown (Davis *et al.*, 1986; 1987; Rodnick *et al.*, 1990; Balon *et al.*, 1990; Biolo *et al.*, 1995) or by reducing protein breakdown alone (Moller-Loswick *et al.*, 1994; Fryburg *et al.*, 1995).

The release of 3-MH provides an index of myofibrillar protein degradation since it is found exclusively in contractile protein (Balon *et al.*, 1990). Post-exercise PRO/CHO/FAT supplementation led to a significant increase in urinary 3-MH excretion on both Days 5 and 6 (POE > PO > PRE). This is in opposition to the findings of Roy and colleagues (1997) who found a decreased urinary 3-MH concentration with post-resistance exercise CHO supplementation versus placebo. However, Roy *et al.* studied healthy males post-resistance exercise whereas the present study examined energy-deficient females post-endurance exercise.

Significantly smaller urinary 3-MH levels during the PRE trial may, in part, be explained by greater glycogen levels prior to endurance exercise. Since protein oxidation is increased during endurance exercise (Phillips *et al.*, 1993), initial elevations in glycogen levels prior to exercise would serve to attenuate protein oxidation during exercise due to decreased BCKAD activity (Wagenmakers, 1989). Secondly, protein synthesis and degradation parallel each other. If protein synthesis was greater after endurance exercise then degradation, as indicated by 3-MH results, might have paralleled synthesis. Thus, net balance between synthesis and degradation may have been more positive with post-exercise supplementation- a finding supported by our N_{BAL} results. In future studies, both protein synthesis and breakdown should be measured simultaneously (Phillips *et al.*,

1997). On the other hand, Tarnopolsky and colleagues found no increase in whole-body leucine oxidation during resisitance exercise (1991) although muscle protein synthesis and degradation are known to both increase post-exercise (Phillips *et al.*, 1997).

It has been suggested that females increase metabolic efficiency in states of energy restriction, as the body seeks to protect and replenish its energy stores (Warren, 1983; Brownell *et al.*, 1987; Mulligan & Butterfield; 1990). When other indices of protein metabolism in the present study are considered, the 3-MH data suggests that females, when engaging in regular endurance activity and consuming lower energy intakes, may have preferentially spared contractile protein for subsequent exercise bouts. Nonetheless, urinary 3-MH concentrations of each trial were similar between Days 5 and 6. This finding is consistent with both rat and human studies employing consecutive days of exhaustive activity (Dohm *et al.*, 1987).

Moller-Loswick and colleagues (1994) used the euglycemic clamp technique across the human leg and forearm to determine that INS attenuated the breakdown of nonmyofibrillar proteins in the peripheral tissues of humans but had no effect on contractile protein as indicated by unchanging 3-MH concentrations. Thus, INS may have attenuated nonmyofibrillar protein breakdown in the present study. The largest plasma INS responses, and thus the most protective of nonmyofibrillar protein, corresponded well with the positive N_{BAL} observed during the post-exercise trials.

A PRO/CHO/FAT supplement immediately post-exercise resulted in a significant increase in plasma INS concentration. The addition of macronutrients (PRO/FAT) to post-exercise CHO supplements has been shown to increase the INS response by some

investigators (Chandler *et al.*, 1994; Spiller *et al.*, 1987; Zawadzki *et al.*, 1992) but not others (Roy *et al.*, 1997). The INS responses observed were significantly different between trials (POE>PO>PRE). Interestingly, the higher post-supplementation INS concentrations may be responsible for the lower plasma potassium concentrations observed during the POE versus PO trial (Tarnopolsky *et al.*, 1997).

Urea excretion was statistically similar between trials and no significant differences were observed for urea excretion between rest (Day 5) and exercise (Day 6) days. Similarly, Tarnopolsky and colleagues (1990) found no significant increase in females' urinary urea over a 24 h collection period that involved both a non-exercise and exercise day. However, males urea N excretion on the exercise day was significantly greater (by 32%) than the non-exercise day. Extending the period of urinary collection over several days would determine if this trend in females may simply be explained by imprecisions in the urine collection process. A longer collection duration for the urinary excretion method is advantageous in assessing MPD over the entire post-exercise recovery period and thus more useful in determining the impact of an intervention upon whole body protein degradation (Sjolin *et al.*, 1989).

Because both urinary urea excretion (Calloway & Kurzer, 1982; Lamont *et al.*, 1987) and energy expenditure (Webb, 1986; Ferraro *et al.*, 1992) are know to fluctuate throughout the menstrual cycle, subjects were tested within the follicular phase (Days 4-12). Although plasma estrogen concentrations were significantly different between trials, the absolute values obtained for the three trials were still within the follicular phase of subjects menstrual cycle. This was further verified by their statistically similar plasma

progesterone levels. Thus differences in plasma metabolites, hormones, oxygen consumption, and energy utilization are unexplainable by differences in menstrual cycle phase.

It has been shown by Wilmore and colleagues (1993) that women tend to underestimate caloric intake when using dietary records by approximately 18%. By providing female subjects with an extra energy trial (~+400 $kcal \cdot d^{-1}$), the present study corrected somewhat for this observed energy deficit. However, an energy deficit existed as females still lost weight on the post-exercise trials. Therefore, this finding demonstrated that a negative E_{BAL} does not necessarily result in a negative balance in PRO.

The finding of no significant difference in TEF between the POE and PO trials is surprising. Hill and colleagues (1993) noted that when a negative energy balance was achieved through a reduced E_{IN}, the primary and short-term effect on energy expenditure was a reduction in the TEF proportional to the reduction in E_{IN}. Furthermore, the INS response following the ingestion of food has been positively correlated with the TEF (Bahr & Ole, 1991). Thus, on the basis of having both the largest daily E_{IN} and post-supplementation INS response, the largest TEF was expected to be observed during the POE trial.

The fact that no significant difference was observed between trials in post-exercise oxidative consumption may be related to a similar exercise duration and intensity between trials. The magnitude of the EPOC may have been slightly greater for the PRE trial, however, this was not significant and requires further investigation. Our results do

suggest that EPOC does not appear to be the major determinant for the differences that we observed in weight change over the short term. Twenty-four hour measurements of the determinants of energy expenditure may be informative.

Since final weight determination for each trial occured on the morning of Day 7, approximately 14 h after the Day 6, 1.5 h ride (65% VO_2max), the observed weight loss for each trial may be attributed largely to decreased glycogen stores. Weight loss was significantly different between trials with subjects losing the most weight during the PRE trial. Future studies should include both pre- and post-exercise biopsies of the vastus lateralis to determine the contribution of glycogen storage loss to loss of body weight. In addition, body composition should be measured before and after each trial to determine the loss of weight attributable to fat oxidation.

A trend towards increased performance times with post-exercise supplementation was found (POE>PO>PRE). The intensity of the performance rides (75% VO_2max) was such that performance was likely indicative of the ability to utilize body glycogen stores. The exhaustive cycling bout was performed ~ 14 hrs after the completion of the 1.5 h ride (65% VO_2max) on Day 6. Thus, it may be that, while on the post-exercise supplementation trials, subjects were better able to replenish their glycogen stores due to (1) elevated INS concentrations observed with post-exercise supplementation, and (2) increased rates of GLUT-4 facilitated transport into muscle tissue closer to completion of the prior exercise bout (Ivy *et al.*, 1988a,b; Zawadzki *et al.*, 1992; Roy *et al.*, 1996a,b). The availability of glucose has been positively correlated with a decreased protein

oxidation as measured by both a decreased urinary urea excretion (Lemon & Mullin, 1980; Roy *et al.*, 1997) and decreased 3-MH excretion (Roy *et al.*, 1997).

Tarnopolsky and colleagues (1995) demonstrated that females do not have the same capacity to increase muscle glycogen stores in response to a pre-exercise increase in % carbohydrates from 57 to 75% of their energy intake. Despite this sex difference in glycogen loading, Tarnopolsky *et al.* (1996) determined that females, receiving only post-exercise CHO/PRO supplementation, have the same rate of post-exercise glycogen resynthesis rate as for males. Such evidence may be a strategy for females to optimally replenish glycogen storage without increasing energy intake and the results of the current study suggest that this may also allow for realtive energy conservation.

CONCLUSIONS.

Our results indicate that consumption of a PRO/CHO/FAT supplement immediately post-endurance exercise may enhance whole-body protein balance in females regularly participating in endurance activity. Weight loss on an energy restricted diet is attenuated when energy is provided immediately following exercise as opposed to an isoenergetic amount given earlier in the day. Neither EPOC nor TEF can account for the apparent energy efficiency seen with extra energy or timing of nutrient provision.

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Table I. Essential and Nonessential Amino Acids for Humans.

Essential Amino Acids[a].

I) Branch Chain Amino Acids.

Valine[G]
Leucine[K]
Isoleucine[G,K]
Lysine[K]
Histidine[G,b]

II) Aromatic Amino Acids

Phenylalanine[G,K]
Tryptophan[G,K]

III) Other Amino Acids

Threonine[G,K]
Methionine[G,K]

Nonessential Amino Acids[a]

Proline[G]
Glutamic Acid[G]
Glutamine[G]
Aspartic acid[G]
Asparagine[G]
Cysteine[G,c]
Tyrosine[G,K,c]
Serine[G]
Glycine[G]
Alanine[G]

[a]G,glucogenic; K, ketogenic; G,K, both.
[b]May only be required in infancy.
[c]Produced from essential amino acids: phe → tyr; met → cys.

(Adapted from Linder, 1991)

Table II: Subject Characteristics.

Subject	Height	Weight	Age	VO2max	65%VO2max	80%VO2max	% Body Fat
1	174	60.3	21.83	45.53	29.6	36.42	17.24
2	168	77	22.75	51.93	33.76	41.54	29.33
3	169	58.7	22	44.43	28.88	35.55	22.4
4	170	70.4	22.75	44.16	28.71	35.33	19.21
5	162.6	56.7	22.5	46.26	30.07	37.01	21.46
6	167	58.8	21.25	48.68	31.64	38.94	23.29
7	172	64.3	23.66	43.99	28.6	35.19	22.45
8	164	63.7	23	41.74	27.13	33.39	24.69
9	165	55	22.08	43.96	28.57	35.17	17.91
10	165	50.9	21.5	52.29	33.99	41.83	21.33
Mean	167.66	61.58	22.33	46.30	30.10	37.04	21.93
SD	3.65	7.65	0.74	3.55	2.31	2.84	3.51
SE	1.15	2.42	0.23	1.12	0.73	0.90	1.11

Table III. Nutritional Analysis.

Diet	Energy Intake (kcal/d)	Protein Intake (g/kg/d)	% PRO	% CHO	% FAT
Habitual	2170.60 ± 511.43	1.40 ± 0.17	16.70 ± 3.89	57.40 ± 6.13	25.60 ± 5.46
POE	2557.80 ± 467.36	1.69 ± 0.22	16.20 ± 3.43	57.60 ± 4.62	26.20 ± 4.08
PRE	2157.20 ± 475.57	1.40 ± 0.19	16.40 ± 3.98	58.00 ± 6.13	25.50 ± 5.82
PO	2157.20 ± 475.57	1.40 ± 0.19	16.40 ± 3.98	58.00 ± 6.13	25.50 ± 5.82

Table IV. Energy Expenditure.

Trial	RMR	TEF (L O2)	EPOC (L O2)
POE	0.234 ± 0.043	5.73 ± 5.53	−0.09 ± 5.56
PRE	0.226 ± 0.037	N/A	2.40 ± 5.26
PO	0.237 ± 0.035	4.40 ± 8.08	−0.20 ± 8.18

Table V: The Thermic Effect of Exercise (TEE).

Trial	Total (kcal)	% PRO	%CHO	%FAT
POE	904.90 ± 322.30	0	83.54 ± 4.04	17.99 ± 2.51
PRE	900.38 ± 185.76	0	84.63 ± 4.16	15.37 ± 4.20
PO	910.55 ± 321.22	0	80.10 ± 1.85	19.90 ± 4.16

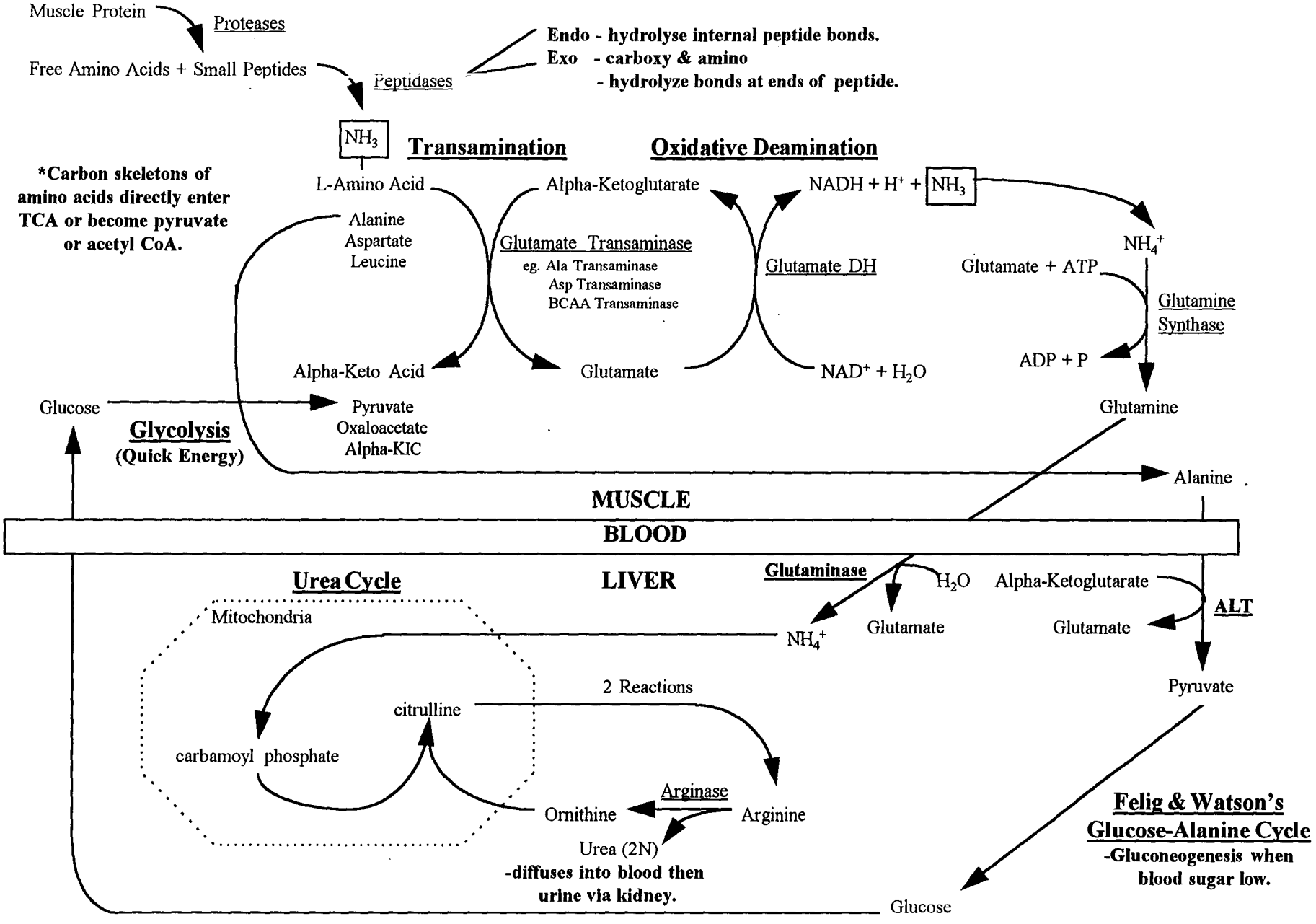


Figure 1. Biological Pathways of Protein Metabolism.

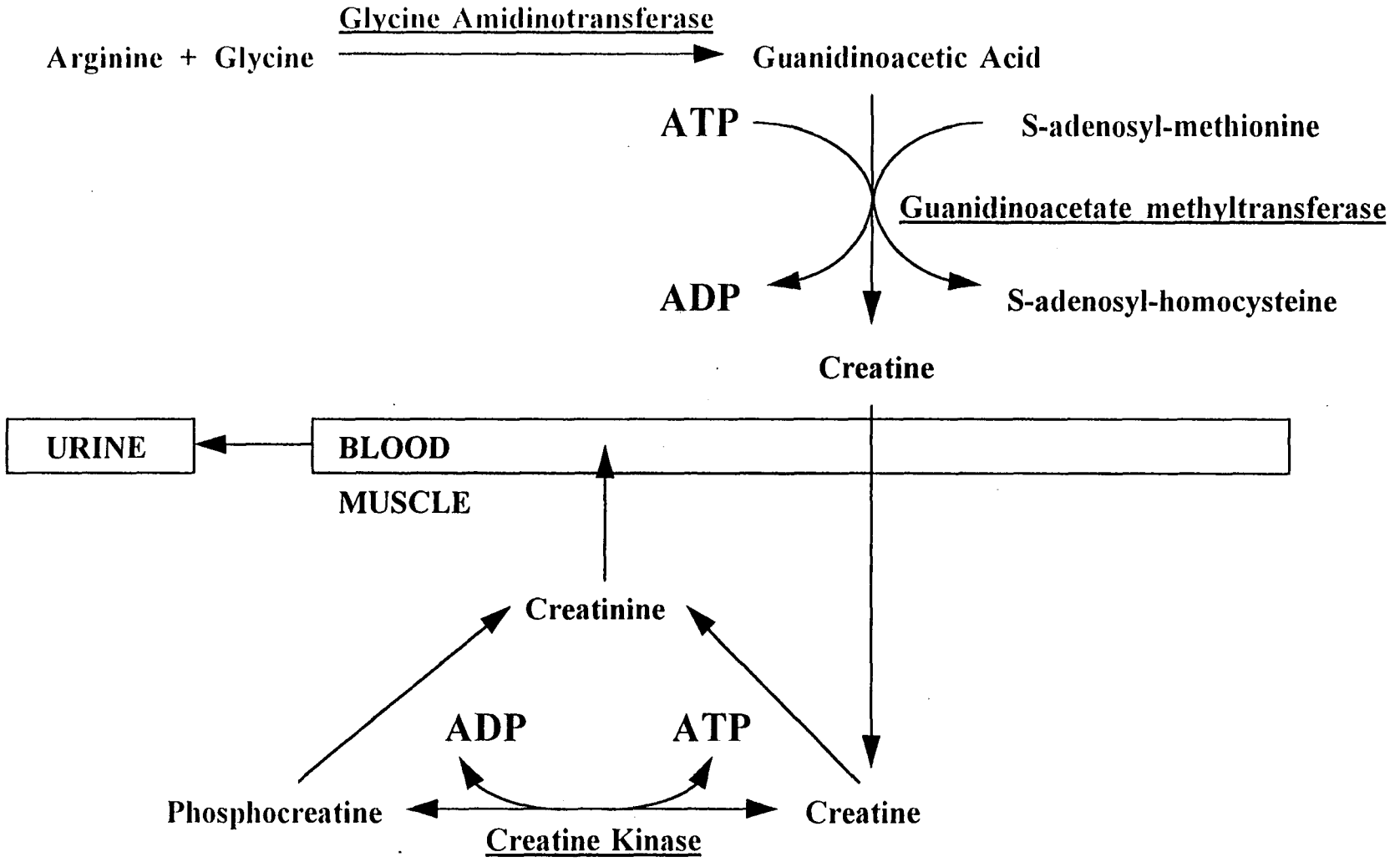


Figure 2. Principal Pathways in Creatinine Metabolism. Adapted from Heymsfield *et al.*, 1983.

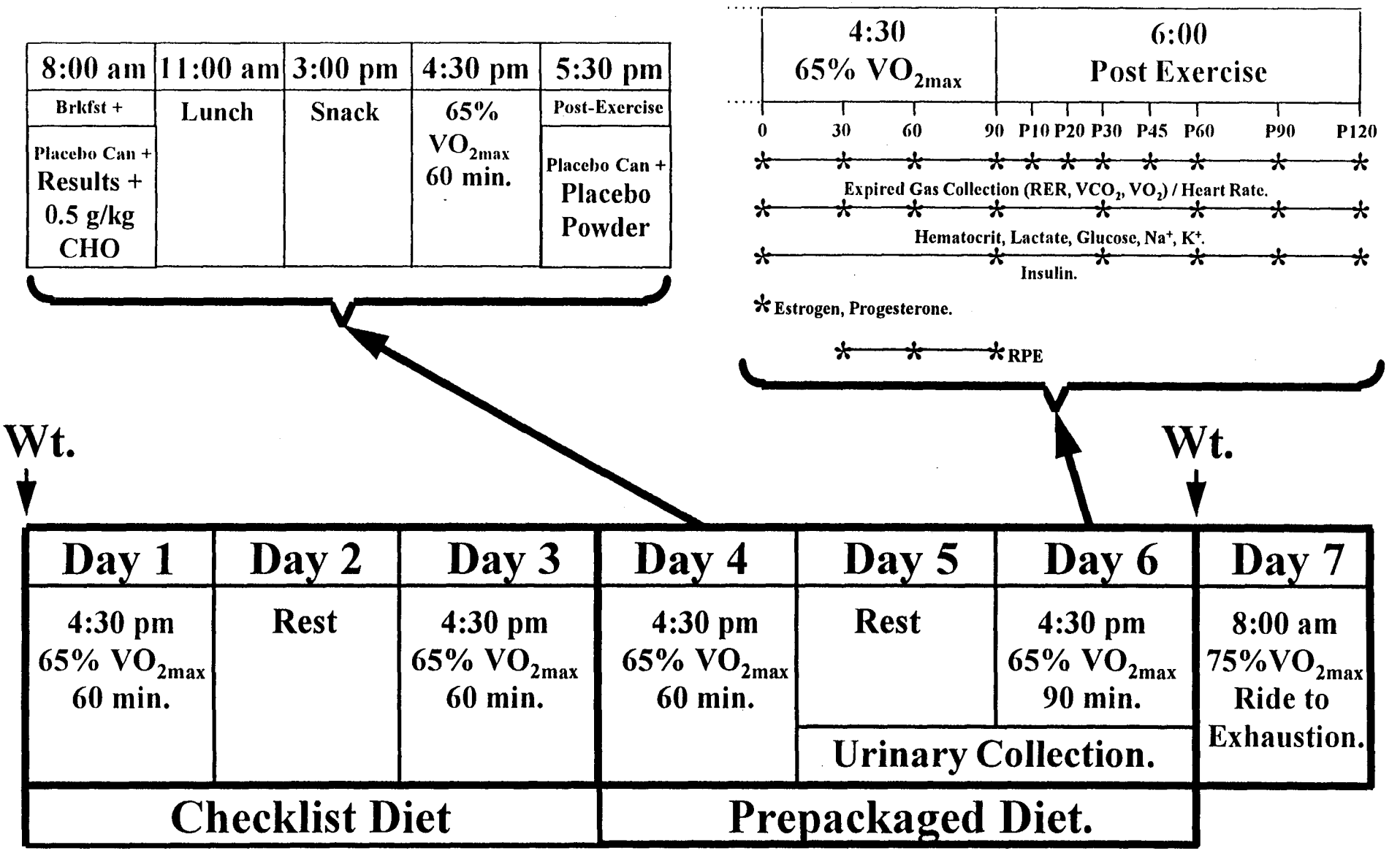


Figure 3. Pre-Exercise Supplementation Trial (PRE).

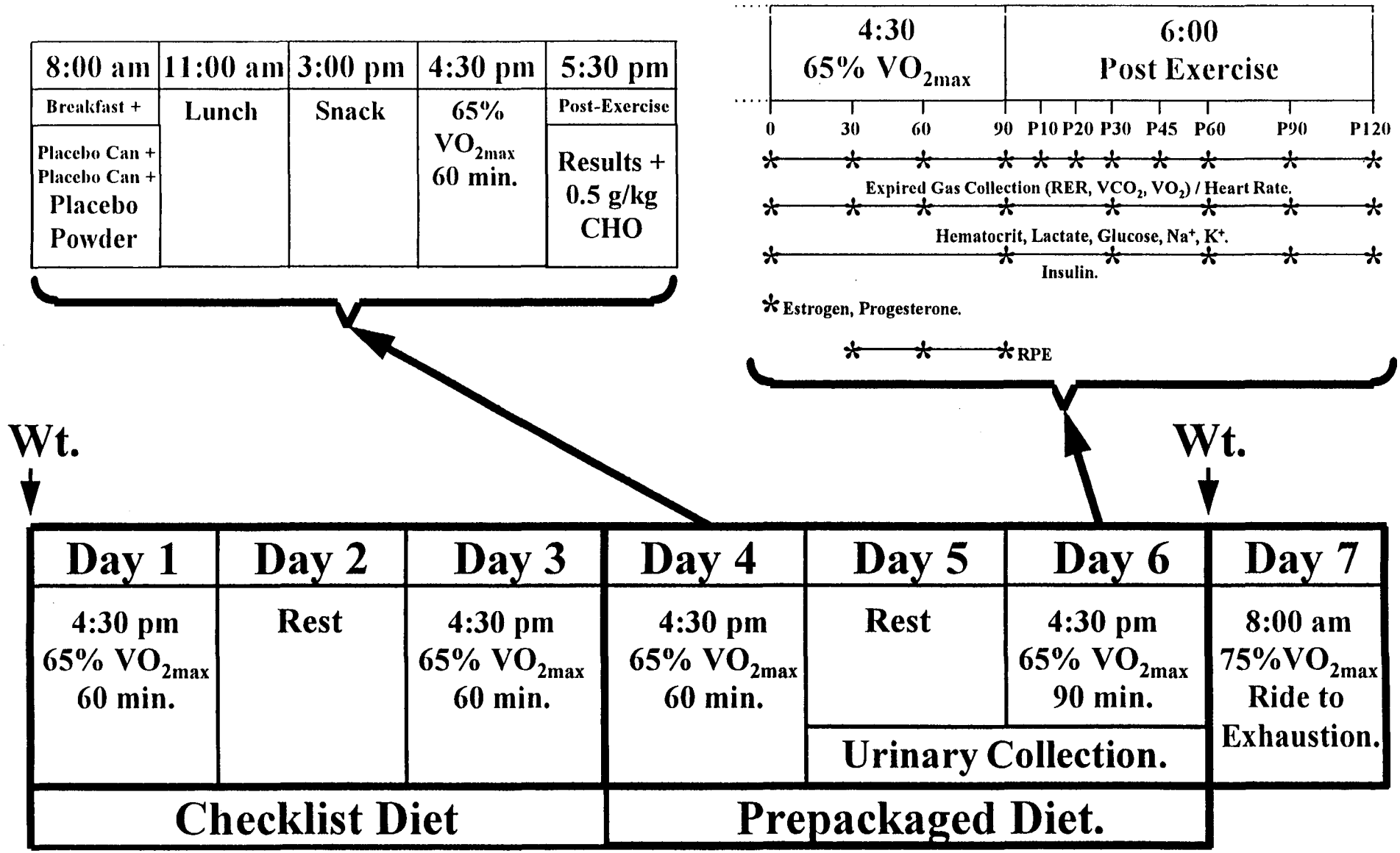


Figure 4. Post-Exercise Supplementation Trial (PO).

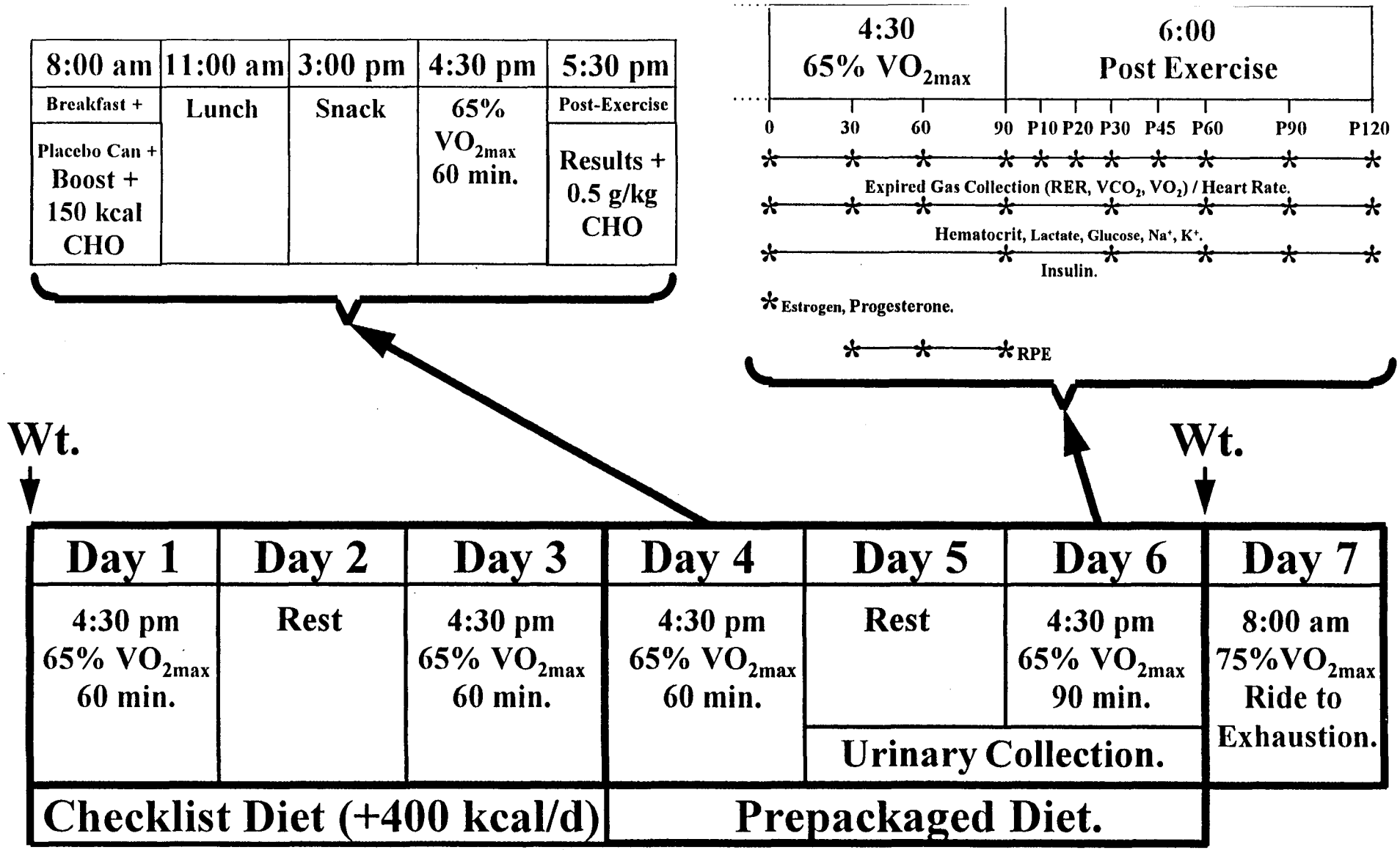


Figure 5. Post-Exercise + Extra Energy Supplementation Trial (POE)

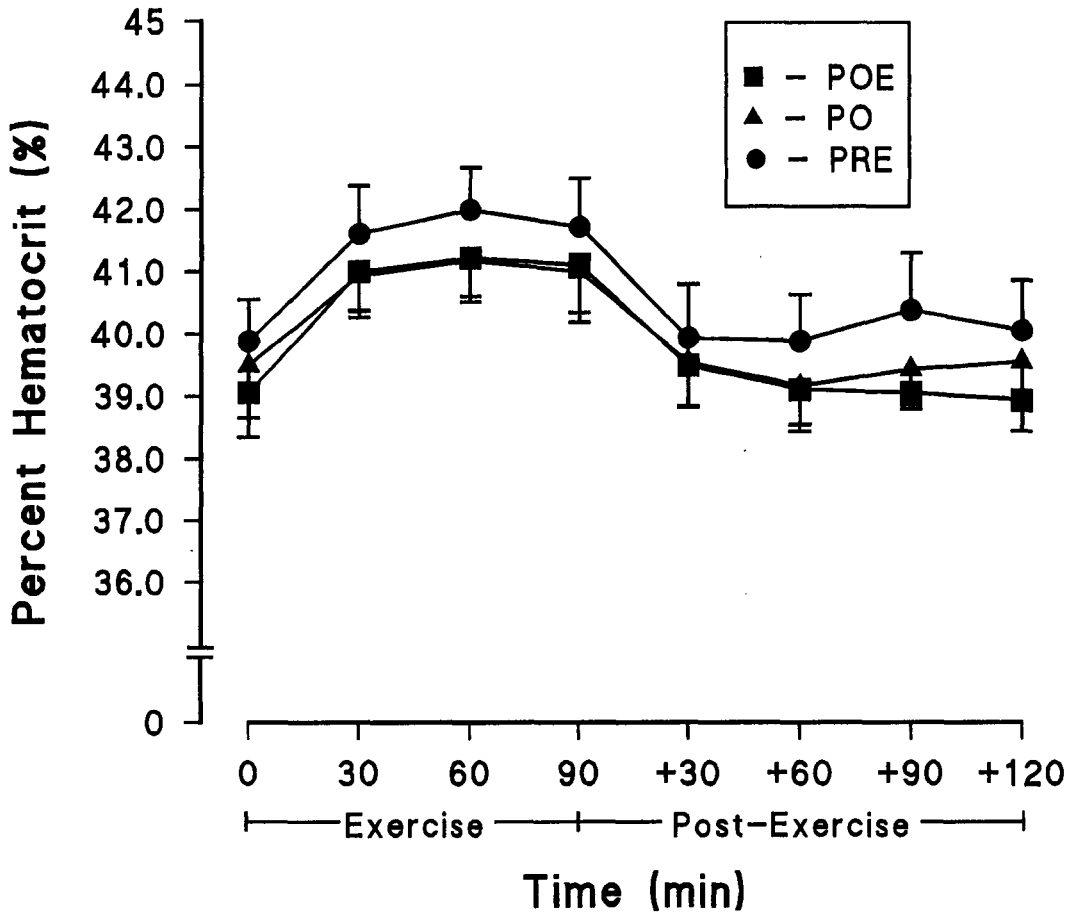


Figure 6. Hematocrit values for POE, PRE, and PO trials with respect to time (mean + SE).

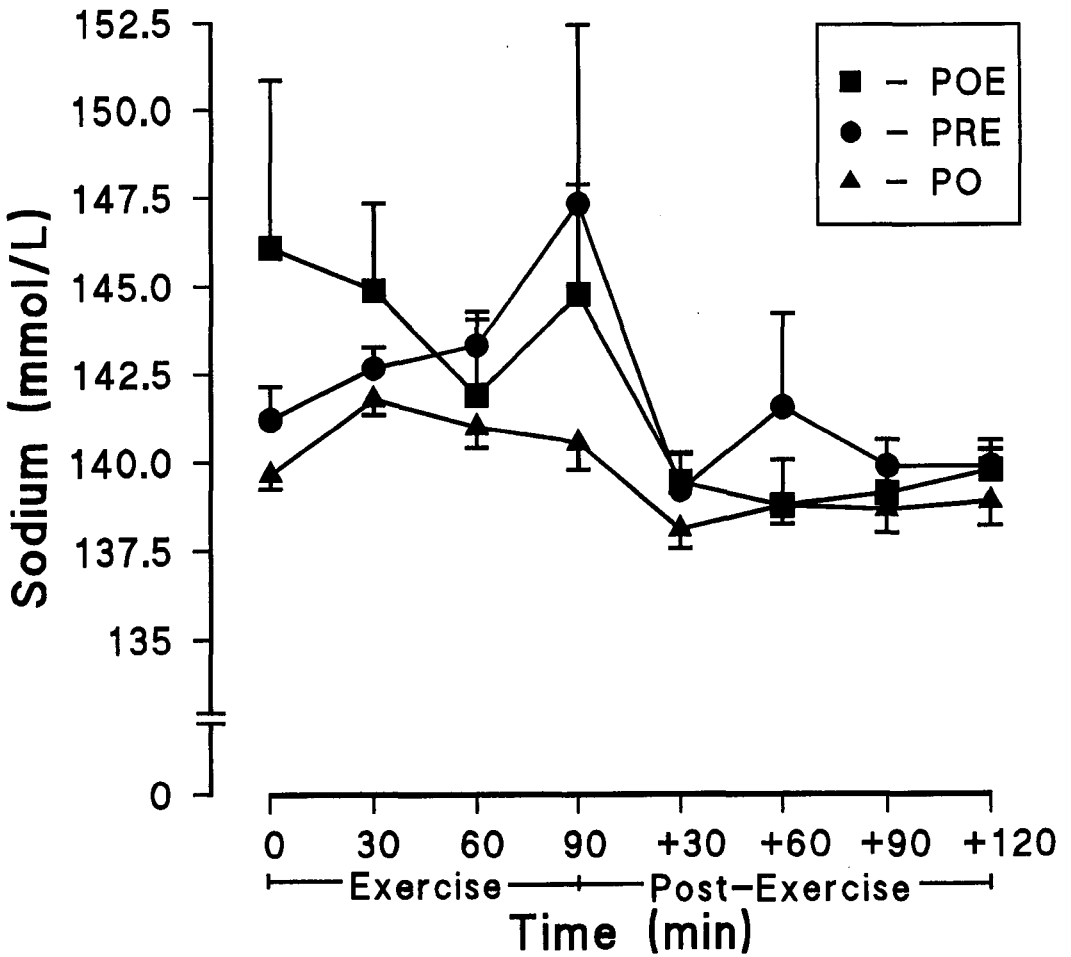


Figure 7. Plasma sodium values for POE, PRE, and PO trials with respect to time (mean + SE).

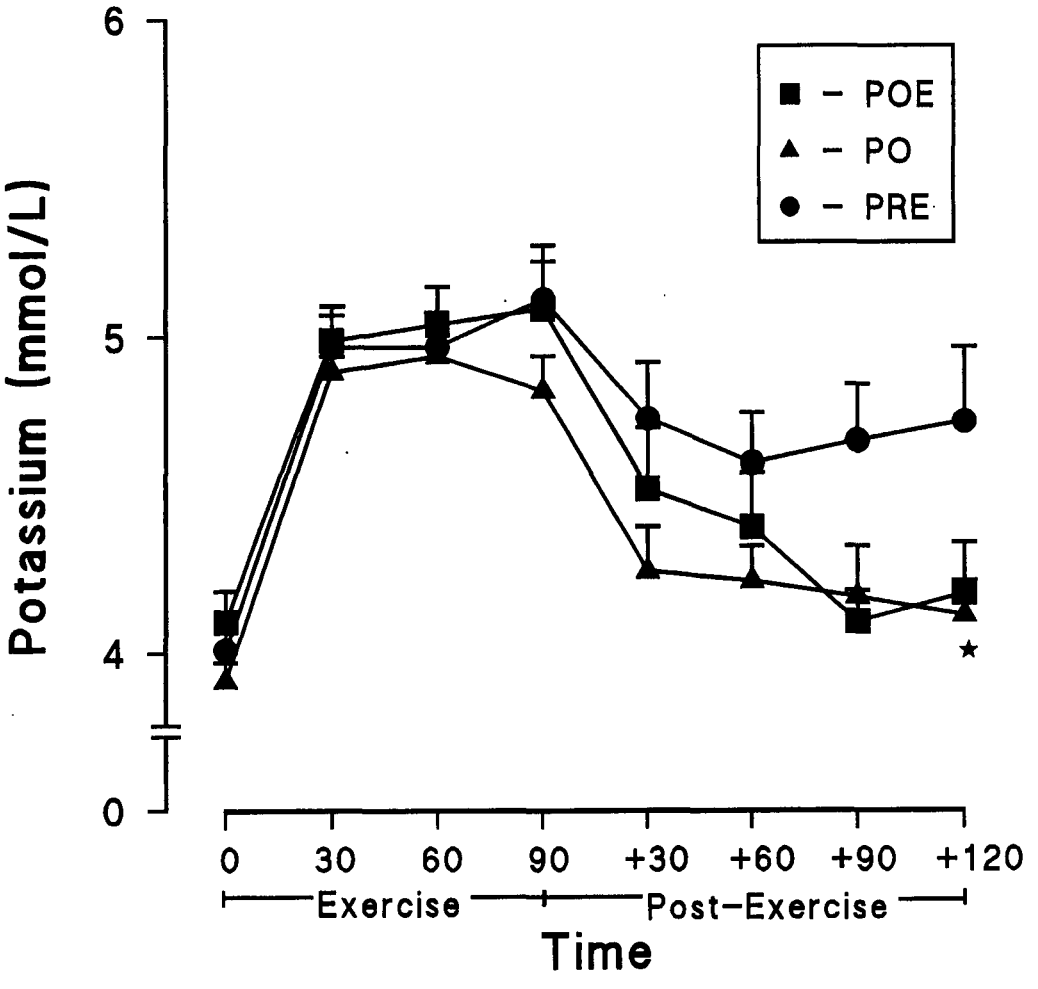


Figure 8. Plasma potassium values for POE, PRE, and PO trials with respect to time (mean + SE). ★$p<0.05$ between the PO and PRE trial.

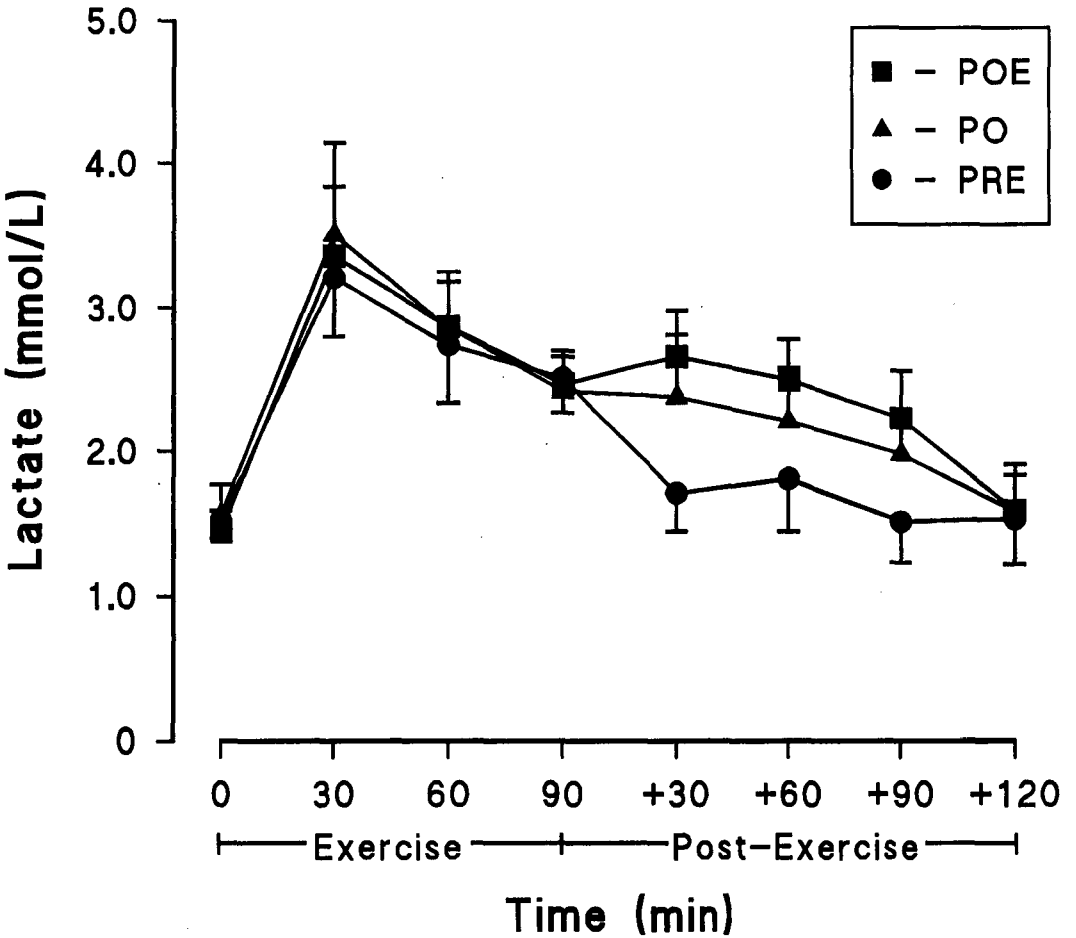


Figure 9. Plasma lactate values for POE, PO, and PRE trials with respect to time (mean + SE).

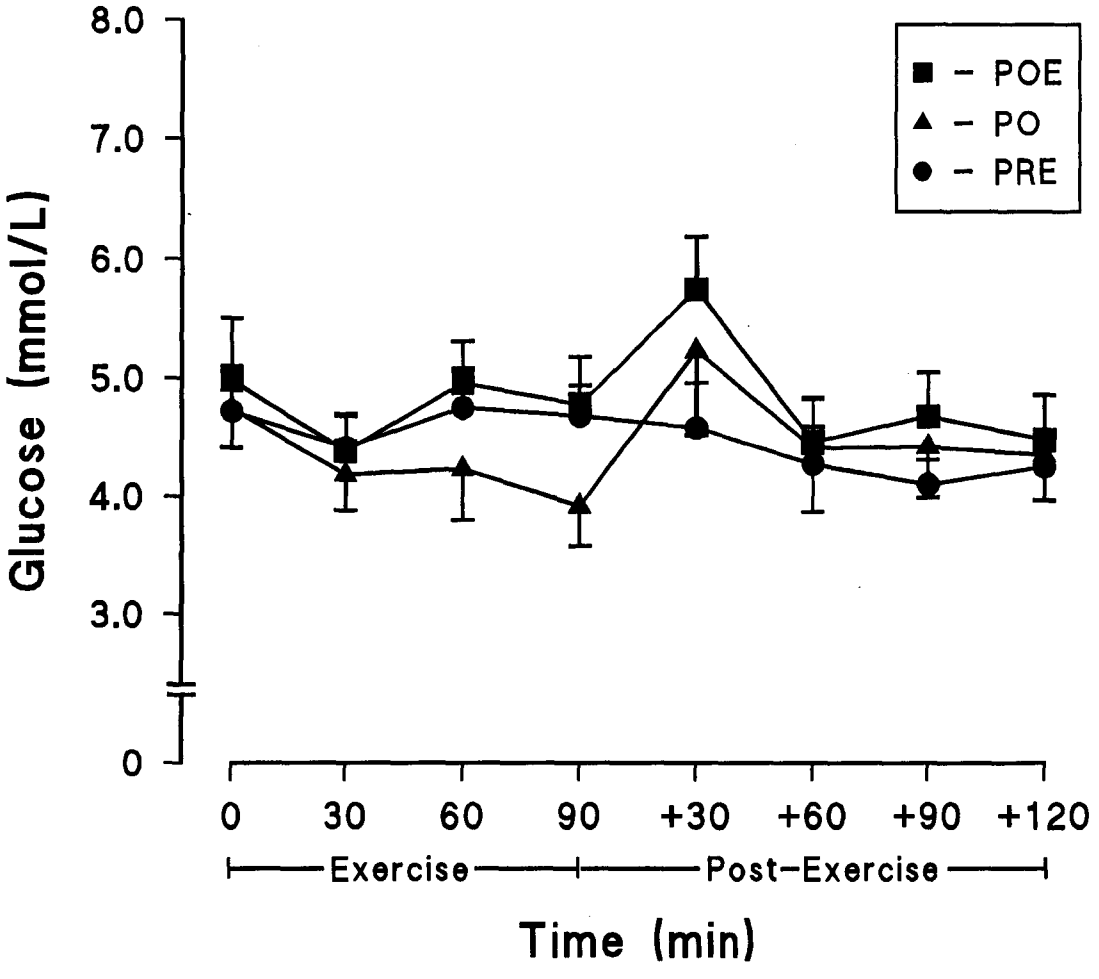


Figure 10. Plasma glucose values for POE, PRE, and PO trials with respect to time (mean + SE).

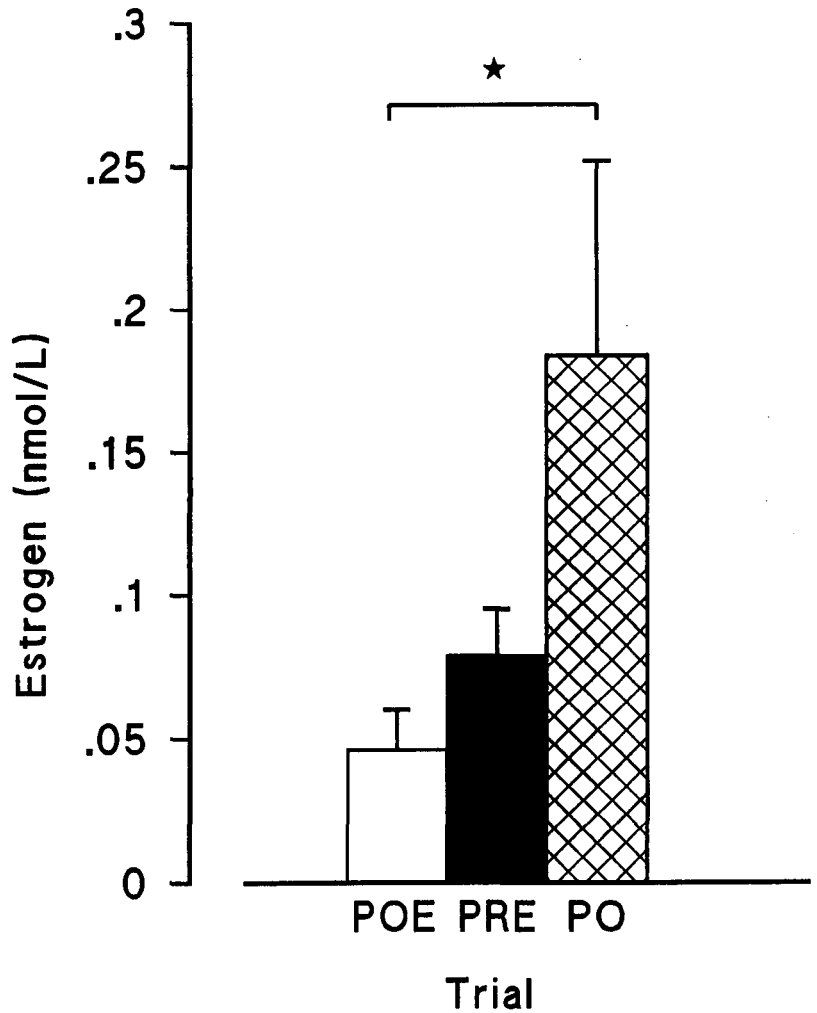


Figure 11. Resting plasma estrogen values for POE, PRE, and PO trials (mean + SE). ★$p<0.05$ between the POE and PO trials.

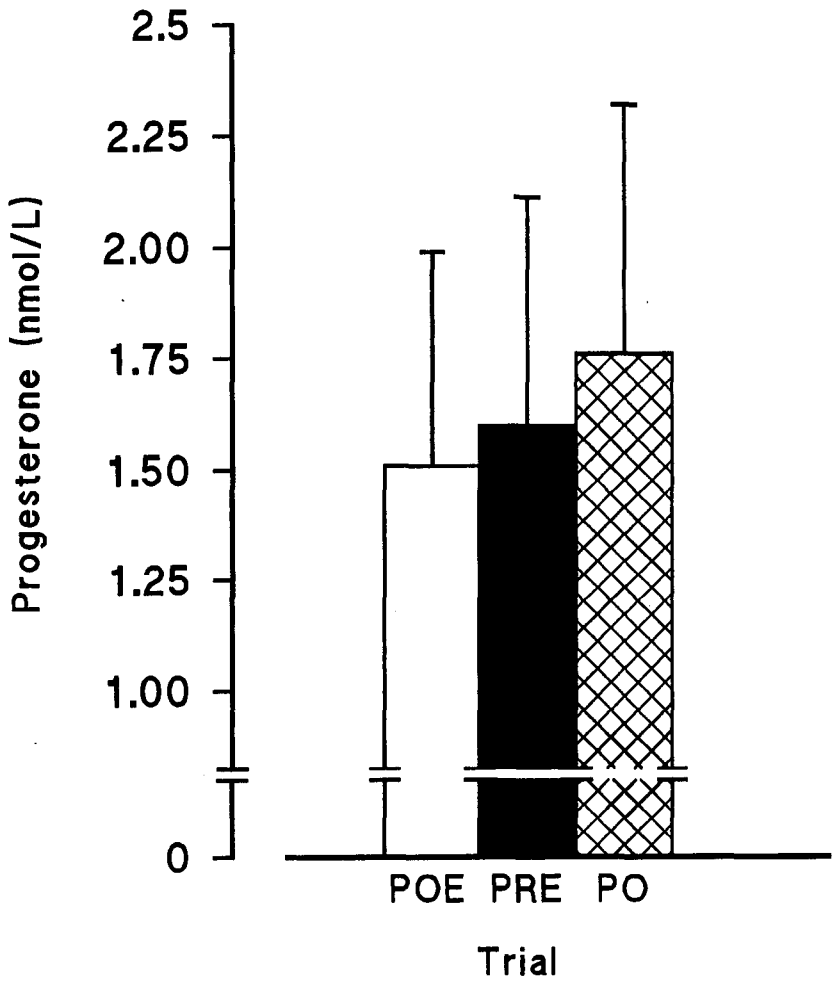


Figure 12. Resting plasma progesterone values for POE, PRE, and PO trials (mean + SE).

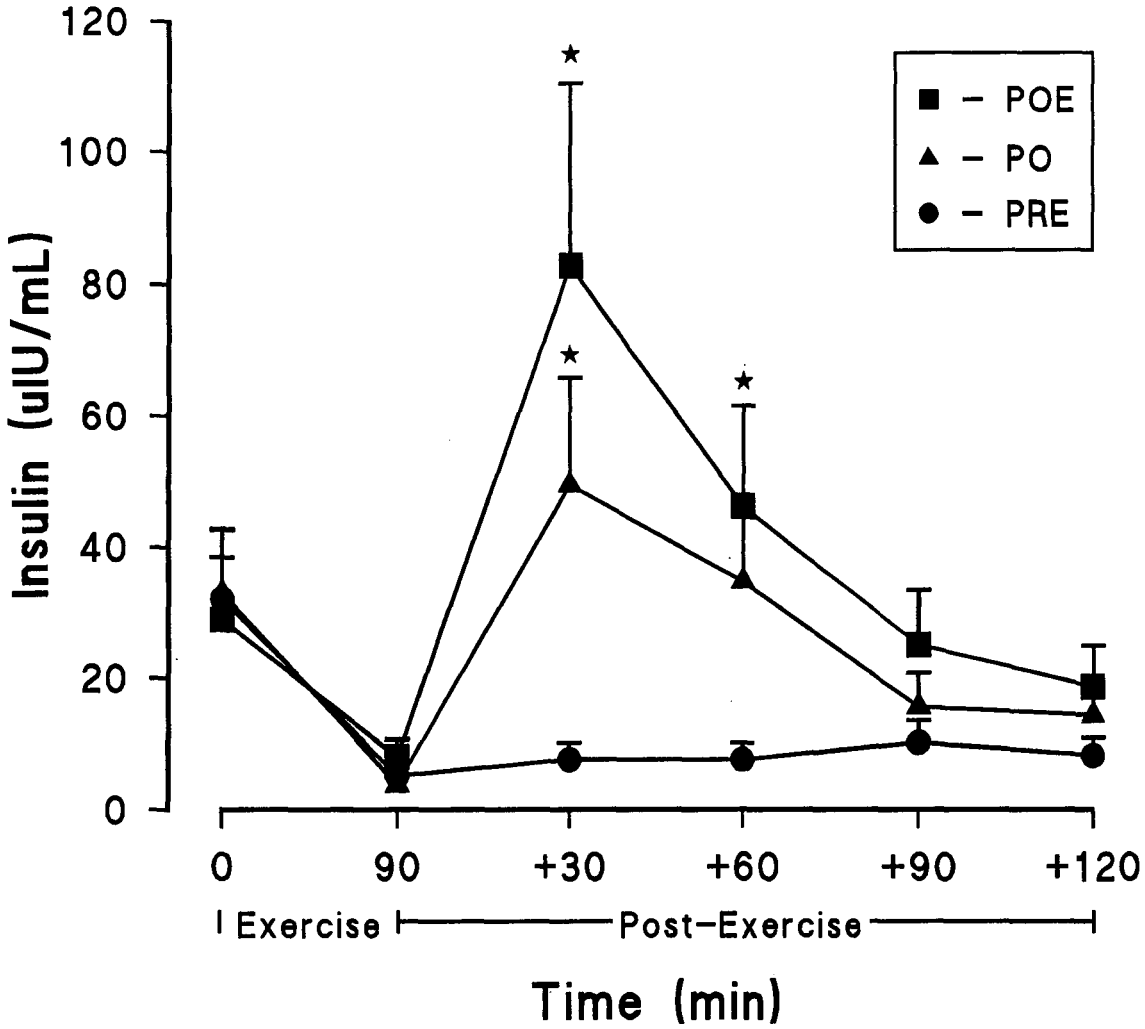


Figure 13. Plasma insulin values for POE, PRE, and PO trials with respect to time (mean + SE). ★p<0.05 between the observed and PRE trial.

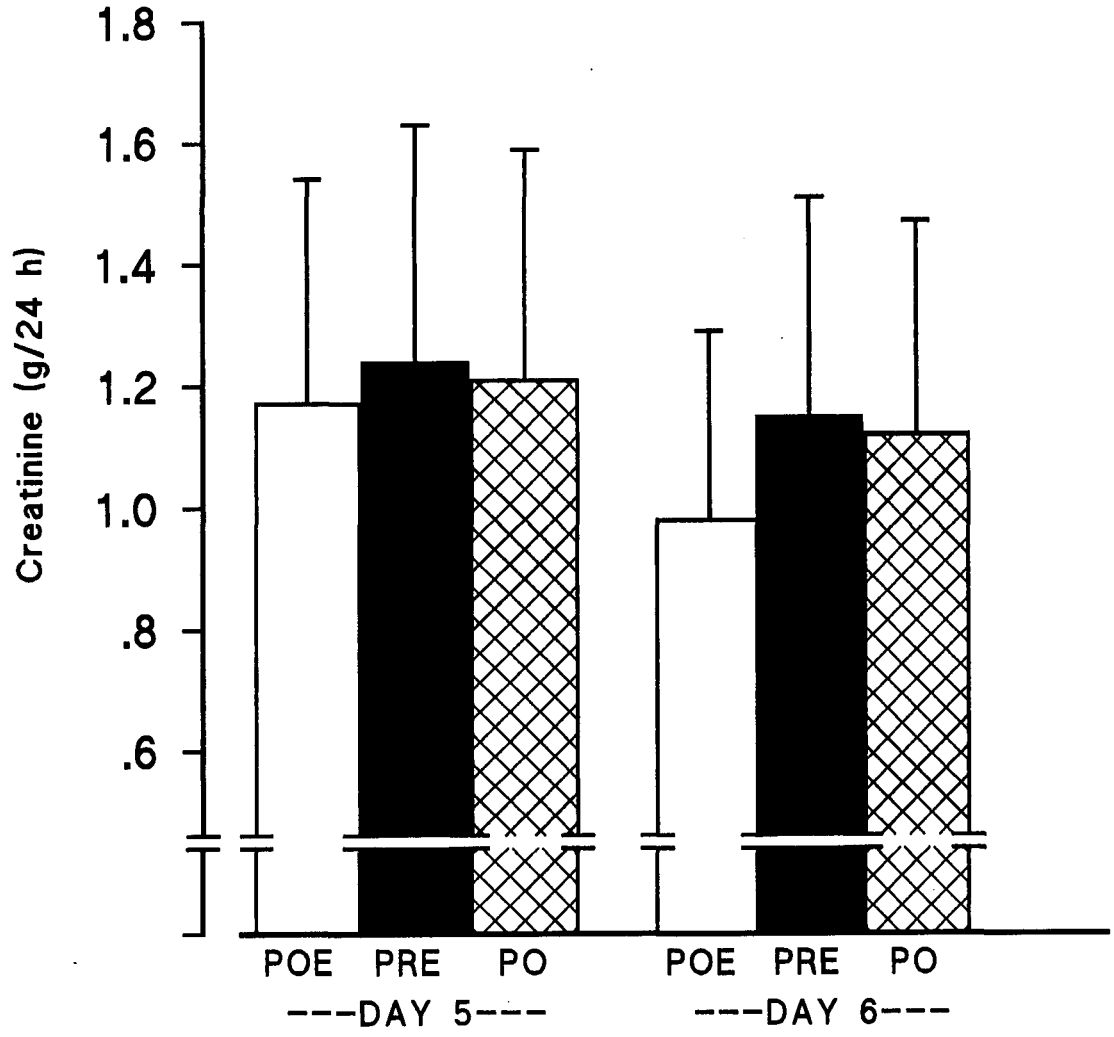


Figure 14. Urinary creatinine values for POE, PRE, and PO trials with respect to day (mean + SE).

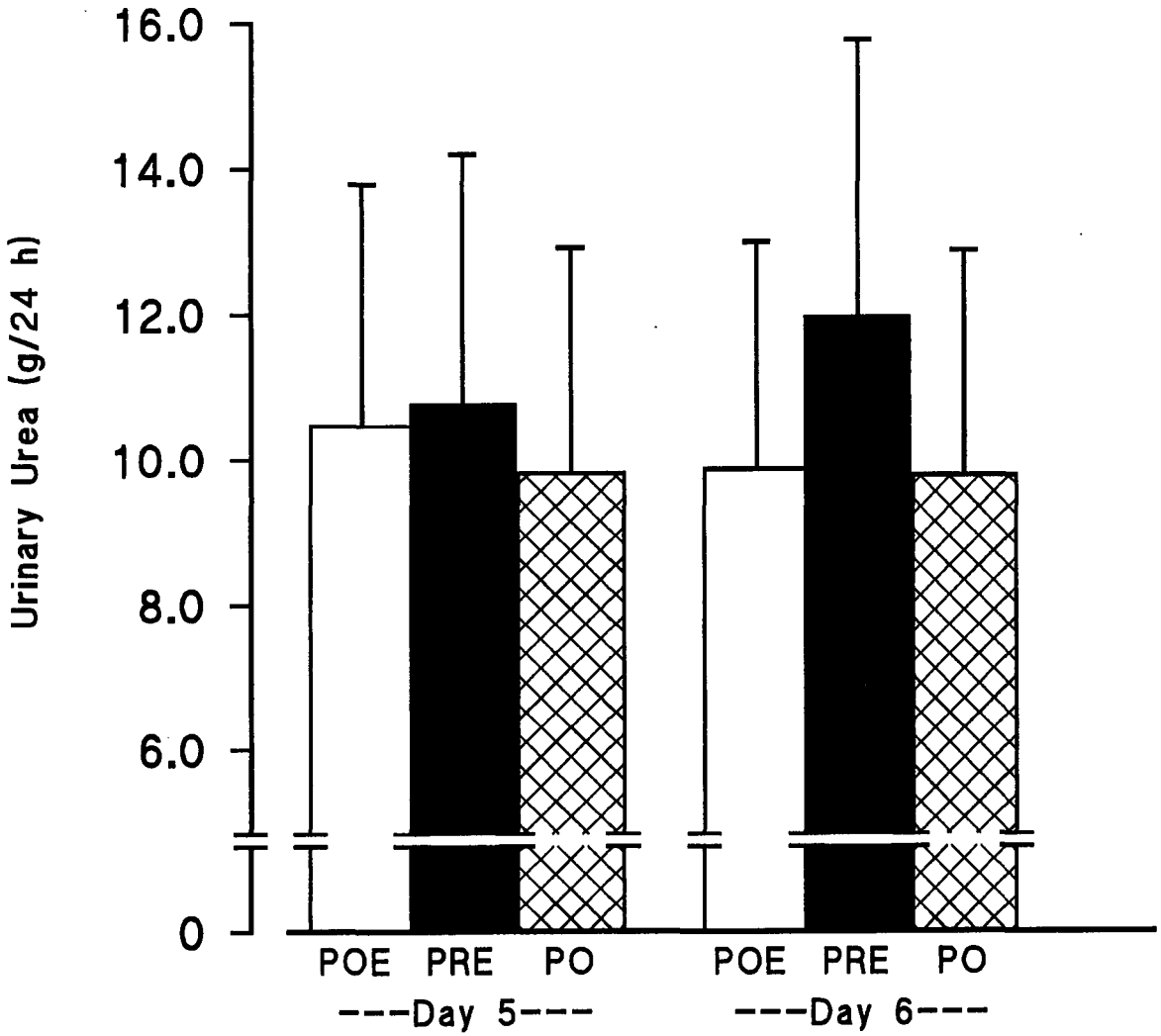


Figure 15. Urinary urea values for POE, PRE, and PO trials with respect to day (mean + SE).

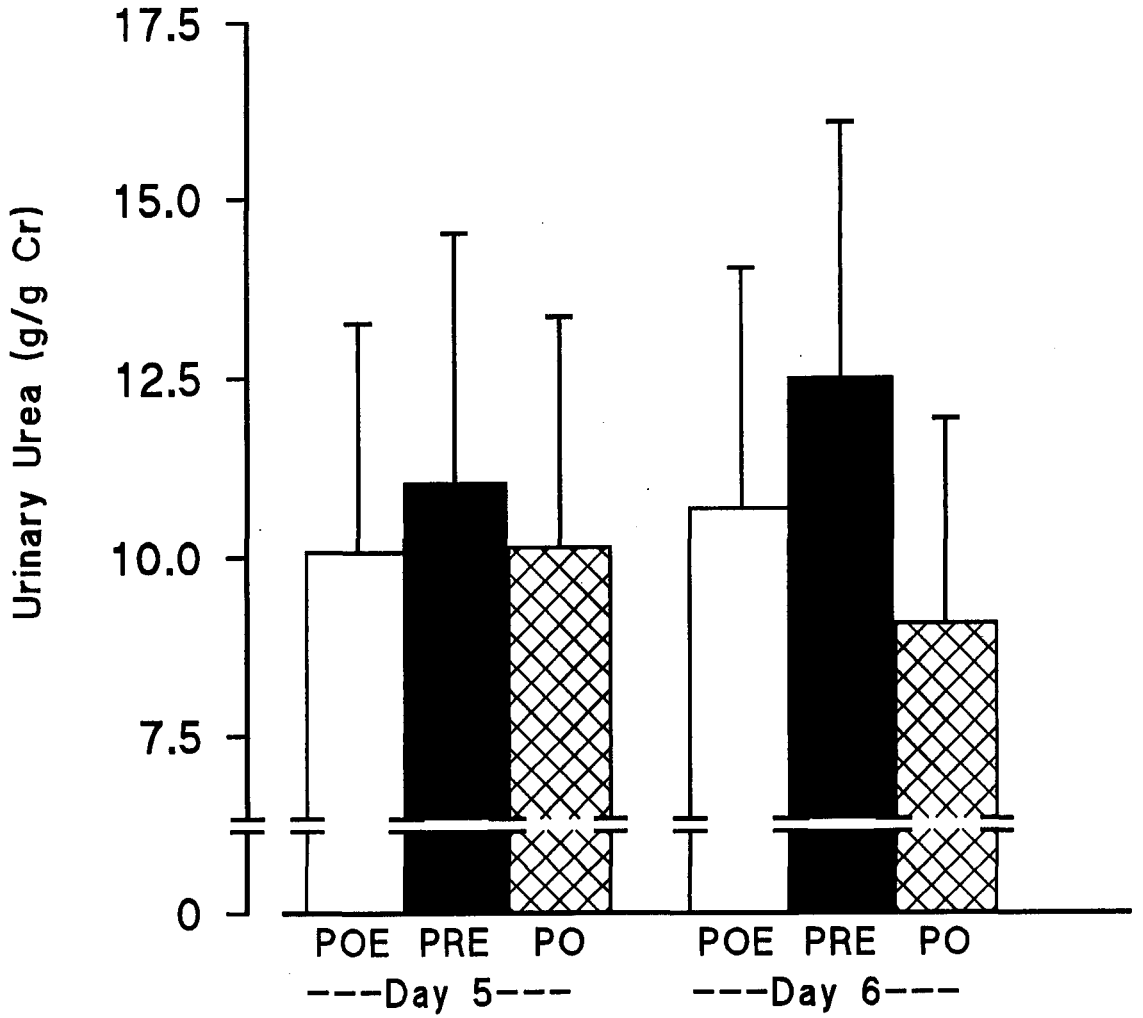


Figure 16. Urinary urea values for POE, PRE, and PO trials with respect to day (mean + SE).

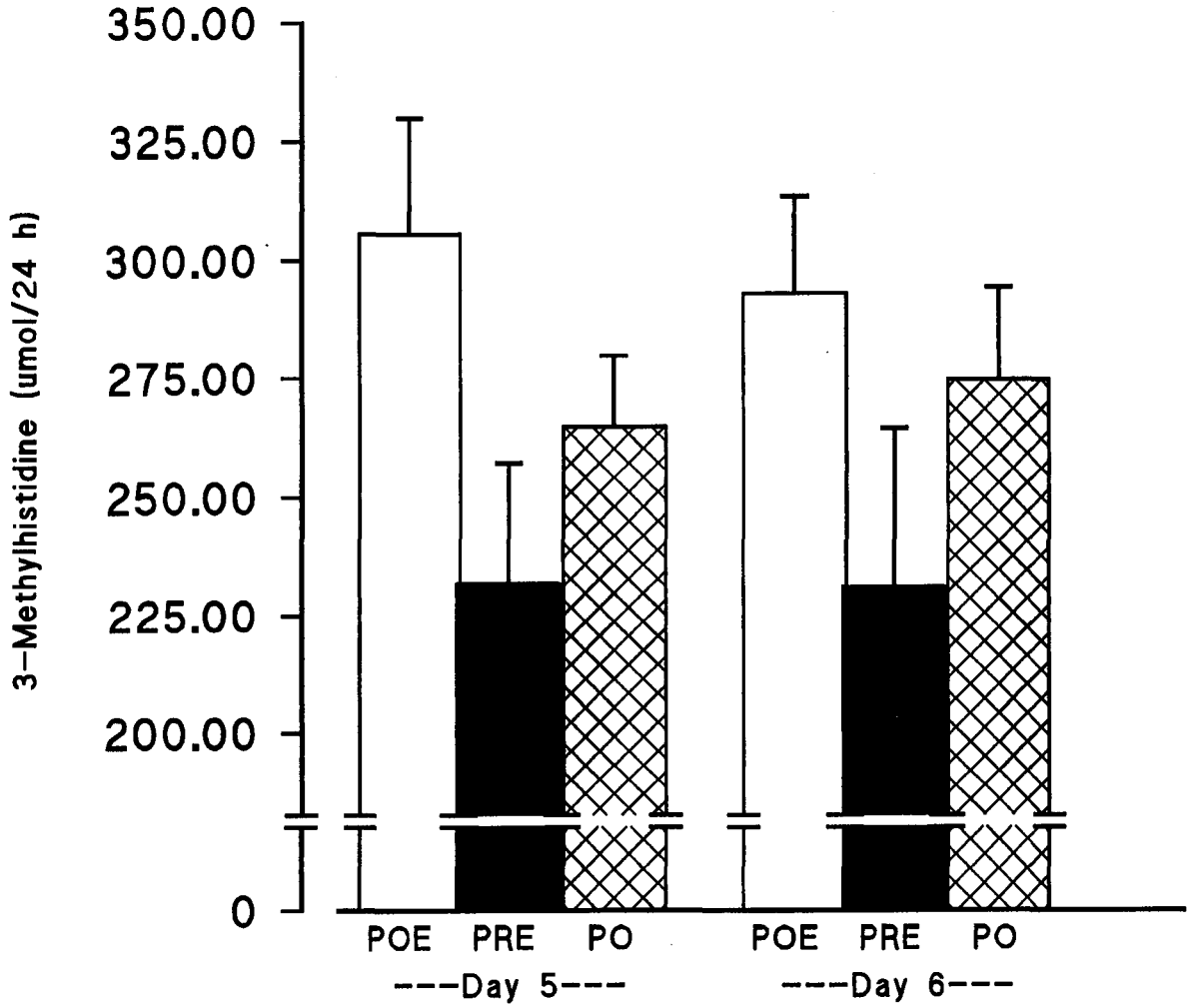


Figure 17. Urinary 3-methyhistidine values for POE, PRE, and PO trials with respect to day (mean + SE).

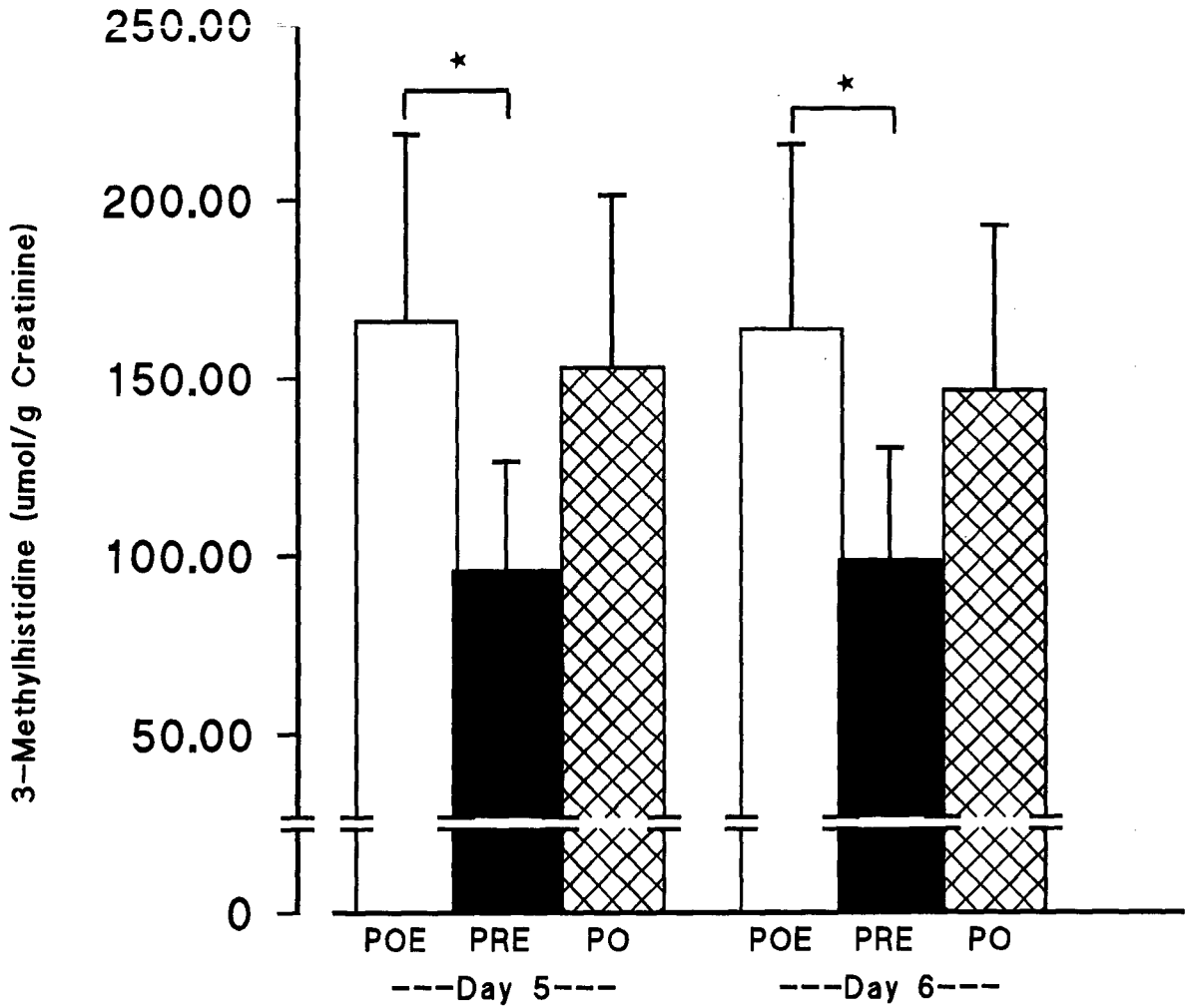


Figure 17. Urinary 3-methyhistidine values for POE, PRE, and PO trials with respect to day (mean + SE). ★p<0.05 between the POE and PRE trials.

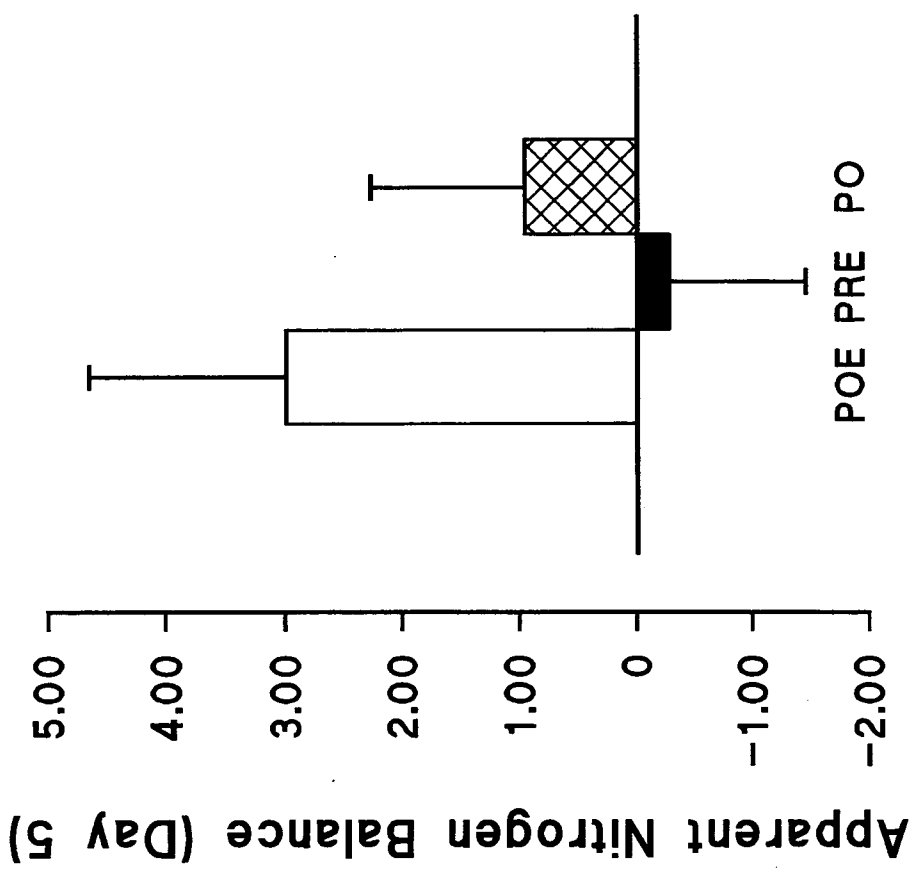


Figure 19. Apparent nitrogen balance for POE, PRE, and PO trials on Day 5 (mean + SE).

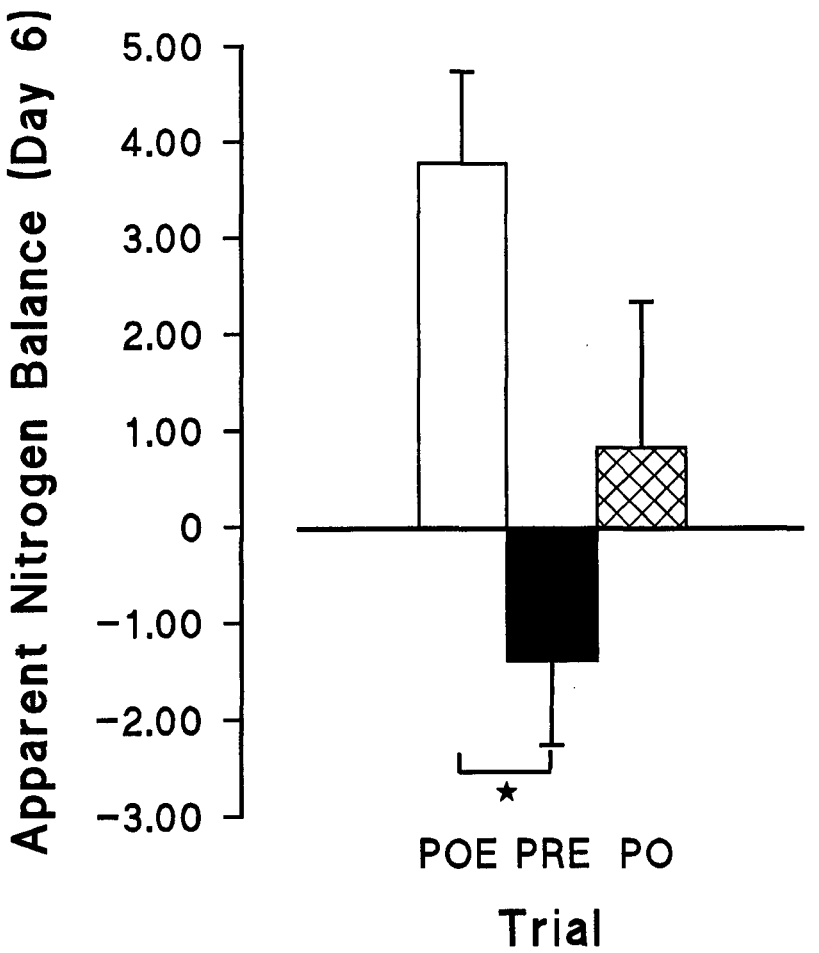


Figure 20. Apparent nitrogen balance for POE, PRE, and PO trials on Day 6 (mean + SE). ★$p<0.05$ between POE and PRE trials.

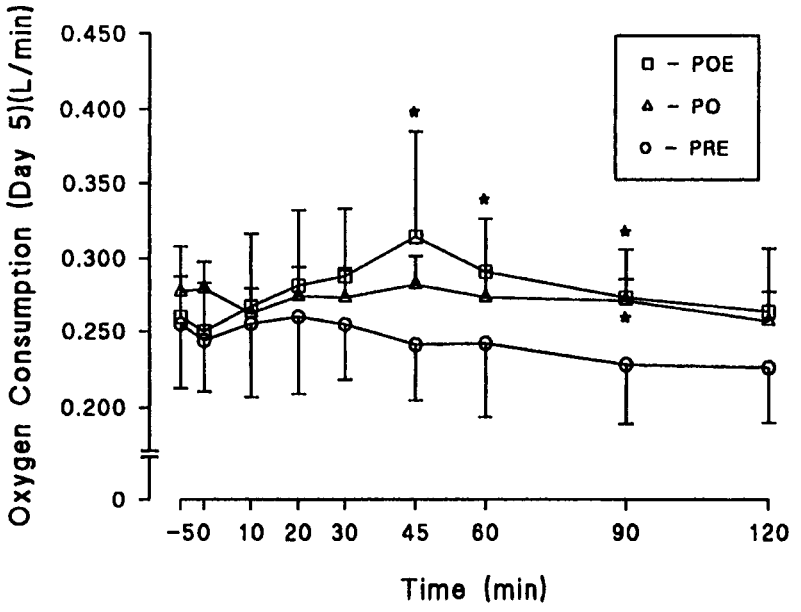


Figure 21. Oxygen consumption for POE, PO, and PRE trials with respect to time (mean + SE). *p<0.05 between observed and PRE trial.

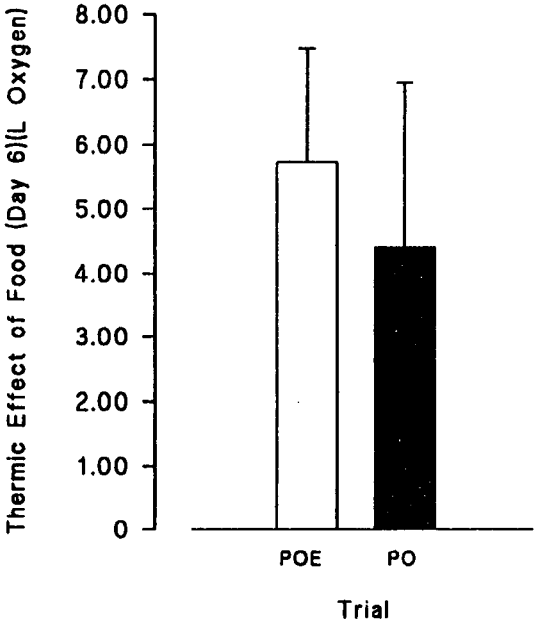


Figure 22. Thermic effect of food for POE and PO trial (Day 5)(mean + SE).

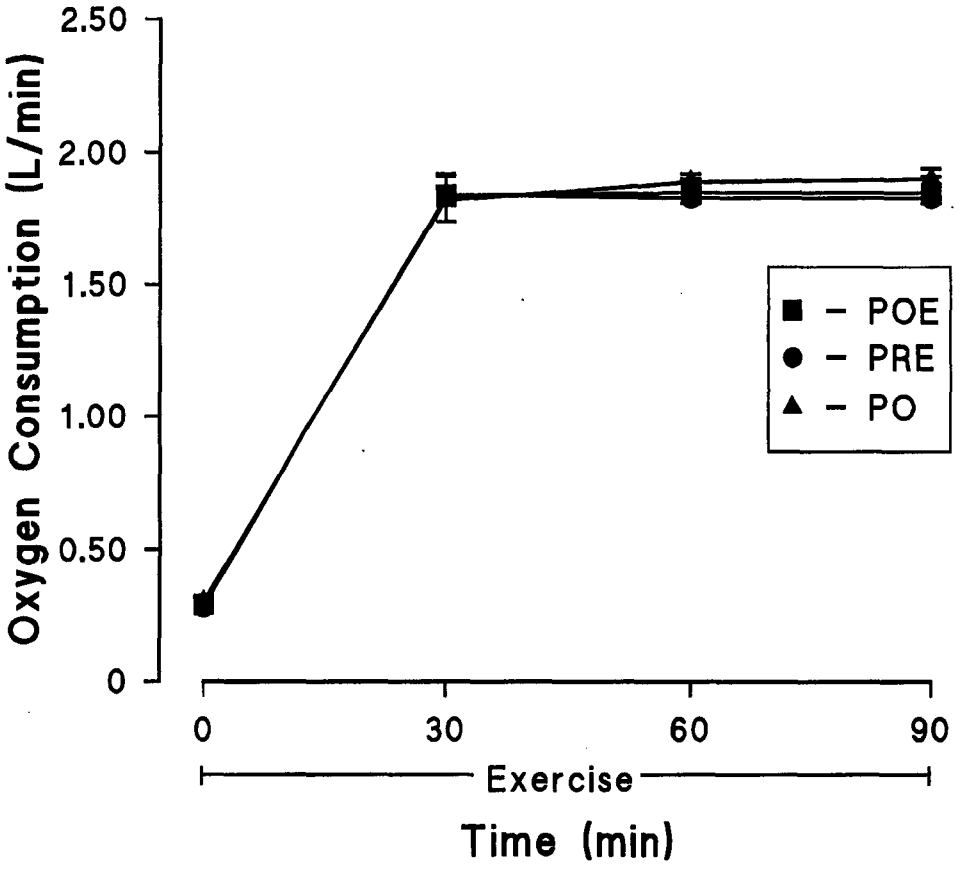


Figure 23. Oxygen consumption during exercise with respect to time (Day 6)(mean + SE).

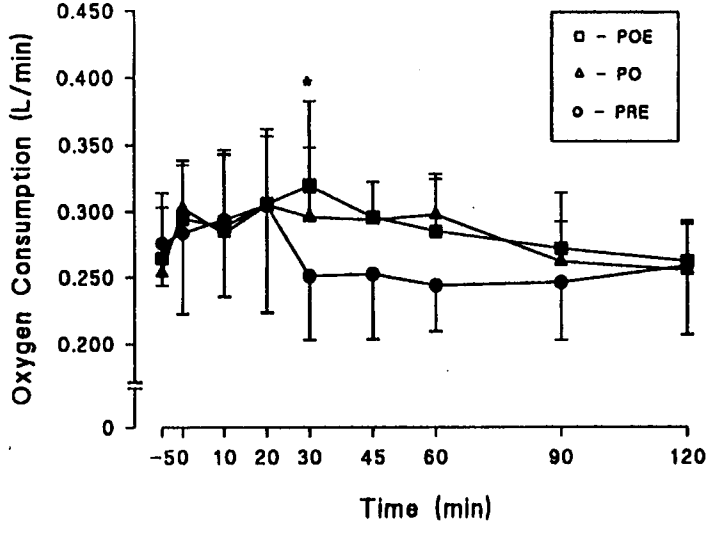


Figure 24. Oxygen Consumption values for POE, PRE, and PO trials with respect to time (Day 6)(mean + SE). *p<0.05 between POE and PRE trials.

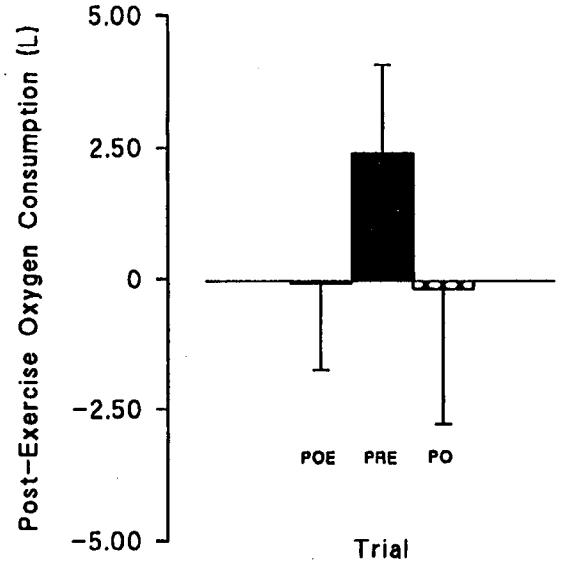


Figure 25. Post-Exercise oxygen consumption for POE, PRE, and PO trials on Day 6 (mean + SE).

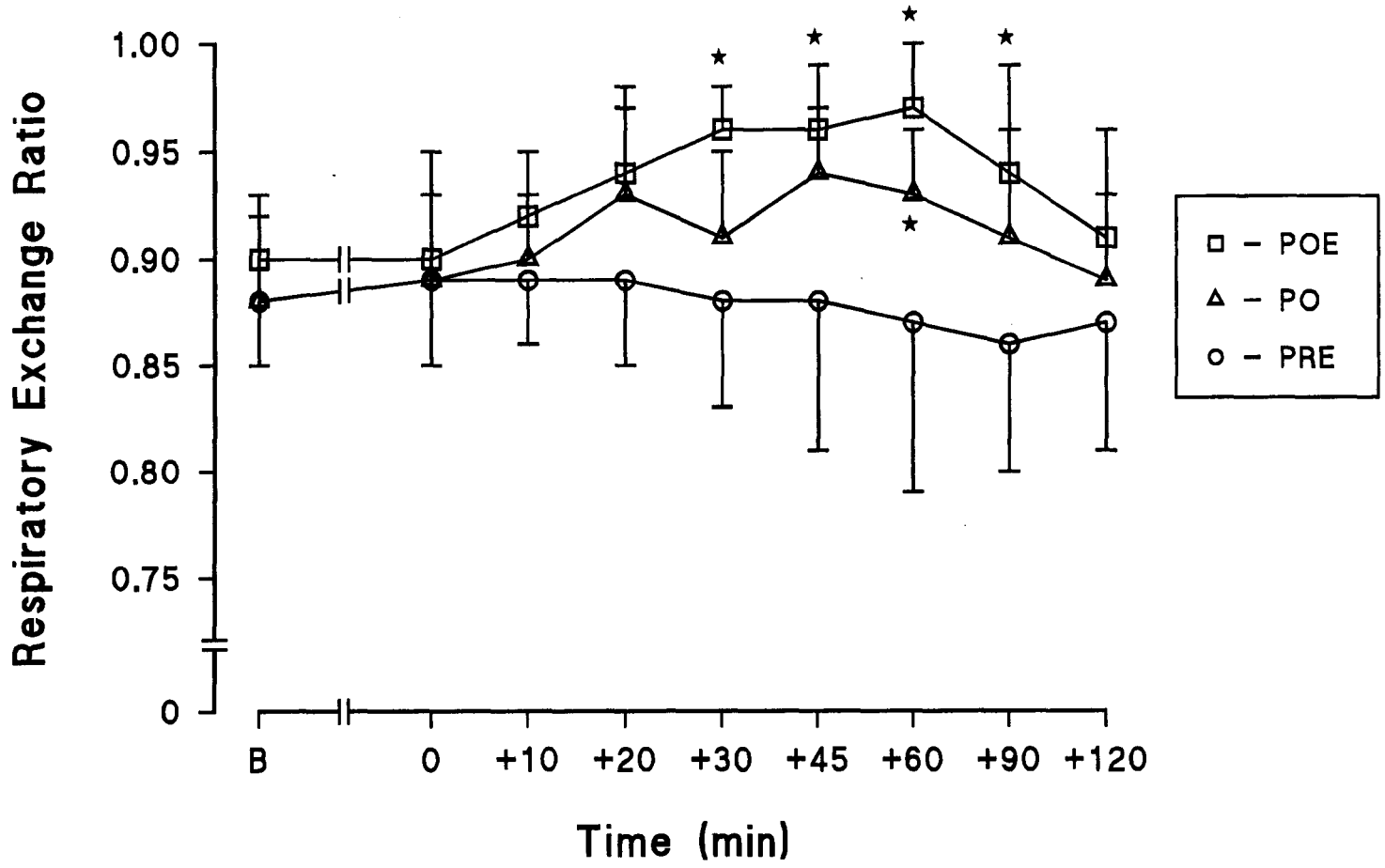


Figure 26. Respiratory Exchange Ratio for POE, PRE, and PO trials with respect to time (Day 5)(mean + SE). ★$p<0.05$ between observed and PRE trials.

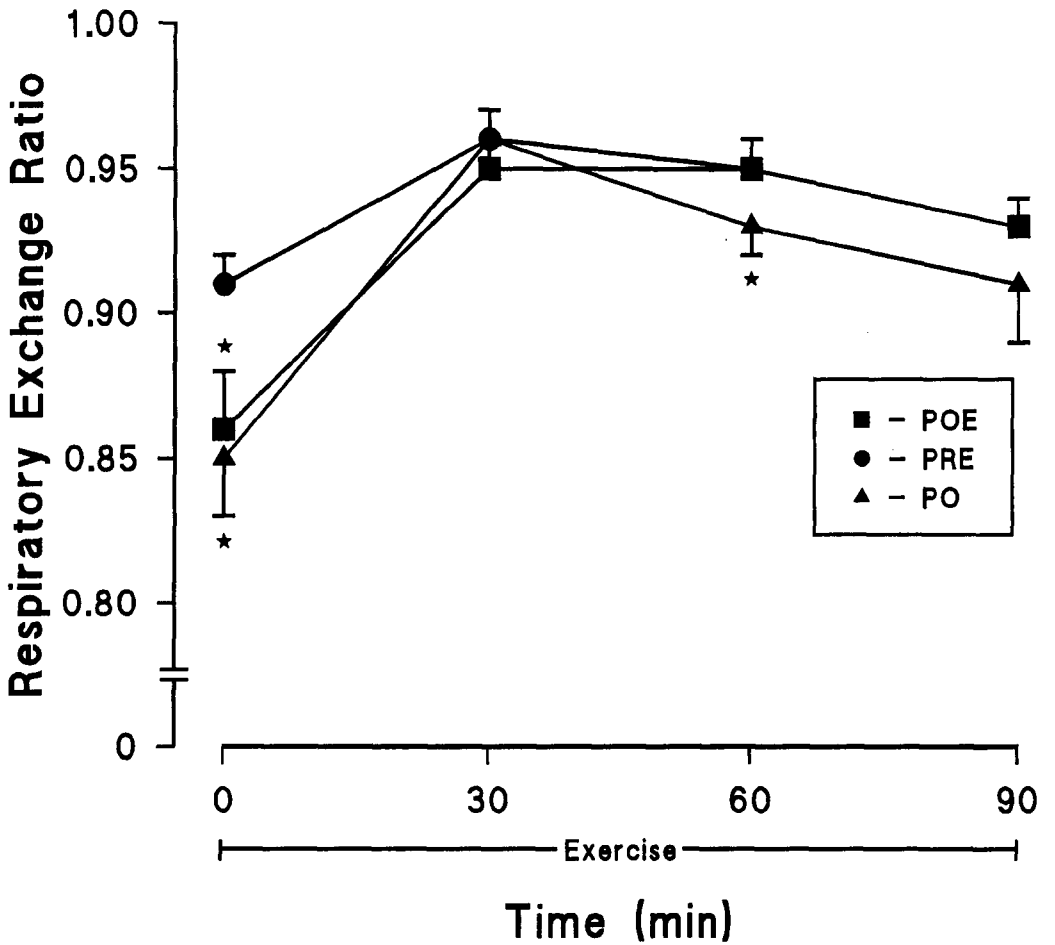


Figure 27. Exercise respiratory exchange ratio for POE, PRE, and PO trials with respect to time (Day 6)(mean + SE). ⋆$p<0.05$ between observed and PRE trials.

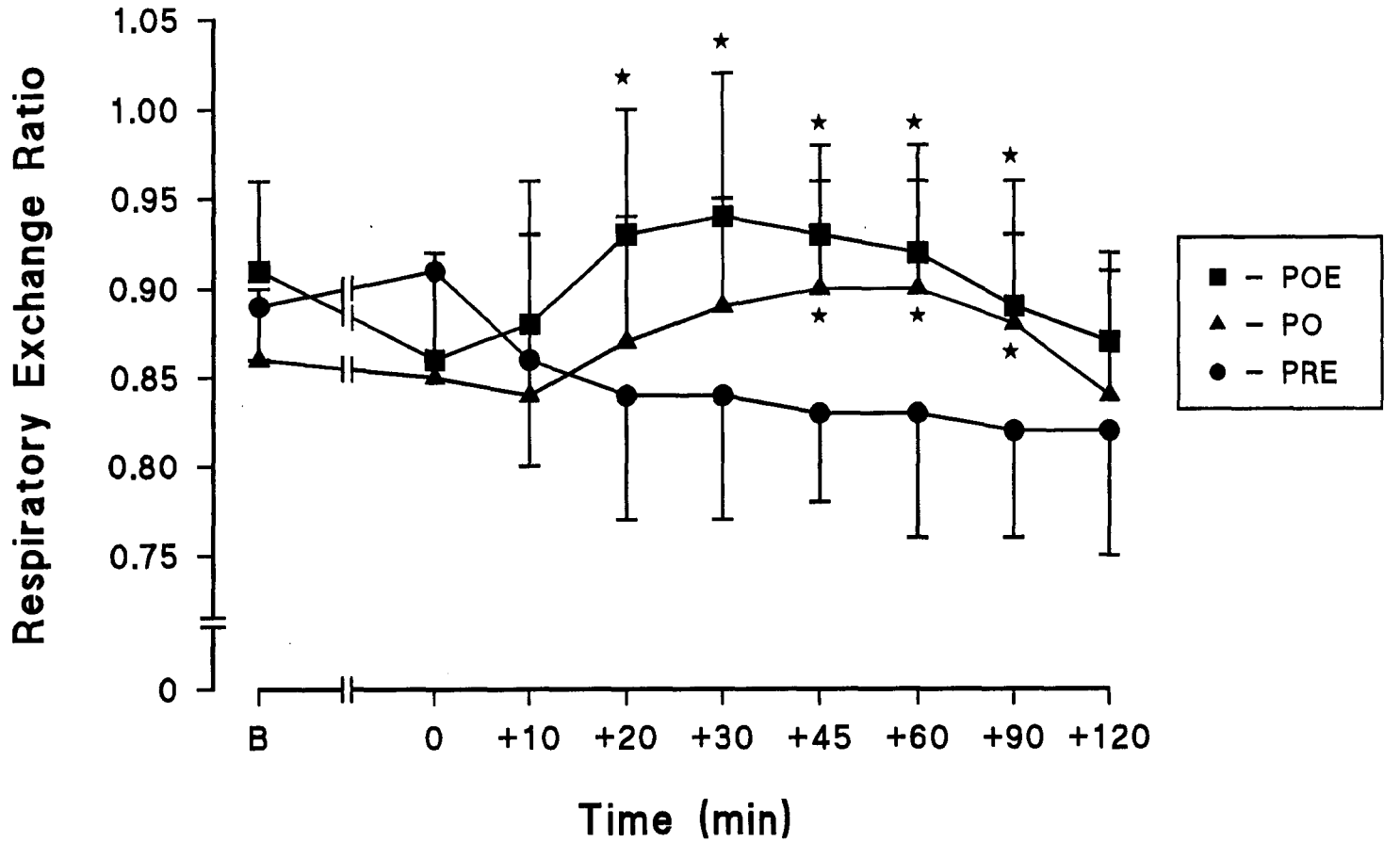


Figure 28. Respiratory exchange ratio for POE, PRE, and PO trials with respect to time (Day 6)(mean + SE). ⋆$p<0.05$ between observed and PRE trials.

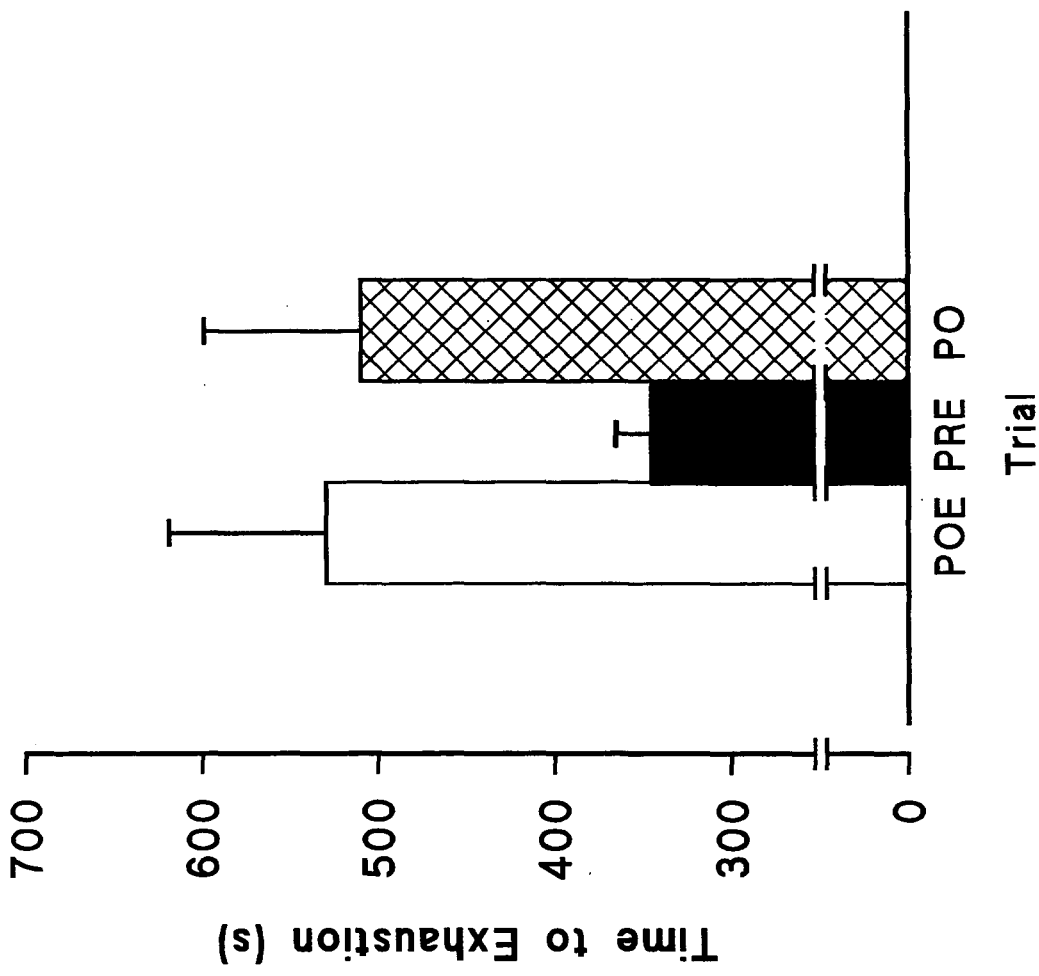


Figure 29. Time to exhaustion for POE, PRE, and PO trials on Day 7 (mean + SE).

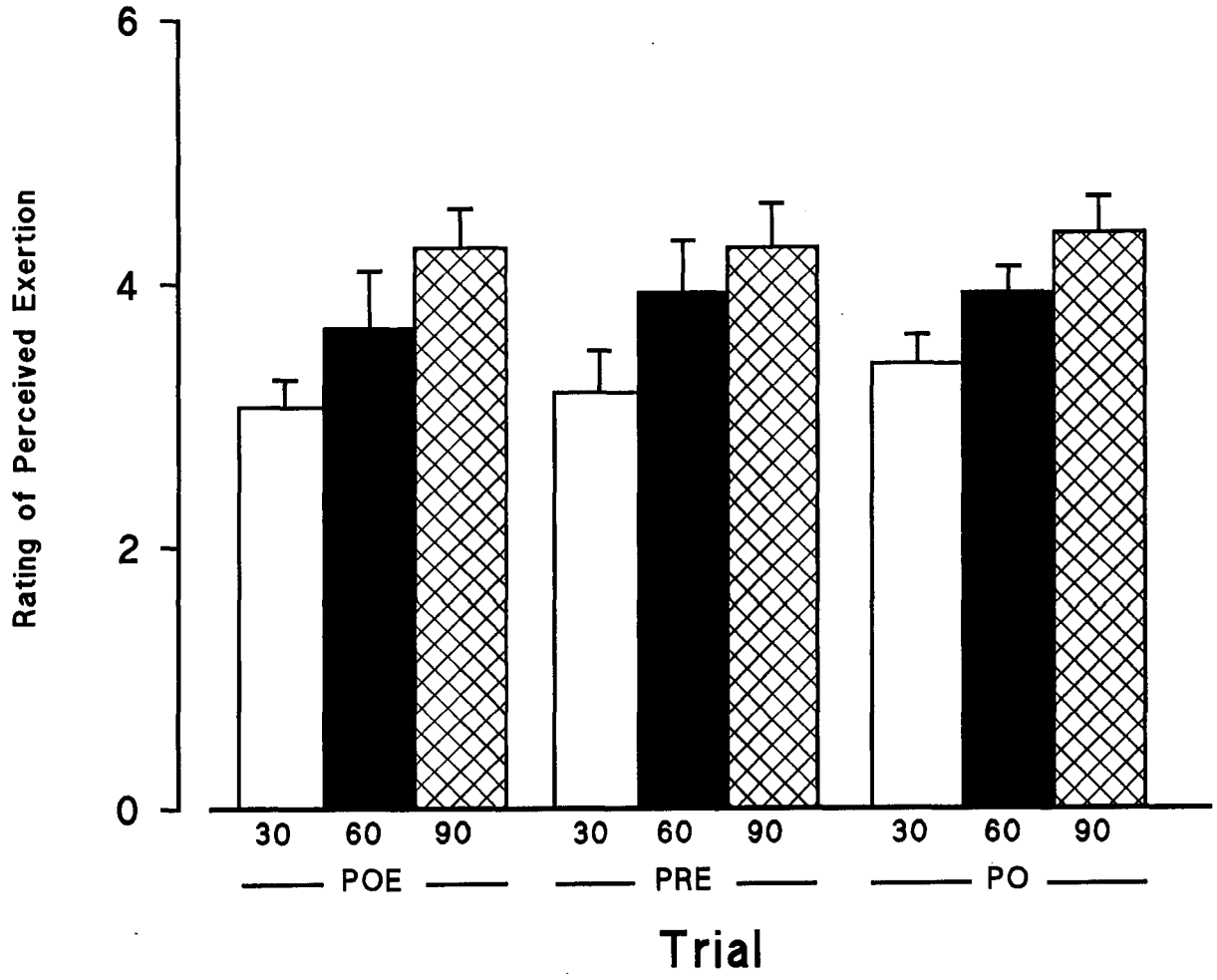


Figure 30. Rating of perceived exertion for POE, PRE, and PO trials with respect to time (Day6)(mean + SE).

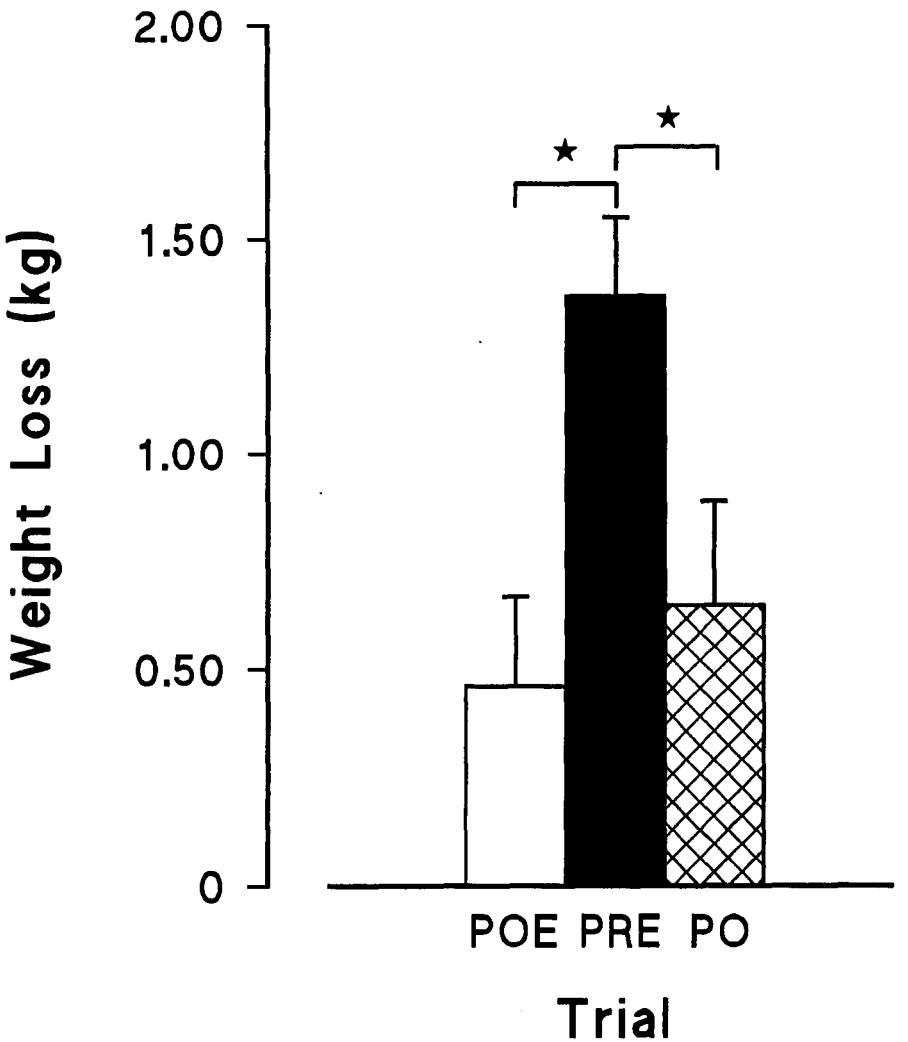


Figure 31. Weight Loss for POE, PRE, and PO trials (mean + SE).
★p<0.05 between observed and PRE trials.

Appendix I: ANOVA Tables.

Appendix 1.0. Hematocrit, 2-Way ANOVA.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Trial (Tr)	2	14.640	16	4.096	3.574	0.052086
Time (T)	7	**23.776**	**56**	**0.656**	**36.258**	**0.000000**
Tr x T	14	0.514	112	0.504	1.020	0.439047

Marked Effects: P < 0.05

Appendix 1.1. Sodium, 2-Way ANOVA.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Trial (Tr)	2	114.875	16	70.927	1.620	0.228800
Time (T)	7	**111.680**	**56**	**23.347**	**4.784**	**0.000287**
Tr x T	14	23.118	112	25.682	0.900	0.560688

Marked Effects: P < 0.05

Appendix 1.2. Potassium, 2-Way ANOVA.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Trial (Tr)	**2**	**1.362**	**16**	**0.212**	**6.409**	**0.009027**
Time (T)	7	**4.140**	**56**	**0.204**	**20.292**	**0.000000**
Tr x T	**14**	**0.275**	**112**	**0.115**	**2.386**	**0.006004**

Marked Effects: P < 0.05

Appendix 1.3. Lactate, 2-Way ANOVA.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Trial (Tr)	2	2.003	16	1.192	1.681	0.217416
Time (T)	7	**10.572**	**56**	**0.688**	**15.366**	**0.000000**
Tr x T	14	0.386	112	0.258	1.495	0.124082

Marked Effects: P < 0.05

Appendix 1.4. Glucose, 2-Way ANOVA.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Trial (Tr)	2	5.342	16	4.112	1.299	0.303654
Time (T)	7	**2.948**	**56**	**0.944**	**3.124**	**0.008306**
Tr x T	14	0.803	112	0.650	1.235	0.263033

Marked Effects: P < 0.05

Appendix 2.0. Estrogen, 1-Way ANOVA.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Trial (Tr)	2	3842.015	18	1036.681	3.706	0.044883

Marked Effects: $P < 0.05$

Appendix 2.1. Progesterone, 1-Way ANOVA.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Trial (Tr)	2	0.161	18	0.089	1.810	0.192200

Marked Effects: $P < 0.05$

Appendix 2.2. Insulin, 2-Way ANOVA.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Trial (Tr)	2	7274.389	16	749.708	9.703	0.001739
Time (T)	5	5862.884	40	258.333	22.695	0.000000
Tr x T	10	1972.630	80	248.214	7.947	0.000000

Marked Effects: $P < 0.05$

Appendix 3.0. Urinary Creatinine (g/24 h), 2-Way ANOVA.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Trial (Tr)	2	0.079	18	0.078	1.015	0.382117
Day (D)	1	0.211	9	0.393	0.538	0.481897
Tr x D	2	0.015	18	0.076	0.196	0.823995

Marked Effects: $P < 0.05$

Appendix 3.1. Urinary Urea (g/24 h), 2-Way ANOVA.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Trial (Tr)	2	13.597	18	5.232	2.599	0.101965
Day (D)	1	0.532	9	5.166	0.103	0.755597
Tr x D	2	4.182	18	5.863	0.713	0.503380

Marked Effects: $P < 0.05$

Appendix 3.2. Urinary Urea (g/g Creatinine), 2-Way ANOVA.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Trial (Tr)	2	24.180	18	11.344	2.132	0.147624
Day (D)	1	1.647	9	14.922	0.110	0.747343
Tr x D	2	8.366	18	6.533	1.280	0.302046

Marked Effects: $P < 0.05$

Appendix 3.3. Urinary 3-MH (Day 5&6)(µmol/24 h), 2-Way ANOVA.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Trial (Tr)	**2**	**22986.22**	**18**	**3982.001**	**5.772**	**0.011563**
Day (D)	1	18.38	9	3458.772	0.005	0.943488
Tr x D	2	615.50	18	4277.612	0.143	0.866970

Marked Effects: $P < 0.05$

Appendix 3.5. Urinary 3-MH (Day 5&6)(µmol/g Creatinine), 2-Way ANOVA.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Trial (Tr)	**2**	**25161.45**	**18**	**4061.619**	**6.194**	**0.008972**
Dayl(D)	1	60.48	9	1993.960	0.030	0.865621
Tr x D	2	107.55	18	2240.336	0.048	0.953250

Marked Effects: $P < 0.05$

Appendix 3.7. Apparent Nitrogen Balance, 2-Way ANOVA.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Trial (Tr)	**2**	**90.27**	**18**	**6.724**	**13.424**	**0.000270**
Dayl(D)	1	0.35	9	7.839	0.044	0.837799
Tr x D	2	4.47	18	5.761	0.776	0.474980

Marked Effects: $P < 0.05$

Appendix 4.0. Post-Supplement Oxygen Consumption (Day 5), 2-Way ANOVA.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Trial (Tr)	**2**	**0.112**	**18**	**0.026**	**4.255**	**0.030671**
Time (T)	**8**	**0.003**	**72**	**0.001**	**4.646**	**0.000132**
Tr x T	**16**	**0.002**	**144**	**0.001**	**2.594**	**0.001423**

Marked Effects: $P < 0.05$

Appendix 4.1. Exercise Oxygen Consumption (Day 6)(L/min), 2-Way ANOVA.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Trial (Tr)	2	0.011	18	0.008	1.217	0.319092
Time (T)	**3**	**18.199**	**27**	**0.042**	**431.009**	**0.000000**
Tr x T	6	0.004	54	0.002	2.028	0.077548

Marked Effects: $P < 0.05$

Appendix 4.2. Excess Post-Exercise Oxygen Consumption (Day 6), 2-Way ANOVA.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Trial (Tr)	**2**	**0.010**	**18**	**0.003**	**3.787**	**0.042399**
Time (T)	**8**	**0.008**	**72**	**0.001**	**5.302**	**0.000031**
Tr x T	16	0.003	144	0.001	1.774	0.40026

Marked Effects: $P < 0.05$

Appendix 4.3. Oxygen Consumption- POE Trial, 2-Way ANOVA.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Trial (Tr)	1	0.005	9	0.008	0.686	0.428899
Time (T)	**8**	**0.005**	**72**	**0.001**	**5.483**	**0.000021**
Tr x T	**8**	**0.002**	**72**	**0.001**	**2.499**	**0.018707**

Marked Effects: $P < 0.05$

Appendix 4.4. Oxygen Consumption- PRE Trial, 2-Way ANOVA.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Trial (Tr)	1	0.023	9	0.008	2.778	0.129926
Time (T)	**8**	**0.005**	**72**	**0.001**	**3.335**	**0.002668**
Tr x T	8	0.001	72	0.001	1.376	0.221894

Marked Effects: $P < 0.05$

Appendix 4.5. Oxygen Consumption- PO Trial, 2-Way ANOVA.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Trial (Tr)	1	0.047	9	0.023	2.090	0.182174
Time (T)	**8**	**0.003**	**72**	**0.001**	**2.235**	**0.003368**
Tr x T	8	0.002	72	0.001	2.114	0.045264

Marked Effects: $P < 0.05$

Appendix 4.6. Post-Supplement Respiratory Exchange Ratio (Day 6), 2-Way ANOVA

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Trial (Tr)	2	0.065	18	0.003	22.939	0.000011
Time (T)	8	0.008	72	0.001	5.767	0.000011
Tr x T	16	0.003	144	0.000	2.835	0.000503

Marked Effects: $P < 0.05$

Appendix 4.7. Exercise Respiratory Exchange Ratio (Day 6), 2-Way ANOVA

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Trial (Tr)	2	0.005	18	0.000	5.386	0.014676
Time (T)	3	0.029	27	0.000	33.709	0.000000
Tr x T	6	0.001	54	0.000	2.199	0.0057025

Marked Effects: $P < 0.05$

Appendix 4.8. Post-Exercise Respiratory Exchange Ratio (Day 6), 2-Way ANOVA.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Trial (Tr)	2	0.065	18	0.011	5.692	0.012147
Time (T)	8	0.008	72	0.001	5.255	0.000034
Tr x T	16	0.008	144	0.001	6.035	0.000000

Marked Effects: $P < 0.05$

Appendix 5.0. Time to Exhaustion (75% VO_{2max}), 1-Way ANOVA.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Trial (Tr)	2	101273.0	18	33508.52	3.022	0.073840

Marked Effects: $P < 0.05$

Appendix 5.1. Rating of Perceived Exertion, 2-Way ANOVA.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Trial (Tr)	2	0.803	18	0.691	1.161	0.335654
Time (T)	**2**	**8.669**	**18**	**0.762**	**11.377**	**0.000640**
Tr x T	4	0.061	36	0.228	0.268	0.896425

Marked Effects: $P < 0.05$

Appendix 5.2. Weight Loss, 1-Way ANOVA.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Trial (Tr)	**2**	**2.304**	**18**	**0.367**	**6.274**	**0.008564**

Marked Effects: $P < 0.05$

Appendix II: Study Data.

Appendix 2.0: Percent Hematocrit

		poe 0	poe 30	poe 60	poe 90	poe pe30	poe pe60	poe pe90	poe pe120	pre 0	pre 30	pre 60	pre 90	pre pe30	pre pe60	pre pe90	pre pe120	po 0	po 30	po 60	po 90	po pe30	po pe60	po pe90	po pe120
2	1	39.5	41.5	42.5	41.5	40.5	40	39.5	40	39.5	41.5	41.5	40.5	40.5	39.5	40	40	39	41.5	42	42	39	39	39.5	39.5
3	1	34	37	37	35.5	35	34.5		35.5	35.5	36	37	37	34.5	35	35	35.5	34.5	36	36.5	36	35	35	35.5	34
4	1	41.5	42.5	42.5	42.5	41	41	40.5	40	41	42	42.5	43	41	40	40	39.5	44	42.5	41	43	41	40	41	42
5	1	40.5	42.5	42	43	40	40	39.5	39.5	41	42.5	42.5	42	39	39.5	40	40	40	40.5	41.5	40	40	39	39.5	40
6	1	39	41	40.5	41	38.5	38	38	38.5	40	41	42.5	40	40	41	41.5	39.5	39.5	41	41	38.5	38.5	39	39	39.5
7	1	39	39	40	40	39	38.5	38.5	38	39	41.5	42	42	38.5	39	39	39.5	38	41	41	41.5	38	38.5	39	38.5
8	1	38	41.5	42.5	42.5	39	39	38.5	39	43	44.5	44.5	45.5	44	43.5	43.5	43.5	41	43	43.5	43.5	41.5	42	41.5	41.5
9	1	40	41.5	42	42	41.5	41	39	40.5	40	42.5	43	43	41	41	44.5	43.5	40	41	41.5	42	42	40	40	41
10	1	40	42.5	42	42	41	40	39	39.5	40	43	42.5	42.5	41	40.5	40	39.5	39.5	42	42.5	42.5	41	40	40	40
	mean	39.06	41.00	41.22	41.11	39.50	39.11	39.06	38.94	39.89	41.61	42.00	41.72	39.94	39.89	40.39	40.06	39.50	40.94	41.17	41.00	39.56	39.17	39.44	39.56
	sd	2.14	1.85	1.82	2.29	1.98	2.01	0.78	1.51	2.01	2.34	2.05	2.37	2.57	2.26	2.72	2.39	2.51	2.02	1.94	2.42	2.20	1.87	1.70	2.35
	se	0.71	0.62	0.61	0.76	0.66	0.67	0.27	0.50	0.67	0.78	0.68	0.79	0.86	0.75	0.91	0.80	0.84	0.67	0.65	0.81	0.73	0.62	0.57	0.78
		0	30	60	90	pe30	pe60	pe90	pe120																
	poe	39.06	41.00	41.22	41.11	39.50	39.11	39.06	38.94																
	pre	39.89	41.61	42.00	41.72	39.94	39.89	40.39	40.06																
	po	39.50	40.94	41.17	41.00	39.56	39.17	39.44	39.56																
		0	30	60	90	pe30	pe60	pe90	pe120																
	sd poe	2.14	1.85	1.82	2.29	1.98	2.01	0.78	1.51																
	sd pre	2.01	2.34	2.05	2.37	2.57	2.26	2.72	2.39																
	sd po	2.51	2.02	1.94	2.42	2.20	1.87	1.70	2.35																

Appendix 2.1: Sodium (mmol/L)

		poe 0	poe 30	poe 60	poe 90	poe pe30	poe pe60	poe pe90	poe pe120	pre 0	pre 30	pre 60	pre 90	pre pe30	pre pe60	pre pe90	pre pe120	po 0	po 30	po 60	po 90	po pe30	po pe60	po pe90	po pe120
2	1	160	164	146	169	140	141	140	140	139	142	144	142	140	139	140	140	139	143	142	143	140	141	141	140
3	1	142	144	144	141	141	140		141	141	142	143	142	136	137	138	139	139	141	140	139	137	137	137	138
4	1	139	141	139	140	138	138	139	140	148	147	145	145	146	144	145	144	142	139	138	140	138	139	139	139
5	1	179	146	128	146	144	143	143	142	142	144	141	144	141	141	141	142	141	143	143	142	140	141	141	141
6	1	142	143	145	144	141	144	141	141	142	143	150	168	139	162	140	141	139	142	142	136	139	139	139	139
7	1	140	142	140	141	136	137	138	141	141	142	141	141	139	138	139	139	140	142	141	142	138	139	141	142
8	1	138	141	142	139	140	138	138	138	139	141	143	142	138	137	137	137	140	143	143	143	139	139	137	139
9	1	137	141	141	142	138	137	138	138	140	141	141	140	136	137	140	139	138	142	141	141	135	137	136	136
10	1	138	142	152	141	137	131	136	137	139	142	142	142	138	139	139	138	139	141	139	139	137	137	137	136
	mean	146.11	144.89	141.89	144.78	139.44	138.78	139.13	139.78	141.22	142.67	143.33	147.33	139.22	141.56	139.89	139.89	139.67	141.78	141.00	140.56	138.11	138.78	138.67	138.89
	sd	14.19	7.36	6.51	9.32	2.46	3.87	2.17	1.72	2.82	1.87	2.87	15.32	3.03	8.00	2.26	2.15	1.22	1.30	1.73	2.30	1.62	1.56	2.00	2.03
	se	4.73	2.45	2.17	3.11	0.82	1.29	0.77	0.57	0.94	0.62	0.96	5.11	1.01	2.67	0.75	0.72	0.41	0.43	0.58	0.77	0.54	0.52	0.67	0.68
		0	30	60	90	pe30	pe60	pe90	pe120																
	poe	146.11	144.89	141.89	144.78	139.44	138.78	139.13	139.78																
	pre	141.22	142.67	143.33	147.33	139.22	141.56	139.89	139.89																
	po	139.67	141.78	141.00	140.56	138.11	138.78	138.67	138.89																
		0	30	60	90	pe30	pe60	pe90	pe120																
	sd poe	14.19	7.36	6.51	9.32	2.46	3.87	2.17	1.72																
	sd pre	2.82	1.87	2.87	15.32	3.03	8.00	2.26	2.15																
	sd po	1.22	1.30	1.73	2.30	1.62	1.56	2.00	2.03																

Appendix 2.2: Potassium (mmol/L)

		poe 0	poe 30	poe 60	poe 90	poe pe30	poe pe60	poe pe90	poe pe120	pre 0	pre 30	pre 60	pre 90	pre pe30	pre pe60	pre pe90	pre pe120	po 0	po 30	po 60	po 90	po pe30	po pe60	po pe90	po pe120
2	1	4.7	5.6	5.2	5.7	4.4	4.2	3.9	4.1	3.9	4.8	4.9	5	4.6	4.6	4.5	4.4	3.8	4.9	5.1	5.1	4	4.1	4.1	3.9
3	1	3.9	5.3	5.4	5.4	4.3	4.3	4	3.7	3.9	5.1	5.5	5.2	5	4.5	4.9	4.4	3.7	5.1	5.2	5.3	4.4	4.3	4.1	4.3
4	1	3.9	4.7	5.5	5.5	5.9	5.5	4	4.1	4.1	4.5	4.6	4.7	4.6	4.7	5.1	5.8	4.3	4.9	4.8	4.8	4.3	4.2	4.2	4.1
5	1	4.5	4.9	4.6	5.2	4.1	4.4	4.1	4.2	4	4.9	4.8	5.1	4.3	4.3	3.9	4.3	3.8	4.8	4.8	4.6	3.8	3.9	3.9	4
6	1	4	5.1	5	5.2	4.4	4.4	4.1	4.1	4.1	5.2	5.3	6.3	4.7	5.1	4.4	4.2	3.8	4.6	4.6	4.6	4.5	4.2	4.1	3.9
7	1	3.9	4.7	4.4	4.2	3.8	3.8	4	3.9	3.8	5	4.8	4.6	4.4	3.9	4	3.9	3.9	4.9	4.9	4.7	3.7	4	3.8	3.9
8	1	4.2	5.1	5.1	5.1	4.5	4.8	4.8	5.4	4.2	5.2	5.3	5.5	5.7	5.5	5.7	6	4	5	5.5	5.3	5.1	4.6	5.4	4.9
9	1	3.9	4.6	4.9	4.8	4.6	4.4	4.2	4.3	3.8	4.6	4.7	4.9	5.4	4.6	4.7	4.7	3.9	4.8	5.1	4.8	4.5	4.9	4.2	4.3
10	1	3.9	4.9	5.3	4.7	4.7	3.8	3.8	3.9	4.3	5.4	4.8	4.8	4	4.2	4.8	4.9	4	5	4.5	4.3	4	3.9	3.8	3.8
	mean	4.10	4.99	5.04	5.09	4.52	4.40	4.10	4.19	4.01	4.97	4.97	5.12	4.74	4.60	4.67	4.73	3.91	4.89	4.94	4.83	4.26	4.23	4.18	4.12
	sd	0.30	0.32	0.36	0.46	0.58	0.52	0.29	0.49	0.18	0.30	0.32	0.52	0.54	0.48	0.55	0.72	0.18	0.15	0.31	0.34	0.43	0.33	0.48	0.34
	se	0.10	0.11	0.12	0.15	0.19	0.17	0.10	0.16	0.06	0.10	0.11	0.17	0.18	0.16	0.18	0.24	0.06	0.05	0.10	0.11	0.14	0.11	0.16	0.11
		0	30	60	90	pe30	pe60	pe90	pe120																
	poe	4.10	4.99	5.04	5.09	4.52	4.40	4.10	4.19																
	pre	4.01	4.97	4.97	5.12	4.74	4.60	4.67	4.73																
	po	3.91	4.89	4.94	4.83	4.26	4.23	4.18	4.12																
		0	30	60	90	pe30	pe60	pe90	pe120																
	sd poe	0.30	0.32	0.36	0.46	0.58	0.52	0.29	0.49																
	sd pre	0.18	0.30	0.32	0.52	0.54	0.48	0.55	0.72																
	sd po	0.18	0.15	0.31	0.34	0.43	0.33	0.48	0.34																

Appendix 2.3: Lactate (mmol/L)

		poe 0	poe 30	poe 90	poe 120	poe pe30	poe pe60	poe pe90	poe pe120	pre 0	pre 30	pre 60	pre 90	pre pe30	pre pe60	pre pe90	pre pe120	po 0	po 30	po 60	po 90	po pe30	po pe60	po pe90	po pe120
2	1	0.7	1.7	1	2.1	1.6	1.5	1.2	0.9	0.8	1.5	1.7	1.4	0.8	0.7	0.6	0.8	0.8	2.1	2	2.1	1.4	1.2	1.1	0.7
3	1	1.4	4.8	2.9	2.3	1.5	2.6	2	1.4	1.2	2.5	2.6	2.5	1	1	1.6	1.1	1.1	2.4	1.9	1.7	1.4	1.5	1.1	1
4	1	1.6	2.1	2.1	2.1	3.2	3.5	3	2.7	2	2.6	2.1	2.3	2.9	2.1	2.4	2.8	2.3	2.4	2.9	2.4	2.4	2.2	2	1.6
5	1	1.7	4.3	4.5	2.3	2.3	1.8	1.3	1.1	1.3	4.4	2.8	2.4	1.2	0.8	0.7	0.9	1.9	3.3	3.3	1.5	1.7	1.3	1.9	0.7
6	1	1.2	2.5	2.2	2.1	2.5	1.8	1.4	1	1.8	2.5	2.2	2.5	1.1	1.2	0.5	0.7	1.1	2.5	1.5	1.6	1.6	1.3	1.1	1.3
7	1	1	2.7	3.7	3.4	2.1	1.7	1.8	1.1	1.3	2.3	1.1	1.9	1.2	1.3	1.2	0.8	0.8	1.2	2.4	2.3	1.4	1.5	1.7	1.1
8	1	1.3	1.9	2	1.8	2.8	2.8	2.1	1.4	1.7	3.5	3	2.4	2.6	2.9	2.4	2	2.1	5.4	4	3	5.2	3.5	3.3	2
9	1	2.1	4.8	4.1	3.3	4.6	3.7	4	2.9	1.6	4.3	4.3	3.3	2.5	4	1.5	1.5	2.3	6.2	4.2	3.9	3.1	3.9	2.9	3.7
10	1	2	5.4	3.3	2.8	3.3	3.1	3.3	1.8	2	5.3	4.9	3.9	2.1	2.3	2.7	3.2	1.6	6.1	3.5	3.3	3.2	3.5	2.7	2.2
	mean	1.44	3.36	2.87	2.47	2.66	2.50	2.23	1.59	1.52	3.21	2.74	2.51	1.71	1.81	1.51	1.53	1.56	3.51	2.86	2.42	2.38	2.21	1.98	1.59
	sd	0.46	1.45	1.14	0.57	0.96	0.83	0.98	0.74	0.40	1.23	1.21	0.73	0.81	1.11	0.84	0.93	0.62	1.88	0.96	0.83	1.28	1.11	0.83	0.95
	se	0.15	0.48	0.38	0.19	0.32	0.28	0.33	0.25	0.13	0.41	0.40	0.24	0.27	0.37	0.28	0.31	0.21	0.63	0.32	0.28	0.43	0.37	0.28	0.32
		0	30	60	90	pe30	pe60	pe90	pe120																
	poe	1.44	3.36	2.87	2.47	2.66	2.50	2.23	1.59																
	pre	1.52	3.21	2.74	2.51	1.71	1.81	1.51	1.53																
	po	1.56	3.51	2.86	2.42	2.38	2.21	1.98	1.59																
		0	30	60	90	pe30	pe60	pe90	pe120																
	sd poe	0.46	1.45	1.14	0.57	0.96	0.83	0.98	0.74																
	sd pre	0.40	1.23	1.21	0.73	0.81	1.11	0.84	0.93																
	sd po	0.62	1.88	0.96	0.83	1.28	1.11	0.83	0.95																

Appendix 2.4: Glucose (mmol/L)

		poe 0	poe 30	poe 60	poe 90	poe pe30	poe pe60	poe pe90	poe pe120	pre 0	pre 30	pre 60	pre 90	pre pe30	pre pe60	pre pe90	pre pe120	po 0	po 30	po 60	po 90	po pe30	po pe60	po pe90	po pe120
2	1	6.95	5.88	4.33	4.83	6.44	6	5.24	4.86	5.54	5.32	5.82	5.68	2.06	1.9	2.94	2.16	3.02	2.53	1.2	2.27	1.39	2.6	3.21	3.65
3	1	3.97	4.13	4.55	2.95	2.6	4.14	4.21	2.87	5.2	5.21	5.11	5.34	4.71	4.91	4.56	4.96	5.36	5.56	4.7	2.33	3.33	3.32	2.32	3.26
4	1	6.47	4.4	7.11	4.97	5.55	5.35	4.27	5.48	4.17	4.23	4.9	4.27	4.89	4.26	3.96	4.77	4.49	3.74	3.4	3.92	5.23	4.41	4.42	4.35
5	1	3.14	3.39	4.32	4.52	5.75	3.12	3.55	3.05	3.27	3.17	3.16	4.09	4.45	4.54	4.4	4.79	5.13	4.61	4.97	4.07	5.16	6.47	4.06	3.84
6	1	4.34	4.49	5.43	5.02	6.84	4.66	4.96	4.26	3.38	3.66	3.98	3.87	4.29	4.46	3.27	4.01	3.78	3.47	3.88	3.72	4.94	4.11	3.9	2.69
7	1	3.42	3.43	3.39	3.41	5.63	3.32	3.24	3.53	3.87	4.27	3.36	3.53	4.56	4.08	4.08	3.46	3.88	4.49	4.66	4.64	4.77	5.27	5.26	5.25
8	1	3.6	5.43	4.98	4.73	5.44	4.21	4.16	4.8	5	5.02	5.77	5.61	5.83	5.11	4.72	4.8	5.91	4.95	5.04	5.27	5.89	5.24	4.39	4.89
9	1	6.34	3.18	4.88	7.23	5.87	3.37	6.65	6.24	5.43	3.59	5.41	4.95	4.31	4.09	4.1	4.25	5.4	3.77	5.34	4.54	8.6	1.8	5.68	4.87
10	1	6.6	5.05	5.67	5.39	7.51	5.98	5.84	5.26	6.66	5.19	5.21	4.74	6.08	5.04	4.85	5.03	5.66	4.51	4.87	4.5	7.73	6.45	6.53	6.37
	mean	4.98	4.38	4.96	4.78	5.74	4.46	4.68	4.48	4.72	4.41	4.75	4.68	4.58	4.27	4.10	4.25	4.74	4.18	4.23	3.92	5.23	4.41	4.42	4.35
	sd	1.57	0.95	1.05	1.21	1.36	1.12	1.10	1.15	1.13	0.81	1.00	0.78	1.14	0.97	0.64	0.94	0.99	0.90	1.29	1.02	2.14	1.63	1.28	1.13
	se	0.52	0.32	0.35	0.40	0.45	0.37	0.37	0.38	0.38	0.27	0.33	0.26	0.38	0.32	0.21	0.31	0.33	0.30	0.43	0.34	0.71	0.54	0.43	0.38
		0	30	60	90	pe30	pe60	pe90	pe120																
	poe	4.98	4.38	4.96	4.78	5.74	4.46	4.68	4.48																
	pre	4.72	4.41	4.75	4.68	4.58	4.27	4.10	4.25																
	po	4.74	4.18	4.23	3.92	5.23	4.41	4.42	4.35																
		0	30	60	90	pe30	pe60	pe90	pe120																
	sd poe	1.57	0.95	1.05	1.21	1.36	1.12	1.10	1.15																
	sd pre	1.13	0.81	1.00	0.78	1.14	0.97	0.64	0.94																
	sd po	0.99	0.90	1.29	1.02	2.14	1.63	1.28	1.13																

Appendix 2.5: Estrogen (nmol/L)

		poe	pre	po
1	1	0.046	0.085	0.280
2	1	0.100	0.052	0.651
3	1	0.007	0.006	0.007
4	1	0.122	0.088	0.184
5	1	0.057	0.172	0.438
6	1	0.094	0.052	0.054
7	1	0.005	0.064	0.030
8	1	0.006	0.135	0.149
9	1	0.013	0.022	0.012
10	1	0.012	0.110	0.033
	mean	0.046	0.079	0.184
	sd	0.045	0.051	0.215
	se	0.014	0.016	0.068
	poe	0.046		
	pre	0.079		
	po	0.184		
	sd pre	0.045		
	sd po	0.051		
	sd poe	0.215		

Appendix 2.6: Progesterone (nmol/L)

		poe	pre	po
1	1	1.5	1.32	2.44
2	1	1.28	1.42	1.57
3	1	1.34	1.72	1.69
4	1	1.41	1.15	1.36
5	1	2.3	1.96	2.54
6	1	1.99	1.83	1.24
7	1	1.2	1.21	1.66
8	1	1.4	1.78	1.54
9	1	1.85	2.36	2.55
10	1	0.84	1.24	1.03
	mean	1.51	1.60	1.76
	sd	0.42	0.39	0.55
	se	0.13	0.12	0.18
	poe	1.51		
	pre	1.60		
	po	1.76		
	sd pre	0.42		
	sd po	0.39		
	sd poe	0.55		

Appendix 2.7: Insulin (uIU/mL)

		poe 0	poe 90	poe pe30	poe pe60	poe pe90	poe pe120	pre 0	pre 90	pre pe30	pre pe60	pre pe90	pre pe120	po 0	po 30	po pe30	po pe60	po pe90	po pe120
2	1	19.86	2.64	56.91	17.89	10.76	7.22	6.25	3.4	4.6	6.37	5.94	5.03	13.13	3.4	53.51	20.2	21.57	18.13
3	1	25.25	11.44	102.64	138.55	25.04	8.78	31.9	5.05	3.72	8.18	4.3	5.19	46.39	3.23	30.8	30.78	13.72	16.91
4	1	52.03	18.37	149.42	77.36	48.04	31.55	117.46	11.45	15.27	15.67	29.84	13.69	33.34	3.02	49.04	34.71	15.62	14.34
5	1	22.77	3.95	36.66	13.71	9.39	5.69	21.69	3.86	6.05	5.75	8.78	7.94	39.71	3.8	62.54	71.83	12.18	9.79
6	1	39.91	17.85	159.79	57.87	48.54	58.87	25.73	6.61	7.21	11.42	13.35	16.66	36.84	3.82	49.46	17.65	14.22	10.17
7	1	7.75	1.31	30.87	10.08	15.94	8.45	9.18	2.22	5.04	3.52	3.45	3.25	22.12	3.96	31.26	17.95	13.39	9.32
8	1	39.71	7.25	101.84	33.5	13.5	13.89	25.98	7.33	15.68	9.67	9.65	8.4	64.33	7.51	69	45.73	11.17	9.8
9	1	22.17	4.7	63.58	35.72	26.74	19.46	20.57	3.09	7.44	4.2	10.18	8.18	24.27	3.55	67.38	46.39	15.48	8.81
10	1	30.47	4.6	43.25	30.92	27.41	14.04	29.64	3.61	3.38	3.82	6.14	5.27	16.36	1.59	31.28	27.12	23.26	31.76
	mean	28.88	8.01	82.77	46.18	25.04	18.66	32.04	5.18	7.60	7.62	10.18	8.18	32.94	3.76	49.36	34.71	15.62	14.34
	sd	13.23	6.42	48.16	40.78	14.81	17.06	33.18	2.88	4.68	4.06	8.01	4.39	16.15	1.57	15.41	17.65	4.12	7.41
	se	4.41	2.14	16.05	13.59	4.94	5.69	11.06	0.96	1.56	1.35	2.67	1.46	5.38	0.52	5.14	5.88	1.37	2.47
		0	90	pe30	pe60	pe90	pe120												
	poe	28.88	8.01	82.77	46.18	25.04	18.66												
	pre	32.04	5.18	7.60	7.62	10.18	8.18												
	po	32.94	3.76	49.36	34.71	15.62	14.34												
		0	90	pe30	pe60	pe90	pe120												
	sd poe	13.23	6.42	48.16	40.78	14.81	17.06												
	sd pre	33.18	2.88	4.68	4.06	8.01	4.39												
	sd po	16.15	1.57	15.41	17.65	4.12	7.41												

Appendix 2.8: Urinary Creatinine (g/24 h)

		poe 5	poe 6	pre 5	pre 6	po 5	po 6
1	1	1.78	0.8	1.87	0.67	3.02	0.83
2	1	2	1.71	1.7	2.21	1.94	1.97
3	1	1.34	0.6	0.73	0.62	0.68	0.65
4	1	0.4	0.76	0.61	0.94	0.4	0.7
5	1	1.3	0.87	1.04	1.68	0.97	0.86
6	1	1.14	1.23	1.61	1.39	1.31	1.31
7	1	0.95	1.05	1.68	1.31	0.77	1.23
8	1	1.17	0.99	1.08	0.92	1.14	0.95
9	1	0.75	0.76	0.96	0.71	1.06	1.05
10	1	0.82	1.06	1.08	1.09	0.81	1.63
	mean	1.17	0.98	1.24	1.15	1.21	1.12
	sd	0.48	0.31	0.44	0.50	0.76	0.42
	se	0.15	0.10	0.14	0.16	0.24	0.13
		5	6				
	poe	1.17	0.98				
	pre	1.24	1.15				
	po	1.21	1.12				
		5	6				
	sd poe	0.48	0.31				
	sd pre	0.44	0.50				
	sd po	0.76	0.42				

Appendix 2.9: Urinary Urea (g/24 h)

		poe 5	poe 6	pre 5	pre 6	po 5	po 6
1	1	13.67	8.64	12.16	7.16	9.42	9.78
2	1	12.33	9.21	9.03	12.1	9.23	15.13
3	1	8.39	8.49	11.24	12.77	10.31	6.79
4	1	5.49	6.21	6.04	12.1	4.7	3.61
5	1	10.2	4.83	8.4	12.14	7.41	4.78
6	1	4.96	9.16	8.13	10.16	8.24	6.09
7	1	7.42	13.05	10.32	11.13	9.84	11.27
8	1	17.04	15.7	15.28	14.47	8.85	12.79
9	1	13.67	12.73	17.05	13.6	17.15	17.26
10	1	11.56	10.71	10.15	14.08	12.95	10.34
	mean	10.47	9.87	10.78	11.97	9.81	9.78
	sd	3.90	3.27	3.34	2.14	3.33	4.49
	se	1.23	1.03	1.06	0.68	1.05	1.42
		5	6				
	poe	10.47	9.87				
	pre	10.78	11.97				
	po	9.81	9.78				
		5	6				
	sd poe	3.90	3.27				
	sd pre	3.34	11.97				
	sd po	3.33	4.49				

Appendix 2.10: Urinary Urea (g/g Creatinine)

		poe 5	poe 6	pre 5	pre 6	po 5	po 6
1	1	7.68	10.8	6.5	10.69	3.12	11.79
2	1	6.16	5.38	5.31	5.47	4.76	7.68
3	1	6.26	14.16	15.4	20.61	15.16	10.44
4	1	13.72	8.17	21.59	17.53	11.76	5.16
5	1	7.85	5.55	8.08	7.23	7.64	5.56
6	1	4.36	7.45	6.05	7.31	6.29	4.65
7	1	7.81	12.43	6.15	8.5	12.78	9.17
8	1	14.56	15.86	14.15	15.72	7.76	13.47
9	1	18.23	16.75	17.76	19.15	16.18	16.44
10	1	14.1	10.11	9.39	12.91	15.99	6.35
	mean	10.07	10.67	11.04	12.51	10.14	9.07
	sd	4.65	4.09	5.76	5.47	4.84	3.94
	se	1.47	1.29	1.82	1.73	1.53	1.24
		5	6				
	poe	10.07	10.67				
	pre	11.04	12.51				
	po	10.14	9.07				
		5	6				
	sd poe	4.65	4.09				
	sd pre	5.76	5.47				
	sd po	4.84	3.94				

Table 2.11. Urinary 3-Methylhistidine (umol/24 h).

	poe5	pre5	po5	poe6	pre6	po6
1	264.16	379.33	343.06	283.42	229.56	261.91
2	441.50	222.19	291.13	373.82	211.14	276.28
3	275.49	163.70	287.54	205.64	178.98	257.39
4	237.64	93.12	246.65	213.95	82.10	212.59
5	364.79	190.31	208.02	278.01	302.91	290.86
6	187.34	319.48	251.44	285.61	141.67	200.18
7	248.94	250.26	221.58	376.83	334.17	206.86
8	329.18	198.26	240.98	267.91	233.22	401.39
9	324.54	244.53	223.95	264.30	158.48	308.31
10	382.10	259.70	334.47	382.17	441.68	330.85
mean	305.57	232.09	264.88	293.17	231.39	274.66
sd	76.87	79.96	47.17	64.30	104.73	62.43
se	24.31	25.29	14.92	20.33	33.12	19.74

Table 2.12. Urinary 3-Methylhistidine (umol/g Creatinine).

	poe 5	pre 5	po 5	poe 6	pre 6	po 6
1	79.15	71.81	72.35	164.78	105.42	102.62
2	71.67	57.32	139.60	69.31	17.77	83.98
3	99.08	113.83	157.20	103.04	106.92	171.42
4	212.94	88.24	322.00	224.74	42.19	208.01
5	143.53	68.28	78.27	152.45	71.83	156.58
6	110.66	172.55	137.10	156.85	72.28	139.55
7	127.83	45.42	111.11	159.67	73.09	44.37
8	133.98	50.71	145.78	135.84	103.05	243.53
9	294.37	170.96	130.42	211.31	172.37	193.17
10	388.31	120.83	235.96	260.67	222.64	121.54
mean	166.15	95.99	152.98	163.87	98.76	146.48
sd	102.78	47.06	74.76	56.72	60.31	60.58
se	52.54	30.36	48.38	51.82	31.23	46.32

Appendix 2.13.: Apparent Nitrogen Balance (Day 5).

	Protein (g/d)			Nitrogen(g/d) = Protein(g/d)/6.25			Urea (g/d)			Cr (g/d)		
	poe 5	pre 5	po 5	poe 5	pre 5	po 5	poe 5	pre 5	po 5	poe 5	pre 5	po 5
1	93.75	78.75	78.75	15	12.6	12.6	13.67	12.16	9.42	1.78	1.87	3.02
2	97.52	83.48	83.48	15.60	13.36	13.36	12.33	9.03	9.23	2	1.7	1.94
3	114.3	86.11	86.11	18.29	13.78	13.78	8.39	11.24	10.31	1.34	0.73	0.68
4	110.7	96.66	96.66	17.71	15.47	15.47	5.49	6.04	4.7	0.4	0.61	0.4
5	85.2	67.26	67.26	13.63	10.76	10.76	10.2	8.4	7.41	1.3	1.04	0.97
6	109.5	79.96	79.96	17.52	12.79	12.79	4.96	8.13	8.24	1.14	1.61	1.31
7	127.2	109.6	109.6	20.35	17.54	17.54	7.42	10.32	9.84	0.95	1.68	0.77
8	112.3	98.24	98.24	17.97	15.72	15.72	17.04	15.28	8.85	1.17	1.08	1.14
9	94.46	82.46	82.46	15.11	13.19	13.19	13.67	17.05	17.26	0.75	0.96	1.06
10	101.2	82.29	82.29	16.19	13.17	13.17	11.56	10.15	10.34	0.82	1.08	0.81
mean	104.61	86.48	86.48	16.74	13.84	13.84	10.47	10.78	9.56	1.17	1.24	1.21
sd	12.40	11.98	11.98	1.98	1.92	1.92	3.90	3.34	3.18	0.48	0.44	0.76
se	3.92	3.79	3.79	0.63	0.61	0.61	1.23	1.06	1.01	0.15	0.14	0.24

Nitrogen Balance = Nitrogen (g/d) - Urea (g/d) - Creatinine (g/d) - Feces [1.394 g/d] - Sweat [0.581 g/d] - Miscellaneous [0.14 g/d]

	Apparent Nitrogen Balance.		
	poe	pre	po
1	-2.57	-3.55	-1.96
2	-0.84	0.51	0.07
3	6.44	-0.31	0.67
4	9.71	6.70	8.25
5	0.02	-0.79	0.27
6	9.31	0.94	1.13
7	9.87	3.42	4.81
8	-2.36	-2.76	3.61
9	-1.42	-6.93	-7.24
10	1.70	-0.18	-0.10
mean	2.99	-0.29	0.95
sd	5.25	3.74	4.13
se	1.66	1.18	1.31

Appendix 2.14: Apparent Nitrogen Balance (Day 6).

	Protein (g/d)			Nitrogen(g/d) = Protein(g/d)/6.25			Urea (g/d)			Cr (g/d)		
	poe 6	pre 6	po 6	poe 6	pre 6	po 6	poe 6	pre 6	po 6	poe 6	pre 6	po 6
1	93.75	78.75	78.75	15	12.6	12.6	8.64	7.16	9.78	0.8	0.67	0.83
2	97.52	83.48	83.48	15.60	13.36	13.36	9.21	12.1	15.13	1.71	2.21	1.97
3	114.3	86.11	86.11	18.29	13.78	13.78	8.49	12.77	6.79	0.6	0.62	0.65
4	110.7	96.66	96.66	17.71	15.47	15.47	6.21	12.1	3.61	0.76	0.94	0.7
5	85.2	67.26	67.26	13.63	10.76	10.76	4.83	12.14	4.78	0.87	1.68	0.86
6	109.5	79.96	79.96	17.52	12.79	12.79	9.16	10.16	6.09	1.23	1.39	1.31
7	127.2	109.6	109.6	20.35	17.54	17.54	13.05	11.13	11.27	1.05	1.31	1.23
8	112.3	98.24	98.24	17.97	15.72	15.72	15.7	14.47	12.79	0.99	0.92	0.95
9	94.46	82.46	82.46	15.11	13.19	13.19	12.73	13.6	17.26	0.76	0.71	1.05
10	101.2	82.29	82.29	16.19	13.17	13.17	10.71	14.08	10.34	1.06	1.09	1.63
mean	104.61	86.48	86.48	16.74	13.84	13.84	9.87	11.97	9.78	0.98	1.15	1.12
sd	12.40	11.98	11.98	1.98	1.92	1.92	3.27	2.14	4.49	0.31	0.50	0.42
se	3.92	3.79	3.79	0.63	0.61	0.61	1.03	0.68	1.42	0.10	0.16	0.13

Nitrogen Balance = Nitrogen (g/d) - Urea (g/d) - Creatinine (g/d) - Feces [1.394 g/d] - Sweat [0.581 g/] - Miscellaneous [0.14 g/d]

	Apparent Nitrogen Balance.		
	poe	pre	po
1	3.45	2.66	-0.13
2	2.57	-3.07	-5.86
3	7.08	-1.73	4.22
4	8.63	0.31	9.04
5	5.82	-5.17	3.01
6	5.02	-0.87	3.28
7	4.14	2.98	2.92
8	-0.84	-1.79	-0.14
9	-0.49	-3.23	-7.23
10	2.31	-4.12	-0.92
mean	3.77	-1.40	0.82
sd	3.05	2.73	4.81
se	0.96	0.86	1.52

Appendix 2.15: Post-Supplement Oxygen Consumption (Day5)(L/min).

	poe5b	poe50	poe510	poe520	poe530	poe545	poe560	poe590	poe5120	pre5b	pre50	pre510	pre520	pre530	pre545	pre560	pre590	pre5120	po5b	po50	po510	po520	po530	po545	po560	po590	po5120
1	0.294	0.282	0.297	0.329	0.349	0.463	0.33	0.27	0.295	0.258	0.267	0.318	0.341	0.298	0.294	0.288	0.266	0.234	0.257	0.257	0.229	0.222	0.28	0.235	0.271	0.302	0.243
2	0.219	0.219	0.244	0.26	0.275	0.261	0.302	0.31	0.211	0.331	0.265	0.3	0.296	0.307	0.289	0.305	0.277	0.263	0.275	0.361	0.297	0.3	0.248	0.338	0.311	0.286	0.334
3	0.247	0.255	0.258	0.275	0.262	0.251	0.245	0.257	0.246	0.256	0.227	0.251	0.267	0.265	0.243	0.233	0.207	0.215	0.289	0.214	0.25	0.287	0.251	0.259	0.277	0.231	0.224
4	0.25	0.272	0.288	0.309	0.288	0.355	0.307	0.228	0.252	0.211	0.227	0.197	0.209	0.205	0.216	0.242	0.198	0.216	0.231	0.175	0.151	0.129	0.124	0.136	0.116	0.163	0.093
5	0.302	0.302	0.329	0.339	0.346	0.388	0.313	0.299	0.321	0.179	0.184	0.181	0.166	0.215	0.184	0.16	0.169	0.155	0.278	0.305	0.303	0.323	0.333	0.333	0.291	0.307	0.276
6	0.355	0.237	0.308	0.321	0.335	0.329	0.308	0.337	0.305	0.281	0.268	0.292	0.281	0.283	0.244	0.3	0.288	0.29	0.337	0.272	0.253	0.29	0.299	0.325	0.268	0.288	0.27
7	0.267	0.266	0.29	0.29	0.3	0.308	0.307	0.27	0.321	0.261	0.253	0.26	0.236	0.251	0.253	0.242	0.235	0.211	0.295	0.306	0.267	0.288	0.288	0.306	0.314	0.301	0.295
8	0.193	0.196	0.158	0.187	0.21	0.24	0.215	0.243	0.231	0.226	0.254	0.197	0.227	0.222	0.193	0.197	0.196	0.2	0.245	0.29	0.241	0.275	0.278	0.309	0.278	0.272	0.284
9	0.26	0.257	0.273	0.282	0.269	0.268	0.308	0.255	0.243	0.264	0.251	0.267	0.278	0.29	0.26	0.259	0.237	0.232	0.324	0.36	0.36	0.366	0.366	0.309	0.317	0.321	0.29
10	0.211	0.211	0.227	0.243	0.245	0.282	0.275	0.264	0.21	0.28	0.243	0.287	0.298	0.23	0.235	0.193	0.209	0.248	0.259	0.252	0.251	0.251	0.284	0.272	0.29	0.239	0.26
mean	0.2598	0.2497	0.267	0.2815	0.2879	0.3145	0.291	0.2733	0.2635	0.2547	0.2437	0.255	0.2599	0.2546	0.2411	0.2419	0.2282	0.2264	0.277	0.2792	0.2622	0.2741	0.2731	0.2822	0.2733	0.271	0.2569
sd	0.0481	0.0335	0.0494	0.0506	0.0454	0.0707	0.0355	0.0329	0.0433	0.0418	0.0332	0.0480	0.0508	0.0362	0.0363	0.0483	0.0391	0.0368	0.0334	0.0566	0.0551	0.0639	0.0638	0.0613	0.0581	0.0477	0.0649
se	0.0152	0.0106	0.0156	0.0160	0.0144	0.0224	0.0112	0.0104	0.0137	0.0132	0.0105	0.0152	0.0161	0.0114	0.0115	0.0153	0.0124	0.0116	0.0106	0.0186	0.0174	0.0202	0.0202	0.0194	0.0184	0.0151	0.0205
	b	0	10	20	30	45	60	90	120																		
poe5	0.2598	0.2497	0.267	0.2815	0.2879	0.3145	0.291	0.2733	0.2635																		
pre5	0.2547	0.2437	0.255	0.2599	0.2546	0.2411	0.2419	0.2282	0.2264																		
po5	0.277	0.2792	0.2622	0.2741	0.2731	0.2822	0.2733	0.271	0.2569																		
	b	0	10	20	30	45	60	90	120																		
sd poe5	0.0481	0.0335	0.0494	0.0506	0.0454	0.0707	0.0355	0.0329	0.0433																		
sd pre5	0.0418	0.0332	0.0480	0.0508	0.0362	0.0363	0.0483	0.0391	0.0368																		
sd po5	0.0334	0.0588	0.0551	0.0639	0.0638	0.0613	0.0581	0.0477	0.0649																		

Appendix 2.16: Oxygen Consumption During Exercise (Day 6)(L/min).

	poe0	poe30	poe60	poe90	pre0	pre30	pre60	pre90	po0	po30	po60	po90
1	0.29	1.72	1.69	1.71	0.30	1.77	1.79	1.76	0.34	1.86	1.91	1.87
2	0.38	2.42	2.47	2.60	0.34	2.45	2.41	2.47	0.33	2.45	2.55	2.68
3	0.24	1.76	1.77	1.73	0.31	1.84	1.82	1.85	0.24	1.80	1.84	1.81
4	0.34	1.57	1.80	1.74	0.38	1.67	1.76	1.68	0.27	1.56	1.79	1.80
5	0.32	1.88	1.90	1.92	0.28	1.78	1.85	1.87	0.36	1.76	1.87	1.83
6	0.28	1.91	1.84	1.90	0.21	1.87	1.88	1.85	0.30	1.76	1.80	1.91
7	0.28	1.71	1.71	1.71	0.28	1.73	1.76	1.76	0.32	1.68	1.74	1.76
8	0.26	1.82	1.86	1.81	0.27	1.76	1.68	1.66	0.30	1.91	1.91	1.91
9	0.30	1.67	1.72	1.74	0.31	1.58	1.64	1.63	0.30	1.70	1.81	1.82
10	0.28	1.92	1.76	1.69	0.16	1.96	1.74	1.76	0.27	1.75	1.64	1.65
mean	0.29	1.84	1.85	1.85	0.28	1.84	1.83	1.83	0.30	1.82	1.89	1.90
sd	0.04	0.23	0.23	0.27	0.06	0.24	0.22	0.24	0.04	0.24	0.25	0.28
se	0.01	0.07	0.07	0.09	0.02	0.08	0.07	0.08	0.01	0.08	0.08	0.09
	0.00	30.00	60.00	90.00								
poe	0.29	1.84	1.85	1.85								
pre	0.28	1.84	1.83	1.83								
po	0.30	1.82	1.89	1.90								
poesd	0.04	0.23	0.23	0.27								
presd	0.06	0.24	0.22	0.24								
posd	0.04	0.24	0.25	0.28								

Appendix 2.17: Post-Exercise Oxygen Consumption (Day 6)(L/min).

	poe6b	poe60	poe610	poe620	poe630	poe645	poe660	poe690	poe6120	pre6b	pre60	pre610	pre620	pre630	pre645	pre660	pre690	pre6120	po6b	po60	po610	po620	po630	po645	po660	po690	po6120
1	0.242	0.281	0.247	0.364	0.268	0.322	0.265	0.207	0.243	0.327	0.298	0.277	0.465	0.275	0.322	0.26	0.234	0.258	0.259	0.339	0.296	0.286	0.283	0.309	0.308	0.315	0.231
2	0.262	0.377	0.41	0.375	0.406	0.286	0.333	0.287	0.254	0.257	0.338	0.338	0.316	0.316	0.283	0.308	0.28	0.221	0.28	0.328	0.32	0.404	0.371	0.325	0.342	0.297	0.337
3	0.213	0.242	0.217	0.242	0.308	0.249	0.239	0.257	0.251	0.29	0.309	0.223	0.204	0.167	0.209	0.192	0.194	0.192	0.262	0.241	0.242	0.242	0.251	0.304	0.274	0.245	0.216
4	0.284	0.338	0.322	0.351	0.374	0.333	0.334	0.351	0.279	0.328	0.362	0.303	0.248	0.238	0.246	0.247	0.298	0.345	0.342	0.265	0.265	0.316	0.289	0.298	0.27	0.261	0.261
5	0.336	0.317	0.28	0.28	0.398	0.29	0.309	0.307	0.318	0.279	0.279	0.287	0.336	0.287	0.331	0.252	0.248	0.271	0.279	0.359	0.349	0.327	0.331	0.325	0.294	0.29	0.285
6	0.27	0.278	0.25	0.27	0.286	0.302	0.312	0.257	0.289	0.231	0.211	0.403	0.39	0.226	0.226	0.262	0.234	0.238	0.129	0.303	0.16	0.205	0.19	0.256	0.323	0.227	0.251
7	0.288	0.275	0.351	0.315	0.348	0.327	0.312	0.296	0.291	0.277	0.277	0.341	0.315	0.312	0.243	0.269	0.294	0.252	0.302	0.317	0.353	0.331	0.358	0.297	0.298	0.273	0.264
8	0.22	0.255	0.246	0.349	0.325	0.269	0.248	0.283	0.236	0.283	0.272	0.201	0.21	0.262	0.262	0.228	0.192	0.221	0.246	0.295	0.259	0.309	0.31	0.279	0.323	0.232	0.248
9	0.284	0.295	0.276	0.259	0.27	0.265	0.266	0.249	0.246	0.249	0.305	0.289	0.254	0.226	0.229	0.223	0.195	0.348	0.178	0.304	0.331	0.357	0.297	0.305	0.305	0.236	0.238
10	0.228	0.277	0.265	0.25	0.208	0.295	0.233	0.225	0.214	0.257	0.164	0.275	0.307	0.178	0.173	0.199	0.293	0.238	0.257	0.274	0.261	0.272	0.278	0.238	0.239	0.242	0.221
mean	0.2647	0.2945	0.2884	0.3055	0.3191	0.2958	0.2851	0.2719	0.2621	0.2758	0.2835	0.2937	0.3045	0.2509	0.2524	0.244	0.2462	0.2584	0.2535	0.3025	0.2836	0.3049	0.2958	0.2936	0.2978	0.2618	0.2552
sd	0.0383	0.0401	0.0579	0.0511	0.0635	0.0263	0.0390	0.0419	0.0312	0.0319	0.0612	0.0582	0.0810	0.0479	0.0490	0.0346	0.0433	0.0514	0.0603	0.0357	0.0590	0.0570	0.0524	0.0283	0.0302	0.0306	0.0355
se	0.0121	0.0127	0.0183	0.0162	0.0201	0.0083	0.0123	0.0133	0.0099	0.0101	0.0194	0.0184	0.0256	0.0152	0.0155	0.0110	0.0137	0.0163	0.0191	0.0113	0.0186	0.0180	0.0166	0.0089	0.0095	0.0097	0.0112
	b	0	10	20	30	45	60	90	120																		
poe6	0.2647	0.2945	0.2884	0.3055	0.3191	0.2958	0.2851	0.2719	0.2621																		
pre6	0.2758	0.2835	0.2937	0.3045	0.2509	0.2524	0.244	0.2462	0.2584																		
po6	0.2535	0.3025	0.2836	0.3049	0.2958	0.2936	0.2978	0.2618	0.2552																		
	b	0	10	20	30	45	60	90	120																		
sd poe6	0.0383	0.0401	0.0579	0.0511	0.0635	0.0263	0.0390	0.0419	0.0312																		
sd pre6	0.0319	0.0612	0.0582	0.0810	0.0479	0.0490	0.0346	0.0433	0.0514																		
sd po6	0.0603	0.0357	0.0590	0.0570	0.0524	0.0283	0.0302	0.0306	0.0355																		

Appendix 2.18: Respiratory Exchange Ratio (Day 5).

	poe5b	poe50	poe510	poe520	poe530	poe545	poe560	poe590	poe5120	pre5b	pre50	pre510	pre520	pre530	pre545	pre560	pre590	pre5120	po5b	po50	po510	po520	po530	po545	po560	po590	po5120
1	0.91	0.93	0.93	0.98	0.99	0.98	1	0.95	0.98	0.92	0.92	0.91	0.91	0.92	0.92	0.93	0.94	0.93	0.9	0.9	0.96	0.96	1	0.95	0.98	0.98	0.96
2	0.88	0.88	0.94	0.95	0.99	0.97	0.99	0.99	0.88	0.92	0.93	0.92	0.88	0.88	0.77	0.77	0.8	0.88	0.88	0.86	0.93	0.95	0.94	0.94	0.94	0.95	0.9
3	0.94	0.96	0.94	0.98	0.98	0.98	0.94	0.92	0.86	0.86	0.92	0.91	0.93	0.92	0.93	0.94	0.93	0.93	0.88	0.98	0.88	0.89	0.87	0.89	0.9	0.9	0.87
4	0.92	0.86	0.94	0.93	0.95	0.99	0.98	0.93	0.91	0.84	0.83	0.91	0.9	0.85	0.91	0.89	0.85	0.8	0.84	0.86	0.9	0.94	0.93	0.93	0.9	0.93	0.83
5	0.88	0.88	0.9	0.93	0.94	0.95	0.97	0.95	0.99	0.84	0.88	0.84	0.85	0.85	0.87	0.87	0.85	0.88	0.84	0.88	0.9	0.89	0.92	0.92	0.89	0.88	0.87
6	0.92	0.92	0.96	0.96	0.97	0.98	0.97	0.97	0.96	0.87	0.92	0.9	0.97	0.95	0.99	0.98	0.95	0.94	0.94	0.86	0.89	0.94	0.97	0.99	0.97	0.94	0.92
7	0.88	0.93	0.9	1	0.98	0.99	0.97	0.81	0.84	0.89	0.88	0.88	0.88	0.82	0.8	0.73	0.8	0.86	0.87	0.91	0.89	0.98	0.96	0.97	0.95	0.79	0.82
8	0.87	0.9	0.9	0.91	0.95	0.94	0.93	0.92	0.86	0.85	0.86	0.83	0.87	0.84	0.83	0.83	0.83	0.78	0.85	0.88	0.86	0.87	0.92	0.9	0.91	0.9	0.89
9	0.85	0.79	0.85	0.86	0.93	0.89	0.92	0.91	0.89	0.88	0.83	0.85	0.83	0.82	0.84	0.93	0.82	0.82	0.87	0.88	0.86	0.9	0.9	0.9	0.92	0.89	0.86
10	0.94	0.94	0.89	0.89	0.94	0.93	0.99	1.01	0.89	0.88	0.91	0.91	0.92	0.91	0.93	0.9	0.87	0.92	0.94	0.92	0.92	0.95	0.97	0.96	0.96	0.92	0.92
mean	0.90	0.90	0.92	0.94	0.96	0.96	0.97	0.94	0.91	0.88	0.89	0.89	0.89	0.88	0.88	0.87	0.86	0.87	0.88	0.89	0.90	0.93	0.94	0.94	0.93	0.91	0.89
sd	0.03	0.05	0.03	0.04	0.02	0.03	0.03	0.05	0.05	0.03	0.04	0.03	0.04	0.05	0.07	0.08	0.08	0.06	0.04	0.04	0.03	0.04	0.04	0.03	0.03	0.05	0.04
se	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01
	b	0	10	20	30	45	60	90	120																		
poe5		0.90	0.90	0.92	0.94	0.96	0.96	0.97	0.94	0.91																	
pre5		0.88	0.89	0.89	0.89	0.88	0.88	0.87	0.86	0.87																	
po5		0.88	0.89	0.90	0.93	0.94	0.94	0.93	0.91	0.89																	
	b	0	10	20	30	45	60	90	120																		
sd poe5	0.03	0.05	0.03	0.04	0.02	0.03	0.03	0.05	0.05																		
sd pre5	0.03	0.04	0.03	0.04	0.05	0.07	0.08	0.06	0.06																		
sd po5	0.04	0.04	0.03	0.04	0.04	0.03	0.03	0.05	0.04																		

Appendix 2.19. Respiratory Exchange Ratio During Exercise (Day 6).

	poe0	poe30	poe60	poe90	pre0	pre30	pre60	pre90	po0	po30	po60	po90
1	0.96	0.99	0.97	0.98	0.92	0.99	0.96	0.98	0.92	0.95	0.95	0.98
2	0.88	0.93	0.91	0.88	0.92	0.93	0.92	0.88	0.85	0.91	0.89	0.86
3	0.94	0.95	0.97	0.97	0.91	1.00	0.98	0.96	0.91	0.98	0.98	0.98
4	0.84	0.98	0.97	0.94	0.83	0.98	0.97	0.96	0.84	0.96	0.97	0.93
5	0.90	0.97	0.96	0.93	0.89	0.95	0.93	0.91	0.84	0.97	0.94	0.94
6	1.00	0.97	0.96	0.94	0.85	0.94	0.95	0.95	0.86	0.97	0.91	0.82
7	0.87	0.91	0.92	0.92	0.89	0.95	0.93	0.90	0.80	0.94	0.91	0.89
8	0.85	0.97	0.95	0.93	0.89	0.95	0.94	0.95	0.82	0.94	0.91	0.86
9	0.87	0.91	0.88	0.87	0.86	0.96	0.93	0.89	0.84	0.94	0.92	0.91
10	0.94	0.98	0.97	0.94	0.92	0.98	0.97	0.97	0.90	1.00	0.95	0.94
mean	0.91	0.95	0.95	0.93	0.89	0.96	0.95	0.93	0.86	0.96	0.93	0.91
sd	0.05	0.03	0.03	0.03	0.03	0.02	0.02	0.04	0.04	0.03	0.03	0.05
se	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02
poe	0.91	0.95	0.95	0.93								
pre	0.89	0.96	0.95	0.93								
po	0.86	0.96	0.93	0.91								
poesd	0.05	0.03	0.03	0.03								
pre sd	0.03	0.02	0.02	0.04								
posd	0.04	0.03	0.03	0.05								

Appendix 2.20: Respiratory Exchange Ratio (Day 6).

	poe6b	poe60	poe610	poe620	poe630	poe645	poe660	poe690	poe6120	pre6b	pre60	pre610	pre620	pre630	pre645	pre660	pre690	pre6120	po6b	po60	po610	po620	po630	po645	po660	po690	po6120
1	0.96	0.96	0.98	1.07	1.1	1.03	0.96	0.94	0.94	0.92	0.98	0.92	0.92	0.87	0.83	0.91	0.93	0.93	0.92	0.98	1.02	0.99	0.96	0.96	0.95	0.98	0.88
2	0.88	0.82	0.77	0.9	0.78	0.85	0.82	0.82	0.81	0.92	0.89	0.88	0.87	0.9	0.9	0.92	0.89	0.88	0.85	0.74	0.68	0.78	0.78	0.81	0.78	0.78	0.76
3	0.94	0.87	0.91	0.93	0.97	0.95	0.95	0.9	0.93	0.91	0.92	0.91	0.9	0.86	0.87	0.87	0.86	0.87	0.91	0.89	0.82	0.87	0.9	0.9	0.95	0.88	0.86
4	0.84	0.85	0.93	0.91	0.9	0.84	0.84	0.86	0.84	0.83	0.85	0.76	0.77	0.76	0.76	0.76	0.76	0.75	0.84	0.83	0.83	0.84	0.9	0.9	0.89	0.93	0.92
5	0.9	0.86	0.84	0.88	0.94	0.94	0.92	0.85	0.84	0.89	0.89	0.88	0.88	0.87	0.83	0.83	0.81	0.81	0.84	0.88	0.83	0.85	0.88	0.89	0.89	0.88	0.84
6	1	0.91	0.88	0.93	0.92	0.95	0.99	0.93	0.93	0.85	0.85	0.85	0.86	0.9	0.86	0.87	0.82	0.82	0.86	0.8	0.8	0.9	0.92	0.96	0.92	0.94	0.89
7	0.87	0.85	0.88	0.92	0.97	0.93	0.89	0.87	0.83	0.89	0.93	0.93	0.74	0.76	0.8	0.72	0.77	0.78	0.8	0.84	0.77	0.77	0.79	0.8	0.8	0.71	0.7
8	0.85	0.73	0.73	0.94	0.92	0.93	0.92	0.93	0.83	0.89	0.85	0.78	0.75	0.75	0.83	0.83	0.73	0.74	0.82	0.83	0.82	0.93	0.94	0.89	0.93	0.88	0.83
9	0.87	0.82	0.86	0.9	0.93	0.9	0.87	0.85	0.82	0.86	0.95	0.82	0.75	0.79	0.75	0.77	0.77	0.74	0.84	0.81	0.85	0.89	0.92	0.88	0.88	0.84	0.82
10	0.94	0.9	1	1	0.99	0.93	1	0.93	0.91	0.92	0.94	0.92	0.92	0.95	0.87	0.86	0.82	0.85	0.9	0.94	0.94	0.86	0.94	0.95	0.98	0.95	0.89
mean	0.91	0.86	0.88	0.93	0.94	0.93	0.92	0.89	0.87	0.89	0.91	0.86	0.84	0.84	0.83	0.83	0.82	0.82	0.86	0.85	0.84	0.87	0.89	0.90	0.90	0.88	0.84
sd	0.05	0.06	0.08	0.07	0.08	0.05	0.06	0.04	0.05	0.03	0.05	0.06	0.07	0.07	0.05	0.07	0.06	0.07	0.04	0.07	0.09	0.07	0.06	0.06	0.06	0.08	0.07
se	0.02	0.02	0.03	0.02	0.03	0.02	0.02	0.01	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.03	0.02	0.02	0.02	0.02	0.03	0.02
	b	0	10	20	30	45	60	90	120																		
poe6	0.91	0.86	0.88	0.93	0.94	0.93	0.92	0.89	0.87																		
pre6	0.89	0.91	0.86	0.84	0.84	0.83	0.83	0.82	0.82																		
po6	0.86	0.85	0.84	0.87	0.89	0.90	0.90	0.88	0.84																		
sd poe6	0.05	0.06	0.08	0.07	0.08	0.05	0.06	0.04	0.05																		
sd pre6	0.03	0.05	0.06	0.07	0.07	0.05	0.07	0.08	0.07																		
sd po6	0.04	0.07	0.09	0.07	0.06	0.06	0.08	0.08	0.07																		

Appendix 2.21: Resting Metabolic Rate.

	poe	pre	po
1	0.244	0.234	0.234
2	0.1825	0.263	0.209
3	0.189	0.215	0.224
4	0.272	0.216	0.341
5	0.255	0.155	0.215
6	0.3215	0.29	0.242
7	0.2275	0.211	0.249
8	0.1805	0.2	0.22
9	0.24	0.232	0.219
10	0.2515	0.248	0.29
MEAN	0.23635	0.2264	0.2443
SD	0.044108	0.036834	0.041258
SE	0.013948	0.011648	0.013047

Appendix 2.22: The Thermic Effect of Food (Day 5)(L Oxygen).

	poe	po
1	5.025	-2.435
2	-2.3725	1.9075
3	2.655	2.035
4	8.37	-9.5425
5	19.0825	16.7075
6	4.5775	0.53
7	6.5525	7.49
8	2.6575	9.04
9	6.5675	15.97
10	4.1875	2.7375
mean	5.73025	4.444
sd	5.531604	8.076405
se	1.749247	2.553984

Appendix 2.23: Post-Exercise Oxidative Consumption (EPOC)(Day6)(L Oxygen).

	poe	pre	po
1	-7.4725	0.1375	4.12
2	6.8175	0.3425	3.45
3	-0.4375	-3.3425	0.3625
4	6.7875	8.815	17.38
5	-1.5975	13.1575	0.455
6	-4.5625	-2.3	-5.3425
7	2.3075	5.425	0.165
8	5.77	2.7825	-9.2575
9	-5.435	-1.2475	-12.4975
10	-3.0675	0.245	-0.8175
mean	-0.089	2.4015	-0.19825
sd	5.264489	5.255048	8.178653
se	1.664777	1.661792	2.586317

Appendix 2.24: Time to Exhaustion (s)

		poe	pre	po
1	1	499	427	506
2	1	247	360	384
3	1	686	416	512
4	1	563	268	476
5	1	267	405	350
6	1	389	376	286
7	1	1185	346	1212
8	1	722	278	383
9	1	331	264	252
10	1	405	320	734
	mean	529.40	346.00	509.50
	sd	282.18	61.60	282.55
	se	89.23	19.48	89.35
	poe	529.40		
	pre	346.00		
	po	509.50		
	sd poe	282.18		
	sd pre	61.60		
	sd po	282.55		

Appendix 2.25: Rating of Perceived Exertion (RPE).

		poe 30	poe 60	poe 90	pre 30	pre 60	pre 90	po 30	po 60	po 90
1	1	4	4	4	5	6	6	4	5	5
2	1	4	3	4.5	4	4	4.5	5	5	5.5
3	1	4	4	4	3	3	4	3	3.5	4
4	1	2	3	4	1	4	4	3	4	4
5	1	3	3.5	4	3	3	3.5	3.5	4	4
6	1	3	3	3.5	3	3	4	3.5	3.5	4
7	1	2.5	3	3	3	3	3	3	3	3.5
8	1	3	3	4.5	3.5	4	4	3	4	4
9	1	3	3.5	5	4.5	5	5	3.5	4.5	4.5
10	1	3	7	6	3.5	6.5	6.5	3	4	6
	mean	3.06	3.67	4.28	3.17	3.94	4.28	3.39	3.94	4.39
	sd	0.63	1.30	0.87	0.97	1.18	1.00	0.65	0.58	0.82
	se	0.21	0.43	0.29	0.32	0.39	0.33	0.22	0.19	0.27
		30	60	90						
	poe	3.06	3.67	4.28						
	pre	3.17	3.94	4.28						
	po	3.39	3.94	4.39						
		30	60	90						
	sd poe	0.63	1.30	0.87						
	sd pre	0.97	1.18	1.00						
	sd po	0.65	0.58	0.82						

Appendix 2.26: Weight Loss per Trial (kg).

		poe	pre	po
1	1	0.3	-0.8	-1.2
2	1	0.5	-1.3	1.2
3	1	-1.3	-0.5	-0.5
4	1	-0.5	-1.1	-0.6
5	1	-1.1	-1.8	-0.9
6	1	0.4	-2.2	-1.7
7	1	-1.1	-2.1	-1
8	1	-0.5	-0.9	-0.4
9	1	-0.6	-1.4	-0.7
10	1	-0.7	-1.6	-0.7
	mean	-0.46	-1.37	-0.65
	sd	0.65	0.56	0.75
	se	0.21	0.18	0.24
	poe	-0.46		
	pre	-1.37		
	po	-0.65		
	sd poe	0.65		
	sd pre	0.56		
	sd po	0.75		

Appendix III: Prepackaged Diet Foodstuffs.

#29039 boost

Macronutrients		
Kilocalories	225.0	Kc
Protein	12.00	Gm
Carbohydrate	35.00	Gm
Fat	5.400	Gm
Alcohol	-	Gm
Fats		
Cholesterol	-	mg
Saturated Fat	-	Gm
Mono Fat	-	Gm
Poly Fat	-	Gm
Carbohydrates		
Dietary Fiber	-	Gm
Soluble Fiber	-	Gm
Insol. Fiber	-	Gm
Sugar	-	Gm
Vitamins		
Vitamin A	-	RE
Beta-Carotene	-	Ug
Vitamin E	-	mg
A-Tocopherol	-	mg
Thiamin B1	-	mg
Riboflavin B2	-	mg
Niacin B3	-	mg
Pyridoxine B6	-	mg
Folate	-	Ug
Cobalamin B12	-	Ug
Pant. Acid	-	mg
Biotin	-	Ug
Vitamin C	-	mg
Vitamin D	-	Ug
Vitamin K	-	Ug
Amino Acids		
Tryptophan	-	mg
Threonine	-	mg
Isoleucine	-	mg
Leucine	-	mg
Lysine	-	mg
Methionine	-	mg
Phenylalanine	-	mg
Valine	-	mg
Misc.		
Weight	55.80	Gm
Moisture	-	Gm
Ash	-	Gm
Caffeine	-	mg
Minerals		
Sodium	-	mg
Potassium	-	mg
Iron	-	mg
Calcium	-	mg
Magnesium	-	mg
Phosphorus	-	mg
Zinc	-	mg
Copper	-	mg
Manganese	-	mg
Selenium	-	mg
Fluoride	-	Ug
Chromium	-	mg

#361 BREAD-WHOLE WHEAT-FIRM-ENRICHED

Macronutrients		
Kilocalories	61.30	Kc
Protein	2.410	Gm
Carbohydrate	11.30	Gm
Fat	1.090	Gm
Alcohol	0.000	Gm
Fats		
Cholesterol	0.000	mg
Saturated Fat	0.100	Gm
Mono Fat	0.200	Gm
Poly Fat	0.300	Gm
Carbohydrates		
Dietary Fiber	2.830	Gm
Soluble Fiber	-	Gm
Insol. Fiber	-	Gm
Sugar	1.000	Gm
Vitamins		
Vitamin A	0.000	RE
Beta-Carotene	-	Ug
Vitamin E	0.225	mg
A-Tocopherol	0.030	mg
Thiamin B1	0.088	mg
Riboflavin B2	0.053	mg
Niacin B3	0.958	mg
Pyridoxine B6	0.047	mg
Folate	13.80	Ug
Cobalamin B12	0.000	Ug
Pant. Acid	0.184	mg
Biotin	1.500	Ug
Vitamin C	0.000	mg
Vitamin D	-	Ug
Vitamin K	-	Ug
Amino Acids		
Tryptophan	32.00	mg
Threonine	78.00	mg
Isoleucine	116.0	mg
Leucine	181.0	mg
Lysine	77.00	mg
Methionine	40.00	mg
Phenylalanine	128.0	mg
Valine	123.0	mg
Misc.		
Weight	25.00	Gm
Moisture	9.570	Gm
Ash	0.585	Gm
Caffeine	0.000	mg
Minerals		
Sodium	159.0	mg
Potassium	44.00	mg
Iron	0.855	mg
Calcium	18.00	mg
Magnesium	23.30	mg
Phosphorus	65.00	mg
Zinc	0.420	mg
Copper	0.086	mg
Manganese	-	mg
Selenium	0.011	mg
Fluoride	-	Ug
Chromium	0.016	mg

#105 BUTTER-REGULAR-PAT

Macronutrients		
Kilocalories	36.00	Kc
Protein	0.040	Gm
Carbohydrate	0.003	Gm
Fat	4.060	Gm
Alcohol	0.000	Gm
Fats		
Cholesterol	11.00	mg
Saturated Fat	2.500	Gm
Mono Fat	1.170	Gm
Poly Fat	0.150	Gm
Carbohydrates		
Dietary Fiber	0.000	Gm
Soluble Fiber	0.000	Gm
Insol. Fiber	0.000	Gm
Sugar	0.000	Gm
Vitamins		
Vitamin A	7.600	RE
Beta-Carotene	4.300	Ug
Vitamin E	0.079	mg
A-Tocopherol	0.079	mg
Thiamin B1	0.000	mg
Riboflavin B2	0.002	mg
Niacin B3	0.002	mg
Pyridoxine B6	0.000	mg
Folate	0.150	Ug
Cobalamin B12	0.006	Ug
Pant. Acid	0.006	mg
Biotin	-	Ug
Vitamin C	0.000	mg
Vitamin D	0.038	Ug
Vitamin K	-	Ug
Amino Acids		
Tryptophan	1.000	mg
Threonine	2.000	mg
Isoleucine	3.000	mg
Leucine	4.000	mg
Lysine	3.000	mg
Methionine	1.000	mg
Phenylalanine	2.000	mg
Valine	3.000	mg
Misc.		
Weight	5.000	Gm
Moisture	0.790	Gm
Ash	0.100	Gm
Caffeine	0.000	mg
Minerals		
Sodium	41.00	mg
Potassium	1.000	mg
Iron	0.010	mg
Calcium	1.000	mg
Magnesium	0.100	mg
Phosphorus	1.000	mg
Zinc	0.003	mg
Copper	0.001	mg
Manganese	0.002	mg
Selenium	0.000	mg
Fluoride	7.500	Ug
Chromium	0.001	mg

#17261 CEREAL-CORN FLAKES-KELLOGG'S

Macronutrients		
Kilocalories	110.0	Kc
Protein	2.000	Gm
Carbohydrate	26.00	Gm
Fat	0.000	Gm
Alcohol	0.000	Gm
Fats		
Cholesterol	0.000	mg
Saturated Fat	0.000	Gm
Mono Fat	0.000	Gm
Poly Fat	0.000	Gm
Carbohydrates		
Dietary Fiber	1.000	Gm
Soluble Fiber	0.000	Gm
Insol. Fiber	1.000	Gm
Sugar	2.010	Gm
Vitamins		
Vitamin A	150.0	RE
Beta-Carotene	0.000	Ug
Vitamin E	0.000	mg
A-Tocopherol	0.000	mg
Thiamin B1	0.375	mg
Riboflavin B2	0.425	mg
Niacin B3	5.000	mg
Pyridoxine B6	0.500	mg
Folate	100.0	Ug
Cobalamin B12	0.000	Ug
Pant. Acid	0.000	mg
Biotin	-	Ug
Vitamin C	15.00	mg
Vitamin D	1.250	Ug
Vitamin K	0.009	Ug
Amino Acids		
Tryptophan	-	mg
Threonine	-	mg
Isoleucine	-	mg
Leucine	-	mg
Lysine	-	mg
Methionine	-	mg
Phenylalanine	-	mg
Valine	-	mg
Misc.		
Weight	30.00	Gm
Moisture	0.590	Gm
Ash	0.658	Gm
Caffeine	0.000	mg
Minerals		
Sodium	330.0	mg
Potassium	35.00	mg
Iron	8.400	mg
Calcium	0.000	mg
Magnesium	0.000	mg
Phosphorus	0.000	mg
Zinc	0.000	mg
Copper	0.020	mg
Manganese	0.019	mg
Selenium	0.001	mg
Fluoride	0.000	Ug
Chromium	0.001	mg

#3939 CRACKER-PREMIUM SALTINE-NO FAT-NABISCO

Macronutrients		
Kilocalories	50.00	Kc
Protein	1.000	Gm
Carbohydrate	12.00	Gm
Fat	0.000	Gm
Alcohol	0.000	Gm
Fats		
Cholesterol	0.000	mg
Saturated Fat	0.000	Gm
Mono Fat	0.000	Gm
Poly Fat	0.000	Gm
Carbohydrates		
Dietary Fiber	-	Gm
Soluble Fiber	-	Gm
Insol. Fiber	-	Gm
Sugar	-	Gm
Vitamins		
Vitamin A	-	RE
Beta-Carotene	-	Ug
Vitamin E	-	mg
A-Tocopherol	-	mg
Thiamin B1	-	mg
Riboflavin B2	-	mg
Niacin B3	-	mg
Pyridoxine B6	-	mg
Folate	-	Ug
Cobalamin B12	-	Ug
Pant. Acid	-	mg
Biotin	-	Ug
Vitamin C	-	mg
Vitamin D	-	Ug
Vitamin K	-	Ug
Amino Acids		
Tryptophan	-	mg
Threonine	-	mg
Isoleucine	-	mg
Leucine	-	mg
Lysine	-	mg
Methionine	-	mg
Phenylalanine	-	mg
Valine	-	mg
Misc.		
Weight	14.20	Gm
Moisture	-	Gm
Ash	-	Gm
Caffeine	0.000	mg
Minerals		
Sodium	115.0	mg
Potassium	15.00	mg
Iron	-	mg
Calcium	-	mg
Magnesium	-	mg
Phosphorus	-	mg
Zinc	-	mg
Copper	-	mg
Manganese	-	mg
Selenium	-	mg
Fluoride	-	Ug
Chromium	-	mg

#549 JAM/PRESERVES-REGULAR

Macronutrients		
Kilocalories	55.00	Kc
Protein	0.000	Gm
Carbohydrate	14.00	Gm
Fat	0.000	Gm
Alcohol	0.000	Gm
Fats		
Cholesterol	0.000	mg
Saturated Fat	0.000	Gm
Mono Fat	0.000	Gm
Poly Fat	0.000	Gm
Carbohydrates		
Dietary Fiber	0.200	Gm
Soluble Fiber	-	Gm
Insol. Fiber	-	Gm
Sugar	12.00	Gm
Vitamins		
Vitamin A	0.000	RE
Beta-Carotene	0.100	Ug
Vitamin E	-	mg
A-Tocopherol	0.018	mg
Thiamin B1	0.000	mg
Riboflavin B2	0.010	mg
Niacin B3	0.000	mg
Pyridoxine B6	0.004	mg
Folate	1.600	Ug
Cobalamin B12	0.000	Ug
Pant. Acid	-	mg
Biotin	-	Ug
Vitamin C	0.000	mg
Vitamin D	0.000	Ug
Vitamin K	-	Ug
Amino Acids		
Tryptophan	0.000	mg
Threonine	0.000	mg
Isoleucine	0.000	mg
Leucine	0.000	mg
Lysine	0.000	mg
Methionine	0.000	mg
Phenylalanine	0.000	mg
Valine	0.000	mg
Misc.		
Weight	20.00	Gm
Moisture	5.800	Gm
Ash	-	Gm
Caffeine	0.000	mg
Minerals		
Sodium	2.000	mg
Potassium	18.00	mg
Iron	0.200	mg
Calcium	4.000	mg
Magnesium	1.000	mg
Phosphorus	2.000	mg
Zinc	0.014	mg
Copper	0.062	mg
Manganese	-	mg
Selenium	0.000	mg
Fluoride	-	Ug
Chromium	-	mg

#5238 MILK-FLUID-SKIM

Macronutrients		
Kilocalories	85.50	Kc
Protein	8.350	Gm
Carbohydrate	11.90	Gm
Fat	0.441	Gm
Alcohol	0.000	Gm
Fats		
Cholesterol	4.410	mg
Saturated Fat	0.287	Gm
Mono Fat	0.115	Gm
Poly Fat	0.017	Gm
Carbohydrates		
Dietary Fiber	0.000	Gm
Soluble Fiber	0.000	Gm
Insol. Fiber	0.000	Gm
Sugar	10.80	Gm
Vitamins		
Vitamin A	149.0	RE
Beta-Carotene	0.000	Ug
Vitamin E	-	mg
A-Tocopherol	0.000	mg
Thiamin B1	0.088	mg
Riboflavin B2	0.343	mg
Niacin B3	0.216	mg
Pyridoxine B6	0.098	mg
Folate	12.70	Ug
Cobalamin B12	0.926	Ug
Pant. Acid	0.806	mg
Biotin	-	Ug
Vitamin C	2.400	mg
Vitamin D	2.630	Ug
Vitamin K	9.800	Ug
Amino Acids		
Tryptophan	118.0	mg
Threonine	377.0	mg
Isoleucine	505.0	mg
Leucine	818.0	mg
Lysine	661.0	mg
Methionine	211.0	mg
Phenylalanine	404.0	mg
Valine	559.0	mg
Misc.		
Weight	245.0	Gm
Moisture	222.0	Gm
Ash	1.860	Gm
Caffeine	0.000	mg
Minerals		
Sodium	126.0	mg
Potassium	406.0	mg
Iron	0.098	mg
Calcium	302.0	mg
Magnesium	27.80	mg
Phosphorus	247.0	mg
Zinc	0.980	mg
Copper	0.027	mg
Manganese	0.005	mg
Selenium	0.007	mg
Fluoride	-	Ug
Chromium	-	mg

#3696 PASTA & CHEESE-PARMESAN-KRAFT*

Macronutrients		
Kilocalories	360.0	Kc
Protein	12.00	Gm
Carbohydrate	38.00	Gm
Fat	16.00	Gm
Alcohol	0.000	Gm
Fats		
Cholesterol	60.00	mg
Saturated Fat	4.000	Gm
Mono Fat	3.530	Gm
Poly Fat	2.000	Gm
Carbohydrates		
Dietary Fiber	0.910	Gm
Soluble Fiber	-	Gm
Insol. Fiber	-	Gm
Sugar	-	Gm
Vitamins		
Vitamin A	99.20	RE
Beta-Carotene	31.00	Ug
Vitamin E	-	mg
A-Tocopherol	0.895	mg
Thiamin B1	0.113	mg
Riboflavin B2	0.191	mg
Niacin B3	2.100	mg
Pyridoxine B6	0.121	mg
Folate	12.40	Ug
Cobalamin B12	0.634	Ug
Pant. Acid	-	mg
Biotin	-	Ug
Vitamin C	3.770	mg
Vitamin D	-	Ug
Vitamin K	-	Ug
Amino Acids		
Tryptophan	-	mg
Threonine	-	mg
Isoleucine	-	mg
Leucine	-	mg
Lysine	-	mg
Methionine	-	mg
Phenylalanine	-	mg
Valine	-	mg
Misc.		
Weight	121.0	Gm
Moisture	84.10	Gm
Ash	-	Gm
Caffeine	0.000	mg
Minerals		
Sodium	1260	mg
Potassium	220.0	mg
Iron	1.630	mg
Calcium	69.20	mg
Magnesium	16.40	mg
Phosphorus	300.0	mg
Zinc	1.490	mg
Copper	0.099	mg
Manganese	-	mg
Selenium	-	mg
Fluoride	-	Ug
Chromium	-	mg

#524 PEANUT BUTTER-SMOOTH TYPE

Macronutrients		
Kilocalories	94.10	Kc
Protein	3.940	Gm
Carbohydrate	3.320	Gm
Fat	8.000	Gm
Alcohol	0.000	Gm
Fats		
Cholesterol	0.000	mg
Saturated Fat	1.530	Gm
Mono Fat	3.770	Gm
Poly Fat	2.300	Gm
Carbohydrates		
Dietary Fiber	1.010	Gm
Soluble Fiber	0.048	Gm
Insol. Fiber	0.960	Gm
Sugar	1.200	Gm
Vitamins		
Vitamin A	-	RE
Beta-Carotene	-	Ug
Vitamin E	3.200	mg
A-Tocopherol	1.120	mg
Thiamin B1	0.022	mg
Riboflavin B2	0.016	mg
Niacin B3	2.090	mg
Pyridoxine B6	0.060	mg
Folate	12.50	Ug
Cobalamin B12	0.000	Ug
Pant. Acid	0.147	mg
Biotin	6.400	Ug
Vitamin C	0.000	mg
Vitamin D	0.000	Ug
Vitamin K	0.018	Ug
Amino Acids		
Tryptophan	38.20	mg
Threonine	135.0	mg
Isoleucine	139.0	mg
Leucine	255.0	mg
Lysine	142.0	mg
Methionine	48.30	mg
Phenylalanine	204.0	mg
Valine	165.0	mg
Misc.		
Weight	16.00	Gm
Moisture	0.227	Gm
Ash	0.526	Gm
Caffeine	0.000	mg
Minerals		
Sodium	76.50	mg
Potassium	115.0	mg
Iron	0.267	mg
Calcium	5.440	mg
Magnesium	25.10	mg
Phosphorus	51.70	mg
Zinc	0.402	mg
Copper	0.089	mg
Manganese	0.246	mg
Selenium	0.002	mg
Fluoride	0.000	Ug
Chromium	0.041	mg

#29041 quaker lowfat tenders

Macronutrients		
Kilocalories	106.0	Kc
Protein	1.500	Gm
Carbohydrate	20.00	Gm
Fat	2.300	Gm
Alcohol	-	Gm
Fats		
Cholesterol	-	mg
Saturated Fat	-	Gm
Mono Fat	-	Gm
Poly Fat	-	Gm
Carbohydrates		
Dietary Fiber	-	Gm
Soluble Fiber	-	Gm
Insol. Fiber	-	Gm
Sugar	-	Gm
Vitamins		
Vitamin A	-	RE
Beta-Carotene	-	Ug
Vitamin E	-	mg
A-Tocopherol	-	mg
Thiamin B1	-	mg
Riboflavin B2	-	mg
Niacin B3	-	mg
Pyridoxine B6	-	mg
Folate	-	Ug
Cobalamin B12	-	Ug
Pant. Acid	-	mg
Biotin	-	Ug
Vitamin C	-	mg
Vitamin D	-	Ug
Vitamin K	-	Ug
Amino Acids		
Tryptophan	-	mg
Threonine	-	mg
Isoleucine	-	mg
Leucine	-	mg
Lysine	-	mg
Methionine	-	mg
Phenylalanine	-	mg
Valine	-	mg
Misc.		
Weight	26.00	Gm
Moisture	-	Gm
Ash	-	Gm
Caffeine	-	mg
Minerals		
Sodium	-	mg
Potassium	-	mg
Iron	-	mg
Calcium	-	mg
Magnesium	-	mg
Phosphorus	-	mg
Zinc	-	mg
Copper	-	mg
Manganese	-	mg
Selenium	-	mg
Fluoride	-	Ug
Chromium	-	mg

#29038 results

Macronutrients		
Kilocalories	234.0	Kc
Protein	17.50	Gm
Carbohydrate	37.40	Gm
Fat	2.900	Gm
Alcohol	-	Gm
Fats		
Cholesterol	-	mg
Saturated Fat	-	Gm
Mono Fat	-	Gm
Poly Fat	-	Gm
Carbohydrates		
Dietary Fiber	-	Gm
Soluble Fiber	-	Gm
Insol. Fiber	-	Gm
Sugar	-	Gm
Vitamins		
Vitamin A	-	RE
Beta-Carotene	-	Ug
Vitamin E	-	mg
A-Tocopherol	-	mg
Thiamin B1	-	mg
Riboflavin B2	-	mg
Niacin B3	-	mg
Pyridoxine B6	-	mg
Folate	-	Ug
Cobalamin B12	-	Ug
Pant. Acid	-	mg
Biotin	-	Ug
Vitamin C	-	mg
Vitamin D	-	Ug
Vitamin K	-	Ug
Amino Acids		
Tryptophan	-	mg
Threonine	-	mg
Isoleucine	-	mg
Leucine	-	mg
Lysine	-	mg
Methionine	-	mg
Phenylalanine	-	mg
Valine	-	mg
Misc.		
Weight	55.30	Gm
Moisture	-	Gm
Ash	-	Gm
Caffeine	-	mg
Minerals		
Sodium	-	mg
Potassium	-	mg
Iron	-	mg
Calcium	-	mg
Magnesium	-	mg
Phosphorus	-	mg
Zinc	-	mg
Copper	-	mg
Manganese	-	mg
Selenium	-	mg
Fluoride	-	Ug
Chromium	-	mg

#13311 RICE CAKE-APPLE CINNAMON-QUAKER

Macronutrients		
Kilocalories	50.00	Kc
Protein	1.000	Gm
Carbohydrate	11.00	Gm
Fat	0.000	Gm
Alcohol	-	Gm
Fats		
Cholesterol	0.000	mg
Saturated Fat	0.000	Gm
Mono Fat	0.000	Gm
Poly Fat	0.000	Gm
Carbohydrates		
Dietary Fiber	0.000	Gm
Soluble Fiber	0.000	Gm
Insol. Fiber	0.000	Gm
Sugar	4.000	Gm
Vitamins		
Vitamin A	-	RE
Beta-Carotene	-	Ug
Vitamin E	-	mg
A-Tocopherol	-	mg
Thiamin B1	0.030	mg
Riboflavin B2	0.000	mg
Niacin B3	0.400	mg
Pyridoxine B6	0.040	mg
Folate	0.000	Ug
Cobalamin B12	0.000	Ug
Pant. Acid	-	mg
Biotin	-	Ug
Vitamin C	-	mg
Vitamin D	-	Ug
Vitamin K	-	Ug
Amino Acids		
Tryptophan	-	mg
Threonine	-	mg
Isoleucine	-	mg
Leucine	-	mg
Lysine	-	mg
Methionine	-	mg
Phenylalanine	-	mg
Valine	-	mg
Misc.		
Weight	13.00	Gm
Moisture	-	Gm
Ash	-	Gm
Caffeine	-	mg
Minerals		
Sodium	0.000	mg
Potassium	25.00	mg
Iron	0.000	mg
Calcium	-	mg
Magnesium	-	mg
Phosphorus	-	mg
Zinc	-	mg
Copper	-	mg
Manganese	-	mg
Selenium	-	mg
Fluoride	-	Ug
Chromium	-	mg

Appendix IV: Assay Principals and Information.

Assay: Blood Glucose (Kit #315, Sigma Diagnostics, St. Louis, MO.)

The enzymatic reactions involved in the assay were as follows:

$$\text{Glucose} + H_2O + O_2 \xrightarrow{\text{Glucose Oxidase}} \text{Gluconic Acid} + H_2O_2$$

Glucose was first oxidized to gluconic acid and hydrogen peroxide, in a reaction catalyzed by glucose oxidase.

$$H_2O_2 + \text{4-Aminoantipyrine} + \text{p-Hydroxybenzene Sulfonate} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine Dye} + H_20$$

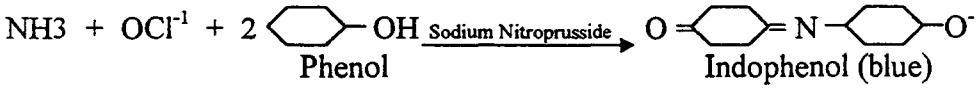
The hydrogen peroxide formed reacted in the presence of peroxidase with 4-aminoantipyrine and p-hydroxybenzene sulfonate to form a quinoneimine dye, with an absorbance maximum at 505 nm. The intensity of the colour produced was directly proportional to the glucose concentration in the sample.

Assay: Urinary Urea Nitrogen (Kit #640, Sigma Diagnostics, St. Louis, MO.)

The described method was based on the following reactions:
Urea was hydrolyzed by urease to ammonia and carbon dioxide.

$$H_2N-\underset{\displaystyle NH_2}{\overset{\displaystyle |}{C}}=O + H_2O \xrightarrow{\text{Urease}} 2\,NH_3 + CO_2$$

Ammonia then reacted with alkaline hypochlorite and phenol in the presence of a catalyst, sodium nitroprusside [$Na_2Fe(CN)_5NO{\bullet}2H_2$)], to form indophenol.



Phenol Indophenol (blue)

The concentraction of ammonia was directly proportional to the absorbance of indophenol, which was measured spectrophotometrically at 570 nm.

Assay: Urinary Creatinine (Kit # 555, Sigma Diagnostics, St.Louis, MO).

Most methods for creatinine measurement are based on the Jaffe[1] reaction, wherein a yellow colour is formed when creatinine is treated with alkaline picrate. Unfortunately the reaction is not specific and a number of substances including proteins in body fluids contribute to the yellow colour observed.

1) Urinary Creatinine + Alkaline Picrate Solution →
Creatinine & Interfering Chromogen Picrate Colour.
2) Stood for 5 minutes at room temperature, absorbance recorded (500 nm).

Slot[2] noted that under acid conditions the creatinine-picrate colour faded faster than the interfering chromogens. The work of these authors served as the basis for the manual method described, where colour derived from creatinine was destroyed at acid pH.

3) Acid Reagent added (Creatinine-Picrate colour faded).
4) Stood for 5 minutes at room temperature, absorbance recorded (500 nm).

The difference in colour intensity measured at or near 500 nm before and after acidification was proportional to the concentration of creatinine.

[1] Jaffe, M. (1886) Uber den Niederschlag, welchen picrinsaure in normalen Harn erzeugt und uber eine neue Reaction des Kreatinins. *Hoppe Seylers Z Physiol Chem* 10: 391.
[2] Slot, C. (1965) Plasma creatinine determination. A new and specific Jaffe reaction method. *Scand J Clin Lab Invest* 17: 381.

Radioimmunoassays: Estrogen, Progesterone, & Insulin (Kits # TKE21, TKPG1, & TKIN5, Coat-A-Count®, Diagnostic Products Corporation, Los Angeles, CA).

The Coat-A-Count® procedures were solid-phase radioimmunoassays, wherein the ^{125}I-labeled hormone competed for a fixed time with the hormone in the subject sample for sites on a hormone-specific antibody. Because the antibody was immobilized to the wall of a polypropylene tube, simply decanting the supernatant suffices to terminate the competition and to isolate the antibody-bound fraction of the radiolabeled hormone. Counting the tube in a gamma counter then yielded a number, which converted, by way of a calibration curve, to a measure of the hormone concentration present in the subject sample.

Assay: Urinary 3 Methyl-Histidine by High Performance Liquid Chromatography (HPLC)(Wassner *et al.*, 1985).

Resolution of components in a liquid chromatographic system is dependent on their differential distribution between a solid stationary phase and a liquid mobile phase. Components of greater concentration in the mobile phase will elute from the column first, followed by those of lower concentration in the mobile phase. Differences in peak maxima between eluted components are a function of the relative difference in their distribution between the phases. Since biopolymers vary in size and shape, solubility, ionic characteristics, hydrophobicity, and affinity for other molecules, discrimination between any one or combination of these properties may serve as the bases for differential distribution within a separation system. The particular "reverse-phase" column fractionation technique for this assay was designed to exploit the hydrophobic properties of 3-methylhistidine (3-MH).

The particular column used for this assay was a C-18 column which refers to the packing material of the column. Such a column is composed of 3 μm-diameter silicon beads to which 18- carbon chains are bonded. These long carbon chains are hydrophobic and therefore retain hydrophobic molecules, like 3-MH, on the column. As the concentration gradient of the eluting solvent becomes more non-polar over time, with the increasing concentration of acetonitrile, the more hydrophobic molecules are eluted from the column. Detection of the eluant was performed with a fluorometer. A known amount of histidinol was introduced into each urine sample to serve as an internal standard for the determination of 3-MH concentration.

Because of the tight packing of the column beads, a powerful pressure-head is required to force the eluant through the matrix. Such high pressures require that the column be reinforced with steel. Although longer and narrower columns are more expensive, they have higher resolving powers than do shorter columns. The current generation of HPLC columns for biopolymers has major advantages over conventional columns in both speed and resolving power.

TABLE 8-1. *Thermal equivalent of oxygen for nonprotein respiratory quotient, including percent kcal and grams derived from carbohydrate and fat*[a]

NONPROTEIN RQ	KCAL PER LITER OXYGEN CONSUMED	PERCENTAGE KCAL DERIVED FROM		GRAMS PER LITER O_2 CONSUMED	
		CARBOHYDRATE	FAT	CARBOHYDRATE	FAT
0.707	4.686	0	100	0.000	.496
.71	4.690	1.10	98.9	.012	.491
.72	4.702	4.76	95.2	.051	.476
.73	4.714	8.40	91.6	.090	.460
.74	4.727	12.0	88.0	.130	.444
.75	4.739	15.6	84.4	.170	.428
.76	4.751	19.2	80.8	.211	.412
.77	4.764	22.8	77.2	.250	.396
.78	4.776	26.3	73.7	.290	.380
.79	4.788	29.9	70.1	.330	.363
.80	4.801	33.4	66.6	.371	.347
.81	4.813	36.9	63.1	.413	.330
.82	4.825	40.3	59.7	.454	.313
.83	4.838	43.8	56.2	.496	.297
.84	4.850	47.2	52.8	.537	.280
.85	4.862	50.7	49.3	.579	.263
.86	4.875	54.1	45.9	.621	.247
.87	4.887	57.5	42.5	.663	.230
.88	4.899	60.8	39.2	.705	.213
.89	4.911	64.2	35.8	.749	.195
.90	4.924	67.5	32.5	.791	.178
.91	4.936	70.8	29.2	.834	.160
.92	4.948	74.1	25.9	.877	.143
.93	4.961	77.4	22.6	.921	.125
.94	4.973	80.7	19.3	.964	.108
.95	4.985	84.0	16.0	1.008	.090
.96	4.998	87.2	12.8	1.052	.072
.97	5.010	90.4	9.58	1.097	.054
.98	5.022	93.6	6.37	1.142	.036
.99	5.035	96.8	3.18	1.186	.018
1.00	5.047	100.0	0	1.231	.000

[a]From Zuntz, N.: Pflugers Arch. Physiol., 83:557, 1901.

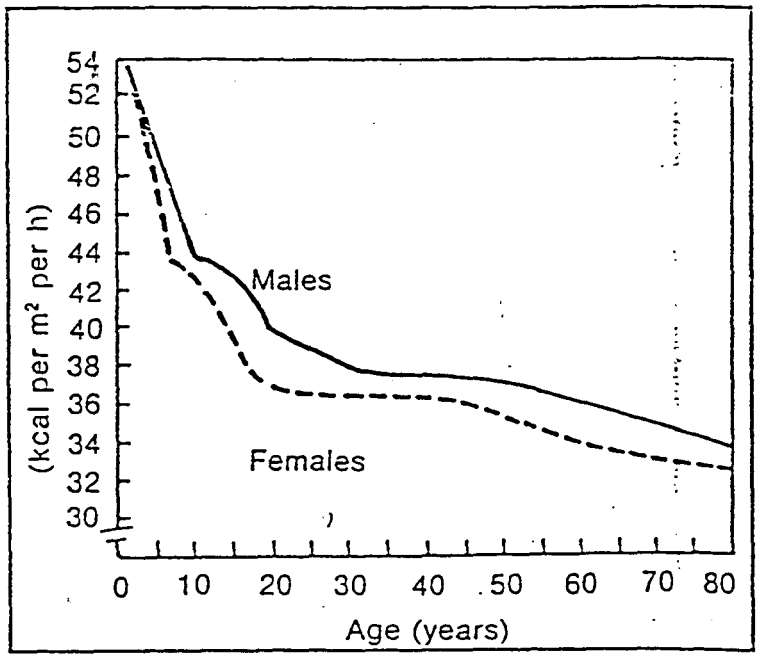


FIG. 9-1. *Basal metabolic rate as a function of age and sex. (Data from Altman, P.L., and Dittmer, D.S.: Metabolism. Bethesda, Md., Federation of American Societies for Experimental Biology*

Appendix V: Sample Consent and Ethics Forms.

Date: January 10, 1997.

Faculty Investigator.

Dr. Mark Tarnopolsky, MD, PhD Dept. of Human Biodynamics x23568

Student Investigator (s).

Michael Bosman, M.Sc Candidate Dept. of Human Biodynamics x27390 or x27037

Katherine Luttmer, B.Kin Candidate Dept. of Kinesiology (905) 308 9000

Type of Project.

Thesis Research: Master's.

Research Sponsor.

Held.

Title of Research Project.

Protein Metabolism and Energy Utilization in Females Participating in Endurance Activity: The Effect of Nutritional Supplement Timing.

Addendum To Ethics Application Form

Part I: Summary of Proposed Research:

a) **Purposed Research:**
A study to determine how the timing and amount of nutritional supplementation (2 trials) will influence protein metabolism following endurance exercise in females. An additional trial will determine how excess energy consumption (+400 kcal) contributes to the forementionned factors. We have been interested in characterizing gender differences in metabolism and, through our research, have found that females have an increased fat untilization and decreased carbohydrate and protein oxidation during long term, endurance exercise when compared to males. We have also examined whether gender differences existed in the capacity for carbohydrate loading in endurance athletes. We found that females did not increase their muscle glycogen in response to a pre-exercise carboyhydrate loading (↑ dietary CHO from 57 to 75% of energy intake), wherease the males increased their glycogen stores by 41% in response to the same dietary manipulation. There are no reports examining the effects of the timing of nutritional supplementation on protein metabolism in women participating in aerobic activity. We have also found that the daily energy intake of females was substantially lower than for males, even when expressed per kg lean body weight. Therefore, some of the changes that we have seen in metabolism may relate to the lower energy intake. We plan to examine the effect of supplemental energy and the timing of a nutritional supplement in female athletes.

b) **Describe in detail what will be done to the subject:**
Fourteen females between the ages of 20 and 24 years will be recruited for participation in the study. They will be active and will have no chronic medical problems nor be on medications except for possibly birth control pills, antihistamines, "puffers" for exercise induced bronchoconstriction or over the counter analgesics as required. The subjects' diet will be recorded for 5 days, (4 week days and 1 weekend day), they must abstain from caffeine 12hrs prior to testing and perform only light physical activity for 18 hours prior to testing. Prior to testing, subjects will be hydrostatically weighed and familiarized with the protocol. In the 3 days prior to each 3-day trial, subjects will consume a diet that, in terms of caloric and nitrogen content, is identical to their normal carbohydrate/protein/fat percentages from their diet records. They will be randomly assigned to receive a can of *Results* (milkshake-type drink) + carbohydrate (0.5 g/kg) either (A) with breakfast prior to exercise, or (B) immediately post-exercise or (C) immediately post-exercise with 400 kcal of *Boost* (a meal supplement) at breakfast during the 3 days of the extra energy trial. During each trial subjects will receive a pre-packaged diet based on their food records. Subjects will exercise on a cycle for 60 min. on Day 1 and 90 minutes on Day 3 of each trial, with Day 2 being a rest day in which the thermic effect of food is measured via expired gas analysis. On Day 3 of each trial, a 22 Ga plastic catheter is inserted into the antecubital vein in the dominant arm. A resting 5 mL blood sample will be drawn. Additional 5 mL blood samples will be taken at +20, +40, +60, and +90 min. of exercise and at +30, +60, +90, and +120 min. of recovery. All blood will be analyzed for insulin, lactate, potassium, hematocrit, sodium, and glucose concentration and, in addition, the

rest sample will also be analyzed for estrogen and progesterone concentration. Urine samples will be collected on days 2 and 3 of testing for 24 hours to monitor urea, creatinine, and 3-methyl histidine excretion. Expired gas measurements will be made at the previous timepoints and, additionally, post-exercise at +10, +20, +45 min. to determine the effect of the 3 interventions on exercise post-oxidative consumption. The trials will be separated by 2-4 weeks (i.e., cross-over design) to allow for recovery and proper timing so that test days fall on the mid-follicular phase of subjects' menstrual cycles. On the morning following Day 3 of each trial, subjects will perform a ride to exhaustion to determine if either of the 3 nutritional interventions had a performance-enhancing effect.

c) **Cite your experience with this kind of research.**

I (Dr. Tarnopolsky) have been involved in studies with similar protocols for over 10 years and have inserted over 2000 of these small plastic arm catheters (like a small IV plastic needle) with no complications whatsoever. I find that subject comfort is enhanced significantly with these catheters vs frequent blood draws (i.e., 1 needle poke/study vs several). We have had extensive experience with the expired-gas apparatus and have had no problems. The diets will consist of the foods that the subjects normally receive plus a sugar drink and a commercially avalable milkshake-type drink.

2. Subjects involved in the Research:

a) Describe the salient characteristics of subjects – number, age range, sex, institutional affiliation or where located. How subjects are to recruited? Describe the relationship between the investigator(s) and the subject(s).

Total number of subjects is 14, all being female. They will be between the ages of 18 and 30 years. They will most likely be undergraduate or graduate students at McMaster University or other faculty, staff or students recruited through word of mouth and posters on campus. They may be friends of some of the investigator(s), some may be students of Dr. Tarnopolsky's, or they may be complete strangers but will be informed that participation in strictly voluntary and participation is in no way related to academic marks.

3. Estimate of the Risks of the Proposed Research:

- Performance test may cause fatigue and occasional nausea post-exercise.
- Blood sampling : Slight bruising may occur at the site of insertion of the catheter. Total amount of blood drawn, 8 x 5 mL = 40 mL /trial (~ 3 tablespoons x 3 trials).

4. Estimate of the Benefits of the Proposed Research:

Studying the effects of the timing of nutritional supplementation on nitrogen balance and protein turnover in females involved in aerobic training has several implications. Females engaging in endurance activity on a regular basis may benefit from post-exercise diet supplementation keeping them in a more positive nitrogen balance. This information could lead to treatment of people with impaired short-term energy production or neuromuscular disease. From a performance perspective a better understanding of the ergogenic effects of supplementation timing, especially between genders, may provide useful information in controlling its use in activities requiring aerobic power. Such information in the field of exercise physiology is currently limited largely to males.

The subjects will receive a measurement of body density, an analysis of their diet, blood work (insulin, glucose, potassium, lactate) and aerobic power while on the aforementionned 3 nutritional interventions as well as remuneration ($200).

5. Plan for obtaining informed consent:

a) Describe the explanation to be given to subjects before they agree to become participants in the project.

This study is to determine and distinguish the effects of nutritional supplementation (Results® + carbohydrate- a milkshake-like drink) both pre- and post-exercise on protein metabolism in females engaging in aerobic activity. In addition, this study will determine the effect of extra daily energy consumption (in the form of Boost®) on protein metabolism. We have been interested in characterizing gender differences in metabolism and have previously shown that the Canadian Recommended Nutrient Intake (RNI) for protein (0.86 $g \bullet kg^{-1} \bullet day^{-1}$) is inadequate for female endurance athletes. Furthermore, we have found that females have an increased fat untilization (↓ RER) and decreased carbohydrate (↓ 25% vs. males) and protein oxidation (↓ urea excretion) during long term, endurance exercise when compared to their male counterparts. We feel that this study will further our understanding of the factors necessary to achieve appropriate protein requirements in women engaging in endurance activity and, in particular, whether post-exercise supplementation and/or extra energy consumption will allow female endurance athletes to derive an ergogenic benefit from nutritional supplementation. There will be an orientation day followed by 3 testing periods ~ 2-4 weeks apart. A 3-day diet will be provided for you (x3 trials) and nutritional supplementation (ie. Results® + carbohydrate- a milkshake-like drink) will be administered in liquid form once and a placebo the other time. In each trial, you will have to cycle for 60 minutes (65% VO_{2max}) on Day 1 and 90 minutes on Day 3. Day 2 is a day of rest with respiratory measurements made intermittently over a 2 hour period via expired gas collection equipment. Additional measurements of respiratory function are made at the same timepoints post-exercise on Day 3 and at 4 timepoints during exercise. Also during Day 3, blood will be drawn 8 different times both during and post-exercise via a small catheter inserted into a vein on your dominant arm. The catheter will be removed upon completion and the total blood

drawn will be about a ¼ cup. The following morning you will perform a ride on the cycle ergometer (80 % VO_{2max}).

6) Steps to be taken to Ensure confidentiality of Data:

The data will be treated as confidential. All data will be kept in a locked cabinet in the back part of the physiology laboratory and Dr. Tarnopolsky will supervise access to the data. The file is my personal file in the lab and only a few senior graduate students and faculty have access to the room.

7) Subject Debriefing:

Subjects will receive a copy of their body density, nutrient analysis and blood work, as well as the final results of the study if they indicate interest on the consent form.

Consent and Information Form.

Protein Metabolism in Females Participating in Endurance Activity: The Effect of Nutritional Supplement Timing and Energy Consumption.

Dr. Mark Tarnopolsky and Michael Bosman, Dept. of Human Biodynamics, McMaster University.

I, _____, consent to participate in a study directed by Dr. M.A. Tarnopolsky designed to examine protein metabolism in endurance-trained females following nutritional supplementation pre- and post-exercise.

OUTLINE:

A study to determine how the timing and amount of nutritional supplementation (2 trials) will influence protein metabolism following endurance exercise in females. An additional trial will determine how excess energy consumption (+400 kcal) contributes to the aforementionned factors. We have been interested in characterizing gender differences in metabolism and, through our research, have found that females have an increased fat utilization and decreased carbohydrate and protein oxidation during long term, endurance exercise when compared to males. We have also examined whether gender differences exist in the capacity for carbohydrate loading in endurance athletes. We found that females did not increase their muscle glycogen in response to a pre-exercise carbohydrate loading (↑ dietary CHO from 57 to 75% of energy intake), whereas the males increased their glycogen stores by 41% in response to the same dietary manipulation. There are no reports examining the effects of the timing of nutritional supplementation on protein metabolism in women participating in aerobic activity. We have also found that the daily energy intake of females was substantially lower than for males, even when expressed per kg lean body weight. Therefore, some of the changes that we have seen in metabolism may relate to the lower energy intake. We plan to examine the effect of supplemental energy and the timing of a nutritional supplement in female athletes.

PROTOCOL:

I am aware that I will be one of 14 female subjects participating in this study. Before beginning the study, I will record my diet for 5 days, 4 of which will be weekdays, 1 being a weekend day. My exercise routine will be recorded and kept constant. I will be given a diet and exercise checklist 3 days prior to each trial and a prepackaged diet for the 3 days of each trial (3 trials total). I will record all physical activity over and above that prescribed for me so that I may repeat such activity for subsequent trials. This is to ensure that variability between trials is minimal.

Prior to arriving at the laboratory for each trial, I will prepare and eat all foods listed on my 3-day diet checklist. During this period, I will exercise for 60 minutes (Days 1 & 3) on a Monarch cycle in the lab at the intensity previously determined for me to be 65% of my VO2max.

On Days 1 through 3 of the actual trial, I will eat all foods prepackaged for me by the investigators at the times specified to verify that I am consuming a diet isoenergetic

with my habitual intake. I am aware that my diet will be manipulated, using both a SUPPLEMENT (Results® + 0.5 g/kg carbohydrate) and PLACEBO, so that SUPPLEMENT consumption will occur either prior to or after exercise. I am also aware that one trial will involve taking an extra 400 kcal per day of energy in a liquid protein/carbohydrate/fat mix (Boost®).

During the trial, I will exercise on a cycle ergometer for 60 minutes on Day 1, and for 90 minutes on Day 3. Day 2 is a rest day but I will have to come into the laboratory for 2 hours to have intermittent respiratory measurements made while at rest (T=0, +10, 20, 30, 45, 60, 90, 120 min) to determine the thermic effect of my food.

On Day 3 of each trial, I will then have a small plastic catheter inserted in a vein in my dominant arm. A pre-exercise 5 mL (1 teaspoon) blood sample will be drawn. I will then cycle (65% VO2max) for 90 minutes with blood samples being taken at T=+30, 60, and 90 min of exercise and T=+30,60, 90, 120 min post-exercise. Respiratory measurements will be made during these timepoints and additionally at 10, 20, and 45 minutes post-exercise to determine post-exercise oxidative consumption (EPOC). The catheter will then be removed and the following morning I will perform a cycle to exhaustion (80% VO2max). A 2-4 week rest period will exist between each of 3 trials.

On Days 2 and 3 of each trial, I will collect 24 hour urine samples for subsequent nitrogen balance and protein turnover analysis. On Day 2, my first urine will not be collected but all other urines, up to and including the first urine on Day 3 will be collected in the REST container. All other urines over the 24 hour period of Day 3 will be collected in the EXERCISE container. I will store all urine in the fridge before taking it to the laboratory within the 48 hour deadline. I will report all episodes of missed urine collection to the investigators.

RISKS:

1) Performance test may cause fatigue and occasional nausea post-exercise.

2) Blood sampling: Slight bruising may occur at the site of insertion of the catheter.
Total amount of blood drawn, 8 x 5 mL = 40 mL

I understand that the results of this study will be made available to the scientific community, although neither my name nor any reference to me will be used in compiling or publishing these results. I understand, however, that I may withdraw from the study at any time without any adverse repercussions, even after signing this form.

I am aware that I will receive a rmuneration of $200 for the completion of the study and knowledge of the results and analysis from my blood, densiometry and diet records.

I have had the study explained to me by either Dr. M.A. Tarnopolsky or Michael Bosman and understand the nature of the investigation and my rights. I am also aware that Dr. Tarnopolsky will be available for the duration of the study to discuss any concerns or problems at 525-9140 ext.23568 or through McMaster Paging at 521-2100 ext.6443.

subject name	signature	date
witness	signature	date