# GENDER DIFFERENCES IN EXERCISE METABOLISM

# GENDER DIFFERENCES IN METABOLIC RESPONSES TO ENDURANCE EXERCISE

By

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## ABSTRACT

While several investigations have reported a higher proportion of lipid oxidation (lower carbohydrate oxidation) in females performing heavy endurance exercise at the same relative intensity as males, some studies have failed to support this. Possible factors contributing to the lack of agreement may be differences in subject training status and diet, or the hormonal status of female subjects related to menstrual phase. All these variables are known to affect substrate metabolism during submaximal exercise. These factors were controlled in the present study by selecting subjects who were matched for level of physical conditioning and performance experience and placing them on eucaloric identical diets for 3 days. The females were tested during the mid-follicular phase of their menstrual cycle. Six males and 6 females ran on a treadmill at ~65% $\dot{V}O_2$ max for a total distance of 15.5 km (range in performance times, 90 to 101 min). Pre and post exercise needle biopsies of vastus lateralis were assayed for glycogen concentration. Plasma glycerol, glucose, free fatty acids and selected hormones (catecholamines, growth hormone, insulin and glucagon) were measured throughout and following the run by sampling from an indwelling venous catheter. Exercise

protein catabolism was estimated from 24 hr (resting and exercise) urinary urea N excretion.

Males were found to have significantly higher respiratory exchange ratios ($\bar{x}$ =0.94 vs 0.87), greater muscle glycogen utilization (by 25%) and greater urea nitrogen excretion (by 30%) than females. Changes in selected hormone concentrations could not explain the greater lipid utilization observed in females. The lower insulin and higher epinephrine levels seen in males could in part explain the greater glycogenolysis and protein catabolism observed in this group. It is concluded that, during moderate intensity long duration exercise, females demonstrate greater lipid utilization and less carbohydrate and protein metabolism than equally trained and nourished males.

## FOREWORD

This thesis is presented in a form similar to that which would be submitted for publication in a scientific journal. This presentation format was approved in 1986 by the Graduate Council of McMaster University to encourage the development of a writing style suitable for career related writing.

Two main sections constitute this thesis. Chapter I includes; a review of the literature on gender differences in substrate metabolism; an examination of the methodological problems associated with this literature; and an examination of the metabolic implications that gender differences in hormonal alterations may have. Chapter II is the thesis research, presented in manuscript form. Appendix I is a presentation of the raw data.

It is hoped that this style of thesis presentation will allow for the preparation of a paper suitable for publication with few revisions.

## TABLE OF CONTENTS

## LIST OF TABLES

TABLES APPEAR AT THE END OF THE
CORRESPONDING SECTION OF TEXT

## LIST OF FIGURES

FIGURES APPEAR AT THE END OF THE CORRESPONDING SECTION OF TEXT

# CHAPTER I

# A GENDER COMPARISON OF SUBSTRATE UTILIZATION: METHODOLOGICAL PROBLEMS AND HORMONAL IMPLICATIONS

## 1.1 INTRODUCTION

Substrate metabolism during endurance exercise has long been a topic of considerable interest. The large body of evidence now available indicates that carbohydrate, fat and in some cases protein, serve as an energy source for adenosine triphosphate generation during aerobic exercise. These energy substrates are found either intra-cellularly (glycogen, triglyceride, amino acids) or are provided from the circulation (glucose, free fatty acids, amino acids). The relative contribution each of these substrates makes to energy production is dependent on several factors including exercise intensity and duration (Gollnick, 1985).

Typically, during steady state endurance exercise of moderate intensity (50 - 70% $\dot{V}O_2$ max), carbohydrate and lipid contribute approximately equally to substrate metabolism (Carlson et al, 1971; Essén et al., 1976; Havel et al, 1967). Exercise of increasing intensity results in proportionally greater carbohydrate utilization (Bergström et al, 1967; Havel et al, 1967; Pernow & Saltin, 1971) while exercise of increasing duration results in proportionally greater lipid

oxidation (Ahlborg et al., 1974; Bergström et al., 1967; Edwards et al., 1934; Essén, 1977).

Traditionally protein was not thought to be a source of energy for the working muscles. This belief was based on data obtained years ago indicating that urinary urea nitrogen excretion (considered the major metabolite of protein catabolism) did not increase above resting levels following exercise (Åstrand and Rodahl, 1986; Cathcart & Burnett, 1926; Fick and Wislicensus, 1865; Young et al, 1966). Recent research, employing more sophisticated and sensitive measuring techniques, (urine and sweat urea N excretion, labeled leucine infusion, 3-methylhistidine (3MH) excretion and whole body N balance) indicates that protein catabolism is increased by endurance exercise (Calles-Escandon, 1984; Décombaz et al, 1979; Dohm et al, 1982; Lemon & Mullin, 1980; Tarnopolsky et al, 1988; Young & Munro, 1978). This increased protein catabolism was found to be most significant when carbohydrate reserves were low (Lemon & Mullin, 1980). Although methodological problems make it difficult to assess, it has been estimated that amino acid oxidation contributes 5% to 15% of the active muscles' total energy needs during long term exercise (Dohm et al, 1983).

Most of the existing knowledge of exercise substrate metabolism has been provided from data obtained on male subjects. With the increased participation of women in endurance-type sports, an interest in the metabolic

response of females during exercise and possible gender differences has developed. The following section will examine the studies that have compared the metabolic responses of men and women participating in aerobic exercise.

The limited amount of research published in the area of gender and substrate metabolism is difficult to assess since few similarities exist in the protocols used. In terms of outcome measurements, most of the conclusive statements made in these studies have been inferred from respiratory exchange ratio (RER) measurements, but the findings have not been consistent. The RER can provide an estimate of the gas exchange at the working muscle (Essén et al, 1977; Jansson, 1982) and can thus indicate the amount of fat and carbohydrate oxidized during exercise (Zuntz, 1901). The ratio of $CO_2$ produced per $O_2$ consumed is termed the respiratory quotient (RQ) when determined from blood gas measurements across the working muscle and is termed the RER when determined from pulmonary ventilation. Since the metabolic fuels, carbohydrate, fat and protein are structurally different, the amount of oxygen ($O_2$) required to completely oxidize these molecules to carbon dioxide ($CO_2$) and water is also different. The RER for lipid oxidation is ~ 0.70 and for carbohydrate oxidation is 1.0. Values lying between these ratios are considered to indicate only the proportion of carbohydrate and lipid metabolised, since

protein is considered to contribute very little to the RER (McArdle et al, 1986). Only under steady state exercise conditions can the RER provide a valid estimate of the amount of carbohydrate and lipid oxidized (Essén et al, 1977; Jansson, 1982).

Investigators, reporting a lower RER in women exercising at the same relative intensity as men, conclude that women derive a greater proportion of their energy from fat oxidation (Blatchford et al, 1985; Brown & White, 1984; Bunt et al, 1986; Froberg & Pedersen, 1984; Nygaard, 1986). Conversely, studies which have failed to find sex differences in RER during exercise infer that no gender differences exist in exercise substrate oxidation (Costill et al, 1979; Graham et al, 1986 ; Powers et al, 1980). Differences across studies with respect to the reported RER's may have been the result of differences in the level of conditioning of the subjects' or the result of protocol differences in exercise intensity, duration, or mode. Sedentary (Blatchford et al, 1985) to well trained (Costill et al, 1979) individuals have been subjects for gender comparisons that examined exercise metabolic response and exercise intensity across the studies have ranged from 35% $\dot{V}O_2$ max (Blatchford et al, 1985) to 80% $\dot{V}O_2$ max (Froberg & Pedersen, 1984). Furthermore, the duration of the exercise bout has ranged from 25 min (Jansson, 1986) to a day of recreational activities (Nygaard et al, 1978) and the types of exercise performed have

included: walking (Blatchford et al, 1985), running (Costill et al, 1979), cycling (Froberg & Pedersen, 1984; Jansson, 1986), and skiing (Nygaard et al., 1978).

Whereas all investigations have used the RER in determining the metabolic requirements of exercise across genders, few studies have included other indices of substrate metabolism. Those which have compared plasma free fatty acid (FFA) response or muscle glycogen utilization provide no conclusive insight into the gender - exercise metabolism controversy, since these substrate indices, like the RER data, yield conflicting results. While some investigators have reported a greater plasma FFA concentration in women engaged in prolonged submaximal exercise at the same relative intensity as men (Blatchford et al, 1985; Nygaard, 1986) (an argument used in support of a greater lipid oxidation for women), others have found no gender difference in plasma FFA response during 25 min and 60 min, of submaximal exercise (Jansson, 1986; Costill et al, 1979 respectively) (an argument used against a metabolic gender difference).

A consistent finding across genders is a similar resting muscle glycogen concentration (Jansson, 1986; Nygaard, 1986). Muscle glycogen depletion following exercise, however, has been reported to be greater in men than women (Nygaard, 1986) or show no gender difference (Jansson, 1986). Perhaps the differing reports are the

result of the different exercise durations employed across studies, since, 60 min of exercise resulted in gender differences in glycogen depletion (Nygaard, 1986) while 25 min did not (Jansson, 1986). Again, the reported differences across studies in plasma FFA response and muscle glycogen utilization may have been the result of differences in the subjects' level of conditioning or the result of protocol differences in exercise intensity, duration, or mode.

Although studies have used both men and women in their investigation of exercise protein catabolism, data collected for these groups have been pooled for analysis (Calles-Escandon et al, 1984; Dolan & Lemon, 1987). It is therefore impossible to determine from these data whether gender differences exist in exercise protein catabolism. It should be pointed out that studies employing both male and female subjects do not constitute a valid gender comparison unless methodological consideration has been given for the selection of comparably trained and nourished subjects. Calles-Escandon et al. (1984) and Dolan & Lemon (1987) did not control for training or dietary difference between their male and female subjects. Thus inferences made from their data would be unfounded with regard to gender differences in exercise protein catabolism.

To our knowledge, no study has systematically compared the exercise protein metabolism between men and women. Investigations on male subjects have found that

prolonged endurance exercise does enhance protein catabolism (Décombaz et al, 1979; Dohn et al, 1982; Haralambie & Berg, 1976; Lemon & Mullin, 1980; Tarnopolsky et al, 1988 , Young & Munro, 1978) and, it has been postulated that the exercise protein catabolism for women would mirror that of men (Lemon and Nagle, 1981). There is some indirect evidence to suggest that this may not be the case. It has been found that male rats increase urinary urea nitrogen (N) excretion following exercise training and exhaustive exercise, while female rats do not (Dohm and Louis, 1978). In a human study of 8 sedentary females, 2 hours of cycle ergometry at 60% $\dot{V}O_2$ max did not enhance protein catabolism, as indicated by 24 hour urinary urea N and 3-Methylhistidine excretion (Plante & Houston, 1984). In contrast, in similar studies on male subjects, protein catabolism was increased as a result of 60 min of cycle ergometry at 61% of $\dot{V}O_2$ max (Lemon and Mullin, 1980) and during 2 hours of cycle ergometry at 60-65% of $\dot{V}O_2$ max (Cerny, 1975).

In summary, the few existing studies of gender differences in exercise substrate metabolism yield conflicting results. Thus it is unclear whether or not gender differences in exercise metabolism actually exist.

The purpose of the following section is to further investigate the factors that may have contributed to the disparate findings in the literature and to provide

suggestions for the improvement of study design.

## 1.2 METHODOLOGICAL PROBLEMS WITH INTERPRETATION OF EXISTING LITERATURE

There are several factors that may have contributed to the conflicting results reported in the gender-exercise metabolism literature. Difficulties with interpretation stem from variability in 1) the subjects' state of physical conditioning, 2) the intensity at which the exercise was performed, 3) the females' hormonal status related to menstrual phase, and 4) the subjects' dietary intakes and composition.

### A. STATE OF PHYSICAL CONDITION

Few would deny that level of physical condition affects substrate utilization during submaximal exercise. Generally, the trained state is characterized by greater fatty acid and lesser carbohydrate utilization at the same relative and absolute exercise intensities (Costill et al, 1979; Gollnick et al, 1972; Gollnick, 1985; Holloszy & Booth, 1976; Holloszy & Coyle, 1984; Rennie & Holloszy, 1977; Saltin et al, 1977).

Certainly, when evaluating gender differences in exercise metabolism the state of physical conditioning is a necessary factor to control. What is difficult to establish, however, are the criteria that would constitute an equal state of training between the sexes. Criteria used to select

and match subjects for level of conditioning include various indices of $\dot{V}O_2$ max: relative to body weight -BW (Costill et al, 1979; Powers et al, 1980), absolute and relative to body weight (Freedson et al, 1979), and relative to lean body weight - LBW (Bunt et al, 1985; Cureton & Sparling, 1980; Sparling, 1979); and similar physical activity and leisure patterns (Brown & White, 1984; Froberg & Pedersen, 1984; Nygaard et al, 1978).

It is not clear which of the many selection variables, if any, should be used to select and match members of the opposite sex for training status. Each of the $\dot{V}O_2$ max expressions have limitations. Since males are typically more muscular and leaner than their female counterparts (Behnke & Wilmore, 1974; Katch & Katch, 1984), their $\dot{V}O_2$ max values when expressed absolutely ($l.min^{-1}$) or relative to body weight ($ml.kg^{-1}BW.min^{-1}$) are normally greater than comparable measures for females (males having a 50% higher absolute and 20% higher relative $\dot{V}O_2$ max than females: Cureton, 1980; Sparling, 1980). Even the most highly trained female athletes are fatter than their athletic male counterparts, thus theoretically, males should have a somewhat higher relative $VO_2$ max (BW) than comparably trained females. Selecting groups of men and women with similar absolute or relative (BW) $\dot{V}O_2$ max values would yield either unequally trained groups of men and women or groups that were morphologically non-representative of their gender (i.e.

males unusually fat and non-muscular or females unusually lean and muscular). On the other hand, to impose typical gender differences in these $\dot{V}O_2$ max expressions would presuppose that men and women within the population are equally trained. There is some evidence to suggest that females may be less fit than males (Cureton, 1981; Sparling, 1980). Therefore, matching groups of men and women based on population norms of $\dot{V}O_2$ max would yield unequally trained groups of men and women.

$\dot{V}O_2$ max expressed relative to lean body weight (LBW) has been suggested by Buskirk and Taylor (1957) to be the most valid physiological predictor of training state. This expression of $\dot{V}O_2$ max takes into account differences in body size and composition, and thus has been suggested to be a valid criterion to use in the selection and matching of groups across genders (Cureton, 1981). This method of equating the sexes may still pose validity problems since the expected gender differences in $\dot{V}O_2$ max relative to LBW remains controversial. Some investigators state that females, equally trained to males, should have a slightly lower (5%) $\dot{V}O_2$ max (LBW) due to their typically lower hemoglobin concentration and (a-v) $O_2$ difference (Cureton, 1981). Others explain that the higher 2,3 diphosphoglyceric acid concentration in females offsets lower hemoglobin concentrations and therefore equally trained members of the

opposite sex should have equal $\dot{V}O_2$ max values when expressed relative to LBW (Pate et al, 1985).

It is apparent that expressions of $\dot{V}O_2$ max pose problems when used to select and match members of the opposite sex. How then can equally trained members of the opposite sex be selected? Amount of endurance training engaged in over the past year has been found to be the best predictor of training equality across genders (Sparling, 1980). Thus, the only valid measure of training equality across genders would be the comprehensive comparison of training and performance histories (Sparling, 1980).

Retrospective accounting of training mileage would seem an insufficient means of assessing training histories, since a subject's perception and recall of work done may be vague. A comprehensive and prospective training log documenting exercise duration, intensity and distance along with health, injuries and competitions would be a sufficient requirement. However, if training background is to be used to select and match members of the opposite sex, one would have to study groups of males and females with an established endurance training program since "sedentary" men are thought to be more active than "sedentary" women (Cureton, 1981).

Another critical design problem that plagues the gender difference studies is that of within-group training differences. While trying to match "equally trained" men and women, several investigators have failed to select subjects

within each sex who were equally trained. Investigators have selected subjects whose running mileage ranged from 28 to 60 $mi.wk^{-1}$ (Powers et al, 1980), 37-120 $mi.wk^{-1}$ (Wells et al, 1980), and 19-113 $km.wk^{-1}$ (Cureton and Sparling, 1980). Furthermore, Nygaard et al. (1978) selected subjects who were novice to internationally ranked skiers. When one considers that these studies involved only 4-6 subjects, one would wonder if the extraordinarily wide within-group training differences did not mask any gender differences that may have existed. In order to examine the effect gender has on exercise substrate metabolism, training differences within a group should be kept within a narrow range.

B. EXERCISE INTENSITY

Although this section is titled exercise intensity a more appropriate title would be "the misuse of the respiratory exchange ratio". The RER can provide a valid estimate of gas exchange at the working muscle (RQ) as long as respiratory measurements of expired carbon dioxide ($CO_2$) reflect that of actual $CO_2$ volumes produced within the body through oxidation (Essén et al., 1977; Jansson, 1982). Hyperventilation and the accumulation of lactic acid both disproportionally raise the RER since both increase $CO_2$ elimination at the lung without a corresponding increase in $O_2$ consumption. The onset of blood lactate accumulation

occurs between 55% and 65% of $\dot{V}O_2$ max in sedentary individuals and 80% of $\dot{V}O_2$ max or higher in well trained athletes (Davis et al, 1976; Michelson and Hagerman, 1982; Wasserman et al, 1973). Although the RER has been validated as reflecting actual substrate utilization during endurance exericse at 55% (Essén et al, 1977) and 65% $\dot{V}O_2$ max (Jansson, 1982), it is questionable whether studies of gender difference employing exercise intensities above 65% $\dot{V}O_2$ max attained a steady state condition, and hence, whether the RER obtained was a valid predictor of fuel consumption at the exercising muscles.

For example, Pate et al, (1985) acknowledge that "it was uncertain whether a steady state condition was attained since the protocol involved changing treadmill running speed or grade every 2 minutes until exhaustion", but concluded based on their RER data, that comparably trained men and women demonstrated similar abilities to metabolize fat during submaximal exercise. Furthermore, Froberg and Pedersen (1984) concluded, based on RER observations, that women exercising to exhaustion utilized a greater proportion of fat than men, even though the men maintained a significantly higher lactate concentration that averaged 8.09 $mmol \cdot l^{-1}$.

Several other investigators have employed protocols of varying intensities (Graham et al, 1981; Wells et al, 1981) or intensities that exceeded 65% $\dot{V}O_2$ max (Costill et al, 1979; Cureton & Sparling, 1980; Graham et al, 1981;

Nygaard, 1986). Since a steady state metabolic condition is a prerequisite for a valid application of the RER for estimation of substrate utilization, it is not surprising that the literature is equivocal regarding gender differences in exercise metabolism.

C. MENSTRUAL PHASE

All gender comparison studies have selected eumenorrheic, non oral contraceptive using women. What has not been consistently controlled for, is the phase of the womens' menstrual cycle during which data were collected.

The menstrual cycle is characterized by two distinct phases, the follicular and luteal stages. The follicular phase begins at the onset of the menstrual bleeding (day 1) and ends at ovulation (~ day 14). The luteal phase begins at ovulation and ends at the onset of menstrual bleeding (~ day 28). The follicular phase is characterized by lower circulating levels of estradiol (25-75 $pg.ml^{-1}$) and progesterone (<1 $ng.ml^{-1}$), the luteal by higher levels of estradiol (100-300 $pg.ml^{-1}$) and progesterone (5-20 $ng.ml^{-1}$). Peak levels of both these hormones occur at ovulation (O'Riordan et al, 1982).

There is some indication that menstrual phase is associated with alterations in substrate metabolism and performance outcome. It has been found that blood glucose concentrations (Lavoie, 1986) and plasma triglyceride

concentrations (de Mendoza at al, 1979) are higher and that the activity of lipoprotein lipase is lower (de Mendoza et al, 1979) during the follicular than luteal phase. Furthermore a greater isometric endurance capacity has been observed during the mid-follicular phase (Petrofsky et al, 1976) and a greater capacity for exhaustive exercise has been observed in the mid-luteal phase (Jurkowski et al, 1981).

Apparently dietary intake is an important variable to control when evaluating the metabolic and hormonal alterations associated with menstrual phase. It was found that glucose loading elicited a greater free fatty acid response in the follicular phase, while fasting elicited an elevated insulin and growth hormone response in the luteal phase (Bonen et al, 1983).

It appears, from the limited data available, that menstrual phase may affect substrate metabolism and that this in turn is influenced by dietary intake. When examining gender differences in substrate metabolism, it would be important to control for the hormonal fluctuations associated with the menstrual cycle. These hormonal fluctuations to a large extent can be controlled by testing all female subjects during the same phase of the menstrual cycle.

## D. DIET AND NUTRITIONAL FACTORS

Although it is known that dietary intake and composition affect substrate utilization and endurance capacity (Bergström et al, 1967, Christensen & Hansen, 1939; Hultman & Bergström, 1967; Hultman & Nilsson, 1971; researchers have given little consideration for controlling this variable when investigating possible metabolic gender differences. Although most investigators have fasted subjects before metabolic testing, few have considered the previous days' nutrient intakes.

Although both genders typically consume 45-55% of their calories as carbohydrate, 34-44% as fat and 12-16% as protein (Brotherhood, 1984), it cannot be assumed that smaller samples of a population will eat consistently within population means and that variability in intake would be similar between the sexes. Wide variations in nutrient intakes do exist within the athletic population (Brotherhood, 1984). In addition, if one considers that females consume energy-reduced diets more often than males (Brotherhood, 1984) and suffer from an increased incidence of eating disorders (Blumenthal et al, 1985), it becomes apparent that dietary intake is an important factor to control in these types of studies. In order to control for potential differences in dietary intake between the sexes, comprehensive dietary logs should be kept by all potential study participants. From dietary logs one can assess whether

gender differences exist in dietary composition and caloric consumption (relative to recommended nutrient intakes for age and sex). To further assure similarity in the quantity and quality of diet, pre-packaged diets could be given to the subjects prior to the testing session.

As a result of the many methodological problems associated with the gender comparison studies, it is difficult to identify what, if any, gender differences exist in exercise substrate metabolism. It is useful to briefly review the nutritional deprivation studies that have compared the metabolic response of males and females during starvation, since they may provide some valuable insight into possible metabolic gender differences.

Starvation is a nutritional stress that is quite commonly compared to the metabolic stress of exercise (Ahlborg et al, 1974; Gontzea et al, 1975; Lemon & Nagle, 1981). The depletion of muscle and liver glycogen stores, the increased rate of FFA mobilization and utilization, and the de novo synthesis of glucose via alanine transamination are all metabolic alterations seen during starvation (Felig et al, 1969; Hultman & Nilsson, 197 ; Marliss et al, 1970; ) as well as during prolonged submaximal exercise (Ahlborg et al, 1974; Gontzea et al, 1975). If exercise substrate metabolism parallels that of starvation, as the above similarities would suggest, and if there are gender differences in metabolic

response to nutritional deprivation, then it would be plausible to hypothesize that there are sex differences in exercise substrate metabolism.

Widdowson (1976) writes "the female is better able to withstand a shortage of food than the male". Both animal and human data support this contention. Using the rat model, it was found that a period of total starvation resulted in a greater lipid metabolism and a lesser protein catabolism in females than in males (Triscari et al, 1980; Widdowson et al, 1956). Following a year of chronic nutritional deprivation, female pigs had a survival rate 4 times greater than that of male pigs (McCance and Widdowson, 1962) and only female pigs have been shown to survive 2 or 3 years of severe caloric deprivation (Widdowson, 1976).

In a human study of 58 obese females and 18 obese males, the males lost more weight, a greater percentage of their initial weight and a greater percentage of their LBW than did the females during the first 14 days of a 30 day fast (Runcie & Hilditch, 1974). When comparing the ratio of urinary ketones to urinary N excretion, it was found that normal weight women catabolised a greater amount of adipose tissue and men a greater amount of structural proteins when energy deprived (Broom et al, 1978).

The fact that there are gender differences in the metabolic response to starvation and nutritional deprivation

lends support to the hypothesis that there are gender differences in exercise substrate metabolism.

## 1.3 HORMONAL REGULATION OF METABOLISM

Investigators purporting a gender difference in exercise substrate metabolism have looked for mechanisms in order to explain this difference. The sex hormones estradiol and testosterone have been reported to be linked to this metabolic gender difference (Brown et al, 1980; Brown et al, 1986; Brown & White, 1984; Dohm et al, 1978). Other hormones that influence fuel mobilization and utilization may also play a part in the sex-linked metabolic differences. This section examines the metabolic significance of gender differences in hormonal response to exercise with reference to the hormones estradiol, testosterone, growth hormone, epinephrine, norepinephrine, insulin and glucagon.

Hormones are chemical messengers that serve an important role during exercise in their ability to regulate and potentiate substrate metabolism (Guyton, 1987). Generally, the concentration of hormones which stimulates the rate of lipolysis, glycolysis and gluconeogenesis, increase during prolonged exercise, whereas lipogenic and glucogenic hormone concentrations decrease (Bunt, 1986; Sutton, 1984). Typically, hormones are released into the circulation by a host gland and act to accelerate or decelerate the rate of chemical reactions at a specific target organ (McArdle et al,

1986). The sex hormones are steroid-based whereas the pancreatic hormones, catecholamines and growth hormone are amino acid derivatives. The steroid based hormones are lipid soluble and act by crossing the cell membrane, binding to cytosolic receptors and influencing the rate of cellular reactions following their translocation into the cell nucleus (O'Riordan et al, 1984). The amino acid based hormones bind with specific cell surface receptors and influence intracellular metabolism through the generation of a second messenger (O'Riordan et al, 1984).

The significance of plasma hormonal concentrations and hormonal alterations during, or as a result of, exercise are difficult to interpret. Since receptor availability and sensitivity determines the activity of most hormones, increases in plasma concentration of a hormone does not necessarily lead to an enhanced hormonal action. If the availability and sensitivity of the receptor specific to that hormone is low, increases in circulating hormonal concentrations will not necessarily lead to the hormone's expected effect. Furthermore, since hormones can act synergistically to potentiate a particular action (i.e. estradiol and cortisol), antagonistically to inhibit a particular action (insulin and glucagon), or both (growth hormone and insulin), information gathered in vitro through the manipulation of only one hormone will provide limited

insight into metabolic significance of a specific hormone in a dynamic intact human system.

The pulsatile nature by which some hormones are secreted, is the final factor that should be considered when evaluating the metabolic significance that hormonal alterations may have during exercise. Estradiol, testosterone, growth hormone and cortisol are among the hormones that are secreted in an episodic fashion throughout the day. Since plasma hormonal concentrations would fluctuate as a result of their secretion pattern, single resting values for these particular hormones may not be representative of true baseline values (Raynaud et al, 1983). If only one resting sample was taken, it would be difficult to assess whether changes from the resting value were the result of the exercise stress or the sampling time.

## A. ESTRADIOL

Estradiol ($E_2$) is the hormone primarily responsible for the development of the secondary sex characteristics in the female. Thus, following puberty females have approximately 5-25 fold greater plasma concentration of $E_2$ relative to males (Martin, 1985; McMaster University Laboratory Services reference range, 1987).

In oophorectomized rats, $E_2$ administration has been shown to: 1) increase the availability and synthesis of free fatty acids and triglycerides (Hamosh and Hamosh, 1975;

Kenargy et al, 1981; Palmer and Davis, 1982); 2) potentiate an exercise-induced increase in lipoprotein lipase activity (Goldberg et al, 1984); and 3) produce a dose dependent increase in the activity of the liver lipogenic enzymes, acetyl CoA carboxylase and fatty acid synthetase and a decrease in the activity of the gluconeogenic rate limiting enzyme phosphoenol-pyruvate carboxykinase (Mandour et al, 1977). Recently Kendrick and coworkers (1987) found that $E_2$ administration had a dose dependent glycogen sparing effect on the cardiac and skeletal muscle of oophorectomized rats running for 2 hours.

In the human female, an acute bout of exercise has been reported to both elevate (Bonen et al, 1979; Jurkowski et al, 1973) and maintain plasma concentrations of $E_2$ (Bunt et al, 1985; Loucks and Horvath, 1984; Kindermann et al, 1982). The plasma $E_2$ concentrations in untrained males have been reported to increase with exercise while variable responses have been reported for trained males (Brown et al, 1980). As a result of training, resting $E_2$ concentrations have been reported to increase in males (Brown et al, 1980; Frey et al, 1983), and decrease (Boyden et al, 1983) or to not change (Brown et al, 1980) in females.

Due to these inconsistent findings, it is difficult to determine the metabolic significance of the altered $E_2$ concentrations. A consistent finding is a greater $E_2$ concentration at rest and during exercise for females than

males (Brown et al, 1983; Bunt et al, 1985; Wallace et al, 1981; ). It has been postulated that these higher $E_2$ levels are in part responsible for the greater lipid oxidation found for females during exercise when compared to males (Walker et al, 1985). If the metabolic significance of $E_2$ is dose dependent, as Kendrick et al (1987) would suggest, the greater $E_2$ concentrations found in females would result in greater lipid utilization and a lesser carbohydrate utilization relative to males. Estradiol could very well play a part in the metabolic gender differences reported during exercise.

B. TESTOSTERONE

Testosterone (T) is secreted primarily by the Leydig cells of the testes, although in both men and women some T is produced by the adrenal gland (O'Riordan et al, 1984). Testosterone is primarily responsible for the development of the secondary sexual characteristics of the male. Metabolically, T increases protein anabolism, enhances glycogen storage, decreases body fat and increases red blood cell production (Guyton, 1987; Lamb et al, 1975).

During exercise, plasma T concentration has been reported to increase in both men and women (Guylielmini et al, 1984; Keizer et al, 1984; Sutton et al, 1973; Vogel et al, 1985) decrease (Kuoppasalmi et al, 1980; Kussi et al, 1984) or not change (Bunt et al, 1985; Loucks & Horvath,

1984). Variations in exercise mode, intensity and duration may have contributed to these reported dissimilarities (Bunt, 1986). It has been found (Brown et al, 1986) that trained males and females both had an enhanced T response to 2 hours of submaximal exercise at 50-55% $\dot{V}O_2$ max, while an untrained group experienced no change from resting values. In both men and women, training has been reported to have no effect on resting T concentrations (Brown et al, 1980; Brown et al, 1983). However, both Bunt (1986) and Dale et al. (1979) found higher resting T concentrations in endurance trained females when compared to controls and increases (Johnson et al, 1983; McConnell & Sinning, 1984; Remes et al, 1979) and decreases (Frey et al, 1983) have been reported for trained men.

It is difficult to identify the metabolic significance of T during exercise since its function in this regard is controversial. Bunt (1986) and Brown et al, (1986) both suggest that the T response during exercise is unimportant but that its metabolic significance may play a part during recovery or at rest, by promoting glycogen storage (Brown et al, 1983). Since males have higher resting and post-exercise T concentrations than females, Brown et al, (1986) postulated that males may have a greater muscle glycogen concentration. They reason that the greater muscle glycogen concentrations would promote a greater carbohydrate

utilization during exercise and thus explain the higher RER values seen for the males when compared to females (Brown et al, 1986). No sex differences in glycogen storage have been reported (Jansson, 1986; Nygaard et al, 1978), however few studies examining this variable exist.

The sex hormones $E_2$ and T most probably influence exercise substrate metabolism through their influence on resting and recovering tissue substrate storage (Bunt, 1986). Sex hormones cannot, however, be totally responsible for the gender-related metabolic differences reported, since it has been shown in one study that these metabolic gender differences in exercise substrate utilization exist in pre-pubescent children - a time when levels of the sex steroids are not significantly different between the genders (Brown et al, 1986).

C. GROWTH HORMONE

Growth hormone is an anterior pituitary hormone whose major function is to promote the growth of the body's cells and tissues through its interaction with the somatomedins (Martin, 1985). Since growth hormone does not function through a target gland but exerts its effects on most body tissues, this hormone has many specific metabolic effects. growth hormone can increase the rate of protein synthesis, decrease protein catabolism, decrease carbohydrate utilization and increase the rate of fatty acid (FA)

mobilization and FA utilization (Haynes, 1986; Macintyre, 1987; Martin, 1985).

Growth hormone's lipolytic effect may be its most important metabolic function since FA mobilization and utilization lower the body's glucose need and hence reduces glycogenolysis and gluconeogenesis (Macintyre, 1987). Generally, during exercise circulating growth hormone concentrations increase (Galbo, 1983; Shephard & Sidney, 1975; Sutton, 1978), however this increase tends to be delayed 5 to 20 minutes (Bunt, 1986; Karagiorgos et al, 1979; Lassarre et al, 1974). Although the exact role growth hormone plays during exercise is not known (Macintyre, 1987), its time lag in response to exercise tends to suggest that its metabolic significance is more involved in potentiating or maintaining blood FFA and glucose concentrations than initiating a shift in substrate metabolism (Bunt, 1986). The finding that FFA mobilization occurs, but is markedly reduced in fasted hypophysectomized rhesus monkeys (Goodman & Knobil, 1959), supports the hypothesis that an increased growth hormone secretion does not initiate FFA mobilization, but does potentiate it. It is possible that growth hormone acts in conjunction with glucocorticoids and other hormones to mediate FFA mobilization (Kostyo & Reagan, 1976).

Women have been reported to have a higher resting growth hormone concentration (Bunt et al, 1986 and; Frantz & Rubkin, 1965) and exhibit a greater exercise response when

compared to males (Bunt et al, 1985; Frantz & Rubkin, 1965; Shephard & Sidney, 1975). These gender differences in growth hormone concentration are thought to be the result of differences in $E_2$ levels since males and females on $E_2$ therapy (Chakmakjian & Bethune, 1968), women using oral contraceptive (Davidson & Holzman, 1973) and females during ovulation (Hansen & Weeke, 1974) have reportedly higher growth hormone levels than controls. However, these findings are not consistent (Bonen et al, 1983; Hansen & Weeke, 1974).

In a well controlled study, it was demonstrated that the resting growth hormone levels of trained men and women were higher than untrained subjects of either gender (Bunt et al, 1986). These authors stated that while resting growth hormone values correlated well with $E_2$ levels, the exercise values did not. In contrast to the findings that females have a greater growth hormone response to exercise than males (Frantz & Rubkin, 1975; Bunt et al, 1985), it was found that females had greater growth hormone values only during the first 30 min of a 60 min exercise stress (Bunt et al, 1986). Following this time period, no gender difference in growth hormone concentration was observed. Expressed as a percent change, males had a greater growth hormone increase than did the females.

Some researchers have concluded that training blunts the growth hormone response during exercise (Bloom et al,

1975; Galbo, 1983). Others have found that training has no significant effect on the growth hormone response of females engaged in prolonged submaximal exercise but enhances the growth hormone response of males (Bunt et al, 1986).

The metabolic implications of possible gender differences in growth hormone response during exercise are unknown. Since the increase in plasma concentration of growth hormone is delayed in response to exercise (Bunt, 1986; Karagiorgos et al, 1979; Lassarre et al, 1974), the higher resting growth hormone concentration found for females may result in greater lipolysis during the first 5-20 min of exercise when compared to males. Higher resting levels of growth hormone in females may "prime" the system for lipid metabolism, thereby requiring lower growth hormone secretion than for males during the initial phase of exercise. Walker et al. (1985) found that trained men tended to utilize a higher percentage of carbohydrate at the onset of exercise than women and that men's RER, although higher than the women's, dropped more steeply. This would indicate that the men experienced a more rapid change in the proportions of carbohydrate and lipid metabolized than did the women. Growth hormone may play a part in this substrate shift since stresses on glucose homeostasis tend to increase growth hormone secretion (Sutton & Lazarus, 1976). The proportionally greater carbohydrate utilization found for the men would deplete glycogen stores more rapidly when compared

to women and hence stimulate growth hormone secretion.

D. CATECHOLAMINES

The catecholamines are a group of related amino acids that include epinephrine and norepinephrine. Epinephrine is produced in the adrenal medulla in response to cortisol and sympathetic nervous system (SNS) stimulation (O'Riordan et al, 1984). Some norepinephrine is produced by the adrenal medulla, but most of the norepinephrine production is by the sympathetic nerve endings in response to SNS stimulation (O'Riordan et al, 1984).

Plasma concentrations of both norepinephrine and epinephrine increase in response to exercise and the magnitude of their increase is positively correlated to exercise intensity (Bloom et al, 1976; Galbo et al, 1975). During exercise of moderate intensity (~ 60 to 70% $\dot{V}O_2$ max) norepinephrine increases in a hyperbolic fashion with greater increases at exercise onset followed by a more gradual increase up to 90 min (Richter et al, 1981). Since norepinephrine increases almost immediately with exercise onset (Galbo et al, 1975; Galbo et al, 1977; Kjaer et al, 1986; Maher et al, 1975), it is thought to stimulate the early release of hepatic glucose (Edwards, 1977). Metabolically norepinephrine promotes FFA mobilization and to a lesser extent, hepatic glycogenolysis (Martin, 1986).

Plasma epinephrine tends to increase later in

exercise than does norepinephrine (Galbo et al, 1977; Kjaer et al, 1986). During moderate intensity exercise, epinephrine does not increase until approximately 30 min after the onset of exercise. From 30 to 90 min of exercise epinephrine increases in an exponential fashion (Richter et al, 1981). The later increase in epinephrine concentration is thought to act as a backup mechanism for plasma glucose maintenance (Galbo et al, 1975). Increasing epinephrine levels promote muscle and liver glycogenolysis (Carlson et al, 1985; Jansson et al, 1984) and to a lesser extent muscle and adipose lipolysis (Martin, 1985; Richter et al, 1982 ).

It has been reported that males have a greater catecholamine response to exercise than do females exercising at the same relative intensity. Sanchez et al. (1980) found that men increased blood epinephrine and norepinephrine concentrations during isometric contractions while women did not. A lower epinephrine concentration in women during the last 20 min of a 60 min continuous exercise bout has been reported when the intensity of exercise was increased from 60% to 75% $\dot{V}O_2$ max (Nygaard, 1986), however no difference in epinephrine response was seen during the less intense exercise (50 and 60% $\dot{V}O_2$ max). It has been shown that amenorreheic and eumenorreheic women have lower plasma norepinephrine concentrations at rest, at 30% $\dot{V}O_2$ max and, at exhaustion when compared to men (Graham, 1986). The reasons

for this gender difference may be the result of differences in the central mechanisms that elicit sympathetic activation (Claustre et al, 1980) or the inhibitory effect of estrogen on catecholamine secretion (Wiechman and Borowitz, 1979).

The greater catecholamine response in males would support the hypothesis of a metabolic gender difference. If norepinephrine stimulates the early release of hepatic glucose as Edwards (1977) suggests, then, the greater norepinephrine response observed for males (Nygaard, 1986; Sanchez et al, 1980) might account for the greater carbohydrate utilization found in males at exercise onset, compared to females (Walker et al, 1985).

## E. PANCREATIC HORMONES

The major hormones secreted by the pancreas are glucagon and insulin. In the islets of Langerhans the alpha cells and beta cells secrete glucagon and insulin respectively. The major regulator of their secretion is plasma glucose concentration. Hypoglycemia increases glucagon secretion while hyperglycemia increases insulin secretion. Insulin mediates the facilitated diffusion of glucose entry into the cells. Thus, when blood glucose levels rise, insulin secretion facilitates glucose entry into the cells, which results in the lowering of blood glucose levels and hence a reduction in pancreatic stimulation of insulin secretion (Sutton, 1984).

During prolonged exercise, plasma concentration of glucose and insulin decrease (Galbo et al, 1979; Pruett, 1970; Sutton, 1978). The reduction in insulin during exercise is thought to be the result of a suppressed insulin secretion (Galbo et al, 1977). Rising epinephrine levels have been shown to suppress insulin secretion (Richter et al, 1981). Metabolically, lower insulin levels may enhance hepatic glucose production, increase the rate of lipid catabolism, or restrain glucose utilization by inactive tissue (Sutton, 1984). As plasma insulin concentrations decline with exercise of long duration, more energy is derived from lipid oxidation.

Insulin response to various intensities and durations of exercise has been reported to be unaltered by the menstrual phase (Sutton et al, 1980; Toth et al, 1987; Yki-Jarvinen, 1984). This would indicate that alterations in the female sex hormones associated with the menstrual cycle, do not affect insulin response. In oophorectomized rats, $E_2$ replacement only slightly reduced insulin concentration, while it markedly reduced basal glucagon concentrations (Mandour et al, 1977).

Glucagon works to increase blood glucose concentrations by increasing hepatic glycogenolysis and gluconeogenesis (Felig et al, 1972; Sutton, 1984). This hormone stimulates hepatic glycogen breakdown by activating adenyl cyclase in the hepatic cell membrane which causes the

formation of cyclic adenosine monophosphate. Following a cascade of events, hepatic glycogen is degraded and dephosphoryolated to glucose and released into the blood (Martin, 1985). Glucagon also increases the rate of hepatic gluconeogenesis by: 1) enhancing the rate-limiting gluconeogenic enzyme that converts pyruvate to phosphoenol pyruvate (Kraus-Friedman, 1984); and 2) increasing the uptake of circulating amino acids by the liver (Ahlborg et al, 1974).

During exercise, circulating glucagon concentrations increase in response to falling glucose concentrations and/or increases in norepinephrine (Galbo et al, 1975; Galbo et al, 1979; Richter et al, 1981). Nearly 75% of the increased hepatic glucose production during exercise is due to increased glucagon levels (Wasserman et al, 1984).

Since $E_2$ has a suppressive effect on the alpha cells of the pancreas (Mandour et al, 1977), one would hypothesize that females would have a reduced glucagon response to exercise when compared to males. It is possible that the greater hepatic lipogenic enzyme activity and decreased hepatic glucogenic enzyme activity seen with $E_2$ replacement in oophorectomized rats is the result of $E_2$ effects on glucagon secretion (Mandour et al, 1977). Further research is needed to explore this hypothesis.

## 1.4 SUMMARY

The present literature is insufficient to state what, if any, gender differences exist in exercise substrate metabolism. From the data on starvation and nutritional deprivation there appears to be support for a metabolic gender difference. If exercise substrate metabolism parallels that of starvation it would be plausible to hypothesize that gender differences in exercise metabolism exist. Some evidence is available to suggest that hormonal alterations during exercise may potentially be responsible for these gender differences.

The purposes of this investigation were to examine the effect of gender on submaximal exercise metabolism and to determine whether possible metabolic differences may be related to gender differences in exercise hormonal response. The design of the study controlled for methodological problems inherent in previous studies.

# CHAPTER II

## GENDER DIFFERENCES IN METABOLIC RESPONSES TO ENDURANCE EXERCISE

### 2.1 INTRODUCTION

Studies investigating the effect of gender on exercise substrate utilization have yielded conflicting results. While some investigators have reported a higher proportion of lipid oxidation in females vs males performing endurance exercise (Blatchford et al, 1985; Brown & White, 1984; Bunt et al, 1986; Froberg & Pedersen, 1984; Jansson, 1986; Nygaard, 1986), others have not (Costill et al, 1979; Graham et al, 1986; Powers et al., 1980). Possible factors contributing to the lack of agreement may be differences in subject training status and diet, or hormonal status of female subjects related to menstrual phase, since, all are known to affect substrate metabolism during submaximal exercise (Bergström et al, 1967; Bonen et al, 1983; Gollnick, 1985; Holloszy & Coyle, 1984; Lavoie et al, 1986).

These potential confounding variables were controlled in the present study by: 1) matching subjects for training status and performance experience; 2) providing eucaloric identical diets for 3 days and; 3) testing the females during the mid-follicular phase of their menstrual cycle.

The purposes of the study were to assess the effect of gender on substrate metabolism during prolonged submaximal exercise and to determine whether possible differences in substrate utilization could be explained by gender differences in the hormonal response to the exercise bout.

## METHODOLOGY

2.2 Subjects. Six male and 6 female endurance trained volunteers were advised of the risks associated with the study and signed written consent forms approved by the University Ethics Committee. In order to select these subjects, a total of 28 females and 19 males were pre-screened to match 2 groups for training status, as evaluated by 6-week training logs and exercise histories. Training status was considered equal if 1) 6-week training logs revealed no statistically significant differences between or within groups with regard to: running distance per week; and running duration per workout; and 2) exercise histories revealed no statistically significant differences between or within groups with regard to: length of participation in endurance running; level of competition; or frequency of competition. The training characteristics of the subjects are shown in Table I.

All female subjects were eumenorrheic with a normal cycle length of 28 to 34 days and were not taking oral

**TABLE I.** SUBJECT'S TRAINING CHARACTERISTICS

| | Male | Female |
|---|---|---|
| Training Distance ($km.wk^{-1}$) | 36.7 ± 1.0 | 37.5 ± 0.8 |
| Training Duration ($min.workout^{-1}$) | 37.5 ± 0.9 | 39.1 ± 1.0 |
| Length of Training (years) | 7.3 ± 0.7 | 8.2 ± 0.5 |
| Most Frequent Racing Event | 10 km run | 10 km run |
| Frequency of Competition (no./year) | 2-4 | 1-4 |

Values for Training Distance, Training Duration, and Length of Training are means ± SE.

contraceptives. No subject was suffering from any apparent eating disorders and no subject was attempting to lose weight during the study period.

2.3 Protocol. Maximal oxygen uptake was determined 2 weeks prior to the metabolic testing session according to the protocol described by Thoden et al. (1982) for treadmill running.

Respiratory measurements were evaluated on an open system. A Zenith IBM-compatible computer was interfaced with: a Validyne pneumotach that measured the volume of expired air; a Rapox oxygen analyser; a Hewlett-Packard carbon dioxide analyser and, a custom heart rate monitor. This system also included a 6 liter gas mixing chamber. Body composition was estimated (Siri, 1956) from measurements of body density determined by hydrostatic weighing (Buskirk, 1961) with residual volumes being measured by the helium dilution method.

For 2 days prior to, and on the test day, subjects ingested a weight-maintaining caffeine free diet (3475 $\pm$ 214 kcal for males and 2308 $\pm$ 178 kcals for females) consisting of 55% carbohydrate, 30% fat, and 15% protein. To ensure accuracy, food was individually packaged and given to each subject. The composition and energy content of the diets were determined from 7 day food records using a computer program for nutrient analysis (ANALYSE, developed at McMaster

University).

On the morning of the test day, subjects arrived at 9 a.m. in a rested state, 11 h post - absorptive. Muscle biopsy samples were taken from the vastus lateralis before and after (alternate legs) a 15.5 km treadmill run (range in performance times, 90 to 101 min) using the Bergström technique (Bergström, 1962). The duration for the treadmill run (minimum of 90 min) was chosen to elicit significant glycogen depletion. Treadmill velocity was set to require approximately 65% of subjects' $\dot{V}O_2$ max. This velocity was pre determined on a 30 min trial run which took place one week prior to the testing session. An indwelling venous catheter with an isotonic saline infusion was used to sample blood pre-exercise (T-0), every 30 min during exercise (T-30, T-60, T-90) and 15 min post-exercise (P-15).

Blood samples were centrifuged within 5 minutes of collection and plasma aliquots were stored at -20°C for future determination of: free fatty acids-FFA (Noma et al, 1973), urea nitrogen (Sigma Diagnostics, W. Germany; Procedure 640), glycerol (Wieland, 1985) epinephrine (Passom & Peuler, 1973), norepinephrine (Passon & Peuler, 1973), insulin (Amersham Corp., Canada), glucagon (Radioassay Systems Laboratories Inc, California), and growth hormone (Schalch & Parker, 1964). Plasma glucose (Beckman Instruments, Toronto) and lactate (Yellow Springs Instrument model 23 L) concentrations were determined immediately after

centrifugation. Respiratory exchange ratios and $\dot{V}O_2$ measurements were determined every 15 min during exercise from 3 min expired gas collections. During the 3 min expired gas collections, RER and $\dot{V}O_2$ data were computer calculated every 15 sec. The average of the final three readings for each 3 min sample was used to determine the data for that time point. The energy, fat, and carbohydrate utilization was calculated from RER (assuming that the RER reflected the non protein RQ) and $\dot{V}O_2$ measurements (Zuntz, 1901).

Twenty-four hour rest and exercise urine samples were collected into sterilized 4 liter jugs treated with 5 ml of glacial acetic acid and assayed for urea N content, correcting for preformed ammonia. Resting urine collections began at 7:00 a.m. the day before the testing session, while exercise urine collections began at 7:00 a.m. on the morning of the testing session. Biopsy samples (ranging from 30 to 90 mg each) were frozen in liquid nitrogen within 45s of excision and after freeze drying, were analyzed for glycogen using the amyloglucosidase flourometric technique (Bergmeyer, 1985). Concentrations were expressed as mmoles of glucose per kg dry weight of tissue sample. For all assays, samples and standards were run in duplicate. All plasma measurements were corrected for changes in plasma volume as calculated from hematocrit values (Van Beaumont, 1984).

2.4 Statistical Procedures. A two way analysis of variance (between and within split-plot design) was used to determine whether significant differences existed between the factors gender and time and for the gender-time interaction. A Tukey A post hoc test was used to identify significantly different means. A probability $P < 0.05$ was taken to indicate significance. Values are given as means $\pm$ standard errors (SE).

## RESULTS

2.5 Descriptive Data (Table II). The two groups did not differ as to: relative exercise intensity, treadmill velocity, or time for completion of the 15.5 km treadmill run. Males were taller, heavier, and leaner than their female counterparts. $\dot{V}O_2$ max was significantly lower in the female group when normalized for total body weight (BW), however, no significant gender difference was found when $VO_2$ max was expressed per kg lean body weight (LBW).

2.6 FFA (Figure 1a) and Glycerol (Figure 1b). There were no significant main effects for gender or time with respect to FFA response during exercise. Post-exercise (P-15) FFA concentrations were significantly higher than T-0 values for both men and women. The magnitude of this increase (at P-15) was significantly greater for the males than for the females. For plasma glycerol concentration there was no main effect for gender. Plasma glycerol increased progressively and

**TABLE II.** SUBJECTS' DESCRIPTIVE DATA

| | Male N = 6 | | Female N = 6 |
|---|---|---|---|
| Age (yr) | 20.0 ± 0.6 | | 21.5 ± 0.8 |
| Height (cm) | 175.3 ± 1.5 | ** | 167.8 ± 1.5 |
| Body Weight (kg) | 66.9 ± 2.1 | ** | 58.4 ± 2.2 |
| Lean Body Weight (kg) | 57.2 ± 1.8 | ** | 45.8 ± 1.7 |
| Percent Body Fat | 14.5 ± 0.9 | ** | 21.6 ± 1.1 |
| $\dot{V}O_2$ max ($l \cdot min^{-1}$) | 4.28 ± 0.14 | ** | 3.42 ± 0.14 |
| $\dot{V}O_2$ max ($ml \cdot kg^{-1} \cdot min^{-1}$-BW) | 63.5 ± 1.1 | * | 57.8 ± 1.8 |
| $\dot{V}O_2$ max ($ml \cdot kg^{-1} \cdot min^{-1}$-LBW) | 74.9 ± 0.9 | | 74.7 ± 1.7 |
| Running Intensity (% of $\dot{V}O_2$ max) | 62.0 ± 0.9 | | 63.7 ± 0.4 |
| Treadmill Speed ($km.hr^{-1}$) | 10.3 ± 0.1 | | 9.8 ± 0.1 |
| Treadmill Duration (min) | 93.1 ± 1.1 | | 97.9 ± 1.3 |
| $\dot{V}O_2$ during exercise ($ml \cdot kg^{-1} \cdot min^{-1}$) | 39.4 ± 0.6 | | 36.8 ± 0.5 |

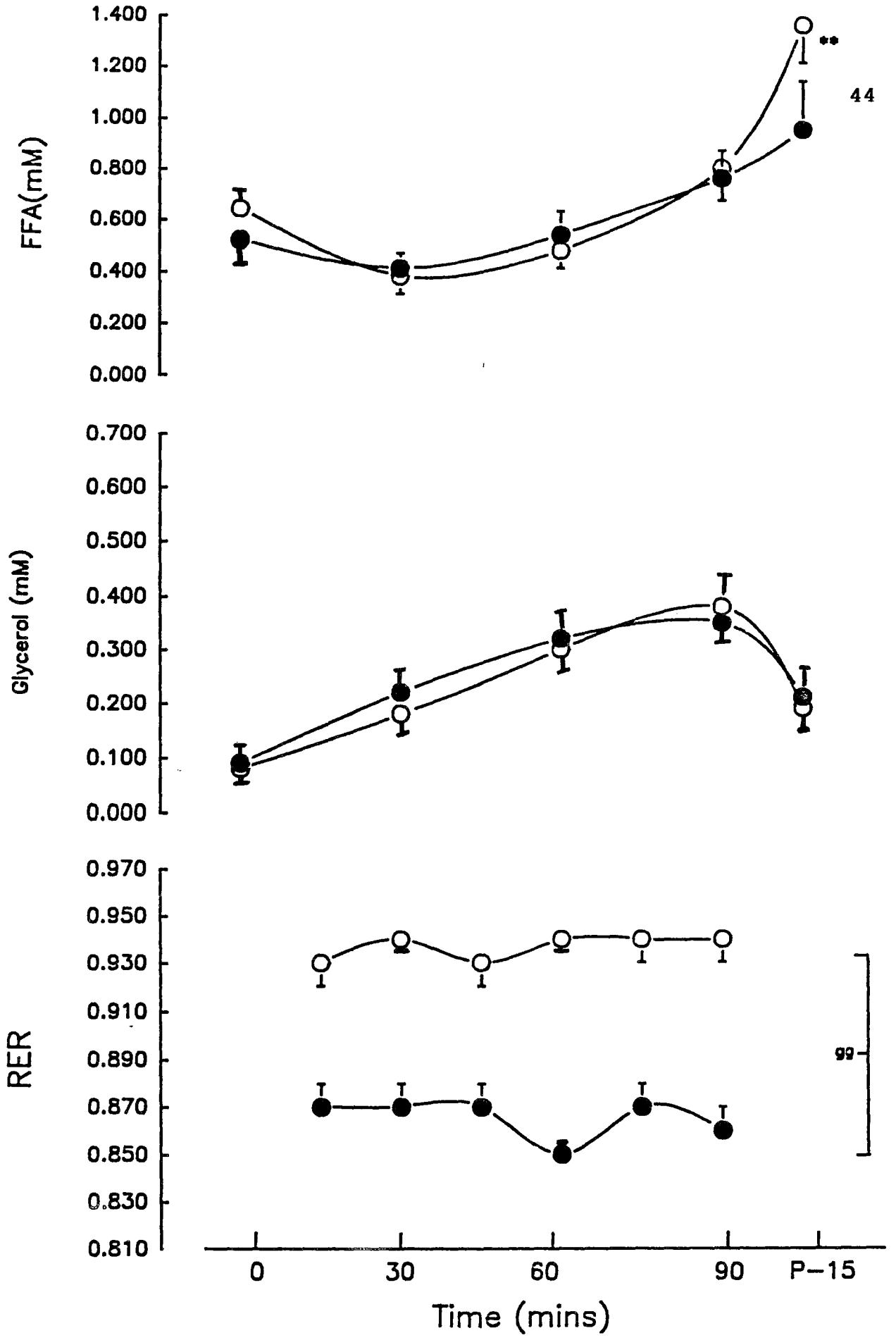
Values are means ± SE. $P<0.05$ *, $P<0.01$ **.

**TABLE III.** CALCULATED FUEL CONSUMPTION

| | Male | | Female |
|---|---|---|---|
| Energy expenditure (kcal) | 1236 ± 53 | ** | 1012 ± 44 |
| Energy expenditure (kcal/kg - BW) | 18.5 ± 0.8 | | 17.3 ± 0.7 |
| Energy expenditure (kcal/kg - LBW) | 21.6 ± 0.9 | | 22.1 ± 0.9 |
| Lipid utilization (g) | 26.9 ± 1.2 | ** | 47.6 ± 2.1 |
| Carbohydrate utilization (g) | 239.7 ± 10.3 | ** | 137.3 ± 6.0 |

FIGURE 1. Plasma concentration of a) free fatty acid (FFA) and b) glycerol for males (O—O) and females (●—●); at rest (0), during 90-min treadmill run (30, 60, 90) and, 15-min post-exercise (P-15); c) RER measurements taken at 15-min intervals during 90-min run. Values are means ± SE.

**: represents a significant ($P<0.01$) gender-time interaction.
gg: represents a significant ($P<0.01$) main effect for gender.


FFA(mM)
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
**
44
Glycerol (mM)
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
RER
0.970
0.950
0.930
0.910
0.890
0.870
0.850
0.830
0.810
99
0
30
60
90
P–15
Time (mins)

significantly during exercise with highest values occurring at T-90 for both groups. P-15 glycerol concentrations although lower than T-90 values were still significantly higher than T-0 concentrations.

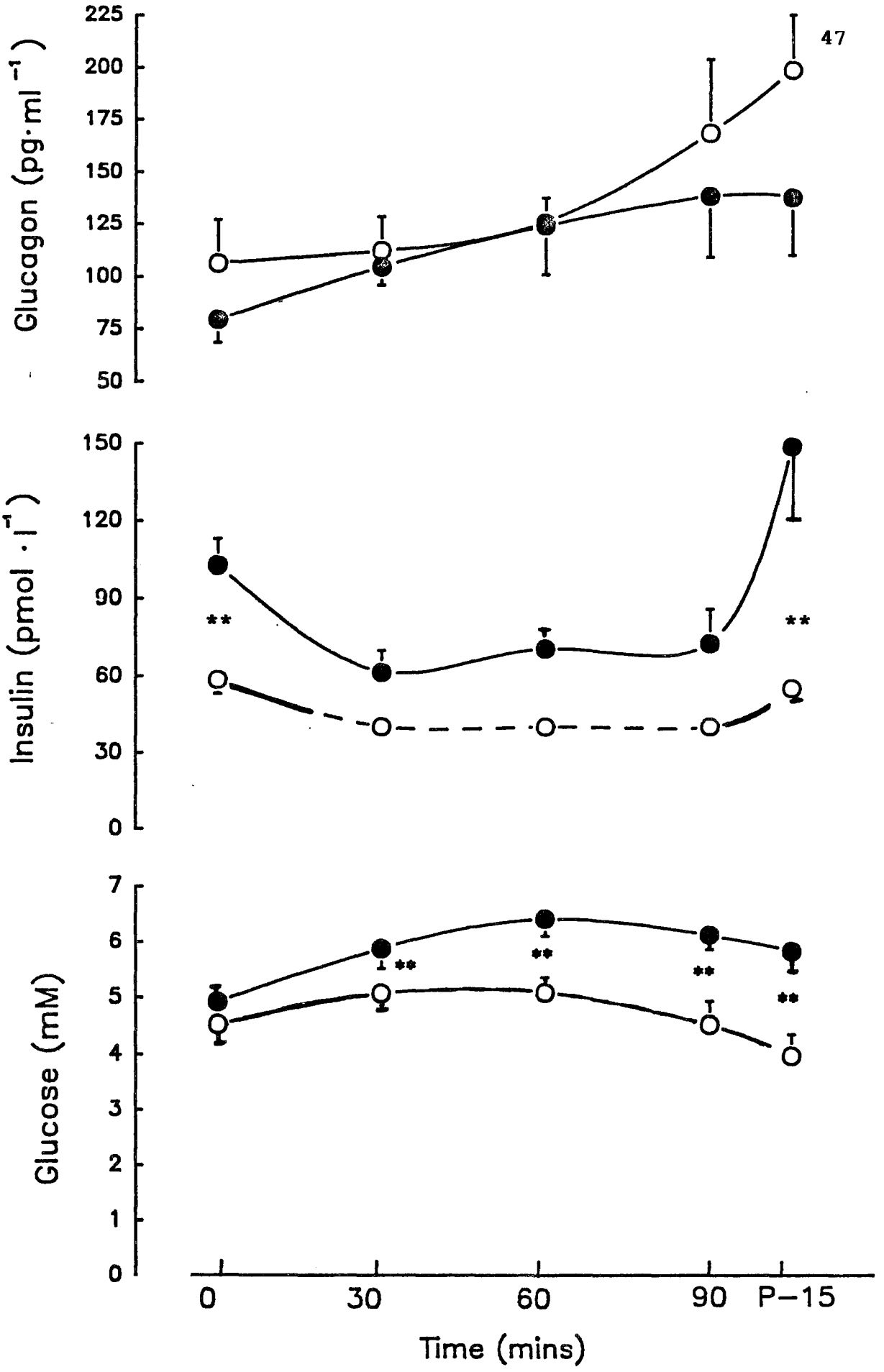
2.7 RER (Figure 1c) and calculated fuel consumption. (Table III) For both groups, RER showed little change with exercise duration. However, there was a main effect for gender. Females maintained a significantly lower RER throughout the exercise than males. Although in absolute terms males utilized significantly more calories than females during the exercise bout, caloric expenditure when expressed per kg BW or kg LBW, did not differ between the groups. Based on RER values, females were estimated to have utilized a significantly greater amount of fat and a significantly lesser amount of carbohydrate than males during the exercise bout.

2.8 Glucagon (Figure 2a), Insulin (Figure 2b), Glucose (Figure 2c) and lactate. With regard to glucagon response, no main effect for gender was found. During exercise no significant increase in glucagon concentration was observed for either sex. Males but not females had a significantly greater plasma glucagon concentration post-exercise when compared to pre-exercise concentrations.

With regard to insulin response, there was a main effect for gender and for time, and a significant gender-time

FIGURE 2. Serum concentration of a) glucagon and plasma concentration of b) insulin and c) glucose for males (O—O) and females (●—●) at rest (0) during 90-min treadmill run (30, 60, 90) and, 15-min post-exercise (P-15). Values are means $\pm$ SE.

* : represents a significant ($P \leq 0.05$) gender-time interaction.
**: represents a significant ($P \leq 0.01$) gender-time interaction.
--: represents an insulin concentration less than 40 $pmol.l^{-1}$ (limitation of the assay).


Glucagon (pg·ml⁻¹)
225
200
175
150
125
100
75
50
Insulin (pmol·l⁻¹)
150
120
90
60
30
0
**
**
Glucose (mM)
7
6
5
4
3
2
1
0
**
**
**
**
0
30
60
90
P–15
Time (mins)

interaction. Plasma insulin concentration (for females when compared to males) was significantly greater at all sampling periods, but this gender difference was only statistically significant at T-0, T-90 and P-15. During exercise plasma insulin concentration fell below resting values for both sexes. For the females, but not the males, P-15 plasma insulin concentration was significantly higher than resting values.

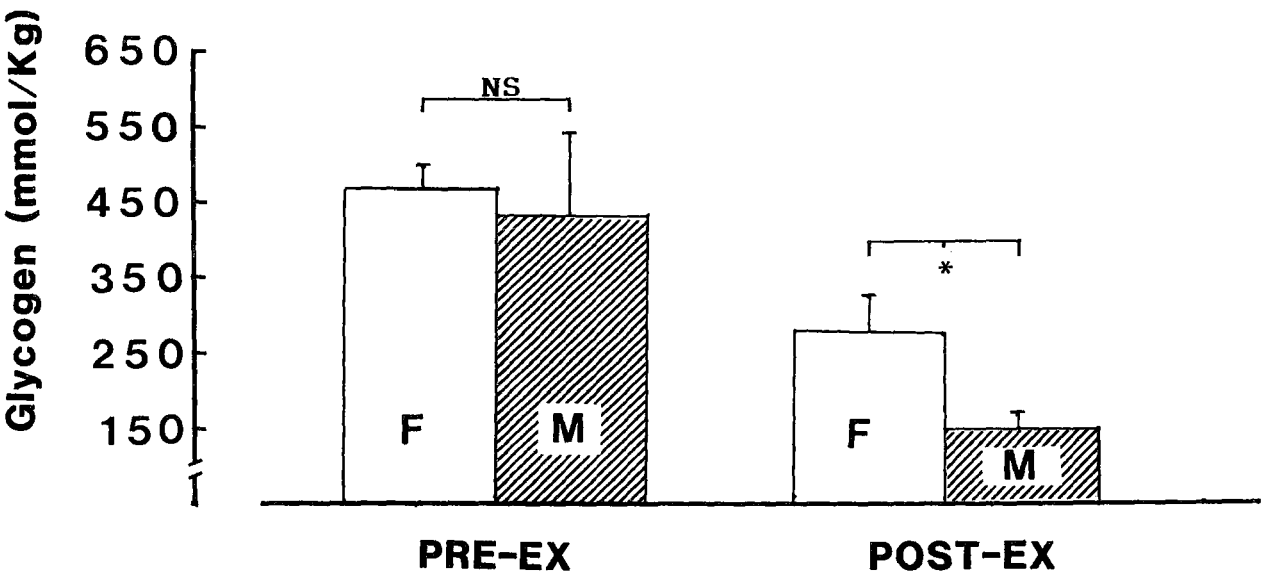
For glucose response there was a main effect for gender, and for time, and a significant gender-time interaction. Females maintained a significantly greater plasma glucose concentration during exercise and P-15, when compared to males. For the females, a significant increase in glucose concentration (above resting values) was found across time, whereas for the males, no significant changes in glucose occurred.

Lactate concentrations did not significantly increase with exercise and, peak values obtained at T-90 did not demonstrate any sex differences (2.85 $\pm$ .20 mM males, 2.57 $\pm$.44 mM females).

2.9 Muscle glycogen (Figure 3). Initial muscle glycogen concentrations revealed no significant gender differences. Post-exercise values demonstrated a significant 25% greater glycogen depletion for the males compared to the females.

FIGURE 3. Muscle glycogen concentration for males (M) and females (F) before (PRE-EX) and following (POST-EX) 15.5 km treadmill run. Values are mean $\pm$ SE.

*: represents a significant ($P \leq 0.05$) gender-time interaction.


Glycogen (mmol/Kg)
650
550
450
350
250
150
NS
*
F
M
F
M
PRE-EX
POST-EX

2.10 Urine urea nitrogen (Figure 4). When expressed absolutely ($g.24h^{-1}$), males had a greater rest day urinary urea nitrogen (N) excretion than females. When expressed relative to body weight ($g.kg^{-1}.24h^{-1}$) however, no significant differences in resting urine urea N excretion was observed across gender. Compared to the resting condition males had a significantly greater (by 32%) urea N excretion during exercise than females. There was no significant effect of exercise on urea N excretion for females.

2.11 Plasma urea nitrogen (Figure 5). Plasma urea nitrogen (N) at all sampling points was significantly higher for the males. During exercise no significant increases in plasma urea N values were observed for either sex.

2.12 Norepinephrine, Epinephrine and Growth Hormone Response (Figure 6)

For norepinephrine response there was no main effect for gender and there was no significant gender-time interaction. However, there was a main effect for time. For both groups, a significant norepinephrine increase above resting values at all exercise time points was observed. P-15 values for both genders were not significantly different from T-0 concentrations.

For epinephrine response there was a main effect for time and a significant gender-time interaction. For the males plasma epinephrine concentration increased

FIGURE 4. Urinary Urea N excretion a) absolute ($g.24h^{-1}$) and b) relative to BW ($g.kg^{-1}.24h^{-1}$) before (REST) and after (EXERCISE) 15.5 km treadmill run. Values are means $\pm$ SE.

* : represents a significant ($P<0.05$ gender-time interaction.

tt: represents a significant ($P<0.01$) main effect for time.

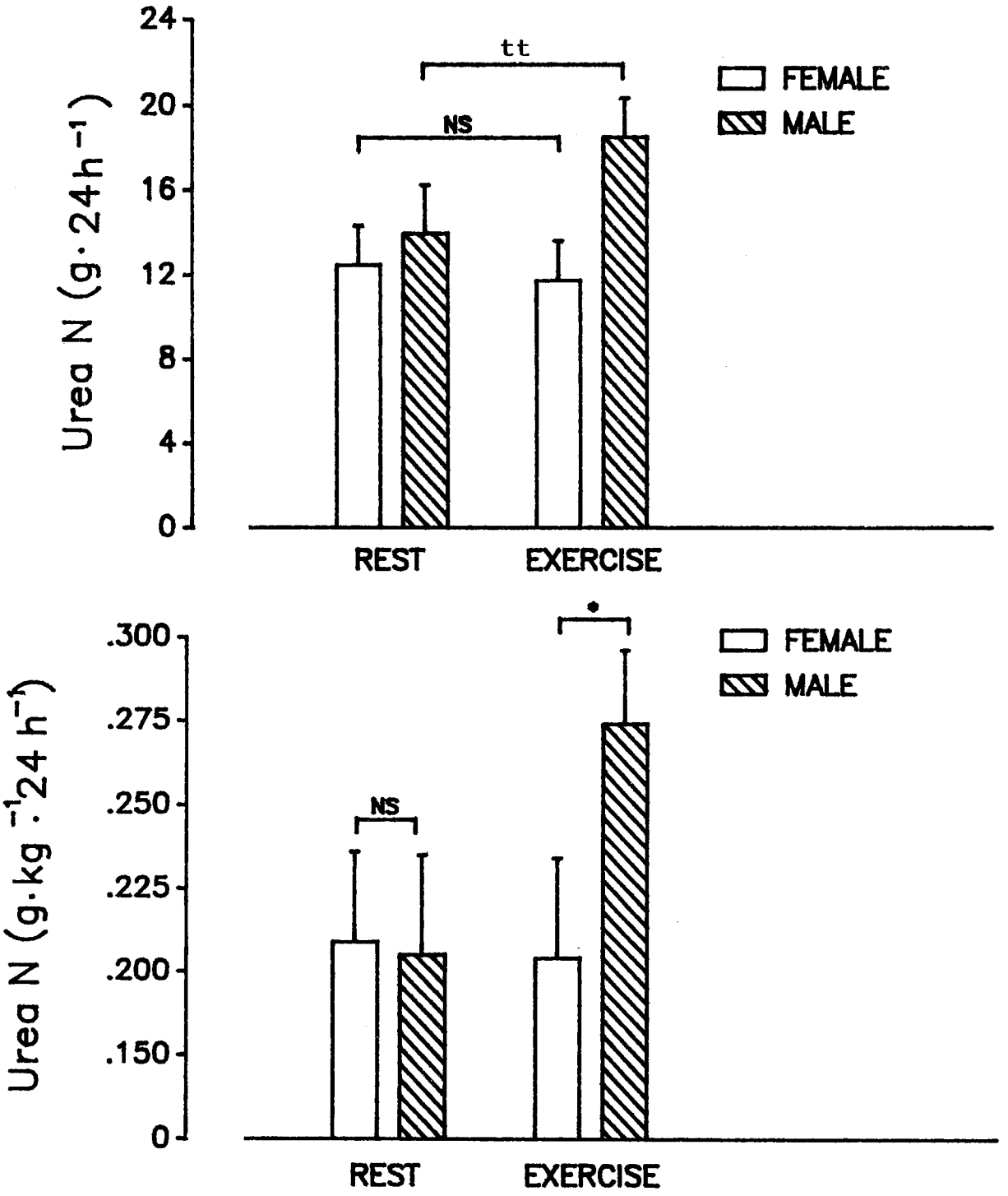

24
20
16
12
8
4
0
Urea N (g·24h⁻¹)
tt
NS
FEMALE
MALE
REST
EXERCISE
.300
.275
.250
.225
.200
.150
0
Urea N (g·kg⁻¹·24 h⁻¹)
*
NS
FEMALE
MALE
REST
EXERCISE

FIGURE 5. Plasma concentration of urea N for males (O—O) and females (●—●), at rest (0), during 90-min treadmill run (30, 60, 90) and 15-min post exercise (P-15). Values are means ± SE.

gg: represents a significant ($P<0.01$) main effect for gender.

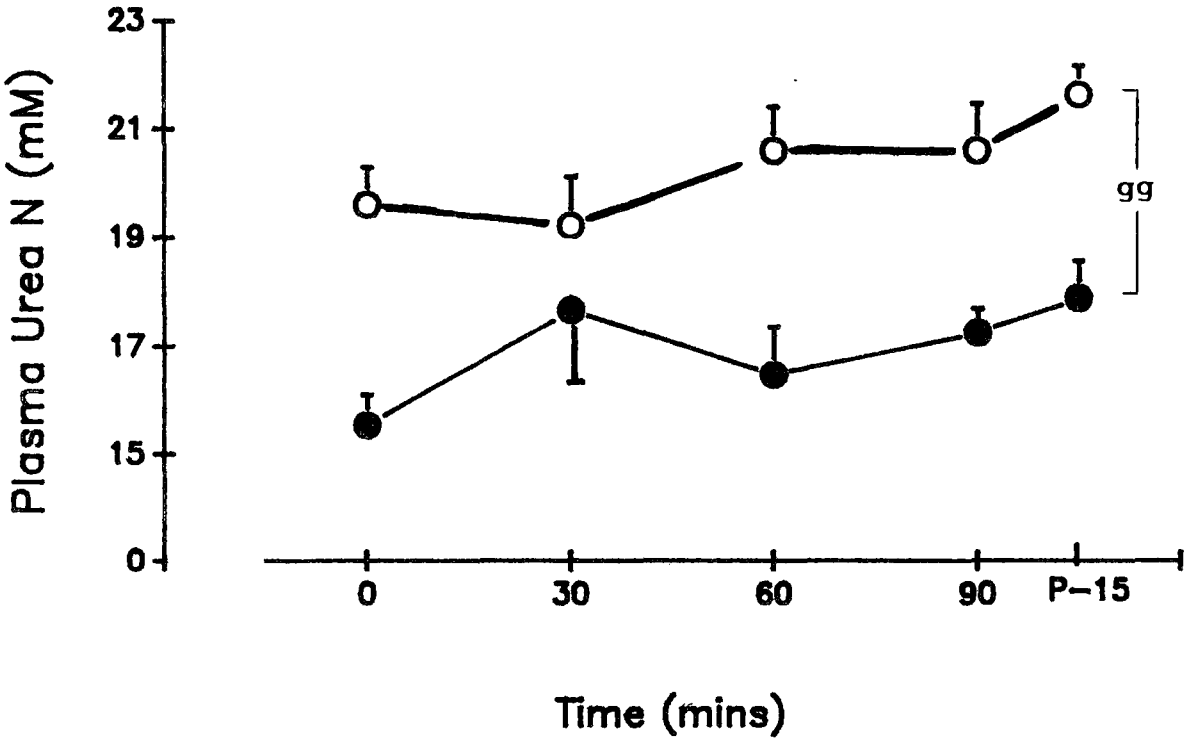
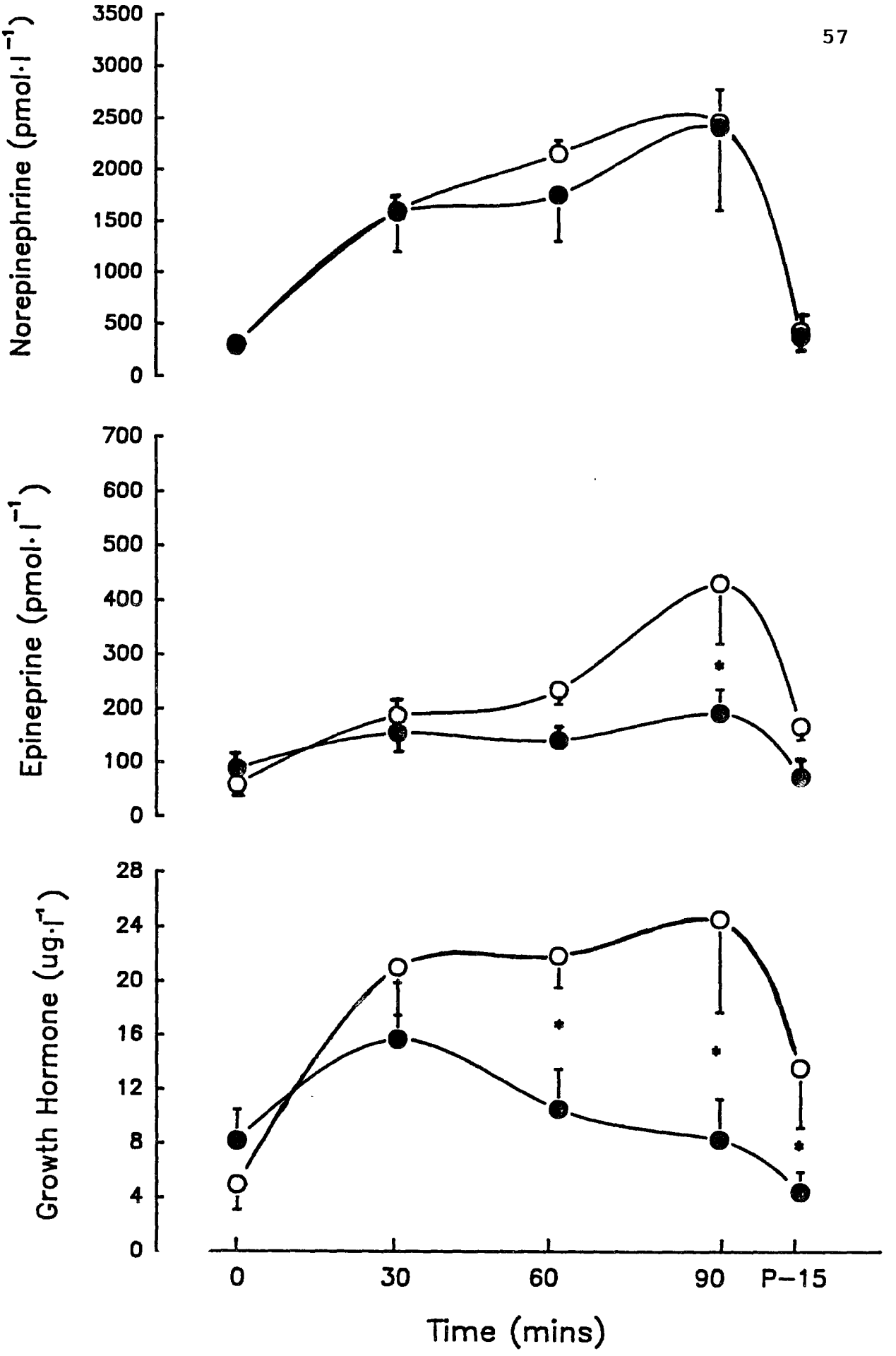

Plasma Urea N (mM)
23
21
19
17
15
0
0
30
60
90
P–15
Time (mins)
gg

FIGURE 6. Plasma concentration of a) NOREPINEPHRINE, b) EPINEPHRINE and c) GROWTH HORMONE for males (O—O) and females (●—●) at rest (0), during 90 min treadmill run (30, 60, 90), and 15-min post-exercise (P-15). Values are means ± SE.

*: represents a significant ($p<0.05$) gender-time interaction.


Norepinephrine (pmol·l⁻¹)
3500
3000
2500
2000
1500
1000
500
0
Epineprine (pmol·l⁻¹)
700
600
500
400
300
200
100
0
Growth Hormone (ug·l⁻¹)
28
24
20
16
12
8
4
0
0
30
60
90
P–15
Time (mins)

significantly above resting values at T-60 and T-90. No significant change from T-0 epinephrine concentrations were found for the females. In general, males had a higher mean epinephrine concentration at all exercise time points and P-15. However, this gender difference was only statistically significant at T-90.

For growth hormone response there was a main effect for time and a signficant gender-time interaction. No significant increase in growth hormone concentration was observed for the females across time. Conversely, growth hormone concentration for males increased significantly above T-0 at all exercise time points, but not P-15. Males had a significantly greater growth hormone concentration than the females at T-60, T-90 and P-15.

## DISCUSSION

Selecting equally trained subjects of the opposite sex is a perplexing methodological problem for studies investigating metabolic differences across gender (Cureton, 1981). Matching on the basis of normalized $\dot{V}O_2$ max expressions does not ensure training equality since, in general, females may not be as well trained as males (Cureton, 1981). Past training and performance histories have been found to best predict training equality between the sexes (Sparling, 1980).

In this study, training logs and performance

histories revealed no significant gender differences in level of training (Table I). Based on this information, the groups were considered equally trained. It was interesting to find that subjects with similar training histories and performance experience had similar $\dot{V}O_2$ max values when expressed per kg LBW.

The significant findings of this study were that males engaged in prolonged submaximal exercise, at a controlled intensity, maintained a higher exercise RER, showed a greater muscle glycogen utilization, and excreted more urinary urea N than equally trained and nourished females. No gender difference was found in exercise plasma FFA or glycerol response.

An obvious question is why the lower RER values observed for the females (reflecting a greater lipid oxidation) was not supported by an increased plasma FFA concentration (which might be reflective of an increased rate of lipid oxidation (Hagenfeldt, 1975; Paul, 1970)). A greater FFA uptake by muscle for females could explain this discrepancy; however, since plasma glycerol concentration is an indicator of adipose tissue lipolysis (Hetenyi et al, 1983), the lack of a greater increase in plasma glycerol concentration for the females would argue against this reasoning. Possible drift outside a steady state zone cannot explain this discrepancy either, since exercise lactate and $\dot{V}CO_2$ did not change over time.

What then could explain the lack of a sex difference in FFA and glycerol response to exercise when the RER indicates a metabolic gender difference? Researchers investigating the effect that endurance training has on substrate metabolism have been perplexed with the finding that, with training, the lowering of the RER was not associated with a concommittant increase in FFA mobilization (Hurley et al, 1986; Koivisto et al, 1982; Winder et al, 1979). A lower exercise RER for trained subjects during submaximal exercise was not associated with increased plasma FFA or glycerol concentrations between trained and untrained males (Hurley et al, 1986). These investigators did, however, find a greater intramuscular triglyceride (IMTG) concentration at rest and a greater IMTG depletion following exercise in the trained males. The quantity of fatty acid released by lipolysis of IMTG could, if oxidized, account for the greater amount of fat oxidized (as indicated by lower RER) in the trained state. The glycerol released by IMTG oxidation may have been oxidized by the active muscle and therefore the change in plasma glycerol concentration would only reflect adipose lipolysis and not IMTG catabolism. It has been shown that women have a greater resting concentration of IMTG than men (Prince et al, 1976). If a greater IMTG storage leads to a greater IMTG utilization during exercise but without a significant increase in plasma

glycerol concentrations (Hurley et al, 1986), then it can be postulated that the greater lipid utilization found for females was the result of a greater IMTG utilization.

No gender difference in resting muscle glycogen concentrations have been reported (Costill et al, 1979; Jansson, 1986; Nygaard, 1978; Nygaard, 1986). The results of this study support these findings (Figure 3a). Whether there is a gender difference in glycogen depletion during exercise remains controversial. Nygaard (1986) reports a greater glycogen depletion in males than females exercising at 50%, 60%, and 75% $\dot{V}O_2$ max for 60 min (20 min at each intensity). Our findings of a 25% greater glycogen utilization in males relative to females lends support to the gender difference hypothesis. In contrast Jansson (1986) found no gender difference during 25 min of exercise at 65% $\dot{V}O_2$ max. Perhaps gender differences in muscle glycogen utilization become evident only following a more prolonged exercise bout when glycogen depletion becomes a limiting factor in endurance exercise.

In the present study females were better able than males to maintain exercise plasma glucose levels. Plasma glucose concentrations during exercise have been reported to be either higher in females than males (Costill et al, 1979; Lavoie et al, 1986) or to exhibit no gender difference (Jansson, 1986; Lavoie et al, 1986). Lavoie and coworkers

(1986) found that females maintained a higher exercise glucose concentration than males during the mid-follicular menstrual phase while no gender difference was observed when females were in the mid-luteal phase of their menstrual cycle. The reason suggested for this metabolic difference between menstrual phase and gender is that the high level of estrogen and progesterone present during the luteal phase suppresses gluconeogenesis and concommittantly lowers plasma glucose levels (Lavoie et al, 1986). The androgen hormones are suggested to have the same metabolic effect on males as high estrogen and progesterone levels have on females (Lavoie et al, 1986). Those who have found no gender difference in exercise glucose response have employed exercise protocols of relatively short duration (25 min.) and have disregarded the hormonal fluctuations associated with the menstrual cycle (Jansson, 1986).

Our findings of an elevated 24 h urinary urea N excretion in males following exercise supports the findings of a greater protein catabolism in males than females during comparable exercise loads (Lemon & Nagel, 1981). The lack of change from resting values in urine urea N excretion for the women would indicate that protein catabolism was not enhanced as a result of the exercise stress. To our knowledge no gender comparison of exercise protein catabolism has been conducted in humans. It has been found that male rats have an increased urinary urea N excretion following exhaustive

exercise while the females do not (Dohm et al, 1978). Furthermore both animal (McCance & Widdowson, 1962; Triscari et al, 1980; Widdowson & McCance, 1956; ) and human (Broom et al, 1978; Runcie & Hilditch, 1974) studies investigating gender differences in substrate utilization during nutritional deprivation and starvation have concluded that males catabolize proportionally more protein and less fat than females.

It cannot be ascertained from urine urea N data whether protein catabolism occurred during exercise or during recovery (Lemon & Nagel, 1981; Viru, 1987). Increases in plasma urea nitrogen (N) have been shown to indicate an enhanced protein catabolism during exercise (Lemon & Mullin, 1980). To this end, our plasma urea N data would argue against an enhanced exercise protein catabolism since no significant gender differences were observed with time. However, the males lost significantly more weight as a result of perspiration (1.0 kg vs 0.6 kg). Since sweat loss is a major vehicle for urea N excretion it is very likely that the males lost a greater amount of urea N than the females. This unequal urea N loss via sweating would disproportionally lower the males urea N concentration when compared to the females. Plasma urea N measures would appear not to be representative of exercise protein catabolism across gender.

The hormonal response to exercise was included to

elucidate the mechanism for potential gender differences in exercise metabolic response. Women have been reported to have greater resting growth hormone concentration (Bunt et al, 1986; Frantz & Rubkin, 1965) and exhibit a greater exercise response when compared to men (Bunt et al, 1985; Frantz and Rubkin, 1965; Shephard & Sidney, 1975). Growth hormone's known lipolytic action would therefore support an enhanced lipolysis in females when compared to males. Our data show greater resting growth hormone values in females, however the males had a significantly greater increase in growth hormone concentration with exercise. In the present study these findings argue against the importance of growth hormone as a prime mediator of FFA metabolism during exercise.

It is possible that growth hormone acts in conjunction with glucocorticoids and other hormones to mediate FFA mobilization (Kostyo & Reagan, 1976) and that gender differences in the interaction of growth hormone with other hormones may have lead to the differences in lipid metabolism. Furthermore, the females higher resting growth hormone concentration may have "primed" their body for a greater lipid metabolism at exercise onset than males, and the greater carbohydrate utilization found for the males may have lead to the concommitant decline in plasma glucose concentration (Fig. 2). Since growth hormone secretion increases in response to hypoglycemia (Sutton & Lazarus,

1976), the males higher exercise growth hormone concentration may have resulted from the greater stress that the exercise bout placed on the males' carbohydrate stores.

Researchers have found a greater catecholamine response to exercise in men when compared to women (Graham, 1986; Nygaard, 1986; Sanchez et al, 1980). We did not find any significant gender differences in norepinephrine concentration in response to exercise. Epinephrine concentration did tend to be greater in the males at T-60, T-90 and P-15, however this gender difference was only significant at T-90. Since epinephrine enhances muscle and liver glycogenolysis, the higher epinephrine concentration found for the men during exercise could in part be responsible for the gender differences found in glycogen utilization.

Insulin concentration was significantly lower in the male group. Plasma insulin concentration falls in response to declining glucose levels. Thus, the significantly lower blood glucose concentration seen in the males would explain the lower insulin levels found for this group. Metabolically, falling insulin concentrations enhance hepatic glucose production, increases the rate of lipid catabolism and/or restrains glucose utilization by inactive tissue (Sutton, 1984). The lower insulin concentrations found for the males would indicate that the males had a greater stress

on carbohydrate stores than the females. This supports the higher RER and lower post exercise muscle glycogen values found for males.

It was surprising to find that glucagon concentration during exercise was not significantly different between the groups. Since estadiol ($E_2$) has a suppresive effect on the alpha cells of the pancreas (Mandour et al, 1977) one would hypothesize that females, having a greater resting and exercise $E_2$ concentration, would have an inhibited increase in glucagon concentration. Plasma glucagon levels provided no insight into the lesser carbohydrate utilization found for females.

The hormonal measurements in this study were not useful in explaining the observed gender difference in lipid metabolism. The lower insulin levels and higher epinephrine levels could in part explain the greater glycogen depletion and protein catabolism observed in males, since these hormonal responses are known to stimulate glycogenolysis and gluconeogenesis (Martin, 1985).

As reflected by similar relative oxygen consumptions per kg BW, the subjects in this study ran at the same relative intensity during the 15.5 km treadmill run. This traditional method of equating power output may disadvantage the males who have a greater muscle mass than females. It has been shown that larger muscles have a higher ratio of energy utilization to force or power output than smaller

muscles (de Haan et al., 1988). Thus gender differences in muscle dimensions may in part explain the observed gender differences in substrate metabolism.

Although the hormonal measures could provide little insight into the mechanisms responsible for the gender differences found it is still possible that the hormones studied are responsible for the gender related differences in substrate utilization during exercise. Hormone levels measured by radioimmunoassay do not necessarily represent the hormones' biological activity (Root, 1973). Biological activity depends not only on circulating concentrations but also on receptor availability and sensitivity within the individual (Bunt, 1986). Females may be more sensitive to the lipolytic effects of a particular hormone while males may be more sensitive to the hormones glycolytic effects. In other words, epinephrine is a hormone that increases glycogenolysis and gluconeogenesis (Guyton, 1987). Epinephrine is also known to increase the activity of Type L hormone-sensitive lipase (L-HSL), an enzyme thought to be responsible for IMTG lipolysis (Oscai, 1983). If females had a greater concentration of, or were more sensitive to the epinephrine effects on Type L-HSL, similar increases in epinephrine would stimulate a greater degree of lipolysis in females compared to males.

## CONCLUSION

Females engaged in prolonged aerobic exercise of moderate intensity (~ 65% $\dot{V}O_2$ max) during the mid-follicular phase of the menstrual cycle derive a greater proportion of energy from fat and catabolize less carbohydrate and protein than equally trained and nourished males.

Changes in plasma growth hormone or glucagon concentrations could not explain the greater lipid utilization observed in females. The lower insulin and higher epinephrine levels seen in males might partially explain the greater glycogenolysis and protein catabolism observed in this group.

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# APPENDIX I

## RAW DATA FOR EACH SUBJECT

I-A Respiratory Exchange Ratio
I-B Free Fatty Acid
I-C Clycerol
I-D Glycogen
I-E Glucose
I-F Lactate
I-G Blood Urea Nitrogen
I-H Urine Urea Nitrogen
I-I Hematocrit Measurements

## SUBJECTS

Six male and 6 female subjects ran on a treadmill at ~65% $\dot{V}O_2$ max for a minimum of 90 minutes. Respiratory exchange measurements were obtained every 15 min during exercise. Free fatty acid, glycerol, glucose, lactate and plasma urea nitrogen were measured at rest (T-0), every 30 min during exercise (T-30, T-60, T-90) and 15 min post-exercise (P-15). Pre and post exercise muscle biopsy samples of the vastus lateralis were assayed for glycogen concentration. Urinary urea N excretion was determined from 24 hour (rest and exercise) urine collections.

## STATISTICS

A two way analysis of variance (between and within split-plot design) was used to determine whether significant differences existed between the factors gender and time and for the gender-time interaction. A Tukey A post hoc test was used to identify significantly different means.

**TUKEY CALCULATION**

The basic computational formula for the Tukey A test is:

$$\text{critical value} = qr \sqrt{\frac{\text{mean square of the within group error}}{n \text{ (per group)}}}$$

where, q = value obtained from Studentized Range Tables with degrees of freedom = r.

n = number of subjects in each group.

r = (number of comparisons, degrees of freedom error).

## APPENDIX I-A

RESPIRATORY EXCHANGE RATIO ($\dot{V}CO_2/\dot{V}O_2$)

| | | T-15 | T-30 | T-45 | T-60 | T-75 | T-90 |
|---|---|---|---|---|---|---|---|
| Females | AT | 0.91 | 0.91 | 0.91 | 0.86 | 0.89 | 0.89 |
| | AC | 0.86 | 0.85 | 0.85 | 0.85 | 0.84 | 0.84 |
| | AE | 0.85 | 0.85 | 0.84 | 0.84 | 0.86 | 0.84 |
| | AA | 0.85 | 0.85 | 0.84 | 0.84 | 0.83 | 0.83 |
| | OR | 0.87 | 0.87 | 0.89 | 0.87 | 0.87 | 0.86 |
| | AO | 0.88 | 0.87 | 0.87 | 0.85 | 0.86 | 0.84 |
| | $\bar{X}$ = | 0.87 | 0.87 | 0.87 | 0.85 | 0.87 | 0.86 |
| | SD= | 0.02 | 0.02 | 0.03 | 0.01 | 0.03 | 0.03 |
| | SE= | 0.01 | 0.02 | 0.02 | 0.00 | 0.01 | 0.01 |
| Males | IA | 0.93 | 0.92 | 0.89 | 0.92 | 0.92 | 0.90 |
| | AI | 0.93 | 0.94 | 0.96 | 0.94 | 0.97 | 0.95 |
| | TE | 0.93 | 0.94 | 0.93 | 0.95 | 0.93 | 0.95 |
| | TC | -- | -- | -- | -- | -- | -- |
| | RI | 0.90 | 0.94 | 0.91 | 0.93 | 0.93 | 0.93 |
| | AU | 0.96 | 0.94 | 0.95 | 0.94 | 0.95 | 0.96 |
| | $\bar{X}$ = | 0.93 | 0.94 | 0.93 | 0.94 | 0.94 | 0.94 |
| | SD= | 0.02 | 0.01 | 0.03 | 0.01 | 0.02 | 0.02 |
| | SE= | 0.01 | 0.00 | 0.01 | 0.00 | 0.01 | 0.01 |

| Source | SS | DF | MS | F | P |
|---|---|---|---|---|---|
| Gender | 0.07 | 1 | 0.07 | 138.68 | <0.001 |
| Time | 0.00 | 5 | 0.00 | 0.31 | |
| Gender Time | 0.00 | 5 | 0.00 | 0.62 | |
| Error | 0.02 | 48 | 0.00 | | |

# APPENDIX I-B

FREE FATTY ACID ($mmol.l^{-1}$)

| | | T-0 | T-30 | T-60 | T-90 | P-15 |
|---|---|---|---|---|---|---|
| Females | AT | 0.82 | 0.62 | 0.96 | 1.25 | 1.50 |
| | AC | 0.48 | 0.49 | 0.54 | 0.62 | 0.60 |
| | AE | 0.68 | 0.48 | 0.52 | 0.91 | 1.48 |
| | AA | 0.26 | 0.30 | 0.41 | 0.50 | 0.57 |
| | OR | 0.54 | 0.32 | 0.32 | 0.71 | 0.52 |
| | AO | 0.34 | 0.27 | 0.48 | 0.56 | 1.04 |
| | $\bar{X}$ = | 0.52 | 0.41 | 0.54 | 0.76 | 0.95 |
| | SD= | 0.21 | 0.14 | 0.22 | 0.28 | 0.46 |
| | SE= | 0.09 | 0.06 | 0.09 | 0.11 | 0.19 |
| Males | IA | 0.60 | 0.20 | 0.44 | 0.60 | 1.72 |
| | AI | 0.78 | 0.24 | 0.35 | 0.66 | 0.98 |
| | TE | 0.60 | 0.38 | 0.42 | 0.74 | 1.36 |
| | TC | 0.74 | 0.68 | 0.83 | 0.91 | 1.70 |
| | RI | 0.61 | 0.34 | 0.34 | 1.40 | 1.52 |
| | AU | 0.53 | 0.46 | 0.48 | 0.50 | 0.86 |
| | $\bar{X}$ = | 0.64 | 0.38 | 0.48 | 0.80 | 1.36 |
| | SD= | 0.10 | 0.17 | 0.18 | 0.32 | 0.36 |
| | SE= | 0.04 | 0.07 | 0.07 | 0.13 | 0.15 |

| Source | SS | DF | MS | F | P |
|---|---|---|---|---|---|
| Gender | .138 | 1 | .138 | .697 | |
| Error | 1.980 | 10 | .198 | | |
| Time | 4.242 | 4 | 1.061 | 27.205 | <.001 |
| Gender Time | .419 | 4 | .105 | 2.692 | .044 |
| Error | 1.565 | 40 | .039 | | |

| | Level of Significance (0.05) | (0.01) |
|---|---|---|
| Critical value | 0.23 | 0.31 |

# APPENDIX I-C

PLASMA GLYCEROL ($mmol.l^{-1}$)

| | | T-0 | T-30 | T-60 | T-90 | P-15 |
|---|---|---|---|---|---|---|
| Females | AT | 0.084 | 0.294 | 0.310 | 0.346 | 0.181 |
| | AC | 0.056 | 0.258 | 0.274 | 0.310 | 0.201 |
| | AE | 0.109 | 0.330 | 0.475 | 0.459 | 0.310 |
| | AA | 0.093 | 0.165 | 0.274 | 0.310 | 0.201 |
| | OR | 0.072 | 0.145 | 0.298 | 0.350 | 0.129 |
| | AO | 0.109 | 0.129 | 0.310 | 0.310 | 0.274 |
| | $\bar{X}$ = | 0.087 | 0.220 | 0.324 | 0.348 | 0.216 |
| | SD= | 0.021 | 0.085 | 0.076 | 0.058 | 0.065 |
| | SE= | 0.009 | 0.035 | 0.031 | 0.024 | 0.027 |
| Males | IA | 0.093 | 0.145 | 0.382 | 0.329 | 0.164 |
| | AI | 0.072 | 0.201 | 0.201 | 0.238 | 0.183 |
| | TE | 0.072 | 0.181 | 0.366 | 0.495 | 0.274 |
| | TC | 0.109 | 0.266 | 0.402 | 0.475 | 0.238 |
| | RI | 0.052 | 0.201 | 0.329 | 0.420 | 0.183 |
| | AU | 0.072 | 0.109 | 0.128 | 0.347 | 0.127 |
| | $\bar{X}$ = | 0.078 | 0.184 | 0.301 | 0.384 | 0.195 |
| | SD= | 0.020 | 0.054 | 0.111 | 0.098 | 0.048 |
| | SE= | 0.008 | 0.022 | 0.045 | 0.040 | 0.020 |

| Source | SS | DF | MS | F | P |
|---|---|---|---|---|---|
| Gender | .001 | 1 | .001 | .071 | |
| Error | .135 | 10 | .014 | | |
| Time | .570 | 4 | .142 | 47.333 | <.001 |
| Gender Time | .009 | 4 | .002 | .667 | |
| Error | .106 | 40 | .003 | | |

## APPENDIX I-D

MUSCLE GLYCOGEN (mmol.kg$^{-1}$ dry tissue)

| | | Pre-Exercise | Post-Exercise |
|---|---|---|---|
| Females | AT | 476.89 | 233.23 |
| | AC | 402.24 | 251.43 |
| | AE | 491.84 | 274.54 |
| | AA | 588.65 | 282.34 |
| | OR | 406.48 | 302.74 |
| | AO | 443.25 | 350.58 |
| | $\bar{X}$ = | 468.23 | 282.48 |
| | SD= | 69.17 | 41.23 |
| | SE= | 28.24 | 16.83 |
| Males | IA | 448.00 | 126.18 |
| | AI | 404.71 | 238.80 |
| | TE | 432.89 | 168.30 |
| | TC | 297.80 | 123.90 |
| | RI | 625.53 | 138.22 |
| | AU | 395.08 | |
| | $\bar{X}$ = | 424.00 | 153.17 |
| | SD= | 107.52 | 45.26 |
| | SE= | 43.87 | 18.48 |

| Source | SS | DF | MS | F | P |
|---|---|---|---|---|---|
| Gender | 333.760 | 1 | 333.760 | 1.094 | .321 |
| Error | 3050.746 | 10 | 305.075 | | |
| Time | 15945.446 | 1 | 15945.446 | 322.143 | <.001 |
| Gender Time | 776.572 | 1 | 776.572 | 15.689 | .002 |
| Error | 494.977 | 10 | 49.498 | | |

| | Level of Significance (0.05) | (0.01) |
|---|---|---|
| critical value | 93.1 | 132.4 |

## APPENDIX I-E

GLUCOSE ($mmol.l^{-1}$)

| | | T-0 | T-30 | T-60 | T-90 | P-15 |
|---|---|---|---|---|---|---|
| Females | AT | 4.7 | 5.3 | 5.8 | 5.4 | 5.7 |
| | AC | 5.4 | 6.6 | 7.2 | 7.0 | 6.5 |
| | AE | 4.6 | 7.2 | 7.4 | 6.6 | 6.0 |
| | AA | 5.0 | 5.9 | 6.6 | 6.3 | 6.0 |
| | OR | 5.3 | 5.6 | 6.0 | 6.0 | 5.8 |
| | AO | 4.5 | 4.7 | 5.4 | 5.8 | 5.0 |
| | $\bar{X}$ = | 4.92 | 5.88 | 6.42 | 6.12 | 5.83 |
| | SD= | 0.38 | 0.90 | 0.78 | 0.65 | 0.49 |
| | SE= | 0.16 | 0.37 | 0.32 | 0.27 | 0.20 |
| Males | IA | 4.7 | 5.5 | 5.8 | 5.3 | 5.2 |
| | AI | 4.2 | 5.6 | 5.6 | 5.2 | 4.7 |
| | TE | 4.2 | 5.0 | 5.3 | 4.4 | 3.0 |
| | TC | 4.4 | 4.1 | 3.9 | 2.8 | 3.0 |
| | RI | 4.6 | 4.8 | 4.7 | 4.0 | 3.4 |
| | AU | 5.0 | 5.4 | 5.2 | 5.4 | 4.5 |
| | $\bar{X}$ = | 4.53 | 5.07 | 5.08 | 4.52 | 3.97 |
| | SD= | 0.31 | 0.56 | 0.69 | 1.01 | 0.95 |
| | SE= | 0.13 | 0.23 | 0.28 | 0.41 | 0.39 |

| Source | SS | DF | MS | F | P |
|---|---|---|---|---|---|
| Sex | 21.600 | 1 | 26.600 | 11.881 | .006 |
| Error | 18.177 | 10 | 1.818 | | |
| Time | 8.422 | 4 | 2.105 | 12.029 | <.001 |
| Sex Time | 4.308 | 4 | 1.077 | 6.154 | <.001 |
| Error | 6.986 | 40 | .175 | | |

| | Level of Significance (0.05) | (0.01) |
|---|---|---|
| Critical value | 0.49 | 0.65 |

## APPENDIX I-F

LACTATE ($mmol.l^{-1}$)

| | | T-0 | T-30 | T-60 | T-90 | P-15 |
|---|---|---|---|---|---|---|
| Females | AT | 0.8 | 2.8 | 2.2 | 2.2 | 1.6 |
| | AC | 1.8 | 1.4 | 1.5 | 2.1 | 1.6 |
| | AE | 1.0 | 3.5 | 3.8 | 4.5 | 2.3 |
| | AA | 1.4 | 1.9 | 2.8 | 2.7 | 1.7 |
| | OR | 1.2 | 0.8 | 0.9 | 1.3 | 1.0 |
| | AO | 1.0 | 1.9 | 2.4 | 2.6 | 1.5 |
| | $\bar{X}$ = | 1.20 | 2.05 | 2.27 | 2.57 | 1.62 |
| | SD= | 0.36 | 0.97 | 1.01 | 1.07 | 0.42 |
| | SE= | 0.15 | 0.04 | 0.41 | 0.44 | 0.17 |
| Males | IA | 1.4 | 1.9 | 2.8 | 3.3 | 2.6 |
| | AI | 2.7 | 2.9 | 2.9 | 3.1 | 1.8 |
| | TE | 0.8 | 0.9 | 1.5 | 1.9 | 0.8 |
| | TC | 1.6 | 1.7 | 1.9 | 3.1 | 1.8 |
| | RI | 1.3 | 1.8 | 2.3 | 3.0 | 1.9 |
| | AU | 1.4 | 1.7 | 2.4 | 2.7 | 1.7 |
| | $\bar{X}$ = | 1.53 | 1.83 | 2.30 | 2.85 | 1.77 |
| | SD= | 0.63 | 0.64 | 0.53 | 0.50 | 0.58 |
| | SE= | 0.26 | 0.26 | 0.22 | 0.20 | 0.24 |

| Source | SS | DF | MS | F | P |
|---|---|---|---|---|---|
| Sex | .198 | 1 | .198 | .198 | |
| Error | 16.780 | 10 | 1.678 | | |
| Time | 12.985 | 4 | 3.246 | 15.028 | <.001 |
| Sex Time | .599 | 4 | .150 | .694 | |
| Error | 8.641 | 40 | .216 | | |

## APPENDIX I-G

PLASMA UREA NITROGEN ($mmol.l^{-1}$)

| | | T-0 | T-30 | T-60 | T-90 | P-15 |
|---|---|---|---|---|---|---|
| Females | AT | 13.94 | 13.07 | 15.83 | 16.22 | 18.19 |
| | AC | 16.24 | 18.23 | 14.92 | 18.62 | 15.22 |
| | AE | 15.91 | 16.22 | 17.20 | 16.22 | 18.78 |
| | AA | 13.59 | 16.46 | 13.23 | 16.28 | 16.92 |
| | OR | 16.70 | 20.00 | 18.76 | 17.65 | 17.96 |
| | AO | 16.75 | 22.00 | 18.77 | 18.36 | 20.12 |
| | $\bar{X}$ = | 15.52 | 17.66 | 16.45 | 17.23 | 17.87 |
| | SD= | 1.40 | 3.14 | 2.21 | 1.12 | 1.67 |
| | SE= | 0.57 | 1.28 | 0.90 | 0.46 | 0.68 |
| Males | IA | 19.29 | 21.04 | 18.98 | 18.98 | 22.49 |
| | AI | 21.39 | 18.83 | 20.16 | 21.18 | 20.75 |
| | TE | 18.96 | 20.94 | 17.80 | 19.37 | 20.39 |
| | TC | 19.68 | 21.27 | 20.87 | 18.21 | 20.21 |
| | RI | 16.82 | 16.64 | 22.67 | 21.62 | 23.13 |
| | AU | 21.41 | 16.46 | 22.91 | 24.07 | 22.67 |
| | $\bar{X}$ = | 19.59 | 19.20 | 20.57 | 20.57 | 21.61 |
| | SD= | 1.72 | 2.23 | 2.02 | 2.16 | 1.30 |
| | SE= | 0.70 | 0.91 | 0.82 | 0.88 | 0.53 |

| Source | SS | DF | MS | F | P |
|---|---|---|---|---|---|
| Gender | 169.445 | 1 | 169.445 | 24.251 | <.001 |
| Error | 69.867 | 10 | 6.987 | | |
| Time | 30.020 | 4 | 7.505 | 2.394 | .065 |
| Sex Time | 13.663 | 4 | 3.416 | 1.090 | |
| Error | 125.396 | 40 | 3.135 | | |

## APPENDIX I-H

URINE UREA NITROGEN ($g.day^{-1}$)

| | | Rest | Exercise |
|---|---|---|---|
| Females | AT | 5.98 | 5.44 |
| | AC | 18.69 | 16.63 |
| | AE | 9.21 | 9.20 |
| | AA | 14.44 | 14.50 |
| | OR | 15.23 | 16.14 |
| | AO | 11.29 | 8.44 |
| | $\bar{X}$ = | 12.47 | 11.73 |
| | SD= | 4.57 | 4.65 |
| | SE= | 1.87 | 1.90 |
| Males | IA | 10.72 | 15.12 |
| | AI | 20.25 | 23.75 |
| | TE | 7.93 | 14.60 |
| | TC | 16.58 | 19.36 |
| | RI | 8.78 | 14.28 |
| | AU | 19.53 | 23.91 |
| | $\bar{X}$ = | 13.97 | 18.50 |
| | SD= | 5.50 | 4.52 |
| | SE= | 2.25 | 1.85 |

| Source | SS | DF | MS | F | P |
|---|---|---|---|---|---|
| Gender | 102.631 | 1 | 102.631 | 2.254 | .161 |
| Error | 455.404 | 10 | 45.540 | | |
| Time | 21.527 | 1 | 21.527 | 21.744 | .001 |
| Gender Time | 41.897 | 1 | 41.897 | 42.320 | <.001 |
| Error | 9.897 | 10 | .990 | | |

| | Level of Significance (0.05) | (0.01) |
|---|---|---|
| Critical value | 1.28 | 1.82 |

## APPENDIX I-I

HEMATOCRIT VALUES FOR EACH SUBJECT

| | | T-0 | T-30 | T-60 | T-90 | P-15 |
|---|---|---|---|---|---|---|
| Female | | | | | | |
| | AC | .38 | .39 | .43 | .42 | .39 |
| | AE | .40 | .41 | .40 | .41 | .40 |
| | AT | .39 | .41 | .40 | .41 | .39 |
| | AA | .41 | .41 | .41 | .41 | .41 |
| | OR | .45 | .44 | .44 | .44 | .44 |
| | AO | .43 | .44 | .43 | .44 | .42 |
| | $\bar{X}$ = | .41 | .417 | .418 | .422 | .408 |

| | | 0 | 30 | 60 | 90 | P |
|---|---|---|---|---|---|---|
| Male | IA | .48 | .50 | .49 | .49 | .49 |
| | AI | .43 | .45 | .46 | .47 | .46 |
| | TE | .38 | .44 | .44 | .44 | .38 |
| | TC | .45 | .46 | .46 | .47 | .46 |
| | RI | .47 | .47 | .45 | .46 | .45 |
| | AU | .46 | .47 | .46 | .46 | .45 |
| | $\bar{X}$ = | .445 | .465 | .460 | .465 | .448 |

Table regarding thermal equivalent of oxygen for nonprotein respiratory quotient, including percent kcal and grams derived from carbohydrate and fat.

Sample calculations of energy expenditure, fat and carbohydrate consumption and, plasma volume changes.

*Thermal equivalent of oxygen for nonprotein respiratory quotient, including percent kcal and grams derived from carbohydrate and fat*

| 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 |

FROM McArdle et al., 1986, pp.127.

## APPENDIX II-A

### CALCULATION OF ENERGY EXPENDITURE FROM RER DATA

Formula Used:

Energy Expenditure = $\dot{V}O_2$ x TE x kcal/l

where: $\dot{V}O_2$ = litres of oxygen consumed per minute

TE = exercise duration in minutes

kcal/l = kcal per litre oxygen consumed (obtained from chart on page 96)

Sample Calculation

RER = 0.87

$\dot{V}O_2$ = 2.4 l/min

TE = 98 min

kcal.l = 4.887

Energy Expenditure (kcal) = 2.4 l/min x 98 min x 4.887 kcal/l

= 1149.4 kcal

## APPENDIX II-A

CALCULATION OF FAT AND CARBOHYDRATE CONSUMPTION FROM RER DATA.

The amount of fat or carbohydrate (CHO) expended per litre of oxygen consumed was estimated from the RER measurements (Zuntz, 1901; chart on page 96). The amount of fat or CHO consumed during the exercise task was calculated from the formula below.

Fat(g) or CHO(g) = $\dot{V}O_2$ x TE (mins) x [Fat(g/l) or CHO(g/l)]

where: $\dot{V}O_2$ = litres of oxygen consumed per minute

TE = exercise duration in minutes

Fat(g/l) or CHO(g/l) = grams of fat or CHO expended per litre of oxygen consumed

Fat(g) or CHO(g) = grams of fat or CHO used during the exercise session.

Sample Calculation of FAT consumption

RER = 0.87

$\dot{V}O_2$ = 2.4 l/min

TE = 98 min

Fat = 0.230 (g/l) (from chart on page 96)

Fat = 2.4 l/min x 98 min x 0.230g/l

Fat = 54 g

Sample Calculation of Carbohydrate consumption

RER = 0.87

$\dot{V}O_2$ = 2.4 l/min

TE = 98 min

CHO = 0.663 g/l

CHO(g) = 2.4 l/min x 98 min x 0.663 g/l

CHO = 155.9 g

## APPENDIX II-B

FORMULA USED TO CALCULATE CHANGE IN PLASMA VOLUME (Van Beaumont, 1972).

$$\% \quad P = 100 \left[ \frac{100}{(100 - Hcta)} \times \frac{(Hcta - Hctb)}{Hctb} \right]$$

where % P = percent change in plasma volume

Hcta = resting hematocrit value

Hctb = hematocrit value of sample you want to determine plasma volume shift from.

**Sample calculation:**

resting Hct value = .40 (Hcta)
exercise Hct value = .45 (Hctb)

$$\% \quad P = 100 \left[ \frac{1}{1 - .40} \times \frac{(.40 - .42)}{.42} \right]$$

$$= 100 \left[ 1.67 \times \frac{(-.02)}{.42} \right]$$

$$= 100 \left[ 1.67 \times (-.05) \right]$$

$$= 8.2\%$$

## APPENDIX III-A

### FREE FATTY ACID ASSAY: UV SPECTROPHOTOMETER METHOD (Noma et al, 1973)

Principle:

$$FFA + Cu^{++} \longrightarrow FFA \text{ --- } Cu$$

$$FFA \text{ --- } Cu + DDC \longrightarrow \text{coloured product}$$

where: FFA - free fatty acid

$Cu^{++}$ - copper

DDC = diethyldithiocarbamic acid

**Reproducibility:** The reproducibility of the 60 duplicate determinations was $\pm$ 13.2%.

## APPENDIX III-B

### GLYCEROL ASSAY: UV-SPECTROPHOTOMETER METHOD (Wieland, 1985)

Principle: The enzymatic determination of glycerol is based on two coupled reactions

$$\text{(1) glycerol} \xrightarrow[GK,\ Mg^{++}]{ATP \rightarrow ADP} \text{glycerol - 3 - phosphate}$$

$$\text{(2) glycerol-3-phosphate} \xrightarrow{NAD^{+} \rightarrow NADH + H^{+}} \text{dihydroxyacetone phosphate}$$

where: Gk - glycerol kinase

GDH - glycerol-3-phosphate dehydrogenase

The increase in NADH concentration measured by change in absorbance at 339, Hg 334 or Hg 365 nm is proportional to the amount of glycerol.

**Reproducibility:** The reproducibility of the 60 duplicate determinations was ± 6.83%.

## APPENDIX III-C

### HUMAN MUSCLE GLYCOGEN ASSAY (Bergmeyer, 1985)

1. AMYLOGLUCOSIDASE FLOROMETRIC METHOD (FREEZE-DRIED MUSCLE)

Principle

$$\text{glycogen} + (n-1)\ H_2O \xrightarrow{\text{amyloglucosidase}} n\ \text{glucose}$$

$$\text{glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{G6P} + \text{ADP}$$

$$\text{G6P} + \text{NADP}^+ \xrightarrow{\text{G6P - DP}} \text{gluconate-6-P} + \text{NADPH} + \text{H}^+$$

where: G6P = glucose-6-phosphate

G6P-DP = glucose-6-phosphate dephosphorylase

The amount of glucose liberated by the hydrolysis of glycogen is proportional to the increase of NADPH, measured by the absorbance change of 339, Hg 334, or Hg 365 nm.

**Reproducibility:** The reproducibility of the 60 duplicate samples was ± 7.43%.

## APPENDIX III-D

UREA NITROGEN ASSAY: UV SPECTROPHOTOMETRIC METHOD
(Sigma Diagnostics, W. Germany, Procedure No. 640)

Principle:

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 \xrightarrow[\text{Alkaline Hypochlorite}]{\text{Phenol Nitroprusside}} \text{indophenol}$$

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

**Reproducibility:** The reproducibility of the 60 plasma duplicate samples was $\pm$ 8.95%. The reproducibility of the 24 urine duplicate samples was $\pm$ 6.4%.