INFLUENCE OF GENDER ON THE PHYSIOLOGICAL RESPONSES TO PROLONGED TREADMILL RUNNING.

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ABSTRACT

The purpose of the three Studies reported in this thesis was to compare and contrast some of the physiological and metabolic responses to prolonged steady pace treadmill running in male and female distance runners. The primary aim was to investigate the possible role of gender and ovarian steroid hormones on the choice of energy substrate during endurance-type exercise.

A standard 60 minute treadmill run, performed at a steady pace corresponding to 70% of each subject's VO_2max formed the exercise challenge in each Study. Circulating concentrations of lactate, glucose, free fatty acids and glycerol during exercise and at rest, as well as R-values were used as indicators of the relative extents of lipid and carbohydrate catabolism and oxidation. Within each Study, male and female subjects were selected on the basis of "training status" with the intention of obtaining equally trained groups.

The first Study examined the metabolic and physiological responses to prolonged steady pace treadmill running in moderately trained, "recreational" men and women. Blood lactate concentration and R-values were markedly higher and blood glucose concentration lower in men than women throughout the 60 minute run; however plasma FFA and glycerol concentrations did not differ between groups. Thus, there appeared to exist a preference for lipid relative to carbohydrate oxidation in women than men, but the underlying mechanisms remained uncertain.

The second and third Studies examined the metabolic and physiological responses to prolonged steady pace treadmill running in highly trained, "elite" standard male and female distance runners. The primary objective was to investigate the validity of the hypothesis that endurance training reduces gender differences in energy substrate metabolism during prolonged submaximal exercise. A trend towards a greater rate of lipid relative to carbohydrate oxidation was apparent in highly trained women as compared to their male counterparts; however, measured metabolic parameters were not significantly different at the 5% level.

In the second Study, the metabolic and physiological responses to prolonged steady pace treadmill running were examined in equally trained, "elite" standard amenorrhoeic and eumenorrhoeic female distance runners in order to establish a possible role for ovarian steroid hormones in the control of energy substrate metabolism. Although the former had markedly lower serum oestradiol levels than the latter, few differences in metabolic parameters were evident between groups, either at rest or during exercise, except for lower plasma FFA and glycerol concentrations in the amenorrhoeic women both pre- and post-exercise. Nevertheless, plasma oestradiol and progesterone concentrations were very much higher in any of these women than would be found in healthy men. A sub-group of the eumenorrhoeic women who performed the 60 minute treadmill test run during both the early follicular and mid-luteal phases of their menstrual cycles exhibited a lower blood lactate concentration and reported a lower perceived rate of exertion during exercise in the mid-luteal phase, when circulating oestradiol and progesterone were at peak levels. Thus, ovarian steroid hormones may exert a regulatory role upon the metabolism of energy substrate during prolonged exercise, provided that their plasma concentrations exceed a "threshold" level and especially when circulating at very high concentrations.

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CHAPTER 1

Introduction

Although men have been competing against one another in power and endurance sports requiring vigorous and exhaustive preparative training for many hundreds of years, competitive women's sport is a relatively recent phenomenon. The amount of scientific data relating to the exercise capacity of women as well as the magnitude and nature of their adaptive responses to training is still relatively sparse in comparison to the in depth knowledge obtained through research on men. In the majority of exercise studies using female subjects it has been assumed that women are simply "feebler" versions of men, so will respond to exercise and training in a similar manner, except that their responses may be "damped" by their smaller physiques. However, it is evident that distinct biological differences exist between the adult male and female which may effect gender-related differences in the nature of acute and long term metabolic responses to exercise and training.

Early studies aimed at comparing the exercise capacities of men and women suggested the existence of a marked sex difference in physical potential. Measurements of aerobic capacity, muscular strength and apparent endurance capacity of women were found to be very much lower in women than in men (Astrand, 1960). A statement by Morehouse and Rasch (1958) that "at puberty, development of ability for strenuous exercise stops, or even declines in girls, while it continues to advance in boys" was widely accepted in the scientific world. However, more recent studies indicate that innate sex differences in exercise capacity and performance potential are actually much smaller than originally thought. This is primarily because at the time of the early studies, in comparison to men, few women participated in vigorous sporting activities so that a bias in training status existed between the sexes. Consequently, the magnitude of gender related differences in exercise potential were probably over-estimated. With little opportunity for equality in employment and the prevailing cultural tradition of a sedentary female role, the habitual level of

activity was also likely to be greater in non-athletic males than their female counterparts.

It is only within the last 20 years that women have been officially permitted to compete in previously male-dominated endurance events such as marathons, ultramarathons and triathlons and there is still much to be learned about the impact of hard physical training on the female body. The importance of matching male and female subject groups on training status (current training volume and intensity as well as training history) has become particularly evident from the findings of recent studies (Cureton, 1980; Bunc and Heller, 1989; Tarnopolsky et al, 1990).

Prior to the 20th Century, women were essentially barred from competitive sport. Cultural and societal stereotyping viewed such activities as a defiance of the natural feminine role, whilst medical "experts" of the time supported the bias with the unfounded rationale that women were physiologically unsuited to strenuous exercise. Although women were encouraged to take light exercise in the form of calisthenics, gymnastics and "bathing" on a recreational basis to promote good health, it was widely assumed that a woman's body would not withstand the physical and psychological stress imposed upon it in training and competition.

At the turn of the 20th Century, through their own insistance, women began to participate in a wider range of sporting activities, in educational institutions and for leisure. Traditionally "male" sports were modified for female participation, or women played their own gender-specific sports which varied according to social class. However, women were still regarded to be of a "delicate physiological and emotional constitution" and were provided with little opportunity to compete against one another in endurance sports such as running, cycling and swimmimg.

The first Modern Olympics, held in Athens in 1896 barred all women from entering, although legend has it that a Greek woman named Melpomene unofficially entered and completed the marathon race in 4½ hours after secretly training for it. The French founder of the Modern Olympics, Baron Pierre de Coubertin had insisted that the games be confined to male participation, claiming that it was "improper" for women to perform in public. Women made their debut appearance in the Paris Olympics of 1900,

when a total of 12 women (amongst 1318 men) were permitted to compete in golf and tennis. During the next 2½ decades a number of other sports such as archery, fencing, golf, yachting and swimming were gradually introduced as women's events into the Olympics, but it was not until the Amsterdam Olympics of 1928 that women were finally allowed to compete in track and field athletics. The longest women's track event was the 800 metres, still regarded by many as too exhausting a distance for women to race and was successfully completed in the winning time of 2 minutes 16.8 seconds by Lina Radka of West Germany. Although the first six women finished within 10 seconds of each other, a number of competitors collapsed before or after the finish line and this somewhat sensationalised incident succeeded in providing adequate confirmation of the hypothesis that women were simply too frail to cope with such a distance. Hence the event was omitted from the games for the next 32 years. Until 1984 when the 3000 metres and marathon were introduced, the 1500 metres was still the longest woman's Olympic track event. Although the first American National Women's Marathon championship, stimulated by the "jogging boom" of the late 1960's was held in 1970, the popularity of recreational running for both sexes did not catch on in other major continents until almost a decade later. In order to fulfil the requirements for inclusion in the Olympic Games, an event must be practised widely in 25 countries on 2 continents and this was not true of the women's marathon until the late 1970's.

Throughout the 1980's the popularity of running for recreation and health benefits increased tremendously in both sexes and the marathon became the ultimate goal for many recreational runners. Once free to run for as long and as far as they wished, women began to train as hard, or harder than many men. Consequently, women's world records in track events have rapidly declined, whilst men's have changed very little over recent years. It is evident that with hard training, women are capable of completing endurance events and of achieving better performance times than many men. Although it has been speculated that women will eventually equal men's world records in many endurance events or even improve upon them, the innate physiological differences between the sexes almost certainly limit such a possibility. An individual's performance potential is largely determined by inherent traits and even though training can potentiate this capacity many fold, women are genetically limited by their physical and physiological characteristics. A difference in the order of approximately

15% still exists between men's and women's world record times in distance running events ranging from the mile to the marathon and in "fun" runs contested by large numbers of male and female recreational runners, a similar sex difference in mean finishing has been shown to exist (Young and Young, 1985).

Nonetheless, given that more and more women and young girls are training and competing in endurance sports and knowing that endurance capacity is maximised only after many months or years of hard training, it would seem that we have yet to observe the limits of female performance capacity as well as the consequences of hard physical training on the female body.

The main objective of the experimental work cited herein was to compare and contrast some of the physiological and metabolic responses to prolonged steady pace treadmill running in men and women. A particular emphasis was placed upon an ascertainment as to whether or not men and women present different patterns of energy substrate metabolism during prolonged steady pace exercise.

Two populations of male and female subjects were studied: moderately trained "recreational" runners and highly trained "elite" standard athletes of either sex. Within each population, men and women were matched as closely as possible with regard to "training status". This was assessed upon past and present physical activity patterns and training regimes, as well as blood lactate measurements during steady pace running at different treadmill speeds.

It has been hypothesised that women are physiologically better suited to endurance exercise than men, owing to their larger body fat stores, which might potentially serve as utilisable energy substrate during prolonged exercise (Ullyot, 1976). Although this hypothesis has been extensively researched in recent years, it is still subject to controversy. The contradictory results of many studies may stem from an inadequate standardisation of factors known to exert a definite influence upon the exercising muscles' choice of energy substrate. A failure to test subjects in the fully post-absorptive state (which influences circulating

concentrations of lipolytic and glycogenolytic hormones), the relative intensity and duration of the exercise challenge and the matching of subjects for "training status" are perhaps the most noticable limitations of many such experiments.

With regard to the experimental work presented herein, the rationale underlying a comparison of exercise-induced metabolic responses in both moderately and highly trained men and women stemmed from evidence suggesting that endurance training may reduce innate gender-related differences in exercise metabolism. Lower respiratory exchange ratio (R) values, lower blood lactate, but higher blood glucose, plasma free fatty acid (FFA) and glycerol concentrations have been measured during submaximal exercise in moderately, or untrained women as compared to men of similar "training status" (Berg and Keul, 1983; Blatchford et al, 1985; Froberg and Pedersen, 1984). Such findings point towards a greater catabolism and oxidation of fat in preference to glycogen in women. In some cases, similar measurements have been shown to be coupled to a smaller extent of intramuscular glycogen utilisation and an increased exercise time to exhaustion in women than in men (Nygaard, 1986; Tarnopolsky et al, 1990).

In contrast, small or insignificant sex differences in R values as well as circulating concentrations of lipid and carbohydrate substrates and metabolites have been measured during submaximal exercise in highly trained male and female athletes (Bransford and Howley, 1979; Costill et al, 1979; Powers et al, 1980; Wallace et al, 1980; Brewer et al, 1988).

A major factor contributing to differences in the physical and physiological characteristics of men and women lies within their circulating sex steroid hormone milieu. Gender-related differences in physique, body composition, the morphology and metabolic properties of skeletal muscle (Simoneaou et al, 1985; Hedberg and Janssen, 1986), adipose tissue (Smith et al, 1979; Despres et al, 1984) as well as basal and exercise-induced concentrations of circulating lipolytic and glycogenolytic hormones all develop during adolescence and are concomitant with an increased synthesis and release of sex steroid hormones (Rebuffe-Scrive et al, 1985).

The results of both animal and human experiments suggest that androgens, oestrogens and progestogens possess regulatory roles in the storage, mobilisation and/or utilisation of energy substrate during prolonged submaximal exercise. Oestrogens in particular have been linked to an enhanced capacity for fatty acid oxidation in women. Exercise-induced metabolic responses as well as circulating concentrations of various lipolytic and glycogenolytic hormones have been shown to differ during the follicular and luteal phases of the menstrual cycle, concommitant with variations in circulating oestrogen and progestogen concentrations (Hall-Jurkowski et al, 1981; Bonen et al, 1983; Lavoie et al, 1987). Exogenously administered contraceptive steroids also appear to affect the metabolism of energy substrate during rest and exercise (Beck, 1973; Kalkhoff, 1975; Skouby et al, 1985; Jensen et al, 1987; Bunt et al, 1990).

If oestrogens and progestogens do serve a regulatory role in the exercising muscle's choice of energy substrate, then the amenorrhoeic (non-menstruating) woman might be expected to exhibit a different metabolic profile during exercise to her eumenorrhoeic ("normally" menstruating) counterpart, since the levels of circulating oestrogen and progestogens are very much lower in the former (Shangold et al, 1979; Fisher et al, 1976). For this reason, the aim of one of the present investigations was to compare the metabolic responses of equally trained amenorrhoeic and eumenorrhoeic women to prolonged steady state treadmill running. Since it is also possible that any metabolic influences of oestrogens and/or progestogens may be concentration dependent, the responses of eumenorrhoeic women to a standard exercise challenge were examined during both the luteal and early follicular phases of their menstrual cycles, when sex steroid hormone levels peak and trough (respectively).

In mixed sex studies concerning exercise metabolism there is often no allowance made for possible gender-related differences in the metabolic and physiological responses to prolonged exercise. It is generally assumed that men and women will respond similarly if exercising at equal relative intensities. However, before men and women can be classified as a single homogeneous subject group for exercise studies they must be shown to respond similarly to an exercise challenge and to long term endurance training.

CHAPTER 2

Review of Literature

2.1 Physical and Physiological differences between men and women

The physical performance capacity of an individual is determined both by nature (inherent qualities) and nurture (environmental influences such as habitual activity patterns and athletic training). Relative to men, the athletic performance capacity of women tends to be limited by their overall size and physique. Although there exists a wide variation in physical characteristics within the distinct male and the female populations and the two Gaussian curves describing any particular physical characteristic of men and women generally overlap, the magnitude of the sex difference in the mean value of such a characteristic is usually statistically significant. Thus, one speaks of the "typical" man or woman, even though some "atypical" men and women may be matched very closely in one or a number of physical characteristics. Given that many of the physiological differences between men and women arise from differences in their physical characteristics, the "typical" man inherently possesses a greater athletic performance potential than his female counterpart.

Prior to adolescence, the physical characteristics of girls and boys are quite similar (Tanner, 1962; Tanner et al, 1966). There is little sex difference in the rate of childhood growth until the age of 9 or 10 when the onset of the adolescent growth spurt in girls results in an accelerated growth rate and an upward deviation in the female growth curve from that of the male. Between the ages of 10 and 14, girls tend to be taller and heavier than boys of the same age since the equivalent growth spurt generally occurs some 2-3 years later in the latter. However, whilst girls usually attain adult height between the ages of 14 and 16, boys are exposed to the anabolic effects of growth hormone for 2-3 years longer than girls before they reach adolescence, as well as rapidly increasing circulating concentrations of androgens during puberty. This means that the growth rate during adolescence is higher and persists for longer in boys than girls,

with most boys achieving their adult height between the ages of 18 to 20 (Tanner, 1962; Tanner et al, 1966).

At full maturity, men are typically in the order of 10% taller and 20% heavier than women and possess a greater lean to fat body mass ratio as a result of their larger and denser skeleton, greater muscle mass and relative paucity of stored fat. From detailed measurements of body size and physique made using a variety of different techniques in a large number of young adults, Behnke and Wilmore (1974) constructed a theoretical model for a "reference" man and woman:

	MAN	WOMAN
Age	20-24	20-24
Height (cm)	174.0	163.8
Weight (kg)	70.0	56.8
Total fat (kg)	10.5	15.4
Storage fat (kg)	8.4	8.5
Essential fat (kg)*	2.1	6.8
Muscle (kg)	31.4	20.5
Bone (kg)	10.5	6.8

* Fat required for normal physiological functioning; the loss of which may lead to a deterioration in health.

Sex differences in physique are strongly influenced by the circulating concentrations of sex steroid hormones. At puberty, neural and humoral signals originating within the central nervous system activate the the male and female gonads from a relative state of childhood quiescence. The testes begin to synthesise large quantities of androgens, the ovaries oestrogens and at a later stage progestogens, which circulate around the body, acting upon various target organs to stimulate the development of secondary sexual characteristics and the proliferation of specific tissues. Although all 3 types of sex steroid hormone are synthesised by both sexes, androgens dominate in the male and oestrogens and progestogens in the female. The anabolic nature of the most potent androgen, testosterone, enhances the capacity for protein synthesis in many tissues, particularly skeletal muscle which hypertrophies in response to resistance loading more readily in males than in females. Testosterone also enhances bone ossification and stimulates haemoglobin synthesis. Sex differences in muscle mass and bone size are especially marked in the upper body, typifying the broad-shouldered, mesomorphic masculine physique. Oestrogens (the most potent of which is Oestradiol 17B) promote fat storage at specific sites in the body,

this adipose tissue frequently being termed "sex-specific fat" on account of its metabolic properties and contribution to the typically endomorphic feminine physique. At full maturity the relative body fat content of the "average" woman is typically in the order of 50% higher than that of her male counterpart, with fat contributing between 25 and 30% of her total body mass in contrast to between 15 and 20% of a man's (Durnin and Womersley, 1974).

In children, the sex difference in exercise and performance capacity is very much smaller than in the adult, primarily as a result of the relative similarities in body size and physique of pre-pubescent girls and boys (Sloane, 1980). However, during adolescence, a gap in athletic performance capacity rapidly develops and widens with increasing age as girls and boys develop there respective adult physiques. As far as strength, speed and endurance are concerned, men generally possess a physiological advantage over women on account of their inherent physical characeristics. In contrast, the relatively high fat to lean body mass ratio of the typical woman tends to limit her performance capacity in sports requiring strength and/or endurance, except in swimming where fat adds bouyancy and tends to increase the metabolic efficiency of motion through water (Holmer, 1974). In weight-bearing activities such as walking, running and jumping, fat merely contributes to the load carried and acts as "dead" weight, since its metabolic role during exercise is negligible. It simply decreases locomotive efficiency by increasing the energy cost required to move the body against opposing gravitational forces (Costill et al, 1971). In sports requiring strength and power, male performance is biassed by their larger limb muscles, since a strong positive correlation exists between the maximal power output of a muscle and its cross sectional area (Ikai and Fukunaga, 1970). In endurance type activities, men yet again possess the physiological advantage of a higher maximal aerobic power output, both in absolute terms and relative to body mass as a result of their larger size and leaner physique.

Maximal aerobic power (VO_2max) is equal to the product of maximal cardiac output (Qmax) and arterio-mixed venous oxygen difference (a-v O_2) and appears to be limited primarily by maximum cardiac output (Saltin et al, 1973). It is evident that part of a training-induced increase in VO_2max is attributable to an increase in the capacity of the exercising muscles to

extract available oxygen from the blood (Pirnay et al, 1972). However, the fact that blood leaving the muscles during intense aerobic exercise is very desaturated and that VO_2max can be increased in an individual almost instantaneously by the reinfusion of his own freeze-preserved blood a few weeks after withdrawal ("blood doping") to result in an elevated circulating haemoglobin concentration (Buick et al, 1980), suggests that oxygen transport is the primary factor limiting VO_2max. VO_2max would not appear to be limited by maximum voluntary ventilation, or the diffusing capacity of the lungs in healthy individuals at sea level (Ekblom and Hermannsen, 1968). Cardiac output is equal to the product of stroke volume and heart rate and a close relationship exists between VO_2max and maximum stroke volume (Saltin et al, 1973). Comparable increases in VO_2max and maximal stroke volume of similar magnitude occurs during longitudinal training studies (Saltin and Astrand, 1967) and some of the highest recorded values of VO_2max have been made in elite endurance athletes whose VO_2max and maximal stroke volume are both in the order of 60% higher than untrained individuals (Arstila and Koivikko, 1966).

A larger or stronger (hypertrophied) heart can achieve a greater stroke volume and hence cardiac output for a given heart rate than a smaller heart, so is a more efficient pump. A woman's heart is typically about 20% smaller than a man's and although maximum heart rate is similar for men and women of the same age, an approximate 30% difference in maximal cardiac output exists between the sexes on account of the difference in stroke volume (Pollock, 1977). For a given submaximal cardiac output, a woman's heart rate tends to be 5-8 beat/min higher (Pate et al, 1977).

Absolute VO_2max (expressed in l min^{-1}) is typically 40-60% higher in men than women (Sparling, 1980). When expressed relative to total body mass (ml kg^{-1} min^{-1}) and lean body mass (ml kg^{-1} min^{-1}) this sex difference is reduced to around 15-25% and 5-15% respectively (Sparling, 1980). Thus, it is evident that a major proportion of the sex difference in VO_2max is governed by differences in body size and composition as well as the size of the organs of oxygen transport. The residual excess in the VO_2max of men over that of women can probably be attributed to the higher blood oxygen transporting capacity of the former. Men on average, possess approximately 6% more red blood cells and 10-15% more haemoglobin per unit volume of blood than women (De Vries, 1980) and therefore a higher arterial oxygen

content and a-v O_2 difference during exercise of a standard submaximal work rate. Thus for a given oxygen uptake, more blood must be delivered to the tissues per unit time, or the extent of oxygen extraction must be higher in women than in men. The portion of the sex difference in VO_2max attributable to differences in haemoglobin concentration has been estimated by withdrawing an appropriate amount of blood from a group of men in order to equate their haemoglobin concentration with that of a group of equally trained women (Cureton et al, 1986). As a result of this procedure, VO_2max declined by approximately 7% in the men 48 hours later. The magnitude of the cardiac output needed to transport 1 litre of oxygen during submaximal exercise requiring an oxygen uptake of 1.5 l min^{-1} has been calculated to be about 9 litres in women (arterial oxygen content of 16.7ml $100ml^{-1}$) and 8 litres in men (arterial oxygen content of 19.2ml $100ml^{-1}$) (Astrand et al, 1964). In spite of the large sex difference in the oxygen transporting capacity of the blood, the magnitude of the resultant affect on VO_2max is not as great as might be expected on account of a woman's lower haematocrit and hence blood viscosity, which facilitates muscle blood flow during exercise. The ratio of Qmax/VO_2max is thus greater in women (Astrand et al, 1964). Women also appear to possess a higher erythrocyte 2,3 DPG content relative to haemoglobin content which may facilitate the desaturation of oxygen at cellular level (Pate et al, 1985).

Endurance training can increase VO_2max in all individuals to an extent which is determined by an interplay between the intensity and duration of training, initial training status and inherent potential (governed by the overall size of their oxygen transport system). Many highly trained elite male and female distance runners have VO_2max values greater than 75 and 65 ml kg^{-1} min^{-1} respectively (Costill and Higdon, 1980). The increase is primarily attributable to cardiac hypertrophy and therefore an increased stroke volume for a given heart rate (Falsetti, 1977), but also an enhancement of the oxygen extracting capacity of the trained muscles. Thus, endurance training increases the maximum cardiac output, arterio-venous oxygen difference across the muscle bed and hence VO_2max (which is equivalent to the product of maximal cardiac output and the mean difference in oxygen content between arterial and venous blood). This means that after training, a given work rate represents a lower relative work intensity (expressed as %VO_2max) for an individual than in the untrained state and therefore exerts less strain on metabolism, the cardiovascular and

thermoregulatory systems whose responses are governed by relative as opposed to absolute work load (Hermansen: et al, 1973).

Studies on highly endurance trained men and women matched for training status suggest the existence of smaller sex differences in VO_2max (per unit total body mass and lean body mass) than in sedentary individuals. In elite male and female athletes training for the same sport, sex differences in VO_2max in the order of 16-18% (ml kg^{-1} min^{-1}) and 3-5% (ml kg lean body mass $^{-1}$ min^{-1}) have been measured (Zwiren et al, 1983; Bunc and Heller, 1989). This suggests that all but a very small proportion of gender related differences in VO_2max are related to differences in the dimensions of the body's oxygen transport system and musculature (the residual excess being most likely attributable to circulating haemoglobin concentration).

Although it would seem apparent that a major portion of the marked sex differences in VO_2max measured in many of the early studies of male and female exercise capacity were attributable to the higher habitual activity level of the "average" untrained male relative to his female counterpart, recent evidence suggests that endurance training does indeed reduce gender-related differences in VO_2max. Since prolonged endurance training tends to reduce adiposity in both sexes, it is possible that the smaller difference in VO_2max existing between highly trained men and women stems from the loss of a large proportion of sex specific and performance-limiting "female" fat in the latter. Indeed, the physique of highly trained female distance runners is very much more masculine in appearence than that of the sedentary woman. The former exhibit a physique which tends towards a higher proportion of mesomorphy and ectomorphy, with a relatively low endomorphic component, similar to that of the moderately trained male. On account of their different hormonal profile, men tend not to accumulate fat stores around the upper limbs and buttocks, but rather the abdomen (a store known as "omental" fat) which interferes to a lesser extent with locomotion (Newsholme and Leech, 1986). In weight-bearing activities such as running and jumping, performance is highly positively correlated with VO_2max per unit body mass (Costill et al, 1981). Thus, by losing a significant amount of fat through endurance training (perhaps most importantly from "sex-specific" sites), women may significantly improve their endurance performance. Elite male and female endurance runners have particularly low body fat contents, normally in the order of 4-10% and 13-19% respectively

(Wilmore and Brown, 1980). However, even in elite male and female athletic populations training for the same event, there still exists an average 9% difference in relative body fat content between the sexes (Wilmore, 1983).

The findings of many recent studies comparing and contrasting gender differences in exercise capacity (especially endurance capacity and exercise metabolism) clearly emphasise the importance of matching male and female subjects for training status as based upon current activity patterns as well as training history (Bunc and Heller, 1989; Tarnopolsky et al, 1990). A number of studies conducted in the late 1970's by Cureton and colleagues suggest that male and female groups can be assumed to be of similar cardiorespiratory "fitness" (although not necessarily endurance fitness) if the gender difference in VO_2max expressed per kg lean body mass is in the order of 5% higher in males (Cureton, 1980).

It is clear, therefore, that inherent physical differences between the sexes result in physiological and hence performance differences. If men and women could be matched on physical and physiological characteristics, then it is likely that this sex bias in exercise performance capacity would be negated. When matched on performance in endurance events, men and women were found to possess similar VO_2max values and relative body fat contents (Pate et al, 1985). However, relative to other members of their sex the women had significantly higher VO_2max values, were significantly leaner and more highly trained in contrast to the men, whose physical and physiological characteristics were typical of the moderately trained male. Thus, as far as exercise performance capacity is concerned, it would seem that elite female performers are comparable to good "club standard" males and that elite males are able to achieve significantly faster race times than elite females.

Although it is true that success in endurance sports requires that an individual possess a high VO_2max (which determines maximal aerobic work rate), endurance performance also depends also upon endurance capacity, which describes the ability to sustain a given submaximal work rate without fatiguing and may be quantified as exercise time to exhaustion. Thus, endurance performance depends upon the size of a person's VO_2max and their ability to sustain an exercise intensity close to their maximum for

prolonged periods of time without evoking gross homeostatic disturbance and depleting the body's glycogen stores. Distance running performance is also influenced by running economy, which is enhanced by training and is particularly high in elite distance runners (Costill et al, 1971). However, amongst equally trained male and female athletes training for the same event, performance differences have been shown to be almost wholly attributable to differences in VO_2max (Bunc and Heller, 1989).

A high VO_2max is a necessary pre-requisite for successful distance running performance and is probably the major determinant of middle distance running performance, where a high aerobic power output must be generated for only a few minutes (Costill and Higdon, 1980). Indeed, some of the highest recorded VO_2max values have been obtained from middle distance runners (Costill et al, 1973). However, in longer distance events, endurance capacity becomes a quantitatively more influential performance-related factor since it is necessary to sustain a high aerobic power output close to maximum for a prolonged duration. Exercise time to exhaustion and performance times have been shown to vary considerably amongst marathon runners with quantitatively similar VO_2max values (Costill, 1971; Saltin, 1973) and endurance performance is more highly correlated with blood lactate parameters than with VO_2max (Jacobs, 1986). Similarly, detraining studies have shown that whilst VO_2max may be maintained close to its pre-training value during the first few weeks of detraining, the aerobic capacity of the trained muscles rapidly declines, concommitant with a decline in endurance capacity (Holloszy and Coyle, 1984).

Endurance capacity during aerobic exercise is essentially governed by the availability of glycogen in the exercising muscles and the rate at which these stores are utilised (Ahlborg et al, 1967). The metabolic efficiency of skeletal muscle is closely related to its aerobic capacity, which may be potentiated by endurance training in both sexes (Holloszy et al, 1970). Slow twitch (type I) muscle fibres possess an inherently greater aerobic capacity than fast twitch (type IIa and IIb) fibres and can derive a relatively greater amount of energy from fat as opposed to carbohydrate (Holloszy and Coyle, 1984). Their capacity for triglyceride storage is also higher, although they store similar amounts of glycogen to fast twitch fibres (Froberg and Mossfeldt, 1971). Elite endurance athletes tend to

possess a surplus of slow twitch fibres (Gollnick et al, 1972; Costill et al, 1976) which are the predominantly active fibres during long slow distance running. However, the oxidative potential of both major fibre types can be increased with training of the appropriate intensity and duration (Janssen and Kaijser, 1977; Dudley et al, 1982).

2.2 Muscular Adaptations to Endurance Training

Endurance training effects a number of biochemical adaptations, in a selective manner within the skeletal muscles recruited during the training exercise which enhance their capacity for aerobic metabolism and the individual's ability to perform the exercise for prolonged periods of time without fatiguing (Costill and Higdon, 1980). Although it was initially believed that endurance capacity was limited by hypoxia within the working muscles (Wassermann and Whipp, 1975) and that training induced increases in endurance capacity could be attributed to an improved delivery of oxygen to the working muscles via an enhanced cardiac output, current opinion suggests that cardiovascular adaptations to training play a relatively minor role in the capacity to endure prolonged submaximal exercise. Oxygen uptake during steady state exercise of a given absolute intensity is generally similar pre and post training (Hagberg et al, 1980), thus if untrained muscles were hypoxic during steady state exercise, training might be expected to increase oxygen uptake at the same absolute exercise intensity. Furthermore, muscle blood flow remains unchanged or may even decrease during exercise of a given absolute intensity after training (Holloszy, 1973).

Endurance-trained muscle possesses a much greater capacity for the aerobic oxidation of energy substrates than untrained muscle. It can metabolise relatively more fat than in the untrained state during steady state exercise of a given absolute or relative intensity (Costill et al, 1977; Henriksson, 1977), is able to conserve its limited glycogen stores more effectively (Hermansen et al, 1967) and consequently produces smaller amounts of lactic acid (Karlsson et al, 1972; Saltin et al, 1976).

The most influential training-induced adaptations effected within the muscle are probably an increase in the number and size of mitochondria (Hoeppeler et al, 1973) and an increase in the number of capillaries per unit cross-sectional area of muscle (Saltin et al, 1976; Ingjer, 1979; Klausen et al, 1981). A greater mitochondrial density leads to a higher total oxidative enzyme activity and an increased potential for the aerobic catabolism of energy substrate (Holloszy et al, 1970). An increased capillary density facilitates the diffusion of oxygen, nutrients and

metabolites between blood and exercising muscle, by lengthening the mean transit time of blood in the muscle capillaries, increasing the surface area for molecular exchange and lowering the diffusion distance (Klaussen et al, 1981). Endurance training also increases temporary stores of oxygen in the muscle via an increase in myoglobin content (Pattengate and Holloszy, 1967). Endurance trained muscle has been shown to possess a 3-4 fold higher oxidative enzyme activity and to be able to metabolise fat at approximately 7 times the rate of untrained muscle in vitro (Costill et al, 1979).

Such adaptations take place gradually and will continue to develop over many years of continued training (Holloszy and Coyle, 1984). They may occur in all types of muscle fibre, but are confined to those recruited in the training exercise (Gollnick et al, 1972; Henriksson, 1977; Saltin et al, 1976). Thus, the extent of the adaptation will vary according to the intensity of the training load, which determines the type of fibre recruited (Dudley et al, 1982; Harms et al, 1983) as well as the total training volume (Costill et al, 1973). The magnitude of training induced increases in mitochondrial protein content is strongly correlated with the total amount of contractile activity and is almost certainly mediated via contractile activity per se as opposed to exogenous humoral factors (Gollnick and Ianuzzo, 1972; Saltin et al, 1976).

Slow twitch (type I) fibres are characterised by an inherently greater number of mitochondria and consequently a higher total oxidative enzyme activity than fast twitch (type II) fibres (Essen et al, 1975; Lowry et al, 1978). The relative distribution of fibre types within a muscle is essentially genetically pre-determined and there is little evidence to suggest that fast and slow twitch fibres are interconvertable through training (Gollnick et al, 1972). However, high intensity "interval" training will recruit fast as well as slow twitch fibres and over a period of months a gradual increase in the number of type IIa (high oxidative-glycolytic fast twitch) fibres coupled to a decrease in the number of type IIb (low oxidative-glycolytic fast twitch fibres) may be observed (Gollnick et al, 1972; Holloszy and Coyle, 1984). This suggests that such a training regime causes type IIb fibres to develop a metabolic profile similar to that of type IIa fibres, so that a continuum from type IIb to type IIa fibres prevails within the muscles of most individuals. Very highly trained

individuals, who have practised an endurance sport for many years often possess an almost undetectable number of type IIb fibres, but a great many type IIa fibres, possibly due to an almost complete conversion of the former to the latter (Holloszy and Coyle, 1984). It would also seem that high intensity endurance training, effecting a recruitment of type II fibres tends to effect a greater extent of mitochondrial proliferation in this particular fibre type relative to type I fibres such that both fibre types ultimately possess similar oxidative enzyme activities (Janssen and Kaijser, 1977).

Endurance training effects few and relatively minor changes in glycolytic enzyme activities (Baldwin et al, 1973; Schantz et al, 1983). Endurance trained muscle appears to possess a relatively low glycolytic capacity and many ultra-distance trained athletes have a subnormal anaerobic capacity and produce little lactate even during high intensity exercise (Holloszy, 1967; Costill et al, 1976). There is little hypertrophy of muscle fibres with endurance training (Fink et al, 1977).

Exercise time to exhaustion (endurance time) is a function of relative work intensity and decreases exponentially with increasing work rate (Tornvall, 1963). This is linked to an exponential increase in the rate of glycogen catabolism in exercising muscle at relative exercise intensities above about 50-55% VO_2max, whilst at lower exercise intensities and at rest, the muscles favour fat as a fuel (Davies and Thompson, 1977). At exercise intensities above 90-95% VO_2max, the muscles metabolise glycogen almost exclusively as energy substrate (Christensen and Hansen, 1939). The kinetics of fat metabolism are such that in contrast to carbohydrates, which may be catabolised both aerobically and anaerobically, ATP can only be resynthesised from fatty acids via aerobic processes (Newsholme and Leech, 1986). Also, a comparatively larger volume of oxygen is required to supply ATP at a given rate from fat as compared to carbohydrate. Therefore, with increasing exercise intensity there comes a point when ATP cannot be resynthesised rapidly enough with fat serving as the major substrate and since less oxygen is required to yield an equivalent amount of ATP from glycogen, the muscles' stores of glycogen rapidly dwindle. If the metabolic rate of the exercising muscles exceeds the rate at which they can synthesise ATP aerobically, energy can still be produced via the anaerobic catabolism of intramuscular glycogen (and phosphocreatine, which is

quantitatively less significant. However, fatigue rapidly ensues as metabolites accumulate, intracellular pH declines and the ATP content of the cell declines (Galbo, 1983).

On account of a greater capacity for aerobic metabolism, endurance trained muscle can continue to rely upon fat as energy substrate at higher relative exercise intensities than untrained muscle, as reflected experimentally by a lower whole body respiratory exchange ratio and a reduction in the rate of glycogen catabolism in the active muscles (Henriksson, 1977; Janssen and Kaijser, 1987). Indeed, elite marathon runners can sustain a running speed corresponding to 85-90% of their VO_2max over 26.2 miles (42.2km) without fatiguing (Pollock, 1977). Also, a training-induced increase in VO_2max will result in a given submaximal work rate corresponding to a lower relative exercise intensity than in the pre-trained state, which further economises on glycogen utilisation and positively influences endurance performance.

The availability of intramuscular glycogen is a major determinant of endurance performance and capacity during prolonged submaximal exercise (Ahlborg et al, 1967). Low or depleted intramuscular glycogen stores severely limit endurance performance since the muscles are forced to rely almost exclusively upon fat for ATP resynthesis. As a result their aerobic power output falls to approximately half of their maximum (Davies and Thompson, 1977). Thus, endurance capacity can be increased by increasing the size of the muscle's glycogen stores prior to exercise and/or decreasing the rate at which these stores are utilised (Karlsson and Saltin, 1971). In contrast to the abundance of storage fat, the stores of glycogen within the body typically amounts to no more than 2% in most individuals and the total energy content of the fat stores of a "typical" 70kg adult male possessing about 9kg of fat (13%) amounts to approximately 5 times that of stored glycogen (Newsholme and Leech, 1983). The energy content of stored triglyceride in the "typical" adult female is about half as much again. Normal muscle glycogen content in a well fed, rested person is equivalent to approximately 80-100mmols of glucose per kg wet weight (or 355-360mmol per kg dry weight), which amounts to 400-500g glucose (approximately 6500kJ) in a person with a total muscle mass of 25-30kg (Newsholme and Leech, 1983).

Generally, for the "typical" individual consuming an "average" western diet consisting of 40-45% carbohydrate and exercising at a relative intensity greater than 70% VO_2max, the active muscles become almost glycogen depleted within 2 hours (Newsholme and Leech, 1983; Costill and Higdon, 1980). Thus, during an endurance event such as the marathon, which is run at a relative exercise intensity equivalent to 70-80% VO_2max of a well-trained recreational/club runner and takes around 3½ hours (Costill, 1970), fatigue attributable to glycogen shortage is likely to limit performance.

The gastrointestinal tract does not readily tolerate food during exercise on account of a sympathetically induced vasoconstriction of arterioles in the region of the splanchnic vascular bed, leading to decreased digestion and absorption of nutrients and water. Thus, ingested nutrients are neither easily digested or utilised by the body which is forced to mobilise intra- and extra-muscular stored fuels for ATP resynthesis. The capacity of endurance trained muscle to utilise relatively more fat as an energy substrate leads to a reduction in the rate of glycogen catabolism so that stores of the latter last longer and the rate of lactic acid production is less (Janssen and Kaijser, 1987). Hence, the onset of fatigue is also limited by the enhanced maintenance of acid-base balance (Kindermann and Simon, 1979).

Blood lactate concentration during prolonged submaximal exercise is governed by a dynamic equilibrium between the rates of production and metabolic degradation. If the former exceeds the latter, lactate and H^+ ions start to accumulate in the muscles and circulation. Blood lactate concentration tends to increase 2 to 3 fold above resting values during steady state exercise at relative intensities below about 60-70% VO_2max in untrained persons, although a considerably higher relative exercise intensity is required to attain a given blood lactate concentration in the trained state Hurley et al, 1984). Blood lactate concentration tends not to rise more than 3-fold above resting values during prolonged "steady state" exercise unless the pathway for lactate clearance becomes saturated, leading to a precipitous rise in blood lactate concentration. This so called "onset of blood lactate accumulation" (OBLA) tends to occur once blood lactate concentration exceeds approximately 4mmol l^{-1} (Kindermann et al, 1979). At lower exercise intensities, a greater proportion of ATP is

resynthesised from lipids as opposed to carbohydrate and less active tissues such as the liver, kidney and resting skeletal muscle remove lactate from the circulation by metabolising it as energy substrate and so preventing its accumulation (Carlsten et al, 1961).

Although training-induced increases in VO_2max are primarily attributable to an improved delivery of oxygen to the exercising muscles (Ekblom, 1969) it would seem unlikely that an elevated blood lactate concentration during "steady state" exercise arises as a result of limited oxygen availability. That the muscles become hypoxic during prolonged "steady state" exercise is central to the controversial "anaerobic threshold" concept which states that the onset of hyperventilation during a graded submaximal exercise bout coincides with the accumulation of lactic acid in the circulation and is effected by a resultant drop in blood pH (Wassermann and Whipp, 1975). Oxygen consumption at a given submaximal work rate is not increased by training (Hagberg et al, 1980) and it appears likely that a higher lactic acid production of untrained muscle is attributable to an increased "glycolytic flux" and increased substrate availabilty to lactate dehydrogenase in the face of a limited capacity for lipid oxidation (Holloszy and Coyle, 1984). The forementioned biochemical adaptations induced by endurance training are likely to reduce lactic acid production during "steady state" exercise by enhancing the muscle's capacity to utilise available oxygen efficiently and to oxidise a relatively greater amount of lipid than untrained muscle.

Performance in endurance events is inversely related to circulating lactate concentration and a strong correlation exists between endurance performance and the exercise intensity required to elicit a given blood lactate concentration in the 1.5 to 4 mM range (LaFontaine et al, 1981). Highly trained endurance athletes exhibit a significantly lower circulating lactic acid concentration at a given relative exercise intensity than untrained persons (Farrell et al 1979) and consequently a greater endurance performance capacity (Costill and Higdon, 1980).

The rate of substrate oxidation by contracting skeletal muscle is governed by the rate of ATP hydrolysis at the cross bridges formed between the actin and myosin filaments and is thus a function of work rate. Provided that the muscle cell's energy requirements do not exceed the

maximum catalytic activities of the rate limiting enzymes and neither oxygen nor substrate are limiting, the rate of mitochondrial respiration is generally proportional to the the intracellular ADP concentration (Jacobus et al, 1982). Although various activating and inhibitory agents are known to regulate the activity of many of the rate limiting enzymes controlling oxidative metabolism, electron transport is tightly coupled to the oxidative phosphorylation of ADP produced in the hydrolysis of ATP (Jacobus et al, 1972). During steady state (submaximal) exercise, the rate of oxidative phosphorylation tends to balance the rate of ATP hydrolysis, leading to a steady state concentration of ADP and thus mitochondrial respiration. Intracellular ATP and phosphocreatine concentrations fall to constant but subnormal levels such that an oxygen deficit is incurred within the muscle (Jacobus et al, 1982). It is likely that a training-induced increase in oxidative enzyme activity lowers the oxygen requirements of the muscle cell for a given rate of ATP resynthesis, such that during exercise of a given submaximal intensity, a similar oxygen uptake post- as pre-training results in a smaller decrease in ATP and phosphocreatine and a smaller rise in ADP, inorganic phosphate and creatine (Holloszy and Coyle, 1984). Additionally, it is probable that after high intensity endurance training, marked increases in the respiratory capacity of type II fibres (with their innately low aerobic capacity in the untrained state) will be a major factor responsible for the lower lactate production and enhanced endurance capacity of the muscle. Exercise intensities which exceeded the respiratory capacity of these fibres prior to training would be more likely to fall within their submaximal (aerobic) capacity after training. In effect therefore, a training-induced increase in oxidative enzyme activity per unit mass of muscle probably results in a given absolute work rate requiring a reduced percentage of the muscle's maximum respiratory capacity, so that both the rate of substrate flux within each muscle cell and the extent of intracellular homeostatic disturbance for balancing a given rate of ATP hydrolysis would be lower in the trained state.

An enhanced capacity for oxidative substrate metabolism in endurance trained muscle leads to a higher rate of fatty acid oxidation and a more economic utilisation of intramuscular and hepatic glycogen (Holloszy et al, 1977). A higher capillary density in the muscle vascular bed will facilitate the delivery of oxygen and free fatty acids (FFA) to the

mitochondria (Klaussen et al, 1981). As a consequence of smaller changes in intracellular ADP, ATP and inorganic phosphate concentration for a given work rate in endurance trained muscle, glycolysis should be activated to a lesser extent and a high rate of fatty acid oxidation will tend to limit carbohydrate utilisation in the muscle by accelerating the rate of citrate production (Randle et al, 1964; Rennie and Holloszy, 1977). Citrate inhibits phosphofructokinase (the enzyme catalysing the "rate limiting" step in the glycolytic pathway) and leads to the accumulation of hexose phosphate compounds; thus glycogenolysis and glucose uptake by the muscle are limited and fatty acid oxidation is further enhanced (Rennie and Holloszy, 1977). This may explain the mechanism underlying the gradual shift towards fat metabolism as indicated by a fall in the whole body respiratory exchange ratio (R) during prolonged steady state exercise (Havel, 1971), an essential adaptation as glycogen stores gradually dwindle.

The relative quantitative importance of the various training-induced biochemical adaptations within the recruited muscle fibres in effecting an enhancement of aerobic metabolism and hence lipid oxidation is presently uncertain. Although the activities of various oxidative enzymes correlate positively with exercise time to exhaustion as well as the capacity for lipid metabolism in skeletal muscle in vitro (Holloszy et al, 1977), such a relationship does not hold in vivo (Costill et al, 1979). This would suggest that the major factor limiting the muscle's capacity for lipid oxidation is not its oxidative enzyme activity; one or a number of other factors evidently impose a greater limitation.

A possible limitation to fatty acid oxidation during exercise is the actual rate of delivery of oxygen and/or FFAs to the muscle cell, both of which are governed by muscle blood flow and hence capillary density. Exercise time to exhaustion and lactate threshold at various submaximal work rates during cycle ergometer exercise has been shown to be highly correlated with capillary density in the recruited muscles, which in turn correlate positively with the number of years vigorous endurance training (Coyle and Coggan, 1988). An enhanced delivery of oxygen and FFAs to the contracting fibres due to the combined effects of a higher VO_2max and muscle capillary density will result in a greater rate of diffusion of

these compounds into the muscle sarcoplasm and higher concentrations in close proximity with the mitochondria. The capacity for lipid oxidation should be further enhanced by training-induced mitochondrial proliferation and elevated oxidative enzyme activities.

Muscle perfusion also depends upon the size of the muscle mass recruited during exercise, which in turn determines the extent of centrally mediated arteriolar vasoconstriction at the entrance to the muscle vascular bed. During dynamic exercise, the accumulation of metabolites effects a locally mediated vasodilatation in the exercising muscles, such that blood is directed through the muscles at the expense of the systemic blood pressure. The latter is maintained by sympathetic vasoconstriction to less active organs such as the gut and kidneys. However, if blood pressure starts to fall, peripheral vascular resistance is elevated by the additional enhancement of vascular tone in the active muscles, which overrides the local vasodilatation resulting in decreased muscle perfusion (Andersen and Saltin, 1985).

Provided that dietary carbohydrate intake is adequate (and especially if it is increased), endurance training also enhances the glycogen storage capacity of the muscles recruited in training. Exercise-induced depletion of muscle glycogen culminates in an increased capacity for glycogen storage or "glycogen supercompensation" phenomenon (Bergstrom and Hultman, 1966). Since the initial glycogen content of the muscles recruited during a bout of prolonged submaximal exercise is highly positively correlated with exercise time to exhaustion (Bergstrom et al, 1967), endurance capacity can be greatly enhanced by training-induced glycogen sparing and supercompensation (Ahlborg et al, 1967; Brewer et al, 1988). Thus, the benefits of endurance training with respect to glycogen economy is a dual one; it not only aids the preservation of glycogen stores during prolonged exercise, but can also increase their initial size.

Endurance training also appears to increase the capacity of the trained muscles to store triglycerides, which may provide a major source of energy during prolonged submaximal exercise (Froberg and Mossfeldt, 1971). Intramuscular triglycerides have been shown to decrease sufficiently during prolonged exercise as to account for a considerable portion of total lipid

oxidation (Essen, 1977). Although intramuscular triglyceride content is generally 2-3 times higher in type I as compared to type II fibres (Essen, 1977), trained skeletal muscle has a larger total triglyceride store than untrained muscle and the extent of intramuscular triglyceride utilisation during prolonged submaximal exercise is significantly greater in trained than in untrained individuals (Lithell et al, 1979). A bout of prolonged submaximal exercise increases muscle lipoprotein-lipase (LPL) activity, which aids the restoration of depleted intramuscular triglycerides with plasma free fatty acids FFA's and triglycerides during the post exercise recovery period (Lithell et al, 1979). Basal LPL activity is higher in endurance-trained than in untrained skeletal muscle and a positive correlation exists between basal muscle LPL activity and the plasma HDL fraction (Nikkila et al, 1978), which may suggest an increased capacity for plasma triglyceride-rich phospholipid degradation in endurance trained persons. Plasma triglycerides are not considered to contribute significantly to ATP resynthesis during exercise (Dufaux et al, 1982); thus the higher HDL cholesterol fraction in endurance trained individuals may result from an enhanced rate of plasma triglyceride catabolism during the post-exercise replenishment of intramuscular triglycerides.

Recent research suggests that a large proportion of the "extra" fat metabolised by endurance-trained muscle is derived from intra-muscular as opposed to adipose tissue triglyceride stores. From simultaneous measurements of whole body respiratory exchange ratio (R) and FFA uptake by working muscle during steady state exercise it has been estimated that plasma FFAs contribute approximately 50% to the total lipid oxidation in untrained persons, whilst the remainder is considered to be derived mainly from intramuscular stores (Essen et al, 1977). Although endurance-trained persons oxidise a significantly greater proportion of fat relative to carbohydrate than untrained persons during prolonged submaximal exercise of similar relative intensity, the muscular extraction of plasma FFAs appears to be no different between trained and untrained groups (Janssen and Kaijser, 1987). This points towards an enhanced oxidation of intramuscular triglyceride in the trained population. Measurements of intramuscular triglyceride and glycogen contents within the same muscle before and after a bout of prolonged submaximal exercise have revealed the existence of a positive correlation between the extent of triglyceride utilisation and training status. In contrast, the extent of glycogen utilisation which is

markedly greater in untrained relative to trained individuals (Lithell et al, 1979).

Plasma concentrations of FFAS and glycerol tend to increase during prolonged submaximal exercise as a result of an enhanced secretion of lipolytic hormones, which mobilise stored lipids from adipose tissue (Galbo, 1983). Although the circulating FFA concentration represents a dynamic equilibrium between the rates of mobilisation and utilisation, plasma glycerol concentration provides a rough indication of the extent of adipose tissue lipolysis, since the extent of glycerol metabolism appears to be minimal during exercise (Hetenyi et al, 1975) and glycerol does not serve as a significant glyconeogenic substrate (Miller et al, 1983). Since the uptake of FFAS by working muscle occurs by diffusion, a close relationship exists between the plasma FFA concentration and the rate of FFA oxidation in muscle (Hagenfeldt, 1975). A training-induced elevation of VO_2max leads to a given submaximal work rate representing a lower relative exercise intensity than in the untrained state, such that the magnitude of the hormonal response to the same absolute work rate and consequently the extent of lipid mobilisation is reduced post training (Rennie et al, 1974; Winder et al, 1979). However, despite the lower plasma FFA concentration, the rate of lipid oxidation by the exercising muscles has been found to be greater (as indicated from R values) (Rennie et al, 1974; Winder et al, 1977). A quantitative study of intramuscular glycogen and triglyceride utilisation, in addition to R values and circulating concentrations of lactate, FFA's and glycerol during submaximal cycle ergometer exercise of the same absolute intensity in a group of individuals pre- and post-training appeared to confirm that the greater utilisation of lipid as energy substrate in the trained state is attributable to an enhanced lipolysis of intramuscular as opposed to extramuscular triglyceride (Hurley et al, 1986). Circulating concentrations of glycerol, FFAS and lactic acid were lower during exercise of the same absolute load after training, as were R values and the extent of muscle glycogen utilisation. However, the decrease in quadriceps muscle triglyceride concentration was significantly greater in the trained state and the authors calculated that the "extra" fat utilisation could feasibly be accounted for by the enhanced muscle triglyceride lipolysis (Hurley et al, 1986). Nevertheless, the precise source of the "extra" fat oxidised by endurance trained muscle remains controversial, in that higher plasma FFA and glycerol concentrations,

concomitant with a higher plasma concentration ratio of FFAs. to glycerol have been measured in certain trained individuals exercising at the same relative exercise intensity as untrained individuals (Friedmann and Kindermann, 1979) which would suggest that the extents of extramuscular lipid mobilsation and utilisation are both greater in the trained state.

Thus, it is evident that a person's "training status" exerts a significant effect on their endurance capacity and that endurance trained persons can continue to exercise at a given relative submaximal work rate longer than untrained persons by making more efficient use of storage fuels and avoiding acid-base disturbances. However, for each and every individual, endurance capacity will be limited if the dietary intake of carbohydrate is insufficient to replenish muscle glycogen stores after exhaustive exercise (Bergstrom et al, 1967; Piehl, 1974). Similarly, endurance capacity can be greatly enhanced by the consumption of more carbohydrate without necessarily increasing the total energy content of the diet (Brewer et al, 1988). Diet thus exerts an important influence on muscle glycogen stores as well as substrate utilisation during exercise. The rate of glycogen resynthesis in glycogen depleted muscle fibres following prolonged submaximal exercise has also been shown to be optimal during the first hour of subsequent rest (Ivy et al, 1988); thus carbohydrates should be consumed as soon as possible after exhaustive exercise in order to enhance recovery.

Not only is the quantity of stored glycogen in the exercising muscles an important determinant of exercise time to exhaustion, but the hepatic store of glycogen also plays a significant role by providing the major source of circulating glucose during prolonged submaximal exercise in the post-absorptive state. The total hepatic glycogen content has been estimated to range from 75-90 grams in the well nourished adult (Wahren et al, 1975) and at least 60% of hepatic glycogen would appear to serve CNS metabolism (Reichard et al, 1961). Whilst many tissues, including skeletal muscle are able to metabolise fat as well as carbohydrate, the CNS depends solely upon carbohydrate as a fuel source and has a limited glycogen storage capacity (Newsholme and Leech, 1983). Hypoglycaemia, defined as a blood glucose concentration below 3mmol l^{-1}, occurs when tissue uptake of glucose exceeds hepatic the output and has been shown to reduce endurance

capacity during prolonged low intensity exercise even when muscle glycogen stores are still plentiful (Pruett, 1971; Rodahl et al, 1964). Although there is a gradual shift towards fat metabolism with increasing exercise time, both hepatic glucose output and muscle glucose uptake increase as exercise progresses, such that the susceptibility to hypoglycaemia is also increased (Rowell et al, 1965; Wahren et al, 1973). However, the resulting fatigue can often be reversed by the ingestion of glucose (Bonen et al, 1971).

Training-induced enhancement of fat metabolism helps limit the onset of hypoglycaemia during prolonged submaximal exercise by reducing the extent to which circulating glucose is utilised as energy substrate by working muscle. Hepatic as well as intramuscular glycogen utilisation is reduced after training (Fitts et al, 1975) and blood glucose concentration tends to remain higher during prolonged submaximal exercise in trained persons who generally maintain a euglycaemic state longer than untrained individuals exercising at the same relative intensity (Bloom et al, 1976; Jansen and Kaijser, 1987). In the event of hepatic glycogen depletion, blood glucose concentration must be maintained via the synthesis of glucose/glycogen from non-carbohydrate precursors such as amino acids (gluconeogenesis).

A high carbohydrate diet is thus vital for the maintenance of adequate muscle and liver glycogen stores during training and preparation for endurance events.

2.3 Making Stored Fuels Available: The Hormonal Response to Exercise

During prolonged submaximal exercise, the ATP concentration within active skeletal muscles tends to remain essentially constant and is maintained primarily via the aerobic catabolism of stored fuels; namely glycogen and triglyceride, stored both intramuscularly and extramuscularly. Before they can be utilised for ATP resynthesis within the muscle cell, extramuscular fuel stores must first be mobilised as utilisable substrate, transported to the muscles via the circulation, and diffuse into the muscle cell where their fate depends upon their specific chemical properties and the availability of oxygen.

The mobilisation of stored fuels during exercise is effected by a marked change in the circulating hormone milieu, namely an increase in the secretion of a number of glycogenolytic and lipolytic ("catabolic") hormones, concommitant with a decreased secretion of "anabolic" hormones. The body is thus rendered in a "catabolic" state to enable the repeated contraction and relaxation of skeletal muscle to continue in the absence of fatigue. The general circulating hormone milieu also varies with food ingestion and is subject to diurnal variation. Eating effects opposing changes in hormone secretion to that of exercise and renders the body in an "anabolic" state to an extent determined by the quantity and composition of food eaten. Fasting effects similar hormonal responses to that of prolonged submaximal exercise.

The patterns of "anabolic" and "catabolic" hormone secretion are closely linked to the control of blood glucose concentration. Avoidance of hypoglycaemia is central to homeostasis at all times on account of the dependency of the central nervous system (CNS) on glucose as energy substrate. The primary mechanism governing the control of blood glucose concentration is the pancreatic hormone insulin, the secretion of which increases in response to a rise in blood glucose concentration and declines when blood glucose falls. Insulin effects the removal of glucose from the blood to cellular storage sites by increasing the permeability of cell membranes to glucose and allowing the glucose to diffuse into the cell. Insulin also exerts a tonic inhibitory influence upon triglyceride lipolysis in adipose tissue (Newsholme and Leech, 1983).

The precise nature and magnitude of the hormonal response to prolonged submaximal exercise depends upon the intensity and duration of exercise in relation to the nutritional status (especially the carbohydrate stores) of the body. A centrally mediated increase in sympathetic nervous activity is evoked merely in anticipation of exercise and the sympathetic neurotransmitter noradrenaline effects an a-adrenoceptor mediated decline in insulin secretion by the pancreatic islet cells, thereby permitting an increase in adipose tissue triglyceride lipolysis as well as stimulating intramuscular and hepatic glycogenolysis (Pruett, 1971; Galbo et al, 1977). Noradrenaline also increases adipose tissue lipolysis directly via its stimulatory action on a-adrenoceptors. The consequent rise in plasma FFA concentration leads in turn to an increase in FFA uptake and turnover in the active muscles (Armstrong et al, 1961; Rodahl and Issekutz, 1965). As exercise continues, the output of catecholamines (mainly adrenaline) from the adrenal medulla, growth hormone (GH) from the anterior pituitary and glucagon from the pancreas increases. These hormones are all potent lipolytic and glycogenolytic stimulants and contribute to the sustained release of FFA's and glucose into the circulation. The extent of their secretion is closely linked to blood glucose concentration (Galbo, 1983) and avoidance of hypoglycaemia during prolonged exercise by the oral administration of glucose can significantly reduce the magnitude of their release. Similarly, their output intensifies when prolonged exercise is performed after the consumption of a low carbohydrate diet which reduces hepatic and intramuscular glycogen stores (Richter et al, 1976). At exercise intensities above approximately 60%VO_2max, plasma cortisol concentration tends to rise (Davies and Few, 1973) and stimulates the catabolism of cellular protein for the provision of precursors for hepatic gluconeogenesis. This functions as a protection against hypoglycaemia and is aided by the stimulatory effect of glucagon on the hepatic uptake of gluconeogenic precursors (Galbo, 1983).

Thus, whilst the exercise-induced increase in plasma FFA concentration is initiated by the sympathetic nervous system, the sustained mobilisation of FFA's and glycogen depends upon a rise in the circulating concentrations of various "catabolic" hormones (catecholamines, GH, glucagon and cortisol). The net result of the hormonal response to prolonged submaximal exercise is thus a marked increase in glycogen and triglyceride mobilisation for use by exercising skeletal muscle, plus an increase in

hepatic glycogenolysis and gluconeogenesis which aid the maintenance of blood glucose concentration and normal CNS metabolism. Muscular uptake of FFAs is a function of their plasma concentration (Armstrong et al, 1961; Hagenfeldt, 1985). Elevation of plasma FFA concentration enhances lipid oxidation even in resting skeletal muscle (Costill et al, 1977); however for any given plasma FFA concentration, FFA turnover is very much greater during exercise than rest (Issekutz et al, 1964). As exercise continues, the muscles tend to "adapt" to fat metabolism (Havel, 1971) on account of dwindling glycogen stores and the inhibitory effect of an increased circulating citrate concentration on phosphofructokinase and glycogen catabolism (Rennie and Holloszy, 1977). In effect therefore, as many tissues as possible are forced to rely upon fat as an energy source so that carbohydrate may be preserved for CNS metabolism.

Effect of endurance-training on the hormonal response to submaximal exercise:

The hormonal response to exercise of a given submaximal intensity is less marked in the endurance trained individual. There is little evidence to suggest that basal concentrations of circulating hormones in the resting individual are modified by training, however plasma concentrations of catecholamines, glucagon and GH have been shown to be significantly lower, and insulin significantly higher in trained cyclists relative to untrained individuals during cycle ergometer exercise at various intensities (Bloom et al, 1976). Endurance trained individuals also tend to present significantly higher plasma concentrations of glucose and lower plasma lactate concentrations during exercise and to metabolise a relatively greater proportion of fat than untrained persons who rely to a greater extent upon hepatic and intramuscular glycogen as energy substrate (Galbo, 1983; Janssen and Kaijser, 1987).

The precise mechanisms underlying these adaptations are uncertain. The enhanced aerobic capacity of endurance-trained skeletal muscle will certainly reduce its requirements for carbohydrate during exercise with the result that homeostasis is stressed to a lesser extent and the magnitude of hepatic and muscle glycogen catabolism is less marked than in the untrained individual. Hormone secretion is controlled via negative feedback

mechanisms, according to the body's overall requirements for a particular hormone. If the response effected by a hormone is beyond, or falls below the requirements of homeostasis then subsequent output of that hormone is decreased or increased appropriately. Adaptation to exercise stress may alter the density of specific hormone receptors in certain tissues, or the substrate affinity of these receptors such that less substrate is required to mediate a response of given magnitude.

Factors governing substrate utilisation during prolonged submaximal exercise:

The choice of fuel for ATP resynthesis during sustained exercise of submaximal intensity is mainly limited to carbohydrate and fat. The extent to which tissue protein is catabolised for energy substrate remains controversial, but appears to be governed by the training status of the individual as well as the nutritional state of the body. The bulk of the literature concerning exercise protein catabolism as evidenced from measurements of urinary nitrogen excretion during and after exercise has suggested that the significance of protein as an energy substrate is minimal during exercise provided that the individual remains in a state of energy balance (Cathcart and Burnett, 1926; Hedmann, 1956; Calles-Eskendon et al, 1984; Wolfe et al, 1984). However a number of studies do not confirm these findings and suggest that the contribution of protein to total exercise energy consumption may be quite marked, especially in untrained individuals and/or those in whom glycogen stores are limiting during exercise.

During the early stages of an endurance training program, previously untrained individuals in positive energy balance with a daily protein intake amounting to at least 1g/kg body mass/day attained a state of negative nitrogen balance at the start of an endurance training programme of cycle ergometer exercise for 120 minutes/day. Nitrogen loss increased to a peak 3-4 days after the start of training and gradually declined thereafter, such that subjects reattained a balance after about 2 weeks of training (Gontzea et al, 1975). A similar pattern of increased protein catabolism followed by a subsequent effort to conserve tissue protein has also been demonstrated during the first few weeks of starvation (Saudek and

Felig, 1976). Additionally, trained individuals consuming insufficient quantities of carbohydrate to adequately restore hepatic and muscle glycogen reserves between training sessions may also assume a state of negative nitrogen balance. Serum and sweat urea nitrogen concentration during the later stages of prolonged submaximal exercise and recovery has been shown to be significantly higher in subjects exercising after the consumption of a low carbohydrate as compared to a high carbohydrate diet (Lemon and Mullin, 1980) which would suggest that the extent of protein catabolism during prolonged submaximal exercise is related to the magnitude of intramuscular and/or hepatic glycogen stores. In the face of depleted glycogen stores, the capacity of the muscles to resynthesise adequate amounts of ATP to sustain a given exercise intensity will be limited such that fatigue and/or hypoglycaemia ensue. An efflux of enzymes from exercising muscle has been measured during prolonged activity (Wilkinson and Robinson, 1974; Kamen et al, 1977) and studies on isolated muscle suggest that this efflux coincides with the onset of fatigue (Thomson et al, 1975). It has been suggested that a fall in ATP concentration in the working muscle due to intramuscular glycogen depletion results in a decrease in membrane permeability (perhaps due to decreased phospholipid resynthesis) culminating in the leakage of protein from muscle cells into the circulation (Wilkinson and Robinson, 1974). Degradation and the subsequent deamination of these enzymes could theoretically provide carbon skeletons as oxidisable substrate.

It would therefore appear that the metabolic situation during prolonged submaximal exercise, especially in the face of limited glycogen stores resembles that of short term starvation which is characterised by protein catabolism (Saudek and Felig, 1976). In both such cases, the depletion of intramuscular and/or hepatic glycogen coincides with an increase in serum urea and a fall in serum amino nitrogen (Heralambie and Berg, 1976) as well as a marked increase in the release of alanine from skeletal muscle (Felig and Wahren, 1971). Given that skeletal muscle protein appears to contain little alanine (Kominz et al, 1954) the theory pertains that "de novo" alanine synthesis, via the transamination of other proteins occurs within the muscles and that this particular amino acid forms an important gluconeogenic substrate (Newsholme and Leech, 1983). It is possible that a decline in insulin secretion and increase in glucocorticoid secretion may play a role in protein degradation. Thus,

carbohydrate loading prior to the performance of prolonged submaximal exercise may also exert a significant protein (as well as a glycogen) "sparing" effect.

The extent to which one or another of fat, carbohydrate and protein contribute as energy substrate for contracting skeletal muscle during prolonged submaximal exercise in any given healthy individual is influenced by the following factors:

i) The intensity of exercise relative to VO_2max,
ii) The duration of exercise,
iii) Whether the exercise is performed at a steady pace, or intermittantly with sudden changes in pace,
iv) The "training status" of the individual,
v) Diet and the magnitude of intramuscular and hepatic glycogen stores.

Exercise intensity governs the rate at which the muscles utilise ATP and therefore the speed at which it must be resynthesised. ATP can be resynthesised more rapidly from carbohydrate than fat, thus the contribution of carbohydrate to energy supply increases exponentially at exercise intensities beyond 70-75% VO_2max (Davies and Thompson, 1977). Additionally, the strain placed upon the cardiovascular system is proportional to relative exercise intensity and at exercise intensities close to VO_2max, the cardiovascular system must compromise between the maintenance of systemic blood pressure and muscle perfusion with the result that the delivery of oxygen and nutrients may be limited by a centrally mediated vasoconstriction to the skeletal muscle vascular bed (Andersen and Saltin, 1985).

With increasing duration of exercise, stores of intramuscular and hepatic glycogen gradually decline resulting in hypoglycaemia and fatigue (Christensen and Hansen, 1939; Wahren et al, 1973). The homeostatic response to hypoglycaemia is one of an increased secretion of various "catabolic" hormones including adrenaline, glucagon, GH and cortisol (Galbo, 1983) the actions of which have been described previously. Exercising muscle is ultimately forced to metabolise a relatively greater proportion of fat (and perhaps protein) than carbohydrate and it would appear that this gradual shift towards fat metabolism during prolonged

submaximal exercise is aided by the increased production of citrate which exerts an inhibitory effect upon glycolysis (Rennie and Holloszy, 1977).

Intermittent exercise is less efficient with regard to glycogen economy than steady pace exercise since a sudden increase in intensity abruptly increases the muscle's demand for ATP and temporarily exceeds the circulatory capacity to supply the muscles with adequate volumes of oxygen (Newsholme and Leech, 1983). If the time lag between increased oxygen demand and supply in the muscles is particularly marked, ATP must be produced via anaerobic glycolysis, which culminates in the rapid utilisation of intramuscular glycogen and the production of lactic acid. A similar situation occurs at the start of exercise if the individual has not "warmed up" adequately and the relative intensity is high. Muscle vasodilatation is effected via a local accumulation of metabolites and so muscle perfusion is limited during the initial stages of exercise.

The influence of training status upon substrate metabolism during prolonged submaximal exercise has been discussed at length in previous chapters. The biochemical adaptations effected within the muscle fibres recruited during the training exercise after many months or years of endurance training greatly enhance the muscle's aerobic capacity, so permitting more fat to be metabolised as energy substrate.

Diet in the preceding days and hours up to the performance of prolonged submaximal exercise can influence substrate metabolism in a number of different ways. Firstly, provided that the individual is in energy balance, the composition of the diet, especially the carbohydrate content, influences the size of intramuscular and hepatic glycogen reserves. These stores can be optimised by a high carbohydrate diet, regular training and a "tapering" in the training load prior to the performance of exercise (Ahlborg et al, 1967; Brewer et al, 1988). A high carbohydrate diet tends to stimulate a hyperinsulinaemic response, characterised by a low circulating concentration of FFAs and a greater contribution of carbohydrate to energy metabolism (Issekutz, 1963; Maughan et al, 1978), but nevertheless increases exercise endurance time (Bergstrom et al, 1967; Brewer et al, 1988).

Inherent qualities, especially concerning the muscle's morphological and biochemical properties will also influence the preferred choice of energy substrate for exercising muscle. Individuals with a predominance of type I ("high oxidative") fibres are potentially the most successful endurance athletes whilst those possessing a relatively greater proportion of type II ("low oxidative") tend to make the best sprinters (Costill and Higdon, 1980; Holloszy and Coyle, 1984). The former possess a greater potential capacity for fat metabolism and tend to produce less lactic acid than the latter during steady state exercise (Gollnick et al, 1972).

2.4 Possible Differences in the Metabolic Responses of Men and Women to Prolonged Submaximal Exercise

Endurance training effects major adaptations within many of the body's organ systems, most notably the cardiovascular system and skeletal muscle, which undergo distinct structural and biochemical changes. More subtle adaptive responses also occur within the thermoregulatory and neuroendocrine systems as well as adipose tissue. The majority of these adaptations are evidently beneficial and serve to limit the magnitude of stress imposed upon the organism, allowing exercise to be performed in the absence of major homeostatic disturbance. The nature of such adaptations are similar in both sexes and when challenged with endurance training programmes of similar intensity and volume, the magnitude of the improvements in cardiovascular and endurance performance appear to be comparable in men and women (Edwards et al, 1974; Kearney et al, 1976; Pedersen and Jorgensen, 1978). It has been stated that "the cellular mechanisms controlling the physiological and biochemical responses to exercise are identical for both sexes; differences are purely quantitative rather than qualitative" (Fox and Matthews, 1980). Although many of the mechanisms underlying these adaptations do appear to be similar (Daniels et al, 1979; Eddy et al, 1977, Burke, 1977), on account of the many physical, physiological and metabolic differences known to exist between the adult male and female it may be unjustifiable to assume that training induced adaptations are both quantitatively and qualitatively similar in the absence of supporting experimental evidence.

Van Aaken, (1976) and Ullyot, (1976) hypothesised that women might be better suited to endurance-type activities than men on account of their smaller physique, smaller muscle mass and more especially, their greater energy stores (as stored triglyceride in adipose tissue). The assumption underlying this hypothesis is that endurance capacity is limited by the body's total energy stores and that adipose triglyceride may be mobilised and converted to oxidisable substrate for ATP resynthesis more readily when stored in large amounts. However, exhaustion during prolonged exercise tends to coincide with glycogen depletion in the recruited muscle fibres (Bergstrom et al, 1967) and there is certainly no correlation between endurance capacity and body fat content [except in channel swimming where

subcutaneous fat serves as insulation and aids bouyancy, (Holmer, 1974)]. In fact, the most successful endurance athletes are generally very much leaner than their sedentary counterparts (Wilmore and Brown, 1980) but are able to sustain a given relative exercise intensity for a prolonged duration on account of training induced biochemical adaptations effected within their skeletal muscle (Holloszy and Coyle, 1984).

Although it is true that the "typical" woman would probably outlive her male counterpart during an enforced fast, the metabolic stress imposed by muscular exercise tends to limit the extent to which stored triglyceride can be used as an energy substrate (Davies and Thompson, 1977). If a woman's abundant fat stores are to confer any beneficial effect on her capacity to endure prolonged exercise, FFAs would have to be mobilised from triglyceride stores within adipose tissue, transported to the necessary sites (muscle cell mitochondria) and be oxidised to produce ATP. Thus, any gender related differences in the relative contribution of fat and carbohydrate to energy metabolism during exercise would be likely to result from one or a combination of the following factors:

i) Sex differences in the morphological and/or metabolic properties of skeletal muscle which potentiate the capacity for fat metabolism.

ii) Sex differences in the capacities of skeletal muscle and/or other organ systems to adapt to endurance training.

iii) Sex differences in the glycogen and/or triglyceride storage capacity of skeletal muscle.

iv) Sex differences in the hormonal response to exercise which potentiate the capacity for fat and/or glycogen catabolism and oxidation.

v) Direct and/or indirect effects of circulating sex steroids on muscle metabolism and/or the circulating hormonal milieu at rest and during exercise.

vi) Sex differences in the biochemical properties of adipose tissue which lead to an increased potential for triglyceride lipolysis and FFA mobilisation in women.

There exists a number of physiological differences between adult men and women which could potentially influence the preferred choice of substrate for exercising skeletal muscle. Most become evident at the time of adolescence, concommitant with an increase in the circulating concentrations of ovarian/testicular sex steroid hormones. However, there remains a marked controversy with regard to the magnitude of such differences as well as their potential significance. Gender-related physiological differences which prevail at rest may either be magnified or diminished during the metabolic challenge of prolonged submaximal exercise and may only apply to the non-specifically trained male and female population.

i) Sex differences in the morphological and/or metabolic properties of skeletal muscle:

Studies on the morphological, histochemical and metabolic properties of male and female skeletal muscle suggest that the latter may possess a greater potential for oxidative metabolism than the former. Such differences have only been reported in the adult and are not apparent in pre-pubescent children (Lundberg et al, 1979; Hedberg and Janssen, 1986) which points to an influential role of the sex steroids on skeletal muscle development and metabolism initiated during adolescence. Although the bulk of the literature supports the concept that there is no difference in the incidence of fast and slow twitch fibres between male and female skeletal muscle (Prince et al, 1977; Komi and Karlsson, 1978; Nygaard, 1981) a number of studies suggest that the relative proportion of type I fibres is greater in female than in male muscle (Jansson, 1980; Simoneaou et al, 1985). The observation that all fibre types are relatively smaller in the skeletal muscle of untrained women than untrained men remains a consistant finding (Costill et al, 1976; Prince et al, 1977) and could serve a potential role in the facilitation of gaseous and metabolic exchange between the exercising muscle and the circulation, since the volume of muscle fibre perfused by each capillary would be less in women. Gender differences in fibre size appear to be absent, or much less marked in endurance trained individuals (Costill et al, 1976) which might suggest that any degree of muscle fibre hypertrophy effected in response to a training stimulus is greater in women than men.

The ratio of the cross sectional area of type I to type II fibres in the vastus lateralis muscle of women has been shown to be higher than in men, resulting in a correspondingly greater ratio of oxidative to glycolytic enzyme activity and perhaps a greater potential for oxidative phosphorylation (Nygaard, 1986). However, an in- vitro study of the oxidative capacities of skeletal muscle from trained men and women actually indicated a greater potential for oxidative metabolism in the former (Costill et al, 1979). The in-vitro activities of selected oxidative enzymes and the capacity for lipid oxidation were both higher in male than female subjects, however there was no difference in the metabolic responses of the same individuals to prolonged submaximal treadmill exercise. An interesting observation that the amount of fat located between skeletal muscle fascicles is markedly greater in sedentary women than in sedentary men (Prince et al, 1977) might play an influential role in substrate metabolism by serving as an easily accessable form of energy substrate.

ii) Sex differences in circulating lipolytic/glycogenolytic hormones:

A sex difference in the circulating concentrations of various lipolytic and glycogenolytic hormones has been observed to exist, both at rest and during exercise and there is evidence to suggest that there may be differences in the magnitude and nature of the hormonal response to exercise between men and women. Basal circulating concentrations of GH and insulin have been reported to be higher in women (Franz and Rabkin, 1965; Nygaard et al, 1984; Bunt et al, 1986) and GH secretion appears to be coupled to the phase of the menstrual cycle, with a greater output during the luteal phase (Franz and Rabkin, 1965). The noradrenaline response to submaximal exercise of a given relative intensity would seem to be similar in men and women (Favier et al, 1983; Tarnopolsky et al, 1990) and the extent of adrenaline, glucagon, cortisol and insulin secretion to be governed essentially by preceding diet and the nutritional state of the body (Galbo, 1983; Tarnopolsky et al, 1990) as opposed to gender-related factors (Friedmann and Kindermann, 1989). However, there have been reports of higher circulating concentrations of lipolytic and glycogenolytic hormones in women than men during the early stages of a prolonged bout of submaximal exercise (Nygaard et al, 1984; Bunt et al, 1986).

Systemic blood pressure tends to be lower in women than in men at rest and during various submaximal exercise intensities, due to a lower total peripheral vascular resistance of the former (Petrofsky et al, 1975; Gleerup-Madsen et al, 1975). Thus, muscle perfusion may be greater in women than men during submaximal exercise.

iii) Sex differences in adipose tissue metabolism and the influence of sex steroid hormones:

Adipocytes at different sites in the body appear to differ both quantitatively and qualitatively in their metabolic properties and to be strongly influenced by a number of the female reproductive hormones. For example, catecholamines have been shown to effect a greater lipolytic response from adipocytes isolated from the abdominal as compared to the femoral region in women (La Fontan et al, 1978; Smith et al, 1979). This is probably attributable to the lower LPL activity of abdominal than femoral adipocytes (Olesky et al, 1974), since adipose tissue LPL activity is inversely related to the lipolytic responsiveness of the tissue and positively correlated with it's capacity to store triglyceride (Zinder et al, 1974). Such differences have not been observed in men and would appear to be strongly influenced by the absolute and relative circulating concentrations of oestradiol, progesterone and prolactin in accordance with the body's energy requirements during pregnancy and lactation (Rebuffe-Scrive et al, 1985). During pregnancy, when maternal energy stores must be optimised, the LPL activity of adipose tissue increases (especially in "sex specific" sites), concomitant with a decline in the in-vitro basal and noradrenaline stimulated lipolytic activity of this tissue. Conversely, the increased energy requirements of lactation appear to be met by a generalised decline in adipose tissue LPL activity, coupled to an increase in the lipolytic activity of isolated adipocytes (Rebuffe-Scrive et al, 1985). A significant positive correlation between circulating oestradiol concentration and adipose tissue lipolytic activity has also been observed by the forementioned authors.

Thus, it would seem that the "sex specific" storage fat of females can be made to be more or less readily mobilisable according to bodily requirements. Women generally possess a greater number of adipocytes than

men, which are larger in size, especially in the gluteal region (Sjostrom et al, 1972) and gluteal subcutaneous fat deposits appear to exhibit a greater response to lipolytic agonists than adipocytes from other sites (Rognum et al, 1982). Adipose tissue lipoprotein-lipase (LPL) activity is very much greater in women than men and correlates positively with plasma HDL levels (Nikkila et al, 1978). Androgens as well as oestrogens influence the deposition, regional distribution, morphological and metabolic properties of adipose tissue and probably also the plasma lipoprotein profile (Despres et al, 1978; Kissebah and Peiris, 1989). Endurance training increases adipose tissue LPL activity and HDL levels in men and women, yet its activity remains higher in untrained women than in trained men (Nikkila et al, 1978). Such a finding might be indicative of a greater capacity for adipose tissue triglyceride storage in women via the catabolism of circulating triglyceride-rich lipoproteins and a subsequent raising of plasma HDL cholesterol levels.

There appears to be no sex difference in the in-vitro basal and adrenaline-stimulated lipolytic activity of isolated adipocyte biopsies obtained from untrained men and women. However, endurance training actually appears to enhance in-vitro adrenaline-stimulated lipolysis to a greater extent in males than females (Despres et al, 1978). An exponential rise in in-vitro adrenaline-stimulated lipolysis with increasing exposure to agonist has been shown to exist in endurance trained individuals; however this steep "dose-response" curve to adrenaline was not evident in untrained individuals of either sex (Crampes et al, 1989). Adherence to an intense endurance training programme tends to reduce body weight and adiposity to a greater extent in men than women (Despres et al, 1978; Krotiewski et al, 1983) although it is possible that women are better able to match the energy demands of training with a compensatory increase in food intake. Oestrogens and progestogens are known to influence appetite and physical activity patterns, especially during pregnancy (Rebuffe-Scrive et al, 1985), so may increase a woman's resistance to weight (especially fat) loss when energy expenditure is increased. Such findings, would thus tend to point to a greater conservation of fat, rather than an enhanced lipolytic potential in women relative to men.

The circulating sex steroid hormone milieu of the adult male and female appear to exert a definite influence on the metabolic response to

exercise (Jurkowski et al, 1978; Bonen et al, 1979 and 1983; Brown et al, 1986). These hormones may exert a direct effect on the morphology and/or biochemical properties of skeletal muscle/adipose tissue, or may do so indirectly by modifying the secretory pattern of other lipolytic and glycogenolytic hormones concerned with the control of substrate metabolism during rest and exercise. Plasma concentrations of ovarian steroids fluctuate throughout the menstrual cycle in the female; are at their lowest (basal) levels during the early follicular phase and attain peak concentrations during the mid luteal phase of the cycle (Bonen et al, 1983). Lower R values have been measured in women exercising during the luteal phase than during the follicular phase of their menstrual cycles (Bonen et al, 1979; Jurkowski et al, 1981) and blood lactate concentration has been shown to be lower and exercise time to exhaustion to be greater during exercise in the luteal phase (Jurkowski et al, 1978; Lavoie et al, 1987). After a low carbohydrate diet, women exhibited lower blood glucose concentrations but higher circulating concentrations of catecholamines, cortisol and glucagon during exercise in the luteal than the follicular phase (Lavoie et al, 1987). Synthetic oestrogens and progestogens in oral contraceptive steroid preparations also appear to influence carbohydrate and lipid metabolism at rest (Hansen and Wecke, 1974; Kim and Kalkhoff, 1975; Jensen et al, 1987) and to facilitate lipid oxidation during exercise by elevating cortisol and GH release (Bunt et al, 1990).

Although such findings indicate a concentration dependent effect of ovarian steroid hormones on exercise metabolism, the findings of some studies suggest that sex steroid hormones may play a more "permissive" role in substrate handling and that any metabolic influences of sex hormones are more likely to be low in threshold and concentration independent (Brown et al, 1986). Unfortunately, it is not easy to assess the metabolic properties of single sex steroid hormones in isolation, since the effective circulating concentration of such a hormone is also influenced by the concentration of other sex steroids and sex hormone binding globulin as well as the density and activities of receptors within a tissue (Bunt, 1986). However, in-vivo experimentation using the ovariectomised, exercising rat injected with various physiological-pharmacological doses of synthetic oestrogens have revealed the existence of a positive dose-response relationship between oestrogen and exercise duration, coupled to a higher post exercise hepatic and intramuscular glycogen content in those

animals receiving the highest dose of hormone (Kendrick et al, 1987). Oestrogen treated ovariectomised rats have also been shown to release a higher proportion of expired $^{14}CO_2$ from administered labelled palmitate relative to labelled glucose than oestrogen deficient rats (Hatta et al, 1988). These results all suggest that oestrogens enhance lipid oxidation in the rat, but whether or not the generalised circulating hormonal milieu of the normal adult female may potentiate fat metabolism during prolonged submaximal exercise is less clear.

iv) The influence of training on sex differences in exercise metabolism:

At present, available evidence would seem to suggest that if a gender related difference in substrate metabolism does exist in the human, it is confined almost exclusively to sedentary or moderately trained individuals and is often undetectable in the highly endurance trained population of male and female athletes (Bransford and Howley, 1979; Costill et al, 1979; Wallace et al, 1980; Powers et al, 1980).

Precisely why endurance training might reduce or negate any gender-related differences in the capacity for lipid metabolism during submaximal exercise is not really understood. The magnitude of many endurance training induced adaptations (especially those effected within skeletal muscle) are so great that they far override existing gender-related differences. Training may remove or reduce the magnitude of one or a number of innate factors which limit the oxidative capacity of skeletal muscle in either sex and may effect metabolic changes in either but not both sexes which serve as a limitation to oxidative metabolism. Alternatively training induced adaptations may differ quantitatively and/or qualitatively between the sexes such that previously existing innate differences are diminished.

2.5 Neuroendocrine Responses and Adaptations to Endurance Training

Endurance training can effect widespread alterations in endocrine control and metabolism, many of which are subtle, asymptomatic and remain undetectable unless the individual is subjected to invasive investigative study. Aberrations in the synthesis, secretion and metabolism of many hormones have been observed to prevail in the highly trained male and female, many of which appear to stem from deviations in hypothalamic functioning (Veldhuis et al, 1985). The hypothalamus is a leading homeostatic organ which receives neural and humoral input signals from all major organ systems and so is constantly informed of the body's physical and emotional state. By detecting metabolic deviations which might potentially disrupt homeostasis, it ultimately reverts the body to a state of balance by initiating appropriate efferent responses. It exerts a dominant control over the pituitary gland, gonads, thyroid, adrenal medulla and cortex as well as playing a major role in autonomic nervous control, blood pressure, glucose homeostasis, thermoregulation, appetite and sexual activity (Newsholme and Leech, 1983).

As yet, it is uncertain as to whether disruptions of neuro-endocrine function effected through endurance training are the direct result of exercise per se, or are effected in a more indirect manner through other training-induced adaptations such as changes in body composition. Even less is understood about the possible homeostatic role and potential outcome of training-induced neuro-endocrine adaptations. Whereas training-induced adaptations in cardiovascular function and skeletal muscle metabolism clearly benefit the organism by enhancing it's capacity to perform the exercise for prolonged duration in the absence of gross metabolic disturbance, the precise physiological purpose of neuro-endocrine adaptations remain uncertain. The role of such changes may be purely protective as opposed to conferring any benefit upon the organism with respect to performance capacity. In the female especially, it is vital that pregnancy is avoided if her energy stores are inadequate to nuture a developing foetus, thus the amenorrhoeic state might serve a contraceptive purpose.

The most obvious disruption to endocrine metabolism in the highly endurance trained individual is that of hypogonadism and reversible infertility. Hypogonadism is clearly manifested in women in the form of menstrual cycle disruption, but is less obvious in the men who have no regular cyclical indicator of reproductive function. However, there have been reports of low sperm counts coupled to depressed plasma testosterone and LH levels in highly endurance trained men (Wheeler et al, 1983; MacConnie et al, 1986).

Over recent years, a steady growth in the popularity of women's endurance sport has been parallelled by an increase in the reported incidence of menstrual dyssfunction in this group and it is now clear that such disturbances are not merely symptomatic of pre-existing neuro-endocrine derangements. Oligomenorrhoea (infrequent menstruation) and amenorrhoea (absent menses) are the most commonly reported menstrual disorders amongst highly active women, however, detailed analyses of fluctuating ovarian and pituitary hormone levels throughout the menstrual cycles of eumenorrhoeic (regulary menstruating) athletic women have revealed the existence of subtle and essentially asymptomatic endocrine changes (Dodson et al, 1975; Ross and Miller, 1978; Sherman and Korenman, 1974). Conditioning exercise appears to effect some degree of change in menstrual function within all women (Prior et al, 1984), ranging from a reduction of molimnal (pre-menstrual) symptoms and dysmenorrhoea (pain during menstruation), through a shortened luteal phase coupled to low plasma progesterone levels, anovulatory cycles and finally oligomenorrhoea or amenorrhoea (Shangold et al, 1979; Bonen et al, 1981; Prior et al, 1984). It appears that exercise effects a gradual depression of the hypothalamic-pituitary-gonadal axis (which mediates the chain of endocrine events culminating in menstruation) the extent of which increases with increasing training volume and/or intensity (Boyden et al, 1983; Bullen et al, 1985).

The precise mechanisms underlying the pathogenesis of exercise-associated amenorrhoea have not been fully elucidated, although the fact that the state is characterised by a persistent depression of circulating oestradiol, progesterone, luteinising hormone (LH) and to a lesser extent follicle stimulating hormone (FSH) (Shangold et al, 1979; Veldhuis, 1985; Kaiserauer et al, 1989), suggests that the disorder stems from aberrations

in neuro-endocrine control at the level of the hypothalamus and/or pituitary gland. It is likely that training-induced hypogonadism in the male is mediated via a similar mechanism to that in the female since the synthesis of androgens and spermatogenesis in men is controlled by the same pituitary hormones that regulate ovarian steroid hormone synthesis and follicular development in women (Santen, 1981).

In the mature woman, reproductive function is controlled via a complex series of interactions between the hypothalamus, anterior pituitary gland and ovaries. A possible "neural oscillator" in the region of the hypothalamus may activate the synthesis as well as the episodic release of gonadotropin releasing hormone (GnRH) into the hypophyseal portal blood from specialised neurones in the arcuate nucleus of the hypothalamus (Knobil, 1980). GnRH synthesis is tonically suppressed in the pre-adolescent child, however the removal of the inhibitory effect at puberty results in an amplification of the GnRH signal and the resultant stimulation of pulsatile pituitary gonadotropin (LH and FSH) secretion (Sherman et al, 1975). The gonadotropins in turn stimulate follicular proliferation and maturation within the ovaries. LH specifically promotes androgen production by the ovarian theca cells, whilst FSH induces an aromatase enzyme in the ovarian granulosa which converts these androgens to oestradiol (Hillier et al, 1980). Thus, as oocytes mature during the folliclular phase of the menstrual cycle, they secrete increasing amounts of oestradiol, which effectively controls pituitary gonadotropin secretion via a negative feedback action on hypothalamic GnRH release (Beaumont et al, 1976; Armstrong, 1980). Once the circulating oestradiol concentration exceeds a specific "threshold" value, it exerts a positive feedback effect on the pituitary gland, culminating in the pre-ovulatory LH surge which triggers ovulation (Yen and Tsai, 1972). As it passes down the fallopian tube, the ripened oocyte becomes the corpus luteum and secretes increasing amounts of oestradiol and progesterone into the circulation (Hillier et al, 1980).

In the amenorrhoeic female athlete or undernourished woman, the primary defect has been located at the level of the hypothalamus where it would seem that the synthesis and/or release of GnRH is suppressed (Veldhuis et al, 1985). The extent of neuro-endocrine disturbance is related to the amount of weight lost through diet and/or training, such

that the pituitary response to exogenous GnRH tends to be normal in all but severely emaciated women in whom it is depressed (Mechlenberg et al, 1977; Katz et al, 1977; Warren, 1977). The neuro-endocrine profile of such an individual resembles that of the pre-pubescent child, with depressed gonadotropin pulsations and persistently low and unvarying plasma oestradiol and progesterone levels (Boyar et al, 1975). The control of GnRH synthesis and release is known to be influenced by a number of neural and humoral stimulants and inhibitors. For example, noradrenaline stimulates GnRH secretion, whilst dopamine and B-Endorphin inhibit it (Kalra and McCann, 1979). The plasma concentrations of adrenocorticotrophic hormone (ACTH), cortisol, melatonin, prolactin, B-Endorphin and its precursor B-Lipotropin, all increase during exercise (Plotsky and Vale, 1984; Bullen et al, 1984), to an extent which depends upon the relative exercise intensity (Carr et al, 1981) and there is evidence to suggest that prolonged training may augment the exercise-induced B-Endorphin response (Carr et al, 1981). A sustained elevation of plasma cortisol (Glass et al, 1987; Ju Hong Ding et al, 1988) and B-Endorphin (Kaye et al, 1982; Hohtari et al, 1988) have been measured in highly trained men and women, the levels being higher in amenorrhoeic as compared to eumenorrhoeic female distance runners. As in the case of exercise related hypogonadism, the abnormality in cortisol secretion appears to be located at or above the hypothalamus and related to a sustained high release of corticotropin releasing factor (CRF) (Gold et al, 1986). Evidence suggests that cortisol and B-Endorphin interfere with GnRH synthesis and/or secretion (Kalra and McCann, 1979) and the opiate antagonist naloxone has been shown to restore near normal LH pulsitilty in some amenorrhoeic runners (Quigley and Yen, 1980; Roper et al, 1981; Veldhuis et al, 1985). The administration of B-Endorphin, ACTH, prolactin, and melatonin (the concentrations of which all increase in the blood and/or CSF during exercise) to experimental animals has also been shown to delay sexual maturation, impair oestrus cyclicity and/or depress luteal function (Knobil, 1974). It is also postulated that exercise-induced hyperthermia may suppress GnRH secretion (Hall et al, 1975).

As yet, the precise physiological influence of exercise per se upon neuro-endocrine functioning remains uncertain. Vigorous training in preparation for competition imposes a continual physical and emotional stress on the body and is associated with various physical and physiological changes which may be of aetiologic significance for the

development of amenorrhoea. Most notably a decrease in body weight and/or a relative increase in lean to fat body mass ratio (Frisch et al, 1981). Thus, whether or not exercise alone can disrupt menstrual cyclicity is hard to qualify. Although the bulk of the literature would suggest that regular exercise at least partially suppresses the hypothalamic-pituitary-gonadal axis (Prior, 1985; Ronkainen et al, 1985) and so effects menstrual cycle changes, the extension of clinical symptoms from irregular menstrual cycles to prolonged amenorrhoea is probably effected via the combined inhibitory influence of a number of physiological stressors coupled to endurance training. It would seem likely that exercise stress acts in synergy with one or a number of other influential factors to disrupt neuro-endocrine functioning, rather than actually inducing such changes by itself.

One of the most common causes of menstrual dysfunction in adolescent girls and young women is extreme dieting and/or weight loss as seen in the pathological eating disorder anorexia nervosa (Beaumont, 1979). Amenorrhoea is a reported symptom of starving women in developing countries (Fishman and Bradlow, 1977) and dietary restriction and/or the undernourished state appears to effect a variety of neuro-endocrine changes in the anorexic woman, the extent of which are related to the magnitude of weight loss (Beaumont, 1979) and gradually normalise as weight is gained (Beaumont and Carr, 1973; Marshall and Fraser, 1981). Sustained hypercortisolism coupled to an absent diurnal variation of cortisol secretion and a high plasma cortisol to androgen ratio is a common feature of anorexia nervosa (Warren and Van de Wiele, 1973; Brown, 1983). Bradycardia, a low bsasdal metabolic rate (BMR), raised serum cholesterol levels as well as a depression of basal plasma TSH and T_3 concentrations but an elevated reverse T_3 concentration are suggestive of hypothyroidism effected via a preferential degradation of T_4 to the less biologically active reverse T_3 in the anorexic woman (Lundberg et al, 1972; Burmon et al, 1977). An elevated plasma GH concentration (Garfinkel et al; 1975; Gold et al, 1980) and abnormal responses to various agonists and antagonists of GH secretion (Sherman and Malmi, 1977; Casper et al, 1977) have been observed in anorexics; the insulin and blood glucose responses to a glucose challenge frequently take on a diabetic feature (Warren and Van de Wiele, 1973) and an attenuated secretory pattern of ADH tends to result in partial diabetes insipidus (Tolis et al, 1982). The pituitary response to a number of exogenously administered hypothalamic releasing hormones (Boyden et al,

1982) and oestrogens (Casenaeuva et al, 1987) is often depressed in addition to the generalised suppression of the synthesis and secretion of endogenous hypothalamic hormones (Veldhuis, 1985).

Thus, the existence of a similar neuro-endocrine profile in anorexic women and low body weight amenorrhoeic athletes (Casenaeuva et al, 1987) would suggest that a low body mass and fat content effected through an inadequate food intake for a given energy expenditure are critical factors implicated in the pathogenesis of "athletic" amenorrhoea. It would seem that a low body mass index (BMI) coupled to a low body fat content is the only single factor actually capable of inducing the prolonged amenorrhoeic state and that other physical/emotional stressors (including exercise) exert a more synergistic influence.

It has been hypothesised that amenorrhoea results from the loss of a "critical" amount of body weight and/or fat, since women have been observed to develop amenorrhoea after a weight loss amounting to 10-15% of their "ideal" body mass, which approximates to the depletion of one third of their body fat stores (Frisch and McArthur, 1974). The bulk of the literature concerning the interrelationships between amenorrhoea, body weight and fat content, combined with a variety of other possible factors implicated in the aetiology of amenorrhoea in otherwise healthy women would suggest that low body weight and body fat content can definitely induce the amenorrhoeic state (Schwarz et al, 1981; Carlberg et al, 1983). The body weights and body fat content of amenorrhoeic athletes are invariably significantly lower than that of eumenorrhoeic athletes training for the same sport (Marcus et al, 1985; Glass et al, 1987). However, there would seem to be no simple relationship between menstrual regularity and body weight or fatness and it would seem that the "critical" threshold weight for regular menstrual cyclicity varies from one woman to another depending upon her exposure to other physical/emotional stressors.

The precise nature of the signal apprising the hypothalamus of the size of body fat stores has not been defined, although it has been speculated to be a decline in metabolic rate as a result of an energy deficit and/or the depletion of adipose tissue triglyceride stores beyond the level which permits a normal peripheral aromatisation of androgens to oestrogens (Frisch et al, 1981). An excessive conversion of androgens to

catecholoestrogens (such as oestrone) is known to occur in very lean women (Adashi et al, 1980) and the latter may exert an inhibitory influence upon GnRH and/or pituitary gonadotropin secretion (Ball et al, 1972; Fishman et al, 1975). Highly trained, lean female athletes with irregular menstrual cycles have recently been shown to metabolise a significantly greater fraction of administered 2-^{3}H oestradiol by 2-hydroxylase oxidation than their fatter eumenorrhoeic team mates, leading to the production of less potent catechol metabolites (Snow et al, 1988).

It would appear that the prolonged duration of amenorrhoea which tends to accompany anorexia nervosa is almost certainly linked to the severe emotional as well as physiological stress experienced by the individual. Emotional stress associated with bereavement, examinations or leaving home has been shown to induce menstrual irregularities in young women in the absence of body weight changes (Litt et al, 1983; Wilson et al, 1984) and there is evidence to suggest that amenorrhoeic runners subjectively experience a greater level of stress related to competition and the pressure to achieve than their eumenorrhoeic counterparts (Schwarz et al, 1981). The incidence of major affective disorders (especially eating disorders) and "compulsive/obsessional" behaviour has also been shown to be greater in amenorrhoeic than eumenorrhoeic female distance runners (Gadpaille et al, 1987). In fact, the similarity in the behaviour patterns displayed by highly motivated male and female endurance runners and individuals suffering from eating disorders as well as the disruption to neuro-endocrine function has lead to the hypothesis that "compulsive running is an analogue of anorexia nervosa" (Yates et al, 1983). The sustained hypercortisolism and raised plasma B-Endorphin levels in highly trained male and female distance runners may well be related to their high degree of self-imposed psychological stress. Interestingly, reversal of the amenorrhoeic state has been reported in anorexics of low body weight after relief from a severely stressful situation (Crisp, 1984) and in many cases amenorrhoea actually preceeds significant weight loss in anorexia nervosa (Morimoto et al, 1980) and may persist for many months after weight is restored (Litt et al, 1986; Fries, 1977), which further supports the concept that the neuro-endocrine system is highly sensitive to changes in emotional stability.

Other factors aside from exercise, low body weight and/or fat content and emotional stress which have been linked to menstrual dysfunction in athletic women include training pre-menarche or shortly after menarche and dietary insufficiency. Vigorous pre-pubertal training has been shown to significantly delay menarche in dancers (Warren et al, 1980), gymnasts, swimmers, and runners (Baker et al, 1981; Stager et al, 1984). In the sedentary and moderately active female populations of Western civilisation, menarche generally occurs between the ages of 11-13 years (Frisch et al, 1981), but may be delayed by as many as 4-6 years in highly active female populations as well as very light and lean women (Frisch et al, 1981; Warren et al, 1983). Training in the early post-menarcheal period before regular ovulatory cycles have been established also increases the adolescent's susceptibility to menstrual dysfunction (Frisch et al, 1981; Marcus et al, 1985; Baker et al, 1981) and can lead to a delay in pubertal progression and prolonged amenorrhoea unless training is significantly decreased and/or there is significant weight gain (Warren et al, 1980). Amenorrhoea is less common in women who started training at a later age, particularly in parous women having borne one or more child (Schwartz et al, 1981; Baker et al, 1981).

Dietary insufficiency, especially of total energy, fat and certain micronutrients has been linked to menstrual dysfunction in female athletes (Brooks et al, 1984; Deuster et al, 1984; Kaiserauer et al, 1989) and deficiencies or excesses of specific macro-/micronutrients are known to modify the metabolism of many hormones including ovarian steroids (Golden et al, 1982; Hill et al, 1984). Since cholesterol is the precursor of steroid hormones, it is possible that too low a fat intake might disrupt the biosynthesis of these hormones which include oestradiol and progesterone. In spite of their high energy expenditures, many female athletes appear to be able to maintain a constant (albeit low) body weight on a remarkably low energy intake (Brooks et al, 1984; Kaiserauer et al, 1989). Although the majority of these athletes are not starving themselves to the same extent as would many anorexic individuals, the combination of hard physical training with a relatively low energy intake might culminate in a similar state of negative energy balance and perhaps comparable metabolic derangements. Decreases in basal metabolic rate (BMR) amounting to as much as 50% have been reported in hospitalised anorexic adolescents (Vaisman et al, 1987) and similar declines have been reported in female

athletes training vigorously without a compensatory increase in food intake (Myerson et al, 1991).

A number of studies of amenorrhoeic and eumenorrhoeic athletes have concluded that the only differential factor apparently separating the groups is that of dietary inadequacy (Deuster et al, 1986; Kaiserauer et al, 1989). Although there was no obvious difference in body weight, body fat content, training experience and current training intensity/volume or age at menarche between groups, the amenorrhoeic athletes consumed significantly less energy, fat and percent fat relative to total energy intake. In contrast to the eumenorrhoeic athletes, the majority of the amenorrhoeic athletes were also vegetarian. A high intake of crude fibre in the form of raw fruit and vegetables can lead to hypercarotinaemia, which may in turn disrupt reproductive function (Kenmann et al, 1983). Hypercarotinaemia has been observed in some amenorrhoeic athletes as well as anorexic women (Schwartz et al, 1981; Marcus et al, 1985) and may therefore be a contributory factor to menstrual dysfunction. The dietary intake of zinc has also been found to be too low in many amenorrhoeic athletes (Brooks et al, 1984; Deuster et al, 1984; Kaiserauer et al, 1989) and the susceptibity to zinc deficiency is potentiated by a high fibre diet which reduces the capacity for the gastro-intestinal absorption of a number of minerals (Kaiserauer et al, 1989). Zinc deficiency has also been associated with abnormal reproductive functioning (Agar, 1981). Many amenorrhoeic but not eumenorrhoeic runners would appear to have an obsessive pre-occupation with diet and weight control and deliberately maintain a low body mass through vigorous exercise and a low energy intake (Gadpaille et al, 1987).

Exposed to the continual stress of hard physical training, the constant emotional strain linked to competition and fear of failure as well as the requirement to maintain a low body weight and body fat content to optimise performance capacity, the competitive female athlete makes a prime candidate for menstrual dysfunction.

Although exercise alone may not induce amenorrhoea, it would certainly appear to be a significant contributory factor forming part of a multifactorial process. It is a well documented fact that menstrual cyclicity may rapidly resume during periods of enforced rest in injured

athletes in the absence of body weight changes (Prior et al, 1982; Warren et al, 1980; Frisch et al, 1984; Shangold et al, 1985). An increase in training intensity and/or volume prior to the competitive season is frequently coupled to menstrual dysfunction and the incidence of amenorrhoea is greatest in runners with the highest weekly milages (Baker et al, 1981; Schwartz et al, 1981; Sanborn et al, 1982; Carlberg et al, 1983). Additionally, the resumption of menstrual cyclicity in anorexic adolescents has been reported to occur at a significantly higher body mass following weight restoral in "active" than in "sedentary" individuals and plasma gonadotropin levels were significantly lower in the former at a comparable BMI to that of the latter (Litt et al, 1986). However, for a given BMI, the body fat content of active individuals would be expected to be lower than that of their sedentary counterparts.

A major difficulty surrounding many of the studies aimed at quantifying the influence of exercise per se upon neuro-endocrine function is related to the fact that they are of a cross-sectional design in which 2 separate populations of individuals are compared. The obvious limitation of this type of study is attached to the difficulty of adequately standardising those factors known to potentially influence menstrual cyclicity between groups. As yet, few longitudinal studies have been completed whereby the effects of training on neuro-endocrine function have been examined in isolation from other influential factors. To date, the most extensive and carefully controlled study of this type was performed by Bullen and colleagues (1985) who "successfully" induced menstrual dysfunction in young women with previously "normal" menstrual cycles by assigning them to a graded running training programme, which over a 5 week period brought their weekly running distance up to 70 miles performed at a relative intensity of 70-80%VO_2max. Of the 28 women who completed the study, 16 lost weight whilst the remainder maintained a near constant body mass. Although luteal phase deficiency, anovulatory cycles and delayed menstruation were documented in women who maintained their weight as well as those who lost it, the incidence of menstrual dysfunction was higher in the "weight loss" group, thereby illustrating the synergistic effects of energy balance and exercise stress. It would seem that menstrual cycle changes ranging from a simple decrease in the severity of molimnal (pre-menstrual) symptoms, to cycles characterised by a shortened luteal phase with low plasma progesterone levels and anovulation occur in almost all

women during vigorous training (Prior et al, 1984). However, oligomenorrhoea and prolonged amenorrhoea tend to affect a much smaller number of particularly susceptible women, especially if they are exposed to other inhibitors of neuro-endocrine function.

It is also necessary to establish standard criteria for classification of the amenorrhoeic and eumenorrhoeic states. There has been some confusion in the literature as to the precise definition of amenorrhoea, which has made it difficult to make meaningful comparisons between studies investigating the incidence of amenorrhoea in different female populations and other possible influential factors. The incidence of menstrual cycle irregularities in the non-athletic and otherwise healthy young adult female population also remains to be established before the influence of exercise per se on reproductive function can be accurately quantified. A proper classification of menstrual status would thus involve a careful documentation of cycle lengths, ovulation and fluctuating pituitary and ovarian hormones in large populations of healthy athletes and non-athletes.

CHAPTER 3

General Methods

All the methods employed in these studies had been approved by the Ethical Advisory Commitee of Loughborough University.

3.1 Subjects:

The subjects were all volunteers and consisted mainly of Physical Education students at Loughborough University and athletes recruited from local Athletics Clubs. The criteria for their selection were generally based upon weekly running milage and training experience, although the menstrual status of female subjects was taken into account when selecting subjects for Study 2.

The subjects of Study 1 were all classed as "moderately trained, recreational" runners who either ran for pleasure and fitness or incorporated running into a general training programme for another sport. The majority also participated at "club" or county level in team sports such as hockey, football, tennis and rugby.

The subjects of Study 2 were all highly trained county standard, national or international middle/long distance runners. None participated in team sports, although some were also triathletes.

All were fully briefed of the intended protocol prior to their participation in a Study and were assured that they could withdraw at any time should they wish to do so.

Subjects unaccustomed to treadmill running were fully familiarised in one or more practise sessions before performing any of the treadmill tests.

3.2 Subject preparation:

Subjects were free to choose the time of day most convenient for themselves to perform the preliminary tests provided that they were at least 3 hours post-absorptive. However, all subjects performed the 60 minute test run between 0730 and 0930 hours following an overnight fast of at 8-10 hours.

The standing height of each subject, barefoot (wall mounted stadiometer; Holtain; measuring to the nearest 0.1cm) was noted at the start of each study and body weight in shorts and t-shirt or nude (as appropriate for the test concerned) (Avery Personal Balance, 120kg; measuring to the nearest 0.1kg) was recorded prior to the performance of each exercise test.

Heart rate was monitored continuously throughout each exercise test via a 3-lead ECG (Rigel Cardiac Memory Monitor 302). Four electrodes (Red Dot 3M, 2255) were used per subject for each test and placed in the following positions:

i) immediately above the sternum,
ii) one on the right and another on the left lower margin of each rib cage,
iii) immediately below the midpoint of the right clavicle (attached via a lead to a circlip on the handrail of the treadmill to act as an earth).

Before applying the electrodes, a Medi Swab (70% isopropyl alcohol) was used to remove dead surface epithelial cells, the skin dried with a tissue (Kleenex Medical Wipes) and then carefully abraded to ensure effective contact with the electrode gel. Each electrode was firmly secured in place with tape (Blenderm 3M).

A microcomputer (BBC Microvitec 653 cub) interfaced to a printer (Canon PW-108A) provided a continuous visual display of heart rate, running velocity and total distance run during each test and enabled a hard copy of this information to be obtained.

Two electric fans (Expelair T-16) were used during exercise tests to facilitate the cooling of subjects.

3.3 The Motorised Treadmill:

A Quinton 24-72 motorised treadmill capable of simulating a range of running speeds up to 6.7ms^{-1} and an elevation level up to 40% was used for all the exercise tests. It was calibrated before the start of each major study by counting the belt revolutions over a set time interval at a series of speeds ranging from 2-6ms^{-1}.

3.4 Preliminary tests:

3.4.1 Oxygen cost of steady pace submaximal running ("Speed-VO_2" test):

The oxygen cost of submaximal treadmill running (running economy) was measured during a 16 minute run on a horizontal treadmill. Subjects ran for 4 minutes at each of 4 different speeds (usually 2.67, 3.11, 3.55 and 4.00ms^{-1} for females and 3.11, 3.55, 4.00 and 4.44 ms^{-1} for males with slight modification for subjects of greater or lesser running ability). Expired air was collected into 150L Douglas bags between the third and fourth minute at each speed for the calculation of oxygen uptake (VO_2) and carbon dioxide production (VCO_2) rates and treadmill speed was increased immediately after each collection. Subjects indicated their perceived rate of exertion (PRE) on a numerical scale (Borg, 1973) halfway through each collection period.

A linear VO_2 versus running speed relationship was established for each subject and the mathematical formula describing the relationship evaluated using linear regression analysis. This enabled running speeds corresponding to a particular VO_2 value to be chosen for each subject.

3.4.2 Maximal oxygen uptake (VO_2max) test:

A modified Taylor treadmill test (Taylor et al, 1955) of uphill running was used to determine maximal oxygen uptake (VO_2max). Subjects were permitted to choose their own warm-up speed which they maintained for 5 minutes before the test began. Treadmill speed was then set at a fixed level chosen with a knowledge of each subjects running ability and his or her performance during the speed-VO_2 test, with the intention that he or she should achieve VO_2max in 8 to 12 minutes. The initial inclination was set at 3.5% and was increased by 2.5% every 3 minutes thereafter until the subject indicated that he or she could continue for only one minute longer. All were verbally encouraged to produce maximal effort as the work load became difficult. Expired air was collected during the last minute at each grade and the last minute of the test. The final collection at the point of exhaustion was taken as peak VO_2 and the heart rate at this point as the maximal heart rate. Each subject's PRE was noted halfway through every collection period.

A maximal heart rate value closely corresponding to the age-predicted maximum, a respiratory exchange ratio (R) value measured during the final minute of the test exceeding 1.1 and a plateauing of VO_2 between the penultimate and final measurements confirmed that subjects had attained VO_2max.

3.4.3 Blood lactate response to steady pace submaximal running ("Speed-Lactate" test):

Using the data obtained from the speed-VO_2 and VO_2max tests, the speeds corresponding to approximately 60, 70, 80 and 90% of each subjects VO_2max were calculated. Subjects then repeated the 16 minute speed-VO_2 test at these calculated speeds. Expired air was collected and PRE noted as previously and duplicate 20ul samples of capillary blood were obtained from the thumb at rest immediately prior to performing the test, and at the end of each expired air collection for the determination of blood lactate concentration. Heart rate was monitored as previously described.

Measurements of blood lactate concentration during submaximal treadmill running at different exercise intensities, together with a knowledge of each subject's training regime, past and present, provided an indication of the subjects' "training status" (Ramsbottom et al, 1987). Since endurance training selectively increases the aerobic capacity of those skeletal muscle fibres recruited during the exercise (Costill et al, 1979; Baldwin et al, 1972), trained muscle relies to a lesser extent upon anaerobic glycolysis for ATP resynthesis than untrained muscle and is capable of metabolising a greater proportion of fat as well as carbohydrate via aerobic processes. Consequently it produces less lactate. Plots of blood lactate concentration (ordinate) against running speed or relative exercise intensity (%VO_2max) (abscissa) were constructed for each subject to estimate the running speeds and relative work intensities at blood lactic acid concentrations equivalent to 2 and 4 $mmoll^{-1}$. These reference concentrations have been suggested as "aerobic" and "anaerobic" lactate thresholds during exercise of increasing intensity (Kindermann et al, 1979) and are more positively correlated with endurance performance and capacity than VO_2max (Jacobs, 1986).

3.5 The 60 minute treadmill test run:

A 60 minute steady pace run on a horizontal treadmill, performed at a relative intensity equivalent to 70% of each subject's VO_2max was used as a metabolic challenge. All such runs were performed between 0730 and 0930 hours with subjects in a fully post-absorptive state after an overnight fast of 8-10 hours. Subjects were asked to consume their "normal" diets, to refrain from vigorous training and from drinking alcohol on the evening before the test run.

After 10-15 minutes relaxation in a warm room, a 10ml sample of venous blood was taken from an antecubital vein and 2x20ul samples of capillary blood from the thumb.

The pre-exercise nude weight of each subject was noted, then each was allowed time to stretch and a 5 minute "warm up" run at a treadmill speed corresponding to approximately 60% of their VO_2max. The treadmill speed was

then adjusted to correspond to 70% VO_2max and the subject continued to run at this same speed for 60 minutes.

A 1 minute collection of expired air was made every 15 minutes (starting at 14 minutes) for the measurement of VO_2, VCO_2, and R. Halfway through the collection period, subjects were asked to indicate their PRE score on the Borg scale. This was followed immediately by the extraction of 2x20ul samples of capillary blood from the thumb for the determination of blood lactate and glucose concentrations. Subjects were allowed free access to water during the test and a note was made of the volume consumed. After 60 minutes running, the treadmill was stopped, a chair provided for the subject and a 10ml sample of venous blood and 2x20ul samples of capillary blood were immediately taken (as pre-exercise). Finally, the nude weight of the subject was noted (after towelling off excess sweat).

3.6 Collection and analysis of expired air:

Expired air was collected into 150L Douglas bags (Harvard Apparatus Ltd). Subjects were handed a noseclip and a mouthpiece connected to a low resistance respiratory valve (Jakeman and Davies, 1979) and light weight smooth bore tubing (Falconia ducting; 4cm diameter, 165cm long) attached to the Douglas bag approximately 45 seconds prior to collection in order to remove atmospheric air from the collecting system.

A small vacuum pump (Charles Austen Pumps Ltd) was used to withdraw a small measured sample of expired air from Douglas bags through silica gel (for the removal of moisture) into an infra-red CO_2 analyser (Mines Safety Appliances Co. Ltd; Model 303) followed by a paramagnetic O_2 analyser (Sybron Taylor Ltd; Model 570A) to measure fractional concentrations of CO_2 and O_2 in expired air samples. Each analyser was zeroed with 100 N_2, spanned using atmospheric air and calibrated using a CO_2/O_2 gas mixture of known concentration (PK Morgan Ltd) twice before each analysis.

Expired air was then evacuated from Douglas bags via an electrically operated vacuum pump (Moulinex 237) and minute ventilation measured via a dry gas meter (Parkinson Cowan Ltd). The temperature of air in the bag was measured during evacuation on an electrical thermometer (Edale Instruments

Ltd; Model C). Barometric pressure was measured at the time of analysis using a wall mounted Fortin barometer (Gallenkamp Ltd) and this, together with air temperature and volume used to convert gas volumes to STPD.

A BBC Basic computer programme (DG Kerwin, 1988) facilitated the calculation of VO_2, VCO_2, R values.

3.7 Blood sampling and the treatment of collected blood:

Capillary blood samples (20uL, Dade Accupette) were immediately deproteinised in 200uL 2.5% perchloric acid, centrifuged and stored at -20C for subsequent analysis. Blood lactate and glucose concentrations were determined using the fluorometric and colourometric methods of Maughan (1982) and Werner (1970) respectively. (Details of the methodology can be found in Appendices 3 and 4).

Packed cell volumes (haematocrits) were determined using a microcentrifuge (Hawksley Ltd) and haemoglobin concentration via the cyanmethemoglobin method (Van Kampen and Zijlstra, 1961) as described in Appendix 7. The remaining venous blood was centrifuged at 4 C for 20 minutes, the plasma was skimmed off and stored at -20 C for subsequent analysis. Plasma FFA and glycerol concentrations were determined via flourometric (Laurell and Tibbling, 1966) and colourometric (Chromy et al, 1977) procedures respectively (described in Appendices 5 and 6). Changes in plasma volume over 60 minutes running were calculated from pre and post exercise haemoglobin and haematocrit concentrations (Dill and Costill, 1974).

3.8 Statistics:

An unpaired, 2 tailed t-test for uncorrelated data was used to compare physical and physiological characteristics, training and performance characteristics, pre-exercise blood lactate and glucose concentrations and the magnitude of change in plasma volume between 2 different groups of subjects in each study. This test was also employed for the comparison of

pre-exercise plasma gonadotropin (LH and FSH) between female subject groups of differing menstrual status in Study 2.

A 2 way analysis of variance (ANOVA) (repeated observations upon a single factor on 2 separate groups of subjects) was used to compare group values and changes in heart rate, respiratory variables and blood glucose and lactate with time during the 60 minute test run as well as plasma FFA and glycerol concentrations between groups and pre- and post-exercise. Pre- and post-exercise plasma sex steroid hormone (oestradiol, progesterone and testosterone) concentrations were also compared between female groups of differing menstrual status in Study 2 using a 2 way ANOVA.

A 2 way ANOVA (repeated measures on a single factor in a single group of subjects) was used to compare changes in heart rate, respiratory variables, blood lactate and glucose concentrations between menstrual cycle phases and over time as well as plasma FFA and glycerol and sex steroid hormone concentrations pre- and post exercise and between menstrual cycle phases in Study 2.

A Mann Whitney U-test for non-parametric statistics was also used to make simple quantitative comparisons of variables between groups in the same Study and between Studies.

All data was expressed as means $\pm$ so and the level of significance was set at 5%.

CHAPTER 4

THE METABOLIC RESPONSES OF MODERATELY TRAINED MALE AND FEMALE RECREATIONAL RUNNERS TO PROLONGED STEADY PACE TREADMILL RUNNING

4.1 Introduction

The aim of this study was to compare and contrast some of the physiological and metabolic responses to prolonged, steady pace treadmill running of male and female recreational runners, and in particular to investigate the possible existence of gender-related differences in substrate metabolism.

Lower respiratory exchange ratios (R), coupled to lower circulating concentrations of blood lactate, but higher concentrations of plasma free fatty acids (FFA) and glycerol have been measured in women as compared to men during submaximal cycle ergometer exercise (Nygaard et al, 1978; Froberg and Pedersen, 1984); during treadmill walking at 35% VO_2max (Blatchford et al, 1985) and submaximal treadmill running (Hardman and Williams, 1983: Tarnopolsky et al, 1990). Muscle biopsy studies suggest that the extent of glycogen utilisation during prolonged submaximal exercise may be significantly lower in females than in males (Nygaard, 1986; Tarnopolsky et al, 1990). There are also reports of women "adjusting" abruptly to fat metabolism during the early stages of submaximal exercise, whilst men rely predominantly upon carbohydrate as energy substrate until a later stage of the exercise (Blatchford et al, 1986).

In contrast, no sex differences in R-values and circulating fat and carbohydrate metabolites were observed during prolonged submaximal treadmill running in studies by Costill et al, (1979) and Powers et al, (1980).

The exercising muscles' choice of energy substrate is governed by a number of factors in the healthy individual, including exercise intensity and duration, skeletal muscle "training status" and recent dietary history.

Thus, the purpose of the present study was to re-examine the metabolic responses of moderately, but essentially equally-trained male and female recreational runners during prolonged steady pace treadmill running of standardised relative intensity and duration in the fully post-absorptive state.

4.2 Methods

Eight male and 8 female physical education students volunteered as subjects for the study. All habitually ran in excess of 16 km per week as well as participating in one or a number of other sports and had been running regularly for at least one year. Many were of county or national standard in team sports such as football, rugby, hockey and tennis but incorporated running into their training programmes.

Each subject was fully informed of the intended protocol, familiarised with treadmill running, then performed 4 exercise tests on different days within a 5 day period. The first 3 preliminary tests, designed to establish the appropriate treadmill speed corresponding to 70% of each subject's VO_2max consisted of the "speed-VO_2" test, a VO_2max test and a "speed-lactate" test (Section 3.3). Subjects were free to choose the time of day most convenient for them to perform the first 3 tests, provided that they had not eaten for at least 3 hours beforehand.

The 4th test formed the exercise challenge and consisted of the 60 minute treadmill run performed at a speed corresponding to 70% of each subject's VO_2max (section 3.3). Subjects were asked avoid training excessively on the day before this run, to abstain from alcohol and to follow their normal diets without specific modification in preparation for the test. All performed the test between 0730 and 0930 hours in the fully post-absorptive state following an overnight fast of 8 to 10 hours.

A 1 minute sample of expired air was collected every 15 minutes for the calculation of VO_2, VCO_2 and whole body respiratory exchange ratio (R).

Capillary samples of blood were obtained from the thumb with the subject at rest, 5 minutes before starting the run and at the end of each expired air collection for the subsequent determination of blood lactate and glucose concentrations. A 10ml sample of venous blood was taken 5 minutes pre-exercise and immediately after the run for the measurement of packed cell volumes (haematocrit), haemoglobin concentration and plasma concentrations of free fatty acids (FFA's) and glycerol.

The nude weight of each subject was taken pre- and post- exercise and a note made of the volume of water consumed during the run noted. Room temperature was maintained between 20 and 23°C during all of the test runs and a wet-dry bulb thermometer used to monitor the relative humidity.

Table 4.1: Physiological Characteristics of Subjects

	Age (yrs)	Height (cm)	Weight (kg)	BMI	VO_2max ($ml kg^{-1} min^{-1}$)	HRmax (bt min^{-1})
M	24 ±2	180.2 ±7.0	75.8 ±7.2	23.4 ±2.1	62.70 ±3.57	190 ±9
F	26 ±6	164.8 ±6.2	59.2 ±6.0	21.8 ±1.8	54.08 ±7.15	185 ±6
	ns	**	**	*	**	ns

Values indicated as mean ± sd of mean.
** $p<0.01$; * $p<0.05$; ns = non significant (Male versus Female subjects).

Table 4.2: Training Characteristics of Subjects

	Weekly running distance (km)	Days run per week	Weeks per year	Total exercise time per week	Years running experience (yrs)
M	41 ±21	5 ±2	47 ±3	343 ±123	3 ±1
F	41 ±11	5 ±2	47 ±2	301 ±99	5 ±2
	ns	ns	ns	ns	ns

Values indicated as mean ± SD of mean.
** $p<0.01$; * $p<0.05$; ns = non significant (Male versus Female subjects).

Table 4.3: Running Economy and Treadmill Speeds equivalent to 70%VO_2max of Subjects

	VO_2 ($ml kg^{-1} min^{-1}$) 3.2ms^{-1}	4.1ms^{-1}	"70%" speed (ms^{-1})
M	40.06 ±2.68	48.70 ±1.63	3.69 ±0.30
F	37.68 ±3.81	47.59 +3.87	3.25 ±0.39
	ns	ns	**

Values indicated as mean ± so of mean.
** $p<0.01$; * $p<0.05$; ns = non significant (Male versus Female subjects).

Table 4.4: Percentage VO_2max and Treadmill Speeds at $2mmoll^{-1}$ and $4mmoll^{-1}$ Blood Lactate concentrations

	2 $mmoll^{-1}$		4 $mmoll^{-1}$	
	Speed (ms^{-1})	$\%VO_2max$	Speed (ms^{-1})	$\%VO_2max$
M	3.32 ±0.33	57.08 ±3.13	4.04 ±0.42	69.29 ±3.25
F	2.70 ±0.53	58.68 ±9.59	3.41 ±0.65	70.20 ±4.23
	**	ns	**	ns

Values indicated as mean ± SD of mean.
** $p<0.01$; * $p<0.05$; ns = non significant (Male versus Female subjects).

Table 4.5: Respiratory and Cardiovascular responses during 60 minute run

		15min	30min	45min	60min
% VO_2max	M	70.38 ±4.17	71.50 ±3.93	72.55 ±4.26	73.44 ±4.31
	F	71.42 ±2.64 ns	71.74 ±5.09 ns	72.21 ±3.89 ns	73.64 ±4.96 ns
Heart rate ($bt\,min^{-1}$)	M	166 ±8	169 ±9	170 ±9	171 ±8
	F	158 ±11 ns	162 ±12 ns	163 ±14 ns	167 ±12 ns
VO_2 ($ml\,kg^{-1}\,min^{-1}$)	M	44.10 ±3.22	44.79 ±2.90	45.46 ±3.38	46.03 ±3.53
	F	38.51 ±4.15 **	38.66 ±4.50 **	38.93 ±4.48 **	39.62 ±3.89 **
VCO_2 ($ml\,kg^{-1}\,min^{-1}$)	M	40.13 ±2.28	39.62 ±2.21	40.63 ±2.54	42.19 ±1.88
	F	30.93 ±4.10 **	30.81 ±4.20 **	30.99 ±4.54 **	31.66 ±4.34 **
R	M	0.92 ±0.10	0.89 ±0.08	0.90 ±0.09	0.92 ±0.11
	F	0.80 ±0.04 **	0.80 ±0.03 **	0.79 ±0.04 **	0.80 ±0.04 **
% Contribution fat to metabolism	M	33.52 ±22.92	38.05 ±25.13	37.84 ±25.95	33.16 ±26.41
	F	67.96 ±13.66 **	70.15 ±11.73 **	70.85 ±14.26 **	69.75 ±14.61 **
Energy expenditure ($kJ\,min^{-1}$)	M	67.87 ±4.83	68.62 ±5.12	69.69 ±5.21	70.95 ±5.22
	F	45.10 ±4.86 **	45.37 ±6.02 **	45.68 ±6.13 **	46.60 ±6.07 **

Values indicated as mean ± sp of mean.
** $p<0.01$; * $p<0.05$; ns = non significant (Male versus Female subjects).

Table 4.6: Metabolic responses during 60 minute run

		Rest	15min	30min	45min	60min
Blood lactate ($mmol l^{-1}$)	M	0.92 ±0.11	2.85 ±0.43	3.02 ±0.33	3.02 ±0.58	3.71 ±1.23
	F	0.79 ±0.13 ns	2.27 ±0.52 *	2.34 ±0.59 *	2.23 ±0.51 *	2.69 ±0.76 *
Blood glucose ($mmol l^{-1}$)	M	4.08 ±0.62	3.88 ±0.51	4.02 ±0.46	4.06 ±0.53	4.19 ±0.58
	F	4.30 ±0.65 ns	4.42 ±0.47 *	4.52 ±0.48 *	4.53 ±0.50 ns	4.59 ±0.28 ns
Plasma FFA ($mmol l^{-1}$)	M	0.28 ±0.10	-	-	-	0.38 ±0.14
	F	0.34 ±0.11 ns	-	-	-	0.47 ±0.13 ns
Plasma glycerol ($mmol l^{-1}$)	M	0.08 ±0.05	-	-	-	0.41 ±0.17
	F	0.10 ±0.04 ns	-	-	-	0.48 ±0.16 ns

Values indicated as mean ± sp of mean.
** $p<0.01$; * $p<0.05$; ns = non significant (Male versus Female subjects).

Table 4.7: Changes in Haemoglobin, Haematocrit and Plasma Volume over 60 minute run

		Pre-exercise	Post-exercise
Haemoglobin (gdl^{-1})	M	15.55 ±1.30	15.82 ±1.26
	F	13.06 ±0.45 **	13.58 ±0.66 **
Haematocrit (%)	M	45.3 ±1.7	45.7 ±2.4
	F	38.7 ±1.8 **	39.1 ±1.1 **
Percentage change in plasma volume	M		-2.32 ±5.16
	F		-4.16 ±5.58 ns
Weight loss (kg)	M		1.59 ±0.19
	F		0.83 ±0.11 **
Percentage loss of body weight	M		2.10 ±0.09
	F		1.40 ±0.17 *

Values indicated as mean ± sd of mean.
** $p<0.01$; * $p<0.05$; ns = non significant (Male versus Female subjects).

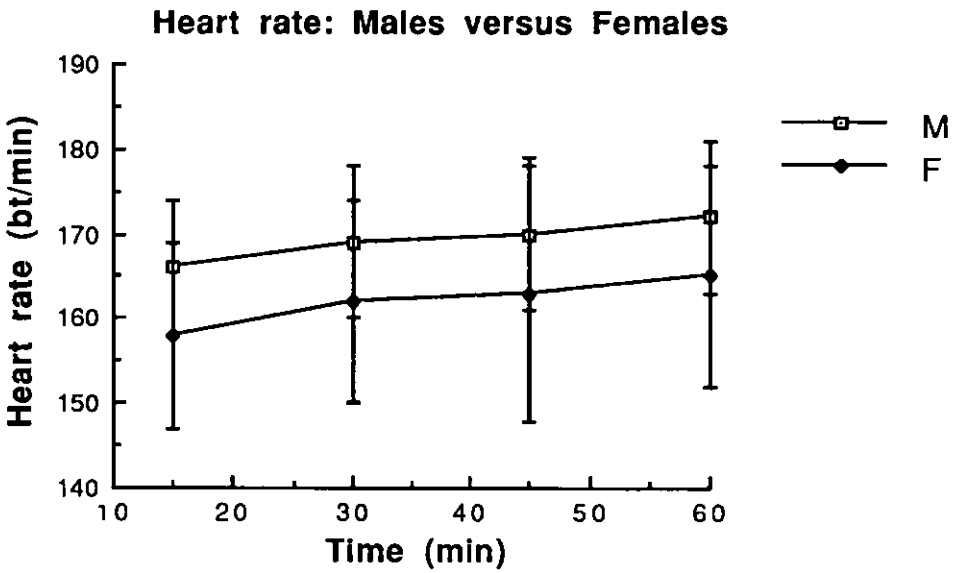
Heart rate: Males versus Females
190
180
170
160
150
140
Heart rate (bt/min)
10
20
30
40
50
60
Time (min)
M
F

Fig 4.1

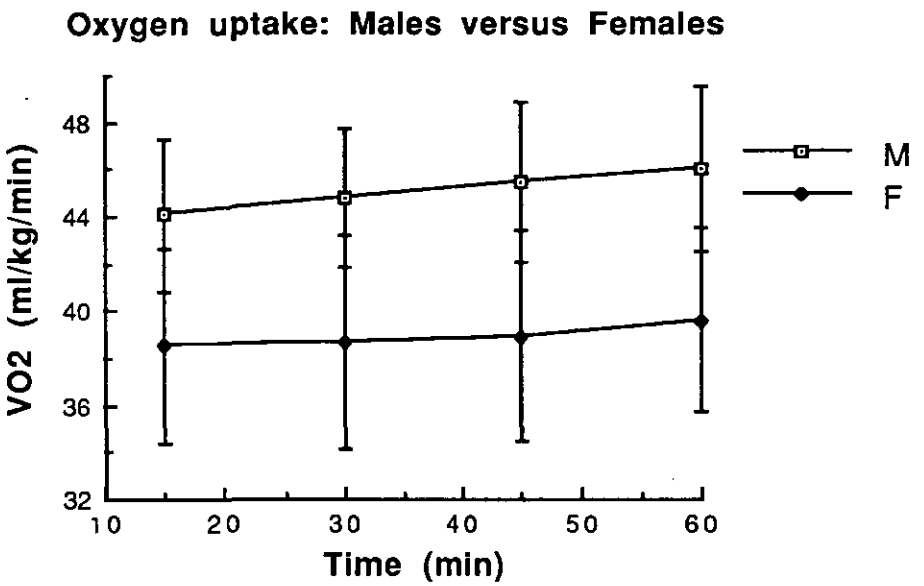
Oxygen uptake: Males versus Females
VO2 (ml/kg/min)
48
44
40
36
32
10
20
30
40
50
60
Time (min)
M
F

Fig 4.2.

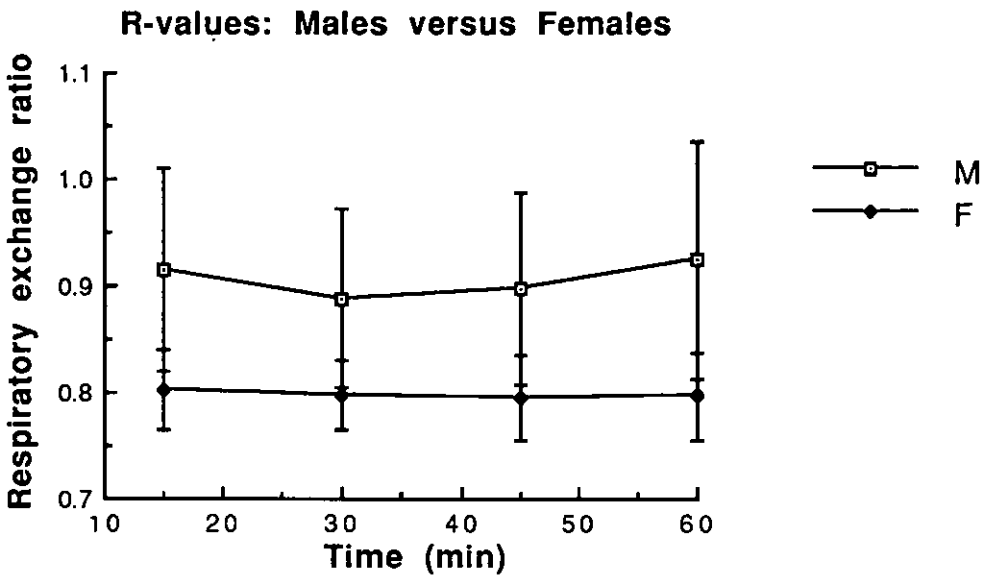
R-values: Males versus Females
Respiratory exchange ratio
1.1
1.0
0.9
0.8
0.7
10
20
30
40
50
60
Time (min)
M
F

Fig 4.3

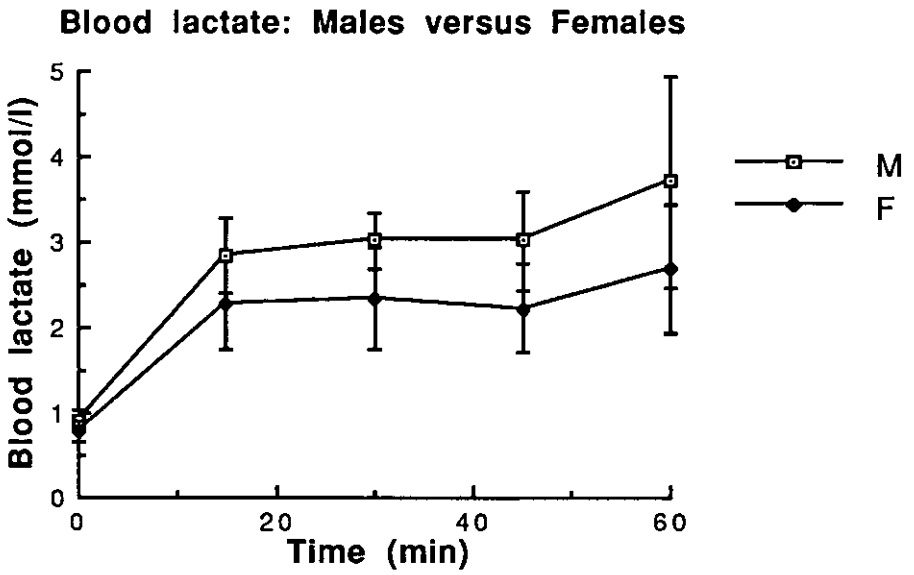
Blood lactate: Males versus Females
Blood lactate (mmol/l)
5
4
3
2
1
0
0
20
40
60
Time (min)
M
F

Fig 4·4

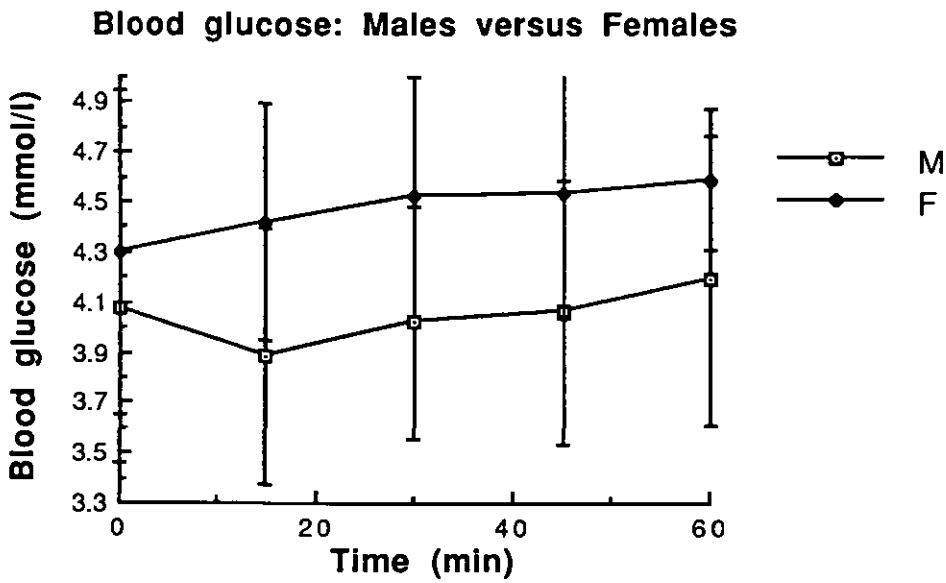
Blood glucose: Males versus Females
Blood glucose (mmol/l)
4.9
4.7
4.5
4.3
4.1
3.9
3.7
3.5
3.3
0
20
40
60
Time (min)
M
F

Fig 4.5

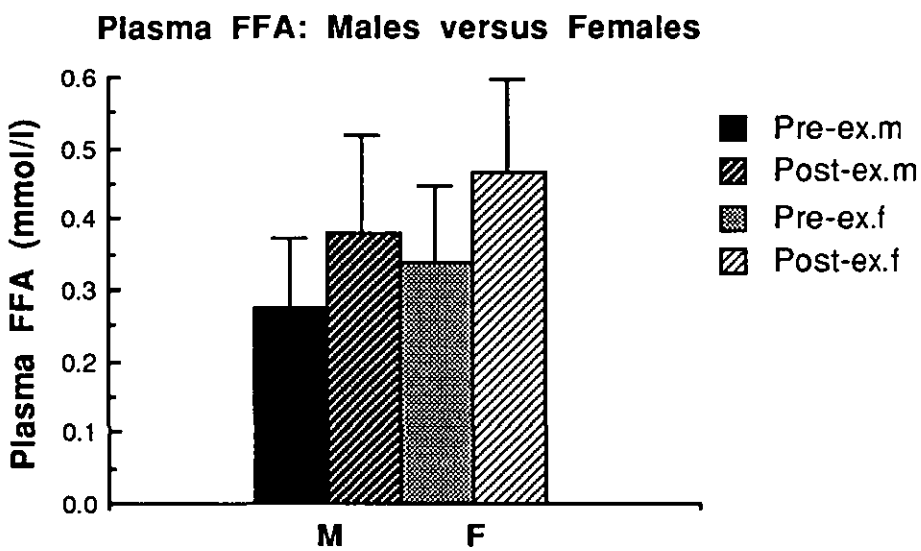
Plasma FFA: Males versus Females
Plasma FFA (mmol/l)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
M
F
Pre-ex.m
Post-ex.m
Pre-ex.f
Post-ex.f

Fig 4.6

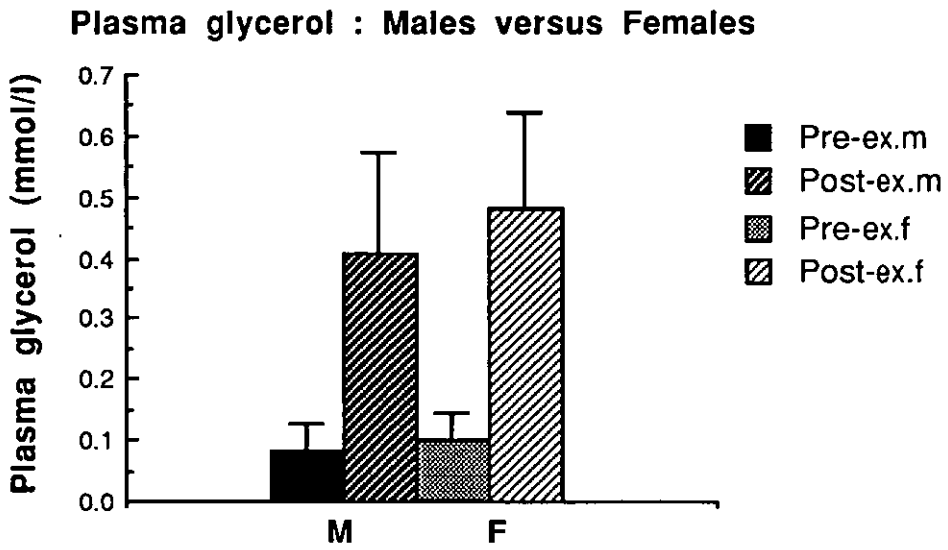
Plasma glycerol : Males versus Females
Plasma glycerol (mmol/l)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
M
F
Pre-ex.m
Post-ex.m
Pre-ex.f
Post-ex.f

Fig 4.7

4.3 Results

The mean and standard error of male and female physiological characteristics are listed in Table 4.1. There was no difference in mean age and maximum heart rate between groups; however the men were 15% taller ($p<0.01$), 28% heavier ($p<0.01$) and had a 15% higher VO_2max per unit body mass ($p<0.01$) than the women. The body mass index (BMI) of the men was 7% higher than that of the women ($p<0.05$) and the former also exhibited a circulating haemoglobin concentration and haematocrit in the order of 17% higher ($p<0.01$) and 19% higher ($p<0.01$) respectively (Table 4.7). There was no difference in running economy between groups (Table 4.3).

Mean treadmill speed corresponding to the 2 and 4 $mmoll^{-1}$ reference blood lactate concentrations was 23% and 19% higher (respectively) in men than in women ($p<0.01$), although there was no difference in $\%VO_2max$ at either blood lactate concentration between groups (Table 4.4). Past and present physical activity patterns (Table 4.2) did not differ between groups, although there was large intra-group variation in many of the measured variables.

All subjects were found to be exercising at a relative intensity close to 70% of their VO_2max during the 60 minute test run (Table 4.5). Mean treadmill speed during the test run (Table 4.3) was 14% higher for men tha women ($p<0.01$), oxygen uptake (VO_2) (Table 4.5 and Figure 4.2) was 15% higher ($p<0.01$) and carbon dioxide production (Table 4.5) in the order of 30% higher ($p<0.01$) for the former. Heart rate (Table 4.5 and Figure 4.1) also tended to be higher in the men than women, although group differences were not significant. Increases in heart rate ($p<0.01$), oxygen uptake ($p<0.01$) and carbon dioxide production ($p<0.01$) were recorded within both groups over the duration of the test.

Blood lactate concentration (Table 4.6 and Figure 4.4) did not differ between groups at rest, but was consistently higher in men than women throughout the exercise period ($p<0.05$). Fifteen minutes after the start of exercise, blood lactate concentration was raised beyond resting values in both groups ($p<0.01$), the mean increments being 2.1 and 1.6 fold in men and women respectively. Blood lactate concentration increased over the duration

of the test run ($p<0.01$) such that after 60 minutes of running, increments above resting values were in the order of 3.0 and 2.4 fold in men and women respectively.

Blood glucose concentration (Table 4.6 and Figure 4.5) tended to be higher at rest in women than in men, although this sex difference was not significant. Throughout the duration of the test run however, blood glucose concentration remained 10-14% higher in women and was significantly higher 15 and 30 minutes after the start of running ($p<0.05$). A gradual increase in blood glucose concentration was observed in both groups as the test progressed, such that it was significantly raised above resting values in women from 30 minutes after the start of running ($p<0.05$), but not in men until after 60 minutes ($p<0.05$).

Pre-exercise plasma FFA concentration (Table 4.6 and Figure 4.6) tended to be higher in women than men both before and after the test, however there was large intra-group variation and group differences were not significant. Plasma FFA concentration was approximately 38% higher post- as compared to pre-exercise in both groups ($p<0.01$). Mean resting plasma glycerol concentration (Table 4.6 and Figure 4.7) did not differ between groups, either pre- or post-exercise and increased to a similar extent (4.1 fold in men and 4.8 fold in women) over the duration of the test ($P<0.01$).

Respiratory exchange ratio (Table 4.5 and Figure 4.3) was in the order of 14% higher for the male than the female subjects throughout the test run ($p<0.01$) and remained essentially constant for both groups throughout the duration of the test.

The percentage contribution of fat and carbohydrate to energy metabolism during the test run (Table 4.5) was calculated from R-values; thus any change in the estimated relative extent of fat and carbohydrate metabolism varied with changes in R. The mean percentage fat oxidised throughout the run was in the order of 70% for women and 35% for men ($p<0.01$), although there was a large degree of inter-subject variation. The total amount of fat metabolised during 60 minutes running was estimated to be 50.4g (0.8g min^{-1}) and 40.9g (0.7g min^{-1}) in women and men respectively

($p<0.01$). Thus the total quantity of fat, as well as the rate of fat metabolism was significantly greater in women.

The mean rate of energy expenditure during the test run (Table 4.5) was in the order of 50% higher for men than women ($p<0.01$) and increased with running time for both groups ($P<0.01$). Total energy expenditure during 60 minutes running was estimated to be 4157±299kJ for men and 2799±375kJ for women ($p<0.01$).

Men lost more weight than women ($p<0.01$), which, over the duration of the test run amounted to a greater percentage of their pre-exercise body mass ($p<0.05$) (Table 4.7). The calculated change in plasma volume during the run (Table 4.7) varied widely between subjects. Most exhibited a decrease of 3 to 4% and the greatest decline was in the order of 9% for a male subject. An increase in plasma volume (<3%) was measured in 3 men but none of the women. Over all however, group differences were not statistically significant.

4.4 Discussion

The higher R-values, higher blood lactate concentration and lower blood glucose concentration of the male relative to the female subjects during the 60 minute run were all suggestive of a greater extent of carbohydrate relative to lipid oxidation in the former (Karlsson et al, 1972; Saltin et al, 1976). A higher circulating lactate concentration in the men was likely to reflect an enhanced extent of intramuscular glycogenolysis over that of the women, whilst a higher blood glucose concentration in the women would suggest a lower rate of glucose uptake by the tissues and/or a greater rate of hepatic glycogenolysis and/or gluconeogenesis relative to the men. Comparable gender-related differences in exercise metabolism have also been noted in previous studies (Nygaard et al, 1978; Froberg and Pedersen, 1984; Blatchford et al, 1985; Tarnopolsky et al, 1990). However, other groups have failed to observe any differences in energy substrate utilisation between exercising men and women (Costill et al, 1979; Powers et al, 1980; Brewer et al, 1988).

Although these measurements pointed towards a relatively greater extent of lipid oxidation during exercise in the female as compared to the male subjects, plasma FFA and glycerol concentration did not differ between groups. The rates of FFA uptake and oxidation by exercising skeletal muscle are closely related to the plasma FFA concentration (Armstrong et al, 1961; Hagenfeldt et al, 1975) which reflects a dynamic balance between the rates of entry and removal of FFAs from the circulation. In contrast, plasma glycerol concentration tends to reflect the total extent of intramuscular plus extramuscular triglyceride catabolism over a short duration (Hetenyi et al, 1983; Miller et al, 1983). The absence of a sex difference in pre- and post-exercise plasma glycerol concentration in the present study suggested a similar extent of lipolysis in men and women; however the origin of the circulating glycerol (intra- versus extramuscular) may have differed between the sexes.

Thus, although it has been hypothesised that women may possess a greater capacity for fat metabolism than men on account of their higher body fat content (Ullyot, 1976), the female subjects of the present study did not appear to catabolise more extramuscular triglyceride than the males

during exercise. The rate of fatty acid oxidation by exercising skeletal muscle depends upon the concentration of fatty acids in the cytoplasm in contact with the mitochondria, the mitochondrial density (and therefore total oxidative enzyme activity) of the muscle fibre and the availability of other energy substrate (Holloszy and Coyle, 1984). Thus, although plasma FFA concentrations were no higher in the female subjects, a greater extent of intramuscular triglyceride catabolism in these subjects would have resulted in the exposure of available mitochondria to a greater FFA concentration for utilisation as energy substrate.

High rates of FFA oxidation in exercising skeletal muscle may lead to the muscle deriving proportionately less of it's ATP from carbohydrate sources through an operation of the "glucose-fatty acid cycle" (Randle et al, 1964; Rennie and Holloszy, 1977). The higher blood glucose concentration measured during exercise in the women certainly suggested that such a mechanism may have been active. However, a number of the hormones released in response to acute and prolonged exercise affect glucose metabolism (Galbo, 1983) and the circulating concentration of such hormones may have differed between the male and female subjects of this Study. Circulating catecholamines and the sympathetic nervous system in particular effect profound changes in blood glucose concentration, via a direct stimulation of hepatic glycogenolysis as well as indirectly through a suppression of insulin release (Porte and Williamson, 1968). An elevation of the glucagon/insulin ratio further enhances hepatic glycogenolysis and gluconeogenesis (Wassermann et al, 1984). The observed rise in blood glucose concentration recorded in women but not men during the early stages of the 60 minute run may therefore have been attributable to a greater exercise-induced catecholamine response in the former.

There was no evidence suggestive of any "adaptation" to fat utilisation with increased exercise duration in either group. R values did not fall, but remained essentially constant in women and tended to increase throughout the last 20 minutes of the test in many of the men. This observed upward drift in the value of R was linked to a marked increase in blood lactate concentration which exhibited a similar time course within the men concerned. Blood lactate concentration increased gradually throughout the test in both groups. Although blood lactate concentration was higher during exercise in men than women, it remained below the 4mmol l^{-1}

"reference" point designated as the "onset of blood lactate accumulation" (Sjodin and Jacobs 1981) in all subjects. A circulatory accumulation of H^+ ions from dissociated lactic acid would lead to an increase in CO_2 production (and hence R values) as the excess protons were buffered using the body's HCO_3^- stores. Thus, although R values did not fall with increasing exercise duration, $\dot{V}CO_2$ increased at a greater rate than did $\dot{V}O_2$ for both groups, which should tend to increase R values. However, the fact that R values remained essentially constant might suggest that there was a small but gradual increase in lipid oxidation as the test progressed.

With regard to the theoretical model of the "reference" man and woman (Behnke and Wilmore, 1974) (Section 2.1), the male subjects of this study were taller, heavier and had a higher BMI.

Sex differences in VO_2max (per unit body mass) were of a similar magnitude to those measured in other studies of "equally" trained men and women (Sparling, 1980; Cureton et al, 1986; Tarnopolsky et al, 1990) and were in the order of 46% higher for the females and 39% higher for the male subjects than the corresponding values typical of untrained men and women (Sparling, 1980).

The mean treadmill speed required to set a relative exercise intensity approximating to 70% VO_2max for each subject and the measured $\dot{V}O_2$ during the 60 minute run were both of the order of 15% higher for men than women. This can be accounted for by the fact that the mean VO_2max (per unit body mass) of the former was also in the order of 15% higher than that of the latter, whilst running economy was similar for both groups.

The 50% difference in estimated energy expenditure between groups during the 60 minute test run was presumably attributable to the combined effects of a greater body weight and faster running speed of the men. Since the energy requirements of treadmill running are essentially governed by force (body mass) and speed, the 28% difference in mean body weight and 14% difference in treadmill speed between groups would feasibly account for a major portion of the higher rate of energy expenditure measured in the men.

Since the ambient temperature and humidity were similar for men and women during the 60 minute run, the greater weight loss of the former was probably attributable to their higher rate of energy expenditure. Sweat rate during exercise is proportional to absolute metabolic rate (Nielsen, 1969). Although men tended to consume more fluid than women during the run, there was no apparent relationship between fluid loss and fluid intake.

A decrease in plasma volume of 4 to 6% was observed in most subjects; however an apparent increase occured in 3 men and 1 woman. Prolonged submaximal exercise in a temporate climate is usually characterised by a haemoconcentration effected mainly through sweat loss (Costill and Fink, 1974; Kolka et al, 1978). A subsequent haemodilution is not usually apparent until a few hours later (Wells et al, 1982; Dickson et al, 1982). However there is evidence to suggest that in trained individuals, protein is moved from the interstitial spaces into the vascular compartment during prolonged exercise, resulting in an increase in the osmotic pressure of the latter and a shift of fluid into the vessels (Senay, 1979).

Heart rate increased significantly in both groups over the duration of the run. The greatest extent of such "cardiovascular drift" were measured in those subjects exhibiting the greatest decline in plasma volume and presumably served to help maintain an adequate cardiac output in the face of a declining central blood volume. A redistribution of cardiac output towards the skin, the loss of fluid in the sweating process and increased filtration of fluid from capillaries perfusing the active skeletal muscles (Rowell et al, 1966) would all lead to a decline in systemic filling pressure and cardiac stroke volume (Saltin and Stenberg, 1964) and hence a compensatory increase in heart rate.

The gradual increase in oxygen uptake with exercise time accounted for the similar rate of increase in the estimated rate of energy expenditure for both groups. The rate and extent of the increase in VO_2 tended to be greater in men and may have been attributable to an increase in deep body temperature (Q10 effect) rather than an increase in the extent of lipid oxidation, since R values did not decline with exercise time for either group. The increase in carbon dioxide production over the duration of the run observed in both groups tended to parallel the increase in blood lactate concentration.

Endogenous sex steroid hormones have been postulated a role in the control of substrate metabolism during exercise by way of their influence on intramuscular triglyceride and glycogen storage (Gillespie and Edgerton, 1970; Knudsen and Max, 1980) as well as via a modification of the relative proportions and absolute concentrations of various "anabolic" and "catabolic" hormones released during exercise (Nygaard et al, 1984). Oestrogens in particular have been shown to potentiate the storage, catabolism and oxidation of lipid in skeletal muscle (Rebuffe-Scrive et al, 1985). Thus, any gender-related differences in the metabolism of energy substrate at rest and especially during a metabolic challenge such as muscular exercise may be effected through differences in circulating "male" and "female" sex steroid hormones. It is noteworthy that all the women of the present Study had regular menstrual cycles and presumably "normal" circulating sex steroid hormone concentrations.

"Training status" exerts a profound influence upon the metabolic responses to submaximal exercise in both sexes. The similarity in past and present physical activity patterns as well as blood lactate concentrations at given relative exercise intensities between the male and female subjects of this Study suggested that there was little difference in skeletal muscle "training status" (oxidative capacity) between groups. However, "training status" is very difficult to quantify precisely. Although it is possible to question subjects about their weekly training regimen and number of years training experience, many recreational athletes (like the subjects in the present study) do not adhere to a rigid training schedule and participate in a number of other different sports to a greater or lesser extent according to desire and season in the year. The extent to which other sports may exert a cross-training effect on the oxidative capacity of the skeletal muscles recruited during steady pace submaximal running as well as VO_2max is not easily quantified. A major problem could reside within the fact that subjects may possess an inherently high VO_2max which they have maximised through participation in sports aside from running, which recruit different muscle groups. In such cases, the speed corresponding to 70%VO_2max of these individuals may exceed the oxidative capacity of their skeletal muscles, so that they fail to maintain a steady state when running for prolonged duration at this speed. If this is the situation, it may be more informative to set the exercise test intensity relative to the speed

at which the subject starts accumulating lactate in the circulation, rather than relative to their VO_2max. Thus, for the purpose of future study it may be advisable to recruit subjects engaged only in running training in order to minimise inter-subject differences in the "training status" of skeletal muscles recruited during treadmill running exercise.

In conclusion, the data collected in this study suggests that a sex difference in the metabolic response to prolonged submaximal exercise probably does exist. However, to elucidate the precise mechanisms underlying any such differences requires the use of sophisticated and invasive techniques such as the muscle biopsy in order to quantify the extents of intramuscular glycogen and triglyceride utilisation during exercise, or a measurement of the turnover of energy substrates and metabolites. Given that a major portion of gender differences in physiological characteristics appear at puberty under the influence of an increased output of gonadal sex steroid hormones, it is possible that any gender-related differences in the capacity to store, mobilise and utilise different types of energy substrate during endurance-type exercise may also be effected through these hormones.

Intense endurance training, especially when combined with an energy restricted diet in lean women can disrupt menstrual cyclicity (Warren and Van de Wiele, 1973). Amenorrhoeic athletes have a hormonal profile similar to that of the pre-pubescent female, characterised by low circulating concentrations of oestrogens, progesterone and gonadotropins which fail to vary in a cyclical manner (Loucks and Horvath, 1984). If the prevailing sex hormone concentration influences substrate utilisation during exercise, then amenorrhoeic women may exhibit a different and perhaps more "masculine" metabolic response to prolonged submaximal exercise than eumenorrhoeic women.

Thus, further research is required into the possible influences of sex steroid hormones on substrate storage, mobilisation and utilisation during prolonged submaximal exercise. An insight into any role for these hormones in the metabolism of energy substrate during exercise may perhaps be obtained through a comparison of exercise-induced metabolic responses between equally trained amenorrhoeic and eumenorrhoeic women.

4.5 Summary

Differences in the metabolic responses of moderately trained men and women to prolonged steady pace treadmill running suggested a relatively greater contribution of lipid than carbohydrate to energy metabolism in the latter. The precise mechanisms underlying such differences could not be elucidated from the techniques employed.

However, there is some dispute as to how evenly matched these men and women were with regard to the oxidative capacity of the skeletal muscles recruited during treadmill running, since running was not the major sport practised by all of the subjects. As a result, the treadmill speed corresponding to 70%VO_2max of some of these individuals may have exceeded the oxidative capacity of their recruited skeletal muscles such that they failed to maintain a near "steady state" when running for prolonged periods at the allocated speeds. Therefore, for the purpose of future studies attempts should also be made to recruit male and female subjects engaging only in the exercise used in the testing procedure in order to minimise the influence of the recruited muscles' "training status" upon their preferred choice of energy substrate.

It is apparent that the focus of future research should involve further qualification of the possible role of sex steroid hormones on substrate storage, mobilisation and utilisation during prolonged submaximal exercise.

CHAPTER 5

THE METABOLIC RESPONSES OF HIGHLY TRAINED "ELITE" EUMENORRHOEIC AND AMENORRHOEIC RUNNERS TO PROLONGED CONSTANT PACE TREADMILL RUNNING

5.1 Introduction

The primary objective of this study was to obtain further insight into a possible influence of ovarian steroid hormones on the metabolism of energy substrate during prolonged submaximal exercise. Gender-related differences in the morphologic and metabolic properties of many organ systems controlling exercise homeostasis can be attributed to influences of circulating sex steroid hormones. These hormones may therefore be directly, or indirectly responsible for effecting gender-related differences in the metabolic responses to prolonged submaximal exercise.

Highly endurance trained women may experience amenorrhoea (cessation of menstrual cycles), coupled to subnormal concentrations of circulating ovarian steroid hormones (Shangold et al, 1979; Fisher et al, 1986). Thus, if ovarian steroid hormones influence the relative extents of lipid and carbohydrate utilisation by exercising skeletal muscle, then the metabolic responses of amenorrhoeic (non menstruating) and eumenorrhoeic (cyclically menstruating) women to submaximal exercise might be expected to differ. Additionally, if any such metabolic effects of these hormones are "dose related", then eumenorrhoeic woman might be expected to exhibit responses of greater magnitude during the luteal phase of their menstrual cycle when circulating oestradiol and progesterone attain peak concentrations.

The objectives of the present study were thus 3-fold:

i) To examine the possible influences of ovarian steroid hormones upon substrate metabolism during prolonged steady pace treadmill running by comparing and contrasting exercised-induced metabolic responses in highly trained amenorrhoeic and eumenorrhoeic female distance runners.

ii) To examine any possible influence of menstrual cycle phase upon substrate metabolism during prolonged steady pace treadmill running by comparing exercise-induced metabolic responses in a group of highly trained eumenorrhoeic female athletes during the early follicular phase ("basal" plasma oestradiol and progesterone concentrations) and mid luteal phase (peak plasma oestradiol and progesterone concentrations) of their menstrual cycles.

iii) To obtain further insight into the factors linked to menstrual cycle disruption in highly trained female athletes.

5.2(a) Methods

A total of 30 female middle/long distance runners volunteered as subjects for the study. All were at least county standard in their best performance distance; 17 were nationally ranked juniors or seniors and 4 were of international standard. Each completed an extensive questionaire (Appendix 8) relating to past and present training and dietary regimens current menstrual status and menstrual history, attitudes towards diet and training as well as subjective stress associated with training, competition, academic work/employment, social and family life. Answers to the questionaire were used to recruit subjects of "appropriate" past and present training characteristics, athletic ability and menstrual status. Those women taking any type of steroid hormone medication were excluded from the study.

Each subject was assigned to one of 2 groups according to menstrual status which was defined as follows:

Eumenorrhoeic (Eu): 11 or 12 menstrual periods during the preceding 12 months.

Amenorrhoeic (Am): Total absence of menstrual periods during the preceding 6 months.

A reproductive endocrine profile of each subject was also made to qualify menstrual status between groups (described below).

On the basis of these classification criteria, 9 volunteers could be neither classified as Eu or Am on account of their very irregular menstrual cycles (fewer than 8 menstrual periods per year). These oligomenorrhoeic women were thus disqualified from the study. Of the remaining 21 subjects, 11 were Am and 10 Eu. However, 3 women were forced to withdraw from the study part way through testing due to injury, thus leaving a total of 9 Am and 9 Eu women to complete the entire study.

Training status:

An attempt was made to select 2 groups of equally, but highly trained subjects. The criteria for selection was that each should currently be engaged in at least 5 hours of vigorous "aerobic" exercise per week, be running at least 50km/week, have been training consistently for at least 4 years and to have achieved success in their chosen event at county, national or international level.

Diet:

Each subject weighed and noted all the food they consumed during 7 consecutive days for an assessment of mean daily energy consumption, macronutrient and micronutrient intakes. The importance of the subjects adhering to their "normal" diets was stressed in an attempt to prevent them from specifically modifying it for the study. Thus, it was hoped that they would provide an honest account of the precise quantitities and types of foods consumed. No attempt was made to exclude women with any history of eating disorders from the study and 4 of the 9 Am subjects admitted to having a medical history of anorexia nervosa.

Body composition:

A detailed assessment of body composition was made in all but 1 subject member of each group who were unavailable at the time of measurement. The relative proportions of fat and lean tissue in the body were calculated via the hydrostatic/underwater weighing (UWW) method for measurement of body density (Siri, 1954) as well as being estimated from measurements of skinfold thickness at 4 standard sites (Durnin and Womersley, 1974). Skinfold thickness was also measured at 3 other sites in order to further examine and compare the pattern of subcutaneous fat distribution between groups. Measurements of waist and hip circumference were also made.

Reproductive hormone status:

Serum concentrations of the ovarian steroid hormones oestradiol 17B (RIA Direct HD 293012, Baxter Healthcare Ltd), progesterone (RIA Gamma B kit, Immunodiagnostics), testosterone (RIA extraction assay STRIA kit) and pituitary gonadotropins (LH and FSH) (WHO LH and FSH assay kit, Chelsea Hospital, London) were measured from venous blood samples taken immediately prior to the 60 minute test run in all subjects. Values thus corresponded to early follicular phase hormone concentrations in all of the Eu participants, but also mid-luteal phase concentrations in the 5 Eu women who offered to perform the test run during both phases of their menstrual cycles. Serum oestradiol, progesterone and testosterone concentrations were also measured from post-exercise venous blood samples for a quantification of the magnitude of change in their circulating concentrations effected by the exercise test.

Exercise tests:

The exercise test protocol employed in the present study was identical to that of Study 1. The standard 60 minute treadmill run formed the metabolic challenge and was preceded by the 3 preliminary tests (section 3.3). An attempt was made to carry out all 4 exercise tests on each subject within a 5-7 day interval and although this was achieved for all the Am subjects, it was rarely possible for the Eu subjects, who were required to perform the 60 minute test run during the early follicular phase of their menstrual cycles (day 2,3 or 4 after the start of menstruation) for the purpose of standardisation of circulating reproductive hormone concentrations. Nevertheless, it was still possible to limit the time span between the subjects' performance of the preliminary tests and the 60 minute test run to no more than 3 weeks, with the test run being performed at the start of the next immediate menstrual cycle subsequent to preliminary testing.

Five of the Eu subjects agreed to perform the 60 minute test run on a second occasion, at a time corresponding approximately to the mid-luteal phase of the same menstrual cycle (20-24 days following the first 60 minute test run). Each of these subjects was provided with a digital display

electrical oral clinical thermometer (Philips HP5311) and instructed to take a reading every morning after their last menstrual period in an attempt to predict the ovulatory rise in basal body temperature (BBT). An abrupt rise in oral temperature amounting to at least 0.3°C which was subsequently maintained throughout the rest of the cycle was designated to signify the onset of the luteal phase (Bullen et al, 1984). However, such an increment was detected by only 1 individual who performed the 60 minute test run for a second time 6 days later. The remaining 4 subjects failed to observe any sustained elevation of oral temperature of a magnitude beyond that of small day to day fluctuations, so were asked to perform the 60 minute test run for a second time 19-21 days after the start of their last menstrual period. It was hoped that subsequent hormonal data derived from the analysis of blood collected from these subjects on the morning of the test run might confirm that they were indeed being tested during the mid-luteal phase of their menstrual cycles.

Sixty minute test run data was thus obtained for 9 Am subjects (in whom reproductive hormones were presumed not to fluctuate cyclically), 9 Eu subjects exercising during the early follicular phase of their menstrual cycles and 5 of the same Eu subjects exercising during the (assumed) mid-luteal phase.

Table 5.1 Physiological Characteristics of Subjects

	Age (yrs)	Height (cm)	Weight (kg)	BMI	VO_2max ($ml kg^{-1} min^{-1}$)	HRmax ($bt min^{-1}$)	VO_2max ($ml kg^{-1} LBM min^{-1}$) (UWW)	VO_2max ($ml kg^{-1} LBM min^{-1}$) (SKF)
Eu	23	169.7	59.67	20.8	59.51	185	75.34	83.01
	±4	±4.9	±4.57	±1.4	±3.34	±5	±3.15	±6.05
Am	24	165.0	50.13	18.3	60.38	185	72.50	75.77
	±6	±4.9	±4.22	±0.8	±7.01	±6	±6.26	±7.10
	ns	*	**	**	ns	ns	ns	ns

Values indicated as mean ± sd of mean.
** $p<0.01$; * $p<0.05$; ns = non significant (Eu versus Am women).

Table 5.2: Physique and Body Composition of Subjects

	%Fat		FFM	SKF thickness (mm)							W/H
	(UWW)	(SKF)	(kg)	B	T	SS	SI	A	C	Th	
Eu	21.6	28.6	47.6	9.1	19.1	17.2	12.3	14.8	11.9	21.4	0.74
	±1.9	±3.5	±3.3	±2.7	±2.8	±5.7	±5.4	±4.4	±3.1	±3.3	±0.02
Am	16.6	18.9	42.3	3.7	11.7	7.5	6.6	6.3	5.6	12.6	0.76
	±2.7	±4.3	±3.0	±0.8	±5.7	±3.6	±1.8	±3.0	±2.1	±4.0	±0.03
	**	**	*	**	**	**	**	**	**	**	ns

Values indicated as mean ± sD of mean.
** $p<0.01$; * $p<0.05$; ns = non significant (Eu versus Am women).

Table 5:3 Reproductive Hormone Profiles of Am versus Eu Subjects (follicular phase) and Eu Subjects (follicular versus luteal phase).

	Pre-exercise		Pre-exercise			Post-exercise		
	LH (IUl^{-1})	FSH (IUl^{-1})	E_2 (pMl^{-1})	P (uMl^{-1})	T (uMl^{-1})	E_2 (pMl^{-1})	P (uMl^{-1})	T (uMl^{-1})
Eu	6.3	4.6	70.0	3.0	2.0	103.2	3.0	2.5
	±1.9	±1.4	±11.3	±0.9	±0.4	±22.4	±1.0	±0.3
Am	3.9	4.7	50.2	2.8	1.9	76.4	2.8	2.1
	±1.8	±0.9	±11.8	±0.7	±0.4	±20.5	±1.1	±0.6
	**	ns	**	ns	ns	**	ns	ns
Eu f (n=5)	5.5	4.5	65.8	3.0	1.9	96.0	3.3	2.3
	±1.8	±1.4	±14.0	±1.1	±0.3	±25.8	±1.2	±0.2
Eu l (n=5)	6.6	4.1	314.0	13.0	2.1	472.6	18.7	2.6
	±1.7	±1.1	±76.8	±11.2	±0.4	±129.2	±16.4	±0.4
	ns	ns	**	**	ns	**	**	ns

Values indicated as mean ± sd of mean.
** $p<0.01$; * $p<0.05$; ns = non significant (Eu versus Am women).
$p<0.01$; # $p<0.05$; ns = non significant (Follicular versus Luteal phase).

Table 5.4: Nutritional Composition of Daily Diet

	Energy intake (kcal)	Fat (g)	Protein (g)	CHO (g)	Iron (mg)	Zinc (mg)
Eu	2484.7 ±673.6	85.6 ±40.2	85.1 ±29.4	362.2 ±75.0	18.7 ±5.4	11.6 ±4.6
Am	1900.0 ±492.0 *	51.4 ±26.3 *	70.8 ±20.4 ns	306.8 ±77.0 ns	18.0 ±6.5 ns	10.2 ±2.8 ns

	Energy intake ($kcalkg^{-1}$)	Fat (gkg^{-1})	Protein (gkg^{-1})	CHO (gkg^{-1})	Iron ($mgkg^{-1}$)	Zinc ($mgkg^{-1}$)
Eu	41.32 ±10.83	1.40 ±0.66	1.38 ±0.48	5.88 ±1.29	0.31 ±0.09	0.19 ±0.08
Am	37.98 ±9.07 ns	1.01 ±0.45 ns	1.41 ±0.35 ns	6.18 ±1.69 ns	0.36 ±0.12 ns	0.20 ±0.05 ns

Values indicated as mean ± sD of mean.
** $p<0.01$; * $p<0.05$; ns = non significant (Eu versus Am women).

Table 5.5: Estimated Daily Energy Balance of Subjects

	Energy exp. in exercise (kcal)	BMR (kcal)	BMR + Energy exp. (kcal)	Energy intake (kcal)	Balance (kcal)
Eu	798	1391	2189	2484.7	+266
	±312	±60	±320	±673.6	±606
Am	718	1266	1983	1900.0	-22
	±310	±68	±353	±492.0	±691
	ns	ns	ns	*	ns

Values indicated as mean ± sd of mean.
** $p<0.01$; * $p<0.05$; ns = non significant (Eu versus Am women).

Table 5·6: Training Characteristics of Subjects

	Weekly running distance (km)	Days run per week	Weeks per year	Total exercise time per week (min)	Years running experience (yr)
Eu	62	6	48	487	8
	±16	±1	±2	±189	±3
Am	67	6	49	504	9
	±24	±1	±1	±211	±5
	ns	ns	ns	ns	ns

Values indicated as mean ± so of mean.
** $p<0.01$; * $p<0.05$; ns = non significant (Eu versus Am women).

Table 5.7: Running Economy and Treadmill Speeds equivalent to 70% VO_2max of Subjects

	VO_2 ($ml kg^{-1} min^{-1}$) 3.20ms^{-1}	4.10ms^{-1}	"70%" speed (ms^{-1})
Eu	36.54 ±1.73	46.86 ±1.47	3.60 ±0.29
Am	34.25 ±3.22	45.48 ±2.87	3.74 ±0.46
	ns	ns	ns

Values indicated as mean ± sd of mean.
** $p<0.01$; * $p<0.05$; ns = non significant (Eu versus Am women).

Table 5.8: Percentage VO_2max and Treadmill Speeds at $2mmoll^{-1}$ and $4mmoll^{-1}$ Blood Lactate Concentrations

	2 $mmoll^{-1}$		4 $mmoll^{-1}$	
	Speed (ms^{-1})	$\%VO_2max$	Speed (ms^{-1})	$\%VO_2max$
Eu	3.77 ±0.42	69.01 ±9.97	4.29 ±0.54	83.82 ±4.68
Am	3.95 ±0.67	73.66 ±4.29	4.56 ±0.59	84.13 ±4.57
	ns	ns	ns	ns

Values indicated as mean ± so of mean.
** $p<0.01$; * $p<0.05$; ns = non significant (Eu versus Am women).

Table 5.9: Respiratory and Cardiovascular responses during 60 minute run

		15min	30min	45min	60min
% VO_2max	Eu	70.16 ±1.60	71.12 ±1.25	71.01 ±1.96	71.18 ±1.64
	Am	70.73 ±2.09 ns	70.84 ±2.05 ns	71.32 ±2.35 ns	71.62 ±2.33 ns
Heart rate ($bt min^{-1}$)	Eu	161 ±9	165 ±8	165 ±9	171 ±6
	Am	154 ±6 ns	160 ±7 ns	163 ±10 ns	165 ±10 ns
VO_2 ($ml kg^{-1} min^{-1}$)	Eu	41.73 ±1.96	42.30 ±1.99	42.25 ±2.54	42.34 ±2.14
	Am	42.69 ±4.88 ns	43.16 ±4.28 ns	43.36 ±4.61 ns	43.53 ±4.60 ns
VCO_2 ($ml kg^{-1} min^{-1}$)	Eu	35.89 ±2.32	35.50 ±2.49	35.28 ±1.89	35.62 ±1.96
	Am	36.38 ±4.74 ns	36.72 ±4.10 ns	36.72 ±4.67 ns	36.26 ±4.72 ns
R	Eu	0.86 ±0.03	0.84 ±0.03	0.84 ±0.03	0.84 ±0.02
	Am	0.85 ±0.06 ns	0.85 ±0.04 ns	0.84 ±0.06 ns	0.83 ±0.06 ns
% Contribution fat to metabolism	Eu	47.54 ±10.12	54.84 ±9.48	55.88 ±8.72	54.46 ±8.08
	Am	50.27 ±13.82 ns	51.00 ±13.91 ns	54.53 ±19.32 ns	58.16 ±17.67 ns
Energy expenditure ($kJ min^{-1}$)	Eu	49.80 ±3.63	51.12 ±4.44	50.83 ±4.30	50.54 ±4.08
	Am	42.48 ±4.86 **	42.77 ±5.34 **	43.03 ±4.74 **	43.22 ±5.47 **

Values indicated as mean ± SD of mean.
** $p<0.01$; * $p<0.05$; ns = non significant (Eu versus Am women).

Table 5.10: Metabolic Responses during 60 minute run

		Rest	15min	30min	45min	60min
Blood lactate ($mmoll^{-1}$)	Eu	0.83 ±0.11	2.53 ±0.77	2.35 ±0.58	2.32 ±1.05	2.27 ±0.62
	Am	0.84 ±0.13 ns	1.88 ±0.35 *	2.02 ±0.61 ns	2.06 ±0.54 ns	2.32 ±0.72 ns
Blood glucose ($mmoll^{-1}$)	Eu	3.92 ±0.47	4.05 ±0.62	4.21 ±0.69	4.40 ±0.66	4.84 ±0.54
	Am	3.99 ±0.33 ns	3.94 ±0.50 ns	4.07 ±0.45 ns	4.15 ±0.38 ns	4.49 ±0.37 ns
Plasma FFA ($mmoll^{-1}$)	Eu	0.48 ±0.19	-	-	-	0.90 ±0.28
	Am	0.31 ±0.10 *	-	-	-	0.59 ±0.18 *
Plasma glycerol ($mmoll^{-1}$)	Eu	0.10 ±0.01	-	-	-	0.55 ±0.19
	Am	0.05 ±0.02 **	-	-	-	0.32 ±0.11 **

Values indicated as mean ± so of mean.
** $p<0.01$; * $p<0.05$; ns = non significant (Eu versus Am women).

Table 5.11: Changes in Haemoglobin, Haematocrit and Plasma Volume over 60 minute run

		Pre-exercise	Post-exercise
Haemoglobin ($g\,dl^{-1}$)	Eu	13.65 ±0.74	14.16 ±0.92
	Am	13.09 ±0.99 ns	13.31 ±0.92 ns
Haematocrit (%)	Eu	39.9 ±2.2	40.9 ±2.0
	Am	38.9 ±1.9 ns	38.4 ±2.8 ns
% change in plasma volume	Eu		-4.96 ±4.33
	Am		-0.79 ±7.50 ns
Weight loss (kg)	Eu		1.02 ±0.23
	Am		0.71 ±0.22 *
% Weight loss	Eu		1.68 ±0.36
	Am		1.41 ±0.40 ns

Values indicated as mean ± sd of mean.
** $p<0.01$; * $p<0.05$; ns = non significant (Eu versus Am women).

Table 5.12: Respiratory and Cardiovascular responses during 60 minute run (Eumenorrhoeic women: Follicular v Luteal phase)

		15min	30min	45min	60min
Heart rate ($bt min^{-1}$)	Fol	161 ±9	165 ±9	170 ±9	174 ±8
	Lut	157 ±9 ns	163 ±7 ns	167 ±7 ns	171 ±6 ns
VO_2 ($ml kg^{-1} min^{-1}$)	Fol	41.47 ±2.47	42.17 ±2.38	41.95 ±3.16	42.09 ±2.45
	Lut	41.58 ±2.66 ns	41.77 ±2.56 ns	42.13 ±2.91 ns	42.38 ±2.79 ns
VCO_2 ($ml kg^{-1} min^{-1}$)	Fol	35.85 ±3.10	35.69 ±3.22	35.16 ±2.26	35.56 ±2.19
	Lut	35.02 ±1.61 ns	36.01 ±2.15 ns	35.62 ±2.26 ns	35.40 ±2.88 ns
R	Fol	0.86 ±0.04	0.84 ±0.03	0.84 ±0.03	0.85 ±0.03
	Lut	0.85 ±0.04 ns	0.86 ±0.05 ns	0.85 ±0.04 ns	0.84 ±0.03 ns

Values indicated as mean ± so of mean.
** $p<0.01$; * $p<0.05$; ns = non significant (Follicular versus Luteal phase).

Table 5.13 : Metabolic responses during 60 minute run
(Eumenorrhoeic women: Follicular v Luteal phase)

		Rest	15min	30min	45min	60min
Blood lactate ($mmoll^{-1}$)	Fol	0.85 ±0.18	2.39 ±1.01	2.24 ±0.76	2.55 ±1.30	2.29 ±0.69
	Lut	0.78 ±0.19 *	2.06 ±0.68 *	1.66 ±0.48 *	1.83 ±0.73 *	1.65 ±0.38 *
Blood glucose ($mmoll^{-1}$)	Fol	3.79 ±0.49	3.85 ±0.45	4.04 ±0.58	4.24 ±0.73	4.81 ±0.66
	Lut	4.06 ±0.25 ns	3.83 ±0.45 ns	4.19 ±0.65 ns	4.29 ±0.68 ns	4.62 ±1.04 ns
Plasma FFA ($mmoll^{-1}$)	Fol	0.41 ±0.22	-	-	-	0.91 ±0.28
	Lut	0.45 ±0.20 ns	-	-	-	0.88 ±0.36 ns
Plasma glycerol ($mmoll^{-1}$)	Fol	0.10 ±0.01	-	-	-	0.54 ±0.24
	Lut	0.11 ±0.03 ns	-	-	-	0.56 ±0.23 ns

Values indicated as mean ± so of mean.
** $p<0.01$; * $p<0.05$; ns = non significant (Follicular versus Luteal phase).

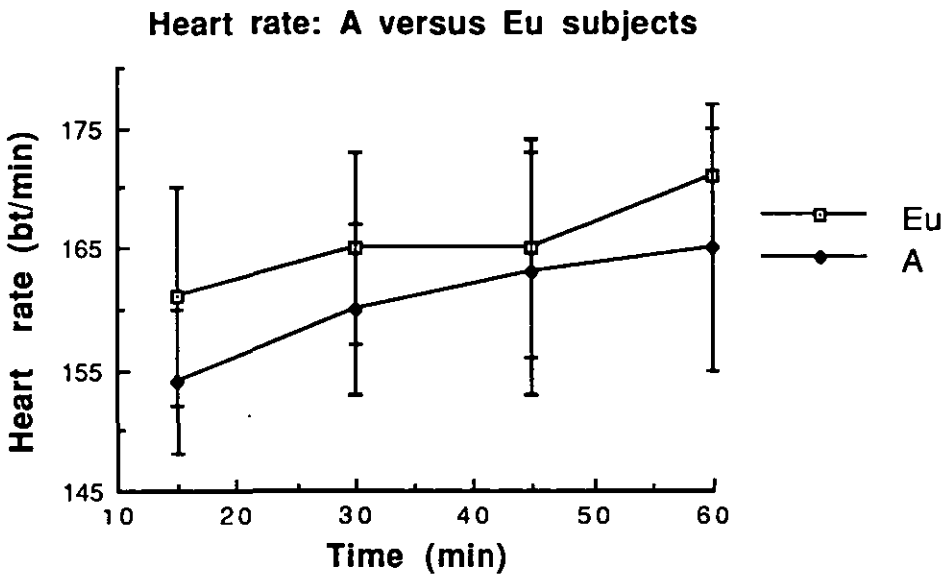
Heart rate: A versus Eu subjects
Heart rate (bt/min)
175
165
155
145
10
20
30
40
50
60
Time (min)
Eu
A

Fig 5.1

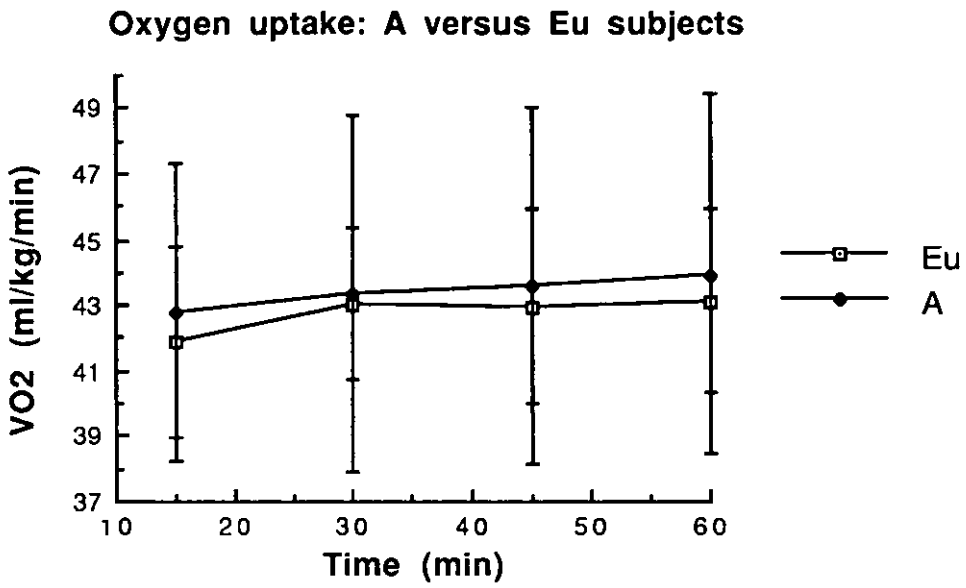
Oxygen uptake: A versus Eu subjects
VO2 (ml/kg/min)
49
47
45
43
41
39
37
10
20
30
40
50
60
Time (min)
Eu
A

Fig 5.2

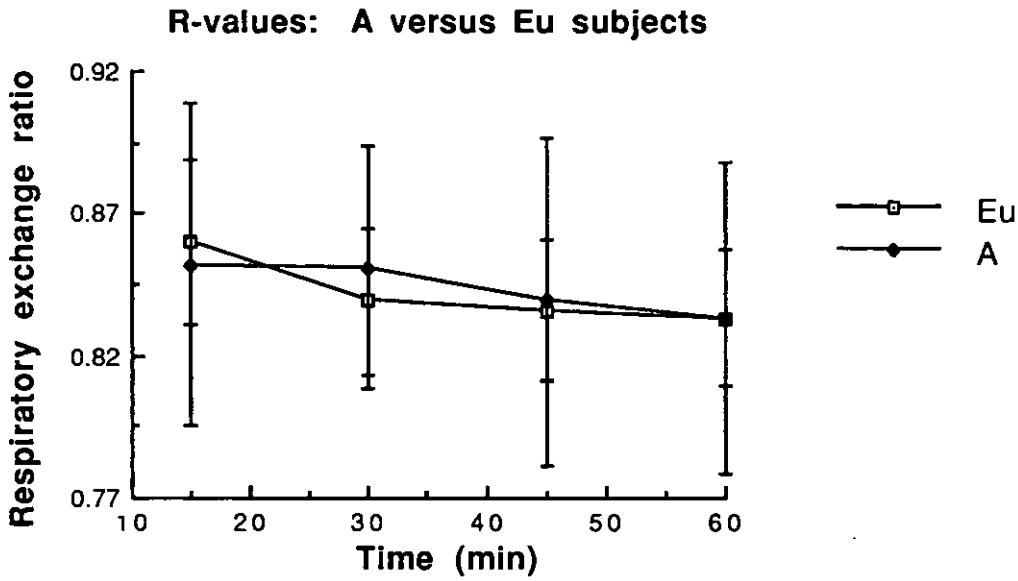
R-values: A versus Eu subjects
Respiratory exchange ratio
0.92
0.87
0.82
0.77
10
20
30
40
50
60
Time (min)
Eu
A

Fig 5.3

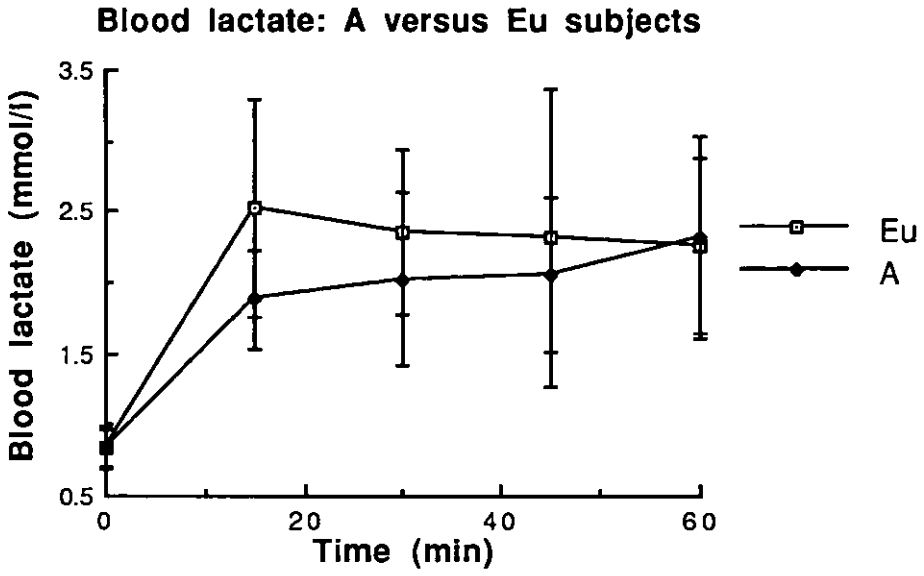
Blood lactate: A versus Eu subjects
Blood lactate (mmol/l)
3.5
2.5
1.5
0.5
0
20
40
60
Time (min)
Eu
A

Fig 5.4

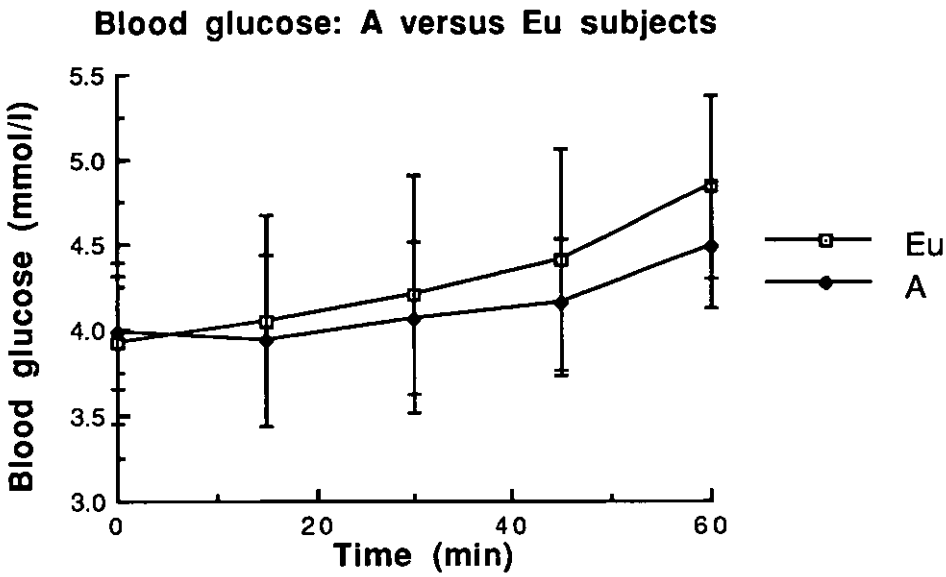
Blood glucose: A versus Eu subjects
Blood glucose (mmol/l)
5.5
5.0
4.5
4.0
3.5
3.0
0
20
40
60
Time (min)
Eu
A

Fig 5.5

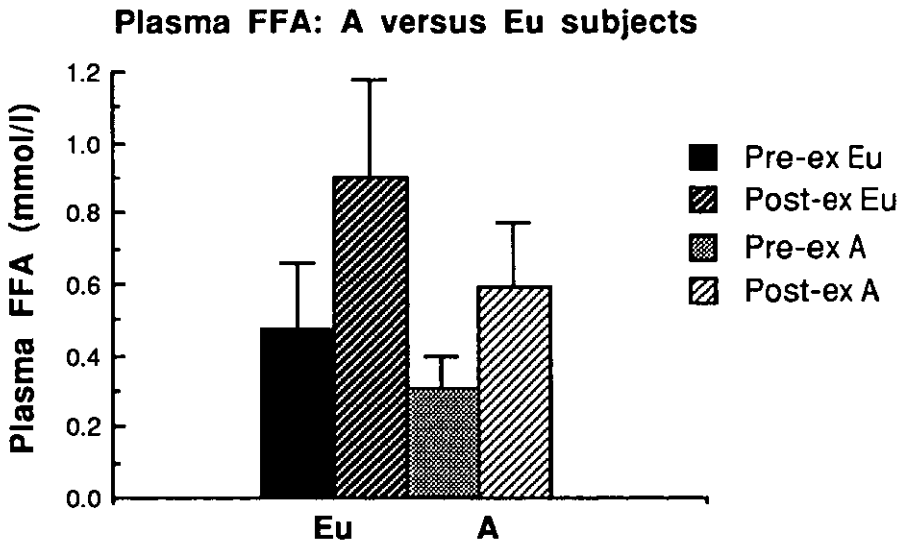
Plasma FFA: A versus Eu subjects
Plasma FFA (mmol/l)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Eu
A
Pre-ex Eu
Post-ex Eu
Pre-ex A
Post-ex A

Fig 5·6

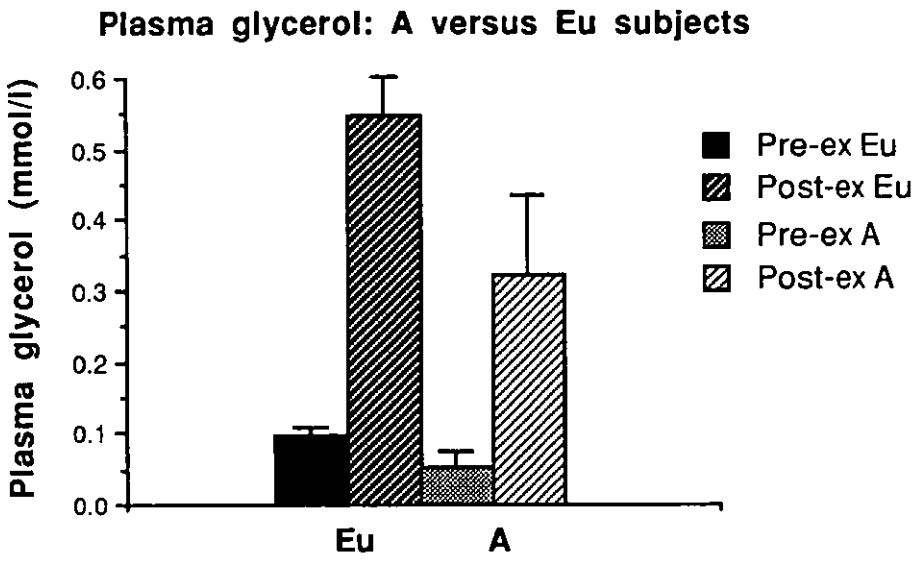
Plasma glycerol: A versus Eu subjects
Plasma glycerol (mmol/l)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Eu
A
Pre-ex Eu
Post-ex Eu
Pre-ex A
Post-ex A

Fig 5·7

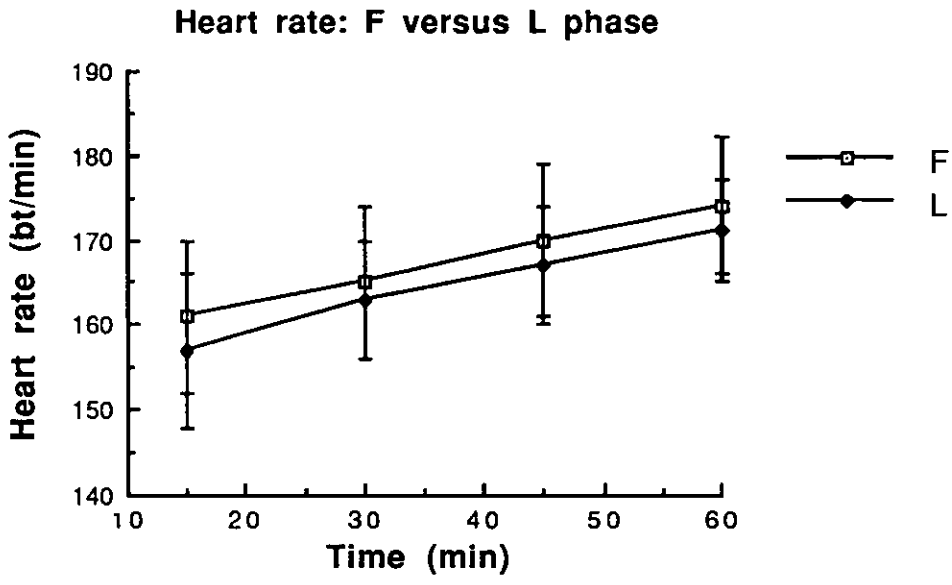
Heart rate: F versus L phase
Heart rate (bt/min)
190
180
170
160
150
140
10
20
30
40
50
60
Time (min)
F
L

Fig 5.8

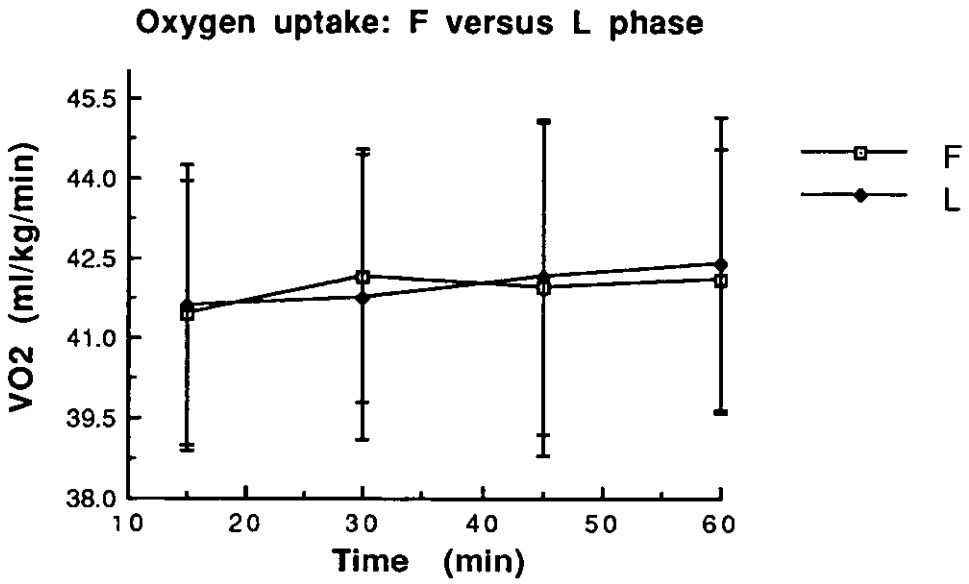
Oxygen uptake: F versus L phase
VO2 (ml/kg/min)
45.5
44.0
42.5
41.0
39.5
38.0
10
20
30
40
50
60
Time (min)
F
L

Fig 5.9

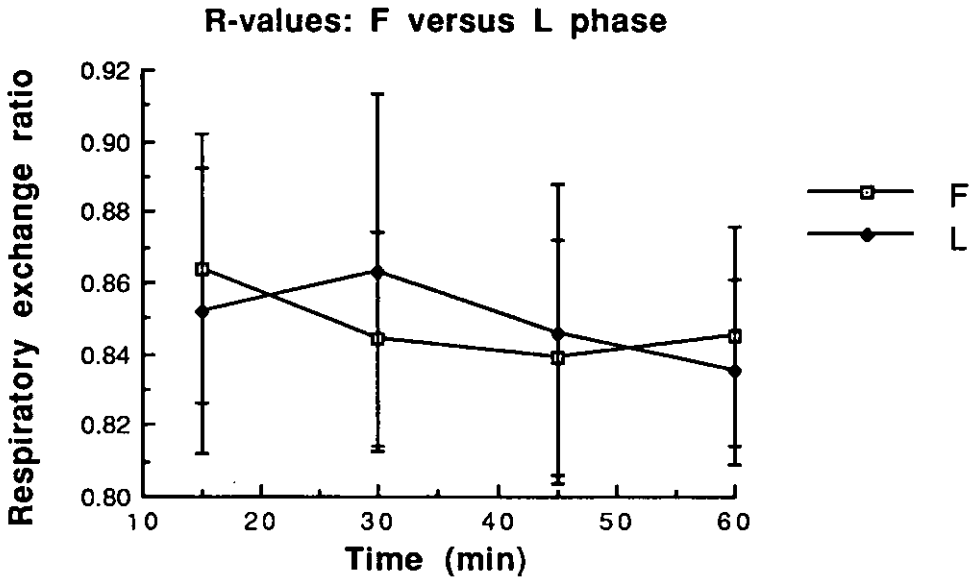
R-values: F versus L phase
Respiratory exchange ratio
0.92
0.90
0.88
0.86
0.84
0.82
0.80
10
20
30
40
50
60
Time (min)
F
L

Fig 5.10

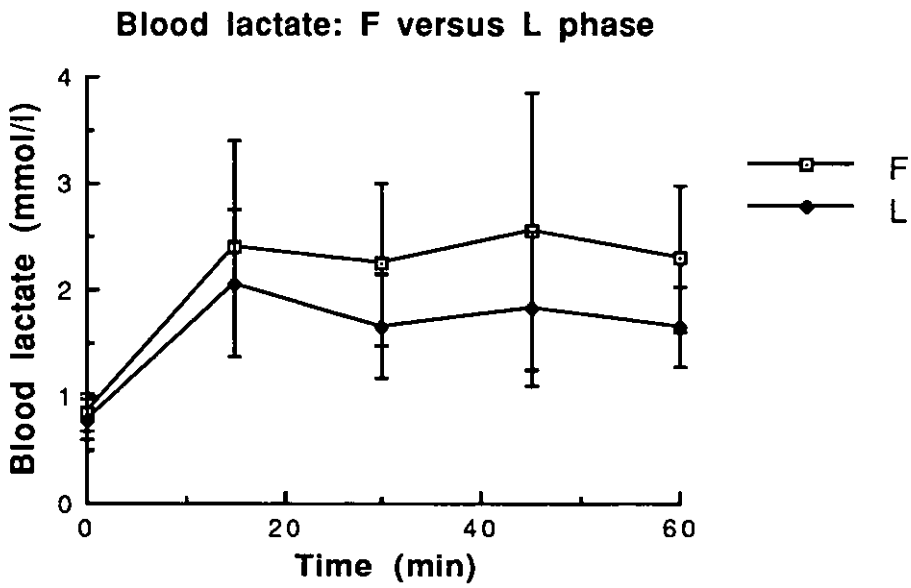
Blood lactate: F versus L phase
Blood lactate (mmol/l)
4
3
2
1
0
0
20
40
60
Time (min)
F
L

fig 5.11

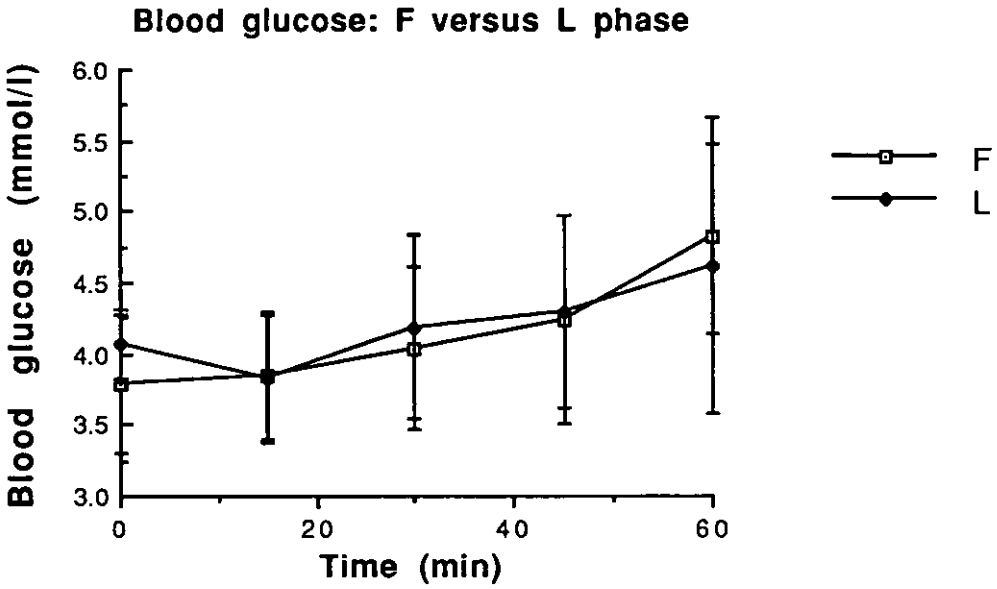
Blood glucose: F versus L phase
Blood glucose (mmol/l)
6.0
5.5
5.0
4.5
4.0
3.5
3.0
0
20
40
60
Time (min)
F
L

Fig 5.12

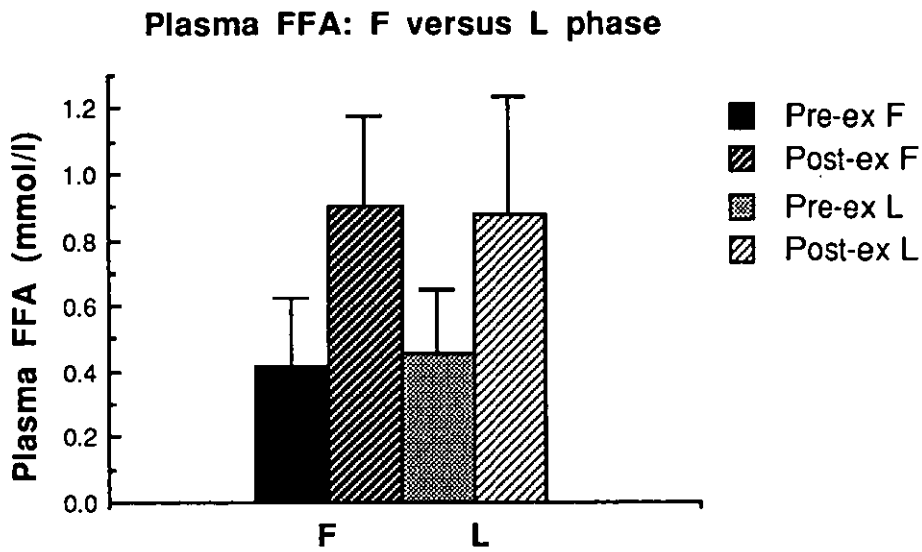
Plasma FFA: F versus L phase
Plasma FFA (mmol/l)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
F
L
Pre-ex F
Post-ex F
Pre-ex L
Post-ex L

Fig 5·13

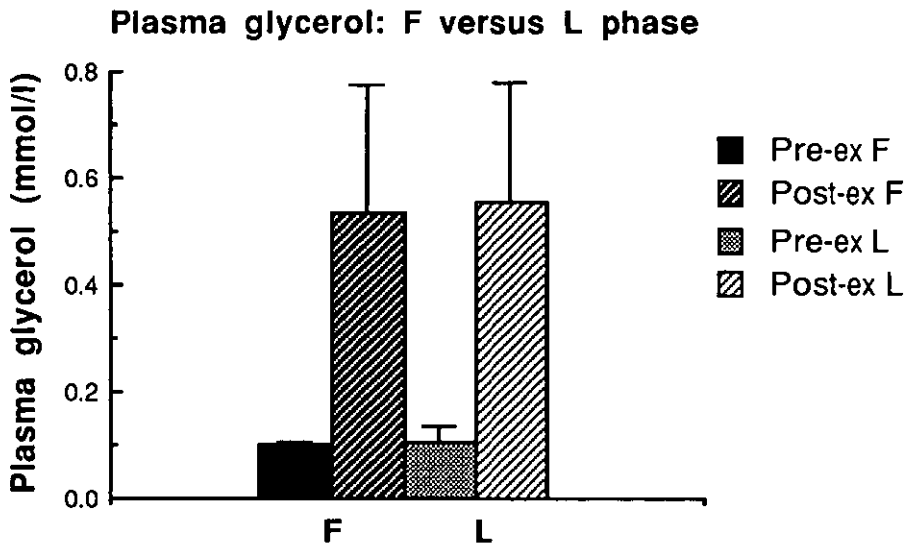
Plasma glycerol: F versus L phase
Plasma glycerol (mmol/l)
0.8
0.6
0.4
0.2
0.0
F
L
Pre-ex F
Post-ex F
Pre-ex L
Post-ex L

Fig 5·14

5.3 Results

i) Physical and Physiological characteristics (Tables 5.1 and 5.2):

Mean age, maximum heart rate and VO_2max (ml kg^{-1} min^{-1} and ml kg^{-1} LBM min^{-1}) were similar for both groups. Eu subjects were 2.8% taller ($p<0.05$) and 19% heavier ($p<0.01$) than Am subjects (Table 5.1); they had a 13.7% higher body mass index (BMI) ($p<0.01$) which was attributable to both a higher fat free mass (FFM) ($p<0.05$) and a higher body fat content ($p<0.01$) (Table 5.2). Percent body fat was in the order of 30% higher for Eu than Am subjects by underwater weighing (UWW) ($p<0.01$) and 51% greater as predicted from skinfold measurements ($p<0.01$). Skinfold thicknesses were greater in Eu subjects at all measurement sites ($p<0.01$). Subcutaneous fat distribution differed markedly between the 2 groups. The greatest differences existed at the biceps, abdomen and calf sites (146%, 135% and 113% greater respectively in Eu than Am subjects) followed by the supraileac, subscapular, thigh and triceps sites (86%, 83%, 70% and 63% greater respectively in Eu than Am subjects). Skinfold measurements over-predicted percent body fat measured via the UWW method in both groups and the greatest discrepancy between the 2 methods existed for the Eu group in whom percent body fat estimated from 4 skinfold thicknesses was approximately 32% greater than the UWW measurement as compared to approximately 14% for the Am group. There was little difference in the mean waist/hip (W/H) circumference ratio between groups. Values of this ratio were highest in those women who attained menarche at the latest age and the highest value was measured in the subject with primary amenorrhoea.

ii) Menstrual status and history (from Questionaire: Appendix 8):

Mean age at menarche did not differ between groups, but at 15 years for both groups was noticably older than "normal" (Frisch et al, 1981). Menarche was "delayed" in all women who started vigorous training prior to menarche, occuring within the age range of 15.4-17.7 years. One subject (age 20.1 years) had never menstruated.

Although the Eu subjects had all menstruated regularly during the 12 months preceding the Study, 3 reported a past history of menstrual irregularity (oligomenorrhoea). The mean duration of amenorrhoea in the 8 subjects with secondary amenorrhoea was 4.1 ± 4.2 years, ranging from 0.7 to 11.2 years.

iii) Serum gonadotropin and sex steroid hormone concentrations (Table 5.3):

Basal (pre-exercise) serum oestradiol was 39.4% higher in Eu subjects during the early follicular phase of their menstrual cycles than in Am subjects ($p<0.01$) (Table 5.3). Mid-luteal phase pre-exercise serum oestradiol of the 5 Eu subjects tested during both phases of their menstrual cycles was 4.8 fold higher than during the early follicular phase ($p<0.01$). Exercise effected an approximate 1.5 fold increase in serum oestradiol ($p<0.01$) for both Am and Eu groups and a rise of similar magnitude was recorded for the subgroup of 5 Eu subjects tested during both menstrual phases. Although serum oestradiol was significantly higher in Eu than Am subjects, the former might still be classified as hypo-oestrogenic on the basis of a group mean basal serum oestradiol concentration (early follicular phase) for this group at the lower level of the "normal" range of 70-370 $pmoll^{-1}$ (RIA Direct HD 293012, Baxter Healthcare Ltd). Mean pre-exercise serum oestradiol concentration of the 5 Eu subjects measured during the luteal phase of their menstrual cycles was in the order of 13% below the lower value of the "normal" range for this phase of 363-1180 $pmoll^{-1}$, (RIA Direct HD 293012, Baxter Healthcare Ltd). Thus, serum oestradiol concentration of the Am subjects was only about 70% of the lower limit of "normal" for the early follicular phase and evidently too low to attain the threshold necessary to trigger ovulation and menstruation.

There was no difference in pre-exercise serum progesterone between Eu subjects during the early follicular phase of their menstrual cycles and Am subjects. Mean serum progesterone concentration lay within the "normal" range of 2-8 $nmoll^{-1}$ (RIA Gamma B kit, Immunodiagnostics - early follicular phase range) for both groups of subjects and exercise effected little change in serum progesterone for either group. Mean pre-exercise serum progesterone was 4.3 fold higher in the 5 Eu subjects during the luteal phase of their menstrual cycles than during the early follicular phase

($p<0.01$). However, the mean value of 11.2 $nmoll^{-1}$ was 30% below the lower limit of the "normal" range for the luteal phase of 16-60 $nmoll^{-1}$ (RIA Gamma B kit, Immunodiagnostics).

Basal serum FSH concentration (measured during the early follicular phase for Eu subjects) was similar in both groups, however basal serum LH was 61.5% higher in Eu subjects. Nevertheless, group mean values for the serum concentrations of both gonadotropins lay within the "normal" range of 1-9 IUl^{-1} FSH, follicular and luteal phases and 2-12 IUl^{-1} LH, follicular and luteal phases (WHO LH and FSH assay kit, Chelsea Hospital, London).

There was no difference in pre- or post-exercise serum testosterone either between groups or between menstrual cycle phases for the 5 Eu subjects and circulating concentrations of this androgen lay within the "normal" range of 0.5-2.5 nMl^{-1} (RIA extraction assay STRIA kit) in both subject groups. Exercise effected a significant increase in serum testosterone concentration ($p<0.05$) in Am subjects and in Eu subjects exercising during both phases of their menstrual cycles.

iv) Diet (Table 5.4):

Total energy intake tended to be greater in Eu than Am subjects, mainly on account of a higher fat intake, which was in the order of 73% higher ($p<0.05$) for the former than the latter (Table 5.4). Fat contributed less to total energy consumption in Am than in Eu females [24% versus 31% of total energy intake respectively; $p<0.05$)]. Whilst all Eu subjects consumed over 40g fat per day, 5 of the Am subjects consumed less than 40g and in one subject fat intake averaged less than 20g per day. There were no group differences in percent total energy intake derived from protein and carbohydrate. Mean daily intakes of iron and zinc did not differ between groups. All subjects took some type of micronutrient supplement (not included in the analysis), most commonly a multivitamin tablet fortified with iron. Some also took extra calcium and multi-mineral tablets. Am subjects tended to exhibit lower haemoglobin concentrations than Eu subjects despite the fact that they were not regularly losing iron through menstruation. It is possible that differences in the major sources of dietary iron (animal versus vegetable; the intestinal capacity of the

latter being lower) between groups resulted in the low haemoglobin concentrations of some of the Am subjects.

When expressed relative to body mass, group mean values for total energy and macronutrient consumption did not differ, although the trend was towards a greater intake of energy and fat in Eu subjects per unit body mass. Crude fibre intake was higher per unit body mass for the Am group on account of a large consumption of raw fruit and vegetables.

Seven of the Am subjects were vegetarian (5 ovo-lacto vegetarian and 2 vegan), but only one Eu subject was vegetarian (ovo-lacto-). All subjects were aware of the effects of diet on body weight, endurance capacity and performance and attempted to eat what they described as a "healthy" diet. However, none of the Eu subjects appeared to strictly control their food intake and manipulate their body weights through diet and exercise in the same way as did the majority of Am subjects. Whilst the diets of the Eu subjects tended to vary widely in content and therefore total energy intake from day to day, most of the Am subjects ate a limited variety of foods and the composition of their diets varied little from one day to another. This would suggest that most of these subjects were consciously controlling their food intakes and total energy consumption. Four of the Am subjects admitted to having had a history of eating disorders and 1 had actually been hospitalised with anorexia nervosa. Almost all of the subjects participating in the study said that they used exercise as a means of weight control. However, whilst it would seem that many of the Am subjects were striving to maintain a low body weight through strict dieting and strenuous exercise, the majority of Eu subjects appeared to eat more freely and probably maintained a constant body weight through vigorous exercise.

v) Training, Competition and Performance (Tables 5.6, 5.7 and 5.8):

There was little difference in weekly running distance, training pace, total time spent per week engaged in vigorous activity, number of years training experience and number of rest days per week between groups (Table 5.6). However, a difference in performance capacity was evident between groups. Three of the Am subjects were of international standard at their best performance distance and 3 were of national standard. Although 3 of

the Eu subjects were national standard athletes, none had competed at international level. Of the 6 remaining county standard Eu athletes, 3 had competed at national level as juniors but admitted to having been unable to sustain sufficient interest and motivation to train sufficiently to extend their performance capacity to senior level. They had gained a large amount of weight during their late teenage years, which tended to preceed a delayed menarche. Running economy was particularly good for the 3 international standard athletes, who all exhibited much lower heart rates whilst running at a given submaximal treadmill speed as compared to the other athletes. Treadmill speed corresponding to 70% VO_2max was also much higher for the international standard athletes on account of their high running economies and high VO_2max values (the latter in excess of 66ml kg^{-1} min^{-1}). Mean differences in running economy and "70%" running speed between the 2 groups were not statistically significant (Table 5.7).

Blood lactate concentration measured during running at various submaximal speeds tended to be lower in the more elite athletes and those who ran the greatest distance per week. However, there was no difference between Am and Eu groups in mean running speed and mean %VO_2max at blood lactate concentrations of 2 and 4 $mmoll^{-1}$ (Table 5.8).

vi) Estimated daily energy expenditure in vigorous exercise (Table 5.5):

Energy expenditure during running training was estimated for each subject from detailed descriptions of weekly running distance, type of training session (e.g interval sessions or long slow distance runs) and approximate running speed during training sessions (obtained from Questionaire: Appendix 8) as well as VO_2 during submaximal treadmill running (Table 5.7). Estimation of energy expenditure in all other vigorous activities aside from running was based upon the assumption that these activities were all performed at a relative exercise intensity equivalent to approximately 70% of each subject's VO_2max. None of the subjects participated in team sports and tended to engage in other predominantly endurance-type sports such as swimming or cycling. Based upon such criteria, the total weekly energy expenditure in vigorous exercise did not differ between groups. However, the latter tended to be slightly higher for the Eu group of subjects (on account of their relatively higher body

weights) and slightly higher when expressed per unit body mass for the Am subjects. Each subject was estimated to expend at least 400kcals/day in vigorous activity and it was calculated that 5 of the subjects (4 of whom were triathletes) expended over 900kcals/day in vigorous activity. When the estimated mean daily energy cost of vigorous exercise was subtracted from mean daily energy intake for each subject, the "net gain" was 1775±630 kcals (27.7±11.3 kcal kg body mass^{-1}) for Eu subjects and 1182±474 kcals (22.7±10.6 kcal kg body mass^{-1}) for Am subjects. This corresponded to a 50% higher absolute net energy gain (or 22% higher gain per unit body mass) for Eu subjects. However, this difference was only statistically significant when expressed in absolute terms ($p<0.05$). Net energy gain was calculated to be less than 1000kcals/day for 1 Eu and 3 Am subjects, 2 of whom had an estimated net energy gain of less than 600kcals (or 10kcal per kg body mass) per day.

There was no difference in mean estimated BMR between groups. However, when estimated BMR values were subtracted from the mean daily energy intake of each subject, the mean energy intake of Eu subjects exceeded their estimated BMR (i.e, they appeared to be in a state of positive energy balance), whilst many of the Am subjects were in apparent negative energy balance. Since BMR was measured according to the assumption that subjects were completely rested during waking hours, yet all were highly active individuals, their energy intakes would appear to be very much below the "optimum" level. However, despite being in an apparent state of negative energy balance, none of the subjects lost any weight over the duration of study and reported to have maintained a near constant body weight over the months preceeding the study.

vii) Stress (from Questionaire: Appendix 8):

Am subjects reported a much higher subjective experience of stress associated with training, competition, familial, social and academic pressures than Eu subjects. Whilst 4 of the Eu subjects reported virtually no stress associated with such factors, all of the Am subjects reported that they were "highly stressed" by the majority of these stressors. The most commonly reported cause of stress in the Am group was associated with training and competition, which tended to take priority over almost all

other parts of their life, including work, social and family life. Most appeared to possess a "single-minded determinedness" to succeed in sport, admitted that they felt "compelled" to train at least once (or more) every day and rarely took days off training for the purpose of recovery. They tended to participate more often and at a higher competitive level than Eu subjects. If absolutely forced to miss training due to injury or illness, many admitted to avoid weight gain through strict dietary control. They would become depressed, irritable and bored, since training and preparation for competition formed their major goal in life and many had few alternative interests. In contrast, most of the Eu subjects seemed to take sport less seriously, competed less often, felt less compelled to train daily ("unless they really felt like it"), were not unduly stressed or "driven" to succeed and seemingly lead a more active social life than the Am subjects. The most common cause of stress within the Eu group of subjects was associated with academic work and social pressures, as opposed to training and competition.

viii) Physiological responses to prolonged submaximal treadmill running:

a) Am subjects versus Eu subjects exercising during the early follicular phase (Tables 5.9, 5,10 and 5.11; Figures 5.1-5.7):

There was no difference in heart rate (Table 5.9; Figure 5.1) and R values (Table 5.9; Figure 5.3) between groups during the 60 minute treadmill run, although the former measurement tended to be slightly higher for Eu subjects throughout the test. Heart rate gradually increased over the duration of the run in both groups ($p<0.01$). Oxygen uptake (Table 5.9; Figure 5.2) and carbon dioxide production (Table 5.9) did not differ between groups and did not change significantly with running time for either group. Eu subjects lost more weight than Am subjects over 60 minutes of running ($p<0.05$) (Table 5.11), although not as a percentage of their pre-exercise body mass. Percentage decline in plasma volume varied widely between subjects and group differences were not significant (Table 5.11). Although plasma volume declined by about 4 to 5% in most subjects, an apparent increase occured in 1 Eu and 2 Am subjects. These increases were especially marked in the 2 Am subjects and as a result, the group mean

value for the A females was reduced to an over all decline of less than 1%. Although there was no difference in the group mean haemoglobin concentrations and haematocrit, these values tended to be lower in Am subjects (Table 5.11).

Blood lactic acid concentration was similar for both groups at rest (Table 5.10; Figure 5.4). After 15 minutes running it had risen above resting values for both groups ($P<0.01$). The mean increase in blood lactate concentration was higher for the Eu subjects ($p<0.05$) and was in the order of 2.3 fold for Am and 3.0 fold for Eu subjects. Between 15 and 60 minutes of exercise, blood lactate concentration tended to decline gradually in Eu subjects and increase in Am subjects, such that at the end of 60 minutes running, values were similar for both groups. However, the extent of change in blood lactate concentration over the duration of the test run was not significant for either group.

There was no difference in blood glucose concentration between groups at rest (Table 5.10; Figure 5.5). A gradual rise occured for both groups as the test progressed and values were significantly raised beyond pre-exercise levels for both groups at the end of 60 minutes running ($p<0.01$). Blood glucose concentration also tended to be higher in Eu than in Am subjects during the test.

The mean estimated rate of energy expenditure was approximately 22% higher for Eu than Am subjects ($p<0.01$) (Table 5.9). There were no group differences in the estimated percentage contribution of fat and carbohydrate to exercise metabolism, or the estimated total amounts of fat and carbohydrate oxidised during the run, although the latter tended to be higher for the Eu subjects.

Plasma FFA concentration was 56% higher for the Eu than the Am group at rest ($p<0.05$) and 52% higher immediately post exercise ($p<0.05$) (Table 5.10; Figure 5.7). The exercise-induced rise in plasma FFA concentration was in the order of 2 fold for both groups ($p<0.01$).

Plasma glycerol concentration was 81% higher for the Eu than the Am group at rest ($p<0.01$) and 70% higher immediately post-exercise ($p<0.01$) (Table 5.10; Figure 5.8). After 60 minutes of exercise it was 5.6 fold

higher than resting values for Eu ($p<0.01$) and 6.2 fold higher for Am subjects ($p<0.01$). The magnitude of the exercise-induced elevation in plasma glycerol concentration was higher for Am than for Eu subjects ($p<0.05$).

The ratio of the plasma concentrations of FFA/glycerol was calculated in order to provide an indication of the extent of triglyceride mobilisation relative to FFA utilisation. This ratio tended to be higher in Am subjects, especially at rest, although there was no difference in group mean values either pre- or post-exercise. The value of this ratio declined in magnitude to approximately one third of the resting value during exercise ($p<0.05$), suggesting the existance of a greater extent of fatty acid utilisation relative to mobilisation during exercise than at rest.

b) Eumenorrhoeic subjects (n=5) exercising during the early follicular phase versus mid-luteal phase (Tables 5.12 and 5.13):

There were no differences in cardiovascular or respiratory parameters, or in energy expenditure for the same group of exercising Eu subjects between menstrual cycle phases. (Table 5.12; Figures 5.8, 5.9 and 5.10). Changes of a similar magnitude in such parameters between menstrual cycle phases were also observed with increasing time during the 60 minute treadmill run. Exercise-induced changes in body weight and plasma volume were also similar between phases.

Pre-exercise blood lactate and blood glucose concentrations did not differ between menstrual cycle phases and blood glucose concentration remained similar throughout the duration of the run in both phases (Table 5.13; Figures 5.11 and 5.12). However, blood lactate concentration was consistently lower at each of the sampling times throughout the 60 minute treadmill run ($p<0.05$). Pre- and post-exercise plasma FFA and glycerol concentrations did not differ between menstrual cycle phases (Table 5.13; Figures 5.13 and 5.14). Interestingly, all subjects scored a lower value on the "perceived rate of exertion" (PRE) chart during exercise in the luteal phase of their cycles.

5.4 Discussion

Over all, the results suggest that there were few differences between Am and Eu runners with regard to their cardiovascular, respiratory and metabolic responses to prolonged steady state treadmill running. The only indications of any possible difference in energy substrate metabolism between the 2 groups resided within the higher blood lactate concentration of the Eu subjects 15 minutes into the 60 minute run and the lower circulating FFA and glycerol concentrations of Am subjects before and after exercise. However, despite their higher plasma FFA concentration, Eu women did not appear to oxidise a greater amount of fat relative to carbohydrate during exercise than Am women. There were no group differences in R-values, or blood glucose concentration during exercise and blood lactate concentration remained similar for both groups after 30 minutes of running. The magnitude of the exercise-induced increase in plasma FFA concentration was similar for both groups and plasma glycerol concentration actually rose to a greater extent beyond pre-exercise levels in Am subjects. The latter was suggestive of a greater exercise-induced extent of triglyceride catabolism in Am as compared to Eu subjects. The ratio of plasma concentrations of FFAs to glycerol ([FFA]/[glycerol]) did not differ between groups either pre- or post-exercise.

Thus, any metabolic effects consequent to the amenorrhoeic state did not apparently exert a marked influence over the regulation of lipid and carbohydrate utilisation during prolonged steady pace exercise. However, even though basal plasma oestradiol concentration was very much lower in Am as compared to Eu subjects, it was also lower in the latter as compared to "average" values reported in the literature for untrained women of a similar age (Bonen et al, 1983; Brown et al, 1986). Nevertheless, basal circulating oestradiol levels were very much higher in even the Am women than would be found in the normal healthy man (Brown et al, 1986). Thus, if oestradiol should play a "permissive" metabolic role, in that the continued exposure to a circulating concentration of this hormone above a specific level leads to transient or long term adaptive changes in the morphologic and/or metabolic properties of skeletal muscle, then it would appear that the basal circulating concentrations of oestradiol of Am subjects exceeded the threshold required to impose such effects. In contrast, the very low

plasma concentrations of oestradiol in men would probably fall far short of the threshold concentration required to exert any metabolic influence.

Therefore, the results of this Study do not exclude the possibility of a regulatory role for oestradiol on substrate metabolism and it's potential to effect gender-related differences in the metabolism of energy substrate by exercising skeletal muscle. Indeed, it is interesting to observe that the highest R-values during exercise were measured within the 3 leannest Am subjects who had the lowest plasma oestradiol concentrations and longest duration of amenorrhoea. A recent study by Vaismann et al (1988) showed the extent of carbohydrate to relative to lipid oxidation in the fasted state to be greater in very lean amenorrhoeic (and presumably hypo-oestrogenic) anorexic women than in healthy eumenorrhoeic women.

The lower blood lactate concentrations and lower PRE scores measured in the 5 Eu subjects during exercise in the mid luteal- as compared to the early follicular phase of their menstrual cycles confirmed the results of studies by Jurkowski and colleagues (1978 and 1981). These authors also recorded greater exercise times to exhaustion in women exercising during the luteal phase of their menstrual cycles. In the present Study however, R-values did not differ between menstrual cycle phases, so it is unlikely that the lower blood lactate concentrations measured during the mid-luteal phase of the menstrual cycle reflected a smaller extent of intramuscular glycogen utilisation in favour of a greater rate of lipid oxidation. Thus, precisely why blood lactate concentration rose to a higher level during exercise in the luteal- than the follicular phase is uncertain, but may well have been influenced in some way by the higher circulating concentrations of ovarian steroid hormones during the luteal phase. Ovarian steroid hormones may therefore exert regulatory metabolic effects in a concentration-dependent manner. Plasma oestradiol and progesterone concentrations were both in the order of 4-5 fold higher during the mid-luteal as compared to the early follicular phase in the 5 Eu subjects tested during both menstrual cycle phases.

It is therefore possible that any metabolic differences between Am subjects and Eu subjects were not evident when the latter were tested during the early follicular phase of their menstrual cycles if group differences in circulating oestradiol and/or progesterone concentrations

were too small. However, the markedly higher plasma concentrations of these hormones measured during the mid-luteal phase of the menstrual cycle relative to basal (early follicular phase) levels may have been sufficiently high as to exert a significant metabolic influence.

The cause of the lower pre- and post-exercise plasma FFA and glycerol concentrations measured in Am subjects relative to Eu subjects is uncertain. Those Am subjects exhibiting the lowest plasma FFA concentrations also had the lowest plasma glycerol concentrations, lowest plasma oestradiol concentrations, the longest duration of amenorrhoea and were the leanest of the group. Most of the Eu subjects exhibited resting plasma FFA and glycerol concentrations within the ranges reported by other groups (Costill et al, 1979; Hardman and Williams, 1983). Thus, the values measured in the Am subjects of the present Study would appear to have been abnormally low.

Oestrogens are known to influence the distribution of body fat stores as well as total body fat content (Olesky et al, 1974; Nikkila et al, 1978; Kissbah and Peiris, 1989). These hormones also appear to regulate the lipolytic response of fat stores located at certain regions of the body to circulating lipolytic and antilipolytic hormones as well as the capacity for triglyceride storage at specific sites around the body (Zinder et al, 1974; Olesky et al, 1974; Smith et al, 1979; Rebuffe-Scrive et al, 1985). For example, it has been observed that adipocytes located in the femoral region in women but not men (a characteristic "female" fat storage site which tends to be much larger in women than men) appear less sensitive to lipolytic stimulants than adipocytes located in other regions, especially during pregnancy when there is a necessity to optimise fat storage and conserve energy (Rebuffe-Scrive et al, 1985; Marin et al, 1987). Thus, the hypo-oestrogenic state may lead to a redistribution of fat storage around the body and/or a relative depletion of specific "female" fat stores. Such effects may in turn lead to alterations in the capacity for lipolysis in women. A relative depletion of "female" fat stores induced through negative energy balance as a result of hard training and/or inadequate energy intake could even trigger the onset of the amenorrhoeic state, since the aromatisation of androgens to oestrogens occurs primarily within adipose tissue (Frisch et al, 1981).

Thus, the rate and extent of basal lipolysis may be less in Am than Eu women as a result of the relatively hypo-oestrogenic state of the former and may help explain their depressed plasma concentrations of FFA's and glycerol. A lower total body fat content would result in there being a reduced number of receptors responsive to circulating lipolytic stimulants. It is also possible that ovarian steroids may "up-regulate" the density of such receptors, increase their substrate affinity. or the efficacy of the substrate-receptor complex. The secretion of lipolytic and/or antilipolytic hormones at rest and during exercise may also differ between Am and Eu women.

Despite their higher plasma FFA concentration, Eu subjects did not appear to oxidise FFAS at a greater rate than their Am counterparts, as evidenced by the similarities in other metabolic variables measured before, during and after the test run.

The gradual increase in blood glucose concentration for both groups may have indicated an adaptation to fat metabolism with increasing exercise duration. If the exercising muscles gradually oxidised more lipid and less carbohydrate, such that the rate of hepatic glycogen catabolism and/or gluconeogenesis exceeded the rate of glucose oxidation, blood glucose concentration would rise, as predicted by the "glucose-fatty acid cycle" (Randle et al, 1964; Rennie and Holloszy 1977). There was a tendency for R values to decline with exercise time, whilst blood lactate concentration remained essentially constant. Alternatively, it is always possible that the observed increase in blood glucose concentration was simply attributable to an output of glycogenolytic hormones which far exceeded the body's requirements for glucose. The output of such hormones is not precisely controlled in a negative feedback manner in accordance with the body's actual requirements for a specific energy substrate with the result that an imbalance frequently exists between supply and demand for blood borne energy substrate (Galbo, 1983).

The higher oxygen uptake and rate of energy expenditure of Eu relative to Am subjects could be attributed to the greater body mass of the former. The size of the recruited muscle mass in Eu subjects was likely to be greater, as was their VO_2max when expressed in absolute terms ($Lmin^{-1}$). The

greater loss of body weight and decrease in plasma volume in the Eu group was also likely to have resulted from their greater rate of energy expenditure.

The higher blood lactate concentration of the Eu group 15 minutes after the start of exercise was difficult to explain. It was not reflected by a difference in R values between groups, so would not appear to have been linked to a relatively greater rate of carbohydrate relative to lipid oxidation. It is possible that the rate of lactate clearance was slower in the Eu subjects, although there is no obvious reason why this might be so since there were no difference in training status and cardiovascular "fitness" between groups. A greater recruited muscle mass by the Eu subjects may lead to a greater circulating lactate concentration if it were not balanced by a correspondingly greater plasma volume.

Post-exercise concentrations of oestradiol and to a lesser extent, testosterone, were markedly higher than pre-exercise values in both groups of subjects. Increases in circulating sex steroid hormone concentrations following vigorous submaximal exercise have been observed in other studies (Jurkowski et al, 1978; Bonen et al, 1979, Shangold et al, 1981) and appear to stem from a decrease in the plasma clearance of these hormones (Keizer et al, 1980). Exercise effects little or no change in plasma gonadotropin concentrations and would not appear to affect the secretory rate of sex steroid hormones (Bonen et al, 1979 and 1981). An approximate 50% depression in the metabolic clearance rate of oestradiol may persist for over 30 minutes following a 10 minute exercise bout performed at 70% VO_2max (Keizer et al, 1981). Thus, any residual physiological effects imposed by exercised-induced increases in circulating steroid hormones may persist for prolonged periods, especially with once or twice daily training sessions. Such effects may include a persistent negative feedback effect on hypothalamic and/or pituitary hormone secretion. An elevation of deep body temperature during exercise could lead to an increase in the unbound and biologically active fractions of circulating sex steroid hormones, since the binding of these hormones to their plasma receptor is temperature dependent, with binding capacity decreasing with increasing temperature, (Lata et al, 1980). In contrast to previous findings, an exercise-induced increase in plasma progesterone concentration was not observed in the present study.

With regard to the "reference woman" devised by Behnke and Wilmore (1974) (Chapter 2, Part 1); Eu subjects were 3.6% taller and 5.0% heavier, but had a 1.9% lower BMI. Amenorrhoeic subjects were 0.7% taller, and 11.7% lighter, with a 13.7% lower BMI. Thus, both Am and Eu subjects were generally lighter relative to their stature than the "average" woman.

The mean VO_2max (per unit body mass) of the 18 subjects participating in this study was in the order of 60% higher than that of the untrained woman (Sparling, 1980), presumably due to the combined effects of genetic constitution (inherent qualities) and vigorous training. The low blood lactate responses of the subjects to steady pace submaximal running at various relative intensities confirmed their high skeletal muscle "training status" (oxidative capacity). Thus, the high performance capacity ("eliteness") of the subjects was evidently attributable primarily to their cardiorespiratory and skeletal muscle "fitness", which enabled them to sustain an exercise intensity close to their maximal "aerobic" running speed for prolonged duration. The absence of a group difference in $\%VO_2max$ corresponding to blood lactate concentrations of 2 and 4 $mmoll^{-1}$ suggested that they were essentially evenly matched on the basis of "training status" (Ramsbottom et al, 1987).

The most striking difference in physical characteristics between the subjects of each group was in physique and body composition. Although Eu subjects were generally taller and might therefore be expected to be heavier than Am subjects, they also had a much higher body mass relative to stature, as evidenced by BMI. Most Eu subjects had a higher lean body mass (LBM) and a higher fat mass (FM) than Am subjects. However, a major proportion of the difference in BMI between groups was attributable to the higher FM of the Eu group, for whom the ratio of LBM/FM (or percent body fat) was much higher.

Estimates of subcutaneous fat thickness from skinfold measurements at different sites on the body surface were also suggestive of group differences in the distribution of subcutaneous fat. Although skinfolds were much thicker at all sites of measurement for the Eu group, the magnitude of group differences were especially large for the biceps, abdominal and calf sites and least for the triceps and thigh. The leanest subjects in particular had a relative paucity of fat in the 3 forementioned

sites. If it is assumed that the extreme leanness of many of the Am females was attributable to a relatively low energy intake in relation to a high energy output through vigorous training, resulting in a low (but constant) body mass, then it would appear that fat accumulates at certain sites of the body more readily than at others. Alternatively, fat from these same sites may be less readily mobilised in the face of a negative energy balance. This would suggest that there are differences between the metabolic properties of adipose tissue stores in different regions of the body, with certain sites possessing a relatively greater resistance to catabolism and/or a greater potential for triglyceride storage. Investigation of such a phenomenon over recent years lends support to the the findings of the present Study (La Fontan et al, 1978; Smith et al, 1979; Rognum et al, 1982).

If it is assumed that the hydrostatic weighing (UWW) method of estimating relative body fat content is superior to all other single methods of measurement (Wilmore, 1983), it would seem that the Durnin and Womersley equation for the prediction of relative body fat content from 4 skinfolds was unsuitable for both female populations of the present Study, but more especially the Eu group. This equation over-estimated percent body fat in all subjects: thus, it would appear that the ratio of subcutaneous ("external") fat to "internally" stored fat was greater than might be expected for the "typical" woman fitting to the age range of the equation. Alternatively, the distribution of subcutaneous fat may have differed from the "norm" upon which the equation was formulated. An alternative equation, based upon different regression coefficients and/or using skinfold thicknesses measured at a different set of sites on the body surface may provide a more accurate prediction of relative body fat content in 2 such "biased" athletic female populations.

Although hydrostatic weighing is generally considered the "gold standard" for measurement of relative body fat content, this method is nevertheless subject to error with regard to the underlying assumption of a "standard" relative density for fat and lean tissue. The magnitude of the error associated with an assumed relative density of "lean" tissue, which includes all body constituents other than fat (but mainly of bone, muscle and water may be particularly large, especially in "atypical" female populations such as the athletes of this study. A lower than expected bone

mass in hypo-oestrogenic women (Drinkwater et al, 1984), or a relatively high muscle mass attributable to training could result in a deviation in the assumed distribution of bone, muscle and water in the body which might be sufficiently large as to markedly raise the prevailing error of the method.

It is perhaps surprising that the discrepancy between the UWW and skinfold methods for estimating percent body fat was greater for the Eu group of female runners, whose relative body fat content was closer to the "average" value for the young adult woman than that of the Am group who were very much leaner than the "typical" young sedentary woman. It is possible that a decrease in relative body fat content induced in the adolescent or young adult female by endurance training is effected through the loss of fat from certain specific areas before others, such that fat is mobilised only from the more resistant sites as negative energy balance continues. Alternatively, it may be that vigorous training and/or a low "net" energy intake during adolescence reduce the potential for fat storage at certain sites within the body, such that the distribution of subcutaneous and perhaps also the relative amounts of "internal" and "externally" stored fat deviate from that of the "typical" woman. If such a hypothesis holds and the body composition measurements reflected "true" readings, then on the basis of the data collected in the present study it would seem that subcutaneous fat is more resistant to mobilisation than "internal" fat. It appeared that the leaner Am females had lost a substantial quantity of subcutaneous in addition to "internal" fat, such that they possessed a more "typical" "internal"/"external" fat mass ratio relative to the Eu females. Alternatively, the mobilisation of subcutaneous fat could occur at a later stage of negative energy balance from the 4 sites of measurement used in the Durnin and Womersley equation.

Although the basal serum oestradiol concentrations of both Am and Eu subjects was lower than might be expected for young adult women (Bonen et al, 1981), it was significantly lower in the former, thereby confirming the findings of other groups (Shangold et al, 1979; Fisher et al, 1986). It is possible that a decline in circulating oestrogen concentration could lead to a reduction in total body fat content and/or a redistribution of adipose tissue in the body. Sex steroid hormones are known to influence the

metabolic properties of adipose tissue in different sites of the body (Olesky et al, 1974; Nikkila et al, 1978; Kissbah and Peiris, 1989) so may effectively "direct" fatty acids to specific sites for storage as triacylglycerol. A relative inhibition of oestrogen synthesis due to vigorous training and/or an inadequate "net" energy intake in young female athletes experiencing delayed menarche may prevent fat being stored in certain "sex specific" sites and the body from attaining the characteristic female physique.

The absence of a difference in plasma progesterone concentration between Am and Eu subjects (early follicular phase) was presumably attributable to the timing of blood sampling in the latter. Progesterone secretion is lowest during the early follicular phase of the menstrual cycle, following degeneration of the corpus luteum released during the previous cycle and prior to maturation of another follicle (Knobil, 1980). The rise in plasma progesterone concentration observed in the 5 Eu subjects from early follicular to mid-luteal phase was very much lower than that observed in age-matched, untrained women (Bonen et al, 1981). Such "luteal insufficiency" is a common feature in highly trained female athletes (Sherman and Korenman, 1974) and an inverse relationship has been observed to exist between luteal phase plasma progesterone concentration and running mileage (Shangold et al, 1979). The fact that only 4 of the 5 Eu subjects who attempted to predict the timing of the mid-luteal phase of their menstrual cycle via measurements of basal body temperature actually observed a sustained increment in this variable might suggest that they were experiencing anovulatory menstrual cycles with luteal insufficiency (Prior et al, 1984).

The lower plasma LH level of the Am group was suggestive of depressed pituitary function, probably effected through reduced pulsatile LHRH secretion from the hypothalamus (Boyar et al, 1985; Veldhuis et al, 1985) and was the likely cause of their amenorrhoea. Regular menstrual cyclicity depends upon the presence of "adequate" and pulsatile gonadotropin secretion during the follicular phase of the cycle (di Zerega and Hodgen, 1981). Perhaps surprisingly, there was no difference in plasma FSH levels between Eu (early follicular phase) and Am subjects. Other studies have shown plasma FSH concentration to be depressed to a greater extent than LH in active women with irregular menstrual cycles (Bonen et al, 1981).

Plasma testosterone concentration could be considered to be "normal" in all subjects. However, plasma testosterone has been shown to be elevated in some amenorrhoeic women (Hale et al, 1983; Chang et al, 1984; Veldhuis et al, 1985).

There is some evidence to suggest that relative to men, women are better able to match the energy demands of vigorous athletic training with a compensatory increase in food intake and may be innately more resistant to fat loss in the face of increased energy expenditure (Despres et al, 1978), perhaps as a consequence of high circulating oestrogen levels. The rates of in-vitro basal and adrenaline-stimulated adipose tissue lipolysis have been shown to increase to a greater extent after training in isolated adipocytes obtained from men than from women (Despres et al, 1978). Higher adipose tissue lipoprotein lipase (LPL) activities have been measured in women than men (Nikkila et al, 1978), which might suggest a greater capacity for triglyceride storage in the former. If it is assumed that ovarian steroid hormones play a role in mediating gender-related differences in the metabolic properties of adipose tissue, then comparable differences may exist between normo-oestrogenic (eumenorrhoeic) and hypo-oestrogenic (amenorrhoeic) women.

The higher W/H circumference ratios measured in the women who experienced a particularly late menarche was probably influenced by a delay in long bone ossification and epiphyseal closure attributable to "subthreshold" circulating oestrogen concentrations during the early teenage years.

A large number of different factors have been associated with menstrual dysfunction in female athletes and can be discussed in turn with regard to the subjects of the present Study:

i) Body weight and composition:

It is evident that the body mass and composition of Am subjects differed markedly from that of Eu subjects. The studies of Frisch and McArthur during the early 1970's linking menarche and the subsequent

maintenance of regular menstrual cyclicity with body weight for height and relative fatness (Frisch and McArthur, 1974) led to the formulation of a "fat hypothesis". This states that menarche is attained only when a "critical" threshold weight for height is achieved in the adolescent girl such that her relative body fat content amounts to approximately 17% of body weight. Percent body fat in the pre-pubertal girl tends to be lower than this figure and a sudden marked weight gain usually preceeds menarche (Frisch and McArthur, 1974). For the subsequent maintenance of regular menstrual cycles, it was suggested that body weight must continue to increase post-menarche until percent body fat amounts to approximately 22% body weight (Frisch and McArthur, 1974). The "energy drain" associated with training immediately post-menarche may therefore limit the subsequent weight gain required to bring body fat stores to their "critical" level for regular menstrual cyclicity, resulting in irregular and perhaps anovulatory menstrual cycles. It would appear that this was probably the case for many Am subjects of the present Study.

Subsequent research has suggested that the "fat hypothesis" may not be so clear cut and that a number of factors aside from body composition may act synergistically to influence the threshold weight and body fat content required for regular menstrual cyclicity within any particular woman. Amenorrhoea occurs in some female athletes who have a relative body fat content in excess of 22% (Baker et al, 1981) whilst others may maintain regular menstrual cycles with a relative body fat content very much lower than 17% (Warren, 1980). Exposure to exogenous and endogenous stressors such as strenuous athletic training (Prior et al, 1984), dietary insufficiency (Brooks et al, 1984; Kaiserauer et al, 1989), anxiety and depression (Litt et al, 1983; Wilson et al, 1984) can all disrupt neuroendocrine function and result in the disturbance of menstrual function in women of "normal" body weight and fatness. However, leanness would certainly appear to increase a woman's susceptibility to amenorrhoea in the face of other stressors (Feicht-Sanborn et al, 1982).

It is likely that every woman has an innate "threshold" weight for "normal" menstrual function, which probably rises according to the extent to which she is exposed to other inhibitory factors. Thus, the high incidence of menstrual cycle disturbances in highly trained female endurance athletes constantly exposed to the physiological stresses of

vigorous training as well as the psychological strain associated with competition is hardly surprising. In order to achieve success, top performers must often train to a level representing the fine borderline between maximising performance and overtraining and in the case of the female endurance runner, compete at a body weight which optimises running efficiency but may be subthreshold for normal neuroendocrine function. Amenorrhoea may be just another symptom of overtraining as opposed to a homeostatic adaptation to endurance training which enhances an organism's capacity to sustain prolonged submaximal exercise.

ii) Athletic training:

Training pre-menarche "delayed" menarche to a greater or lesser extent in all the subjects of the present Study. Late menarche has also been reported to occur in other athletic female populations, especially ballet dancers (Warren et al, 1980), gymnasts (Warren et al, 1983), swimmers (Stager et al, 1984) and runners (Baker et al 1981). It is associated with the failure to attain a "threshold" body weight for height, or relative fatness to trigger the chain of hormonal events across the hypothalamic-pituitary-ovarian axis which culminate in ovulation and menstruation (Frisch and McArthur, 1974; Frisch et al, 1978). Since all but one of the subjects in this study were post-menarcheal, on the basis of the "fat hypothesis", the Am subjects would at some time have attained a body mass or relative fatness sufficient to trigger ovarian function. However, strenuous athletic training in women of "normal" weight for height and relative fatness can effect menstrual cycle irregularities even in the abscence of weight loss or significant changes in body composition (Bullen et al, 1984). It would also seem that the magnitude of the body weight and/or fat "threshold" for menstruation is higher in athletic than sedentary female populations and probably rises in parallel with the total volume of training above a certain intensity performed over a given time scale (Feicht et al, 1978; Baker et al, 1981; Schwarz et al, 1981).

Four of the Am subjects in the present study reported that menstruation tended to resume after a few weeks if they were forced to reduce, or cease training due to injury, or if they gained as little as 2kg in weight whilst continuing to train. These women were of a higher body

weight and exhibited higher circulating concentrations of oestradiol and LH than the Am women, who had experienced prolonged amenorrhoea of duration greater than 3 years. The latter were the lightest and leanest subjects of the Study, had all lost weight prior to becoming amenorrhoeic and had failed to menstruate when forced to cease training for more than 2 weeks through injury. It is probable that these particularly lean women would have to gain a substantial amount of weight in order to restore menstrual function.

Of the 9 Am women in this Study, 5 had never established regular menstrual cycles. They formed the leanest in the group and had all trained vigorously pre- or almost immediately post-menarche. The remaining 4 had not trained so close to menarche and although they had at one time menstruated with some degree of regularity, their current amenorrhoeic state was associated with a loss in body weight attributable to training and/or a deliberate reduction in food intake.

The Eu women with a history of menstrual irregularity had all begun vigorous athletic training pre- or almost immediately post-menarche and were generally able to associate their present menstrual regularity with a gain in body weight which they had subsequently maintained. However, one particularly lean Eu subject with a BMI of 18.7 (but for whom percent body fat was not measured) had a history of menstrual irregularity prior to 3 full term pregnancies and although now lighter in weight and training more intensely than before bearing children, she reported a regular menstrual cycle and had a basal serum oestradiol concentration higher than all but one of the other Eu subjects. Parity has been associated with a "maturation process" of the hypothalamic-pituitary-ovarian axis and a protective effect against menstrual cycle irregularities (Dale et al, 1981; Baker et al, 1981). Similarly, starting vigorous training at an age after experiencing a regular menstrual cycle for a few years also appears to decrease a woman's susceptibility to amenorrhoea (Baker et al, 1981), possibly via a mechanism involving the "facilitation" of neural and humoral pathways regulating ovarian function through constant repetition (Veldhuis et al, 1985). It would appear that the establishment of regular ovulatory menstrual cycles following menarche may take up to 2 years in the adolescent female, since the positive feedback effect of oestradiol on the pituitary pre-ovulatory LH surge is a relatively late maturational event (Beaumont, 1979) and that

the region of the hypothalamus responsible for GnRH synthesis is particularly sensitive during early adolescence to inhibitory neural and/or humoral input linked to the stress of vigorous athletic training. Interestingly, none of the Eu subjects of the present study reporting a history of regular menstrual cycles had begun regular vigorous athletic training until at least 2 years post-menarche.

Of the 5 Eu subjects who agreed to perform the 60 minute test run during both phases of their menstrual cycles, only one actually observed a sustained increase in oral temperature on wakening, which occured at around the expected time of ovulation (day 12 of her cycle). This subject reported a history of regular menstrual cycles, had higher basal serum oestradiol and LH levels than any other subject and had a luteal phase serum progesterone concentration approximately 8 fold higher than that measured during the early follicular phase of the same cycle. In contrast, the rise in serum progesterone concentration from early follicular to mid-luteal phase of the same cycle was in the order of only 2-4 fold in the other 4 subjects. Thus, it is possible that these 4 subjects were experiencing anovulatory menstrual cycles which were coupled to their "lower than average" basal oestradiol levels. The single Eu subject with an apparently "normal" menstrual cycle trained more vigorously, was leaner and had a lower BMI than many of the other Eu subjects. However, unlike the other 4 Eu subjects, she had performed little vigorous activity on a regular basis until 5 years post-menarche, when at the age of 17 she began serious athletic training.

Anovulatory menstrual cycles and/or a shortened luteal phase are commonly and unknowingly experienced by many highly active women with apparently regular menstrual cycles (Shangold et al, 1979; Prior et al, 1982). However, such subtle menstrual cycle aberrations are revealed only through the daily measurement of circulating pituitary and ovarian hormone concentrations throughout one or more cycles (Bullen et al, 1985). Although this type of analysis was not performed in the present study, the fact that basal plasma oestradiol and LH levels were lower than "average" in all of the women under study might suggest that a large number of the Eu participants of this study experienced "abnormal" menstrual cycles.

iii) Diet:

The major factor differentiating amenorrhoeic from eumenorrhoeic runners in a number of studies has been that of nutritional inadequacy and potential energy deficit (Brooks et al, 1984; Deuster et al, 1986; Kaiserauer et al, 1989). These studies suggested that amenorrhoeic runners consumed significantly less energy, fat and red meat than eumenorrhoeic runners and eumenorrhoeic sedentary women.

Although the total average daily energy intake as well as the daily energy intake per kg body mass tended to be less for the Am group, only the former was statistically significant. However, whereas none of the Eu females consumed fewer than 1800 kcal/day (or<30kcal/kg/day), 5 of the 9 Am females consumed less than 1800kcal/day and 2 consumed fewer than 1300kcal/day (<30kcal/kg/day) which might be considered too low even for a sedentary woman. After subtracting the estimated daily energy expenditure in vigorous exercise from the mean daily energy intake for each subject, the "net basal energy intake" was also less for Am subjects when expressed in absolute terms but not relative to body mass. Estimation of basal metabolic rate (BMR) from height and weight by the formula of DuBois suggested that most Am subjects were actually consuming less energy than their BMR even before accounting for the extra energy expended during training and normal daily activity and that many of the remaining subjects were consuming only enough energy to maintain energy balance and body weight if they remained totally sedentary. Nevertheless, despite their apparently negative energy balance, all subjects were maintaining a near constant body mass. These findings confirm the work of others (Warren et al, 1980; Kaiserauer et al, 1989).

It is well documented that the body adapts to a negative energy balance effected through food restriction via a reduction in BMR (Owen et al, 1979) as an energy conservation mechanism. This is normally achieved through a depressed peripheral conversion of thyroxine (T_4) to the more active 3,5,3'-triiodothyronine (T_3) and coupled to a relative increase in reverse T_3 synthesis (Bleech and Moore, 1982). This effectively hypothyroid state appears to aid the conservation of body protein as well as economising on the rate of fuel utilisation (Gardner et al, 1979). Conversely, eating, especially protein foods, tends to accelerate metabolic

rate (Rothwell and Stock, 1979 and 1981; Dauncey and Bingham, 1983). An energy imbalance effected through heavy training, coupled to an inadequate compensatory energy intake would thus represent a form of undernutrition and an initially negative energy balance during the early stages of training may lead to a new "steady state" of low energy balance characterised by a low, but constant body mass. Recent research has actually confirmed the hypothesis that amenorrhoeic runners have a lower resting metabolic rate than eumenorrhoeic runners and sedentary women (Myerson et al, 1991).

An acute 24 hour fast has been shown to reduce circulating gonadotropin levels in normally menstruating women (Bonen et al, 1983) and it is possible that weight loss alone may stimulate menstrual cycle changes. Interestingly, it has been suggested that a decline in metabolic rate resulting from an energy deficit may function as a suppressor of hypothalamic function and so culminate in generalised neuroendocrine disturbance (Frisch et al, 1981).

The majority of Am and Eu subjects consumed adequate or excess amounts of micronutrients with respect to WHO recommendations (even without extra supplementation provided by multivitamin and mineral tablets). However, the intake of one or more macronutrients was seemingly too low in a number of Am subjects, especially with regard to fat. Most of the Am subjects consumed less than 50g fat/day, derived mainly from vegetable sources. It is possible that too low a fat intake could lead to the malabsorption of fat soluble vitamins and/or a deficiency of essential fatty acids, culminating in disorders of metabolism. A strict vegetarian diet could also limit the synthesis of sex steroid hormones, the precursor of which is cholesterol. The consumption of large quantities of raw vegetables has been known to result in B-carotine "poisoning" and an excess intake of B-carotene may lead to disorders in oestrogen metabolism (Kenmann et al, 1983).

The daily protein consumption of all but 2 subjects was in excess of 1g/kg body mass (or greater than 60g/day) which is well within the WHO recommendations. However, since most of the Am subjects derived almost all of their protein from cereals, pulses and nuts as opposed to animal products, it is possible that their diets may be deficient in one or a number of essential amino acids. To be nutritionally sound, a vegan diet

requires careful planning so that different protein sources are combined in such a way that an adequate intake of all essential amino acids is attained. Adherence to a time-consuming training schedule could easily leave the athlete with insufficient time (or will) to plan and prepare nutritious meals. Alternatively, they may be unsure as to how a strict vegetarian diet ought to be planned if it is to include an adequate intake of essential nutrients. Repeated performance of strenuous exercise on a daily basis, especially when coupled with a diet containing inadequate carbohydrate can lead to the catabolism of tissue protein for energy substrate and consequently muscle wasting, increased susceptibility to infection and general debility (Gontzea et al, 1975). Thus, although the protein intake of most subjects was in excess of the WHO recommendations of 1g/kg/day it is possible that they may require a higher protein and carbohydrate intake during periods of intense training in order to provide an adequate safety margin against deficiencies.

The most common cause of amenorrhoea in adolescent girls and young women is anorexia nervosa (Beaumont, 1979) and certainly a number of the Am subjects of this study had received therapy for this disorder. The incidence of eating disorders in female athletes has been found to be higher than in sedentary populations (Gadpaille et al, 1987), probably as a result of the pressure to remain lean in order to optimise performance capacity. However, both anorexics and highly motivated athletes possess a similar "type A" personality profile, characterised by compulsive and obsessive behaviour patterns and the desire to achieve in order to gain self-acceptance (Gadpaille et al, 1987). It has been suggested that "compulsive running is an analogue of anorexia" (Yates et al, 1983) and indeed, many of the neuro-endocrine disturbances characteristic of anorexia nervosa are common to highly trained male and female athletes (Luger et al, 1987). Thus, it may be difficult to single out "true" anorexic individuals amongst a group of highly motivated endurance runners due to the apparent overlap in psychological as well as physiological characteristics between the two states.

By convention, the eating patterns of many athletes might be considered to be "disordered" since they attempt to fit their meals around their training schedules (and in many cases a full time occupation). They

may deliberately avoid eating certain foods which they have found through experience to limit their training and performance capacity and may consume large quantities of carbohydrate foods in place of fatty and/or high protein foods to maximise their endurance capacity and accelerate recovery. The extreme leanness of many endurance runners in vigorous training may therefore be as much attributable to eating behaviour patterns as to training per se if they choose to refrain from eating for 3 or more hours prior to training and do not feel able to tolerate food until a few hours afterwards. In effect they leave little time in which to actually consume an adequate diet on top of a heavy training programme. Although they cannot be said to be suffering from a psychological eating disorder, the consequential effects on metabolism are likely to be similar.

iv) Stress:

It was evident from the answers provided by subjects on the part of the questionaire relating to "stress exposure" as well as "attitudes towards training, competition, eating and body image" that the psychological behaviour patterns of the Am subjects in this study differed quite considerably from that of the Eu subjects. The former displayed many more obsessive and compulsive traits, admitted to carefully controlling their body weights through diet and exercise lest they should become "fat and useless at running". Their desire to achieve athletically often appeared to extend beyond any other of their needs, such that their lives were effectively "ruled" by their training regimes. By contrast, the Eu subjects displayed a more relaxed attitude to training and competition and unlike the Am subjects, expressed the desire for a social life and to pursue interests aside from running. Given the existence of a complex relationship between stress and neuro-endocrine function it is hardly surprising that the Am subjects were experiencing disordered endocrine balance since they exposed themselves to far more physical and psychological stress than the Eu subjects.

Amenorrhoeic subjects had attained higher levels of success in athletics than Eu subjects, especially at senior level. This was perhaps due to their extreme motivation and single minded determinedness to succeed. The similarity in innate physiological qualities of Am and Eu

subjects (e.g VO_2max kg LBM^{-1} and blood lactate responses during submaximal exercise), combined with the fact that both groups were similarly successful at junior level would suggest that the Eu subjects possessed the potential to extend their performance qualities to senior level. However, they may have failed to do so through lack of motivation and/or will to suppress natural adolescent weight gain. Since the major difference in physical characteristics between groups was that of body mass and body fat content, it is likely that the extra weight of the Eu subjects hindered their performance capacity, but "protected" them against gross menstrual dysfunction. In contrast, the Am subjects appeared to consciously or subconsciously adopt a subthreshold weight for normal menstrual function and remain successful performers. Such findings might suggest that normal fertility and supreme success in certain sports are mutally exclusive.?

5.5 Summary

A comparison of the cardiovascular, respiratory and metabolic responses to prolonged steady state treadmill running of Am and Eu runners suggested that there was essentially no difference between groups when the latter were tested during the early follicular menstrual cycle phase. Thus, the hypothesis that oestrogens potentiate lipid oxidation and therefore account for possible gender-related differences in the relative contribution of lipid and carbohydrate to energy metabolism during exercise might appear at first sight to be unsupported by the findings of the present study. However, plasma oestradiol levels were still very much higher in even the Am subjects of this Study than would be expected in the "typical" man despite being depressed beyond "normal" levels in both groups of women tested. Therefore, the findings of this Study do not exclude a role for oestrogens in mediating differences in the metabolism of energy substrate metabolism during exercise between men and women, especially if the circulating concentration of oestradiol must be raised beyond a specific threshold level to exert it's metabolic effects.

Although circulating FFA concentration was higher in the Eu than the Am subjects, the former exhibited no apparent enhancement of the capacity for lipid oxidation relative to the latter. The mechanisms underlying the higher plasma glycerol and FFA concentrations of Eu as compared to Am subjects is uncertain. One can only speculate that it may be linked to the higher body fat content of Eu subjects, to differences in the regional distribution of adipose tissue and/or the metabolic properties of adipose tissue between Am and Eu women, to a higher circulating concentration of ovarian hormones, or perhaps to a higher circulating concentration of lipolytic hormones in Eu than Am subjects.

Acting as their own controls, Eu subjects appeared to produce less lactic acid and to subjectively experience less stress when exercising at the same relative intensity during the luteal as compared to the follicular phase of their menstrual cycles. Thus, it is possible that oestrogens and/or progestogens may influence exercise metabolism in a concentration-dependent manner.

The possible effects of ovarian sex steroid hormones on substrate metabolism during exercise is still uncertain and difficult to assess in the human subject in isolation of the many other factors known to exert an influence. Although there is some suggestion that athletic performance capacity and metabolic responses vary according to the phase of the menstrual cycle (Pahlke et al, 1977; Hall-Jurkowski et al, 1981; Bonen et al, 1981; Lavoie et al, 1987), reports are conflicting (Drinkwater, 1984) and often bear more association with subjective symptoms linked to the pre-menstrual phase and menstruation itself.

If any influence of ovarian steroid hormones on energy substrate metabolism cannot wholly account for the gender-related differences observed in so many studies, then it is possible that androgens may exert a more important influence. These predominantly "male" hormones control a variety of metabolic effects in the body, including a potentiation of glycogen storage capacity (Gillespie and Edgerton, 1967).

There were marked differences between Am and Eu subjects with regard to physique and body composition, dietary habits, energy balance and even personality profile. All such factors, to a greater or lesser extent may have contributed to the differences in menstrual status between groups. The precise cause of the neuro-endocrine disturbances observed in highly trained female athletes is presently uncertain. It appears to revolve around a multifactorial process involving body weight and composition, age at onset of training in relation to menarcheal age, training intensity and volume, dietary content, energy balance and the exposure to endogenous and exogenous stress. However, the relative contribution of each of these factors to the amenorrhoeic state and whether or not any single factor can trigger such endocrine abnormalities requires further study.

Although there were no differences in assessed training characteristics between groups, there was a tendency for Am subjects to engage in a greater total training volume, to exhibit a greater running economy and to be able to sustain a higher treadmill speed corresponding to a higher percentage of their $\dot{V}O_2max$ for a given steady state blood lactate concentration. Thus, the combined effects of such group differences, in addition to differences in motivational aspects would probably account for

the enhanced performance capacity of Am as compared Eu subjects in competition.

Vigorous endurance training has been shown to exert an inhibitotry effect upon the hypothalamic-pituitary-gonadal axis in men as well as women, culminating in depressed plasma testosterone levels in the former (MacConnie et al, 1986). Thus, highly trained male endurance athletes may respond in a metabolically different manner to untrained men during endurance-type exercise if they possess lower than "normal" plasma testosterone levels. Therefore, if vigorous endurance training depresses circulating sex steroid hormone concentrations in both sexes and if ovarian hormones potentiate lipid oxidation and/or inhibit carbohydrate utilisation, whilst testicular hormones potentiate carbohydrate utilisation and/or inhibit lipid oxidation by exercising skeletal muscle, then the magnitude of any gender-related differences in the preference for energy substrate during prolonged submaximal exercise may be reduced or non existent within such a population.

CHAPTER 6

THE METABOLIC RESPONSES OF HIGHLY TRAINED "ELITE" MALE RUNNERS TO PROLONGED STEADY PACE TREADMILL RUNNING

6.1 Introduction

The aim of this study was to examine the influence of "training status" upon the magnitude of possible gender-related differences in the physiological and metabolic responses to prolonged steady pace treadmill running.

There is evidence to suggest that endurance training may reduce innate gender related differences in the metabolism of energy substrate during prolonged submaximal exercise (Bransford and Howley, 1979; Powers et al, 1980; Wallace et al, 1980). However, it is also possible that in comparison to non-specifically trained men and women, highly trained men and women competing in the same event are more evenly matched with regard to the "training status" of the skeletal muscles recruited during the experimental exercise protocol.

The moderately trained female recreational runners of Study 1 appeared to oxidise a relatively greater proportion of fat than their "equally" trained male counterparts during 60 minutes of steady pace treadmill running. Whether or not such a gender-related difference also prevails between highly endurance trained female distance runners and their "equally" trained male counterparts was an objective of the present study. Hence, the physiologic and metabolic responses of a group of highly trained, "elite" standard male distance runners were measured in the present Study for the sake of a comparison with the responses of the highly trained, "elite" standard female runners of Study 2.

6.2 Methods

Nine male subject volunteers participated in the study. The criteria for their selection were based upon those set for the selection of the female subjects of Study 2; i.e, that they were county, national or international calibre middle or long distance runners currently running at least 50km per week in training and having a background experience amounting to at least 4 years of serious running training. An attempt was made to match the "training status" of these men as closely as possible to that of the women of Study 2 on the basis of past and present physical activity and training patterns. In this way, any bias prevailing from gender related differences in endurance "fitness" between male and female groups which might significantly influence metabolic responses to prolonged steady pace exercise should be reduced to a minimum.

The standard protocol of a 60 minute treadmill run performed at a relative intensity of 70% VO_2max was employed as an exercise challenge. An estimation of relative body fat content was made from the measurement of skinfold thicknesses at 4 standard sites using the equation of Durnin and Womersley (1974).

Differences in all measured variables between male, Am and Eu female subjects were evaluated using the Mann Whitney U-test for non-parametric statistics.

Table 6.1: Physiological Characteristics of Subjects

	Age (yrs)	Height (cm)	Weight (kg)	BMI	VO_2max ($mlkg^{-1}min^{-1}$)	HRmax ($btmin^{-1}$)	VO_2max ($mlkg^{-1}LBMmin^{-1}$) (SKF)
F(Eu)	23	169.7	59.67	20.8	59.51	185	83.01
	±4	±4.9	±4.57	±1.4	±3.34	±5	±6.05
F(Am)	24	165.0	50.13	18.3	60.38	185	75.77
	±6	±4.9	±4.22	±0.8	±7.01	±6	±7.10
M	27	175.4	66.60	21.7	70.71	181	76.98
	±6	±6.8	±5.00	±1.7	±3.53	±5	±3.38
	ns	**	**	**	**	ns	ns
	ns	ns	##	ns	##	ns	#

Values indicated as mean ± sD of mean.
** $p<0.01$; * $p<0.05$; ns = non significant (Men versus Am Women).
$p<0.01$; # $p<0.05$; ns = non significant (Men versus Eu Women).

Table 6.2 Physique and Body Composition of Subjects

	%Fat (SKF)	FFM (kg)	SKF thickness (mm) B	T	SS	SI
F(Eu)	28.6 ±3.5	47.6 ±3.3	9.1 ±2.7	19.1 ±2.8	17.2 ±5.7	12.3 ±5.4
F(Am)	18.9 ±4.3	42.3 ±3.0	3.7 ±0.8	11.7 ±5.7	7.5 ±3.6	6.6 ±1.8
M	8.2 ±2.4 ** ##	77.0 ±3.4 ** ##	2.7 ±0.3 ** ##	5.8 ±1.6 ** ##	6.8 ±0.8 * ##	4.4 ±0.4 ** ##

Values indicated as mean ± SD of mean.
** $p<0.01$; * $p<0.05$; ns = non significant (Men versus Am Women).
$p<0.01$; # $p<0.05$; ns = non significant (Men versus Eu Women).

Table 6.3: Training Characteristics of Subjects

	Weekly running distance (km)	Days run per week	Weeks per year	Total exercise time per week (min)	Years running experience (yr)
F (Eu)	62	6	48	487	8
	±16	±1	±2	±189	±3
F (Am)	67	6	49	504	9
	±24	±1	±1	±211	±5
M	75	7	49	508	9
	±13	±1	±1	±246	±4
	ns	ns	ns	ns	ns
	ns	ns	ns	ns	ns

Values indicated as mean ± so of mean.
** $p<0.01$; * $p<0.05$; ns = non significant (Men versus Am Women).
$p<0.01$; # $p<0.05$; ns = non significant (Men versus Eu Women).

Table 6.4: Running Economy and Treadmill Speeds equivalent to 70% VO_2max of Subjects

	VO_2 ($mlkg^{-1}min^{-1}$)			"70%" speed
	$3.20ms^{-1}$	$4.10ms^{-1}$	$5.45ms^{-1}$	(ms^{-1})
F(Eu)	36.54	46.86	-	3.60
	±1.73	±1.47		±0.29
F(Am)	34.25	45.48	-	3.74
	±3.22	±2.87		±0.46
M	-	42.95	60.62	4.57
		±2.74	±3.64	±0.36
		ns		**
		ns		##

Values indicated as mean ± SD of mean.
** $p<0.01$; * $p<0.05$; ns = non significant (Men versus Am Women).
$p<0.01$; # $p<0.05$; ns = non significant (Men versus Eu Women).

Table 6.5: Percentage VO_2max and Treadmill Speeds at $2mmoll^{-1}$ and $4mmoll^{-1}$ Blood Lactate Concentrations

	2 $mmoll^{-1}$		4 $mmoll^{-1}$	
	Speed (ms^{-1})	$\%VO_2max$	Speed (ms^{-1})	$\%VO_2max$
F(Eu)	3.77 ±0.42	69.01 ±9.97	4.29 ±0.54	83.82 ±4.68
F(Am)	3.95 ±0.67	73.66 ±4.29	4.56 ±0.59	84.13 ±4.57
M	4.74 ±0.51	69.83 ±5.16	5.31 ±0.48	82.78 ±3.14
	*	ns	*	ns
	##	ns	#	ns

Values indicated as mean ± sd of mean.
** $p<0.01$; * $p<0.05$; ns = non significant (Men versus Am Women).
$p<0.01$; # $p<0.05$; ns = non significant (Men versus Eu Women).

Table 6.6: Respiratory and Cardiovascular responses during 60 minute run

		15min	30min	45min	60min
% VO_2max	F(Eu)	70.16	71.12	71.01	71.18
		±1.60	±1.25	±1.96	±1.64
	F(Am)	70.73	70.84	71.32	71.62
		±2.09	±2.05	±2.35	±2.33
	M	69.88	70.52	70.82	70.87
		±2.17	±1.41	±1.53	±1.46
		ns	ns	ns	ns
		ns	ns	ns	ns
Heart rate ($btmin^{-1}$)	F(Eu)	161	165	165	171
		±9	±8	±9	±6
	F(Am)	154	160	163	165
		±6	±7	±10	±10
	M	152	155	158	161
		±5	±4	±5	±5
		ns	ns	ns	ns
		ns	ns	ns	ns
VO_2 ($mlkg^{-1}min^{-1}$)	F(Eu)	41.73	42.30	42.25	42.34
		±1.96	±1.99	±2.54	±2.14
	F(Am)	42.60	43.16	43.36	43.53
		±4.88	±4.28	±4.61	±4.60
	M	49.38	49.87	50.04	50.13
		±2.16	±2.78	±2.02	±3.07
		**	**	**	**
		##	##	##	##
VCO_2 ($mlkg^{-1}min^{-1}$)	F(Eu)	35.89	35.50	35.28	35.62
		±2.32	±2.49	±1.89	±1.96
	F(Am)	36.38	36.72	36.72	36.26
		±4.74	±4.10	±4.67	±4.72
	M	43.12	43.20	43.39	43.46
		±3.44	±3.15	±3.20	±3.85
		**	**	**	**
		##	##	##	##
R	F(Eu)	0.86	0.84	0.84	0.84
		±0.03	±0.03	±0.03	±0.02
	F(Am)	0.85	0.85	0.84	0.83
		±0.06	±0.04	±0.06	±0.06
	M	0.87	0.86	0.86	0.87
		±0.04	±0.03	±0.04	±0.04
		ns	ns	ns	ns
		ns	ns	ns	ns

** $p<0.01$; * $p<0.05$; ns = non significant (Men versus Am Women).
$p<0.01$; # $p<0.05$; ns = non significant (Men versus Eu Women).

Table 6.7: Energetics and Substrate Metabolism during 60 minute run

		15min	30min	45min	60min
% Contribution fat to metabolism	F(Eu)	47.54 ±10.12	54.84 ±9.48	55.88 ±8.72	54.46 ±8.08
	F(Am)	50.27 ±13.82	51.00 ±13.91	54.53 ±19.32	58.16 ±17.67
	M	43.29 ±13.04	47.17 ±11.09	45.28 ±14.39	45.68 ±14.39
		ns ns	ns ns	ns ns	ns ns
Energy expenditure ($kJmin^{-1}$)	F(Eu)	49.80 ±3.63	51.12 ±4.44	50.83 ±4.30	50.54 ±4.08
	F(Am)	42.48 ±4.86	42.77 ±5.34	43.03 ±4.74	43.22 ±5.47
	M	65.70 ±6.18	66.62 ±6.81	66.61 ±5.86	66.50 ±6.52
		** ##	** ##	** ##	** ##

Values indicated as mean ± SD of mean.
** $p<0.01$; * $p<0.05$; ns = non significant (Men versus Am Women).
$p<0.01$; # $p<0.05$; ns = non significant (Men versus Eu Women).

Table 6.8: Metabolic Responses during 60 minute run

		Rest	15min	30min	45min	60min
Blood lactate ($mmoll^{-1}$)	F(Eu)	0.83 ±0.11	2.53 ±0.77	2.35 ±0.58	2.32 ±1.05	2.27 ±0.62
	F(Am)	0.84 ±0.13	1.88 ±0.35	2.02 ±0.61	2.06 ±0.54	2.32 ±0.72
	M	0.87 ±0.11	2.97 ±0.42	2.84 ±0.44	2.71 ±0.56	2.47 ±0.45
		ns	**	**	*	ns
		ns	ns	ns	ns	ns
Blood glucose ($mmoll^{-1}$)	F(Eu)	3.92 ±0.47	4.05 ±0.62	4.21 ±0.69	4.40 ±0.66	4.84 ±0.54
	F(Am)	3.99 ±0.33	3.94 ±0.50	4.07 ±0.45	4.15 ±0.38	4.49 ±0.37
	M	3.95 ±0.31	3.90 ±0.38	4.11 ±0.38	4.04 ±0.35	4.13 ±0.61
		ns	ns	ns	ns	ns
		ns	ns	ns	ns	#
Plasma FFA ($mmoll^{-1}$)	F(Eu)	0.48 ±0.19	-	-	-	0.90 ±0.28
	F(Am)	0.31 ±0.10	-	-	-	0.59 ±0.18
	M	0.31 ±0.05	-	-	-	0.43 ±0.08
		ns				ns
		#				#
Plasma glycerol ($mmoll^{-1}$)	F(Eu)	0.10 ±0.01	-	-	-	0.55 ±0.19
	F(Am)	0.05 ±0.02	-	-	-	0.32 ±0.11
	M	0.08 ±0.04	-	-	-	0.48 ±0.22
		*				*
		ns				ns

Values indicated as mean ± sd of mean.
** $p<0.01$; * $p<0.05$; ns = non significant (Men versus Am Women).
$p<0.01$; # $p<0.05$; ns = non significant (Men versus Eu Women).

Table 6.9: Changes in Haemoglobin, Haematocrit and Plasma Volume over 60 minute run

		Pre-exercise	Post-exercise
Haemoglobin ($g\,dl^{-1}$)	F(Eu)	13.65 ±0.74	14.16 ±0.92
	F(Am)	13.09 ±0.99	13.31 ±0.92
	M	14.49 ±0.85 ** ##	15.41 ±1.32 ** ##
Haematocrit (%)	F(Eu)	39.9 ±2.2	40.9 ±2.0
	F(Am)	38.9 ±1.9	38.4 ±2.8
	M	43.7 ±2.5 ** ##	45.1 ±2.9 ** ##
% change in plasma volume	F(Eu)		-4.96 ±4.33
	F(Am)		-0.79 ±7.50
	M		-8.01 ±4.28 ns ns
Weight loss (kg)	F(Eu)		1.02 ±0.23
	F(Am)		0.71 ±0.22
	M		1.54 ±0.23 ** ##
% Weight loss	F(Eu)		1.68 ±0.36
	F(Am)		1.41 ±0.40
	M		2.32 ±0.28 ** ##

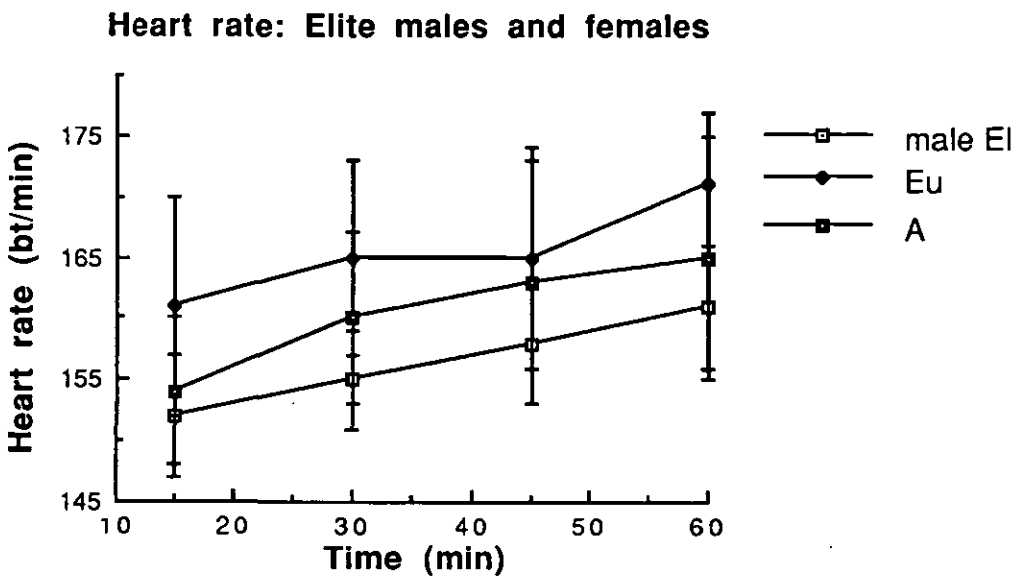
Heart rate: Elite males and females
Heart rate (bt/min)
175
165
155
145
10
20
30
40
50
60
Time (min)
male El
Eu
A

Fig 6.1

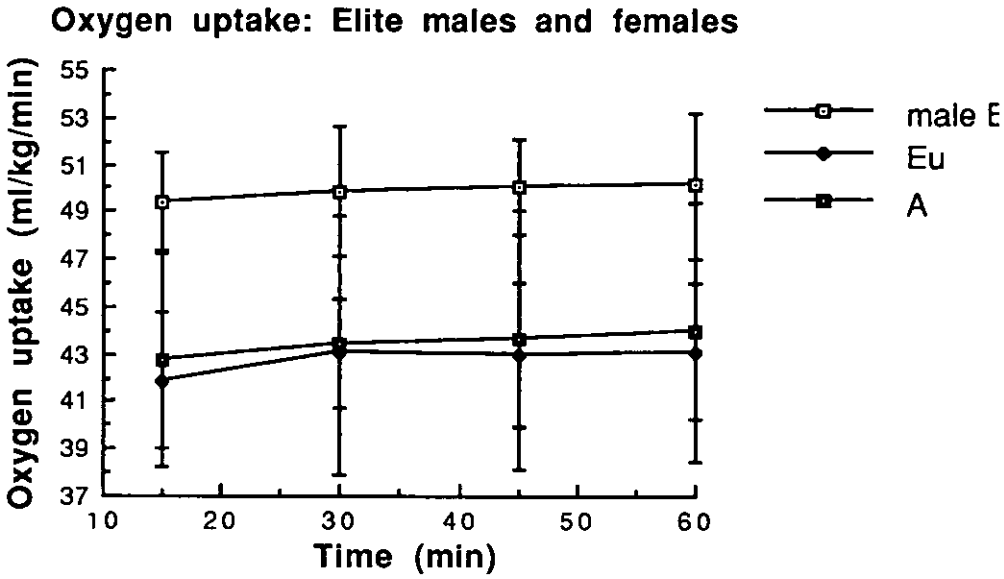
Oxygen uptake: Elite males and females
Oxygen uptake (ml/kg/min)
55
53
51
49
47
45
43
41
39
37
10
20
30
40
50
60
Time (min)
male E
Eu
A

Fig 6.2

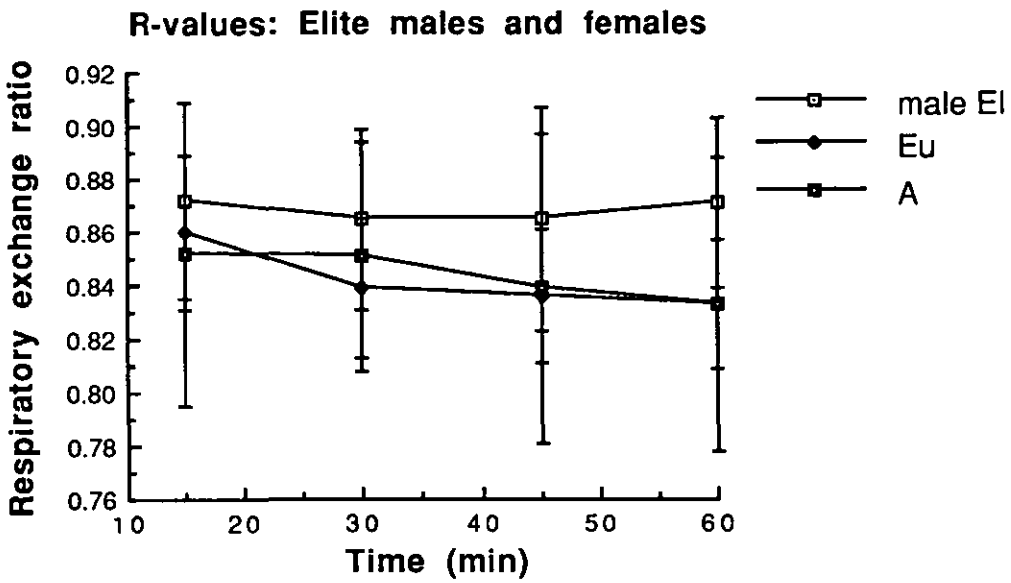
R-values: Elite males and females
Respiratory exchange ratio
0.92
0.90
0.88
0.86
0.84
0.82
0.80
0.78
0.76
10
20
30
40
50
60
Time (min)
male El
Eu
A

fig 6·3

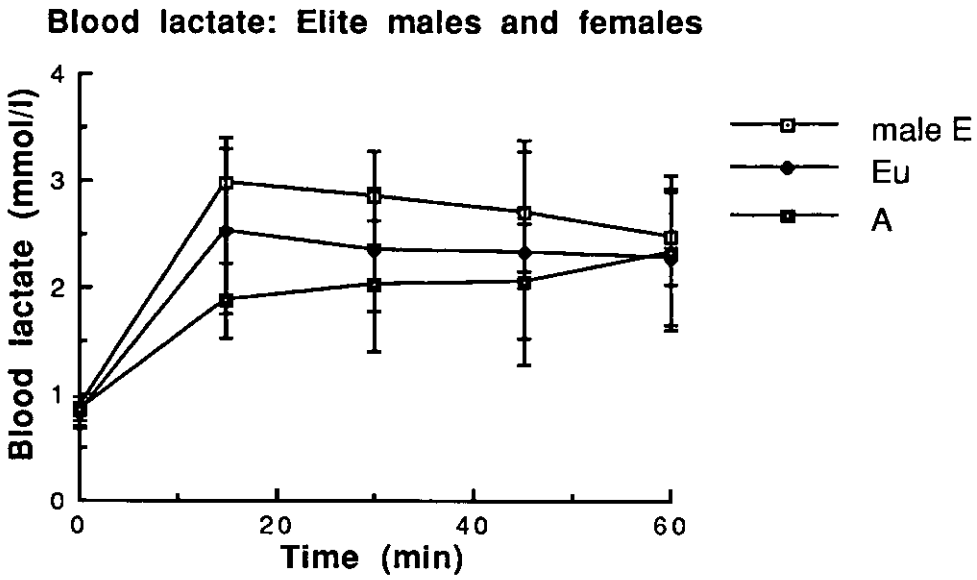
Blood lactate: Elite males and females
Blood lactate (mmol/l)
4
3
2
1
0
0
20
40
60
Time (min)
male E
Eu
A

fig 6·4

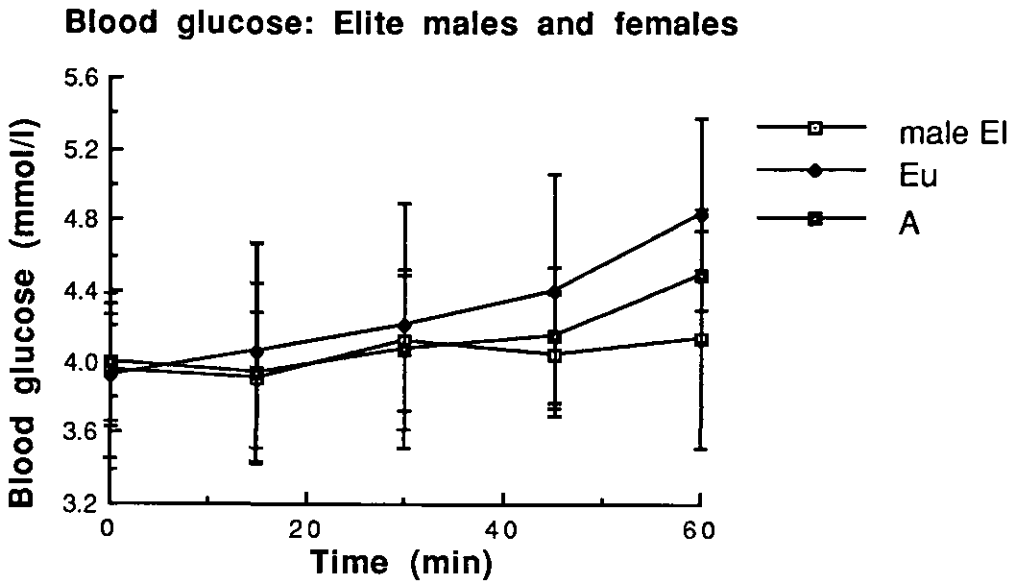
Blood glucose: Elite males and females
Blood glucose (mmol/l)
5.6
5.2
4.8
4.4
4.0
3.6
3.2
0
20
40
60
Time (min)
male El
Eu
A

Fig 6.5

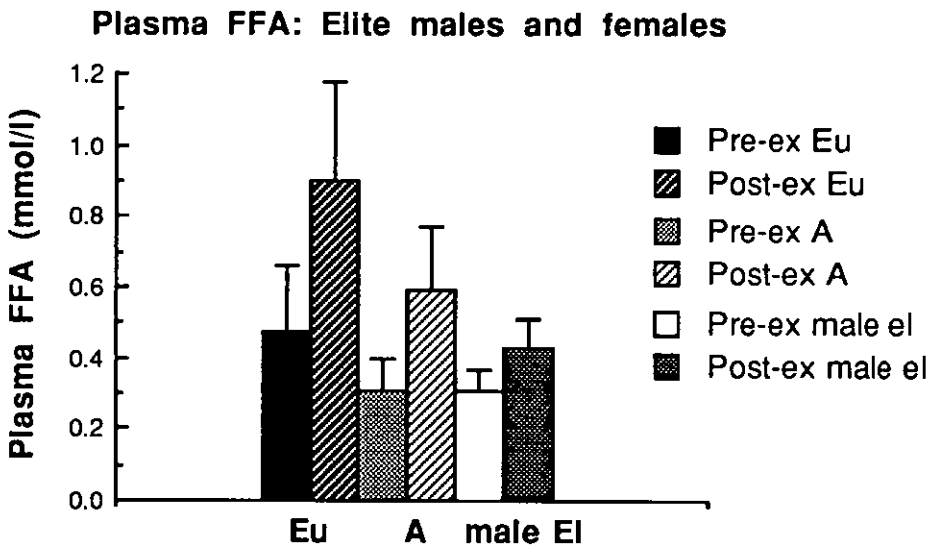
Plasma FFA: Elite males and females
Plasma FFA (mmol/l)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Eu
A
male El
Pre-ex Eu
Post-ex Eu
Pre-ex A
Post-ex A
Pre-ex male el
Post-ex male el

fig 6.6

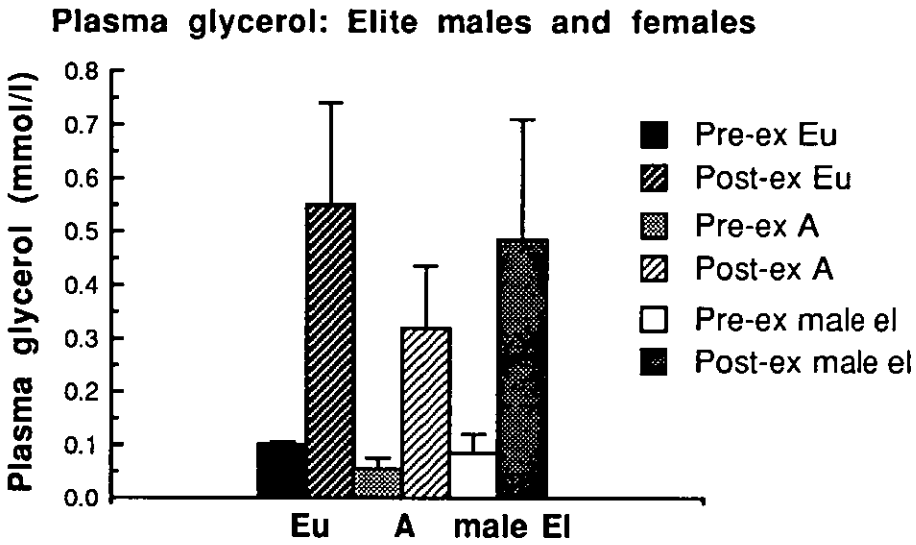
Plasma glycerol: Elite males and females
Plasma glycerol (mmol/l)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Eu
A
male El
Pre-ex Eu
Post-ex Eu
Pre-ex A
Post-ex A
Pre-ex male el
Post-ex male el

Fig 6.7

6.3 Results

6.3.1 Physical and Physiological characteristics (Tables 6.1 and 6.2):

There were no differences in age or maximum heart rate between the male, Eu and Am female subjects groups (Table 6.1). The men were taller than the Am women ($p<0.01$), but not the Eu women. The former were 12% heavier than Eu ($p<0.01$), 33% heavier than Am women ($p<0.01$), had an 18.5% greater BMI than Am women ($p<0.01$), but there was no difference in the BMI of men and Eu women. The VO_2max. (per unit total body mass) of the men was 18.8% higher than that of Eu women ($p<0.01$) and 17.1% higher than that of Am women ($p<0.01$). On the basis of body composition measurements estimated from skinfold thicknesses, Eu women were estimated to have a 3.5 fold higher percent body fat ($p<0.01$) and a 3.2 fold higher body fat content ($p<0.01$) than men and Am women to have a 2.3 fold higher percent body fat ($p<0.01$) and a 1.8 fold higher body fat content than men ($p<0.01$) (Table 6.2).

VO_2max per unit lean body mass (the latter estimated as fat free mass from measurements of skinfold thicknesses) was generally between 70 and 80$ml kg^{-1} min^{-1}$ with a mean value in the order of 77$ml kg^{-1} min^{-1}$ for the male subjects (Table 6.1). This value closely corresponded to values of this same parameter calculated for both female groups using estimates of fat free body mass obtained via the hydrostatic weighing procedure. However, the marked discrepancy in the estimates of percent body fat for Eu women made via the hydrostatic weighing as compared to the skinfold method (body fat content appeared to be much higher using the former method) resulted in a much higher calculated value of VO_2max per unit lean body mass being obtained for this particular group from the latter method. On account of the fact that the quantification of body composition for male subjects was an estimate made from skinfold measurements only and not the hydrostatic weighing method, the accuracy of the estimates of fat and "lean" mass for this group is questionable.

Skinfold thickness was less at all sites of measurement for the male subjects as compared to both female groups and most notably over the triceps muscle (Table 6.2). Relative to men, the skinfold thicknesses of Am

women were 40 to 100% greater ($p<0.01$ for triceps, supraileac and biceps sites and $p<0.05$ for the subscapular site) and 150 to 240% greater for Eu women ($p<0.01$ for all 4 sites). The pattern of fat distribution for male subjects was closer to that of the leaner Am women than the Eu women.

There was a trend towards a higher running economy in men relative to that of both groups of women, but this did not attain the prescribed level of significance (Table 6.4).

6.3.2 Training status (Tables 6.3 and 6.5):

Weekly running distance tended to be greater for men than women of both groups (Table 6.3). The total time engaged in vigorous exercise per week was in the order of approximately 8 hours for all 3 groups. All subjects ran 6 or 7 days per week, 48 to 50 weeks of the year and had been training for an average of 9 years.

The running speeds corresponding to the reference blood lactate concentrations of 2 and 4 $mmoll^{-1}$ were both in the order of 20% higher for the men than the Am ($p<0.05$) and Eu ($p<0.01$) women, however there was no difference in relative exercise intensity (%VO_2max) at both such speeds between any of the groups (Table 6.5).

6.3.3 Physiological responses to the 60 minute treadmill test run (Tables 6.6, 6.7, 6.8 and 6.9):

Oxygen uptake, carbon dioxide production (Table 6.6), running speed corresponding to 70% VO_2max (Table 6.5) and rate of energy expenditure were all higher for the male than the 2 female groups. Oxygen uptake was in the order of 16% higher in men than women ($p<0.01$); "70%" running speed was approximately 27% and 22% higher for men than for Eu and Am females respectively ($p<0.01$; both female groups) and the estimated total energy expenditure during the run was 27% and 56% higher for men than for Eu and Am women respectively ($p<0.01$, both female groups).

Heart rate tended to be slightly higher in women than in men throughout the 60 minute run (Table 6.6), whilst R values tended to be higher in men than women (Table 6.7). The estimated total quantity of fat metabolised during the run as well as the percentage contribution of fat to exercise metabolism did not differ between between men and either group of women, however the amount of carbohydrate metabolised over the run was very much higher for men than women ($p<0.01$) (Table 6.7).

Pre-exercise blood lactate concentration did not differ between any of the groups, or between men and Eu women during the test run; however it was higher in men than Am women 15 ($p<0.01$), 30 ($p<0.01$) and 45 ($p<0.05$) minutes after the start of the test (Table 6.8). An increase in blood lactate concentration from rest to 15 minutes occured in all groups ($p<0.01$), but thereafter it tended to decline gradually in men and remain essentially constant in both groups of women throughout the remainder of the test. Thus, values were similar in all groups after 60 minutes of running.

Blood glucose concentration was similar for all groups at rest and changed very little from pre-exercise values for any group up to 30 minutes after the start of the test run (Table 6.8). Thereafter, it gradually increased in both groups of women ($p<0.01$) until the end of the test, but maintained an essentially constant value in the men such that it was higher in Eu women than in men at the end of the test ($p<0.05$).

Pre-exercise plasma FFA concentration was similar in men and Am women, but lower in men than in Eu women ($p<0.05$) (Table 6.8). It increased in all groups over the duration of the run ($p<0.01$) and was again higher in Eu women relative to both Am women and men at the end of the test ($p<0.05$). The magnitude of the exercise-induced increase in plasma FFA concentration was greater for both groups of women than men ($p<0.05$). Plasma glycerol concentration tended to be slightly lower in men than in Eu women both pre- and post exercise, yet higher than for Am women both pre- and post-exercise ($p<0.05$) (Table 6.8). Post-exercise plasma glycerol concentration was higher than pre-exercise values for all groups ($p<0.01$) and the magnitude of this exercise-induced increase did not differ between groups.

Pre-and post-exercise haemoglobin concentration and haematocrit were higher in men than women ($P<0.01$) (Table 6.9). Men lost more weight than Eu or Am women ($p<0.01$) and the magnitude of weight loss relative to body mass was also greater for men than for both groups of women ($p<0.01$). The percentage decline in plasma volume was correspondingly greater for men than women ($p<0.01$).

6.4 Discussion

In contrast to the marked gender-related differences in the metabolic responses to prolonged steady state treadmill running observed in moderately trained "recreational" runners (Study 1), comparable differences were not evident between the highly trained "elite" male and female runners of the present Study. Although there was a trend towards higher R values and blood lactate concentration during exercise in highly trained men than "equally" trained women, differences were not significant at the 5% level.

However, blood glucose was significantly higher in Eu women than men towards the end of the test run and tended to be higher in Am women than men. This finding may have reflected a slower rate of carbohydrate oxidation in the women over that of the men such that the rate of hepatic glycogenolysis would exceed the rate of blood glucose oxidation in the former causing blood glucose to increase. A higher rate of fatty acid oxidation in exercising skeletal muscle would lead to a suppression of glucose utilisation and intramuscular glycogen catabolism as described by the "glucose-fatty acid cycle" (Randle et al, 1964; Rennie and Holloszy, 1977). This metabolic cycle may have occured to a greater extent in the female than the male subjects of the present Study.

The magnitude of exercise-induced rises in plasma glycerol concentration were similar for all groups, however, the higher plasma FFA concentration for Eu women than men may have permitted a greater extent of FFA oxidation to occur in the former. Although the rate of lipid relative to carbohydrate oxidation tended to be greater in both groups of women than in men, the total quantity of fat metabolised during 60 minutes running was generally higher for male subjects on account of their greater total energy expenditure. The total quantity of carbohydrate metabolised by the males was thus very much higher than that of the females. Over all however, the relationship between concentrations of circulating lipid metabolites and R values was poor within groups.

The cause of the large rise in blood lactate concentration during the first part of the run in men as compared to Am women is uncertain and was not reflected in R values which remained essentially constant throughout

the duration of the run in men as well as in women. It is possible that by recruiting a much larger muscle mass, men produced a relatively greater amount of lactic acid than Am women, or that the rate of lactate clearance was greater in the latter. However, a greater circulating blood volume might be expected to dilute any "extra" lactic acid produced by males relative to females and there is no evidence to suggest the existence of a sex difference in lactate clearance capacity.

The higher VO_2, VCO_2 and "70%" running speed of men than women during the 60 minute treadmill run could be attributed to the higher VO_2max of the former. The greater rate of energy expenditure of men was due to a combined effect of their higher running speed and body mass. Differences in body mass between the 2 groups of women accounted for the greater energy expenditure between men and Am women as compared to Eu women.

The greater extent of weight loss (in both absolute terms and relative to body mass) as well as the greater magnitude of decline in plasma volume for men than women could be explained on the basis of a greater total energy expenditure and exercise intensity relative to body mass in the former. Although men tended to consume more water whilst running, they still lost a greater amount of body fluids and were visibly sweating more profusely than the women.

With regard to the "reference" man described by Behnke and Wilmore (1974) (Section 2.1), the highly trained male subjects of this study were 0.8% taller, 4.8% lighter, with a 6.1% lower BMI, a 46% lower percentage body fat and a 29.4% higher fat free mass. Their VO_2max (per unit body mass) was in the order of 57% higher than that of the "typical" untrained man (Sparling, 1980).

The male, like the female athletes of this study were very much leaner than untrained men and women, with a lower body fat content but higher lean body mass (Wilmore, 1983). It would also appear that relative to untrained men and women, the magnitude of leanness of the male athletes was greater than that of the female athletes. Based upon an "average" relative body fat content of 14 to 16% for men and 26 to 28% for women

(Behnke and Wilmore, 1974), the percentage body fat of the male athletes was in the order of 45-50% lower than average, that of the Eu female athletes 20-25% lower and that of the Am female athletes 35-40% lower than average. Such values confirm previous findings (Behnke and Wilmore, 1980). The BMI of all 3 groups of athletes was also lower than that of the "average" man or woman.

The 18% difference in VO_2max per unit body mass between male and female athletes was of a similar magnitude to gender-related differences of this same variable measured within the untrained population (Sparling, 1980). When expressed relative to fat-free body mass (estimated from skinfold thicknesses), the value of VO_2max was similar for the male, Eu female and Am female athletes under study. It has been stated that men and women can be assumed to be of equal "cardiovascular fitness" if the difference in VO_2max expressed per unit lean body mass between them amounts to no more than 5% in favour of males (Cureton, 1981). Therefore, on the basis of the similarities in VO_2max per unit fat free mass, past and present training characteristics and blood lactate concentrations during steady state submaximal treadmill running at a given relative intensity, the male and female athletes of this study could be said to be essentially evenly matched with regard to "training status".

From a comparison of gender-related differences in energy substrate metabolism during prolonged submaximal treadmill exercise within a highly and a moderately trained population of male and female distance runners, it can be concluded that there was an apparent trend towards a greater extent of lipid oxidation in women than in men, with the latter relying to a greater extent upon substrate derived from the catabolism of intramuscular and hepatic glycogen stores. However, the magnitude of observed differences were very much greater within the moderately, as compared to the highly trained population. This might suggest that endurance training does reduce the magnitude of gender-related differences in substrate metabolism during prolonged submaximal exercise. Underlying mechanisms may involve sex steroid hormones, although it is not possible to make valid conclusions from the data collected in the present series of Studies. However, if ovarian and testicular steroid hormones exert opposite facilitatory and/or

inhibitory effects on lipid and carbohydrate metabolism and the synthesis and secretion of these hormones are suppressed through intense endurance training in both sexes, this could contribute to the reduction in gender-related differences in carbohydrate and lipid oxidation observed in highly trained endurance athletes.

Alternatively, it may be simply that highly trained men and women practising the same sport are more closely matched with regard to the "training status" of the skeletal muscles recruited during the exercise challenge than non-specifically trained men and women. There may exist a limitation to fatty acid oxidation within untrained men but not untrained women which is removed by endurance training. Perhaps untrained male skeletal muscle is less well perfused than untrained female skeletal muscle such that the muscle's capacity for lipid oxidation is limited by the supplies of circulating FFAs and/or oxygen. A training-induced stimulation of capillary growth within the muscles would thus help overcome any perfusion limitation.

A more accurate means of testing the hypothesis that training reduces/negates gender-related differences in substrate metabolism during prolonged submaximal exercise would be to train a population of "equally" untrained men and women by prescribing them a similar training stimulus in a longitudinal study, then to measure their metabolic responses to a standardised exercise test pre- and post-training.

6.5 Summary

A marked gender-related difference in the metabolism of energy substrate during prolonged steady state treadmill running was not evident within the population of highly trained distance runners examined in this Study. This contrasts the observations made within the moderately trained men and women of Study 1 where women apparently oxidised lipid much more readily than men. Thus, the hypothesis that "endurance training reduces innate gender-related differences in the metabolism of energy substrate during exercise" would appear to hold within the realms of the experimental protocol employed in the present series of Studies.

Highly trained "elite" standard male and female distance runners differed from the moderately trained "recreational" male and female runners with regard to the following characteristics:

a) Physical and Physiological characteristics: "Elite" athletes were lighter relative to stature, very much leanner and had a 10-15% higher VO_2max (per unit body mass) than "recreational" runners of the same sex.

b) Physiological and Metabolic responses to prolonged steady pace running: "Elite" athletes experienced less homeostatic disturbance during 60 minutes of treadmill running than "recreational" athletes, as evidenced from the magnitude and pattern of change of measured respiratory and metabolic variables.

c) Training and Performance characteristics: "Elite" athletes adhered to a strict and fairly rigid running training programme which concentrated upon a high total training volume as well as interval work ("track sessions"). All trained at least once daily, 6-7 days per week and many supplemented their running training with other endurance sports such as swimming and cycling. In contrast, "recreational" runners did not generally plan a specific running training programme and as a result, their running training volume tended to fluctuate week by week, depending upon the extent to which they participated in "team" sports and their time or desire to train. The "training status" of the skeletal muscles recruited during running in these individuals was thus difficult to qualify and it is possible that the

treadmill speed corresponding to 70% VO_2max of some of these subjects was too high with respect to the aerobic capacity of the skeletal muscles recruited in the test. These individuals may have trained their cardiovascular systems (potentiated their VO_2max values) to a greater extent through participation in sports aside from running which require the recruitment of different muscle groups. Consequently, the moderately trained men and women may not have been as evenly matched with regard to "training status" as the highly trained male and female athletes under study.

CHAPTER 7

General Discussion

The respiratory and metabolic responses to prolonged steady pace treadmill running of both moderately trained "recreational" male and female runners and highly trained "elite" standard male and female runners exhibited trends towards gender-related dissimilarities in substrate metabolism during a metabolic challenge. It would appear that the exercising skeletal muscles of the female subjects preferentially oxidised fat, whilst those of the male subjects metabolised a relatively greater proportion of carbohydrate as energy substrate during a prolonged bout of steady pace treadmill running. However, the magnitude of this gender-related difference was apparently much greater for moderately trained than highly trained male and female runners matched for "training status".

Estimates of the percentage contribution of lipid and carbohydrate to exercise metabolism from R values suggested that female subjects generally derived over 50% of their energy from fatty acid oxidation, whilst the male subjects tended to derive over 50% of their energy from the oxidation of carbohydrates. The mean percentage contribution of fat to energy expenditure during a 60 minute treadmill run was estimated to be in the order of 65-70% for female recreational runners, 50-55% for "elite" female runners, 30-35% for male recreational runners and 40-45% for "elite" male runners. Thus, the preference for lipid as energy substrate was greater in highly trained women than in moderately trained women, but greater in highly trained men than moderately trained men. Although blood lactate concentration attained during the 60 minute treadmill run was lower for highly trained men and women as compared to their moderately trained counterparts, it was very much higher for moderately trained men than moderately trained women and values tended to be higher in highly trained men than in highly trained women. These observations are suggestive of a greater extent of intramuscular glycogen catabolism in men than women. Blood glucose concentration was generally higher in women than men of similar training status, providing perhaps another indication of the

existence of a relative preference for fat than carbohydrate as energy substrate during exercise in women than men.

The results of these studies confirm the findings of earlier studies comparing exercise metabolism in adult men and women (Nygaard et al, 1978; Hardman and Williams, 1983; Froberg and Pedersen, 1984; Blatchford et al, 1986; Nygaard, 1986; Tarnopolsky et al, 1990). In these particular studies, the women apparently possessed an innately greater capacity for lipid oxidation than men, who tended to rely to a greater extent upon limited intramuscular and hepatic glycogen stores. In agreement with the work of other groups (Bransford and Howley, 1979; Costill et al, 1979; Powers et al, 1980; Wallace et al, 1980), the results obtained in the present Studies also suggest that training reduces or negates gender-related differences in substrate metabolism during prolonged submaximal exercise.

It is evident that the high performance capacity of the "elite" male and female distance runners of Studies 2 and 3 was attributable to:

i) Their high VO_2max, which when expressed per unit body mass was in the order of 50-60% higher than that of untrained men and women (Sparling, 1980) and 10-15% higher than the moderately trained recreational male/female runners of Study 1. The sex difference in VO_2max existing between similarly trained subjects of both Studies was in the order of 15-20%, a value which matches the magnitude of the corresponding sex difference in VO_2max of untrained individuals (Sparling, 1980).

ii) The high aerobic capacity ("training status") of the athletes' skeletal muscles recruited during treadmill running. For a given blood lactate concentration, highly trained "elite" runners were able to sustain a submaximal running pace requiring a 20% higher percentage of their VO_2max than the moderately trained "recreational" runners. The treadmill speeds corresponding to steady state blood lactate concentrations of 2 and 4 $mmoll^{-1}$ were in the order of 30-40% higher in highly trained as compared to moderately trained male and female distance runners, these speeds being approximately 20% higher in males than females of similar training status.

iii) The marked leanness and high running economy of highly trained relative to moderately trained runners.

The magnitude and pattern of change of respiratory and metabolic variables measured during the 60 minute treadmill test run in "recreational" and "elite" runners would suggest that this test imposed less physiological strain upon the former. Although all subjects were exercising at the same relative intensity; blood lactate concentration, R-values, $\dot{V}O_2$ and $\dot{V}CO_2$ remained essentially constant in the highly trained male and female groups, but steadily increased in the moderately trained groups. This would suggest that the latter failed to maintain a homeostatically steady state throughout the duration of the test. Regular and vigorous endurance training over many years effects a great many adaptive changes in a number of different organ systems (described in detail in sections 2.1-2.5) which enable the individual to perform the training exercise for prolonged periods of time in the absence of marked homeostatic disturbance. As a result, the magnitude of exercise induced hormonal responses are lower in more highly trained individuals (Bloom et al, 1976; Galbo, 1983) since they are subjected to a lower degree of metabolic stress at a given relative exercise intensity than lesser trained individuals.

It is likely that by increasing their running training volume, the "recreational" runners of Study 1, like the "elite" runners of Study 2 would become better adapted to running long distances in the face of minimal metabolic stress. However, the former would probably never achieve the same performance potential as the latter, simply because they were probably not endowed wth the necessary genetic qualities. Increased training may increase the endurance capacity of the "recreational" runners to the same degree as that of the "elite" runners, but not their maximal aerobic running speed (governed by $\dot{V}O_2max$) and hence performance capacity.

The results of the present series of Studies as well as previous research along the same lines suggest that gender probably does exert an influence upon exercise metabolism. However, the precise underlying mechanisms are uncertain. Recent evidence suggests that women may derive

any "extra" fat for exercise metabolism from triglyceride stored within the exercising muscles (Tarnopolsky et al, 1990) rather than from extramuscular triglyceride stores. The absence of marked differences in the concentrations of circulating lipid metabolites pre- and post-exercise between male and female subjects in the preceding Studies would suggest the occurance of a similar total extent of triglyceride mobilisation in men and women; however, the origin of the lipid oxidised during the run (intramuscular versus extramuscular) could not be deduced.

In order to make an accurate quantification of substrate utilisation during prolonged exercise it would be necessary to incorporate the use of more refined and invasive techniques than those employed in the present Studies. Muscle biopsies would aid quantification of the extent of intramuscular glycogen and triglyceride utilisation during exercise and it may be useful to make a direct measure of respiratory quotient across the vascular bed of the exercising muscles. A quantification of FFA turnover during exercise might also prove informative, as would measurements of the various circulating lipolytic and glycogenolytic hormones (which control the rate and extent of substrate mobilisation during exercise) before, during and after exercise. Supreme accuracy in the quantification of substrate metabolism evidently requires the simultaneous use of a wide variety of different invasive techniques and may well remain confined to the experimental animal. The ethical feasibilty of such human experimentation is questionable and it is doubtful that "elite" or regularly competing athletes would be willing to volunteer as subjects in this type of study.

Diet is known to exert a profound influence on substrate metabolism, both at rest and during exercise. The quantity and composition of the food eaten in the few hours preceding exercise will significantly affect the concentration of various anabolic and catabolic hormones (Galbo, 1983), and the composition of food eaten (in relation to the amount and type of physical exercise performed) during the days preceeding an exercise test will influence the intramuscular and hepatic stores of glycogen (Bergstrom et al, 1967). A high fat/low carbohydrate diet leads to an increase in the circulating concentrations of FFA's and glycerol, an increase in the contribution of fat to energy metabolism during submaximal exercise and a decrease in endurance capacity due to a lowering of intramuscular glycogen

stores. In contrast, a high carbohydrate/low fat diet leads to a relatively high oxidation of carbohydrate and an increase in endurance capacity due to the optimisation of muscle glycogen stores (Krogh and Lindhardt, 1920; Rennie and Johnson, 1974; Maughan et al, 1978). There is also evidence to suggest that females "adapt" more readily to fat utilisation during submaximal exercise after a high fat diet than males (Hede and Raa Andersson, 1985). The mechanisms underlying these observations may involve a dietary-induced change in insulin secretion since a high fat diet has been shown to lower plasma insulin concentration (Galbo, 1983) and a removal of the tonic inhibitary effect of insulin on adipose tissue lipolysis would lead to an increase in plasma FFA and glycerol concentrations. Thus, although all subjects performed the 60 minute test run after an overnight fast, a more definitive approach to this type of study might also include the standardisation of subjects' diets and exercise regimes during the 48 hours preceding the 60 minute run.

The potential influence of sex steroid hormones on substrate metabolism also requires further qualification and might prove to be especially interesting with regard to the amenorrhoeic, hypo-oestrogenic female athlete who tends to typify the most successful distance runner. If supreme success in long distance running and "intact" neuro-endocrine function are mutually exclusive, yet ovarian steroid hormones facilitate lipid metabolism and hence potentiate endurance capacity by economising on glycogen utilisation during prolonged exercise, it might be interesting to study the effects of hormone replacement therapy on endurance capacity and performance in hypo-oestrogenic female distance runners.

Although gender related differences in substrate metabolism appear to be less marked in highly trained, as compared to untrained or moderately trained men and women, the extent to which training per se reduces such differences is uncertain. The "elite" athletes presently under study were very highly trained runners who are likely to have maximised their aerobic capacity through running training, whilst many of the "recreational" runners under study did not follow a rigid running training programme, but tended to practise a wide variety of sports, incorporating the use of different muscle groups. Thus, the treadmill speed corresponding to 70% VO_2max of many of the latter may have been too high for them to attain a homeostatically steady state if they had essentially achieved their high

VO_2max values through participation in sports other than running. It is noteworthy that most of the male recreational runners of Study 1 played team sports to county or even national level, whilst the female subjects of the same study played fewer sports aside from running. Thus it is possible that the male and female "recreational" runners were not as evenly matched with regard to the "training status" of the skeletal muscles recruited during treadmill running as were the "elite" male and female distance runners under study. In this case, a more accurate gender comparison might have been obtained by setting relative exercise intensity according to the subjects' running speeds at their "onset of blood lactate accumulation" (OBLA) (Sjodin and Jacobs, 1979) rather than their VO_2max.

As yet, few longitudinal studies with the aim of quantifying the extent of lipid and carbohydrate oxidation during prolonged submaximal exercise by the same groups of men and women, before and after training have been conducted. Since a great many different factors are known to influence the muscles' preferred choice of energy substrate during exercise, an adequate standardisation of such factors between trained and untrained groups is not easily achieved and an accurate quantification of any gender related influence on substrate metabolism during exercise obtained in cross sectional studies remains questionable.

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APPENDIX 1

Lactic Acid Assay

The methodology was adapted from that of Olsen (1971) and involves the conversion of lactate to pyruvate with the formation of NADH. The latter is then quantified by its native fluorescence:

$$\text{Lactate} + \text{NAD} \xrightarrow{\text{LDH}} \text{pyruvate} + \text{NADH}$$

Each 20uL sample of capillary blood was deproteinised in 200uL perchloric acid, mixed thoroughly, centrifuged and stored at -20 C for subsequent analysis.

Reagents:

- Perchloric acid: 2.5% w/v
- Hydrazine buffer (1.1 M, pH 9.0): 1.3g hydrazine sulphate, 5.0g hydrazine hydrate and 0.2g disodium ethylenediaminetetraacetic acid (EDTA) in 100ml distilled water.
- Reaction mixture: 2mg NAD and 10uL LDH per ml hydrazine buffer. (prepared immediately prior to use).
- Diluent: 0.07 MHCl.

Standards:

A range of concentrations from 0.5 to 10mmoll^{-1} made from 1.0M Sodium L-lactate stock solution.

Procedure:

1) Samples removed from freezer and thawed for 60 min at room temperature.
2) Samples mixed thoroughly and centrifuged for 2 min.
3) 20uL supernatant or standard pipetted into clean glass tube and 200uL reaction mixture added to each tube, mixed and allowed to incubate 30min at room temperature. (Samples analysed in duplicate; standards in triplicate).
4) 1ml diluent added to each tube and mixed. Fluorescence (arbitary units) of samples read against standards and blank (diluent) in flourimeter (Perkin Elmer Ltd).
5) Concentration of lactate in sample calculated using the equation of the straight line relating flourescence to lactate concentration.

Reference:

Olsen, C. (1971): An enzymatic, fluorometric micromethod for the determination of acetoacetate, B hydroxybutyrate, pyruvate and lactate. Clin. Chim. Acta. 33: 293-300.

APPENDIX 2

Glucose assay

The assay is based upon the conversion of glucose to a coloured complex, the concentration of which is quantified in solution photometrically:

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconate} + H_2O_2$$

$$H_2O_2 + \text{ABTS} \xrightarrow{\text{POD}} \text{coloured complex} + H_2O$$

Reagents: (Boehringer Mannheim GmbH Diagnostica Ltd)

1) Standard - Glucose: 9.1mg100ml^{t-1} (=0.505mmoll^{-1})
2) Phosphate buffer: 100mmoll^{-1}, pH 7.0
3) POD: $\geq$ 0.8Uml^{-1}
4) GOD: $\geq$ 10.0Uml^{-1}
5) ABTS [di-ammonium 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonate)] 1.0mgml^{-1}

Procedure:

1) Samples removed from freezer and thawed for 60 min at room temperature.
2) Samples mixed thoroughly and centrifuged for 2 min.
3) 20uL supernatant or standard pipetted into clean glass tube (Samples analysed in duplicate; standards in triplicate). 1ml reaction mixture added and the contents thoroughly mixed. Tubes were left to incubate 20 min at room temperature.
4) Absorbance of samples and standards measured photometrically (wavelength Hg 436nm) in a cuvette (1cm light path) against a blank (reaction mixture).
5) Concentration of glucose in samples calculated as follows:

$$C = 5.55 \times \frac{A\ \text{sample}}{A\ \text{standard}} \qquad (\text{mmoll}^{-1})$$

Reference:

Werner, W., Rey, H.G. & Wielinger, H. (1970): Uber die Eigenshaften eines neuen chromogens fur die blutzuckerbestimmung nach der GOD/POD-methode. Z. Anal. chem. 252: 224-228.

APPENDIX 3

Free Fatty Acid Assay

The procedure is adapted from the method of Chromy et al, (1977). It involves the extraction of FFA's from plasma using a solvent (CHM) and their conversion to a coloured complex, the concentration of which is quantified in solution photometrically:

Reagents:

1) Standards: a series of dilutions of palmitic acid solution: (0.2-1.0mmol l^{-1}).
2) Extraction solvent (CHM): chloroform (560mll^{-1}), n-heptane (420ml/l^{-1}) and methanol (20mll^{-1}).
3) Stable copper reagent: sodium citrate (7.5gl^{-1}), triethanolamine (67gl^{-1}), copper nitrate (32.5gl^{-1}) and sodium chloride (125gl^{-1}) in distilled water.
4) TAC: 100mg 2-thiozolylazo-p-cresol in 1 litre of ethanol.

Procedure:

1) Samples removed from freezer and thawed for 60 min at room temperature.
2) 100uL sample or standard pipetted into glass tubes containing CHM (3ml/tube) (samples analysed in duplicate; standards in triplicate).
3) 1ml stable copper reagent added to each tube.
4) Tubes (tightly capped) shaken vigorously for 2 minutes, then centrifuged (4 rpm) for 8 minutes.
5) 1 ml upper phase transferred to glass tube containing TAC and mixed well.
6) Absorbance of samples and standards measured photometrically (wavelength 578nm) in a cuvette (1cm light path) against a blank (reaction mixture).
5) Concentration of FFA in samples calculated using the equation of the straight line relating flourescence to lactate concentration.

Reference:

Chromy, V., Gergel, J., Voznicek, J., Krombholzova, L. & Musil, J. (1977): Assay of serum free fatty acids by extraction-photometric procedure. Clin. Chim. Acta. 80: 327-332.

APPENDIX 4: Glycerol Assay

The procedure is adapted from Laurell and Tibbling (1966) and involves the precipitation of glycerol from plasma and its conversion to a compound, the concentration of which is quantified in solution fluorometrically:

Reagents:

1) Standards: a series of dilutions of glycerol solution: (0.05-0.8mmol l^{-1}).
2) Zinc sulphate solution (0.087mmoll^{-1}).
3) Barium hydroxide solution (0.083mmoll^{-1}).
4) Hydrazine-HCl buffer (1M); pH 9.4.
5) Reaction mixture: 12mg ATP, 20mg NAD dissolved in 0.2ml distilled water; 100ul cysteine solution (0.2mmoll^{-1} NaOH), 700ul H-HCl buffer, 1ul glycerokinase and 5ul glycerin-3-phosphate dehydrogenase.

Procedure:

1) Samples removed from freezer and thawed for 60 min at room temperature.
2) 0.25ml zinc sulphate pipetted into small eppendorf centrifuge tubes.
3) 100uL sample or standard added to each tube (samples analysed in duplicate; standards in triplicate).
4) 0.25ml barium hydroxide added to each tube and mixed immediately.
5) Samples and standards chilled (-20 C) for 5 minutes.
6) 200ul supernatant pipetted into clean glass tubes.
7) 100ul reaction mixture added to each tube.
8) Incubate 60 minutes at room temperature.
9) 1ml diluent added to each tube and fluorescence (arbitary units) of samples read against standards and blank (diluent) in flourimeter (Perkin Elmer Ltd).
10) Concentration of glycerol in sample calculated using the equation of the straight line relating flourescence to lactate concentration.

Reference:

Laurell, S. & Tibbling, G. (1966): An enzymatic-flourometric micromethod for the determination of glycerol. Clin. Chim. Acta. 13: 317-322.

EXERCISE AMENORRHOEA STUDY

Menstrual dysfunction is common in women who regularly participate in vigorous exercise, especially competitive distance running. We are interested not only in what causes these disturbances, but also whether they are associated with other metabolic changes, both during exercise and at rest. We would therefore be grateful if you could complete the following questionnaire, which will be kept strictly confidential to all but the few people concerned with the investigation.

NAME:..... DATE OF BIRTH:.....

HEIGHT:..... WEIGHT:.....

ADDRESS:..... TELEPHONE NUMBER:.....

MARITAL STATUS:..... OCCUPATION:.....

TRAINING:

1) HOW OLD WERE YOU WHEN YOU STARTED TRAINING ?.....
2) DO YOU TRAIN HARD ALL YEAR ROUND ?.....
   ESTIMATE THE NUMBER OF WEEKS INTENSE TRAINING PER YEAR (max 52).....
3) HOW MANY DAYS DO YOU TRAIN PER WEEK ? (max 7).....
4) WHAT IS YOUR AVERAGE WEEKLY RUNNING MILEAGE ?.....
5) DO YOU RUN MORE THAN ONCE PER DAY ?.....
   IF YES, HOW MANY TIMES PER DAY ?.....
   ON HOW MANY DAYS PER WEEK DO YOU RUN MORE THAN ONCE PER DAY ?.....
6) DO YOU REGULARLY PARTICIPATE IN ANY OTHER SPORTS (e.g swimming/cycling /weight-training/team sports) ?.....
   IF YES, LIST THESE ACTIVITIES.....
   .....
   .....
7) WHY DO YOU RUN ? (tick which applicable):
   a) For competition.....
   b) Health reasons (to keep "fit").....
   c) To improve/maintain body image.....
   d) Other (please specify; you may have several reasons).....
   .....
   .....
8) WOULD YOU EVER SAY THAT YOU FEEL "COMPELLED" TO RUN ?.....
   HOW DO YOU FEEL IF YOU ARE FORCED TO MISS TRAINING DUE TO ILLNESS/INJURY (eg: depressed/irritable/unable to cope).....
   .....
   .....
9) HOW IMPORTANT IS RECOVERY TO YOU ?.....
   .....
   DO YOU TAKE "REST" DAYS ON WHICH YOU DO NO VIGOROUS EXERCISE ?.....
   DO YOU EVER INTENTIONALLY TAKE MORE THAN 2 TO 3 DAYS OFF TRAINING FOR THE PURPOSE OF RECOVERY ?.....

IN THE TABLE BELOW, FOR EACH DAY COULD YOU PLEASE WRITE DOWN ALL THE VIGOROUS EXERCISE YOU TAKE ?

	Type of exercise (eg: run/swim/cycle)	Time spent (minutes)	Distance covered (miles/metres/km)
DAY 1:			
DAY 2:			
DAY 3:			
DAY 4:			
DAY 5:			
DAY 6:			
DAY 7:			

STRESS:

1) DO YOU EXPERIENCE ANY PRESSURES/STRESSES SPECIFIC TO RUNNING /.....
   IF YES, PLEASE EXPLAIN.....
   .....
2) RATE HOW YOU EXPERIENCE THE FOLLOWING ON A SCALE OF 1 to 10:

   a) Athletic pressures.....1 2 3 4 5 6 7 8 9 10
   b) Academic pressures.....1 2 3 4 5 6 7 8 9 10
   c) Familial pressures.....1 2 3 4 5 6 7 8 9 10
   d) Social pressures.....1 2 3 4 5 6 7 8 9 10

MENSTRUATION:

1) AT WHAT AGE DID YOU HAVE YOUR FIRST PERIOD ?.....
2) DO YOU HAVE REGULAR MONTHLY PERIODS ?.....
   IF NO, PLEASE EXPLAIN ANY MENSTRUAL ABNORMALITIES.....
   .....
3) HOW MANY PERIODS HAVE YOU HAD DURING THE LAST 12 MONTHS ?.....
4) WHAT ARE THE DATES OF THE START AND FINISH OF YOUR LAST PERIOD ?.....
   .....
5) DID YOU HAVE REGULAR PERIODS BEFORE STARTING VIGOROUS ATHLETIC TRAINING?
   .....

6) HAVE YOU EVER BEEN PREGNANT ?.....
IF YES, WAS THIS PREGNANCY FULL TERM / MISCARRIAGE / OTHER ?.....
.....

7) ARE YOU USING ORAL CONTRACEPTIVES (the "Pill") AT PRESENT ?.....

8) IF YES, FOR HOW LONG HAVE YOU BEEN TAKING THEM ?.....
WHAT ARE THEY CALLED ?.....
IF NO, HAVE YOU EVER USED ORAL CONTRACEPTIVES ?.....
IF YES, WHEN DID YOU FINISH TAKING THEM ?.....

9) PLEASE NAME ANY OTHER MEDICATION YOU ARE TAKING.....
.....

DIET:

1) DO YOU FOLLOW ANY SPECIAL DIET (e.g vegan, ovo-lacto vegetarian etc)?...

2) IF YES, PLEASE DESCRIBE YOUR DIET ?.....
.....
.....
.....

3) HAS RUNNING CAUSED YOU TO LOSE WEIGHT ?.....

4) IS YOUR WEIGHT STABLE, OR DOES IT FLUCTUATE ?.....
IF IT FLUCTUATES, BY HOW MANY POUNDS/KGS IS THIS, AND IN WHAT SPACE OF TIME ?.....
.....

5) DO YOU USE RUNNING AS A MEANS OF WEIGHT CONTROL ?.....

WOULD YOU BE WILLING TO PARTICIPATE IN A STUDY INVOLVING THE MEASUREMENT OF BODY COMPOSITION, HORMONAL PROFILES, VARIOUS ASPECTS OF AEROBIC RUNNING PERFORMANCE AND CAPACITY AND AN ANALYSIS OF DIETARY INTAKE ?.....

INDIVIDUAL SUBJECT DATA : STUDY 1 : "MODERATELY" TRAINED ♂ & ♀ RUNNERS

PHYSIOLOGICAL CHARACTERISTICS OF SUBJECTS:

Subject	Age (yr)	Height (cm)	Weight (kg)	BMI	VO_2max ($mlkg^{-1}min^{-1}$)	HRmax ($btmin^{-1}$)
m1	25	183.2	69.9	20.8	63.08	194
m2	22	174.5	67.3	22.1	67.37	202
m3	25	171.8	80.4	27.2	59.06	186
m4	25	185.7	80.6	23.4	60.09	175
m5	23	188.0	79.0	22.4	65.14	185
m6	25	171.0	71.9	24.6	63.17	188
m7	22	188.2	88.0	24.8	57.41	197
m8	21	179.2	69.4	21.7	66.28	195
	24	180.2	75.8	23.4	62.70	190
	±2	±7.0	±7.2	±2.1	±3.57	±9
f1	23	166.6	55.2	19.9	49.80	185
f2	29	170.0	61.0	21.1	46.33	183
f3	27	165.2	62.8	23.0	49.71	192
f4	27	174.8	62.9	20.6	59.84	182
f5	18	160.7	50.2	19.4	68.45	191
f6	25	160.0	56.5	22.1	52.14	186
f7	21	165.7	69.4	25.3	50.37	183
f8	39	155.0	55.3	23.0	55.97	174
	26	164.8	59.2	21.8	54.08	185
	±6	±6.2	±6.0	±1.8	±7.15	±6

TREADMILL SPEEDS DURING 60 MINUTE RUN:

Subject	Speed(ms^{-1})	Subject	Speed(ms^{-1})
m1	3.90	f1	2.96
m2	3.82	f2	2.99
m3	3.40	f3	2.84
m4	3.42	f4	3.70
m5	3.88	f5	3.93
m6	3.48	f6	3.33
m7	3.40	f7	3.23
m8	4.20	f8	3.03
	3.69		3.25
	±0.30		±0.39

OXYGEN UPTAKE AT 2 SUBMAXIMAL RUNNING SPEEDS (RUNNING ECONOMY):

	Oxygen uptake ($ml kg^{-1} min^{-1}$) at:				
	$3.2 ms^{-1}$	$4.1 ms^{-1}$		$3.2 ms^{-1}$	$4.1 ms^{-1}$
m1	40.77	50.97	f1	38.31	49.11
m2	42.15	49.54	f2	35.65	45.66
m3	39.02	48.98	f3	32.69	42.47
m4	39.67	49.06	f4	42.86	54.61
m5	39.49	48.57	f5	40.63	48.95
m6	39.25	49.34	f6	36.54	47.26
m7	44.72	47.79	f7	33.18	43.30
m8	35.43	45.34	f8	41.59	49.38
	40.06	48.70		37.68	47.59
	±2.68	±1.63		±3.81	±3.87

TRAINING CHARACTERISTICS OF SUBJECTS:

Subject	Distance run per week (km)	No. days run per week	No. weeks per year	Total exercise time per week (min)	No. years running
m1	56	6	50	540	4
m2	38	4	45	280	4
m3	16	5	48	300	2
m4	32	6	47	220	2
m5	64	6	50	380	5
m6	24	5	45	360	2
m7	24	4	42	180	1
m8	72	6	50	480	3
	41	5	47	343	3
	±21	±2	±3	±123	±1
f1	32	5	48	260	3
f2	37	5	48	280	4
f3	45	6	47	234	6
f4	56	6	50	540	6
f5	30	6	48	240	6
f6	29	5	48	270	2
f7	38	4	45	300	2
f8	56	6	45	280	8
	41	5	47	301	5
	±11	±2	±2	±99	±2

%VO_2max & TREADMILL SPEEDS AT 2mmoll^{-1} BLOOD LACTATE CONCENTRATION:

Subject	Speed at 2mmoll^{-1} [lactate] (ms^{-1})	%VO_2max at 2mmoll^{-1} [lactate]
m1	3,60	61,25
m2	3,53	58,75
m3	3,04	58,75
m4	3,03	56,75
m5	3,14	53,55
m6	3,06	51,50
m7	3,21	57,80
m8	3,94	58,25
	3,32	57,08
	±0,33	±3,13
f1	2,22	52,50
f2	2,28	54,00
f3	2,65	54,50
f4	3,69	82,00
f5	3,18	56,65
f6	2,61	56,70
f7	2,80	58,10
f8	2,14	55,00
	2,70	58,68
	±0,53	±9,59

$\%VO_2max$ & TREADMILL SPEEDS AT $4mmol l^{-1}$ BLOOD LACTATE CONCENTRATION:

Subject	Speed at $4mmol l^{-1}$ [lactate]	$\%VO_2max$ at $4mmol l^{-1}$ [lactate]
m1	4,04	67,71
m2	4,57	72,04
m3	3,86	70,67
m4	3,67	66,57
m5	3,83	63,81
m6	3,77	68,16
m7	3,76	72,27
m8	4,81	73,09
	4,04	69,29
	±0,42	±3,25
f1	2,84	65,39
f2	2,47	70,65
f3	3,46	72,17
f4	3,97	74,22
f5	4,55	71,85
f6	3,12	75,87
f7	3,24	63,83
f8	3,63	67,64
	3,41	70,20
	±0,65	±4,23

PERCENTAGE MAXIMAL OXYGEN UPTAKE DURING 60 MINUTE RUN:

Subject	15min	30min	45min	60min
m1	78.49	77.45	79.42	78.63
m2	66.05	67.65	67.39	70.12
m3	74.52	76.89	76.67	80.43
m4	67.84	67.92	67.78	69.18
m5	70.50	71.02	72.95	75.98
m6	67.15	67.58	69.36	70.61
m7	68.74	71.98	72.51	70.17
m8	69.74	71.52	74.32	72.45
	70.38	71.50	72.55	73.44
	±4.17	±3.93	±4.26	±4.31
f1	72.15	69.68	72.32	74.71
f2	73.97	71.16	73.22	74.37
f3	71.04	71.37	73.45	72.47
f4	71.92	76.37	78.23	77.14
f5	67.71	62.93	65.28	63.90
f6	74.37	80.23	73.96	77.17
f7	72.86	72.60	72.90	79.53
f8	67.33	69.57	68.28	69.81
	71.42	71.74	72.21	73.64
	±2.64	±5.09	±3.89	±4.96

HEART RATE ($bt min^{-1}$) DURING 60 MINUTE RUN:

Subject	15min	30min	45min	60min
m1	173	185	185	188
m2	166	170	170	174
m3	170	175	178	173
m4	165	163	162	164
m5	155	163	159	163
m6	156	158	163	170
m7	180	172	179	180
m8	162	162	167	165
	166	169	170	172
	±8	±9	±9	±9
f1	162	166	167	169
f2	163	164	167	169
f3	173	178	179	181
f4	159	165	167	167
f5	142	144	142	143
f6	168	170	175	174
f7	156	162	166	169
f8	141	143	139	149
	158	162	163	165
	±11	±12	±14	±13

CARBON DIOXIDE PRODUCTION (mls/kg/min) DURING 60 MINUTE RUN:

Subject	15min	30min	45min	60min
m1	42.480	41.786	42.831	41.760
m2	37.910	37.510	37.366	39.584
m3	37.495	36.914	37.397	41.087
m4	39.989	38.731	39.910	45.225
m5	38.019	37.885	38.965	40.580
m6	40.595	40.726	43.071	43.757
m7	43.883	43.139	43.500	43.630
m8	40.678	40.247	41.967	41.867
	40.131	39.615	40.626	42.187
	±2.283	±2.210	±2.542	±1.876
f1	28.063	27.829	28.670	28.573
f2	27.311	26.538	26.626	26.596
f3	28.392	27.742	27.929	28.671
f4	35.893	36.607	38.245	39.051
f5	37.495	35.020	36.772	36.127
f6	32.614	34.689	32.587	34.324
f7	26.498	26.401	26.291	29.524
f8	31.203	31.890	30.762	30.436
	30.930	30.840	30.985	31.663
	±4.100	±4.201	±4.544	±4.340

OXYGEN UPTAKE ($ml\,kg^{-1}min^{-1}$) DURING 60 MINUTE RUN:

Subject	15min	30min	45min	60min
m1	49,511	48,851	50,095	49,596
m2	44,495	45,577	45,402	47,236
m3	44,008	45,405	45,275	47,444
m4	40,764	40,812	40,724	41,567
m5	45,917	46,258	47,518	49,488
m6	42,419	42,690	43,816	44,604
m7	39,463	41,321	41,627	40,286
m8	46,225	47,405	49,257	48,023
	44,100	44,790	45,464	46,031
	±3,223	±2,897	±3,381	±3,526
f1	35,932	34,699	36,017	37,205
f2	34,267	32,966	33,919	34,451
f3	35,313	35,476	36,508	36,019
f4	43,037	45,702	46,812	46,159
f5	46,347	43,075	44,681	43,737
f6	38,780	41,844	38,564	40,239
f7	36,701	36,566	36,719	40,060
f8	37,685	38,938	38,214	39,070
	38,508	38,658	38,929	39,618
	±4,154	±4,503	±4,475	±3,893

RESPIRATORY EXCHANGE RATIO DURING 60 MINUTE RUN:

Subject	15min	30min	45min	60min
m1	0,858	0,855	0,855	0,842
m2	0,852	0,823	0,823	0,838
M3	0,852	0,813	0,826	0,866
m4	0,981	0,949	0,980	1,088
m5	0,828	0,819	0,820	0,820
m6	0,957	0,954	0,983	0,981
m7	1,112	1,044	1,045	1,083
m8	0,880	0,849	0,852	0,872
	0,915	0,888	0,898	0,924
	±0,096	±0,084	±0,090	±0,111
f1	0,781	0,802	0,796	0,768
f2	0,797	0,805	0,785	0,772
f3	0,804	0,782	0,765	0,796
f4	0,834	0,801	0,817	0,846
f5	0,809	0,813	0,823	0,826
f6	0,841	0,829	0,845	0,853
f7	0,722	0,722	0,716	0,737
f8	0,828	0,819	0,805	0,779
	0,802	0,797	0,794	0,797
	±0,038	±0,033	±0,040	±0,041

BLOOD LACTATE CONCENTRATION ($mmol\,l^{-1}$) DURING 60 MINUTE RUN:

Subject	Rest	15min	30min	45min	60min
m1	0,86	3,01	2,79	2,48	3,01
m2	1,04	2,03	2,75	3,05	3,11
m3	0,98	3,06	2,98	3,03	3,91
m4	0,92	2,98	3,11	3,61	3,71
m5	0,71	2,94	3,07	3,05	3,86
m6	0,97	3,06	2,78	2,99	3,14
m7	1,03	3,34	3,77	3,89	4,02
m8	0,86	2,37	2,87	2,04	2,42
	0,92	2,85	3,02	3,02	3,71
	±0,11	±0,43	±0,33	±0,58	±1,23
f1	0,94	3,22	2,74	2,41	3,13
f2	0,88	2,01	2,71	2,67	3,11
f3	0,68	1,79	2,44	2,21	2,54
f4	0,91	2,25	2,40	2,31	2,79
f5	0,64	2,03	1,46	1,48	1,52
f6	0,90	2,89	2,98	3,06	3,98
f7	0,64	2,18	2,57	2,01	2,48
f8	0,76	1,78	1,39	1,68	1,97
	0,79	2,27	2,34	2,23	2,69
	±0,13	±0,52	±0,59	±0,51	±0,76

PLASMA FREE FATTY ACID & GLYCEROL CONCENTRATIONS BEFORE & AFTER 60 MINUTE RUN:

Subject	[FFA](mmoll^{-1})		Inc	[Glycerol](mmoll^{-1})		Inc
	Pre	Post		Pre	Post	
m1	0.221	0.583	x2.64	0.057	0.522	x9.16
m2	0.480	0.518	x1.08	0.181	0.595	x3.29
m3	0.338	0.478	x1.41	0.096	0.425	x4.43
m4	0.251	0.341	x1.36	0.101	0.371	x3.67
m5	0.182	0.185	x1.02	0.064	0.142	x2.22
m6	0.200	0.244	x1.22	0.028	0.218	x7.79
m7	0.283	0.388	x1.37	0.053	0.597	x11.26
m8	0.248	0.278	x1.12	0.075	0.381	x5.08
	0.275	0.377	1.40	0.082	0.406	5.86
	±0.096	±0.141	±0.52	±0.046	±0.166	±3.19
f1	0.370	0.674	x1.82	0.067	0.661	x9.87
f2	0.202	0.308	x1.52	0.075	0.354	x4.72
f3	0.243	0.542	x2.23	0.088	0.478	x5.43
f4	0.505	0.578	x1.14	0.176	0.466	x2.65
f5	0.381	0.481	x1.26	0.114	0.727	x6.38
f6	0.330	0.398	x1.21	0.114	0.328	x2.88
f7	0.223	0.292	x1.31	0.030	0.281	x9.37
f8	0.438	0.448	x1.02	0.124	0.547	x4.41
	0.337	0.465	1.44	0.099	0.480	5.71
	±0.108	±0.132	±0.40	±0.044	±0.159	±2.71

BLOOD GLUCOSE CONCENTRATION ($mmol\,l^{-1}$) DURING 60 MINUTE RUN:

Subject	Rest	15min	30min	45min	60min
m1	4,16	4,28	4,42	4,49	4,61
m2	4,94	4,77	4,43	4,34	4,34
m3	3,24	3,60	3,98	4,06	4,15
m4	4,60	3,92	3,98	4,01	4,64
m5	3,47	3,24	3,39	3,28	3,30
m6	3,92	3,86	4,42	4,67	4,63
m7	3,62	3,27	3,28	3,28	3,28
m8	4,67	4,07	4,27	4,38	4,58
	4,08	3,88	4,02	4,06	4,19
	±0,62	±0,51	±0,46	±0,53	±0,58
f1	4,26	4,44	4,55	4,63	4,99
f2	4,84	4,97	5,16	5,34	4,41
f3	4,86	4,85	4,62	4,73	4,80
f4	3,58	3,88	4,03	3,99	4,30
f5	4,68	4,50	4,34	4,37	4,41
f6	4,02	4,14	4,82	4,86	4,90
f7	3,22	3,70	3,72	3,77	4,30
f8	4,99	4,86	4,95	4,61	4,59
	4,30	4,42	4,52	4,53	4,59
	±0,65	±0,47	±0,48	±0,50	±0,28

ESTIMATED PERCENTAGE FAT AND CARBOHYDRATE UTILISATION DURING 60 MINUTE RUN:

Subject		15min	30min	45min	65min
m1	Fat	48.085	49.145	49.184	53.732
	CHO	51.914	50.854	50.815	46.267
m2	Fat	50.297	60.416	60.410	55.155
	CHO	49.702	39.583	39.589	44.884
m3	Fat	50.149	63.967	59.306	45.327
	CHO	49.850	36.032	40.693	54.672
m4	Fat	6.340	16.897	6.550	0.000
	CHO	93.659	83.102	93.449	100.000
m5	Fat	58.553	61.779	61.487	61.479
	CHO	41.446	38.220	38.512	38.520
m6	Fat	14.271	15.274	15.624	6.290
	CHO	85.728	84.725	84.375	93.709
m7	Fat	0.000	0.000	0.000	0.000
	CHO	100.000	100.000	100.000	100.000
m8	Fat	40.489	39.845	50.122	43.291
	CHO	59.510	48.717	49.877	56.708
Mean %	Fat	33.523	38.048	37.835	33.159
Mean %	CHO	66.477	61.952	62.165	66.841
	S.E	±22.923	±25.132	±25.949	±26.406

ESTIMATED RATE OF ENERGY EXPENDITURE DURING 60 MINUTE RUN ($kJmin^{-1}$):

Subject	15min	30min	45min	60min
m1	69,739	68,790	70,541	69,654
m2	60,290	61,383	61,163	63,818
m3	71,226	72,924	72,897	76,996
m4	67,857	67,500	67,766	70,627
m5	72,668	73,075	75,087	78,208
m6	62,692	63,045	64,696	66,228
m7	73,556	76,011	76,591	74,665
m8	64,943	66,191	68,812	67,364
	67,871	68,615	69,694	70,945
	±4,825	±5,122	±5,208	±5,221

Subject	15min	30min	45min	60min
f1	39,354	38,166	39,574	40,652
f2	41,612	40,104	41,092	41,635
f3	44,221	44,223	45,356	45,028
f4	54,156	57,164	58,716	58,161
f5	46,440	43,185	44,888	43,979
f6	44,012	47,315	43,804	45,788
f7	49,940	49,761	49,900	54,677
f8	41,753	43,055	42,137	42,866
	45,186	45,371	45,683	46,598
	±4,863	±6,015	±6,128	±6,357

ESTIMATED PERCENTAGE FAT AND CARBOHYDRATE UTILISATION DURING 60 MINUTE RUN:

Subject		15min	30min	45min	65min
f1	Fat	75,320	67,882	69,895	80,138
	CHO	24,679	32,177	30,104	19,861
f2	Fat	69,591	66,759	73,962	78,739
	CHO	30,408	33,240	26,037	21,260
f3	Fat	67,184	75,058	81,163	69,924
	CHO	32,815	24,941	18,836	30,075
f4	Fat	56,773	68,464	62,780	52,592
	CHO	43,227	31,536	37,220	47,408
f5	Fat	65,445	63,893	60,556	59,324
	CHO	34,554	36,106	39,443	40,675
f6	Fat	54,003	60,336	52,700	49,819
	CHO	45,996	39,663	47,299	50,180
f7	Fat	96,826	96,899	98,956	91,311
	CHO	3,173	3,100	1,043	8,688
f8	Fat	58,559	61,865	66,779	76,159
	CHO	41,440	38,134	33,220	23,840
Mean %	Fat	67,962	70,145	70,849	69,750
Mean %	CHO	32,038	29,855	29,151	30,250
	S.E	±13,656	±11,730	±14,263	±14,614

ESTIMATED QUANTITY OF FAT AND CARBOHYDRATE UTILISED DURING 60 MINUTE RUN:

Subject	Fat (ghr^{-1})	CHO (ghr^{-1})	Subject	Fat (ghr^{-1})	CHO (ghr^{-1})
m1	51,63	129,01	f1	45,06	37,15
m2	73,34	94,67	f2	46,30	40,25
m3	62,65	117,56	f3	51,09	42,09
m4	7,95	223,56	f4	53,48	80,24
m5	70,86	103,38	f5	43,32	59,37
m6	12,86	197,36	f6	44,55	37,63
m7	0,00	265,45	f7	75,61	7,21
m8	48,22	126,66	f8	43,56	51,18
	40,94	157,23		50,37	39,78
	±29,60	±63,12		±10,84	±15,14

FLUID BALANCE DURING 60 MINUTE RUN:

Subject	Hct Pre	Hct Post	Hb Pre (gdl^{-1})	Hb Post (gdl^{-1})	Fluid intake (ml)	Weight loss (kg)	%Weight loss	%change plasma volume
m1	46.3	48.2	15.27	15.60	-	1.38	1.97	-5.58
m2	46.3	48.8	16.98	17.35	-	1.37	2.03	-6.68
m3	46.2	45.5	17.57	17.13	15	1.70	2.11	+3.90
m4	44.7	45.0	13.96	14.63	80	1.77	2.20	-5.10
m5	45.5	43.5	14.86	14.67	10	1.68	2.13	+5.01
m6	45.9	47.0	15.97	17.20	-	1.45	2.21	-9.04
m7	46.3	46.0	15.79	15.71	-	1.87	2.13	+1.07
m8	41.3	41.5	14.00	14.26	134	1.53	2.21	-2.16
	45.3	45.7	15.55	15.82		1.59	2.10	-2.32
	±1.7	±2.4	±1.30	±1.26		±0.19	±0.09	±5.16
f1	37.7	38.5	15.27	15.60	123	0.94	1.71	-3.29
f2	38.3	39.0	16.98	17.35	36	0.94	1.54	-4.65
f3	36.2	38.3	17.57	17.13	-	0.75	1.19	-5.18
f4	37.8	39.0	13.96	14.63	-	0.81	1.28	-3.03
f5	38.7	40.8	14.86	14.67	43	0.63	1.26	-14.86
f6	40.2	40.7	15.97	17.20	-	0.77	1.36	-4.40
f7	39.0	38.0	15.79	15.71	-	0.95	1.37	-3.75
f8	42.0	38.3	14.00	14.26	-	0.82	1.49	+5.85
	38.7	39.1	13.06	13.58		0.83	1.40	-4.16
	±1.8	±1.1	±0.45	±0.66		±0.11	±0.17	±5.58

ODIVIDUAL SUBJECT DATA : STUDY 2(a) : "HIGHLY" TRAINED ♀ RUNNERS

PHYSICAL & PHYSIOLOGICAL CHARACTERISTICS OF SUBJECTS:

EUMENORRHOEIC FEMALES:

Subject	Age (yrs)	Height (cm)	Weight (kg)	VO_2max ($mlkg^{-1}min^{-1}$)	HRmax ($btmin^{-1}$)	B.M.I	VO_2max ($mlkg^{-1}LBMmin^{-1}$) (UWW)	(SKF)
EF	27	167.6	57.55	66.16	190	20.6	81.88	93.84
CD	21	172.4	64.26	58.94	178	21.7	75.75	88.23
VL	25	162.6	60.10	59.63	181	22.7	74.66	80.26
CS	21	177.8	66.90	55.56	183	21.2	70.93	76.21
MW	18	164.3	60.65	57.44	185	22.5	75.41	85.86
KS	19	169.2	55.20	55.19	181	19.7	72.88	77.84
LF	18	171.1	56.95	60.42	195	19.4	75.29	83.57
BC	27	174.8	62.90	66.28	192	20.6	75.97	78.24
TD	29	167.8	52.55	62.08	187	18.7	-	-
	23	169.7	59.67	59.51	185	20.8	75.34	83.01
	±4	±4.9	±4.57	±3.34	±5	±1.4	±3.15	±6.05

AMENORRHOEIC FEMALES:

Subject	Age (yrs)	Height (cm)	Weight (kg)	VO_2max ($mlkg^{-1}min^{-1}$)	HRmax ($btmin^{-1}$)	B.M.I	VO_2max ($mlkg^{-1}LBMmin^{-1}$) (UWW)	(SKF)
HT	20	161.8	45.00	67.44	181	17.2	77.42	79.06
AH	27	165.1	49.80	51.70	176	18.3	62.95	69.40
KA	25	171.2	53.15	59.37	181	18.2	69.05	69.68
CB	23	170.7	57.20	58.46	185	19.4	72.09	77.02
CT	17	160.8	44.75	64.71	194	17.3	74.63	75.16
GT	18	160.1	48.90	51.63	182	19.1	-	-
MN	19	160.7	50.15	69.72	190	19.4	82.50	87.04
LR	36	170.8	53.80	53.83	183	18.4	66.12	66.29
CN	27	162.5	46.90	66.53	189	17.8	78.20	82.54
	24	165.0	50.13	60.38	185	18.3	72.50	75.77
	±6	±4.9	±4.22	±7.01	±5	±0.8	±6.26	±7.10

PHYSIQUE AND BODY COMPOSITION OF SUBJECTS:

EUMENORRHOEIC SUBJECTS:

Subject	% fat (UWW)	%fat (SKF)	lean mass (kg)	SKF thickness (mm) B	I	SS	SI	A	C	Th	W/H
EF	19,2	29,5	46,5	8	22	20	10	16	12	18	0,73
CD	22,3	33,2	50,0	12,5	17	25	23	10	10	21	0,76
VL	20,1	25,7	48,0	5,5	19	12	9,5	13	10	23	0,73
CS	21,7	27,1	52,4	10	18	13	10	14	11	22	0,72
MW	24,2	33,1	46,2	13	24,5	25	14,5	24	13	27	0,75
KS	24,3	29,1	41,8	9,5	18	17	14	17	17	22	0,75
LF	19,8	27,7	45,7	8	17	15	13	12	15	16	0,75
BC	20,8	23,1	49,8	6,5	17	10,5	4,5	12	7	22	0,72
	21,6	28,6	47,6	9,1	19,1	17,2	12,3	14,8	11,9	21,4	0,74
	±1,9	±3,5	±3,3	±2,7	±2,8	±5,7	±5,4	±4,4	±3,1	±3,3	±0,02

AMENORRHOEIC SUBJECTS:

Subject	% fat (UWW)	%fat (SKF)	lean mass (kg)	SKF thickness (mm) B	I	SS	SI	A	C	Th	W/H
HT	17,3	14,7	39,2	3	7	5	6	6	2,5	9	0,82
AH	20,1	25,5	40,9	4,5	23	10	8	12	6,5	18	0,73
KA	14,0	14,8	45,7	3	8	4	6	3,5	3,5	11	0,72
CB	20,0	24,1	46,4	5	11,5	15	9,5	9	8	19	0,76
CT	12,7	13,9	38,8	2,5	6,5	5	5,5	3,5	3,5	8	0,78
MN	14,8	19,9	43,7	4	11	8	7,5	7,5	7	13	0,76
LR	17,7	18,8	43,8	4	9,5	7,5	7	4,5	6,5	13	0,75
CN	15,8	19,4	39,9	3,5	17	5,5	3,5	4,5	7	10	0,72
	16,6	18,9	42,3	3,7	11,7	7,5	6,6	6,3	5,6	12,6	0,76
	±2,7	±4,3	±3,0	±0,8	±5,7	±3,6	±1,8	±3,0	±2,1	±4,0	±0,03

PERFORMANCE CHARACTERISTICS OF SUBJECTS:

EUMENORRHOEIC FEMALES:

Subject	"70%" speed (ms^{-1})	VO_2 at: 3.2ms^{-1}	VO_2 at: 4.1ms^{-1}	HR at: 3.2ms^{-1}	HR at: 4.1ms^{-1}
EF	4.10	35.04	47.22	132	159
CD	3.60	34.74	46.60	135	161
VL	3.42	36.18	46.34	147	166
CS	3.35	36.33	46.37	157	180
MW	3.17	40.71	50.29	157	183
KS	3.41	36.28	46.72	152	171
LF	3.77	36.28	45.07	140	164
BC	3.70	35.95	47.38	148	168
TD	3.89	37.33	45.73	133	153
	3.60	36.54	46.86	145	167
	±0.29	±1.73	±1.47	±10	±10

AMENORRHOEIC FEMALES:

Subject	"70%" speed (ms^{-1})	VO_2 at: 3.2ms^{-1}	VO_2 at: 4.1ms^{-1}	HR at: 3.2ms^{-1}	HR at: 4.1ms^{-1}
HT	4.33	31.97	43.98	108	143
AH	3.38	33.17	45.50	137	168
KA	3.61	36.43	47.31	140	170
CB	3.67	33.88	43.77	148	169
CT	3.75	37.87	50.06	143	178
GT	2.96	39.74	48.86	165	181
MN	4.16	31.48	43.58	113	149
LR	3.48	34.10	45.39	152	174
CN	4.31	29.64	40.84	110	143
	3.74	34.25	45.48	135	163
	+0.46	+3.22	+2.87	+19	+14

TRAINING CHARACTERISTICS OF SUBJECTS:

EUMENORRHOEIC FEMALES:

Subject	Distance run per week (km)	No. days train per week	No. weeks per year	Total exercise time per week (min)	No. years training
EF	80	7	50	532	9
CD	67	7	46	368	9
VL	48	7	48	895	12
CS	45	6	46	521	8
MW	56	6	48	258	5
KS	52	6	48	309	6
LF	76	6	48	582	7
BC	48	6	50	521	11
TD	90	6	50	396	4
	62	6	48	487	8
	±16	±1	±2	±189	±3

AMENORRHOEIC FEMALES:

Subject	Distance run per week (km)	No. days train per week	No. weeks per year	Total exercise time per week (min)	No. years running
HT	112	7	50	459	7
AH	68	7	51	320	10
KA	60	7	52	956	12
CB	40	7	50	668	7
CT	72	6	50	317	4
GT	48	7	45	560	4
MN	64	6	48	322	7
LR	40	7	50	383	20
CN	96	7	52	554	13
	67	6	49	504	9
	±24	±1	±1	±211	±5

NUTRITIONAL COMPOSITION OF DIET:

EUMENORRHOEIC FEMALES:

Subject	Energy intake (kcal/day)	Fat (g)	Protein (g)	CHO (g)	Iron (mg)	Zinc (mg)
EF	1920,0	65,9	67,2	272,1	14,9	13,3
CD	3698,4	157,0	126,0	473,9	26,0	18,3
VL	2933,0	87,2	113,5	449,0	19,7	5,4
CS	1686,1	57,0	50,3	258,2	13,0	8,6
MW	2051,9	39,5	88,0	356,5	22,8	14,1
KS	1819,3	47,2	43,6	361,1	12,4	7,0
LF	2757,1	127,5	68,2	312,0	17,8	8,7
BC	2468,9	70,9	113,4	420,9	26,7	17,5
TD	2051,9	118,1	95,5	206,1	15,3	11,7
	2484,7	85,6	85,1	362,2	18,7	11,6
	±673,6	±40,2	±29,4	±75,0	±5,4	±4,6

AMENORRHOEIC FEMALES:

Subject	Energy intake (kcal/day)	Fat (g)	Protein (g)	CHO (g)	Iron (mg)	Zinc (mg)
HT	1670,6	17,0	68,3	332,1	20,0	10,3
AH	1278,4	37,5	38,6	209,1	9,7	5,4
KA	2492,4	59,4	72,4	444,2	18,5	11,9
CB	2560,0	99,7	92,1	343,5	16,5	14,2
CT	1857,9	50,1	62,8	306,0	12,8	9,8
GT	1274,5	37,4	61,9	183,9	18,4	6,7
MN	1636,5	32,1	69,7	285,0	15,7	10,5
LR	2394,6	85,4	110,7	314,0	33,2	13,1
CN	2494,4	59,4	72,4	444,2	18,5	11,9
	1900,0	51,4	70,8	306,8	18,0	10,2
	±492,0	±26,3	±20,4	±77,0	±6,5	±2,8

%VO_2max & TREADMILL SPEEDS AT 2 & 4 mmoll^{-1} BLOOD LACTATE CONCENTRATIONS:

EUMENORRHOEIC FEMALES:

Subject	Speed (ms^{-1})		%VO_2max	
	2mmoll^{-1}	4mmoll^{-1}	2mmoll^{-1}	4mmoll^{-1}
EF	4,27	5,18	74,63	89,13
CD	3,61	4,16	69,02	80,12
VL	3,62	4,12	68,46	77,13
CS	3,50	3,94	71,13	80,27
TD	4,49	5,24	78,94	89,67
MW	3,19	3,70	70,63	79,81
KS	3,48	4,08	71,41	84,17
BC	3,69	3,97	73,80	88,62
LF	4,09	4,23	73,10	85,43
	3,77	4,29	69,01	83,82
	±0,42	±0,54	±9,97	±4,68

AMENORRHOEIC FEMALES:

Subject	Speed (ms^{-1})		%VO_2max	
	2mmoll^{-1}	4mmoll^{-1}	2mmoll^{-1}	4mmoll^{-1}
HT	4,57	5,25	74,52	87,89
AH	2,94	3,70	72,00	84,27
KA	5,09	5,61	80,17	89,97
CB	4,19	4,56	81,24	88,82
CT	4,30	4,72	69,99	78,43
GT	5,21	4,58	71,68	85,13
MN	3,91	4,34	73,17	82,23
LR	3,83	4,18	69,72	76,44
CN	3,51	4,09	70,41	83,87
	3,95	4,56	73,66	84,13
	±0,67	±0,59	±4,29	±4,57

ENERGY BALANCE OF SUBJECTS:

EUMENORRHOEIC FEMALES:

Subject	Distance run ($kmwk^{-1}$)	Pace ($minkm^{-1}$)	Total exercise time ($minwk^{-1}$)	Energy exp. [($kcalday^{-1}$)]	BMR [($kcalday^{-1}$)]	Balance [($kcalday^{-1}$)]
EF	80	4.4	532	912	1361	353
CD	67	4.6	368	647	1427	1624
VL	48	4.9	895	1453	1353	127
CS	45	4.9	521	841	1509	-664
MW	56	4.6	258	417	1369	266
KS	52	4.8	309	437	1361	21
LF	76	4.5	582	858	1377	522
BC	48	4.6	521	931	1452	86
TD	90	4.4	396	683	1311	58
	62	4.6	487	798	1391	+266
	±16	±0.2	±189	±312	±60	±606

Subject	Distance run ($kmwk^{-1}$)	Pace ($minkm^{-1}$)	Total exercise time ($minwk^{-1}$)	Energy exp. [($kcalday^{-1}$)]	BMR [($kcalday^{-1}$)]	Balance [($kcalday^{-1}$)]
HT	112	4.1	459	647	1196	-176
AH	68	4.7	320	412	1262	-396
KA	60	4.6	956	1401	1336	-245
CB	40	4.7	668	989	1377	194
CT	72	4.4	317	472	1188	198
GT	48	4.8	560	677	1229	-631
MN	64	4.1	322	523	1245	-132
LR	40	4.7	383	562	1336	497
CN	96	4.1	554	778	1221	495
	67	4.5	504	718	1266	-22
	±24	±0.3	±211	±310	±68	±391

PERCENTAGE MAXIMAL OXYGEN UPTAKE DURING 60 MINUTE RUN:

EUMENORRHOEIC FEMALES:

Subject	15min	30min	45min	60min
EF	69,04	70,15	71,89	70,24
CD	68,88	70,97	68,65	70,53
VL	69,74	70,89	68,92	69,94
CS	71,49	72,36	72,28	72,30
MW	69,25	70,37	70,41	70,52
KS	73,14	72,89	72,92	73,00
LF	68,71	71,19	69,92	70,31
BC	71,92	72,37	74,40	74,32
TD	69,29	68,96	69,71	69,47
	70,16	71,12	71,01	71,18
	±1,60	±1,25	±1,96	±1,64

AMENORRHOEIC FEMALES:

Subject	15min	30min	45min	60min
HT	70,30	71,77	74,11	74,23
AH	71,02	71,14	73,19	72,43
KA	72,58	72,64	72,32	72,41
CB	71,10	71,40	71,42	73,15
CT	74,03	73,14	73,52	73,47
GT	68,04	68,08	68,12	68,38
MN	67,89	67,45	67,52	67,81
LR	72,42	72,49	71,79	72,86
CN	69,24	69,46	69,87	69,90
	70,73	70,84	71,32	71,62
	±2,09	±2,05	±2,35	±2,33

OXYGEN UPTAKE ($ml kg^{-1} min^{-1}$) DURING 60 MINUTE RUN:

EUMENORRHOEIC FEMALES:

Subject	15min	30min	45min	60min
EF	45,677	46,411	47,562	46,471
CD	40,598	41,830	40,462	41,570
VL	41,586	42,272	41,097	41,705
CS	39,720	40,203	40,159	40,170
MW	39,777	40,421	40,444	40,507
KS	40,366	40,228	40,245	40,289
LF	41,515	43,013	42,246	42,481
BC	43,274	43,545	44,766	44,718
TD	43,015	42,810	43,276	43,127
	41,725	42,304	42,251	42,338
	±1,958	±1,990	±2,544	±2,140

AMENORRHOEIC FEMALES:

Subject	15min	30min	45min	60min
HT	47,410	48,402	49,980	50,061
AH	36,717	37,779	37,839	37,446
KA	43,091	43,126	42,936	42,990
CB	41,565	41,740	41,752	42,763
CT	47,905	47,329	47,575	47,542
GT	35,129	37,762	37,958	37,933
MN	47,333	47,026	47,075	47,277
LR	38,984	39,021	38,645	39,221
CN	46,065	46,212	46,485	46,504
	42,689	43,155	43,361	43,526
	±4,879	±4,277	±4,607	±4,602

HEART RATE ($bt min^{-1}$) DURING 60 MINUTE RUN:

EUMENORRHOEIC FEMALES:

	15min	30min	45min	60min
EF	170	173	175	178
CD	148	152	158	161
VL	155	160	163	171
CS	168	173	178	181
MW	166	169	175	177
KS	174	176	176	178
LF	157	160	162	164
BC	159	165	167	167
TD	154	156	159	164
	161	165	165	171
	±9	±8	±9	±6

AMENORRHOEIC FEMALES:

	15min	30min	45min	60min
HT	152	159	164	164
AH	150	156	158	160
KA	153	156	156	155
CB	161	163	167	172
CT	154	162	167	167
GT	147	153	150	152
MN	151	155	157	160
LR	167	178	184	187
CN	154	161	166	168
	154	160	163	165
	±6	±7	±10	±10

RESPIRATORY EXCHANGE RATIO DURING 60 MINUTE RUN:

EUMENORRHOEIC FEMALES:

	15min	30min	45min	60min
EF	0,887	0,887	0,813	0,828
CD	0,917	0,858	0,894	0,893
VL	0,842	0,814	0,829	0,829
CS	0,850	0,842	0,843	0,860
MW	0,823	0,820	0,818	0,816
KS	0,848	0,825	0,829	0,829
LF	0,872	0,847	0,826	0,821
BC	0,834	0,801	0,817	0,846
TD	0,865	0,854	0,852	0,850
	0,860	0,839	0,836	0,841
	±0,029	±0,026	±0,025	±0,024

AMENORRHOEIC FEMALES:

	15min	30min	45min	60min
HT	0,928	0,894	0,939	0,919
AH	0,851	0,851	0,838	0,831
KA	0,851	0,831	0,821	0,820
CB	0,853	0,855	0,881	0,841
CT	0,763	0,800	0,778	0,786
GT	0,816	0,805	0,805	0,777
MN	0,892	0,886	0,795	0,826
LR	0,915	0,913	0,893	0,908
CN	0,801	0,822	0,801	0,785
	0,852	0,851	0,839	0,833
	±0,057	±0,043	±0,058	±0,055

CARBON DIOXIDE UPTAKE ($ml kg^{-1} min^{-1}$) DURING 60 MINUTE RUN:

EUMENORRHOEIC FEMALES:

Subject	15min	30min	45min	60min
EF	40,515	41,167	38,668	38,478
CD	37,228	35,890	36,146	37,122
VL	35,015	34,409	34,069	34,573
CS	33,762	33,851	33,854	34,546
MW	32,736	33,145	33,083	33,054
KS	34,230	33,188	33,363	33,400
LF	36,201	36,432	34,895	34,877
BC	36,091	34,880	36,574	37,831
TD	37,208	36,560	36,871	36,658
	35,887	35,502	35,280	35,615
	±2,320	±2,486	±1,890	±1,959

AMENORRHOEIC FEMALES:

Subject	15min	30min	45min	60min
HT	43,996	43,271	46,931	46,006
AH	31,246	32,150	31,709	31,118
KA	36,670	35,838	35,250	35,252
CB	35,455	35,688	36,784	35,964
CT	36,522	37,863	37,013	37,368
GT	28,665	30,398	30,398	29,474
MN	42,221	41,665	41,665	39,051
LR	35,670	35,626	35,626	35,613
CN	36,898	37,986	37,986	36,506
	36,375	36,721	36,721	36,261
	±4,740	±4,095	±4,667	±4,718

BLOOD LACTATE CONCENTRATION DURING 60 MINUTE RUN:

EUMENORRHOEIC FEMALES:

	Rest	15min	30min	45min	60min
EF	0,64	1,38	1,40	0,98	1,35
CD	1,14	3,83	3,20	4,41	2,53
VL	0,78	1,54	1,68	2,13	2,19
CS	0,83	2,87	2,84	3,20	3,26
MW	0,84	2,35	2,08	2,05	2,10
KS	0,88	2,71	2,81	2,84	2,72
LF	0,67	2,65	2,20	1,64	1,53
BC	0,91	2,25	2,40	2,31	2,79
TD	0,82	3,22	2,50	1,33	1,97
	0,83	2,53	2,35	2,32	2,27
	±0,15	±0,77	±0,58	±1,05	±0,62

AMENORRHOEIC FEMALES:

	Rest	15min	30min	45min	60min
HT	0,91	1,68	2,15	2,09	2,31
AH	0,99	1,71	1,68	1,96	1,63
KA	0,68	1,68	1,28	1,27	1,48
CB	1,08	2,34	2,55	3,16	3,09
CT	0,73	2,23	1,66	1,62	3,73
GT	0,81	2,43	2,04	2,53	2,10
MN	0,87	1,62	1,78	1,90	2,30
LR	0,73	1,74	3,33	2,19	2,44
CN	0,75	1,53	1,69	1,82	1,80
	0,84	1,88	2,02	2,06	2,32
	±0,13	±0,35	±0,61	±0,54	±0,72

BLOOD GLUCOSE CONCENTRATION DURING 60 MINUTE RUN:

EUMENORRHOEIC FEMALES:

	Rest	15min	30min	45min	60min
EF	3,70	3,53	3,68	3,71	3,77
CD	3,19	3,47	3,77	3,88	4,63
VL	3,52	3,97	4,19	4,25	5,06
CS	4,15	3,71	3,57	3,87	5,09
MW	4,40	4,57	4,98	5,49	5,52
KS	4,37	4,41	4,65	4,85	4,96
LF	3,84	3,54	3,51	4,28	4,97
BC	3,58	3,88	4,03	3,99	4,30
TD	4,56	5,33	5,48	5,30	5,30
	3,92	4,05	4,21	4,40	4,84
	±0,47	±0,62	±0,69	±0,66	±0,54

EUMENORRHOEIC FEMALES:

	Rest	15min	30min	45min	60min
HT	3,37	3,59	3,65	3,88	3,95
AH	4,54	2,89	3,44	3,57	4,21
KA	3,80	4,52	4,77	4,41	4,56
CB	4,12	4,15	4,12	4,37	4,74
CT	4,17	4,17	4,35	4,40	5,02
GT	4,21	4,10	4,23	4,41	4,30
MN	3,75	3,60	3,50	3,54	4,30
LR	3,98	4,01	4,36	4,48	5,01
CN	4,01	4,20	4,25	4,28	4,32
	3,99	3,94	4,07	4,15	4,49
	±0,33	±0,50	±0,45	±0,38	±0,37

PLASMA FFA & GLYCEROL CONCENTRATIONS BEFORE & AFTER 60 MINUTE RUN:

EUMENORRHOEIC FEMALES:

Subject	[FFA](mmol l^{-1})		Inc.	[Glycerol](mmol l^{-1})		Inc.
	Pre	Post		Pre	Post	
EF	0.765	1.363	×1.78	0.104	0.773	×7.43
CD	0.238	0.671	×2.82	0.087	0.286	×3.29
VL	0.396	0.742	×1.87	0.099	0.474	×4.79
CS	0.239	0.807	×3.38	0.091	0.417	×4.58
MW	0.419	0.942	×2.25	0.109	0.887	×8.14
KS	0.428	0.578	×1.35	0.100	0.472	×4.72
LF	0.558	0.921	×1.65	0.095	0.603	×6.35
BC	0.520	0.730	×1.40	0.086	0.638	×7.42
TD	0.718	1.345	×1.87	0.110	0.380	×3.45
	0.476	0.900	×2.04	0.098	0.548	×5.57
	±0.186	±0.281	+0.67	±0.009	±0.194	±1.81

EUMENORRHOEIC FEMALES:

Subject	[FFA](mmol l^{-1})		Inc.	[Glycerol](mmol l^{-1})		Inc.
	Pre	Post		Pre	Post	
HT	0.313	0.335	×1.07	0.031	0.126	×4.06
AH	0.207	0.624	×3.01	0.051	0.317	×6.22
KA	0.156	0.347	×2.22	0.066	0.476	×7.21
CB	0.436	0.605	×1.39	0.041	0.270	×6.59
CT	0.327	0.636	×1.94	0.073	0.347	×4.75
GT	0.402	0.779	×1.94	0.094	0.447	×4.76
MN	0.345	0.876	×2.54	0.047	0.410	×8.72
LR	0.204	0.640	×3.14	0.033	0.301	×9.12
CN	0.353	0.491	×1.39	0.047	0.205	×4.36
	0.305	0.593	×2.07	0.054	0.322	×6.20
	±0.096	±0.179	+0.73	±0.020	±0.113	±1.88

ESTIMATED RATE OF ENERGY EXPENDITURE DURING 60 MINUTE RUN ($kJmin^{-1}$):

EUMENORRHOEIC FEMALES:

Subject	15min	30min	45min	60min
EF	52,716	54,949	54,080	54,988
CD	52,689	53,607	52,252	52,709
VL	49,691	50,281	48,975	49,880
CS	54,333	56,433	54,901	51,212
MW	46,188	46,212	46,364	46,639
KS	46,383	48,345	49,013	49,005
LF	47,299	48,776	48,364	47,559
BC	53,747	56,674	58,240	57,758
TD	45,193	44,842	45,287	45,114
	49,804	51,124	50,831	50,540
	±3,626	±4,435	±4,295	±4,083

AMENORRHOEIC FEMALES:

Subject	15min	30min	45min	60min
HT	43,179	43,745	45,612	45,500
AH	37,451	36,134	38,375	37,494
KA	46,904	47,002	46,324	46,305
CB	49,341	48,635	49,152	52,265
CT	41,937	46,050	44,421	45,061
GT	32,983	32,360	33,149	32,906
MN	43,125	43,065	42,285	43,130
LR	44,827	46,094	43,144	43,224
CN	42,578	41,849	44,794	43,068
	42,481	42,770	43,028	43,217
	±4,859	±5,343	±4,742	±5,467

ESTIMATED PERCENTAGE FAT AND CARBOHYDRATE UTILISATION DURING 60 MINUTE RUN:

EUMENORRHOEIC FEMALES:

Subject		15min	30min	45min	60min
EF	Fat	38,05	37,13	63,79	58,60
	CHO	61,95	62,87	36,21	41,14
CD	Fat	27,69	48,12	35,81	36,01
	CHO	72,31	51,88	64,19	63,99
VL	Fat	53,85	63,55	58,17	58,35
	CHO	46,15	36,45	41,83	41,65
CS	Fat	50,88	53,76	53,37	52,10
	CHO	49,12	46,24	46,63	47,90
MW	Fat	60,43	61,48	62,20	62,82
	CHO	39,57	38,52	37,80	37,18
KS	Fat	51,55	59,81	57,89	58,06
	CHO	48,45	40,19	42,11	41,94
LF	Fat	43,14	51,94	59,23	61,04
	CHO	56,86	48,06	40,77	38,96
BC	Fat	56,45	68,20	62,40	52,26
	CHO	43,55	31,80	37,60	47,74
TD	Fat	45,83	49,54	50,07	50,86
	CHO	54,17	50,46	49,93	49,14
Mean %	Fat	47,54	54,84	55,88	54,46
Mean %	CHO	52,46	45,16	44,12	45,54
	S.E	±10,12	±9,48	±8,72	±8,08

ESTIMATED PERCENTAGE FAT AND CARBOHYDRATE UTILISATION DURING 60 MINUTE RUN:

AMENORRHOEIC FEMALES:

Subject		15min	30min	45min	65min
HT	Fat	23,90	35,65	20,43	26,99
	CHO	76,10	64,35	79,57	73,01
AH	Fat	50,48	52,53	55,08	57,61
	CHO	49,52	47,47	44,92	42,39
KA	Fat	50,56	57,50	61,83	61,19
	CHO	49,44	42,50	38,17	38,81
CB	Fat	49,94	49,00	35,96	62,17
	CHO	50,06	51,00	64,04	37,83
CT	Fat	81,80	68,47	76,62	73,48
	CHO	18,20	31,53	23,38	26,52
GT	Fat	62,68	66,75	66,67	76,75
	CHO	37,32	33,25	33,33	23,25
MN	Fat	36,34	39,25	70,29	59,36
	CHO	63,66	60,75	29,71	40,64
LR	Fat	28,51	29,15	35,69	32,16
	CHO	71,49	70,85	64,31	67,84
CN	Fat	68,18	60,72	68,16	73,75
	CHO	31,82	39,28	31,82	26,25
Mean %	Fat	50,27	51,00	54,53	58,16
Mean %	CHO	49,73	49,00	45,47	41,84
	S.E	±18,82	±13,91	±19,32	±17,67

ESTIMATED QUANTITY OF FAT AND CARBOHYDRATE UTILISED DURING 60 MINUTE RUN:

EUMENORRHOEIC FEMALES:

Subject	Fat (gh^{-1})	CHO (gh^{-1})
EF	41,76	96,66
CD	30,42	117,51
VL	45,31	72,81
CS	44,40	90,81
MW	44,59	62,59
KS	42,72	73,32
LF	40,29	78,16
BC	52,80	80,20
TD	34,50	81,08
	41,87	83,68
	±6,45	±16,14

AMENORRHOEIC FEMALES:

Subject	Fat (gh^{-1})	CHO (gh^{-1})
HT	18,51	115,17
AH	31,41	60,74
KA	41,97	69,54
CB	39,22	87,14
CT	51,81	39,25
GT	34,92	36,85
MN	34,24	73,86
LR	21,63	107,44
CN	45,48	49,02
	35,47	71,00
	±10,73	±28,09

FLUID BALANCE DURING 60 MINUTE RUN:

EUMENORRHOEIC FEMALES:

Subject	Hct		Hb		Fluid	Weight	%Weight	%change
	Pre	Post	Pre	Post	intake	loss	loss	plasma volume
			(gdL^{-1})		(ml)	(kg)		
EF	40,8	40,3	13,56	14,26	302	0,96	1,69	-4,09
CD	44,3	44,5	14,78	14,86	-	1,48	2,20	-0,85
VL	41,6	44,0	14,56	15,99	5	1,13	1,89	-12,71
CS	38,0	39,5	13,46	14,34	180	0,70	1,03	-8,42
MW	41,0	40,5	14,41	14,38	-	0,78	1,26	+1,11
KS	38,8	40,0	12,80	13,20	28	0,90	1,54	-4,95
LF	38,3	40,5	12,98	13,68	-	1,08	1,83	-8,49
BC	37,8	39,0	13,09	13,05	-	1,19	1,89	-1,65
TD	38,8	39,5	13,20	13,68	-	0,95	1,78	-4,60
	39,9	40,9	13,65	14,16		1,02	1,68	-4,96
	±2,2	±2,0	±0,74	±0,92		±0,23	±0,36	±4,33

AMENORRHOEIC FEMALES:

Subject	Hct		Hb		Fluid	Weight	%Weight	%change
	Pre	Post	Pre	Post	intake	loss	loss	plasma volume
			(gdL^{-1})		(ml)	(kg)		
HT	40,5	38,5	15,00	14,16	-	0,61	1,36	+9,54
AH	39,5	39,5	13,27	13,79	-	0,53	1,07	-3,73
KA	38,5	38,6	12,47	12,98	-	0,40	0,75	-4,12
CB	39,0	40,0	12,91	13,35	110	0,90	1,50	-4,89
CT	40,0	40,3	13,34	13,60	-	0,80	1,77	-2,38
GT	37,8	32,5	12,06	11,51	-	0,51	1,04	+13,72
MN	40,5	41,5	13,64	14,49	-	0,93	1,78	-7,42
LR	34,5	35,4	11,58	12,39	-	1,05	1,95	-7,81
CN	39,7	38,9	13,49	13,68	-	0,69	1,49	-0,04
	38,9	38,4	13,09	13,31		0,71	1,41	-0,79
	±1,9	±2,8	±0,99	±0,92		±0,22	±0,40	±7,50

IDIVIDUAL SUBJECT DATA: STUDY 2A: "HIGHLY" TRAINED EUMENORRHOEIC ♀ RUNNERS (FOLLICULAR V LUTEAL PHASE)

HEART RATE ($bt min^{-1}$) DURING 60 MINUTE RUN:

FOLLICULAR PHASE:

	15min	30min	45min	60min
EF	170	173	175	178
CD	148	152	158	161
VL	155	160	163	171
CS	168	173	178	181
MW	166	169	175	177
	161	165	170	174
	±9	±9	±9	±8

LUTEAL PHASE:

EF	167	170	173	177
CD	148	152	156	162
VL	148	160	165	169
CS	158	164	169	171
MW	164	169	174	176
	157	163	167	171
	±9	±7	±7	±6

OXYGEN UPTAKE ($ml kg^{-1} min^{-1}$) DURING 60 MINUTE RUN:

FOLLICULAR PHASE:

Subject	15min	30min	45min	60min
EF	45,677	46,411	47,562	46,471
CD	40,598	41,830	40,462	41,570
VL	41,586	42,272	41,097	41,705
CS	39,720	40,203	40,159	40,170
MW	39,777	40,421	40,444	40,507
	41,472	42,167	41,945	42,085
	±2,470	±2,376	±3,159	±2,450

LUTEAL PHASE:

EF	46,214	46,637	47,202	47,294
CD	39,687	40,131	40,137	40,983
VL	41,226	41,263	41,882	41,810
CS	40,811	40,301	40,910	40,257
MW	39,982	40,511	40,524	40,543
	41,584	41,769	42,131	42,377
	±2,661	±2,661	±2,908	±2,787

CARBON DIOXIDE UPTAKE ($ml\,kg^{-1}min^{-1}$) DURING 60 MINUTE RUN:

FOLLICULAR PHASE:

Subject	15min	30min	45min	60min
EF	40,515	41,167	38,668	38,478
CD	37,228	35,890	36,146	37,122
VL	35,015	34,409	34,069	34,573
CS	33,762	33,851	33,854	34,546
MW	32,736	33,145	33,083	33,054
	35,851	35,692	35,164	35,555
	+3,099	+3,099	+3,099	+3,099

LUTEAL PHASE:

EF	37,387	38,428	38,564	39,916
CD	35,401	37,161	35,602	34,385
VL	34,712	35,527	35,097	36,417
CS	34,689	36,231	36,492	33,666
MW	32,905	32,692	32,338	32,637
KS	35,019	36,008	35,164	35,404
	+1,614	+2,148	+2,262	+2,877

RESPIRATORY EXCHANGE RATIO DURING 60-MINUTE RUN:

FOLLICULAR PHASE:

EF	0,887	0,887	0,813	0,828
CD	0,917	0,858	0,894	0,893
VL	0,842	0,814	0,829	0,829
CS	0,850	0,842	0,843	0,860
MW	0,823	0,820	0,818	0,816
	0,864	0,844	0,839	0,845
	±0,038	±0,030	±0,033	±0,031

LUTEAL PHASE:

EF	0,809	0,824	0,817	0,844
CD	0,892	0,926	0,887	0,839
VL	0,873	0,861	0,838	0,871
CS	0,877	0,899	0,892	0,816
MW	0,810	0,807	0,798	0,805
	0,852	0,863	0,846	0,835
	±0,040	±0,050	±0,042	±0,026

BLOOD LACTATE CONCENTRATION DURING 60 MINUTE RUN:

FOLLICULAR PHASE:

	Rest	15min	30min	45min	60min
EF	0,64	1,38	1,40	0,98	1,35
CD	1,14	3,83	3,20	4,41	2,53
VL	0,78	1,54	1,68	2,13	2,19
CS	0,83	2,87	2,84	3,20	3,26
MW	0,84	2,35	2,08	2,05	2,10
	0,85	2,39	2,24	2,55	2,29
	±0,18	±1,01	±0,76	±1,30	+0,69

LUTEAL PHASE:

EF	0,64	0,96	1,01	1,14	1,26
CD	1,09	2,03	1,98	2,94	2,03
VL	0,62	2,18	1,37	1,38	1,37
CS	0,74	2,79	2,22	2,16	2,09
MW	0,83	2,33	1,72	1,53	1,50
	0,78	2,06	1,66	1,83	1,65
	+0,19	±0,68	±0,48	±0,73	±0,38

BLOOD GLUCOSE CONCENTRATION DURING 60 MINUTE RUN:

FOLLICULAR PHASE:

	Rest	15min	30min	45min	60min
EF	3,70	3,53	3,68	3,71	3,77
CD	3,19	3,47	3,77	3,88	4,63
VL	3,52	3,97	4,19	4,25	5,06
CS	4,15	3,71	3,57	3,87	5,09
MW	4,40	4,57	4,98	5,49	5,52
	3,79	3,85	4,04	4,24	4,81
	±0,49	±0,45	±0,58	±0,73	±0,66

LUTEAL PHASE:

EF	3,94	3,91	4,29	3,94	3,87
CD	3,72	3,51	4,15	4,19	4,38
VL	4,33	4,35	4,33	4,35	4,66
CS	4,04	2,94	3,19	3,59	3,81
MW	4,27	4,42	5,00	5,40	6,37
	4,06	3,83	4,19	4,29	4,62
	±0,25	±0,45	±0,65	±0,68	±1,04

PLASMA FFA & GLYCEROL CONCENTRATIONS BEFORE & AFTER 60 MINUTE RUN:

FOLLICULAR PHASE:

Subject	[FFA](mmol l^{-1}) Pre	Post	Inc.	[Glycerol](mmol l^{-1}) Pre	Post	Inc.
EF	0.765	1.363	×1.78	0.104	0.773	×7.43
CD	0.238	0.671	×2.82	0.087	0.286	×3.29
VL	0.396	0.742	×1.87	0.099	0.474	×4.79
CS	0.239	0.807	×3.38	0.091	0.417	×4.58
MW	0.419	0.942	×2.25	0.109	0.887	×8.14
	0.411	0.905	2.42	0.098	0.536	5.65
	±0.215	±0.275	±0.68	±0.009	±0.238	±2.05

LUTEAL PHASE:

EF	0.734	0.865	×1.18	0.151	0.553	×3.64
CD	0.300	0.693	×2.31	0.084	0.402	×4.79
VL	0.525	1.411	×2.69	0.100	0.517	×5.17
CS	0.238	0.449	×1.89	0.094	0.367	×3.90
MW	0.459	0.976	×2.13	0.105	0.936	×8.91
	0.451	0.879	2.04	0.107	0.555	×5.28
	±0.196	±0.358	±0.56	±0.026	±0.227	±2.12

TRAINING CHARACTERISTICS OF SUBJECTS:

Subject	Distance run per week (km)	No. days run per week	No. weeks per year	Total exercise time per week (min)	No. years running
M1	69	7	50	835	9
M2	74	7	50	782	17
M3	80	6	47	300	5
M4	96	7	50	360	4
M5	80	6	48	300	9
M6	78	6	48	280	9
M7	56	7	50	791	4
M8	88	6	50	290	11
M9	56	7	48	632	10
	75	7	49	508	9
	±13	±1	±1	±246	±4

$\%VO_2max$ & TREADMILL SPEEDS AT 2 & 4 $mmol l^{-1}$ BLOOD LACTATE CONCENTRATIONS:

Subject	Speed at 2$mmol l^{-1}$ [lactate] (ms^{-1})	$\%VO_2max$ at 2$mmol l^{-1}$ [lactate]
M1	4,35	61,04
M2	4,26	68,16
M3	4,71	69,92
M4	5,67	70,14
M5	4,97	71,78
M6	5,12	80,64
M7	4,33	66,37
M8	5,08	69,88
M9	4,18	70,51
	4,74	69,83
	±0,51	±5,16

Subject	Speed at 4$mmol l^{-1}$ [lactate]	$\%VO_2max$ at 4$mmol l^{-1}$ [lactate]
M1	5,15	77,08
M2	4,97	83,80
M3	5,21	79,75
M4	5,36	83,24
M5	4,77	83,97
M6	5,57	84,33
M7	5,24	81,63
M8	6,44	88,34
M9	5,08	82,89
	5,31	82,78
	±0,48	±3,14

INDIVIDUAL SUBJECT DATA : STUDY 2(b) : "HIGHLY" TRAINED ♂ RUNNERS

PHYSIOLOGICAL CHARACTERISTICS OF SUBJECTS:

Subject	Age (yr)	Height (cm)	Weight (kg)	BMI	VO_2max ($mlkg^{-1}min^{-1}$)	HRmax ($btmin^{-1}$)
M1	23	180.1	76.6	23.6	71.31	190
M2	34	167.6	67.6	24.1	67.62	182
M3	25	185.4	64.2	18.7	68.77	182
M4	26	172.2	63.8	21.5	73.69	176
M5	36	167.8	61.0	21.7	65.02	181
M6	24	168.2	61.0	21.6	72.89	188
M7	21	177.5	65.4	20.7	74.76	184
M8	27	183.6	69.7	20.6	74.57	175
M9	27	176.1	70.4	22.7	67.76	176
	27	175.4	66.6	21.7	70.71	181
	±6	±6.8	±5.0	±1.7	±3.53	±5

	Skinfold thicknesses (mm)				% fat (SKF)	lean mass (kg)	VO_2max ($mlkg^{-1}LBM^{-1}$)
	B	T	SS	SI			
M1	2.4	3.9	5.9	4.1	5.60	72.34	75.54
M2	3.0	7.9	7.4	4.2	9.34	61.29	74.59
M3	2.5	4.5	6.8	4.1	6.68	59.91	73.69
M4	2.8	8.3	6.1	4.1	8.71	80.72	80.72
M5	3.1	6.9	8.1	5.3	13.60	75.25	75.25
M6	2.9	5.9	5.9	4.3	8.75	55.66	79.88
M7	2.5	5.9	5.9	4.3	7.12	80.49	80.49
M8	2.6	5.5	6.7	4.2	7.37	80.50	80.50
M9	2.5	3.8	6.7	4.1	6.15	72.20	72.20
	2.7	5.8	6.8	4.4	8.15	76.98	76.98
	±0.3	±1.6	±0.8	±0.4	±2.40	±3.38	±3.38

TREADMILL SPEEDS DURING 60 MINUTE RUN & RUNNING ECONOMY:

Subject	Speed(ms^{-1})	Oxygen uptake ($mlkg^{-1}min^{-1}$) at:	
		$4.10ms^{-1}$	$5.45ms^{-1}$
M1	4.69	43.62	58.60
M2	4.29	44.29	63.41
M3	4.66	41.11	58.12
M4	4.64	43.71	63.08
M5	4.02	44.61	63.87
M6	4.54	45.97	61.33
M7	4.62	42.39	63.78
M8	5.34	36.63	52.84
M9	4.34	44.19	60.52
	4.57	42.95	60.62
	±0.36	±2.74	±3.64

PERCENTAGE MAXIMAL OXYGEN UPTAKE DURING 60 MINUTE RUN:

Subject	15min	30min	45min	60min
M1	68,93	70,65	69,94	70,24
M2	72,41	70,05	73,29	70,53
M3	69,83	69,88	69,89	69,94
M4	72,20	71,20	69,64	70,83
M5	72,93	71,68	73,64	71,00
M6	66,45	68,06	70,00	69,13
M7	68,55	70,13	70,69	71,50
M8	68,97	73,12	70,40	74,32
M9	68,66	69,88	69,92	70,31
	69,88	70,52	70,82	70,87
	±2,17	±1,41	±1,53	±1,46

HEART RATE ($bt min^{-1}$) DURING 60 MINUTE RUN:

Subject	15min	30min	45min	60min
M1	158	158	165	167
M2	155	157	158	159
M3	152	153	155	158
M4	147	149	150	150
M5	151	158	159	160
M6	160	161	164	167
M7	151	153	158	164
M8	147	152	154	160
M9	145	154	155	161
	152	155	170	161
	±5	±4	±9	±5

OXYGEN UPTAKE ($ml kg^{-1} min^{-1}$) DURING 60 MINUTE RUN:

Subject	15min	30min	45min	60min
M1	49,153	50,382	49,875	50,089
M2	48,965	47,365	49,456	47,693
M3	48,021	48,056	48,060	48,101
M4	53,203	52,464	51,319	52,195
M5	47,422	46,606	47,881	46,166
M6	48,432	49,610	51,028	50,391
M7	51,249	52,426	52,846	53,454
M8	51,431	54,524	52,501	55,418
M9	46,521	47,354	47,377	47,641
	49,377	49,869	50,038	50,128
	±2,159	±2,783	±2,018	±3,068

CARBON DIOXIDE PRODUCTION ($ml kg^{-1} min^{-1}$) DURING 60 MINUTE RUN:

Subject	15min	30min	45min	60min
M1	43,322	42,763	42,506	45,964
M2	42,632	42,829	41,851	41,387
M3	42,643	42,962	42,629	42,617
M4	47,888	46,975	47,095	46,823
M5	38,497	37,724	39,167	32,532
M6	39,997	40,407	40,699	42,096
M7	46,111	45,608	47,120	46,612
M8	47,464	47,643	48,037	47,913
M9	39,554	41,918	41,439	42,163
	43,123	43,203	43,394	43,456
	±3,444	±3,154	±3,199	±3,853

RESPIRATORY EXCHANGE RATIO DURING 60 MINUTE RUN:

Subject	15min	30min	45min	60min
M1	0,881	0,849	0,852	0,918
M2	0,871	0,900	0,846	0,868
M3	0,888	0,894	0,887	0,886
M4	0,900	0,890	0,918	0,897
M5	0,812	0,809	0,818	0,810
M6	0,826	0,814	0,798	0,835
M7	0,900	0,870	0,892	0,872
M8	0,923	0,874	0,915	0,865
M9	0,850	0,885	0,875	0,885
	0,872	0,865	0,865	0,871
	±0,037	±0,034	±0,042	±0,032

BLOOD LACTATE CONCENTRATION ($mmol l^{-1}$) DURING 60 MINUTE RUN:

Subject	Rest	15min	30min	45min	60min
M1	0,83	2,80	2,60	2,97	3,05
M2	1,03	2,58	2,56	2,53	1,91
M3	0,85	3,11	2,63	2,28	2,98
M4	0,87	3,04	2,74	2,44	2,56
M5	0,85	3,07	3,02	2,70	1,98
M6	0,69	2,32	2,43	1,68	2,05
M7	0,78	3,61	3,79	3,63	2,55
M8	0,88	2,70	2,53	3,04	2,93
M9	1,03	3,53	3,26	3,13	2,23
	0,87	2,97	2,84	2,71	2,47
	±0,11	±0,42	±0,44	±0,56	±0,45

BLOOD GLUCOSE CONCENTRATION ($mmol l^{-1}$) DURING 60 MINUTE RUN:

Subject	Rest	15min	30min	45min	60min
M1	4.19	4.56	4.72	4.40	4.65
M2	4.31	4.03	4.40	4.57	4.62
M3	4.29	4.29	4.36	3.88	4.61
M4	3.50	3.60	3.64	3.59	3.22
M5	3.90	3.84	4.03	4.17	4.45
M6	3.69	3.24	3.52	3.53	3.45
M7	4.20	3.81	3.95	3.93	3.32
M8	3.90	3.83	4.22	4.26	4.40
M9	3.58	3.92	4.13	4.07	4.46
	3.95	3.90	4.11	4.04	4.13
	±0.31	±0.38	±0.38	±0.35	±0.61

PLASMA FFA & GLYCEROL CONCENTRATIONS BEFORE & AFTER 60 MINUTE RUN:

Subject	[FFA]($mmol l^{-1}$)		Inc.	[Glycerol]($mmol l^{-1}$)		Inc.
	Pre	Post		Pre	Post	
M1	0.283	0.343	×1.21	0.056	0.294	×5.25
M2	0.291	0.496	×1.70	0.093	0.406	×4.37
M3	0.336	0.564	×1.68	0.079	0.529	×6.70
M4	0.276	0.388	×1.41	0.082	0.380	×4.63
M5	0.276	0.500	×2.03	0.101	0.898	×8.98
M6	0.390	0.418	×1.07	0.161	0.774	×4.81
M7	0.300	0.388	×1.29	0.038	0.235	×6.18
M8	0.399	0.433	×1.09	0.067	0.519	×7.75
M9	0.244	0.336	×1.38	0.081	0.317	×3.91
	0.311	0.430	×1.43	0.084	0.484	×5.84
	±0.053	±0.077	+0.32	±0.035	±0.224	±1.70

ESTIMATED RATE OF ENERGY EXPENDITURE DURING 60 MINUTE RUN ($kJ min^{-1}$):

Subject	15min	30min	45min	60min
M1	75.506	76.897	76.140	77.514
M2	66.212	66.341	66.534	66.665
M3	61.866	62.015	61.926	61.975
M4	68.264	67.244	66.087	66.909
M5	56.135	56.147	57.783	55.174
M6	58.544	59.814	61.328	61.048
M7	67.370	68.493	69.354	69.879
M8	72.175	75.771	73.576	71.783
M9	65.223	66.885	66.726	67.256
	65.700	66.623	66.606	66.497
	±6.178	±6.806	±5.862	±6.523

ESTIMATED PERCENTAGE FAT AND CARBOHYDRATE UTILISATION DURING 60 MINUTE RUN:

Subject		15min	30min	45min	65min
M1	Fat	39,976	51,319	50,009	27,515
	CHO	60,024	48,681	49,991	72,484
M2	Fat	43,668	48,511	52,194	56,827
	CHO	56,332	51,489	47,806	43,173
M3	Fat	37,650	35,635	37,986	38,391
	CHO	62,350	64,365	62,014	61,609
M4	Fat	33,209	35,154	27,614	34,525
	CHO	66,791	64,846	72,386	65,475
M5	Fat	65,567	65,210	62,164	79,479
	CHO	34,433	34,790	37,836	20,521
M6	Fat	59,318	63,336	69,369	56,002
	CHO	40,682	36,664	30,631	43,998
M7	Fat	33,660	43,956	36,416	43,195
	CHO	66,340	56,044	63,584	56,805
M8	Fat	25,711	42,630	28,489	49,129
	CHO	74,289	57,370	71,511	50,871
M9	Fat	50,764	38,796	42,293	26,065
	CHO	49,236	61,204	57,707	73,935
Mean %	Fat	43,290	47,172	45,282	45,681
Mean %	CHO	57,710	52,828	54,718	54,718
	S.E	±13,035	±11,092	±14,390	±14,390

ESTIMATED QUANTITY OF FAT AND CARBOHYDRATE UTILISED DURING 60 MINUTE RUN:

Subject	Fat (ghr^{-1})	CHO (ghr^{-1})
M1	50,34	156,35
M2	47,69	142,59
M3	36,16	136,93
M4	34,21	159,51
M5	59,69	63,52
M6	58,13	73,05
M7	42,23	147,16
M8	41,77	168,69
M9	44,10	134,82
	46,04	131,40
	±8,86	±37,47

FLUID BALANCE DURING 60 MINUTE RUN:

Subject	Hct		Hb		Fluid	Weight	%Weight	%change
	Pre	Post	Pre	Post	intake	loss	loss	plasma volume
			(gdl^{-1})		(ml)	(kg)		
M1	40,2	41,4	13,49	14,08	82	1,85	2,42	-6,10
M2	46,4	48,0	15,74	17,54	196	1,70	2,51	-12,95
M3	42,6	42,3	13,72	13,94	385	1,27	1,99	-1,07
M4	42,1	43,8	14,27	15,37	-	1,75	2,76	-9,90
M5	44,4	46,0	14,41	15,19	-	1,15	1,89	-7,82
M6	47,8	49,8	15,59	17,20	-	1,55	2,56	-12,87
M7	41,4	41,8	13,60	14,01	352	1,40	2,14	-3,55
M8	42,8	46,3	14,34	15,33	0	1,65	2,39	-12,20
M9	45,6	46,2	15,22	15,96	130	1,55	2,21	-5,66
	43,7	45,1	14,49	15,41		1,54	2,32	-8,01
	±2,5	±2,9	±0,846	±1,324		±0,23	±0,28	±4,28