THE EFFECTS OF OESTRADIOL ADMINISTRATION ON ENERGY METABOLISM DURING PROLONGED EXERCISE IN AMENORRHEIC ATHLETES

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Dissertation submitted for the degree of
Master of Science

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1996

FTS THESIS
612.044 CLA
30001005131240
Clark, Sally Ann
The effects of oestradiol
administration on energy
metabolism during prolonged

SUMMARY

To delay the onset of fatigue in endurance exercise it is advantageous to prolong muscle glycogen depletion. Oestradiol (the female sex hormone) has been shown to increase muscle glycogen content at rest and increase the availability of fats as a fuel source during exercise therefore delaying the onset of fatigue.

The effects of oestradiol administration on energy metabolism during sub-maximal exercise were examined by administering oestradiol using a transdermal oestradiol therapy system in five amenorrheic athletes. The subjects completed two exercise trials at a workload of 70% VO_2 max for a period of two hours. Prior to each exercise trial they underwent three weeks of either an oestradiol or a placebo treatment. A three-week washout period elapsed before the other treatment was administered. In all trials heart rates and perceived exertion were monitored, respiratory gases were collected, blood samples were taken from a vein in the antecubital space and analysed for metabolites and hormones. Pre and post exercise muscle biopsies were taken in two subjects and were analysed for glycogen and lactate.

Results from this study suggest that moderate elevation of serum oestradiol concentrations in amenorrheic athletes has no effect on substrate utilisation during two hours of sub-maximal exercise. There were no significant differences in heart rates, perceived exertion or respiratory exchange ratios, blood and plasma metabolites including glucose, lactate and free fatty acids; or plasma insulin, cortisol and catecholamines. It therefore appears that raising oestradiol concentrations to those achieved in the present study is not advantageous for an amenorrheic athlete during two hours of sub-maximal exercise.

ACKNOWLEDGMENTS

I would like to acknowledge the support of my principal supervisor, Dr. Iva Martin, for her professional guidance and encouragement. I also acknowledge the support of my co-supervisor Dr. Steve Selig for his assistance throughout my candidature.

I would sincerely like to thank Dr. Mark Febbraio for his valued support and for taking the time to teach me many of the analytical skills necessary to complete this thesis.

Many thanks to Associate Professor Mick Carey, Mr Rod Snow and fellow post-graduate students for their ongoing encouragement and for providing a fun and positive environment to work in.

To Dr Glenn Ward for his expert advice and for taking the time to screen all subjects who participated in the present study.

To Dr. Joe Proietto, Sue Fabris, and Michael Clarke for allowing me to use their respective laboratories and for providing me with their technical assistance.

I would like to thank Steve Fraser, Dr. Stuart Malcolm and Dr. Andrew Garnam for all their help during the exercise trials.

To the subjects who participated in the study I thank you for your time and enthusiasm for without you this research could not be possible.

Finally, to my family and friends who have provided me with the encouragement, love and support whilst completing this thesis.

God grant me the serenity to accept the things I cannot change,
the courage to change the things I can,
and the wisdom to know the difference.

Reinhold Niebuhr

DECLARATION

This thesis is original and previously unpublished work conducted in the Human Performance Laboratory, Department of Physical Education and Recreation at Victoria University of Technology. With the exception of isotope analyses, which were conducted at St Vincent's Hospital, Royal Melbourne Hospital, Western General Hospital and Royal Melbourne Institute of Technology, all analyses were conducted in the Department of Chemistry and Biology, Victoria University of Technology. The invasive procedures were, however, conducted by qualified medical personnel and the catecholamine analysis and muscle analysis was done in collaboration with Dr. Mark Febbraio and Mr Rod Snow respectively. This thesis is the result of work performed by the author.

Sally. A. Clark

PREFACE

The results reported in this dissertation which have been presented at the following scientific metting.

Clark, S.A., Selig, S., Febbraio, M.A., and Martin, I.K. The effect of oestradiol on exercise metabolism in amenorrheic athletes. Proc. 10th. Biennial Conference, Cumberland College of Health Sciences. Lidcome, Australia, pg 42, 1995.

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CHAPTER ONE

INTRODUCTION

In recent years there has been a marked increase in women participating in physical activity for either recreational or competitive purposes. Both volume and intensity of training have increased in female athletes. This has coincided with an increase in the awareness of exercise-related changes in reproductive function of females, prompting researchers to investigate the effect of exercise on the menstrual cycle and the effect of the menstrual cycle on athletic performance. Menstrual dysfunction in athletes has been associated with changes in body composition (Carlberg et al., 1983; Schwartz et al., 1981), menstrual cycle history (Cavanaugh et al., 1989), training regimes (Bullen et al., 1985), dietary practices (Byran Pedersen et al., 1991; Kaiserauer et al., 1989) and altered hormonal status (Baer, 1993; Boyden et al., 1983). From the literature it would appear that there is no single factor which can account for the reproductive disturbances seen amongst some female athletes.

The effect of the menstrual cycle on athletic performance is of interest to exercise physiologists, coaches, and athletes. Physiological parameters including maximal aerobic capacity tests, ratings of perceived exertion, blood lactate concentrations, respiratory exchange ratios (RER), strength tests and various measures of energy metabolism have been determined during different phases of the menstrual cycle. Studies in women which compared the mid-follicular and mid-luteal phases of the menstrual cycle (low and high oestradiol levels respectively) reported improved endurance exercise performance during the mid-luteal phase (Nicklas et al., 1989). The mechanisms that are thought to be responsible for enhanced performance in the luteal phase of the menstrual cycle include increased muscle glycogen levels prior to exercise (Hackney, 1990) and an increase in the availability and oxidation of free

fatty acids (FFA) in the contracting muscle (Hackney et al., 1990). It is well known that the work capacity of the contracting muscle for endurance exercise is improved with increased glycogen availability (Bergstrom et al., 1967) as well as a high level of FFA oxidation (Costill, 1977). For an endurance athlete it would appear more favourable to compete during the luteal phase of the menstrual cycle when oestradiol levels are high.

Amenorrheic athletes are characterised by having chronically low circulating levels of the ovarian hormones oestrogen (primarily oestradiol) and progesterone (Wilmore et al., 1992). In terms of performance, amenorrheic athletes may be at a disadvantage during prolonged exercise events because of a reduced ability to store muscle glycogen prior to exercise, and a reduced capacity for mobilising fats to use for energy during exercise.

The purpose of the present study, therefore was to determine the effect of oestradiol administration on energy metabolism during prolonged exercise in amenorrheic athletes.

It is also known that amenorrheic athletes are at increased risk of suffering from loss of bone density (Drinkwater et al., 1984), causing a higher incidence of stress fractures (Clark et al., 1988) leading to premature osteoporosis (Parker Jones, 1985) and possibly infertility (Shangold and Levine, 1982). Although these issues are not the subject of this study, the present research may encourage female athletes to monitor any changes which occur to their menstrual status, and seek medical advice which would prove to be beneficial for both their long term health and current exercise performances.

1.1. Limitations to the Study

Recruitment of subjects for the present study proved to be a major problem. The criteria to select subjects were rigid, requiring the subjects to have two or less menstrual cycles per year and not to be taking the oral contraceptive pill. Currently, oral contraceptives are commonly prescribed by medical practitioners for amenorrheic athletes because the oestrogenic component is thought to help protect the athlete from stress fractures and premature osteoporosis. Consequently, only five subjects completed the study and that this small sample size severely compromised the statistical power of the study. In addition, of the five subjects, only two subjects agreed to muscle biopsies and therefore the interpretation of muscle metabolites was limited.

CHAPTER TWO

REVIEW OF LITERATURE

2.1. The Control of the Menstrual Cycle

Although a normal menstrual cycle is typically described as being 28 days in duration, Loucks and Horvath, (1985) defined eumenorrhea as normal menstrual cycles of 23-35 days duration, counting from the first day of menses to the first day of the succeeding cycle.

The menstrual cycle is regulated by hormones of the hypothalamic - pituitary - ovarian (HPO) axis and is divided into two phases (Fig. 2.1). The pre-ovulatory phase, in which the follicles develop is called the follicular phase (FP). The first four to six days of the follicular phase coincide with menstruation. Following ovulation, at about day fourteen the ovary enters the luteal phase (LP) which is characterised by the presence of a functional corpus luteum (Wells, 1991).

The timing of ovulation and the total length of the menstrual cycle shows considerable variation between women, and may vary from cycle to cycle in an individual (Loucks, 1990b). The length of the follicular phase appears to be more variable than the length of the luteal phase, thereby influencing the exact timing of ovulation and the total menstrual cycle duration. To accurately determine the duration of a menstrual cycle requires the analysis of frequent blood, urine or saliva samples for gonadotropin and gonadal steroid hormone concentrations (Keizer and Rogol, 1990). A reasonable assessment of menstrual function, however, may also be obtained from basal body temperature measurements (Prior et al., 1990a).

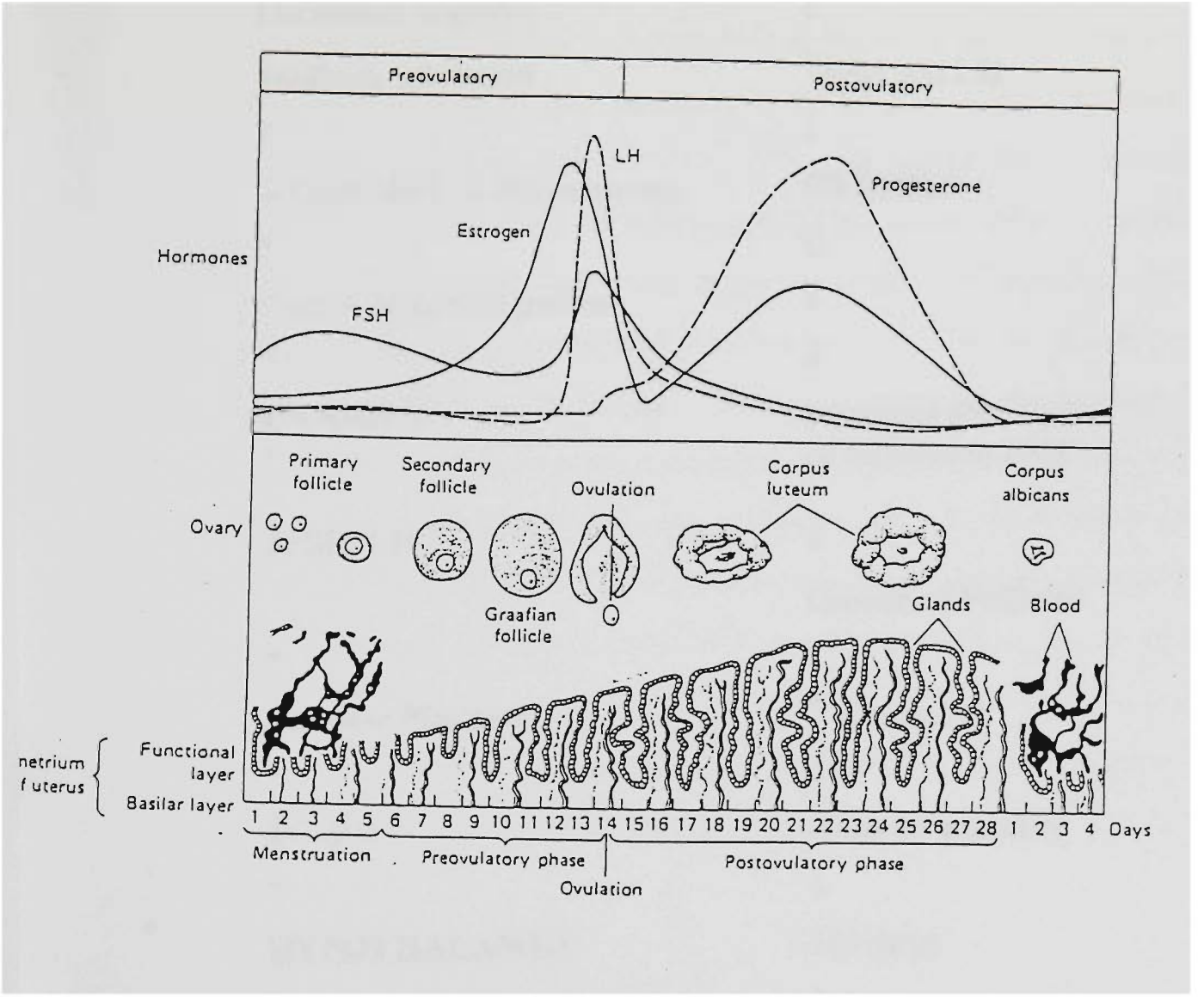

Fig. 2.1 The Menstrual Cycle. Taken from Villie, C.A., Solomon, E.P., and Davis, P.W. Biology, CBS College Publishing, pg 939, 1985.

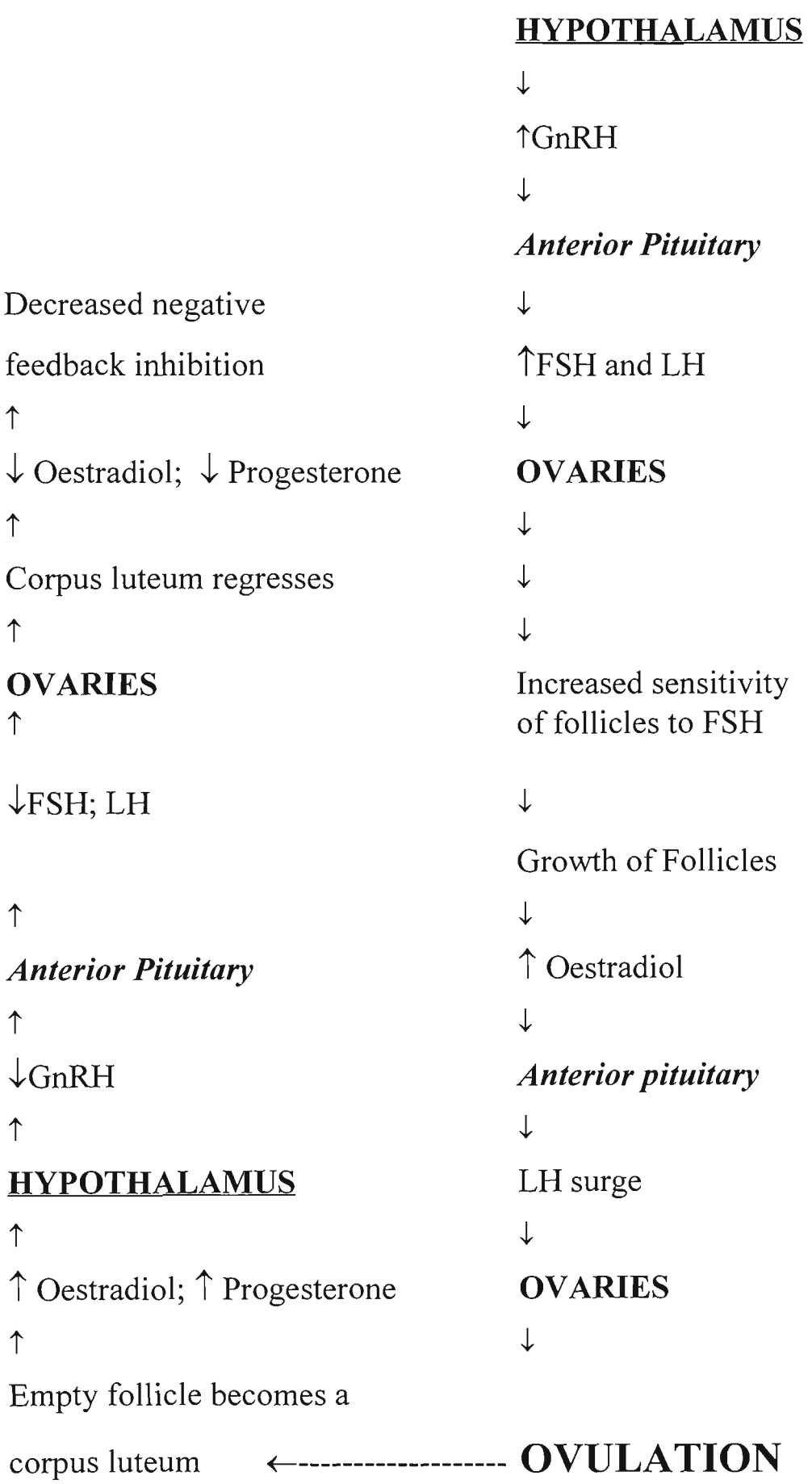

Fig 2.2 The sequence of events in the endocrine control of the ovarian cycle. Taken from Van DeGraff and Fox, pg. 837, 1992.

The changes which take place in the ovary during the menstrual cycle are controlled by complex interactions between the hypothalamus, anterior pituitary gland and the ovaries (Fig 2.2). During the follicular phase of the menstrual cycle, the hypothalamus secretes gonadotropin releasing hormone (GnRH) in a pulsatile fashion. GnRH stimulates the anterior pituitary gland to release luteinising hormone (LH) and follicle stimulating hormone (FSH). FSH and LH are transported via the circulatory system to the ovaries, whereby they stimulate the follicles in the ovary to mature, with only one follicle normally reaching maturity (Wells, 1991). The mature follicle produces oestrogen, primarily beta-oestradiol (Bonen and Keizer, 1984). Prior to ovulation there is a sudden rise in oestradiol, triggering further LH secretion, which superimposes on the cyclic secretions of LH, resulting in an LH surge. The LH surge is responsible for stimulating the release of the ovum (ovulation) and stimulating the portion of follicle that remains in the ovary to develop into the corpus luteum (Villee et al, 1985). The corpus luteum functions as a temporary endocrine gland that secretes both oestrogen and progesterone. The increased levels of oestrogen and progesterone inhibit GnRH pulsations, which decrease FSH and LH secretion so that no further follicles will mature. The corpus luteum begins to degenerate if the ovum is not fertilised and the levels of oestrogen and progesterone fall, stimulating a rise in the gonadotropin hormones (LH and FSH), and resulting in menstruation once again (Villee et al., 1985).

2.2. Exercise and the Menstrual Cycle

2.2.1. Amenorrhea

Amenorrhea is a term used to describe the absence of menstruation. It may be sub-classified into primary and secondary amenorrhea. Primary amenorrhea is the delayed onset of menarche beyond the age of sixteen (Loucks and Horvath, 1985).

Secondary amenorrhea is the cessation of menstruation which occurs after menses have initially been established.

There appears to be no clear agreement among researchers upon the precise definition of secondary amenorrhea (Stager, 1984). The criterion used to assess secondary amenorrhea is a menstrual cycle longer than 90 days (Loucks and Horvath, 1985); however, variations do exist, with some researchers preferring to use the criterion of a menstrual cycle longer than 180 days (Prior and Vigna, 1986). These variations in definitions in the literature are a limitation when interpreting results in which amenorrheic women are subjects. For the purpose of this study, the criterion used for amenorrhea was a menstrual cycle longer than 180 days.

Secondary amenorrhea is often termed athletic amenorrhea because of its relatively high prevalence amongst the athletic population (Carlberg et al., 1983). Several reasons for this occurrence have been examined and it appears that athletic amenorrhea is not induced by exercise alone and that other possible mechanisms may exist, which will be discussed in detail subsequently.

2.2.2. Epidemiology and Aetiology of Changes in Menstrual Cycle Status in Athletes

Determination of the incidence of amenorrhea amongst athletic women has been previously assessed through the use of questionnaires. Shangold and Levine, (1982), distributed a questionnaire to all females who had entered the 1979 New York City marathon and they received 394 responses in total (21% of participants). They reported that 24% of all the respondents had oligomenorrhea/amenorrhea during training, and 19% of all the respondents had experienced these problems prior to training. It is recognised that limitations exist when using questionnaires to obtain information in regards to the incidence of amenorrhea amongst the athletic

population. Information collected via questionnaires is often obtained retrospectively and limitations include the individual's inability to recall information and lack of awareness of changes in menstrual cycle during training (Bonen and Keizer, 1984). Past results obtained from questionnaires however, have established the prevalence of several predisposing factors regarding athletic menstrual cycle irregularity.

The strongest evidence to indicate that amenorrhea in athletes is related to exercise can be found in the work of Bullen et al. (1985). A group of eumenorrheic women who exercised regularly increased their training volume and intensity over a period of two months. Among this group half of the subjects were required to maintain their weight whilst the other half were required to lose weight. The authors reported a significant change in menstrual function in nearly all the subjects. During the two months, fifty three menstrual cycles were analysed and of those, only five were reported to be normal and four of those five were in subjects in the weight maintenance group. In contrast, when increases in exercise intensities were only moderate, changes in menstrual function did not occur (Bonen, 1992). It is also important to note that loss of body weight in conjunction with a large increase in exercise training possibly exacerbates the problem of menstrual cycle alterations.

Changes in body composition is one mechanism which has been held responsible for athletic amenorrhea. Frisch and McArthur, (1974) hypothesised a critical ratio of body fat (17%) is necessary for normal menstrual function to occur, whilst resumption of normal menstrual function following its loss required an increase to 22% body fat. This critical fat ratio hypothesis was developed because it was learnt that adipose tissue was a site of conversion of androgens to oestrogens and for certain oestrogens to other forms of oestrogens. Therefore, if the amount of adipose tissue was decreased, total oestrogen levels would also decrease. The critical fat ratio hypothesis has received criticism from many researchers. It is based on retrospective analysis of observations from growth and development studies (Sinning and Little, 1987) and it

uses the Mellits-Cheek (1970) equation to estimate body fat levels, which has been proven to be inaccurate (Trussell, 1978). Sanborn et al. (1987) compared body fat levels of amenorrheic and eumenorrheic athletes who were matched for somatotype and training load. They reported no difference between the two groups for body fat composition. Bonen and Keizer (1984) postulated that the link between weight loss and secondary amenorrhea which maybe a useful clinical tool but, in actual fact, it is the change in endocrine milieu associated with weight loss rather than fat loss that may stimulate menstrual cycle changes.

The level of maturity of the reproductive system is possibly another factor responsible for maintaining normal menstrual cycle status. A previous pregnancy, early age of menarche and menstrual regularity prior to exercise training are all signs of reproductive maturity and that reproductive maturity is protective against athletic amenorrhea (Loucks and Horvath, 1985). This proposal has been further supported by the relationship between the age of the athlete and the incidence of amenorrhea. Baker et al. (1981b), reported that in a group of runners, 66% of those under the age of 29 (n=12) years were amenorrheic whilst only 9% of women over the age of 30 (n=11) years were amenorrheic. However this study was conducted on a small sample size and may not be representative of the population.

Delayed menarche is considered a sign of reproductive immaturity and has been associated with the incidence of amenorrhea. In a group of 41 cross country runners there was no significant difference between the amenorrheic and eumenorrheic runners for the number of miles run per week, age when training began, skinfold measurements and the number of years they had been training (Wakat et al., 1982). There was however, a difference in the mean age of menarche, with the amenorrheic runners having a higher mean age at menarche. Delayed menarche could possibly represent some sort of endocrine fragility before engaging in heavy training or it could be interpreted as the involvement of an athlete in heavy training prior to puberty

which may continue to affect the endocrine function (Wakat et al., 1982). Evidence for the latter is provided by the relationship between delayed menarche and premenarchal training (Warren, 1980).

Another mechanism thought responsible for athletic amenorrhea may be hypocaloric diets, which has been termed energy drain. Myerson et al. (1991), observed that female athletes did not have a large enough energy intake to match energy output yet they maintained their weight. It is possible that the females under-reported their food intake or, alternatively, their basal metabolic rate (BMR) was reduced, with associated diminished follicular development. A reduced BMR and amenorrhea in runners may be part of an adaptive syndrome to conserve energy and maintain stable weight in response to the caloric demands of a high level training program that is not compensated by an increase in caloric intake (Myerson et al., 1991). This theory was further supported by Graham et al. (1989) who observed a 17% decrease in oxygen consumption at rest among amenorrheic women compared to eumenorrheic women. Amenorrhea in athletes may be an energy conserving strategy similar to that seen in anorexics (Casper et al., 1991).

There has been growing evidence of changes occurring in the menstrual cycle amongst athletes which can not be defined as amenorrhea but may have implications for the health of the female (Prior et al., 1990b). These exercise-related changes in menstrual function include oligomenorrhea (menstrual cycle lasting between 36-90 days), luteal phase deficiency (luteal phase less than 12 days) and anovulation (absence of egg release) (Prior and Vigna, 1986). Luteal phase deficiency has been observed amongst athletic females who regard themselves as having regular menstrual function (Beintins et al., 1991; Loucks et al., 1989). Loucks et al. (1989) observed that regular cyclic females who participated in frequent bouts of aerobic exercise all had shortened luteal phases by two days, with three subjects having luteal phases less

than ten days. The females who had a shortened luteal phase also had lower progesterone levels during the luteal phase and an extended follicular phase. Loucks, (1990a) in her review claimed that the observed extended follicular phase meant that the overall duration of the menstrual cycle appeared normal and therefore symptomatically the subjects appeared indistinguishable from the sedentary women. The health implications for females suffering shortened luteal phases are unknown. Prior et al. (1990b), observed that females training for a marathon did not increase their prevalence of amenorrhea even though the training did bring about menstrual cycle disturbances including anovulatory cycles and shortened luteal phases. These changes in menstrual function were associated with a decreased production of progesterone and a decrease in spinal bone density. Prior et al. (1990b) concluded that inadequate production of progesterone was associated with accelerated bone loss despite normal production of oestradiol and preservation of what appeared as a normal cycle. It is possible that these changes in the menstrual cycle may be the earliest forms of reproductive dysfunction with severe hypo-oestrogenic amenorrhea at the other end of the spectrum of abnormalities (Warren, 1992).

There is still a lack of evidence for the mechanisms responsible for luteal phase deficiency and whether luteal phase deficiency is an adaptation to physical training in the more robust female or is just an intermediate step which may progress to secondary amenorrhea (Loucks, 1990a). This phenomenon further highlights the need to assess and monitor hormonal patterns in women involved in metabolic studies to ensure their menstrual status can be defined accurately.

2.3. Endocrine Mechanisms Responsible for Changes in Menstrual Cycle Function in Athletes

The normal menstrual cycle is dependent on accurate timing of gonadotropin releasing hormone (GnRH) pulses from the hypothalamus. If the GnRH pulse is

disrupted then there is insufficient stimulus for the pituitary gland to release LH and FSH resulting in either irregular or complete cessation of menstruation (Highet, 1989). Amenorrheic athletes have been observed to have reduced concentrations of LH and FSH (Fisher et al., 1986) which is hypothesised to be a result of a decrease in amplitude in GnRH secretion (Fisher et al., 1986) Evidence to support this is the observation that LH concentrations increased in amenorrheic women after a bolus of GnRH was administered (Loucks et al., 1989). If the GnRH pulse is disrupted and LH is inhibited, there may not be adequate stimulus for ovulation to occur. Without ovulation the corpus luteum cannot develop and so levels of oestrogen and progesterone remain at relatively low levels. Chronically low levels of oestradiol have been observed in amenorrheic athletes compared with eumenorrheic athletes (Baker et al., 1981b; Fisher et al., 1986; Wilmore et al., 1992) which is consistent with the observation of decreased LH release amongst amenorrheic women.

The disruption in GnRH activity is thought to be caused by a hypothalamic neurotransmitter, corticotropin - releasing hormone (CRH). CRH is released from the hypothalamus in response to stress and other stimuli, and has been observed to inhibit the release of GnRH from the hypothalamus in rats (Gambacciani et al., 1986). Increased plasma and urinary cortisol concentrations serve as a marker for increased CRH production. Elevated concentrations of plasma and urinary cortisol amongst amenorrheic athlete have been observed at rest (Ding et al., 1988), and during exercise (Kanaley et al., 1992a) compared with eumenorrheic athletes. The increased values for cortisol which are observed amongst amenorrheic athletes are consistent with greater CRH production.

Several groups have observed that GnRH release may also be inhibited by endorphins (Grossman and Sutton 1985; Laatikainen et al., 1986). Endorphins are produced in the pituitary gland in response to CRH stimulation along with adrenocorticotropic hormone (ACTH) and other active peptides (Findling and Tyrell, 1991). This

inhibition of the hypothalamic secretion of GnRH results in decreased levels of LH (Fisher et al., 1986). Laaitikainen et al. (1986) observed higher beta-endorphin levels at rest in amenorrheic women compared with eumenorrheic women. Contrary to these findings, Dixon et al. (1984) observed no changes to LH and FSH secretion amongst amenorrheic runners when they were given naloxone which is the opioid receptor antagonist. If opioids inhibit gonadotropin secretion in amenorrheic athletes, then an increase in gonadotropins in response to naloxone would have been anticipated (Dixon et al., 1984).

Psychological stress has been observed to elevate dopamine or endogenous opiates and other neurotransmitters which have a role in regulating the reproductive system (Loucks and Horvath, 1985). Athletes engaging in competition regularly may exhibit higher psychological stress and thereby contribute to disrupting the menstrual cycle pattern.

2.4. Energy Metabolism During Exercise

The immediate source of energy for muscular contraction is the high energy phosphate, adenosine 5'- triphosphate (ATP). The breakdown of its high energy bond to form adenosine 5'- diphosphate (ADP) and inorganic phosphate (Pi) releases chemical energy that can be used for work by the contracting muscle. The ATP compound is held in a limited amount in the muscle and needs replenishing if muscle contraction is required for exercise lasting more than 2-3 seconds. During muscle contraction many energy transduction pathways exist and contribute to replenishing ATP stores. Muscle cells can produce ATP by any one or combination of three metabolic pathways. The breakdown of another high energy phosphate, creatine phosphate (CP) and the degradation of glycogen to lactate are two of the metabolic

pathways which lead to resynthesis of ATP. Both pathways function under anaerobic conditions. The third metabolic pathway which requires the presence of oxygen is the aerobic pathway. Aerobic production of ATP occurs inside the mitochondria of the cell and it involves the interaction of two pathways called the tricarboxylic acid cycle and the electron transport chain. The energy produced in these two pathways enables ADP and Pi to reform ATP (Powers and Howley, 1990). The relative contribution of each pathway to energy production depends upon numerous factors including intensity and duration of exercise (Vϕllestad and Blom, 1985), individual training state (Hurley et al., 1986), pre-exercise diet (Bergstrom et al., 1967) and hormonal status (Hackney et al., 1990).

The relative amount of carbohydrate to fat utilisation by humans during low and moderate intensity exercise, but not in maximal exercise, can be indirectly determined from ventilatory exchange which calculates the non-nitrogen R-value or respiratory exchange ratio (RER) (Wasserman and Whipp, 1983). RER is the ratio of the volume of expired carbon dioxide to the volume of oxygen taken up by the lungs per unit of time. The oxidation of carbohydrate results in a RER value of 1.0. The oxidation of fats has a RER value of 0.7 because the process requires more oxygen than for carbohydrate (Powers and Howley, 1990). During sub-maximal exercise it is unlikely that the energy provided comes from only one pathway and therefore the exercising RER value would be between 0.7 and 1.

During prolonged, submaximal exercise, the primary sources of energy for the contracting muscle are carbohydrates, fats, and to a lesser extent amino acids (Fig 2.3)

(Hultman and Harris, 1988). Carbohydrates have a finite storage capacity and a limited regeneration capacity during exercise, therefore the depletion of carbohydrate as a fuel source is associated with fatigue (Coyle et al., 1986). Carbohydrate is stored as glycogen in the body in two forms. Glycogen is stored in skeletal muscles in close proximity to the excitation-contraction mechanism so it can be readily broken down by a process called glycogenolysis. Glycogen is also stored in the liver. Liver glycogen is broken down to glucose (glycogenolysis) and transported to the contracting muscle via the blood. In addition, glucose can be formed in the liver by a process called gluconeogenesis which uses non-carbohydrate metabolic precursors such as lactate and glycerol (Felig & Wahren, 1975).

In contrast with carbohydrate, there is a very large supply of free fatty acids (FFA) that can be mobilised during exercise at low to moderate intensity. Fats provide 37 kJ/gm of energy while carbohydrates only provide 16 kJ/gm. However the maximal energy output from lipid metabolism is lower than either carbohydrate or phosphagen degradation (Hargreaves, 1992).

The mechanisms which control the usage of both these substrates will be outlined in the subsequent sections.

2.4.1. Muscle Glycogen Utilisation During Exercise

Muscle glycogen breakdown is controlled by two enzymes, 1) phosphofructokinase (PFK) and 2) glycogen phosphorylase (Hultman and Harris, 1988). Glycogen phosphorylase exists in two enzymatically interconvertible forms, a and b. The b-isomer is active in the presence of AMP, whereas the a-isomer is active in the absence of AMP (Chasiotis et al., 1982). The increase in glycogenolysis in skeletal

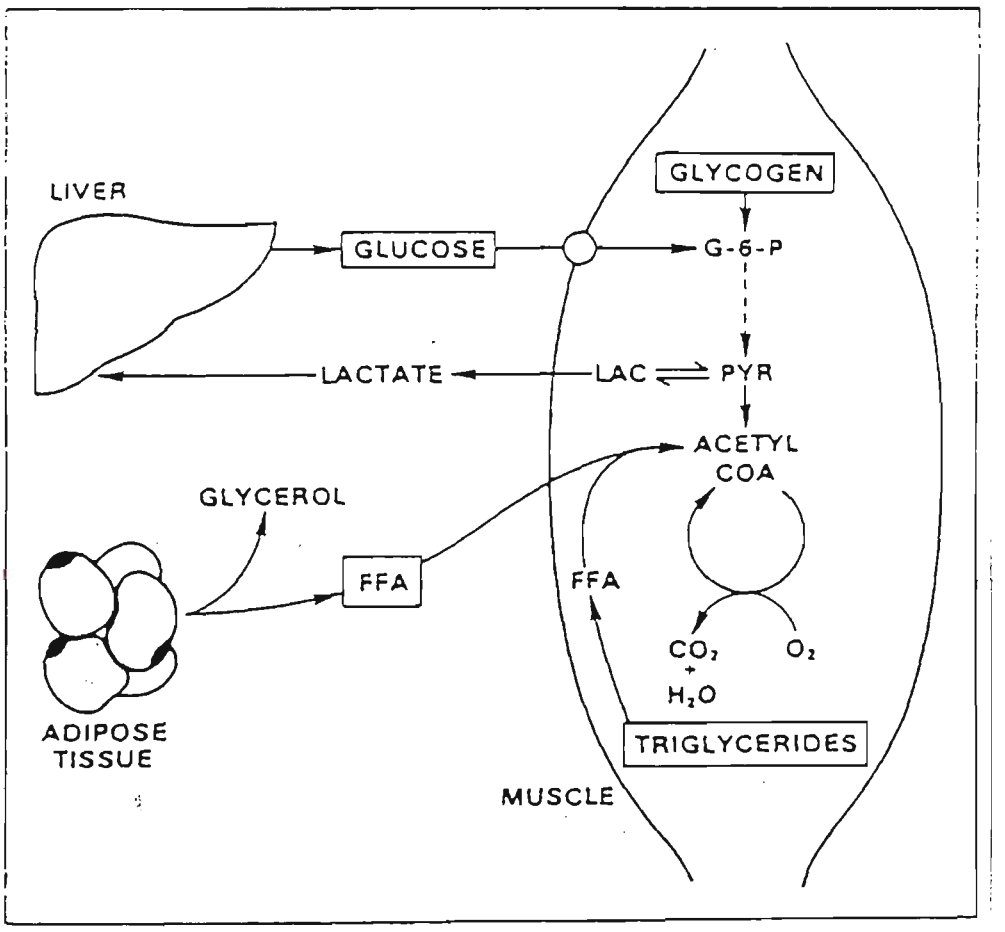

Fig 2.3. A schematic diagram of the muscle cell and its intracellular and extracellular fuel sources. Taken from; Richter, E.A., Ruderman, N.B., and Schneider, S.H. Diabetes and Exercise. The Am. J. of Med. 70:202, 1981.

muscle is initiated by the transformation of phosphorylase b to a and this transformation occurs by hormonal mechanisms mediated by cAMP, by increased [Ca^{2+}] in the sarcoplasm during muscle contraction and by increased levels of inorganic phosphate (Chasiotis et al., 1983).

Hormonal activity, in particular that of adrenaline, is observed to exert a powerful influence on phosphorylase activity (Chasiotis et al., 1983). Adrenaline induces a transformation of phosphorylase b to a in skeletal muscle by a sequence of reactions starting with an increase in activity of adenyl cyclase. The resulting increase in cAMP concentration activates a protein kinase which then catalyses the conversion of phosphorylase b kinase to the active form (Chasiotis et al., 1983). Evidence to support the action of adrenaline on glycogenolysis is the observation that adrenodemedullation reduces muscle glycogen breakdown during moderate exercise (Richter et al., 1981c) and when levels of adrenaline are returned to normal by infusion there is an increase in the rate of glycogenolysis (Richter et al., 1981b). In humans, when adrenaline was infused into the single leg during double leg exercise there was an increase in the rate of glycogenolysis (Jansson et al., 1986). In contrast Chesley et al. (1995) observed no increase in the rate of glycogenolysis when adrenaline was infused in humans during aerobic exercise and hypothesised that allosteric regulators may be more important in controlling the rate of muscle glycogen breakdown and not adrenaline (Lavoie et al., 1992). The differences observed in the literature are probably a result of differences in adrenaline doses, the intensity and type of contractile activity performed.

Another hormone which is observed to influence muscle glycogenolysis to a lesser extent is insulin (Costill et al., 1977). When plasma insulin levels are elevated prior to exercise there was a decrease in blood glucose and plasma FFA levels and a greater reliance on muscle glycogen compared when exercising under fasting conditions, i.e. with low insulin levels (Costill et al., 1977).

The level of glycogen present in muscle before exercise has been observed to alter muscle glycogen utilisation during exercise (Hargreaves et al., 1995; Hespel and Richter 1990; Richter and Galbo 1986). Originally it was thought that muscle glycogen availability could not alter muscle glycogen utilisation because the enzyme controlling glycogenolysis has a very low Michaelis constant (Km of 1-2mM glucose units) for its substrate glycogen and therefore the breakdown of muscle glycogen can not be influenced by pre-exercise levels (Newsholme and Leech, 1983). It has been observed that muscle glycogen is not found in a homogenous solution but rather as particles distributed unevenly with in a cell and therefore, test tube results may have little meaning and the actual Km of phosphorylase for muscle glycogen in vivo may be higher (Richter et al., 1986). More recently, Hargreaves et al. (1995) observed that during 40 minutes of exercise at 65-70% peak pulmonary O_2 uptake when pre-exercise muscle glycogen availability was increased, there was an increase in muscle glycogenolysis. A possible explanation for this, is that the substrate glycogen binds to phosphorylase which increases its catalytic activity, so that as glycogen stores decrease during exercise so does the glycogenolytic rate (Johnson, 1992).

The availability of other substrates, such as blood-borne glucose and FFA, may influence muscle glycogenolysis during exercise (Coyle et al., 1986; Hespel and Richter 1990; Turcotte et al., 1995). The effect of elevated blood glucose concentration on muscle glycogen utilisation has been observed by either ingesting carbohydrate prior to (Coyle et al., 1985) or during exercise (Coyle et al., 1986). When blood borne glucose levels were elevated by ingesting carbohydrate four hours prior to exercise, the rate of muscle glycogenolysis increased (Coyle et al., 1985) which the authors suggested was a result of greater muscle glycogen availability since insulin levels had returned to resting values prior to the commencement of exercise. Glucose ingestion 30-60 minutes prior to exercise is observed to increase blood glucose levels and increase muscle glycogenolysis at the onset of exercise (Costill et al., 1977). The rise in blood glucose just prior to exercise results in an rapid increase in insulin. The release of insulin results in metabolic changes which facilitate the rapid uptake of glucose into the muscle and hence lowers levels of blood glucose which has been observed after the first 10-20minutes of exercise (Costill et al., 1977). Insulin also acts to inhibit FFA availability which therefore would explain the greater reliance on carbohydrate metabolism. The detrimental effect of carbohydrate ingestion prior to exercise has not always been observed, if the increase in insulin is lower, for example after fructose ingestion (Hargreaves et al., 1987).

Ingesting carbohydrates during exercise does not cause a large insulin response and it does not alter the rate of muscle glycogenolysis (Coyle et al., 1986). During prolonged exercise when muscle glycogen levels decrease, there is an increase in ability of muscles to take up blood borne glucose (Hespel and Richter, 1990) and

therefore by ingesting carbohydrates, blood glucose levels are maintained and there is a high rate of carbohydrate oxidation which is observed to increase time to fatigue (Coyle et al., 1986; Coggan and Coyle, 1987).

When levels of FFA are increased by heparin administration there is a decrease in the rate of muscle glycogenolysis (Costill et al., 1977; Hickson et al., 1977) and in contrast, when plasma FFA levels are decreased with nicotinic acid there is an accelerated rate of glycogen degradation during exercise (Bergstrom et al., 1969). It is thought that increasing the rate of FFA oxidation results in a citrate-mediated inhibition of PFK, which causes the accumulation of G-6-P, and therefore inhibits glucose uptake (Rennie and Hollozsy, 1977).

More recently, it has been observed that the accumulation of glucose and G-6-P in the muscle was not associated with decreased muscle glucose uptake when arterial levels of FFA were elevated both at rest and during exercise (Hargreaves et al., 1991). Furthermore, the observation that when muscle glycogen content is decreased during exercise, there is an increase in glucose uptake without any effect on plasma FFA oxidation, suggests that regulation within pathways of carbohydrate metabolism takes precedence over regulation between pathways of lipid and carbohydrate metabolism (Turcotte et al., 1995).

2.4.2. Liver Glycogen Utilisation During Exercise

During exercise of long duration (>2 hours), blood borne glucose becomes an important carbohydrate source when muscle glycogen levels are compromised. Although muscle glycogen is the primary storage form of carbohydrate it lacks the enzyme glucose-6-phosphate and therefore cannot release glucose into the blood stream (Felig and Wahren, 1975). The glucose derived from the liver can be released

into the blood stream and therefore during exercise is an important carbohydrate source. During prolonged exercise there is an increase in the uptake of glucose by the contracting muscle and therefore liver glycogen is broken down to glucose and released into the blood stream to maintain blood glucose levels (Wahren et al., 1971). Glucose production in the liver can either be from the direct breakdown of liver glycogen (glycogenolysis) or by the formation of glucose using other metabolic precursors such as alanine, lactate and pyruvate (gluconeogenesis). The contribution of both these pathways is under hormonal control and alters throughout exercise (Ahlborg et al., 1974; Wahren et al., 1971). After four hours of low intensity exercise (30% VO_2 max) gluconeogenesis contributed 45% of the glucose release from the liver compared with 20-25% at rest and after 40 minutes (Ahlborg et al., 1974). This is in contrast with the earlier work of Wahren et al. (1971) who reported that the contribution of glucose production from gluconeogenesis decreased during exercise, however in this study subjects exercised for only 40 minutes which may explain the difference. The liver has a lower storage capacity for glycogen compared with total skeletal muscle and its ability to maintain blood glucose may be limited when muscles are removing glucose from blood at high rates, and blood glucose levels decrease, resulting in hypoglycaemia (Ahlborg et al., 1974).

2.4.3. Fat utilisation During Exercise

During prolonged exercise lipids are an important source of fuel. The onset of exercise results in a fall in plasma FFA concentration. This results mostly from a temporary imbalance between the rate of FFA uptake by muscle and FFA released by adipose tissue. As exercise continues there is a gradual rise in plasma FFA because of an increase in mobilisation from adipose tissue. The major site of triglyceride (TG) storage is the cytoplasm of adipose cells. Small amounts are also present in the cytoplasm of other cells including skeletal muscle and in the blood (Holloszy, 1990). FFA are taken up by the muscle after being esterified in the sarcoplasmic reticulum to

form TG which are then incorporated into lipid droplets in muscle (Linder et al., 1976; Tan et al., 1977). It was observed that between 70-90% of fatty acids entering muscle cells from circulation are esterified to TG (Linder et al 1976; Tan et al., 1977) with the remaining FFA entering the cell forming a pool of free (non esterified) fatty acids (Terjung et al.,1982; Terjung et al 1983). A combination of the non esterified fatty acids and the FFA released from TG by lipolysis then undergo beta oxidation in the mitochondria to release energy (Oscai et al., 1990).

Lipolysis of triglycerides stored in adipocytes is stimulated by sympathoadrenal factors and low plasma insulin values (Bulow and Madsen, 1986). The decline of plasma insulin during exercise is accompanied by increases in lipolysis, partly as a result of reduced antagonism of the stimulatory actions of catecholamines and glucagon (Martin and Wahren, 1993). Catecholamines stimulate adenylate cyclase and cAMP which activates the hormone sensitive TG lipase, and therefore the subsequent hydrolysis in the adipose tissue (Gorski, 1990). This enables fatty acids to be mobilised in excess of FFA utilisation by muscle therefore resulting in an increase in arterial FFA content. A subsequent increase of FFA concentration in the circulating blood causes an increase in muscle uptake and utilisation of fatty acids (Bulow and Madsen 1986).

It has become evident that another source of lipids besides blood-borne FFA exists with the observation that trained endurance athletes oxidised more fats during exercise compared with the untrained (Hurley et al., 1986). It appears the rate at which muscle oxidises FFA is determined not only by plasma FFA concentration but by the concentration of FFA in the cytoplasm to which the mitochondria are exposed (Hurley et al., 1986).

The regulation of the breakdown of muscle triglyceride is not understood at rest or during exercise (Gorski, 1990). Mobilisation, however, appears to be under both

adrenergic and noradrenergic control. An increase in lactic acid inhibits muscle TG lipolysis, whilst the reduction of carbohydrate during exercise appears to enhance the mobilisation of muscle triglycerides (Gorski, 1990).

2.5. Energy Metabolism and Fatigue

During prolonged exercise at moderate to high intensity, carbohydrates are a major source of fuel for the contracting muscle. At the onset of exercise, muscle glycogen is the primary source of carbohydrates used for energy (Felig and Wahren, 1975). At workloads between 65-85% VO_2 max, muscle fatigue is highly correlated with muscle glycogen depletion (Bergstrom et al., 1967).

The development of the muscle biopsy technique enabled the confirmation of the relationship between endogenous muscle glycogen and the ability to sustain exercise. The relationship between muscle glycogen and exercise performance has been further illustrated by several techniques which manipulate either the pre-exercise levels of glycogen or alter the reliance on muscle glycogen as a fuel source by altering the exogenous fuel sources such as free fatty acids and glucose (Coyle et al., 1986; Coggan and Coyle, 1987; Costill, 1977).

Bergstrom et al. (1967) reported a significant correlation between initial glycogen levels and exercise performance. In their study subjects exercised to exhaustion and then they consume either a i) mixed , ii) high fat and protein, or iii) high carbohydrate diet for three days. After the three days the subjects exercised again until exhaustion. The high carbohydrate diet elevated resting levels of glycogen compared with the other two diet regimes and increased exercise time to exhaustion.

When blood glucose levels are maintained by ingestion of carbohydrates during exercise there is a distinct shift to energy derived from blood glucose and therefore

delaying the use of muscle glycogen (Coyle, 1991). The lowering of blood glucose during the later stages of prolonged exercise results in the inability of skeletal muscle to take up sufficient glucose to offset the reduced muscle glycogen availability (Coyle, 1991).

Why glycogen depletion coincides with fatigue when large amounts of fatty acids are still available is unclear (Felig and Wahren, 1975). One proposed mechanism is that glycogen provides glucose moieties that are subsequently metabolised to pyruvate. The pyruvate is carboxylated to oxaloacetate which is used to maintain the citric acid cycle function. When glycogen is depleted, citric acid cycle intermediates such as oxaloacetate decrease, the processing of acetyl groups from beta oxidation of fatty acids is impaired and therefore there is insufficient formation of ATP. This mechanism has been supported by the findings of reduced levels of citric acid cycle intermediates during exercise to fatigue (Sahlin et al., 1990).

In addition to low glycogen levels, other mechanisms have been proposed to contribute to fatigue during exercise. The inability to sustain force output during prolonged sub-maximal exercise could be caused by any one of a number of processes either involved in the activation of the muscle or in the translation of the neural signal into force generation within the muscle (Green, 1991), and further research is required to examine and understand these mechanisms.

2.6. Sex Steroids and Metabolism

2.6.1. Oestrogen

Oestrogens, particularly oestradiol, play an important role in reproductive function. Differential sex characteristics among males and females, and maintenance of

reproductive organs are the result of differing ratios of oestrogens to androgens. The major oestrogen produced in females is oestradiol (E2) whilst oestrone (E1) and oestriol (E3) have a less potent effect (Bunt, 1990). Oestradiol is responsible for the changes in secondary sexual characteristics that take place at puberty, control of the menstrual cycle and also contributes to the function of other metabolic processes including the modulation of bone reabsorption (Balfour and Heel, 1990). The production rates and blood concentrations vary at different phases of the menstrual cycle (Shangold, 1984). During the early follicular phase of the menstrual cycle, concentrations of oestrogen are low, rising to their highest just prior to ovulation. After ovulation both the hormones oestradiol and progesterone are produced by the corpus luteum and decline at the end of the luteal phase if fertilisation has not occurred (Villee et al., 1985). Oestrogen is secreted by the ovary and a small amount is produced from the adrenals (Bunt, 1990). Muscle and fat tissue are able to convert androgens to oestrogens, specifically oestrone (Keizer et al., 1982). However, oestrone produced by this pathway is negligible compared with that produced by the ovaries. Most of the oestrogens circulating in the blood are bound to proteins including sex steroid binding protein (SBP) and albumin. Only a small fraction (1-3%) is present in the unbound state, and it is this fraction which is thought to be biologically active (Keizer et al., 1982). Basal body temperatures vary between the luteal and follicular phases of the menstrual cycle (Stephenson et al., 1982b) and this may influence the ratio between bound and unbound steroid concentrations (Bonen and Keizer, 1984). Higher body temperature which have been reported during the luteal phase of the menstrual cycle may cause steroids to dissociate from their protein

causing an increase in free fraction and presumably biological activity (Bonen and Keizer, 1984).

Progesterone, another ovarian steroid has been observed to have an antioestrogenic effect (Carrington and Bailey, 1985). It is therefore difficult to compare the menstrual cycles between females because of the variation in progesterone levels altering the ratio of oestrogen to progesterone. For example when oestrogen levels are high during the luteal phase of the menstrual cycle so to are levels of progesterone which may counteract the oestradiol actions (Bunt, 1990).

2.6.2. Metabolic Effects of Oestradiol

The metabolic effects of oestradiol appear to be direct on tissues such as liver, muscle and adipose (Carrington and Bailey,1985; Hackney, 1990; Matute and Kalkhoff, 1973), or indirect by influencing other metabolic hormones such as insulin and glucagon (Mandour et al., 1977; Matute and Kalkhoff, 1973).

The metabolic effects of oestradiol have been well documented through the use of rodent studies (Kendrick and Ellis, 1991; Kendrick et al 1987; Matute and Kalkhoff, 1973; Rooney et al., 1993). In humans, information in regards to the metabolic actions of oestradiol have been observed in studies which compare phases of the menstrual cycle in eumenorrheic women (Hackney et al., 1990; Jurowski et al., 1981; Lavoie et al., 1987 Nicklas et al., 1989) and by observing the effects of synthetic oestradiol in the form of the contraceptive pill (Bemben et al., 1992).

The extent to which oestradiol exerts metabolic effects at rest, and during exercise are outlined in section 2.6.3. and 2.6.4.

2.6.3. Effects of Oestradiol on Muscle Glycogen levels at Rest

Administration of oestradiol in rodents increases glycogen content in the liver (Matute and Kalkhoff, 1973) and skeletal muscle tissue (Carrington and Bailey, 1985) which is thought to occur because of an oestradiol-mediated suppression of hepatic gluconeogenesis whilst promoting liver glycogen deposition (Matute and Kalkhoff, 1973). When oestradiol is administered in combination with progesterone there is also an increase in muscle glycogen (Carrington and Bailey, 1985) and liver glycogen content (Matute and Kalkhoff, 1973) however not to the same magnitude as when oestradiol only is administered (Carrington and Bailey, 1985).

The metabolic effects of oestradiol on resting muscle glycogen content in females was observed by comparing the mid-follicular with the mid-luteal phase of the menstrual cycle when concentrations of oestradiol are low and high respectively. When concentrations of oestradiol were elevated there was an observed increase in resting muscle glycogen content after 36 hours of no strenuous exercise and consumption of a mixed diet (Hackney, 1990). In addition, muscle glycogen repletion during three days recovery after exhaustive exercise was observed to be greater during the mid-luteal phase of the menstrual cycle when oestradiol levels are elevated (Nicklas et al., 1989).

2.6.4 Effects of Oestradiol on Muscle Glycogen and Lipid Utilisation During Exercise

The well documented metabolic effect of oestradiol on muscle glycogen at rest led researchers to investigate metabolic actions of oestradiol during exercise. During prolonged sub-maximal exercise, the administration of oestradiol decreased liver and skeletal muscle glycogenolysis in female ovariectomized (Kendrick et al, 1987) and male (Kendrick and Ellis, 1991) rats by altering the temporal pattern of glycogen depletion (Rooney et al., 1993) despite there being no difference in pre-exercise muscle and myocardial glycogen content (Kendrick and Ellis, 1991). This altered pattern of glycogen depletion is thought to be mediated by a shift towards lipid

metabolism which is supported by increased circulatory levels of FFA (Kendrick and Ellis, 1991) and muscle TG levels (Rooney et al., 1993) at rest, and during prolonged exercise.

Hackney et al. (1990), examined substrate utilisation in women during sixty minutes of exercise at 70% maximal VO_2 and under three different hormone conditions. Subjects were tested in the mid-follicular phase (low oestradiol and progesterone), at ovulation (high oestradiol and low progesterone) and during the mid-luteal phase (high oestradiol and progesterone). The authors reported lower ($P<0.05$) RER values and greater fat oxidation (g/min) during ovulation compared with mid-follicular but not mid-luteal phase, suggesting an increased utilisation of lipids when oestradiol levels are elevated. It is however, not possible to determine whether the rate of muscle glycogenolysis was different between the phases of the menstrual cycle because muscle samples were not obtained.

Lavoie et al. (1987) measured the substrates glucose, lactate, FFA, and glycerol during ninety minutes of exercise at 60% max VO_2 after a carbohydrate deplete diet during the mid-luteal and the mid-follicular phase of the menstrual cycle. The prolonged exercise bout and the carbohydrate deplete diet were designed to reduce liver glycogen content and increase the metabolic demands of the muscle and consequently increase the potential metabolic influences of the sex hormones. When oestradiol levels were higher, plasma glucose levels were lower after 70 and 90 minutes of exercise and when oestradiol and progesterone levels were decreased (during the mid-follicular phase) plasma lactate levels were increased. Lavoie et al. (1987) suggested that lowered liver glycogen stores due to a low carbohydrate diet may have increased gluconeogenesis in the liver and that the ovarian hormones may inhibit that action hence the lowered blood glucose levels during the exercise.

There is conflicting evidence in relation to the menstrual cycle effect on plasma lactate levels. Lactate levels have been reported to be elevated in the follicular phase of the menstrual cycle during exercise (Bullen et al., 1984; Jurowski, 1981), and in recovery (McCracken et al., 1994) whilst others have reported no difference (DeSouza et al., 1990; Lamont, 1986).

The metabolic actions of oestradiol and progesterone during exercise have been further examined by investigating the effect of the oral contraceptive pill. There have been many advances in oral contraceptive formulations which is beyond the scope of this thesis to define, however for further information the reader is referred to the review by Bemben, (1993).

During mild (40% VO_2) and intense (85% VO_2) exercise plasma FFA and blood glucose concentrations were higher amongst subjects on oral contraceptives (OC) compared with the controls. However, it is important to note that in the same study there was no difference in RER values between the two groups and the subjects only exercised for thirty minutes which may not be long enough to demonstrate an greater fat utilisation. Bemben et al., (1992) completed a similar study, however subjects exercised for 90 minutes at 50% VO_2. Calculations from RER values indicated that OC users utilised significantly lower levels of carbohydrate during exercise compared with controls, despite no difference in FFA utilisation. Whether this discrepancy can be accounted for by differences in protein utilisation could not be determined in this study.

During exercise the OC users had a higher growth hormone (GH) response and utilised significantly less carbohydrate compared with controls (Bemben et al. 1992). It has been postulated that OC users demonstrate a greater ability to spare

carbohydrate than controls because of a contra-insulin effect of GH. The carbohydrate sparing mechanism may be a compensatory mechanism for decreased hepatic glucose output as observed in animals (Matute and Kalkhoff, 1973). These studies further highlight that the female sex steroid hormones do exert a strong influence on endocrine and metabolic actions during exercise.

2.6.5. The Effect of Sex Steroid Hormones on Other Hormones Controlling Metabolism

Oestradiol also exerts its influence by altering the levels of other hormones (Matute and Kalkhoff, 1973; Mandour et al., 1977; Sutton et al., 1980). Oestradiol administration has been observed to increase plasma insulin concentration (Matute and Kalkhoff, 1973) and decrease glucagon in relation to insulin (Mandour et al., 1977) and therefore increase the rate of enzymes involved in hepatic lipogenesis (Mandour et al., 1977). Progesterone does not appear to alter the ratio of insulin to glucagon and consequently was observed to have no effect on fasting plasma glucose and triglyceride content (Mandour et al., 1977).

Sutton et al. (1980) studied the effect of exercise during the luteal and follicular phase of the menstrual cycle on plasma catecholamine response in eumenorrheic women. Plasma concentrations of adrenaline were increased during exercise in the follicular phase compared with the luteal phase of the menstrual cycle when levels of oestradiol were low. Plasma concentrations of glucose and lactate were observed to be higher in the follicular phase which is consistent with an increased stimulation of muscle and liver glycogenolysis (Sutton et al., 1980). It is possible that oestradiol may exert a metabolic effect during exercise by altering the catecholamine response.

2.6.6. Summary of the Metabolic Effects of Oestradiol

The metabolic effects of oestradiol have been observed in humans by comparing the different phases of the menstrual cycle and by observing the effects of synthetic oestradiol. Oestradiol has been observed to exert a direct metabolic effect by increasing resting glycogen content in liver of rodents (Matute and Kalkhoff, 1973) and skeletal muscle of humans (Hackney, 1990). It also promotes a shift towards lipid metabolism during exercise (Kendrick and Ellis, 1991). Finally oestradiol has been observed to exert an indirect metabolic effect by altering the activities of other glucoregulatory hormones such as catecholamines, insulin and glucagon.

2.7. Energy Metabolism in the Amenorrheic Female

Substrate metabolism has been investigated in the amenorrheic athlete (De Souza et al., 1990; Kanaley et al., 1992b). In both studies amenorrheic women were compared with eumenorrheic women for hormone and substrate responses during maximal and submaximal exercise. De Souza et al. (1990) reported no difference between amenorrheic and eumenorrheic women for heart rate, plasma lactate, and RER values. The authors concluded that amenorrheic athletes therefore suffer no performance disadvantages as a result of their menstrual status. Similarly, Kanaley et al. (1992b) compared amenorrheic and eumenorrheic women during 90 minutes of exercise at 60% max VO_2. They reported that there was no difference amongst the two groups for substrate utilisation which was determined by indirect calorimetry respiratory exchange ratios (RER). The authors suggested that because the amenorrheic subjects were well trained they may have adapted their use of fuel for energy by utilising more fat. This adaptive mechanism may have masked any effect of the sex hormones. It is worth considering the problems associated with comparing the

two separate groups. When one compares the two phases of the menstrual cycle, the subject acts as her own control. In a comparison of amenorrheic women to eumenorrheic women, there is no control. Consequently further studies are needed to better define the role of changes in sex steroid hormone levels in energy substrate utilisation amongst amenorrheic women.

CHAPTER THREE

METHODOLOGY

3.1. Subjects

3.1.1. Recruitment of Subjects

Subjects were recruited in the present study by a variety of methods. Initially letters were sent to every Victorian athletics, cycling and triathlon club and followed up by a telephone call. A letter was written to the Victorian Institute of Sport requesting assistance and was followed up with a meeting with the executive director. Letters were written to physicians at Prahran, Olympic Park and McKinnon Sports Medicine Clinics requesting the physicians to refer any interested patients that met the criteria. Notices were placed at Victoria University of Technology, Royal Melbourne Institute of Technology, The University of Melbourne and Monash University. In addition notices were placed at leisure facilities such as The State Swimming Centre and Kew Recreation Centre. The study gained media attention and requests for participation were printed in The Age newspaper and announced on radio. Despite the intensive recruitment process only five volunteers finally were recruited to participate in this study.

3.1.2. Selection Criteria

Amenorrhea was defined as a menstrual cycle greater than 180 days without any menstrual bleeding in the three months prior to testing. None of the subjects was taking an oral contraceptive pill or had done so in the eighteen month period prior to testing. All subjects were required to answer a questionnaire regarding menstrual cycle and exercise training histories (Appendix A). In addition, all subjects were

screened by an endocrinologist to ensure that there were no contraindications for oestradiol treatment. A single blood sample was taken for determination of serum oestradiol concentration for each subject (Appendix D).

The author attempted to recruit subjects with a Max VO_2 greater than 45 $ml.kg.min^{-1}$, however, as a result of recruitment problems subjects with a lower VO2 max were recruited if they were partcipating in regular endurance exercise.

3.1.3. Subject Instructions

Prior to participation in the study each subject was informed of the possible risks associated with the study and was required to sign an informed consent form (Appendix A). Subjects were free to withdraw from the study at anytime. The study was approved by the Victoria University Human Research Ethics Committee.

Subjects were required to report to the laboratory on three separate occasions. On their first visit, subjects performed a maximal aerobic capacity test on the cycle ergometer, measurements of skinfolds (Appendix B), and measurements of height and weight. The next two visits to the laboratory, subjects were to report in before 8am after an overnight fast and having refrained from tobacco, alcohol, caffeine and strenuous physical activity for the 24 hours prior to the exercise trial. Subjects were instructed to record their diet and all physical activity for the period of 72 hours prior to each exercise trial. The record was copied and handed back to the subjects to be followed again before subsequent trials in an effort to minimise substrate variation. During the nine weeks of the study the subjects were also instructed to maintain their level of training in terms of volume and intensity. Following the exercise trials subjects who had undergone muscle biopsies were instructed to refrain from cycling or running for 24 hours and swimming for 72 hours to avoid any complications.

3.2. Study Design

The study was of a double blind cross over design with neither the subjects nor the experimenter knowing the treatment order.

The study protocol consisted of two exercise trials. Preceding each trial, the subjects underwent a treatment for a period of three weeks. The first trial occurred at the end of the three week treatment period. Each subject then went through a washout period for another three weeks before starting the second treatment. At the completion of the second three week treatment period the subject completed the second exercise trial. The subjects were randomly allocated to treatments, with some subjects receiving the oestradiol treatment first and others receiving the placebo first. The administration of the oestradiol to the subject involved using a transdermal oestradiol system. A full description of this therapy is outlined in section 3.3.

3.3. Transdermal Oestradiol Therapy System

The transdermal oestradiol delivery system (Trademark Estraderm Ciba Geigy Pharmaceutical Company) is a rate controlled system that delivers oestradiol through the skin and is commonly prescribed for the treatment of menopausal symptoms. The system consists of a thin adhesive patch containing a drug reservoir, where oestradiol is held in ethanolic solution. The two layers surrounding the drug reservoir consist of

1) an adhesive backing layer

2) a rate controlling microporous membrane.

To apply the patch to the skin, the subjects removed the backing and pressed the patch into place. The patch was worn on either the abdomen or buttocks. The oestradiol was delivered at a constant rate to the skin surface to be absorbed into the systemic system for circulation. Currently three sizes of delivery system are available with nominal delivery rates of 0.025, 0.05, and 0.1 $mg.day^{-1}$. The recommended initial dose for the treatment of menopausal symptoms is 0.05$mg.day^{-1}$ for a period of four

weeks (3 weeks on, one week off) with the patches being changed twice a week (Balfour and Heel, 1990). With this information and after consultation with the endocrinologist, G.M.Ward (Personal Communication) the dose of 0.05mg.day^{-1} was chosen for the subjects in the present study. The subjects changed their patch every four days with the last patch applied the day prior to the exercise trial. The placebo patch used in the study consisted of all the components of the oestradiol patch with the exception of the active ingredient of oestradiol. Subjects were advised to rotate the site of application of the patch to prevent skin irritation.

3.4. Measurement of Maximal Aerobic Capacity

3.4.1. Equipment

Maximal oxygen uptake (VO_2 max) tests were conducted on a friction-braked bicycle ergometer (Monark Ergomedic 814E) using open circuit spirometry. Expired air was directed by a Hans - Rudolf valve and plastic tubing into a mixing chamber and through a ventilometer (Pneumoscan 830). Aliquots of the expired air were pumped from the mixing chamber through O_2 (Applied electrochemistry S - 3A) and CO_2 (Applied electrochemistry CD - 3A) analysers. VO_2 and RER were calculated using standard equations (Consolazio et al., 1963). During all maximal aerobic capacity tests the subject's nose was occluded by a nose clip.

3.4.2. VO_2 Max Protocol

All VO_2 max tests consisted of an incremental test to volitional fatigue. The criteria used to determine maximal aerobic capacity were 1) a plateau or decrease in VO_2 with an increase in work 2) RER > 1.1 or 3) volitional fatigue.

Subjects remained stationary for three minutes so resting data could be collected. Subjects then began cycling at 100 watts with the resistance increasing by 50 watts every three minutes for twelve minutes and then by 25 watts for every minute thereafter until volitional fatigue.

3.4.3. Prediction of Submaximal Work Intensities

The relationship between steady state submaximal VO_2 ($ml.kg.min^{-1}$) values and corresponding workloads was determined using a linear regression equation. From these equations a workload which would elicit 70% of VO_2 max was calculated. The criterion for a correlation was set at $R > 0.98$ (workload vs VO_2).

3.5. Sub-Maximal Exercise Testing

At each thirty minute interval during the two hour exercise trial, subjects provided a rating of perceived exertion (RPE) by using the 20 point Borg Scale (Borg, 1982) and expired air was collected over a period of 90 seconds.

Expired air samples were collected in Douglas bags. The fraction of CO_2 and O_2 were subsequently measured on the gas analysers previously mentioned. The volume of expired air was measured using a gas meter (Parkinson Cowan) and calibrated against a Tissot Spirometer. These analyses were used to confirm that the subjects were actually working at 70% Max VO_2.

Heart rate was also measured at thirty minute intervals using a three lead electrocardiograph (ECG). Subjects were allowed *ad libitum* consumption of water during their first trial. The volume consumed was recorded and subject consumed the same quantity during the second trial.

3.6. Collection of Blood and Muscle Samples

Blood samples were collected from an indwelling teflon catheter (Jelco 20G) which was inserted into a vein in the antecubital space. Following the insertion of the catheter the subject remained lying supine for at least fifteen minutes before two resting samples were taken five minutes apart. The catheter was kept patent by filling it with a small amount of 0.9% NaCl after each blood sample collection. Blood was collected every thirty minutes during the two hour exercise trial. A total of 140 ml of blood was collected during the two hour period.

In two of the subjects muscle samples were obtained from the vastus lateralis muscle using 5mm (Stille) needles (Bergstrom, 1962) modified to include suction (Evans et al., 1982). An incision was made approximately 10 cm proximal to the lateral epicondyle of the femur after a local anaesthetic (1% Xylocaine) was injected subcutaneously over the incision site. Two biopsies were taken from the one incision and an effort was made to angle the needle away from the previous biopsy site in order to avoid the influence of acute trauma on the successive biopsy specimen (Costill et al., 1977). Samples were quickly frozen and stored in liquid nitrogen (lN_2) until analysis. The estimated time from the cessation of exercise to freezing in post exercise samples was 20 seconds.

3.7. Blood Analysis

3.7.1. Serum Oestradiol and Progesterone

After sampling 10 ml of blood was placed in a sterile tube and was allowed to clot at room temperature. At the completion of the trial the blood was centrifuged at 1200 rpm and the serum was removed and stored at -80°C. Oestradiol and Progesterone were measured using a standard technique (Ciba Corning ACS Oestradiol competative chemiluminescent immunoassay).

The assays were performed by technical staff at St Vincent's and Western General Hospital.

3.7.2. Glucose

After sampling, approximately 2 ml of blood were placed in a fluoride heparin tube, mixed, placed on ice and centrifuged at 4°C for ten minutes at 4000 rpm. The plasma was transferred to another tube and stored at - 80°C until analysis using an automated glucose analyser employing the glucose oxidase method (YSI model 23AM).

3.7.3. Blood and Plasma Lactate

Blood was placed in a lithium heparin (anticoagulant) tube and mixed. A 500µl aliquot of blood was transferred to a tube containing 1 ml of 3M perchloric acid (PCA), mixed, place on ice and spun in a centrifuge at 4000 rpm for 10 minutes. The supernatant was transferred to another tube and was stored at -80° C until analysis. Anticoagulated blood was centrifuged and a 500 µl aliquot of the plasma was transferred into a tube containing 1 ml of 3M PCA and treated as for blood lactate. Plasma and blood lactate analysis was performed on a spectrophotometer (Shimadzu UV - 120) using an enzymatic technique (Appendix C) (Lowry & Passonneau, 1972).

3.7.4. Cortisol and Insulin

Plasma was separated from anticoagulated blood by centrifugation and stored at - 80°C until analysis.

Plasma cortisol was determined by Radioimmunoassay as prescribed in the ICN Biomedical Immuchemistry Cortisol Coated tube ^{125}I Radioimmunoassy Kit (Cat No. 60380-03).

Plasma insulin concentration was determined by radioimmunoassay as prescribed in the Incstar Insulin ^{125}I Radioimmunoassay Kit (Cat. No. 06130). Each sample was counted for one minute on a gamma counter (LKB Wallac 1277 Gamma Master).

3.7.5. Catecholamines and Free Fatty Acids

A preservative was prepared by dissolving 2.25g of ethleneglycol - bis-(betaaminoethylether)N, N '- tetraacetic acid (EGTA) and 1.5g reduced glutathione (GSH) in 25ml of normal saline (0.9% sodium chloride w/v) and adjusted to 6-7.4 pH with 5-10M NaOH. Aliquots of this preservative (20µl per 1ml of whole blood) were placed in tubes and stored on ice. The appropriate volume of blood was added to the tubes, mixed gently, and centrifuged at 4°C for fifteen minutes at 1500rpm (900g). The supernatant was transferred to another tube and stored at -80°C until analysis. Samples were analysed for plasma catecholamines by a modification of the single isotope [^{3}H] radioenzymatic assay of Passon & Peuler, (1973) as described in the Amersham Catecholamine research assay system (code TRK 995). Each sample was counted for ten minutes on a beta counter (Packard Tri-Carb 4000).

Free fatty acids (FFA) were determined by a modification of the enzymatic colorimetric method for determination of non-esterified acids (NEFAC) of Miles et al. (1983) as described in the Wako NEFAC kit (code no. 990-75401).

3.8. Muscle Sample Analysis

The portion of muscle sample collected was freeze dried for 48 hours, weighed, powdered and portioned out for glycogen and lactate analysis. The first portion (approximately 2 mg) was used for the analysis of lactate using standard enzymatic techniques with flurometric detection. The second portion (approximately 1mg) was used for the measurement of glycogen.

3.8.1. Lactate Determination

A portion of powdered muscle was extracted according to the procedure of Harris et al. (1974). The tubes containing the samples were placed on ice, and 250µl of ice cold 0.5M PCA/1mM ethlenediaminetetra-acetic acid (EDTA). The tubes were periodically mixed on a vortex for a period of 10 minutes. The samples were then spun in a centrifuge (Heraeus Sepatech Biofuge 28RS) at 28,000 RPM (51,000 g) at 0°C for two minutes. After centrifugation, 200µl of supernatant was transferred to a pre-cooled, Eppendorf tube. The extract was neutralised with 50µl of 2.1M ice-cold potassium bicarbonate ($KHCO_3$), mixed and kept on ice for five minutes to ensure complete precipitation of perchlorate ions. The samples were then mixed again and spun in a centrifuge as described before. The supernatant was transferred to another eppendorf tube and was analysed immediately.

Lactate was analysed using standard enzymatic techniques with fluorometric detection (Turner Model 112) according to the procedure of Lowry and Passonneau (1972). (Appendix C).

3.8.2. Glycogen Determination

The portions of powdered muscle were hydrolysed in 250 µl of 2M hydrochloric acid (HCl) at 100°C for two hours with periodical agitation. The samples were subsequently neutralised by addition of 750µl 0.67M sodium hydroxide (NaOH) before analysis. The extract was then analysed for glucose according to the procedure of Passonneau and Lavderdale (1974), (Appendix C).

3.9. Statistical Analyses

Two way (time by treatment) analysis of variance with repeated measures (ANOVA) was used to compare data between trials. When analysis revealed a significant interaction, simple main effects and Newman Keuls post hoc analyses were used to locate differences. A BMPD computer software program was used to compute these statistics. Student t-tests was also used for paired comparisons. The level of probability to reject the null hypothesis was set at $P<0.05$. All data are reported as means ± the standard error of the mean (SE).

CHAPTER FOUR

RESULTS

4.1. Subjects

The physical characteristics of the subjects are presented in Table 4.1 and include the menstrual cycle characteristics. The subjects consisted of three triathletes, one rower and one ballet dancer. Two of the five subjects were involved in physical training prior to menarche, whilst the other three subjects began physical training after menarche. The five subjects reported menstrual irregularities after either changing their physical training regime, or substantially increasing the number of hours spent training. The subjects that had previously been on the contraceptive pill had stopped taking the oral contraceptive pill for at least two years prior to the commencement of the study. At the time of the subjects' involvement in the study, the subjects spent a mean time of 16.8 ± 3.0 hours in training per week.

4.2. Treatment

4.2.1. Oestrogen and Progesterone

Resting concentrations of oestradiol and progesterone are presented in Fig. 4.1. Resting mean concentrations of oestradiol were higher ($P<0.05$) for all subjects after the three week period wearing the treatment patch compared with the control patch (114 ± 17 vs 81 ± 6.7 $pmol.l^{-1}$). Resting concentrations of progesterone were not statistically different between the TRE (1.46 ± 0.89) and CON (1.04 ± 0.33 $pmol.l^{-1}$). When oestradiol and progesterone are expressed as a ratio (E_2 : Pg) there was no difference ($P=0.06$) between the TRE and CON treatments.

Physical Characteristic	**Mean ± S.D.**	**Range**
Age (yr)	24.6 ± 3.9	20 - 30
Age at menarche (yrs)	13.6 ± 1.1	13 - 15
No. of menstrual cycles per year	1.8 ± 1.6	0 - 3
Training hours per week (hrs)	16.8 ± 6.7	15 - 25
Height (cm)	174.6 ± 6.3	174 - 185
Weight (kg)	61.42 ± 8.0	49 - 71
Skinfolds* (mm)	70.5 ± 7.2	69.6 - 77.9
BMI ($kg.m^{-1}$)	20.2 ± 1.1	19-21.5
VO_2 max ($ml.kg^{-1}.min^{-1}$)	48.43 ± 1.6	35.87 - 56.12
VO_2 max ($l.min^{-1}$)	3.01 ± 0.6	1.75 - 3.37

Table 4.1. Physical characteristics, menstrual history and training volume of the study subjects (n=5).

* Sum of seven sites (See Appendix B)

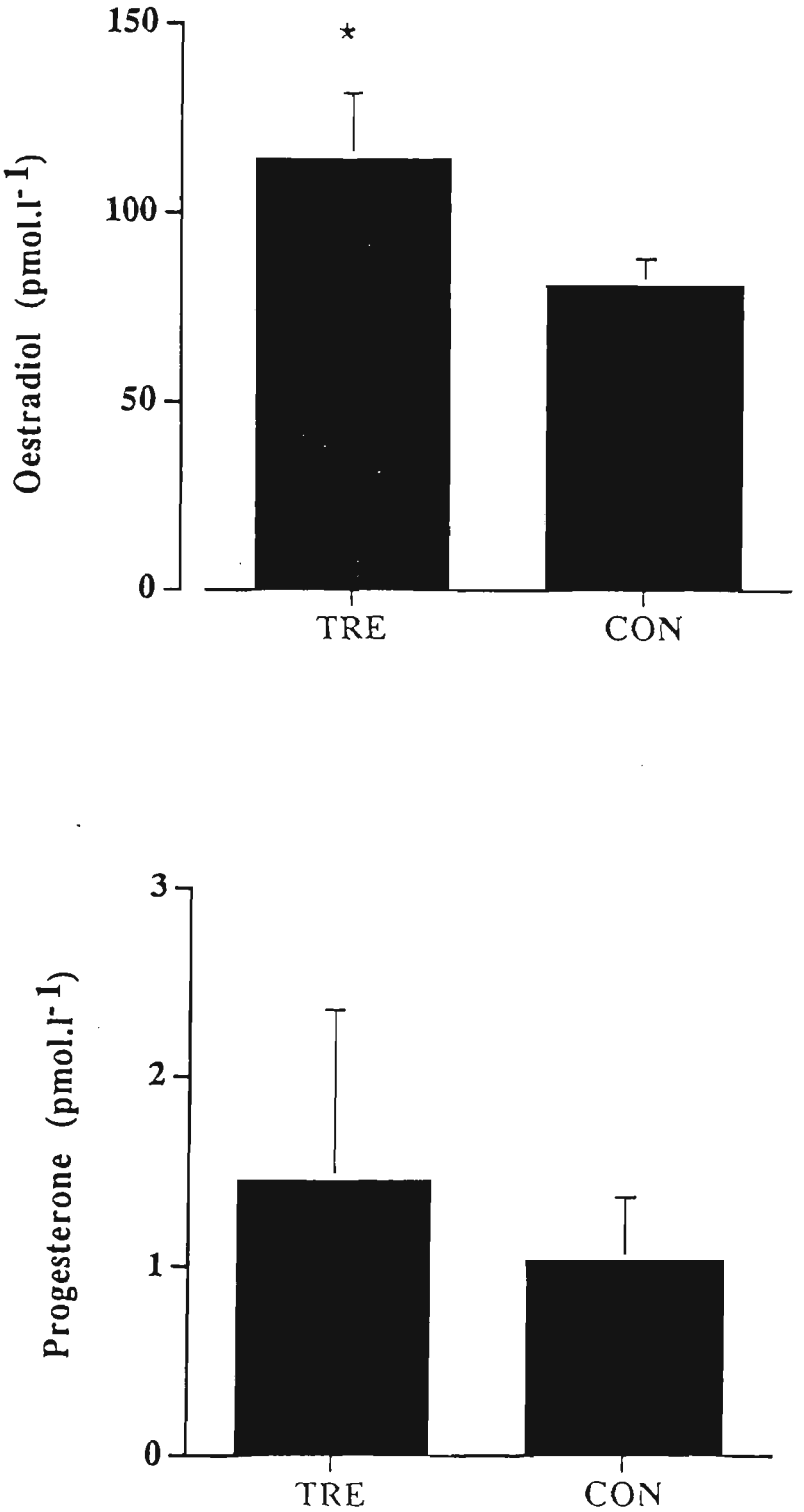

Fig. 4.1. Serum oestradiol and progesterone concentrations at rest under treatment (TRE) and control (CON) conditions Values are means ± SE, (n=5) .

* indicates difference at ($P<0.05$).

4.3. Parameters of Exercise Intensity

4.3.1. Heart Rate

The exercise heart rates are presented in Fig. 4.2. The heart rate values tended to increase (P=0.05) during the two hours of exercise. There was however, no difference in heart rate response between TRE and CON conditions.

4.3.2. Rating of Perceived Exertion (RPE)

Ratings of perceived exertion (Borg Scale, see appendix B) are presented in Fig. 4.3. There was no difference in RPE either between treatments or with respect to time.

4.3.3. Respiratory Exchange Ratio (RER)

The values for respiratory exchange ratio (RER) are presented in Fig. 4.4. There was no difference for RER values between the TRE and CON treatments. RER values tended to decrease (P=0.06) during the two hours of exercise however this was not significant.

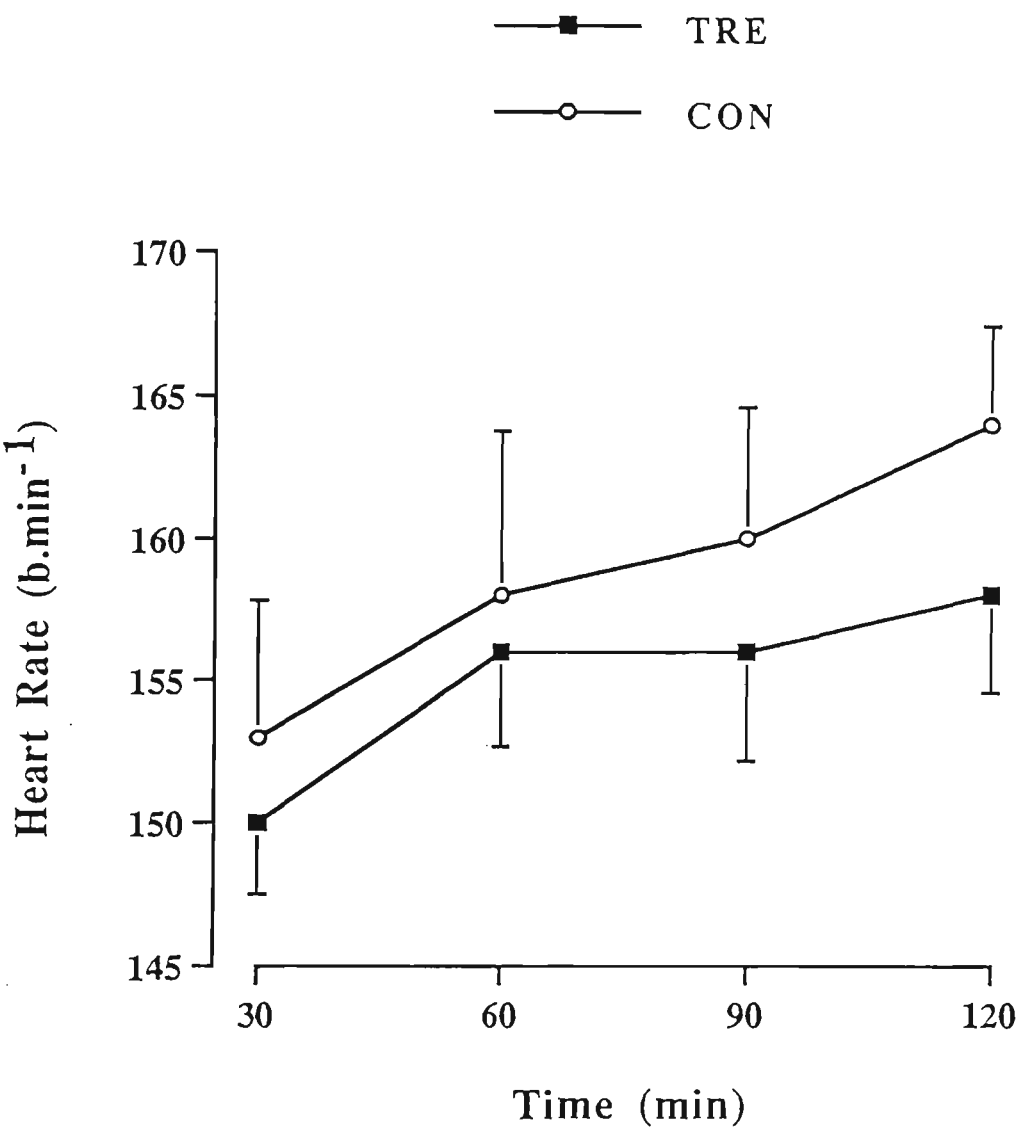

Fig. 4.2. Heart rate values during exercise under treatment (TRE) and control (CON) conditions. Values are means ± SE, (n=5).

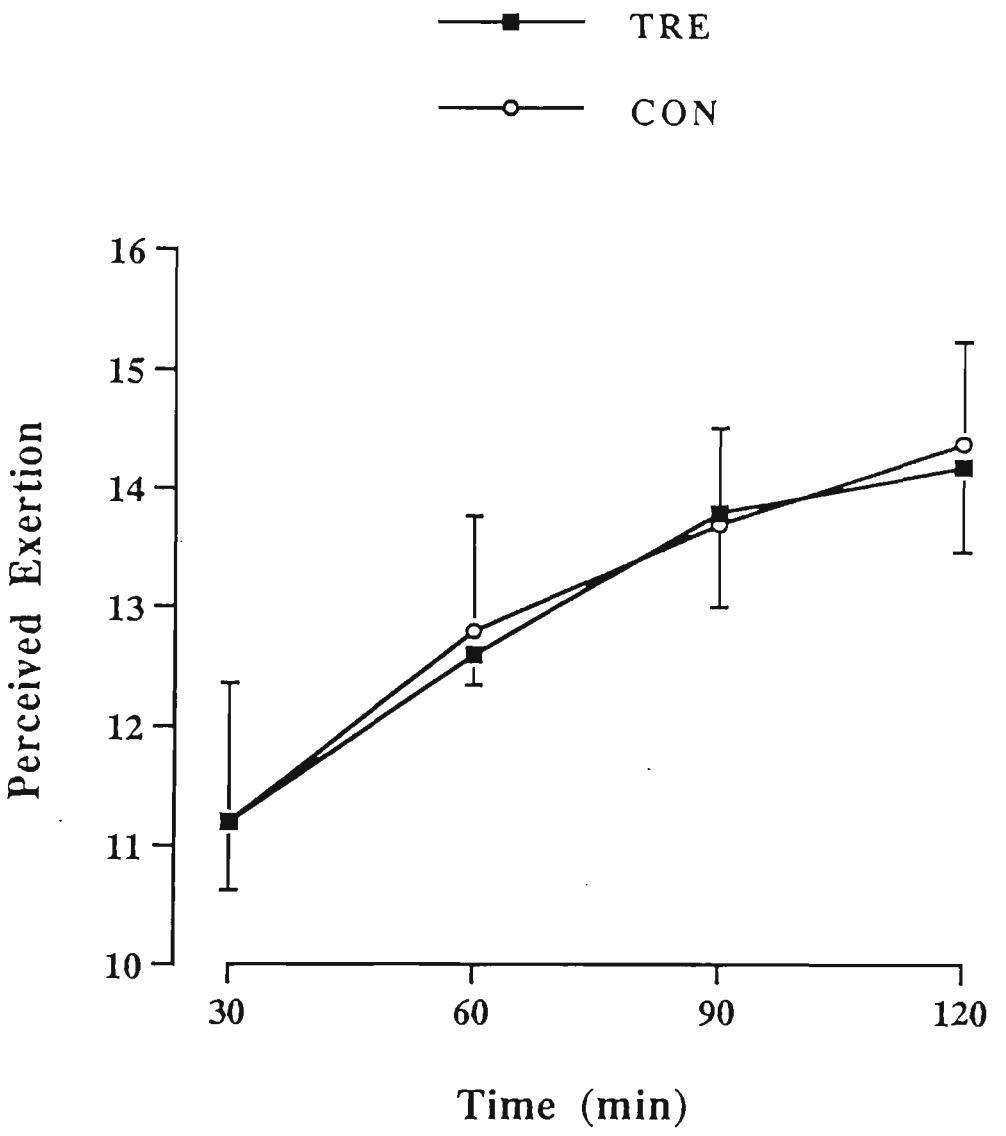

Fig. 4.3. Ratings of perceived exertion (Borg Scale) during exercise under treatment (TRE) and control (CON) conditions. Values are means ± SE, (n=5).

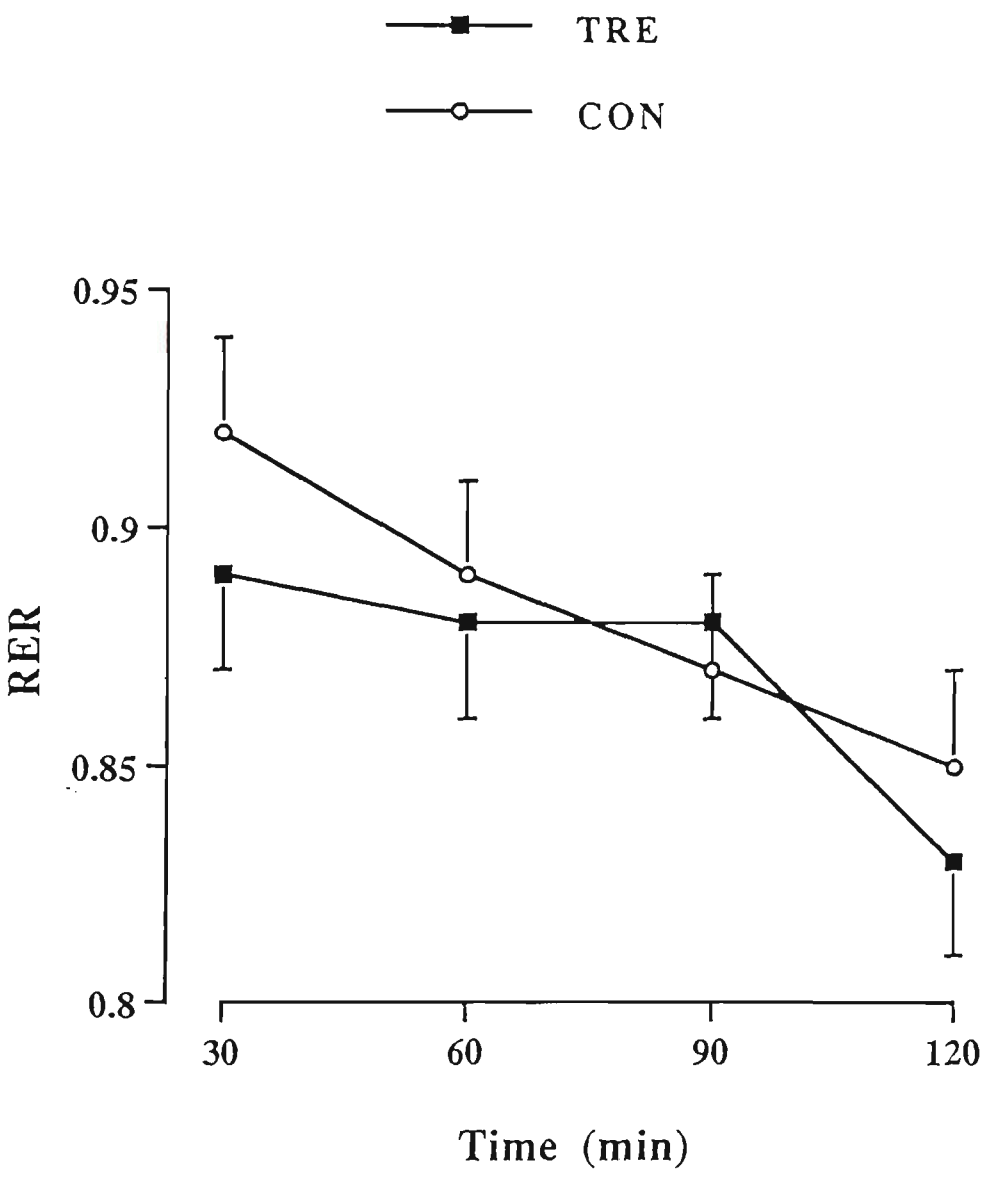

Fig. 4.4. Respiratory exchange ratio values during exercise under treatment (TRE) and control (CON) conditions. Values are means ± SE, (n=5).

4.4. Blood and Plasma Metabolites

4.4.1. Plasma Glucose

Plasma glucose concentrations are presented in Fig. 4.5 and Table 4.2. Plasma glucose concentration remained relatively stable during the two hour exercise period for all subjects and in all exercise trials. There was no difference for plasma glucose between the two treatments.

4.4.2. Blood and Plasma Lactate

Blood and plasma lactate concentrations are presented in Fig. 4.6 and Table 4.2. There was no difference between the TRE and CON trials for either plasma or blood lactate concentrations. The moderate increase seen in mean concentrations of plasma and blood lactate at thirty minutes of exercise (Fig 4.6) during the control trial is largely a result of one subject (see Appendix D) having a very sharp rise in lactate which is reflected by the large standard error of mean. This particular subject exercised at the same relative workload, however, she was not as familiar with riding a bike compared with the others.

4.4.3. Free Fatty Acids (FFA)

The mean plasma FFA concentrations are presented in Fig. 4.7 and Table 4.2. Plasma FFA concentrations increased ($P < 0.05$) during the two hour exercise period but there was no difference between the TRE and CON conditions.

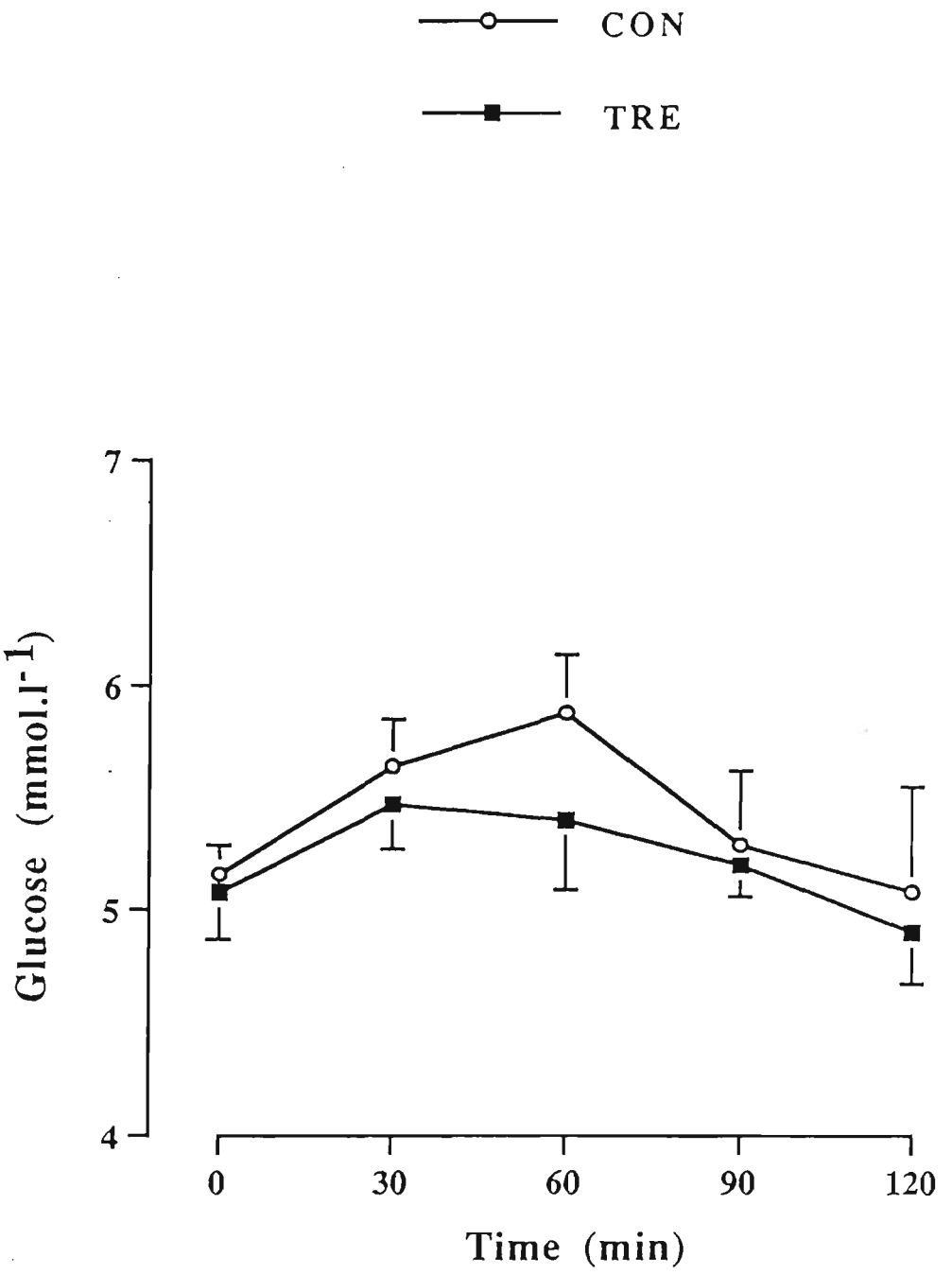

Fig. 4.5. Plasma glucose concentrations during exercise under treatment (TRE) and control (CON) conditions. Values are means ± SE, (n=5).

	TRIAL	PRE - EX	POST - EX
Plasma Lactate	TRE	1.9 ± 0.5	2.6 ± 0.5
($mmol.l^{-1}$)	CON	1.5 ± 0.1	2.6 ± 0.7
Blood Lactate	TRE	1.7 ± 0.4	2.2 ± 0.5
($mmol.l^{-1}$)	CON	1.4 ± 0.1	2.5 ± 0.8
Plasma Glucose	TRE	5.08 ± 0.21	4.91 ± 0.23
($mmol.l^{-1}$)	CON	5.16 ± 0.13	5.08 ± 0.47
Plasma FFA	TRE	0.33 ± 0.09	0.76 ± 0.16
($mmol.l^{-1}$)	CON	0.27 ± 0.04	0.87 ± 0.30

Table 4.2. Blood and plasma metabolites before exercise (PRE-EX) and after (POST-EX) exercise under treatment (TRE) and control (CON) conditions.

Values are means ± SE, (n=5).

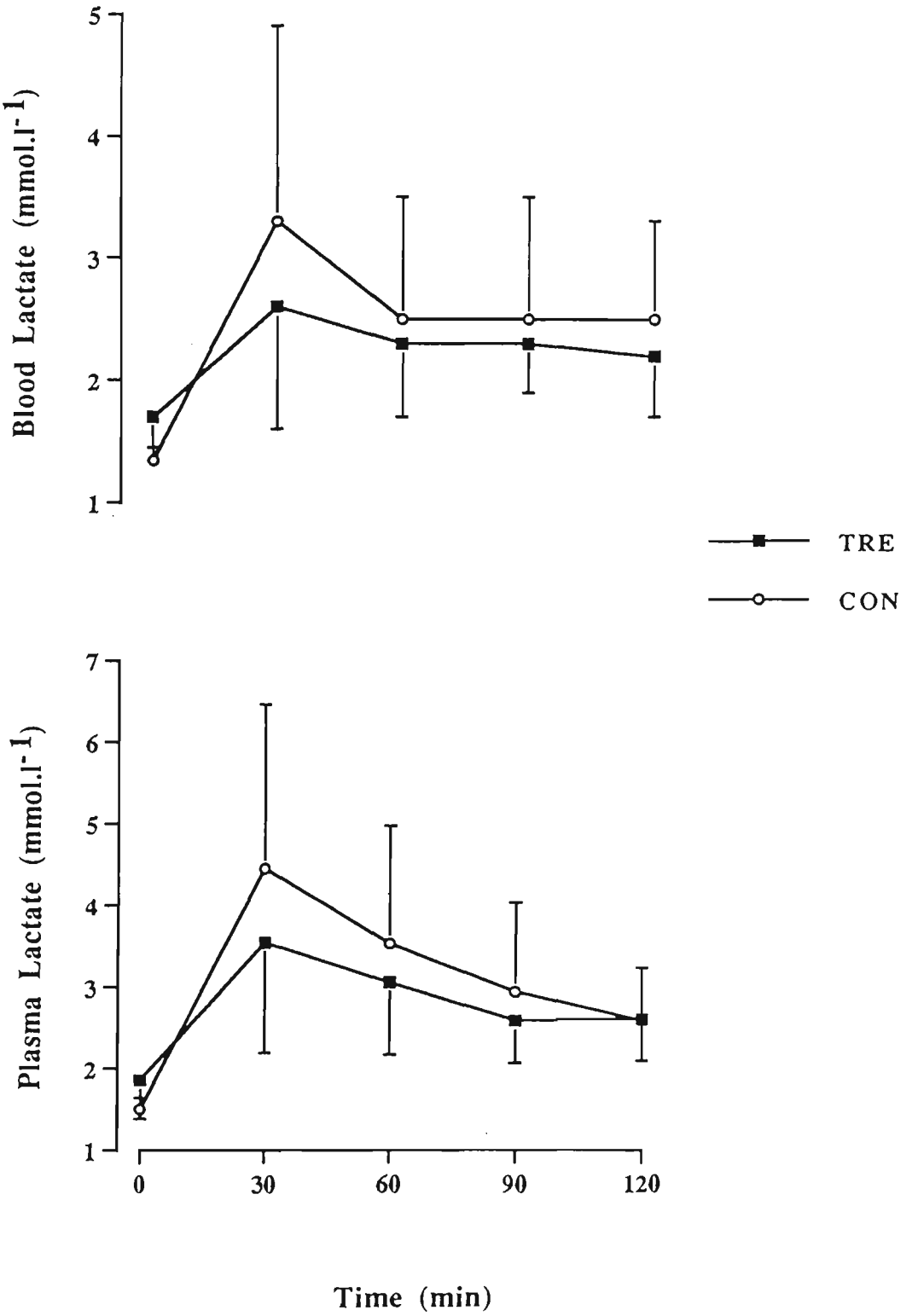

Fig. 4.6. Plasma and blood lactate concentrations during exercise under treatment (TRE) and control (CON) conditions. Values are means ± SE, (n=5).

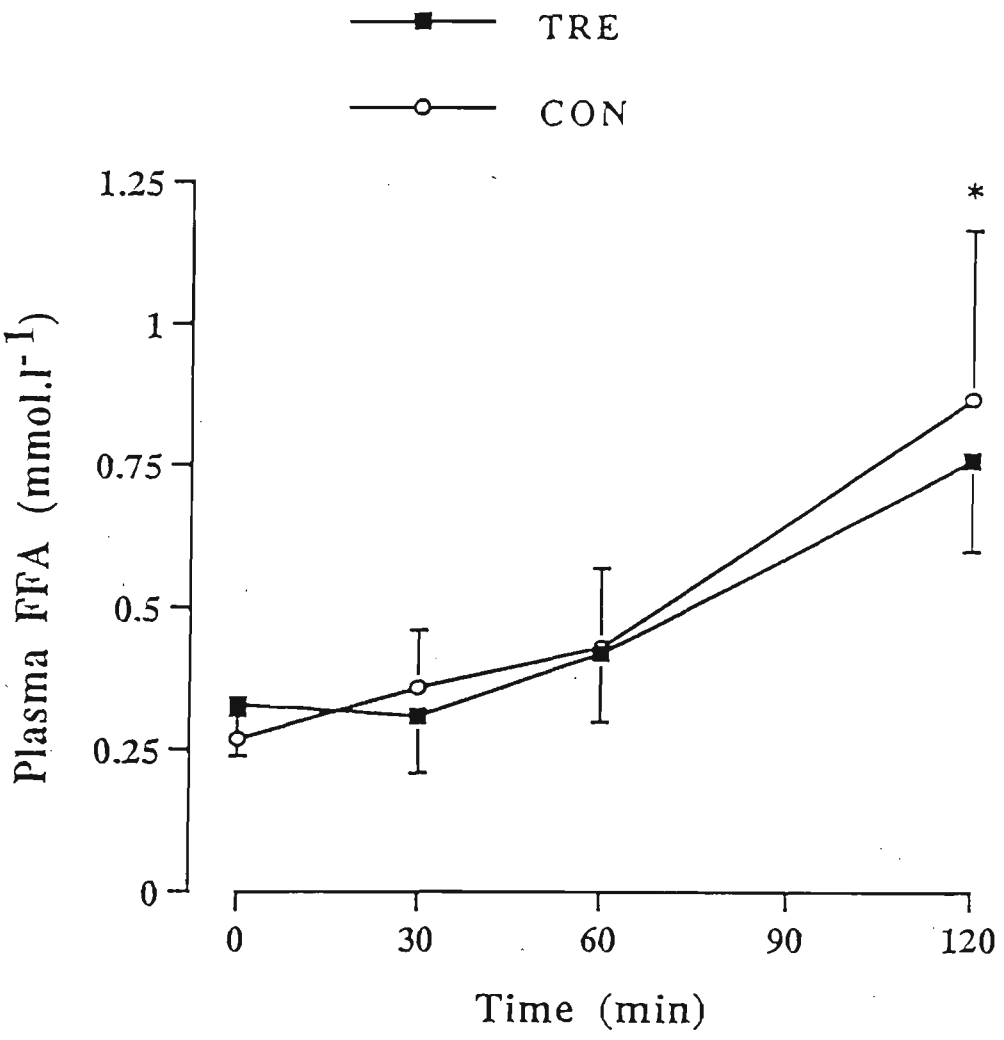

Fig. 4.7. Plasma free fatty acid (FFA) concentrations during exercise under treatment (TRE) and control (CON) conditions. Values are means ± SE, (n=5).

* Denotes difference from 60 min, 30 min and rest.

4.5. Hormones

4.5.1. Insulin

Plasma insulin concentrations are presented in Fig. 4.8. and Table 4.3. There was no difference in plasma insulin concentrations during two hours of exercise or between the TRE and CON conditions.

4.5.2. Cortisol

Plasma cortisol concentrations are presented in Fig. 4.9 and Table 4.3. There was no difference in plasma cortisol concentrations during the two hours of exercise or between the TRE and CON conditions.

4.5.3. Catecholamines

Plasma adrenaline and noradrenaline concentrations are presented in Fig. 4.10 and 4.11 respectively. Adrenaline concentrations increased ($P < 0.05$) during exercise. There was no difference in plasma noradrenaline during exercise or for either adrenaline and noradrenaline between treatments.

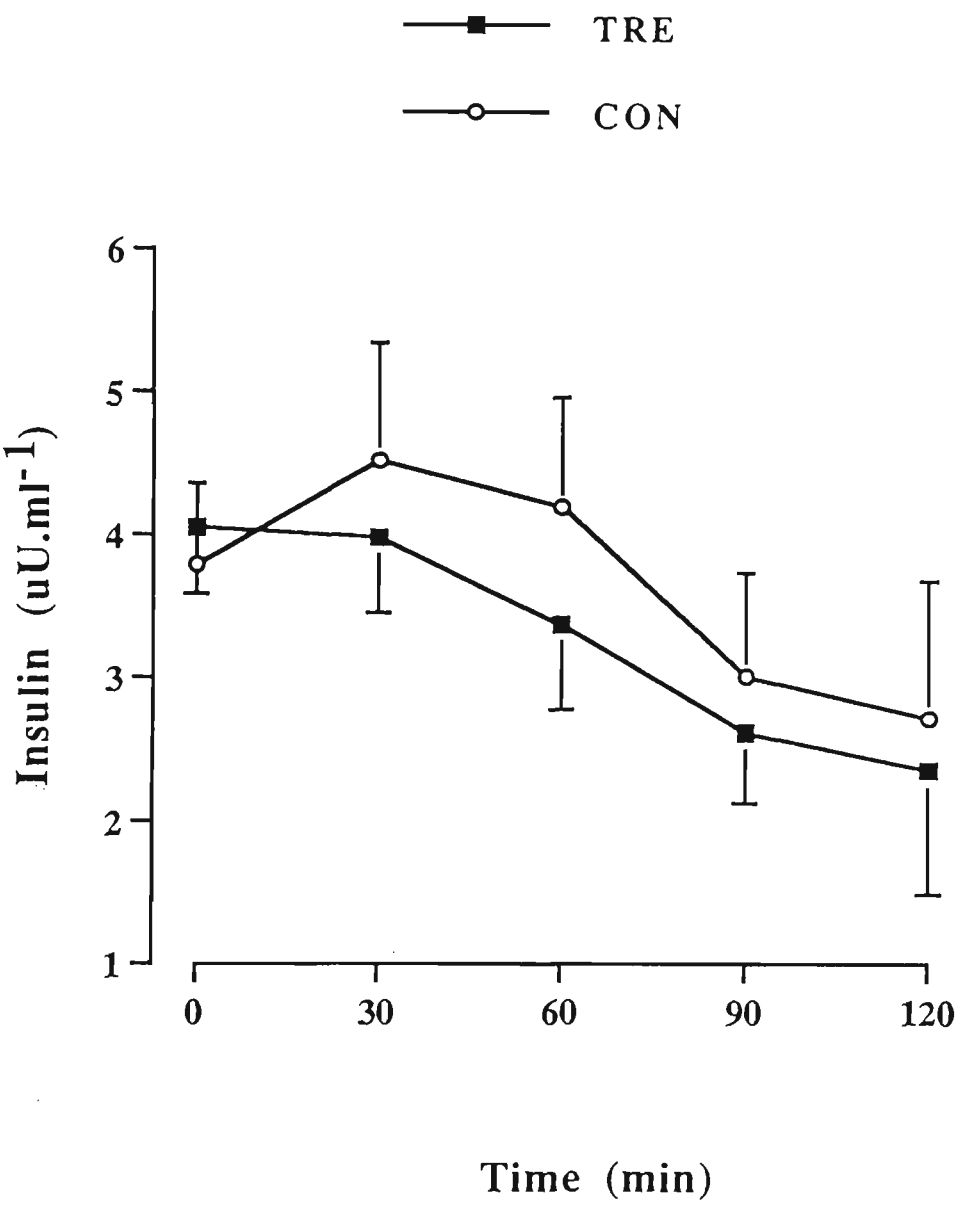

Fig. 4.8. Plasma insulin concentrations during exercise under treatment (TRE) and control (CON) conditions. Values are means ± SE, (n=5).

	TRIAL	PRE - EX	POST - EX
Insulin	TRE	4.05 ± 0.47	2.36 ± 0.87
($\mu U.ml^{-1}$)	CON	3.79 ± 0.57	2.72 ± 0.96
Cortisol	TRE	609.1 ± 61.5	631.6 ± 54.8
($nmol.l^{-1}$)	CON	588.9 ± 80.6	758.6 ± 17.4
Adrenaline	TRE	0.16 ± 0.05	1.04 ± 0.5
($nmol.l^{-1}$)	CON	0.09 ± 0.01	1.4 ± 0.5
Noradrenaline	TRE	0.83 ± 0.22	8.32 ± 2.64
($nmol.l^{-1}$)	CON	1.09 ± 0.20	7.84 ± 0.84

Table 4.3. Plasma hormone concentrations before (PRE-EX) and after (POST-EX) exercise under treatment (TRE) and control (CON) conditions.

Values are means ± SE, (n=5)

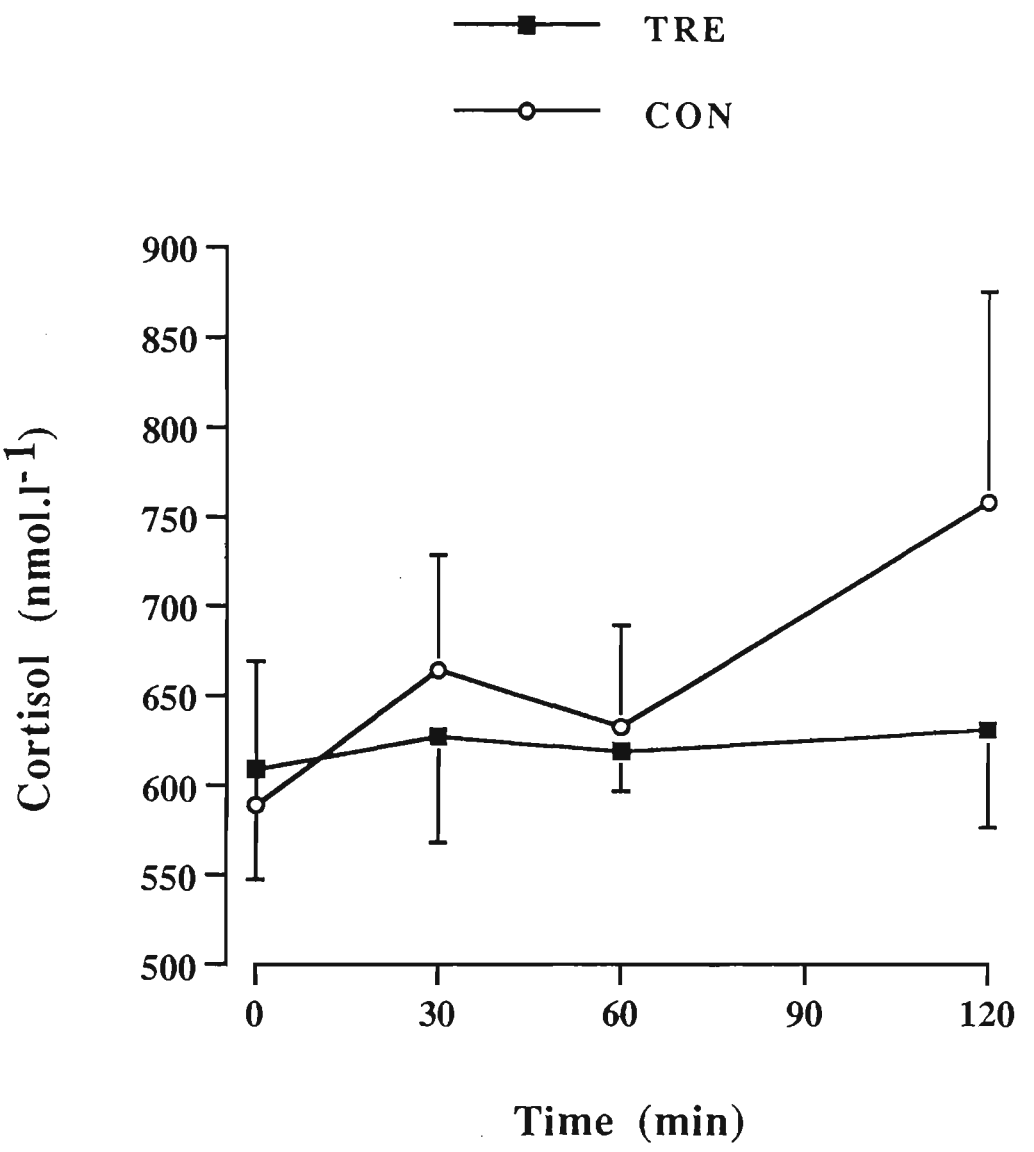

Fig. 4.9. Plasma cortisol concentrations during exercise under treatment (TRE) and control (CON) conditions. Values are means ± SE, (n=5).

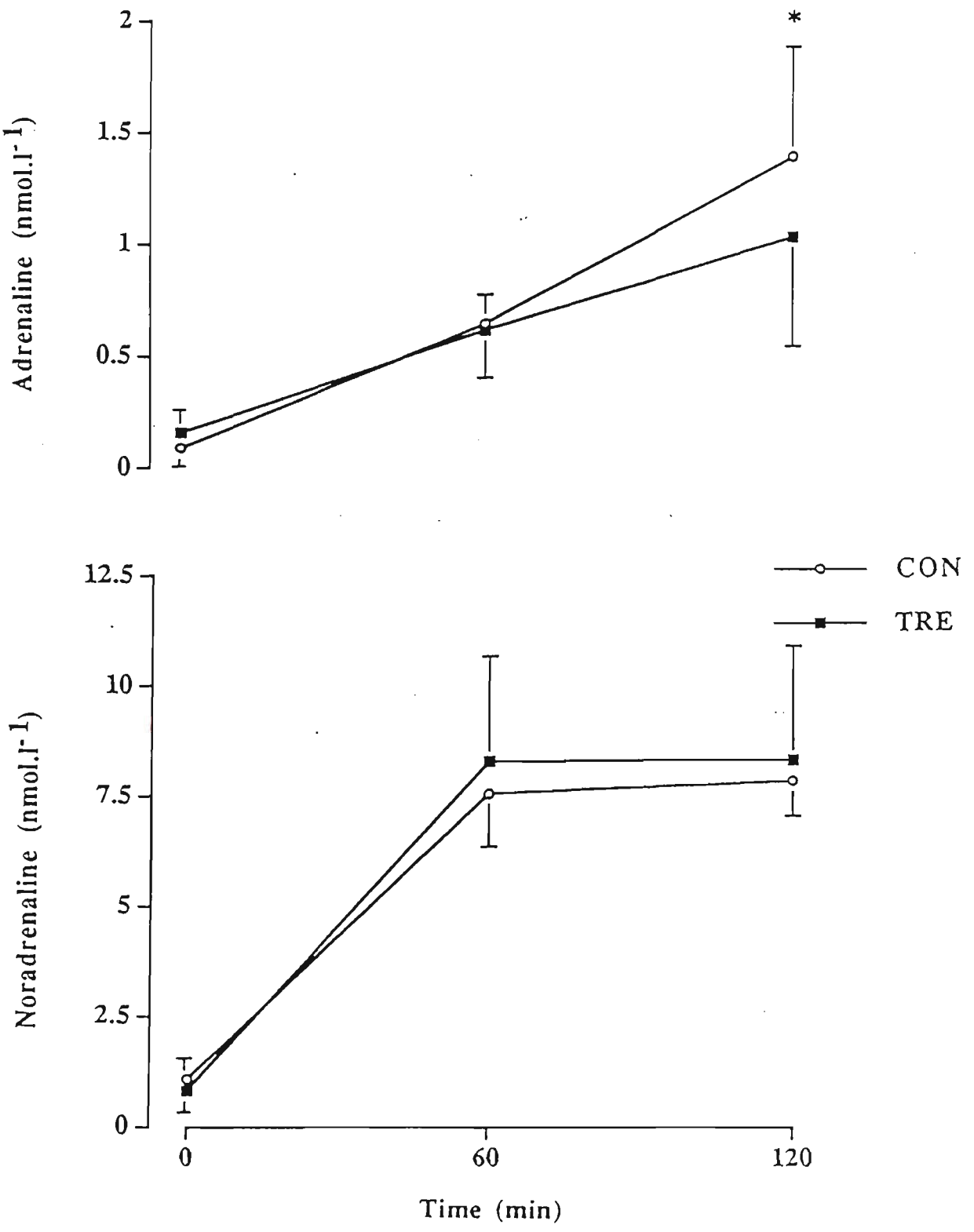

Fig. 4.10. Plasma adrenaline and noradrenaline concentrations during exercise under treatment (TRE) and control (CON) conditions. Values are means ± SE, (n=5).

* Denotes difference from 60 min and rest.

4.6. Muscle Metabolites

4.6.1. Muscle Glycogen

The pre and post exercise muscle glycogen concentrations (n=2) are presented in Table 4.4. Subject three had a higher pre-exercise concentration for glycogen (721 mmol.kg dw^{-1}) under TRE conditions compared with (395 mmol.kg dw^{-1}) CON conditions. Subject five had a lower pre-exercise concentration for glycogen (401 mmol.kg dw^{-1}) under TRE conditions compared with (451 mmol.kg dw^{-1}) under CON conditions. The rate of glycogen utilisation is also presented in Table 4.5. Subject three utilised 672 mmol.kg.dw^{-1} (TRE) compared with 326 mmol.kg dw^{-1} (CON). Subject five utilised 265 mmol.kg dw^{-1} (TRE) compared with 378 mmol.kg dw^{-1} (CON).

4.6.2 Muscle Lactate

The pre and post exercise muscle lactate concentrations are presented in Table 4.5. The muscle lactate concentrations for the two subjects were similar during all exercise trials and there was no difference between the treatments.

Subject Three	TRIAL	PRE-EX	POST-EX	Δ Glycogen
Glycogen	TRE	721	49	672
mmol.kg dw^{-1}	CON	395	69	326
Subject Five	**TRIAL**	**PRE-EX**	**POST-EX**	**Δ Glycogen**
Glycogen	TRE	401	137	265
mmol.kg dw^{-1}	CON	451	73	378

Table 4.4. Muscle glycogen concentrations before (PRE-EX) and after (POST -EX) exercise and muscle glycogenolysis (Δ) under (TRE) and (CON) conditions.

Subject Three	TRIAL	PRE - EXERCISE	POST - EXERCISE
Lactate	TRE	3.3	3.2
mmol.kg dw^{-1}	CON	2.5	2.6
Subject Five	**TRIAL**	**PRE - EXERCISE**	**POST - EXERCISE**
Lactate	TRE	3.5	4.7
mmol.kg dw^{-1}	CON	4.4	4.7

Table 4.5. Muscle lactate concentrations before (PRE-EX) and after (POST-EX) under (TRE) and (CON) conditions.

CHAPTER FIVE

DISCUSSION

The purpose of the present study was to determine whether being amenorrheic altered the pattern of energy metabolism during sub-maximal endurance exercise. This was investigated by raising the concentration of oestradiol in amenorrheic athletes to concentrations similar to those of their eumenorrheic counterparts.

The administration of oestradiol using the medium dose patch significantly increased the resting concentrations of oestradiol from 81 ± 7 to 114 ± 17 pmol. l^{-1}. The expectation was that oestradiol concentrations would be raised to levels similar to those that occurred during the follicular phase of the menstrual cycle (Balfour and Heel, 1990). Such moderate elevations of oestradiol were unlikely to be associated with side-effects such as fluid retention, which would have jeopardised the continued participation of the subjects in the study. The mean oestradiol concentrations after three weeks oestradiol therapy in the present study (114 ± 17 pmol. l^{-1}) were similar to the mean reported oestradiol levels reported for eumenorrheic women (153.4 ± 71.3 pmol. l^{-1}) in the follicular phase (De Souza et al., 1990). These concentrations contrast with those seen in eumenorrheic women in the mid-luteal phase where oestradiol levels average 549.2 pmol. l^{-1} . It is important to note that in the present study the mean of the differences in oestradiol values between the two trials was 33.12 pmol. l^{-1} whereas the difference in oestradiol values between the two phases of the menstrual cycle in De Souza et al., (1990) study was 395.8 pmol. l^{-1}. This may help to explain

why in the present study there was no significant difference in the way substrates were metabolised during two hours of sub-maximal exercise under treatment and control conditions.

It has been suggested that progesterone, the other ovarian steroid, exerts an anti-oestrogenic effect and that it is the ratio of oestradiol to progesterone that determines the metabolic action of oestradiol (Kenagy et al., 1981; Puah and Bailey, 1985). In the present study, despite elevating oestradiol, the ratio of oestradiol to progesterone was not different between the treatment (TRE) and control (CON) conditions. The unchanged ratio of the two hormones may help to explain why there was no difference in energy metabolism under TRE and CON conditions.

In the present study there was no difference in any of the parameters of exercise intensity when comparing TRE with CON. The elevations in heart-rate during exercise, were similar for the two trials, in agreement with studies conducted to compare the mid-luteal with the early-follicular phases of the menstrual cycle (De Souza et al., 1990; Jurowski et al., 1981; Nicklas et al., 1989). Ratings of perceived exertion (RPE) in the present study were not different between TRE and CON in accord with the studies by Stephenson et al. (1982), who observed no difference in RPE at the same workload during five different phases of the menstrual cycle, and De Souza et al., (1990) who observed no difference for RPE between the mid luteal and early follicular phase of the menstrual cycle during sub-maximal and maximal exercise.

Blood glucose is normally the sole source of energy for the central nervous system and several other tissues and therefore homeostasis is directed towards maintaining blood glucose concentration within a narrow range (Bjorkman, 1986). Maintenance of blood glucose during exercise is predominantly due to glycogen breakdown to glucose in liver and the production of glucose via gluconeogenic precursors. The extent to which oestradiol affects these two processes directly is not well understood. The concentration of glucose in blood plasma in the present study was not altered by the change in oestradiol levels. Bonen et al. (1991) who observed no difference in glucose concentrations during exercise between the luteal and follicular phases of the menstrual cycle. These results, however, contrast with those of Lavoie et al. (1987) who observed lower blood glucose concentrations at 70 and 90 minutes of moderate intensity exercise during the luteal phase compared with the follicular phase of the menstrual cycle. In the study by Lavoie et al. (1987), subjects fasted overnight after adhering to a 24-hour low-carbohydrate diet, and are likely to have had a lower pre-exercise liver glycogen content than in the present study. Gluconeogenesis would therefore be an important mechanism to maintain blood glucose. The authors hypothesised that oestradiol may have exerted its effect by inhibiting gluconeogenesis, thereby lowering blood glucose concentrations. The present study is inconclusive with regard to the effects of oestradiol administration on the homeostasis of blood glucose, since subjects were glycogen replete prior to exercise and the blood glucose concentrations were maintained throughout exercise.

In the present study the lactate response to exercise was similar between TRE and CON which contrasts the findings of Jurowski et al. (1981) and Lavoie et al. (1987) who observed lower concentrations of lactate in the luteal phase of the menstrual cycle when oestradiol is high. The differences in these findings compared with the present study may be due to the different exercise protocols used by Jurowski et al. (1981) and the different pre-exercise diet regimes used by Lavoie et al. (1987).

Jurowski et al. (1981) tested trained athletes during heavy (90%VO_2 max) and maximal (100% VO_2 max) exercise and reported higher lactate concentrations in the follicular phase compared with the luteal phase of the menstrual cycle. These authors postulated that oestrogen mediated a shift towards FFA metabolism in the luteal phase, which even during intense exercise resulted in decreased lactate concentrations. However, during exercise at 100% VO_2 max, it is unlikely that there would be any shift towards fat metabolism (Powers and Howley, 1993). Indeed, Robertson and Higgs (1983) observed no difference in blood lactate during treadmill running at 90% and 100% VO_2 max during the mid-follicular and mid-luteal phases and day one of menses.

It is well documented that the metabolic effect of oestradiol is to cause a shift in metabolism during exercise at lower intensities towards FFA metabolism through increased lipid synthesis and enhanced lipolysis in muscle and adipose tissue (Bunt, 1990; Hackney et al., 1990; Jurowski et al., 1981). In the present study there was no difference in the resting or exercising concentrations of plasma FFA between the TRE and CON trials. It appears unlikely that these results would have represented increased muscle triglyceride (TG) lipolysis and a concurrent increase in fat oxidation, i.e. increased turnover of FFA, without altering plasma FFA concentrations. If such a shift towards increased muscle TG utilisation had occurred, then RER values would be expected to have been lower for TRE which was not the case. This similarity in FFA concentrations between TRE and CON are in agreement with others whereby there was no difference in resting plasma FFA (Bonen et al., 1991) or exercising plasma FFA concentrations (Lavoie et al., 1987) during the luteal phase compared with the follicular phase of the menstrual cycle.

Oestradiol may alter metabolism by exerting an influence on other regulatory hormones. In the present study, the effects of oestradiol administration did not have a measurable effect on plasma concentrations of insulin, cortisol or catecholamine

responses. Amenorrheic women are observed to have higher resting cortisol levels compared with eumenorrheic women (De Souza et al, 1988; Ding et al., 1988; Kanaley et al., 1992). The cortisol levels in the present study are similar to those observed by others for amenorrheic women. The elevated cortisol concentrations in amenorrheic women may be associated with disturbances in the hypothalamic-pituitary-adrenal function (Kanaley et al., 1992) however the site(s) of the disturbance is/are yet to be determined. The difference in cortisol levels between amenorrheic and eumenorrheic women does not appear to be related to changes in oestradiol concentrations because there has been no difference observed in cortisol levels during the early follicular, late follicular and mid luteal phase of the menstrual cycle (Kanaley, 1992). The concentration of cortisol is observed to increase during moderate exercise and exert metabolic actions by antagonising the effects of insulin (Martin and Wahren, 1993). A similar trend in cortisol concentrations during the two hours of sub-maximal exercise was observed in the present study however the increases in TRE and CON were not statistically significant.

During exercise insulin concentrations commonly decline and catecholamine and glucagon concentrations increase resulting in the stimulation of glycogenolysis, gluconeogenesis and lipolysis (Martin and Wahren, 1993). In the present study neither the exercise stimulus nor the oestradiol therapy had any effect on insulin response. These results are similar to those observed by Bonen et al. (1991). It is possible that no significant decrease in insulin concentrations was observed over the two hour exercise period because of the small sample size. Oestradiol has been observed to increase the molar ratio of insulin to glucagon when moderate to high doses (10 or 100 nmol/kg/day) were infused but not when using low doses (1 nmol/kg/day) (Mandour et al., 1977). The magnitude to which these hormones exert any metabolic action appears to be dependent on the relative molar ratio between insulin and glucagon rather than the absolute concentrations of either hormone (Mandour et al., 1977). An increase in the insulin to glucagon molar ratio is observed to increase

hepatic lipogenesis and inhibit gluconeogenesis (Mandour et al., 1977). In the present study, it is unknown the effect oestradiol had on the molar ratio of insulin to glucagon because plasma glucagon was not measured. However, given the lack of differences between treatment and control conditions, it is unlikely that the ratio of insulin to glucagon would have changed significantly.

The observed increase in plasma catecholamines during the two hours of exercise was expected as exercise is a strong stimulus for catecholamine release. There was however no difference in adrenaline or noradrenaline responses under different oestradiol concentrations which is in contrast with the observations of Sutton et al. (1980) who reported higher adrenaline concentrations when oestradiol concentrations are low. Increased concentrations of adrenaline result in an increase in circulatory glucose due to an increase in glycogenolysis (Chasiotis et al., 1983). Evidence to support this is the increase in glucose and lactate concentrations during the follicular phase of the menstrual cycle when adrenaline concentrations were elevated (Sutton et al., 1980). In the present study despite an increase in adrenaline during exercise there was no change in either plasma glucose or lactate concentrations.

Initial muscle glycogen stores are thought to be a limiting factor in the ability to sustain endurance exercise (Bergstrom et al., 1967). In the present study permission to obtain muscle samples was given only by two of the subjects and therefore the interpretation of the results is limited. Subject number three had a greater muscle glycogen content at rest for TRE compared with CON, which is similar to the findings of Hackney, (1990) who observed greater resting muscle glycogen content during the menstrual cycle when oestradiol concentrations are high. This subject also utilised more glycogen during the two hour exercise period when pre-exercise muscle glycogen levels were high. It is well documented that an elevated pre-exercise glycogen content, increases the rate of muscle glycogen utilisation during sub-maximal exercise (Chesley et al., 1995; Hargreaves et al., 1995; Hespel and Richter,

1992). Subject number five, had an elevated pre-exercise content for muscle glycogen under the CON treatment compared with the TRE. During exercise this subject utilised more glycogen during the control trial and once again this may be related to pre-exercise muscle glycogen levels. Muscle lactate values for the same two subjects were also measured. When resting muscle glycogen content was higher corresponding with greater utilisation there also appeared to be higher muscle lactate levels, as would be expected.

It is known that when muscle glycogen availability is increased, there is an increase in muscle glycogenolysis and that oestradiol exerts an effect on metabolism by increasing resting muscle glycogen content. In addition, oestradiol promotes the release and utilisation of lipids. What is not known is which metabolic pathway is favoured under the control of oestradiol during exercise. It is interesting to point out that the one subject who showed a large increase in plasma oestradiol also showed a lower RER during exercise suggesting the lipid pathway is favoured.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

In the present study moderate elevation of plasma oestradiol concentrations in amenorrheic athletes did not alter substrate utilisation during two hours of exercise. It therefore appears that raising oestradiol concentration to that achieved in the present study is not advantageous for amenorrheic athletes during two hours of sub-maximal exercise.

It may, however, be possible that some plasma oestradiol threshold needs to be exceeded or the ratio of oestradiol to progesterone to be altered before differences in energy metabolism can be observed. It is also possible that under other exercise performance parameters, such as exercise until exhaustion, an altered metabolic response may be observed.

Recommendations for further studies include the administration of higher doses of oestradiol and changing the exercise performance parameters, for example, exercise to exhaustion.

REFERENCES

Ahlborg, G., Felig, P., Hagenfeldt, L., Hendler, R., Wahren, J. Substrate turnover during prolonged exercise in man. *Journal of Clinical Investigation*, 53:1080, 1974.

Baer, J.T. Endocrine parameters in amenorrheic and eumenorrheic adolescent female runners. *Int. J. Sports Med.* 14(4):191-195, 1993.

Baker, E.R. Menstrual dysfunction and hormonal status in athletic women: A review. *Ferility and Sterility,* 36(6):691-695, 1981a.

Baker, E.R., Mathur, R.S., Kirk, R.F., and Williamson, H.O. Female runners and secondary amenorrhea: correlation with age, parity, mileage and plasma hormonal and sex hormone-binding globulin concentration. *Ferility and Sterility*, 36:183, 1981b.

Balfour, J.A., and Heel, R.C. Transdermal Estradiol. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficiency in the treatment of menopausal complaints. *Drugs*, 40(4):561-582, 1990.

Beitins, I.Z., McArthur, J.W., Turnbull, B.A., Skrinar, G.S., and Bullen, B.A. Exercise induces two types of human luteal dysfunction: confirmation by urinary free progesterone. *Journal of Clinical Endocrinology and Metabolism,* 72(6):1350-1358, 1991.

Bemben, D.A., Boileau, R.A., Bahr, J.M., Nelson, R.A., and Misner, J.E. Effects of oral contraceptives on hormonal and metabolic responses during exercise. *Medicine and Science in Sports and Exercise*, 24(2):434-441, 1992.

Bemben, D.A. Metabolic effects of oral contraceptives. Implications for exercise responses of premenopausal women. *Sports Medicine*, 16(5): 295-304, 1993.

Bergstrom, J. Muscle electrolytes in man. *Scand. J. Lab. Invest. Suppl.* 68:1-110, 1962.

Bergstrom, J., Hermansen, L., Hultman, E., and Saltin, B. Diet, muscle glycogen and physical performance. *Acta Physiol, Scand,* 71:140-150, 1967.

Bergstrom, J., Hultman, E., Jorfeldt, L., Pernow, B., and Wahren, J. Effect of nicotinic acid on physical work capacity and on metabolism of muscle glycogen in man. *Journal of Applied Physiology,* 26:170-176, 1969.

Bjorkman, O. Fuel metabolism during exercise in normal and diabetic men. *Diabetes Metabolism Reviews*, 1:483-496, 1986.

Bonen, A., Ling, W.Y., MacIntyre, K.P., Neil, R., McGrail, J.C., and Belcastro, A.N. Effects of exercise on serum concentrations of FSH, LH, progesterone, and estradiol. *European Journal of Applied Physiology,* 42:15-23,1979.

Bonen, A., Haynes, F.J., Watson-Wright, W., Sopper, M.M., Pierce, G.N., Low, M.P., and Graham, T.E. Effects of menstrual cycle on metabolic responses to exercise. *Journal of Applied Physiology*, 55(5):1506-1513, 1983.

Bonen, A., and Keizer, H.A. Athletic menstrual cycle irregularity: Endocrine response to exercise and training. *The Physician and Sports Medicine*, 12(8):78-91, 1984.

Bonen, A., Haynes, F.W., and Graham, T.E. Substrate and hormonal responses to exercise in women using oral contraceptives. *Journal of Applied Physiology*, 70(5): 1917-1927, 1991.

Bonen, A. Recreational exercise does not impair menstrual cycles:A prospective study. *International Journal of Sports Medicine*, 13(2):110-120, 1992.

Bonen, A., Exercise induced menstrual cycle changes: A functional, temporary adaptation to metabolic stress. *Sports Medicine,* 17(6):373-392, 1994.

Borg, G.A. Psychophysical bases of perceived exertion. *Medicine and Science in Sports and Exercise*, 14:377, 1982.

Boyden, T.W., Pamenter, R.W., Stanforth, P., Rotkis, T., and Wilmore, J. Sex steroids and endurance running in women. *Ferility and Sterility,* 39(5):629-631, 1983.

Byran Pedersen, A., Bartholmew, M.J., Dolence, L.A., Aljadir, L.P., Netteburg, K.L., Lloyd, T. Menstrual differences due to vegetarian and nonvegetarian diets. *American Journal of Clinical Nutrition*, 53:879-885, 1991.

Bullen, B., Skrinar, G.S., Beitins, I.Z., Carr, D.B., Reppert, S.M., Dotson, C.O., Fenci, M., Gervino, E.V., and McArthur, J.W. Endurance training effect on plasma hormonal responsiveness and sex hormone excretion. *Journal of Applied Physiology,* 56(6):1453-1463, 1984.

Bullen, B.A., Skrinar, G.S., Beitins, I.Z., Merong, G., Turnbull, B.A., and McArthur, J.W. Induction of menstrual disorders by strenous exercise in untrained women. *The New England Journal of Medicine,* 312(21):1349-1353, 1985.

Bulow, J. and Madsen, J. Regulation of fatty acid mobilisation from adipose tissue during exercise. *Scand. J. Sports Sci.* 8(1):19-26, 1986.

Bunt, J.C. Hormonal alterations due to exercise. *Sports Medicine,* 3:331-345, 1986.

Bunt, J.C. Metabolic actions of estradiol: Significance for acute and chronic exercise responses. *Medicine and Science in Sport and Exercise*, 22(3):286-290, 1990.

Carlberg, K.A., Buckman, M.T., Peake, G.T., and Riedesel, M.L. A survey of menstrual function in athletes. *Eur. J. Appl. Physiol*, 51:211-222, 1983.

Carrington, L.J., and Bailey, C.J. Effects of natural and synthetic estrogens and progestins on glycogen deposition in female mice. *Hormone Research*, 21:199-203, 1985.

Casper, R.C., Scholler, D.A., Kushner, R., Hnilicko, J., and Trainer Gold, S. Total daily energy expenditure and activity level in anorexia nervosa. *American Journal of Clinical Nutrition,* 53:1143-1150, 1991.

Cavanaugh, D.J., Kanonchoff, A.D., and Bartels, R.L. Menstrual irregularities in athletic women may be predictable based on pre-training menses. *The Journal of Sports Medicine and Physical Fitness*, 29(2):163-169, 1989.

Chasiotis, C., Sahlin, K., and Hultman, E. Regulation of glycogenolysis in human muscle at rest and during exercise. *Journal of Applied Physiology,* 53(3):708-715, 1982.

Chasiotis, D., Hultman, E., and Sahlin, K. Acidotic depression of cyclic AMP accumulation and phosphorylase b to a transformation in skeletal muscle of man. *Journal of Physiology*, 335:197-204, 1983.

Chesley, A., Hultman, E., and Spriet, L.L. Effects of epinephrine infusion on muscle glycogenolysis during intense aerobic exercise. *American Journal of Physiology*, 268: E127-E134, 1995.

Clark, N., Nelson, M., and Evans, W. Nutrition education for elite female runners. *Physician and Sports Medicine*,16:124-136, 1988.

Coggan, A.R., and Coyle, E.F. Reversal of fatigue during prolonged exercise by carbohydrate infusion or ingestion. *Journal of Applied Physiology*, 63(6): 2388-2395, 1987.

Conlee, R.K. Muscle glycogen and exercise endurance: A twenty year perspective. *Exercise and Sport Science Reviews*, Vol 15, 1987.

Consolazio, C.G., Johnson, R.E., and Pecora, L.J. Physiological measurements of metabolic functions in man, New York, McGraw-Hill, 1963.

Constantin-Teodosiu, D., Cederblad, G., and Hultman, E. PDC activity and acetyl group accumulation in skeletal muscle during prolonged exercise. *Journal of Applied Physiology*, 73:2403-2407, 1992.

Costill, D.L., Coyle, E., Dalsky, G., Evans, W., Fink, W., and Hoopes, D. Effects of elevated plasma FFA and insulin on muscle glycogen usage during exercise. *Journal of Applied Physiology,* 43(4):695-699, 1977.

Costill, D.L., Fink, W.J., Getchell, L.H., Ivy, J.L., and Witzmann, F.A. Lipid metabolism in skeletal muscle of endurance-trained males and females. *Journal of Applied Physiology*, 47(4):787-791, 1979.

Costill, D.L. Carbohydrates for exercise: Dietary demands for optimal performance. *International Journal of Sports Medicine*, 9:1-18, 1988.

Coyle, E.F., Coggan, A.R., Hemmert, M.K., Lowe, R.C., and Walters, T.J. Substrate usage during exercise following a preexercise meal. *Journal of Applied Physiology*, 59(2):429-433, 1985.

Coyle, E.F., Coggan, A.R., Hemmert, M.K., and Ivy, J.L. Muscle glycogen utilisation during prolonged strenous exercise when fed carbohydrate. *Journal of Applied Physiology*, 61(1):165-172, 1986.

Coyle, E.F. Carbohydrate metabolism and fatigue. In: Muscle fatigue: Biochemical and physiological aspects. Edited by G. Atlan, L. Beliveau and P. Bouissou. Paris Masson, pg 153-164, 1991.

Dale, E., Gerlach, D.H., and Wilhite, A.L. Menstrual dysfunction in distance runners. *Obstetrics and Gynecology*, 54(1):47-53, 1979.

De Souza, M.J., Maguire, M.S., Rubin, K.R., and Maresh, C.M. Effects of menstrual phase and amenorrhea on exercise performance in runners. *Medicine and Science in Sport and Exercise*, 22(5):575-580, 1990.

Ding, J., Sheckter, C., Drinkwater, B., Soules, M., and Bremner, W. High serum cortisol levels in exercise-associated amenorrhea. *Annals of Internal Medicine*, 108:530-534, 1988.

Dixon, G., Eurman, P., Stern, B.E., Schwartz, B., and Rebar, R.W. Hypothalamic function in amenorrheic runners. *Fertility and Sterility,* 42(3):377-383, 1984.

Drinkwater, B.L., Nelson, K., Chesnut, C.H., Bremner, W.J., and Shainholtz, S.S. Bone mineral content of amenorrheic and eumenorrheic athletes. *New England Journal of Medicine*, 311:277-281, 1984.

Drinkwater, B.L., Bruemner, B., Chesnut, C.H. Menstrual history as a determinant of current bone density in young athletes. *JAMA*, 263:545-548, 1990.

Evans, W.J., Phinnet, S.D., and Young, V.R. Suction applied to a muscle biopsy maximises sample size. *Medicine and Science in Sports and Exercise*, 14(1):101-102, 1982.

Felig, P., and Wahren, J. Fuel homeostasis in exercise. *New England Journal of Medicine*, 293:1078-1084, 1975.

Findling, J.W., and Tyrell, J.B. Anterior Pituitary gland. In: Basic and Clinical Endocrinology. Ed. F.S. Greenspan, 3rd Ed. Prentice-Hall, London, Ch 6, pg 79-132, 1991.

Fisher, E.C., Nelson, M.E., Frontera, W.R., Turksoy, R.N., and Evans, W.J. Bone mineral content and levels of gonadotropins and estrogens in amenorrheic running women. *Journal of Clinical Endocrinology and Metabolism*, 62:1232, 1986.

Fisherman, J., Boyar, R.M., and Hellman, L. Influence of body weight on estradiol metabolism in young women. *Journal of Clinical Endocrinology Metabolism*, 41:989-991, 1975.

Frisch, R.E., and McArthur, J.W. Menstrual cycles: Fatness as determinant of minimum weight for height necessary for their maintenance or onset. *Science,* 185:949-951, 1974.

Galbo, H., Christensen, N.J., and Holst, J.J. Glucose induced decrease in glucagon and epinephrine response to exercise in man. *Journal of Applied Physiology*, 42: 525-530, 1977a.

Galbo, H., Christensen, N.J., and Holst, J.J. Catecholamines and pancreatic hormones during blockade in exercising man. *Acta. Physiol. Scand.* 101:428-437, 1977b.

Gorski, J. Muscle triglyceride metabolism during exercise. *Canadian Journal of Physiology and Pharmacol.* 70:123-131, 1990.

Graham, T.E., Viswanathan, M., Van Dijk, P., Bonen, A., and George, J.C. Thermal and metabolic responses to cold by men and by eumenorrheic and amenorrheic women *Journal of Applied Physiology,* 67:282-290, 1989.

Green, H.J. How important is endogenous muscle glycogen to fatigue in prolonged exercise? *Canadian Journal of Physiology and Pharmacology*, 69:290-297, 1991.

Grossman, A., and Sutton, J.R. Endorphins: What are they? How are they measured? What is their role in exercise? *Medicine and Science in Sports and Exercise*, 17(1), 1985.

Hackney, A.C., Curley, C.S., and Nicklas, B.J. Female sex hormone influences on physiological responses to submaximal exercise. *Medicine and Science in Sports and Exercise*, S22, 1990.

Hackney, A.C. Effects of the menstrual cycle on resting muscle glycogen content. *Hormone Metabolism Research*, 22:647, 1990.

Hargreaves, M., Costill, D.L., Fink, W.J., King, D.S., and Fielding, R.A. Effect of pre-exercise carbohydrate feedings on endurance cycling performance. *Medicine and Science in Sports and Exercise*, 19: 33-36, 1987.

Hargreaves, M., Kiens, B., and Richter, E.A. Effect of increased plasma free fatty acid concentrations on muscle metabolism in exercising men. *Journal of Applied Physiology*, 70(1):194-201, 1991.

Hargreaves, M. Fuels for exercise: Implications for sports nutrition. *Australian Sports Commision*, August, 1992.

Hargreaves, M., McConnell, G., and Proietto, J. Influence of muscle glycogen on glycogenolysis and glucose uptake during exercise in humans. *Journal of Applied Physiology*, 78(1):288-292, 1995.

Harris, R.C., Hultman, E., and Nordesjo, L.O. Glycogen, glycolytic intermediates and high energy phosphates determined in biopsy samples of musculus quadriceps femoris of man at rest. Methods and variance of values. *Scand. J. Clin. Lab. Invest.* 33:109-120, 1974.

Hespel, P., and Richter, E.A. Glucose uptake and transport in contracting perfused rat muscle with different pre-contraction glycogen concentrations. *Journal of Physiology*, 427:347-359, 1990.

Hickson, R.C., Rennie, M.J., Conlee, R.K., Winder, W.W., and Holloszy, J.O. Effect of increased plasma fatty acids on glycogen utilisation and endurance. *Journal of Applied Physiology*, 43(5):829-833, 1977.

Highet, R. Athletic amenorrhea: An update on aetiology, complications and management. *Sports Medicine*, 7:82-108, 1989.

Hohtari, H., Salmininen- Lappalainen, K., and Laatikainen, T. Response of plasma endorphins, corticotropin, cortisol, and lutenizing hormone in the corticotropin-releasing hormone stimulation test in eumenorrheic and amenorrheic athletes. *Fertility and Sterility*, 55(2):277-279, 1991.

Holloszy, J.O. Utilisation of fatty acids during exercise. In Biochemistry of Exercise VII, Vol 21, pg 319-328. Edited by Taylor, A.W., Gollnick, P.D., Green, H.J., Ianuzzo, G.D., Noble, E.G., Metivier, G., and Sutton, J.R., 1990.

Hultman, E. and Harris, R.C. Carbohydrate Metabolism. Principles of Exercise Biochemistry. Medicine and Sport Science, 27: 78-119. Poortmans J.R. (ed), 1988.

Hurley, B.F., Nemeth, P.M., Martin III, W.H., Hagberg, J.M., Dalsky, G.P., and Holloszy, J.O. Muscle triglyceride utilisation during exercise:Effect of training. *Journal of Applied Physiology*, 60(2):562-567, 1986.

Jansson, E., Hjemdahl, P., and Kaijser, L. Epinephrine-induced changes in muscle carbohydrate metabolism during exercise in male subjects. *Journal of Applied Physiology*, 60(5):1466-1470, 1986.

Johnson, L.N. Glycogen phosphorylase: control by phosphorylation and allosteric effectors FASEB J. 6:2274-2282, 1992.

Jurowski, J.E., Jones, N.L., Toews, C.J., and Sutton, J.R. Effects of menstrual cycle on blood lactate, O_2 delivery, and performance during exercise. *Journal of Applied Physiology*, 51(6):1493-1499, 1981.

Kaiserauer, S., Snyder, A.C., Sleeper, M., and Zierath, J. Nutritional, physiological, and menstrual status of distance runners. *Medicine and Science in Sport and Exercise,* 21(2):120-125, 1989.

Kanaley, J.A., Boileau, R.A., Bahr, J.M., Misner, J.E., and Nelson, R.A. Cortisol levels during prolonged exercise: The influence of menstrual phase and menstrual status. *International Journal of Sports Medicine*, 13:332-336, 1992a.

Kanaley, J.A., Boileau, J.A., Misner, J.E., and Nelson, R.A. Substrate oxidation and GH responses to exercise are independent of menstrual phase and status. *Medicine and Science in Sport and Exercise*, 24(8):873-880, 1992b.

Keizer, H.A., Kuipers, H., Verstappen, T.J., and Janssen, E. Limitations of concentration measurements for evaluation of endocrine status of exercising women. *Canadian Journal of Sport Science*, 7(2):79-84, 1982.

Keizer, H.A., and Rogol, A.D. Physical exercise and menstrual cycle alterations What are the mechanisms? *Sports Medicine*, 10(4):218-235, 1990.

Kenagy, R., Weinstein, I., and Heimberg, M. The effects of 17 Beta estradiol and progesterone on the metabolism of free fatty acid by perfused livers from normal female and ovariectomized rats. *Endocrinology,* 108:1613-1621, 1981.

Kendrick, Z.V., Steffen, C.A., Rumsey, W.L., and Goldberg, D.I. Effect of estradiol on tissue glycogen metabolism in exercised oophorectomized rats. *Journal of Applied Physiology,* 63(2):492-496, 1987.

Kendrick, Z.V., and Ellis, G.S. Effect of estradiol on tissue glycogen metabolism and lipid availability in exercised male rats. *Journal of Applied Physiology*, 71(5):1694-1699, 1991.

Kim, H., and Kalkhoff, K. Sex steroid influence on triglyceride metabolism. *The Journal of Clinical Investigation,* 56:888-896, 1975.

Laaitikainen, T., Virtanen, T., and Apter, D. Plasma immunoreactive beta endorphin in exercise associated amenorrhea. *American Journal of Obstetrics and Gynecology.* 54:94-97, 1986.

Lamont, L. Lack of influence of the menstrual cycle on blood lactate. *The Physician and Sports Medicine,* 14(11):159-163, 1986.

Lavoie, J., Dionne, N., Helie, R., and Brisson, G.R. Menstrual cycle phase dissociation of blood glucose homeostasis during exercise. *Journal of Applied Physiology*, 62(3):1084-1089, 1987.

Lavoie, C., Peronnet, F., and Chiasson, J.L. Role of the sympathoadrenal system in the regulation of glycogen metabolism in resting and skeletal muscles. *Hormone Metabolism Research*, 24:266-271, 1992.

Linder, C., Chernick, S.S., Fleck, T.R., and Scow, R.O. Lipoprotein lipase and uptake of chlomicron triglyceride by skeletal muscle in rats. *Am. Journal of Phsiology*, 231:860-864, 1976.

Loucks, L.B., and Horvath, S.M. Exercise induced stress responses of amenorrheic and eumenorrheic runners. *Journal of Clinical Endocrinology and Metabolism,* 59:1109-1119, 1984.

Loucks, A.B., and Horvath, S.M. Athletic amenorrhea: A review. *Medicine and Science in Sport and Exercise,* 17(1):56-72, 1985.

Loucks, A.B., Mortola, J.F., Girton, L., and Yen, S.S.C. Alterations in the hypothalamic-pituitary-ovarian and the hypothalamic-pituitary-adrenal axis in athletic women. *Journal of Clinical Endocrinology and Metabolism*, 68:402-411, 1989.

Loucks, A.B. Effects of exercise training on the menstrual cycle: Existence and mechanisms. *Medicine and Science in Sports and Exercise*, 22(3):275-280, 1990a.

Loucks, A.B. Athletics and menstrual dysfunction in young women. In Perspectives in Exercise Science and Sports Medicine Volume 2. Edited by C.V. Gisolfi and D.R. Lamb, Benchmark press, Indianapolis, 1990b.

Lowry, O.H., and Passonneau, J.V., Flexible System of Enzymatic Analysis. New York:Academic Press, 1972.

Mandour, T., Kissebah, A.H., and Wynn, V. Mechanism of oestrogen and progesterone effects on lipid and carbohydrate metabolism: alteration in the insulin: glucagon molar ratio and hepatic enzyme activity. *European Journal of Clinical Investigation,* 7:181-187, 1977.

Martin, I.K., and Wahren, J. Glucose metabolism during physical exercise in patients with noninsulin-dependent (type II) diabetes. In Advances in Experimental Medicine

and Biology. Edited by C.G. Ostenson, S. Efendic and M. Vranic, Volume 334, Plenum Press, New York, 1993.

Matute, M.L., and Kalkhoff, R.K. Sex steroid influence on hepatic gluconeogenesis and glycogen formation. *Endocrinology*, 92:762-768, 1973.

McCartney, N., Spriet, L.L., Heigenhauser, G.J.F., Kowalchuk, J.M., Sutton, J.R., and Jones, N.L. Muscle power and metabolism in maximal intermittent exercise. *Journal of Applied Physiology*, 60:1164-1169, 1986.

McCracken, M., Ainsworth, B., and Hackney, A.C. Effects of the menstrual cycle phase on the blood lactate responses to exercise. *European Journal of Applied Physiology*, 69:174-175, 1994.

Mellits, E.D., and Cheek, D.B. The assessment of body water and fatness from infancy to adulthood. Monogram of Social Research in Child Development. 35,12-26, 1970.

Miles, J., Glasscock, R., Aikens, J., Gerich, J., and Haymond, M. A microflurometric method for the determination of free fatty acids in plasma. *Journal of Lipid Research*, 24:96-99, 1983.

Myerson, M., Gutin, B., Warren, M.P., May, M.T., Contento, I., Lee, M., Pi-Sunyer, F.X., Pierson, R.N., and Brooks-Gunn, J. Resting metabolic rate and energy balance in amenorrhiec and eumenorrheic runners. *Medicine and Science in Sport and Exercise*, 23(1):15-22, 1991.

Newsholme, E.A., and Leech, A.R. Biochemistry for the Medical Sciences. New York: Wiley, 1983.

Nicklas, B.J., Hackney, A.C., and Sharp, R.L. The menstrual cycle and exercise: Performance, muscle glycogen and substrate responses. *International Journal of Sports Medicine,* 10(4):264-269, 1989.

Noakes, T.D., and and Van Gend, M. Menstrual dysfunction in female athletes. *South Africa Medicine Journal*, 73:350-355, 1988.

Oscai, L.B., Essig, D.A., and Palmer, W.K. Lipase regulation of muscle triglyceride hydrolysis. *Journal of Applied Physiology*, 69:1571-1577, 1990.

Parker Jones, K., Ravniker, V.A., Tulchinsky, D., and Schiff, I. Comparison of bone density in amenorrheic women due to athletics, weight loss and premature menopause. *Obstetrics and Gynecology,* 66:5-8, 1985.

Passon, P.G., and Peuler, J.D. A simplified radiometric assay for plasma norepinephrine and epinephrine. *Anal. Biochem*, 51:618, 1973.

Passonneau, J.V. and Lavderdale, V.R. A comparison of three methods of glycogen measurement in tissues. *Analytical Biochemistry*, 60:404-412, 1974.

Powers, S.K. and Howley, E.T. Exercise Physiology: Theory and application to fitness and performance. Wm.C. Brown Publishers, Dubuque, 1990.

Prior, J.C., and Vigna, Y.M. The therapy of reproductive system changes associated with exercise training. In The Menstrual Cycle and Physical Activity edited by Puhl, J.L., and Brown, C.H. Human Kinetics Publishers, Inc, 1986.

Prior, J.C., Vigna, Y.M., Schulzer, M., Hall, J.E., and Bonen, A. Determination of luteal phase length by quantitative basal temperature methods: Validation against the mid cycle LH peak. *Clin. Invest. Med*, 13:123-131, 1990a.

Prior, J.C., Vigna, Y.M., Schechter, M.T., and Burgess, A.E. Spinal bone loss and ovulatory disturbances. *New England Journal of Medicine*, 323:1221-1227, 1990b.

Puah, J.A., and Bailey, C.J. Effect of ovarian hormones on glucose metabolism in mouse soleus muscle. *Endocrinology*, 117:1336-1340, 1985.

Randle, P.J., Garland, R.B., Hales, C.N., and Newsholme, E.A. The glucose-fatty acid cycle: Its role in insulin sensitivity and metabolic disturbances of diabetes mellitus. *Lancet,* 1:785-789, 1963.

Ravussin, E., Bogardus, C., Scheidegger, K., Lagrange, B., Horton, E. Effect of elevated FFA on carbohydrate and lipid oxidation during prolonged exercise in humans. *Journal of Applied Physiology*, 60(3):893-900, 1986.

Ren, J.M., and Hultman, E. Regulation of phosphorylase a activity in human skeletal muscle. *Journal of Applied Physiology*, 69(3):919-923, 1990.

Rennie, M.J., and Holloszy, J.O. Inhibition of glucose uptake and glycogenolysis by availability of oleate in perfused skeletal muscle. *Biochem. J.* 168:161-170, 1977.

Richter, E.A., Ruderman, N.B., and Schneider, S.H. Diabetes and exercise. *American Journal of Medicine*, 70:201-209, 1981a.

Richter, E.A., Sonne, B., Christensen, N.J., and Galbo, H. Role of epinephrine for muscular glycogenolysis and pancreatic hormone secretion in running rats. *American Journal of Physiology*, 240: E526-E532, 1981b.

Richter, E.A., Galbo, H., and Christensen, N.J. Control of exercise induced muscular glycogenolysis by adrenal medullary hormones in rats. *Journal of Applied Physiology*, 50(1):21-26, 1981c.

Richter, E.A., Garetto, L.P., Goodman, M.G., and Ruderman, N.B. Enhanced muscle glucose metabolism after exercise: Modulation by local factors. *American Journal of Physiology*, 246:E476-E482, 1984.

Richter, E.A., and Galbo, H. High glycogen levels enhance glycogen breakdown in isolated contracting skeletal muscle. *Journal of Applied Physiology*, 61(3):827-831, 1986.

Roades, R., and Pflanzer, R., Human Physiology. Sanders College Publishing, 1989.

Robertson, L.A., and Higgs, S.L. Menstrual cycle variations in physical work capacity, post exercise blood lactate and percieved exertion. *Canadian Journal of Applied Sport Science*, 8:220, 1983.

Rooney, T.P., Kendrick, Z.V., Carlson, J., Ellis, G.S., Matakevich, B., Lorusso, S.M., and McCall, J.A. Effect of estradiol on the temporal pattern of exercise-induced tissue glycogen depletion in male rats. *Journal of Applied Physiology*, 75(4):1502-1506, 1993.

Ruffin, M.T., Hunter, R.E., and Arendt, E.A. Exercise and secondary amenorrhea linked through endogenous opiods. *Sports Medicine*, 10(2):65-71, 1990.

Sahlin, K., Katz, A., and Broberg, S. Tricarboxylic acid cycle intermediates in human muscle during prolonged exercise. *American Journal of Physiology*, 259:C834-C841, 1990.

Sanborn, C.F., Albrecht, B.H., and Wagner, W.W., Athletic amenorrhea: Lack of association with body fat. *Medicine and Science in Sports and Exercise,* 19(3):207-212, 1987.

Sanborn, C.F., Martin, B.J., and Wagner, W.W. Is athletic amenorrhea specific to runners? *American Journal of Obstetrics and Gynecology,* 143:859-861, 1982.

Schwartz, B., Cumming, D.C., Riordan, E., Selye, M., Yen, S.S.C., and Rebar, R.W. Exercise associated amenorrhea: a distinct entity? *American Journal of Obstetrics and Gynecology,* 141:662, 1981.

Schweiger, Laessle, Schwiger, Hermann, F., Riedel, W., and Pirke, K. Caloric intake, stress, and menstrual function in athletes. *Fertility and Sterility,* 49(3):447-450, 1988.

Sinning, W.E., and Little, K.D. Body composition and menstrual function in athletes. *Sports Medicine,* 4:34-45, 1987.

Shangold, M.M. Exercise and the adult female: Hormonal and Endocrine effects. *Exercise and Sport Science Reviews*, 12:53-80, 1984.

Shangold, M.M., and Levine, H.S. The effect of marathon training upon menstrual function. *American Journal of Obstetrics and Gynecology*, 143:862-869, 1982.

Stager, J.M. Reversibility of Amenorrhea in athletes. *Sports Medicine*, 1:337-340, 1984.

Stephenson, L.A., Kolka, M.A., and Wilkerson, J.E. Perceived exertion and anaerobic threshold during the menstrual cycle. *Medicine and Science in Sports and Exercise*, 14(3):218-222, 1982a.

Stephenson, L.A., Kolka, M.A., and Wilkerson, J.E. Metabolic and thermoregulatory responses to exercise during the human menstrual cycle. *Medicine and Science in Sports and Exercise*, 14(4):270-275, 1982b.

Sutton, J.R., Jurowski, J.E., Keane, P., Walker, W.H.C., Jones, N.L., and Toews, C.J. Plasma catecholamines, insulin, glucose and lactate responses to exercise in relation to the menstrual cycle. *Medicine and Science in Sport and Exercise,* 12:83-84, 1980.

Sutton, J.R., Farrell, P.A., and Harber, V.J., Hormonal adaptation to physical activity. Exercise, Fitness, and Health. Human Kinetics Books, 1990.

Tan, M.H., Sata, T., and Havel, R.J. The significance of lipoprotein lipase in rat skeletal muscles. *Journal of Lipid Research*, 18:363-370, 1977.

Tarnopolsky, L.J., MacDougall, J.D., Atkinson, S.A., Tarnopolsky, M.A., and Sutton, J.R. Gender differences in substrate for endurance exercise. *Journal of Applied Physiology*, 68(1):302-308, 1990.

Terjung, R.L., Budohoski, L., Nazar, K., Kobryn, A., and Kaciuba-Uscilko, H. Chylomicron triglyceride metabolism in resting and exercising fed dogs. *Journal of Applied Physiology*, 52:815-820, 1982.

Terjung, R.L., Mackie, B.G., Dudley, G.A., and Kaciuba-Uscilko, H. Influence of exercise on chylomicron triacylglycerol metabolism: plasma turnover and muscle uptake. *Medicine and Science in Sport and Exercise*, 15:340-347, 1983.

Trussell, J. Menarche and fatness: reexamination of the critical body composition hypothesis. *Science*, Vol 200, 1978.

Turcotte, L.P., Hespel, P., and Richter, E.A. Circulating palmitate uptake and oxidation are not altered by glycogen depletion in contracting skeletal muscle. *Journal of Applied Physiology,* 78(4):1266-1272, 1995.

Van De Graff, K.M. and Fox, S.I. Concepts of Human Anatomy and Physiology. Third Edition. WmC. Brown Publishing, Dubuque, 1992.

Villee, C.A., Solomon, E.P., and Davis, P.W., Biology. CBS College Publishing, 1985.

Vϕllestad, N.K., and Blom, P.C.S. Effect of varying exercise intensity on glycogen depletion in human muscle fibres. *Acta Physiol. Scand*, 125:395-405, 1985.

Wahren, J., Felig, P., Ahlborg, G., and Jorfeldt, L. Glucose metabolism during leg exercise in man. *The Journal of Clinical Investigation*, Vol 50, 2715-2725, 1971.

Wahrenberg, H., Engfeldt, P., Bolinder, J., and Arner. P. Acute adaptation in adrenergic control of lipolysis during physical exercise in humans. *American Journal of Physiology, Endocrinology and Metabolism*, 16:E383-E390, 1987.

Wakat, D.K., Sweeney, K.A., and Rogol, A.O. Reproductive system function in women cross-country runners. *Medicine and Science in Sports Exercise*, 14(4):263-269, 1982.

Warren, M.P. The effect of exercise on pubertal progression and reproductive function in girls. *Journal of Clinical Endocrinology*, 51: 1150, 1980.

Warren, M.P. Amenorrhea in endurance runners. *Journal of Clinical Endocrinology and Metabolism*, 75(6):1393-1397, 1992.

Wasserman, D.H., and Whipp, B.J. Coupling of ventilation to pulmonary gas exchange during non steady-state work in men. *Journal of Applied Physiology*, 54(2):587-593, 1983.

Wells, C.L. Women sport and performance: a physiological perspective, 2nd edition. Human Kinetics books, Champaign, Illinois, 1991.

Williams, M.H., Nutritional aspects of human physical and athletic performance. Second Edition. Charles Thomas Publisher, Springfield, Illinois, USA, 1985.

Wilmore, J.H., and Costill, D.L., Training for sport and activity. Third Edition, Wm C. Brown Publishers, 1988.

Wilmore, J.H., Wambsgan, K.C., Brenner, M., Broeder, C.E., Paijmans, I., Volpe, J.A. and Wilmore, K.A. Is there energy conservation in amenorrheic compared to eumenorrheic distance runners? *Journal of Applied Physiology*, 72(1):15-22, 1992.

Yahiro, J., Glass, A.R., Fears, W.B., Ferguson, E.W., and Vigersky, R.A. Exaggerated gonadotropin response to luteinizing hormone-releasing hormone in amenorrheic runners. *American Journal of Obstetrics and Gynecology*, 156:586-591, 1987.

APPENDIX A

Subject Correspondence

SUBJECT INFORMATION SHEET

To delay the onset of fatigue in endurance events it is advantageous to prolong muscle glycogen depletion. Oestradiol (the female sex hormone) has been shown to increase muscle glycogen content and increase the availability of fat as a fuel source therefore delaying the onset of fatigue. Initially you will answer questionnaires in relation to your menstrual cycle, physical training , cardiovascular and blood sampling risk factors. All responses will be numerically coded and remain confidential. Measurements will be taken for height, weight, and body composition. Body composition will be determined by taking skin fold measurements at eight different sites. To assess your levels of aerobic fitness an incremental exercise test until exhaustion will be performed on a cycle ergometer.

The next two visits to the lab will consist of an exercise trial which will be performed under a placebo and treatment condition. It is very important that you arrive at the laboratory well rested and in a fasted state prior to each trial. In order to ensure this, you need to avoid strenuous exercise twenty four hours prior to the trial and refrain from eating breakfast. Dietary guidelines will be given for you to follow in the three days prior to each test and once again it is very important that they are adhered to. The exercise trial consists of cycling for two hours at 70% max VO_2. Prior to the exercise a small catheter will be placed into an arm vein. The catheter consists of a needle and teflon tubing. The tubing is fed over the top of a needle (which has punctured the vein) and into the vein. A tap (stopcock) is placed into the tubing so the flow of blood along the tubing can be altered at will. This procedure allows the taking of multiple blood samples without the need for multiple vena puncture (puncturing of the vein). Each time a blood sample is taken a small volume of sterile heparinised saline will be injected to clear the catheter and keep patent. Catheterisation of subjects is slightly discomforting and can lead to the possibility of bruising and infection. In our experience this occurs rarely. Prompt action will alleviate any serious medical problem.
In addition, before and after exercise we will take a small sample of muscle from the thigh muscle using a special needle biopsy technique. Two small incisions, one for each sampling time will be made in the skin overlying the muscle under a local anaesthetic by a doctor. A biopsy needle is inserted into the belly of the muscle and a piece of muscle about the size of a rice grain is removed. At this time you may experience a pressure in the muscle and a tendancy for the muscle to cramp but this lasts only a couple of seconds. The incision is closed with special closures and will heal over the next week. It is common to experience some local soreness in the next 24-48 hours. In extreme cases local bruising and swelling may occur; however, the use of sterile procedures and the application of an ice pack and pressure bandage minimises the chance of this.
During the exercise you will be wearing a mouthpiece/valves (similar to a snorkel) system to collect the air you have expired. We will also be monitoring your heart rate using a heart rate monitor.

The results of this study will provide important new information on the metabolic responses in ammenorrheic athletes when administered oestradiol during prolonged exercise and the possible implications for performance.

Could you please answer the attached questionnaires and if you are happy with everything sign the consent form. I remind you that you are free to withdraw from any phase of the study at anytime and that all your results are strictly confidential. Please dont hesitate to ask questions if you have any. Thank you for agreeing to assist with us in our research.

QUESTIONNAIRE

1. Name:

2. Address

Postcode:

3. Telephone No: (B) (H)

4. Date of Birth:

5. Are you currently using oral contraceptive pills?

6. Have you ever used oral contraception, if so for how long where you taking them for and how long has it been since you stopped taking it?

.....

.....

.....

7. How old were you when you first menstruated?

.....years.....months.

8. Would you describe your menstrual cycle as being regular (10-12 cycles per year)?

Yes [ ] No [ ]

9. If you answered no to question eight, how many times would you menstruate per year and how long has it been since you last menstruated?

.....

.....

.....

10. Have you ever had any irregularities with your menstrual cycle before you began athletic training? If yes, please explain.

.....

.....

.....

11. How old were you when you first began to train for trialthlons?

.....years.

12. What form of exercise were you participating in before triathalon training if any at all

.....

.....

.....

.....

13. How many hours are you currently training per week?

.....

14. What is the distance that you would cover on average during one weeks training?

Running.....km

Swimming.....km

Cycling.....km

15. Is there any other form of training eg. weights that you do each week? If so, what form does this training take and how much time is spent doing it?

.....

.....

.....

16. How many hours sleep do you get on average per night?

.....hours.

17. Describe a typical days diet including all food, beverages and quantites.

Breakfast:.....

.....

Lunch:.....

.....

Dinner.....

.....

.....

Snacks:.....

.....

HEALTH AND FITNESS SERVICES
RISK FACTOR ASSESSMENT QUESTIONNAIRE

NAME: ..... DATE: ..... ADDRESS: .....

AGE: ..... (Years) SEX: M/F ....

WEIGHT: ..... (kg) HEIGHT: ..... (cm) POSTCODE: .....

TELEPHONE: (Work) ..... (Home) ..... FAX: .....

MEDICAL HISTORY:

In the past have you ever had (tick No or Yes)

	NO	YES		NO	YES
Stroke	☐	☐	Congenital Heart Disease	☐	☐
Myocardial infarction (heart attack)	☐	☐	Disease of Arteries/Veins	☐	☐
Angina Pectoris	☐	☐	Asthma	☐	☐
Heart Murmur	☐	☐	Other Lung Disease (eg. emphysema)	☐	☐
Heart Rhythm Disturbance	☐	☐	Epilepsy	☐	☐
Rheumatic Fever	☐	☐	Injuries to back, knees, ankles	☐	☐

List any prescribed medications being taken

.....

.....

Other illness (Give details).....

.....

.....

ALLERGIES: Do you have any allergies NO ☐ YES ☐

If yes, give details: .....

SYMPTOMS DURING OR AFTER EXERCISE

As a result of exercise, have you ever experienced any of the following:

	NO	YES		NO	YES
Pain or discomfort in the chest, back, arms, or jaw	☐	☐	Palpitations (heart rhythm disturbance) or racing heart rate	☐	☐
Severe shortness of breath or problems with breathing during mild exertion	☐	☐	Pain in the legs during mild exertion	☐	☐
Dizziness, nausea or fainting	☐	☐	Severe heat exhaustion (ie heat stroke)	☐	☐

CARDIOVASCULAR RISK FACTORS:

Do you have (tick No, Yes or circle ?)	NO	YES	DON'T KNOW	
High Blood Pressure	☐	☐	?	
High Blood Cholesterol/Triglycerides	☐	☐	?	
Smoking Habit	☐	☐	Ex. Smoker	Average/day ...
Diabetes	☐	☐	?	
Do you drink alcohol regularly	☐	☐		Average/day ...

FAMILY MEDICAL HISTORY:

Have members of your immediate family ever had any of the following conditions: (tick No, Yes or circle ?).
If you answer Yes or ?, write beside this the member of the family affected (F = father, M = mother, B = brother, S = sister, GM = grandmother, GF = grandfather).

	NO	YES		FAMILY MEMBER	AGE (Years)	ALIVE NOW? (Y/N)
Myocardial infarction (heart attack)	☐	☐	?			
Angina Pectoris	☐	☐	?			
Stroke	☐	☐	?			
High Blood Pressure	☐	☐	?			
High Blood Cholesterol/Triglycerides	☐	☐	?			
Diabetes	☐	☐	?			
Cancer	☐	☐	?			

PERSONAL LIFESTYLE:

A. **Exercise**

List the sports, exercises or physically active hobbies (eg. gardening or playing with the kids) that you are currently engaged in:

Sports/ Activity	Day(s) of week Sa-Su-Mo-Tu-We-Th-Fr	Time of the day eg. 6 p.m.	Approx. duration eg. 30 minutes
		TOTAL	

B. **Nutrition**

List a typical day's eating pattern.

Breakfast	Lunch	Dinner	Snacks	Drinks

C. **Rest/Recreation**

How many hours sleep do you usually have? ..... hours.

On average how much time do you spend each day on passive hobbies or just relaxing ..... minutes/hours.

Do you feel that you usually get enough restful sleep and time to relax? Yes No

CLIENT DECLARATION

I declare that the above information is to my knowledge true and correct, and that I have not omitted any information that is requested on this form

S _____ 99

D

OFFICE USE ONLY

CLEARANCE TO UNDERGO AN EXERCISE TEST

This person has been cleared to undergo a fitness test:

☐ Without medical supervision

☐ With medical supervision

☐ A fitness test is not advisable at this time

Signed: Dr/Mr/Mrs/Ms .....

(Circle appropriate title: physician exercise physiologis

VICTORIA UNIVERSITY OF TECHNOLOGY

STANDARD CONSENT FORM FOR SUBJECTS
INVOLVED IN EXPERIMENTS

CERTIFICATION BY SUBJECT

I, ...
of.....
certify that I have the legal ability to give valid consent and that I am voluntarily giving my consent to participate in the experiment entitled :

"..... .....
....."

being conducted at Victoria University of Technology by :

.....

I certify that the objectives of the experiment, together with any risks to me associated with the procedures listed hereunder to be carried out in the experiment, have been fully explained to me by :

Miss Sally Clark

and that I freely consent to participation involving the use on me of these procedures.

Procedures

Maximal aerobic capacity testing
Blood Sampling by catherisation
Answering of Questionnaires
Underwater weighing technique
Muscle sampling by needle biopsy

I certify that I have had the opportunity to have my questions answered and that I understand that I can withdraw from the experiment at any time and that this withdrawal will not jeopardise me in any way.

I have been informed that the confidentiality of the information I provide will be safeguarded.

Signed : .....)
)
Witness other than the experimenter :) Date : .....
)
.....)

APPENDIX B

Description of Skinfold Sites
Borg Scale
Body Mass Index Equation

Skinfold Sites

Bicep - The skinfold is raised with the left thumb and index finger on the marked mid-acromiale-radiale line so that the fold runs vertically. The subject stands with the arm relaxed and extended, and the fold is located on the most anterior aspect of the surface of the right arm.

Tricep - The skinfold is raised with the left thumb and the index finger on the marked posterior mid-acromiale-radiale line. The fold is vertical and parallel to the line of the upper arm. The skinfold is taken on the most posterior surface of the arm over the triceps muscle when viewed from the side.

Subscapular - The left thumb palpates the inferior angle (apex) of the scapula and with the thumb still in position the index finger reaches over the top of the thumb which is then reposition to raise a skinfold just inferior to and slightly lateral to the apex. This fold runs obliquely downward toward the lateral aspect of the body.

Suprailiac - This skinfold is raised immediately superior to the iliac crest on the ilio-axilla line. Palpate the superior edge of the right ilium of the subject using the fingers of the right hand. Align the fingers with the ilio-axilla. Using the left hand raise the skinfold immediately above the right hand. The fold runs slightly downwards toward the medial aspect of the body.

Abdominal - This is a vertical fold raised 5 cm from the right hand of the mid point of the navel.

Thigh - The fold is raised parallel to the long axis of the femur at the mid-point of the distance between the inguinal fold and the superior anterior border of the patella.

Calf - With the subject seated a vertical fold is raised on the medial aspect of the calf at a level where it has maximal circumference.

BORG SCALE

6	
7	Very Very Light
8	
9	Very Light
10	
11	Fairly Hard
12	
13	Somewhat Hard
14	
15	Hard
16	
17	Very Hard
18	
19	Very Very Hard

Body Mass Index Equation

Body mass (kg) ÷ The square of the height (m)

APPENDIX C

Analytical Techniques

MUSCLE LACTATE DETERMINATION

Principle

Lactate + NAD^+ ----------> pyruvate + NADH + H^+

lactate dehydrogenase

Pyruvate + Hydrazine ----------> pyruvate hydrazone

MUSCLE GLYCOGEN DETERMINATION

Principle

Glucose + ATP ----------> ADP + G-6-P

hexokinase

G-6-P + NADP ----------> 6-P-Gluconolactone + NADPH

G-6-P dehydogenase

* 500 and 50μM lactate standards and 500 and 250μM glucose standards are used in the assay

* assays are run with a set of NADH standards (50, 100, 200 400μM)
- read absorbance of these on spectrophotometer at 340 nm and adjust concentration according to the MEC ($\times 10^{-3}$) (6220)
- record actual standard concentration and construct a standard curve (NADH concentration and fluorescence).

* if the metabolite standard fluorescence's are within 10% error of predicted standard fluorescence, the assay is successful.

BLOOD AND PLASMA LACTATE

Principle:- The enzyme lactate dehydrogenase (LDH) catalyses the transfer of hydrogen from the cofacter NADH to pyruvic acid to produce lactic acid. However, the equilibrium highly favors the reduction of pyruvic acid:

$$\text{Lactic acid} + NAD^{+} \xleftarrow{\text{LDH}} \text{Pyruvic acid} + NADH$$

$$Keq = 3.6 \times 10^{11}\ M^{-1}$$

In the presence of an excess of the cofactor NAD+ and lactate dehydrogenase activity, and at a pH of 9.6, nearly all of the lactate, is deproteinized whole blood, is converted to pyruvate. To ensure the reaction goes to completion, pyruvic acid, in the presence of hydrazine, is removed by converting it to pyruvatehydrazone. The appearance of NADH yields an observable method to quantify the concentration of lactate originally present. The concentration of NADH was measured spectrophotometrically at wavelength of 340 nm using a spectrophotometer (Shimadzu UV - 120-02) in conjunction with flow cell apparatus (Sigma Technical Bulletin, No 826 UV, Oct 1986).

A set of standards ranging in concentration from 1mM to 10mM was run with each anaylsis in conjunction with a reagent blank.

Calculations for Determining Metabolite Concentrations

Calculation for the metabolites is as follows:

$$\text{no. moles} = \frac{\text{sample fluorescence}}{\text{standard Fluores.}} \times \text{Std. Molarity(M)} \times \text{vol. of sample}$$

You must correct for the fact that you have 250 µl of extract one, which you then transferred 200µl to 50µl of $KHCO_3$ to make 250µl of extract two. You must also take into consideration the weight of the extract. Examples of a method for calculation is as follows:

CALCULATIONS TO DETERMINE MUSCLE GLYCOGEN CONCENTRATION

Extraction

A known weight of muscle was placed into 0.5 ml of 2M HCL. This was cooked for two hours. After cooking 1.5ml of 0.667M NaOH was added. Therefore, a known weight of muscle was extracted in 2 ml of fluid. For glycogen assay the standard was 0.5 mM glucose.

Calculations

10µl of standard contains $10 \times 10^{-6} \times 0.5 \times 10^{-3}$ moles of glucose and gives × fluorescence units (FU).

10µl of sample extract contains y moles and produces z FU. Therefore

$y = z / \times \times 10 \times 10^{-6} \times 500 \times 10^{-6}$ moles.

Muscle sample contains $y \times 200$ moles / weight of muscle.

Convert units to $mmol.kg^{-1}$ dry weight.

APPENDIX D

Individual Data

SUBJECT ONE												
	Control						Treatment					
Time	R1	R2	30	60	90	120	R1	R2	30	60	90	120
Physiological Parameters												
VO2	**	**	38.98	37.96	40.69	42.91	**	**	35.71	35.21	34.27	34.86
VCO2	**	**	2.19	2.19	2.19	2.28	**	**	1.94	2.05	1.87	1.85
RER	**	**	0.92	0.91	0.85	0.84	**	**	0.9	0.91	0.88	0.83
P.E.	**	**	13	14	14.5	15	**	**	12	13	13	13
H.R.	**	**	153	165	169	170	**	**	149	155	158	163
Plasma/Blood Metabolites												
Glucose (mmol/L)	4.9	4.77	5.4	4.98	4.76	4.04	4.57	4.6	5.42	5.59	5.24	4.59
Bld Lac (mmol/L)	1.43	1.44	1.82	1.58	1.45	1.76	1.7	1.67	2.08	2.11	2.13	2.05
Pla Lac (mmol/L)	1.79	1.82	2.26	2.09	2.22	2.02	2.24	2.35	2.9	2.59	2.43	2.59
FFA (mmol/L)	0.27	**	0.315	0.407	**	0.843	0.067	**	0.111	0.18	**	0.376
Hormones												
Adren (nmol/L)	0.13	**	**	0.42	**	0.72	0.03	**	**	0.14	**	0.18
Noradren (nmol/L)	0.36	**	**	3.81	**	6.55	0.07	**	**	1.68	**	1.65
Insulin (uU/ml)	2.548	2.585	2.782	1.982	1.367	out	2.709	2.846	3.27	2.737	2.121	0.807
Cortisol (nmol/L)	883.99	**	707	588.93	**	570.5	793.1	**	724.43	639.17	**	573.84
Oestradiol (pmol/L)	96.09	**	**	**	**	**	137.99	**	**	**	**	**
Prog (nmol/L)	1	**	**	**	**	**	0.6	**	**	**	**	**

SUBJECT TWO												
	Control						Treatment					
Time	R1	R2	30	60	90	120	R1	R2	30	60	90	120
Physiological Parameters												
VO2	**	**	34.31	35.54	34.38	35.07	**	**	37.49	40.06	40.08	40.65
VCO2	**	**	2.13	2.17	1.98	1.97	**	**	2.08	2.16	2.16	2.11
RER	**	**	0.96	0.94	0.89	0.87	**	**	0.86	0.84	0.84	0.8
P.E.	**	**	8	10	11	11	**	**	11	13	13	13
H.R.	**	**	167	172	167	170	**	**	158	166	168	168
Plasma/Blood Metabolities												
Glucose (mmol/L)	**	5.01	5.14	5.97	5.62	6.69	**	4.6	5.06	5.07	5.54	5.43
Bld Lac (mmol/L)	1.46	1.91	**	1.99	1.9	2.19	1.73	1.65	1.97	1.99	1.93	1.54
Plac Lac (mmol/L)	1.61	1.75	3.67	3.14	2	2.62	1.97	2	1.95	2.14	1.99	**
FFA (mmol/L)	0.157	**	0.172	0.226	**	0.513	0.215	**	0.172	0.27	**	0.678
Hormones												
Adren (nmol/L)	0.08	**	**	1.06	**	3.2	0.19	**	**	1.35	**	2.9
Noradren (nmol/L)	1.05	**	**	10.7	**	10.96	1.27	**	**	11.79	**	12.11
Insulin (uU/ml)	5.487	5.163	7.072	6.286	5.059	5.864	4.811	4.093	3.982	2.577	2.131	2.366
Cortisol (nmol/L)	574.359	**	667.23	789.04	**	1200.3	418.98	**	623.08	692.65	**	788.373
Oestradiol (pmol/L)	86.89	**	**	**	**	**	102.47	**	**	**	**	**
Prog (nmol/L)	0.5	**	**	**	**	**	0.5	**	**	**	**	**

SUBJECT THREE												
	Control						Treatment					
Time	R1	R2	30	60	90	120	R1	R2	30	60	90	120
Physiological Parameters												
VO2	**	**	35.42	35.6	35.8	40.85	**	**	40.65	40.88	43.43	41.37
VCO2	**	**	1.79	1.8	1.74	1.95	**	**	2.05	2.07	2.25	1.97
RER	**	**	0.84	0.84	0.81	0.8	**	**	0.84	0.85	0.87	0.8
P.E.	**	**	9	11	13	15	**	**	9	12	17	17
H.R.	**	**	137	140	148	152	**	**	143	152	160	159
Plasma/Blood Metabolites												
Glucose (mmol/L)	**	5.48	6.31	6.46	6.45	5.46	**	5.48	6.22	6.45	5.47	4.71
Bld Lac (mmol/L)	0.985	0.985	1.155	0.937	1.131	1.155	0.743	0.718	1.349	1.446	1.979	1.712
Plac Lac (mmol/L)	0.851	1.107	1.481	1.014	1.06	1.084	0.571	0.396	1.375	1.737	1.817	1.923
FFA (mmol/L)	0.367		0.537	0.472	**	1.21	0.583	**	0.433	0.483	**	0.648
Hormones												
Adren (nmol/L)	0.11	**	**	0.45	**	0.59	0.28	**	**	0.61	**	1.14
Noradren (nmol/L)	1.64	**	**	8.19	**	8.19	1.02	**	**	15.3	**	16.49
Insulin (uU/ml)	3.256	2.83	2.873	3.147	2.514	2.305	3.288	3.557	2.486	1.99	1.41	0.442
Cortisol (nmol/L)	561.65	**	420.11	452.29	**	552.18	564.42	**	582.12	583.685	**	735.66
Oestradiol (pmol/L)	72.52	**	**	**	**	**	100.47	**	**	**	**	**
Prog (nmol/L)	0.8	**	**	**	**	**	0.4	**	**	**	**	**
Muscle												
Gly (mmol/kg/dw)	394.5	**	**	**	**	68.61	720.51	**	**	**	**	48.48
Lac (mmol/kg/dw)	2.5	**	**	**	**	2.6	3.25	**	**	**	**	3.2

SUBJECT FOUR												
	Control						Treatment					
Time	R1	R2	30	60	90	120	R1	R2	30	60	90	120
Physiological Parameters												
VO2	**	**	28.49	27.17	27.93	29.36	**	**	23.09	23.55	25.99	25.38
VCO2	**	**	1.3	1.17	1.25	1.23	**	**	1.09	1.1	1.21	1.12
RER	**	**	0.91	0.85	0.88	0.83	**	**	0.96	0.94	0.94	0.89
P.E.	**	**	14	14	14	15	**	**	12	13	13	14
H.R.	**	**	157	151	149	160	**	**	153	147	145	151
Plasma/Blood Metabolites												
Glucose (mmol/L)	**	5.41	5.9	6.34	4.84	4.75	**	5.54	5.19	5.31	4.99	5.44
Bld Lac (mmol/L)	1.276	1.3	9.833	6.415	6.439	5.542	2.658	3.167	6.391	4.5	3.918	4.136
Plac Lac (mmol/L)	1.64	1.61	12.41	9.14	7.26	4.965	3.39	3.39	8.826	6.588	4.64	4.594
FFA (mmol/L)	0.224	**	0.146	0.143	**	-0.0087	0.283	**	0.167	0.291	**	0.728
Hormones												
Adren (nmol/L)	0.08	**	**	0.8	**	1.49	0.22	**	**	0.55	**	0.63
Noradren (nmol/L)	1.18	**	**	5.79	**	6.19	1.14	**	**	4.9	**	5.24
Insulin (uU/ml)	2.916	3.469	4.223	4.21	1.749	0.835	4.579	6.303	4.607	5.173	3.354	2.826
Cortisol (nmol/L)	533	**	754.11	703.09	**	705.19	669.23	**	773.033	614.777	**	548.246
Oestradiol (pmol/L)	81.35	**	**	**	**	**	114	**	**	**	**	**
Prog (nmol/L)	1.04	**	**	**	**	**	1.46	**	**	**	**	**

SUBJECT FIVE												
	Control						Treatment					
Time	R1	R2	30	60	90	120	R1	R2	30	60	90	120
Physiological Parameters												
VO2	**	**	30.51	32.79	28.35	30.1	**	**	26.6	**	28.61	29.32
VCO2	**	**	1.64	2.16	1.85	1.96	**	**	1.76	**	1.84	1.84
RER	**	**	0.94	0.92	0.93	0.91	**	**	0.91	**	0.88	0.86
P.E.	**	**	12	15	16	16	**	**	12	12	13	14
H.R.	**	**	153	165	166	168	**	**	148	160	153	150
Plasma/Blood Metabolites												
Glucose (mmol/L)	**	5.11	5.45	5.69	4.79	4.44	**	5.19	5.47	4.59	4.8	4.34
Bld Lac (mmol/L)	1.276	1.3	1.834	1.591	1.64	1.591	1.737	1.155	1.058	1.64	1.567	1.494
Plac Lac (mmol/L)	1.349	1.664	2.488	2.221	2.124	2.221	1.446	1.47	2.633	2.173	2.027	1.93
FFA (mmol/L)	0.315	**	0.644	0.924	**	1.79	0.508	**	0.648	0.867	**	1.35
Hormones												
Adren (nmol/L)	0.05	**	**	0.48	**	1	0.06	**	**	0.42	**	0.4
Noradren (nmol/L)	1.2	**	**	9.15	**	7.35	0.59	**	**	7.7	**	6.05
Insulin (uU/ml)	5.08	**	5.67	5.4	4.39	3.82	4.4	**	5.56	4.38	4.11	5.33
Cortisol (nmol/L)	391.78	**	773.69	630.93	**	765.14	599.834	**	434.44	565.44	**	511.743
Oestradiol (pmol/L)	91.45	**	**	**	**	**	165.12	**	**	**	**	**
Prog (nmol/L)	2.3	**	**	**	**	**	0.8	**	**	**	**	**
Muscle												
Gly (mmol/kg/dw)	451	**	**	**	**	72.9	401.69	**	**	**	**	136.6
Lac (mmol/kg/dw)	4.35	**	**	**	**	4.7	3.5	**	**	**	**	4.7

Subject One

FILING INSTRUCTION Jul 15 1993 4:24 pm Replaces previous Page 1 /93

Year: 1993
Date: 28,06
Time: 1525

Plasma,serum,blood								Units	Range
Prolactin	37	-	-	-	-	-	-	mIU/L	See Below
Progestrone	4.7	-	-	-	-	-	-	nmol/L	See Below
Oestradiol	80	-	-	-	-	-	-	pmol/L	See Below

Prolactin Reference Range from 1/9/1989.
Normal 65 - 455 Raised >620 mIU/L.

Progesterone Reference Range :

Female	Follicular	1 - 4 nmol/L
	Peri-Ovulatory	2 - 15 nmol/L
	Luteal	11 - 55 nmol/L
	Mid Luteal	20 - 57 nmol/L
	Pregnancy(6-14Wk)	30 -140 nmol/L
	Post menopausal	0 - 3 nmol/L
Male		2 - 6 nmol/L

New Oestradiol Reference Range from 1/1/1993

Female	Follicular	30 - 600 pmol/L
	Luteal	35 - 700 pmol/L
	Post-Menopausal	<60 pmol/L
Male		60 - 200 pmol/L

E2 levels New assay approximately 0.66xOld assay.

Directors : Drs.F.Alford & G.Ward Phone : 288-3576 Signed :

179-2034 - - - - - -

Subject Two

IG INSTRUCTION Feb 1 1994 11:23 am Single report

: 215-2019 Serum Date: 03,08,1993 Time 1110 HR.

t	Result	Units	Ref. Range
Prolactin	116	mIU/L	(See Below)
Follicle Stimulating Hormone	5.1	mU/mL	(See Below)
Luteinizing Hormone	3.6	mU/mL	(See Below)
Oestradiol	56	pmol/L	(See Below)

Prolactin Reference Range from 1/9/1989.
Normal 65 - 455 Raised >620 mIU/L.

GONADOTROPHINS	REF.RANGE	FSH mU/ml	LH mU/ml
Follicular	(F)	2.3 - 8.8	2.2 - 6.2
Luteal	(F)	1.2 - 5.5	0.9 - 5.3
Post Menopause	(F)	>= 12	>= 7.4
Male		1.2 -11	0.9 - 9.0

New Oestradiol Reference Range from 1/1/1993

Female	Follicular	30 - 600 pmol/L
	Luteal	35 - 700 pmol/L
	Peri-Menopausal	<200 pmol/L
	Post-Menopausal	<60 pmol/L
Male		60 - 300 pmol/L

The interpretation of isolated Oestrogen levels in the absence of corresponding gonadotrophin levels can be misleading.

irectors : Drs.F.Alford & G.Ward Phone : 388-3576 Signed :

Subject Three

FILING INSTRUCTION Oct 13 1993 9:43 am Replaces previous Page 1 /93

Year:	1993		
Date:	11,10		
Time:	1500		
Plasma,serum,blood		Units	Range
Prolactin	56	mIU/L	See Below
FSH	4.3	mU/mL	See Below
Lut.Hormone	3.0	mU/mL	See Below
Oestradiol	36	pmol/L	See Below

Prolactin Reference Range from 1/9/1989.
Normal 65 - 455 Raised >620 mIU/L.

GONADOTROPHINS	REF.RANGE	FSH mU/ml	LH mU/ml
Follicular	(F)	2.3 - 8.8	2.2 - 6.2
Luteal	(F)	1.2 - 5.5	0.9 - 5.3
Post Menopause	(F)	>= 12	>= 7.4
Male		1.2 -11	0.9 - 9.0

New Oestradiol Reference Range from 1/1/1993

Female	Follicular	30 - 600	pmol/L
	Luteal	35 - 700	pmol/L
	Peri-Menopausal	<200	pmol/L
	Post-Menopausal	<60	pmol/L
Male		60 - 200	pmol/L

The interpretation of isolated Oestrogen levels in the absence of corresponding gonadotrophin levels can be misleading.

Directors : Drs.F.Alford & G.Ward Phone : 288-3576 Signed :

284-2030

Subject Four

IG INSTRUCTION Feb 1 1994 11:30 am Single report

: 330-2022 Serum Date: 26,11,1993 Time 1310 HR.

t	Result	Units	Ref. Range
Prolactin	179	mIU/L	(See Below)
Follicle Stimulating Hormone	7.4	mU/mL	(See Below)
Luteinizing Hormone	4.1	mU/mL	(See Below)
Oestradiol	105	pmol/L	(See Below)

Prolactin Reference Range from 1/9/1989.
Normal 65 - 455 Raised >620 mIU/L.

GONADOTROPHINS	REF.RANGE	FSH mU/ml	LH mU/ml
Follicular	(F)	2.3 - 8.6	2.2 - 6.2
Luteal	(F)	1.2 - 5.5	0.9 - 5.3
Post Menopause	(F)	>= 12	>= 7.4
Male		1.2 -11	0.9 - 9.0

New Oestradiol Reference Range from 1/1/1993

Female	Follicular	30 - 600	pmol/L
	Luteal	35 - 700	pmol/L
	Peri-Menopausal	<200	pmol/L
	Post-Menopausal	<60	pmol/L
Male		60 - 200	pmol/L

The interpretation of isolated Oestrogen levels in the absence of corresponding gonadotrophin levels can be misleading.

irectors : Drs.F.Alford & G.Ward Phone : 288-3576 Signed :

Subject Five

Oct 19 1993 9:41 am Single report

SID: 287-2002 Serum Date: 14,10,1993 Time 0915 HR.

Test	Result	Units	Ref. Range
Prolactin	81	mIU/L	(See Below)
Follicle Stimulating Hormone	7.2	mU/mL	(See Below)
Luteinizing Hormone	4.0	mU/mL	(See Below)
Oestradiol	61	pmol/L	(See Below)

Prolactin Reference Range from 1/9/1989.
Normal 65 - 455 Raised >620 mIU/L.

GONADOTROPHINS	REF.RANGE	FSH mU/ml	LH mU/ml
Follicular	(F)	2.3 - 8.8	2.2 - 6.3
Luteal	(F)	1.2 - 5.5	0.9 - 5.3
Post Menopause	(F)	>= 12	>= 7.4
Male		1.2 -11	0.9 - 9.0

New Oestradiol Reference Range from 1/1/1993

Female	Follicular	30 - 600 pmol/L
	Luteal	35 - 700 pmol/L
	Peri-Menopausal	<200 pmol/L
	Post-Menopausal	<60 pmol/L
Male		60 - 200 pmol/L

The interpretation of isolated Oestrogen levels in the absence of corresponding gonadotrophin levels can be misleading.

Directors : Drs.F.Alford & G.Ward Phone : 288-3576 Signed :