Hormonal responses in women as a function of physical exercise and training

Sportwetenschappelijke Onderzoekingen 4

HORMONAL RESPONSES IN WOMEN AS A FUNCTION OF PHYSICAL EXERCISE AND TRAINING

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CHAPTER VI

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Exercise-induced changes in estradiol metabolism and their possible physiological meaning. Med. Sport 14: 125-140, Karger, Basel, 1981.

Keizer, H.A., Kuipers, H., Verstappen, F.T.J. and E. Janssen.
Limitations of concentration measurements for evaluation of endocrine status of exercising women.
Can. J. Appl. Sport Sci. 7: 79-84, 1982.

ABBREVIATIONS

ACTH = adrenocorticotropic hormone
Δ4-A = androstenedione
BBT = basal body temperature
DHEA = dehydroepiandrosterone
DHEA-S = dehydroepiandrosterone sulfate
dpm = desintegrations per minute
E_2 = estradiol
GnRH = gonadotropin releasing hormone
FSH = follicle stimulating hormone
IU = international unit
K_{ass} = affinity constant
LH = Luteinizing hormone
M = molar
MCR = metabolic clearance rate
mmol = millimol (10^{-3}mol)
nmol = nanomol (10^{-9}mol)
μmol = micromol (10^{-6}mol)
pmol = picomol (10^{-12}mol)
MPWC = maximal physical working capacity
PRL = prolactin
SHBG = sex hormone binding globulin
T = testosterone
TRH = thyrotropin releasing hormone
$\dot{V}O_2max$ = maximal oxygen uptake

CHAPTER 1

INTRODUCTION

It is only since the early seventies that women are allowed to compete in athletic activities associated with intense physical exercise. Until then it was thought that such activities as marathon running and cross-country skiing were harmful to the female body although direct evidence for this idea was lacking. As a result of the growing interest to participate in such demanding types of exercise, the volume and intensity of the training of women increased tremendously, improving their performance level. Consequently the time devoted to training in elite class women athletes is not different from their male counterparts. Despite these alterations, there still remains a significant difference between the absolute performance of male and female athletes, probably due to hormonal, social and cultural factors. Also the level of selection and competition might play a role (Harris, 1977; Wells, 1977).

Parallel to the increased participation in various types of athletic activities in women a growing scientific interest in responses to exercise can be observed. Especially the relation between physical exercise and the changes in plasma concentration of sex hormones - induced by the menstrual cycle itself or by physical exercise - receives more and more attention. Several reasons can be given for this growing scientific interest. Firstly, the influence of the phase of the menstrual cycle on maximal physical performance and related physiological parameters is still controversial. Secondly, the exercise induced changes in sex hormone levels might be responsible for menstrual cycle disturbances.

- The phase of the menstrual cycle and physical performance.

It has been suggested that in women maximal athletic performance is dependent on the phase of the menstrual cycle, although conflicting results are reported about this relation. Several authors suggested a post-menstrual, pre-ovulatory performance optimum and a decreased maximal performance in the pre-menstrual or luteal phase (Millahn and Drecoll, 1960; Pahlke and Smitka, 1977; Schoene et al, 1981). Others showed an increased

physical performance in the luteal phase as compared to the follicular phase (Muller-Limroth and Lohaus, 1963; Jurkowski et al, 1978). To complete the confusion several authors could n't find any effect of the phase of the menstrual cycle on maximal physical performance (Drinkwater, 1973; Verstappen et al, 1981; Kuipers, 1983).

Maximal physical performance in prolonged physical exercise is not only dependent on the maximal oxygen uptake ($\dot{V}O_2max$) of a subject, but also on the blood lactate response in relation to the workload and substrate turn-over in skeletal muscle.

Since the blood concentrations of estradiol-17β (E_2) and progesterone (P) shift markedly during the menstrual cycle their normal physiological alterations may possibly affect substrate utilization. In women this has been suggested by several authors (Merimee et al, 1969; Reinke et al, 1972; Morrow et al, 1981). Such differences may become more apparent during exercise, when large alterations in substrate turn-over occur to meet the increased metabolic demands of skeletal muscle. The scarce data dealing with this topic are conflicting. Schwantiz and co-investigators (1978) reported a higher plasma glucose concentration in the luteal phase as compared to the follicular phase in young women during a bicycle ergometer ride. On the other hand, Bonen and co-investigators (in press) could not find any effect at all of the phase of the menstrual cycle on the blood glucose response to exercise in young women.

Data on exercise-induced changes in blood lactate in relation to the phase of the menstrual cycle are very scarce. Jurkowski and co-investigators (1978) reported a higher blood lactate content during submaximal and maximal exercise in the follicular phase of the menstrual cycle as compared to the luteal phase in normally menstruating women. On the contrary Verstappen and co-investigators (1981) and Kuipers (1983) did not observe any difference between the phases of the cycle.

The experimental design of most of the afore mentioned studies i.e. the lack of data on the plasma concentration of ovarian steroid hormones and the wide variety of exercise protocols used, makes it impossible to draw definite conclusions regarding the relation between the phase of the menstrual cycle, the glucose and lactate responses and maximal athletic performance. If it is true that the phase of the menstrual cycle indeed can affect maximal physical performance and/or the response of physiological

variables to exercise, it is tempting to speculate that the associated alterations in circulating sex-hormone levels might be responsible for this phenomenon.

- Exercise-induced changes in plasma sex hormone levels.

Recently it has been shown that physical exercise can be a powerful stimulus for increasing the circulating levels of sex hormones. For example, exercise induced increments of estradiol-17β (E_2) and progesterone (P) have been observed in young untrained women (Jurkowski et al, 1978; Bonen et al, 1979). The magnitude of these increments seems to be dependent on the phase of the menstrual cycle in which the exercise was performed; it was reported to be most pronounced in the luteal phase (Juwkowski et al, 1978; Bonen et al, 1979). In contrast to trained women (see below) no data are available on changes in androgen concentration as a result of physical exercise in untrained women. Yet the influence of the phase of the menstrual cycle on androgen responses to exercise is unknown.

It is incompletely understood whether the hormonal responses to physical exercise are different in trained and untrained women. Bonen and co-investigators (1979) did not find an exercise-induced increase in plasma E_2 levels in women after a training period of three months. However, these findings seem to be in contrast with data from Schmitt and co-investigators (1981) who found distinct increments in plasma E_2 levels after a marathon run in trained women. The plasma testosterone (T) levels are reported to rise during exercise in trained women (Shangold et al, 1981; Baker et al, 1982). During exercise the concentration of some hormones, for example prolactin (PRL), was found to rise more pronounced in trained than in untrained women (Brisson et al, 1980; Shangold et al, 1981).

Detailed information about the possible origin of the exercise-induced increments in plasma hormone concentration or the variability in the hormonal response to exercise in different phases of the menstrual cycle is not available due to the scanty number of investigations and the small number of subjects participating in these studies.

Interpretation of the afore mentioned data is difficult because the total plasma concentration of a hormone is the net result of production and metabolic clearance. The plasma E_2 concentration in young women for example results from secretion by the ovaries and to a minor extent by the adrenal

cortex, peripheral conversion of androgens and degradation which takes place in hepatic and extra-hepatic tissues. Thus, an enhanced plasma concentration can be attributed to increased glandular secretion, decreased degradation, increased peripheral conversion or combinations of these three mechanisms.

An increased secretion rate of sex hormones by the ovaries is likely to result from stimulation by the gonadotropins: the follicle stimulating hormone (FSH) and luteinizing hormone (LH), which are secreted by the anterior pituitary gland. From the literature it is not clear whether the pituitary is indeed involved in the increased plasma concentration of sex hormones during exercise, because LH concentrations were reported to be unaffected (Jurkowski et al, 1978; Bonen et al, 1979), increased (Bonen et al, in press) or decreased (Brisson et al, 1981) under these circumstances. In addition no data are available about the effect of physical exercise on the episodic secretion pattern of gonadotropin releasing hormone (GnRH), LH and FSH.

For T the situation is even more complicated, because in women a considerable part of the plasma concentration of this hormone is derived from adrenal secretion (Abraham, 1974). However, data on the adrenal contribution to the plasma concentration of androgen during exercise are lacking. Also the exercise-induced changes in degradation or conversion rate of ovarian and adrenal steroids have not been investigated until now.

Another complicating factor concerning the interpretation of changes in hormone concentrations is that they do not necessarily represent the biological activity. The major part of the circulating plasma steroids is bound to specific proteins, which render them inactive (Pearlman et al, 1967; Kato and Horton, 1968; Vermeulen, 1973). The free fraction and/or the fraction that is bound to proteins with low binding affinity is responsible for the biological activity. Physical exercise does not only increase the concentration of sex hormones, but also body temperature. Consequently this might change the biological activity of hormones, because the dissociation rate is temperature dependent (Vermeulen and Verdonck, 1968; Vigersky et al, 1979; Lata et al, 1980). In addition, a rise in free concentration tends to increase the degradation rate (Vermeulen and Ando, 1979).

In summary it appears from the afore mentioned studies that comprehensive knowledge of the hormonal responses to exercise in women is lacking.

Yet, the underlying mechanisms for the exercise-induced increments in sex-hormone levels are incompletely understood. More detailed knowledge is of interest because:

- it is well recognized that the incidence of menstrual cycle disorders is significantly higher in athletes than in control-subjects (Dale et al, 1979; Speroff and Redwine, 1980; Baker et al, 1981).
- it is not known whether a decreased metabolic clearance rate or an increased ovarian and/or adrenal secretion rate is responsible for the observed increases in circulating levels of sex hormones.

The objectives of the present study were:

1 to evaluate the influence of the phase of the menstrual cycle, if any, on maximal physical performance and related physiological parameters.

2 to collect more detailed information about the hormonal responses to exercise in relation to the phase of the menstrual cycle in women with various levels of physical fitness. This aspect of the study was aimed at setting a valid hypothesis for the origin of the exercise-induced changes in sex hormone levels.
This may also lead to a better insight into the reported menstrual cycle disturbances in women-athletes.

3 to differentiate between changes in degradation rate (measured as the metabolic clearance rate) and production rate as a possible cause of the exercise-induced increments in sex-hormone levels.

The effects of the phase of the menstrual cycle on physical performance was investigated in three studies (I to III). In study I 19 untrained students volunteered, while in study II 6 highly trained marathon runners participated. In study III the influence of a training period on maximal physical performance was evaluated in 8 previously untrained women. In these studies also the hormonal responses to prolonged physical exercise were investigated.

The possible mechanisms of the increases in plasma hormone concentrations were investigated in study IV and V. In study IV, the post-exercise responses of gonadotropin and E_2 pulsatile secretion patterns were compared to the pre-exercise period in 7 women in the follicular phase of their menstrual cycle. In study V the degradation rate of E_2, measured as the metabolic clearance rate (MCR), in relation to the workload was investigated in 15 physical education students in the follicular phase of their menstrual

cycles.

The motivation of the choice of various hormones measured in this investigation is given in chapter II.

CHAPTER II

SYNTHESIS, SECRETION, TRANSPORT, CONVERSION AND DEGRADATION OF STEROIDS

2.1 INTRODUCTION

Throughout reproductive life the normal human ovary is capable of synthesizing estrogens, progestins, and androgens. However, the secretion rate and consequently the blood concentration of estrogens and progestins fluctuates markedly according to the phase of the menstrual cycle. On the other hand, the plasma concentration of sex steroids does not simply reflect glandular (ovarian) and/or adrenal secretion. As discussed in chapter I, the plasma concentration is determined by production from different sources (ovaries and adrenals), peripheral conversion and degradation. This makes deductions about glandular secretion rates from single concentration measurements difficult, if not impossible.

Because the afore mentioned mechanisms are closely related and, in addition controlled by neuro-endocrine processes, a short description of the processes related to changes in plasma concentrations of steroids during the menstrual cycle can be considered to be appropriate. Therefore, these aspects will be discussed in the following sections.

2.2 GLANDULAR STEROIDOGENESIS

All steroid hormones are basically of similar structure with relatively minor chemical differences. Nevertheless, these slight differences are responsible for a striking variation in biologic activity. The sex steroids are divided into three main groups according to the number of carbon atoms. Corticoids and progestins are derived from a C-21 skeleton, the pregnane nucleus. The C-19 series includes all the androgens and is based on the androstane nucleus, whereas the estrogens are derived from the estrane nucleus, a C-18 skeleton. Cholesterol serves as a common precursor for the biosynthesis of the steroid skeleton of these steroids (fig 2.1). The rate limiting step in steroidogenesis is the conversion of cholesterol to preg-

nenolone, which is stimulated in the ovary by LH and in the adrenal cortex by adreno corticotropic hormone (ACTH). During steroidogenesis the number of carbon atoms in cholesterol or any other steroid molecule can be reduced, but never increased. In contrast to the adrenal gland which is capable of synthesizing all classes of steroids, the ovary is deficient of 21-hydroxylase and 11-beta-hydroxylase enzymes. Consequently the ovary is unable to produce glucocorticoids and mineralocorticoids. A simplified scheme of the major biochemical pathways involved in androgen and estrogen synthesis is depicted in fig 2.1.

2.2.1 Ovarian steroidogenesis.

The human ovary is capable of synthesizing all three classes of sex steroids dependent on the phase of the menstrual cycle. The selection of specific biochemical pathways leading to synthesis of estrogens, androgens or progestins is not a chance event but a matter of cell types involved or, in other words the stage of follicle development. Therefore, a short description of the different phases of the menstrual cycle, the development of the follicle in relation to blood-levels of sex hormones, sites of hormone production and regulation of these phenomena will be given.

2.2.1.1 Phases of the menstrual cycle.

The length of the menstrual cycle is the difference between two dates; the onset of the previous and the onset of the recent menstruation. The median length of the menstrual cycle in women with a chronological age between 20 and 25 years (the age of the majority of the subjects who volunteered in the present study) is reported to be about 28 days, with 5th and 95th percentiles of 22 and 42 days respectively (Treloar et al, 1967; Vollman, 1977).

The menstrual cycle can be divided into two distinct phases:

- the follicular phase or the interval between the onset of bleeding and the ovulation. Its length can be extremely variable. In women (chronological age 20-30 yrs), the median length of the follicular phase is reported to be about 13 and 22 days in cycles with a length of 25 and 35 days, respectively (Vollman, 1977). In the early part of the follicular phase, the blood concentration of sex steroids is derived from a large pool of small follicles, but in the next part (mid and late follicular phase) it

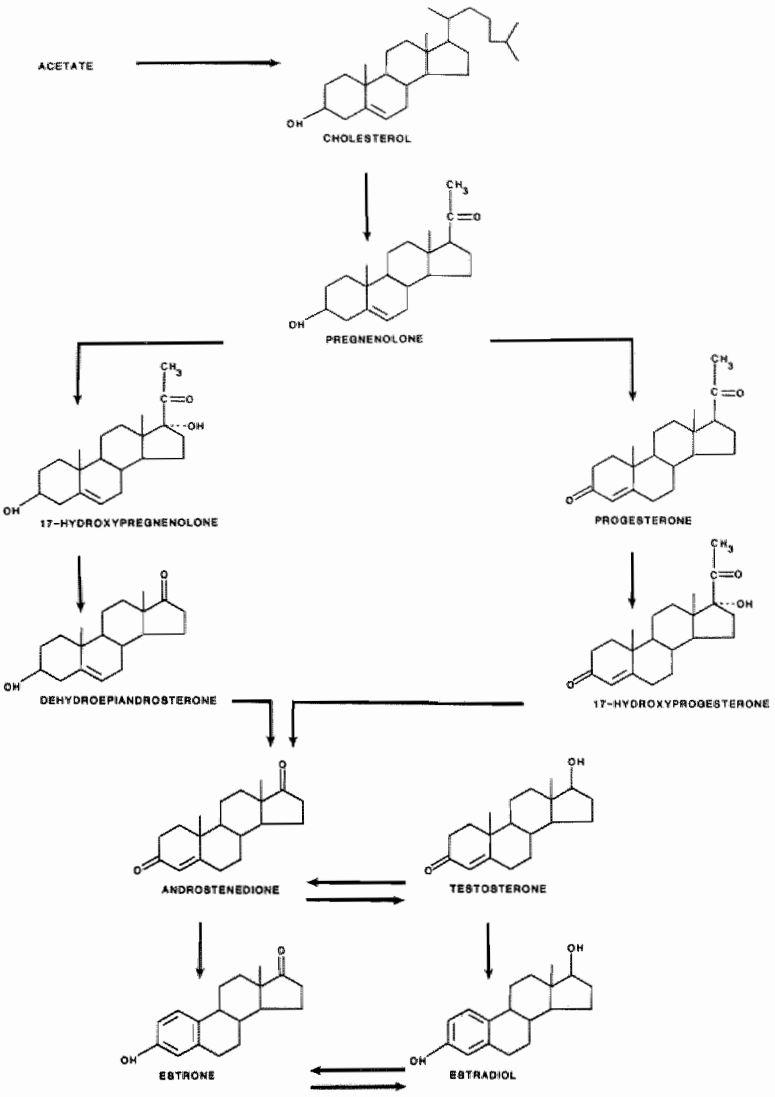


Figure 2.1

A simplified scheme of the major biochemical pathways involved in the synthesis of sex hormones.

is actually the dominant follicle that determines the phase of the cycle by its hormone production and hence the blood concentration.

- the luteal phase or the interval between ovulation and onset of the next menses. In the normal menstrual cycle its duration is remarkably constant, i.e. 12-14 days.

The ovulation itself is a very short event as revealed by ultrasonographical studies (Wetzels, 1983). Preceding the ovulation some hormonal changes occur, the most conspicuous being the LH surge, which takes place about 1 day before the ovulation.

The follicular and luteal phases can be subdivided into an early, mid and late phase.

The hormone concentrations in peripheral blood fluctuate with different frequencies. In principal three types of secretion patterns may be distinguished:

- low-frequency changes, which represent changes in the mean daily plasma hormone concentration during the menstrual cycle. These changes are called "trigintan" or "circatrigintan" (Williams, 1981) and reflect the well-known cyclic changes in plasma levels of sex steroids during the menstrual cycle (fig. 2.2).
- high-frequency changes, superimposed on the low-frequency changes. To date it is well recognized that many hormones including LH, FSH, E_2, PRL and P are secreted in a characteristic pulsatile pattern (Yen et al, 1972 a and b; Santen and Bardin, 1973; Korenman and Sherman, 1973; Lenton et al, 1978; Lenton et al, 1979; Backstrom et al, 1982). These changes have been called "circhoval" since they repeat themselves approximately every hour (Knobil, 1974).
- changes of intermediate frequency, called "diurnal or circadian" because they recur every 24 hours. In women, diurnal changes are found for LH, FSH, E_2 and PRL (Backstrom et al, 1982).

In the next paragraphs these low, intermediate and high-frequency changes of the blood concentrations of ovarian and adrenal sex steroids and gonadotropins will be briefly discussed.

2.2.1.2 Changes in plasma sex hormone levels during the normal menstrual cycle.

Gonadotropins

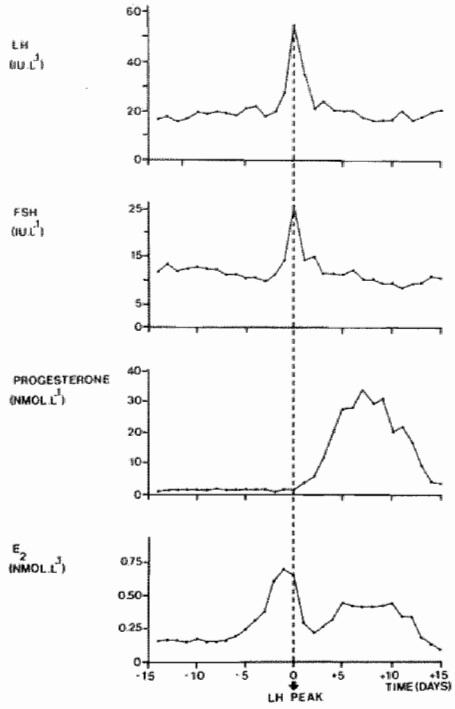
High frequency changes in plasma concentration of gonadotropins are due to changes in the episodic secretion pattern of gonadotropin releasing hormone (GnRH) (Yen et al, 1972 a and b; Santen and Bardin, 1973; Shaw, 1978). In women, the frequency and magnitude of the gonadotropin pulses vary both between and within individuals (with the phase of the menstrual cycle). The frequency of the LH pulses has been reported to increase significantly from the early follicular to the late follicular phase of the cycle (from about one pulse per 2 h to 1½-2 per 2 h). During the luteal phase this frequency is much lower than during any stage of the follicular phase (Yen et al, 1972 a and b; Shaw et al, 1974; Shaw, 1978; Lenton et al, 1979; Backstrom et al, 1982). The pattern of changes of the plasma FSH concentration has been reported to be very similar to that of LH throughout the menstrual cycle (Yen et al, 1972 a and b; Backstrom et al, 1982). The number of pulses of FSH increases from about one per 3 hours in the early follicular phase to about 1-2 per 2 hours in the mid and late follicular phases, while it drops to very low levels in the luteal phase (Backstrom et al, 1982). It is believed that during spontaneous cycles the varying LH and FSH levels result from complex positive and negative feedback actions of ovarian sex steroids on hypothalamic GnRH secretion and the pituitary cell responses to this hormone (Shaw, 1978). The cyclic changes and normal values of plasma gonadotropin levels are depicted in fig. 2.2. From this graph it can be seen that the LH concentration rises slightly during the follicular phase, followed by a pronounced increase during the mid-cycle surge. Thereafter it declines sharply, followed by a slight decrease during the luteal phase. The mean FSH levels begin to rise in the late luteal phase, but show a gradual decrease during the follicular phase, followed by a modest mid-cycle peak, preceding ovulation. Thereafter a gradual decline is seen during the early and mid-luteal phases.

Estradiol-17β (E_2).

In women the concentration of E_2 in peripheral blood fluctuates from hour to hour (Korenman and Sherman, 1973; Lenton et al, 1978; Backstrom et

al, 1982). In most cases (about 80%) the LH pulses are followed by a pronounced

Figure 2.2
Cyclic variations in plasma luteininizing hormone (LH), follicle stimulating hormone (FSH), progesterone (P) and estradiol-17β (E_2) during the menstrual cycle.



change in plasma E_2 concentration within 50 min (Backstrom et al, 1982). The magnitude and frequency of the E_2 pulses has been reported to fluctuate with the phase of the menstrual cycle; in the early follicular phase about three pulses per 6 hours could be observed, increasing to 4-6 pulses per 6 hours in the late follicular phase and decreasing to about 2 pulses per 6 hours in the midluteal phase (Lenton et al, 1978; Backstrom et al, 1982). The largest sample-to-sample variation (often more than 50%) was found in women near midcycle (Lenton et al, 1978; Backstrom et al, 1982). During the day the mean plasma E_2 concentration was highest between 8.00 and 9.00 a.m., whereafter it decreases (Lenton et al, 1978; Backstrom et al, 1982).

The low frequency or cyclic changes in plasma levels follow a characteristic pattern, due to secretion of the dominant follicle and/or corpus luteum. During the menses and the early follicular phase the E_2 concentration of the blood remains low (fig 2.2). In the mid-follicular phase the E_2 concentration graduallly increases, followed by a more rapid rise. The peak concentration in peripheral blood is reached in the late follicular phase, usually one day before the LH concentration peak. Concomitantly with the rise in LH the E_2 concentration decreases. Since ovulation occurs approximately 24-36 hours after the LH peak, the drop in E_2 concentration precedes ovulation. During the luteal phase, the E_2 concentration rises again, reaching a maximum approximately 5-8 days after ovulation, i.e. in the mid-luteal phase. In the late luteal phase the E_2 concentration decreases sharply.

Progesterone (P).

High frequency changes in plasma levels could only be observed in the luteal phase of the menstrual cycle (Korenman and Sherman, 1973; Backstrom et al, 1982). Backstrom and co-investigators (1982) found about 3 pulses per 6 hours with a relatively small amplitude. The low frequency changes and normal values of plasma progesterone levels during the menstrual cycle are depicted in figure 2.2. The small amounts of P present in the circulating blood during menses and the follicular phase are mainly derived from extra-glandular conversion of adrenal pregnenolone and pregnenolone sulfate, and secretion of small amounts of P by the adrenal cortex. At the beginning of the LH surge, the plasma P concentration gradually increases, followed by a more pronounced increase during the luteal phase which parallels the increase in E_2. Peak concentrations are reached in the mid-luteal phase, whereupon the P concentration decreases sharply in the late-luteal phase, if no conception had occurred.

Prolactin (PRL).

In women with a normal menstrual cycle high frequency (pulsatile) changes in prolactin concentration were described by several investigators (Ehara et al, 1973; Backstrom et al, 1982). The pulse frequency has been reported to increase from 1.5 per 6 hours in the early follicular phase, to

2.5 per 6 hours in the late follicular phase (Backstrom et al, 1982). In the luteal phase the number of pulses was found to decrease to about 1 pulse per 6 hours (Backstrom, 1982). During the day the mean prolactin level in the blood follows a diurnal pattern, being highest during sleep and decreasing within 1-3 hours thereafter (Williams, 1981). Throughout the cycle no consistent changes in circulating levels of prolactin have been found throughout the cycle by some investigators (McNeilly and Chard, 1974; Ehara et al, 1973; Jaffe et al, 1973), whereas others found an increased plasma prolactin concentration in the late follicular phase with a modest peak during ovulation (Vekemans et al, 1977; Lenton et al, 1979; Backstrom et al, 1982). In women the normal values of plasma prolactin levels are between 0.06 and 0.5 $IU.l^{-1}$.

Testosterone (T).

To date no pulsatile changes in plasma androgen (including testosterone and androstenedione) concentration have been reported. In contrast, a diurnal secretion pattern of T has been found; the highest values being recorded in the morning (Vermeulen, 1973).

The plasma T concentration has been reported to be fairly constant during the menstrual cycle (Williams, 1981), albeit that a gradual increase of plasma T concentration was found during the follicular phase with a rather sharp peak paralleling the LH peak, followed by a gradual decline during the luteal phase (Abraham, 1974; Apter et al, 1978). Plasma T is derived from various sources (table 2.1); about 50% arises from glandular secretion, while the remainder arises from peripheral conversion. Abraham (1974) showed that from the glandular T production 40-66% is derived from the adrenal cortex, and about 33% from the ovary, both in the follicular and luteal phase of the menstrual cycle. About 50% of plasma T is derived from conversion of androstenedione (Δ4-A) in various tissues (Horton and Tait, 1966; Longcope et al, 1976; Frisch et al, 1980). The normal values of plasma testosterone levels during the menstrual cycle are depicted in table 2.2.

Androstenedione (Δ4-A).

No high frequency changes or episodic secretion pattern of Δ4-A has been described, but low frequency changes were reported during the menstru-

al cycle (Williams, 1981; Judd and Yen, 1973). A minimum concentration is reached directly after menses, which is followed by an increase during the follicular phase. About 70% higher levels are reached during the late follicular phase. Parallel with the LH peak a more pronounced increase in Δ4 has been observed, whereafter the plasma concentration decreases.

A considerable part (table 2.1) of the plasma Δ4-A concentration is derived from the adrenal gland (Abraham, 1974). Adrenal Δ4-A secretion exhibits a diurnal pattern with a peak in the morning and a minimum in the late evening. There seems to be no variation in adrenal secretion during the menstrual cycle (Abraham, 1974). Normal values for plasma Δ4-A concentrations are given in table 2.2.

Tabel 2.1
Relative contribution (%) of ovarian and adrenal secretion and peripheral conversion to the blood production rate of T, Δ4-A and DHEA-S during a normal menstrual cycle. (according to data from Abraham, 1974; Farber et al, 1977; De Jong, 1982).

	OVARY			Adrenal cortex	Peripheral conversion
	follicular	"ovulation"	luteal		
T	10%	20%	10%	20-30%	50%
Δ4-A	20%	65%	55%	30-55%	5%
DHEA-S	2%		2%	98%	0%

Dehydroepiandrosterone sulfate (DHEA-S).

Conflicting results have been reported about the variation of plasma DHEA-S during the menstrual cycle. Several authors did not show any distinct effect of the phase of the menstrual cycle on plasma DHEA-S levels (Abraham, 1973; Williams, 1981), whereas others showed significantly higher DHEA-S levels near mid-cycle (Cattaneo et al, 1975; Abraham, 1974). The latter is somewhat curious because DHEA-S is almost exclusively derived from the adrenal cortex (Abraham, 1974).

The plasma concentration of DHEA-S shows a wide, interindividual variation. The mean concentration was reported to be 4.1 $\mu mol.l^{-1}$ with a range of 2-9 $\mu mol.l^{-1}$ (Buster and Abraham, 1972; Metcalf, 1976). In addition it

has been shown that the circulating levels of DHEA-S decrease with age (Drucker and David, 1980; De Peretti and Forest, 1978).

Table 2.2.
Reference values (mean and standard deviations) of androstenedione (Δ4-A), dehydroepiandrosterone sulphate (DHEA-S) and testosterone (T) in plasma of normal women, in the follicular, ovulatory and luteal phases of the menstrual cycle. (According to data from Faiman et al 1976 and Hollanders, 1981).

	PHASE OF THE CYCLE		
Hormone	Follicular	Ovulatory	Luteal
Δ^4-A ($nmol.l^{-1}$)	5.9 ±1.9	6.6 ±2.7	6.0 ±2.2
DHEA-S ($\mu mol.l^{-1}$)	6.7 ±2.5	7.5 ±2.5	7.8 ±3.6
T ($nmol.l^{-1}$)	1.2 ±0.3	1.4 ±0.4	1.0 ±0.4

2.2.1.3 Sites of ovarian hormone synthesis.

As mentioned before, the plasma concentration of several sex hormones and gonadotropins changes markedly from hour to hour and during the normal menstrual cycle. In women measurement of sex steroid concentrations in blood, simultaneously sampled in a peripheral vein and a vein draining the ovary, revealed that the ovary containing the dominant follicle is responsible for the rise in peripheral E_2 and P levels (Mikhail, 1970; Baird and Fraser, 1975; McNatty et al, 1976). This is supported by other investigations showing that excision of the ovary containing the dominant follicle or the corpus luteum results in a rapid decline in serum E_2 and P levels (Aedo et al, 1980 a and b). Besides, in women with a normal menstrual cycle, estrogen pre-treatment affects gonadotropin release by the pituitary gland (Shaw et al, 1975; Shaw, 1978). This further suggests that the ovary containing the dominant follicle or the corpus luteum coordinates the sex steroid hormone synthesis in relation to hypothalamic and pituitary cen-

tres. Although a detailed description of the mechanisms involved in ovarian steroidogenesis is far beyond the scope of this thesis, the most important processes leading to follicle maturation and hence appropriate steroid synthesis during the follicular and luteal phases of the menstrual cycle, will be briefly summarized here.

Follicle growth and steroidogenesis in the follicular phase.

The human ovary contains follicles in several stages of development. According to the developmental stage three main groups of follicles can be distinguished:

- the small, non-growing follicle (also called the primordial or primary follicle), in which the oocyte and its surrounding cells are at rest.
- the pre-antral follicle (also called the secondary follicle) in which the oocyte and its surrounding (granulosa) cells grow and differentiate, thus forming one or more layers around the oocyte. At this stage, the zona pellucida forms and a theca layer differentiates. Fluid begins to collect between the granulosa cells, at last forming the fluid filled cavity: the antrum.
- the antral follicle (also called the tertiary or Graafian follicle) in which the oocyte has terminated its growth, while the surrounding cells continue to differentiate. This follicle containes a full grown oocyte, many granulosa cells, a fluid filled antrum and outside the basement membrane a well developed theca layer.

The initiation of follicular growth is a continuous process, which is independent of gonadotropin influence. Even without pituitary support, the primary follicle can reach the next stage of development, it becomes a secondary or pre-antral follicle. Although the stimulus for this initiation of follicular growth is unclear, the further development of the secondary follicle is greatly dependent on both gonadotropins and ovarian steroidogenesis. From now on, the secondary or pre-antral follicle begins to concentrate FSH in its developing antrum. This is important because particularly FSH is necessary for further development of the follicle in the early follicular phase. In this phase differences in antral fluid concentration of FSH can be found between the follicles, as was shown in experiments with human ovarian tissue in vitro (McNatty and Sawers, 1975; McNatty, 1978; McNatty et al, 1979). From these experiments it appeared that FSH induces

the following events in human granulosa cells:
- an increase in mitotic activity and hence proliferation of granulosa cells.
- an increase in synthesis of aromatizing enzymes.
- stimulation of FSH receptor synthesis.
- stimulation of LH receptor synthesis.

These FSH stimulated events are of particular importance because thecal cells are supposed to synthesize androgens (mainly Δ4-A and to a lesser extend T). These cells, however, cannot aromatize the C-19 steroids to estrogens. After synthesis Δ4-A and T diffuse through the basement membrane into the FSH-stimulated granulosa cells, where they can be aromatized to estrogens (Channing, 1969; Moon et al, 1978; McNatty et al, 1979). However, the role of the androgens at this stage of follicular development is complex. At relatively low concentrations androgens enhance their own aromatization, but at higher levels the capacity for aromatization is overwhelmed and consequently the follicle becomes androgenic and ultimately atretic (McNatty et al, 1979; Hillier et al, 1980). Therefore, the follicle which is best capable of concentrating FSH and responding to this hormone will survive and eventually ovulate. Consequently the concentration of estrogens increases in the antral fluid of this follicle, while that of androgens and PRL decreases (the follicle becomes more "estrogenic"). This process will take place at the beginning of the mid-follicular phase of the menstrual cycle, the follicle is at its next stage of development and is called a tertiary or antral follicle.

The rising E_2 concentration in both the follicle and the peripheral blood is important for two reasons:
- it maintains follicular growth and enzyme induction, independent of FSH
- it inhibits FSH release which will hamper further development of the non-dominant follicle. In the antral fluid of these follicles high concentrations of androgens and PRL are found before they become atretic.

If in the late-follicular phase, just before ovulation, the production of E_2 in the follicle becomes so high that the plasma concentration exceeds 0.7 nmol.l^{-1} for a period of at least 50 hrs (Young and Jaffe, 1976), the hypothalamus will respond with an outpouring of gonadotropin releasing hormone (GnRH), resulting in the LH surge. This will also lead to inhibition of granulosa cell mitosis as has been shown in experiments with human

ovarian tissue in vitro (Delforge et al, 1972, McNatty and Sawers, 1975), the resumption of oocyte maturation (McNatty, 1978; Hillier et al, 1980), and initiation of granulosa cell luteinization (Baird et al, 1975). Simultaneously with the mid-cyclic LH-rise an increase in FSH concentration of the blood can be observed. The functional importance of this FSH surge is thought to be a further induction of LH receptors in the follicle. To produce a normal corpus luteum, the rising concentration of P in the late follicular phase seems to play an additional, facilitating role in the regulation of the LH mid-cycle surge (Shaw, 1975; Shaw, 1978).

Steroidogenesis in the luteal phase.

After rupture of the follicle and release of the ovum, the granulosa cells increase in size and a characteristic vacuolization associated with the accumulation of a yellow pigment, lutein, appears. During the early luteal phase (the first 3 days after ovulation) the granulosa cells enlarge and become more vascularized. From now on the predominant pathway of steroidogenesis in the corpus luteum is the delta-4 pathway, which means that not only estrogens and androgens, but also P is synthesized. Recently the importance of the vascularization for P synthesis was investigated by Carr and co-investigators (1982). They suggested that the rate of P secretion is determined by two factors:

- stimulation of cholesterol side-chain cleavage by cAMP-requiring mechanisms. This occurs throughout the ovarian cycle as a consequence of the action of FSH and LH.
- availability of adequate quantities of cholesterol as a precursor. A considerable amount of cholesterol has to be derived from the low density lipoproteins (LDL) in the blood. Until the corpus luteum is vascularized, the granulosa cells are not exposed to adequate quantities of LDL.

Eight to 9 days after ovulation, the peak of vascularization has been reached. This is associated with high synthesis rates of all three classes of steroids. In this period plasma gonadotropin levels reach the lowest values. In the late luteal phase (10-12 days after the ovulation) the corpus luteum reaches a phase of regression, resulting in a sharp decline in steroidogenesis. The factors contributing to this regression are incompletely understood, but from experiments with rhesus monkeys (Karsch et al, 1973; Karsch and Sutton, 1976; Stouffer et al, 1977) and isolated human

luteal cells (Williams et al, 1981) there is considerable evidence that high estrogen levels may initiate luteolysis, which is further mediated by an increased prostaglandin F ($PGF_2\alpha$) synthesis (Auletta et al, 1978; Fritz and Speroff, 1982).

2.2.1.4 Neuro-endocrine control of ovarian steroidogenesis.

The changes in hormonal levels are regulated by feedback mechanisms. In the early follicular phase there is a negative inhibitory effect on gonadotropin release initiated by the relatively low levels of E_2 (0.1 - 0.2 $nmol.l^{-1}$). It should be noticed that this negative feedback is not only determined by the absolute level of E_2, but also by the time of exposure and the sensitivity of the individual. The site of action of E_2 in this negative feedback appears to be at the hypothalamic and pituitary level. At the level of the hypothalamus, E_2 decreases GnRH secretion (Nillius and Wide, 1970), while at the level of the pituitary the inhibitory effect is exerted by a decreased sensitivity to GnRH (Shaw, 1978).

If E_2 synthesis has reached such high levels that the plasma E_2 concentration rises over 0.7 $nmol.l^{-1}$ and this concentration is maintained for approximately 50 hrs a positive feedback on gonadotropin release developes (Hohlweg and Junkman, 1932; Nillius and Wide, 1971; Leyendecker et al, 1972; Monroe et al, 1972; Yen and Tsai, 1972; Shaw, 1975 a and b; Young and Jaffe, 1976; Shaw, 1978). A positive feedback is only noticed in the estrogen primed hypothalamus-pituitary (Shaw, 1978).

Progesterone may also have negative or positive feedback effects on the hypothalamus-pituitary axis. The negative feedback action of P might be responsible for the short duration of mid-cycle gonadotropin surge (Shaw, 1978). Its positive feedback action has been thought to facilitate the mid-cyclic FSH surge but again only in estrogen primed subjects (Shaw et al, 1975).

Summarizing the mechanisms leading to steroidogenesis it is obvious that E_2 plays a key role in this process. The role of this hormone is essential in the following events:

- in response to the decline in E_2 concentration in the late-luteal phase the FSH concentration will rise, which initiates the beginning of the next cycle.

- estradiol acts synergistically with FSH in maintaining follicular sensitivity to FSH by inducing FSH receptors.
- estradiol induces LH receptors in granulosa cells, and hence acts synergistically with FSH.
- estradiol induces and maintains the mitotic and biosynthetic activity of granulosa cells.
- ovulation is triggered by the LH surge, which in turn is triggered by the rapid rise in plasma E_2 concentration (positive feedback on the hypothalamic-pituitary axis).
- regression of the corpus luteum is initiated by its own estradiol production.

2.2.2 Regulation of adrenal steroidogenesis.

Adrenal steroidogenesis is stimulated by ACTH, circulating cyclic AMP (cAMP) and possibly PRL (Parker et al, 1978; Forest, 1978; Trager, 1977; Nelson, 1980). Of these factors ACTH is far out the most important one. About 90% of the ACTH originates from the anterior pituitary gland and about 10% from the lobus posterior and the eminentia medianis (Nelson, 1980). ACTH exerts glandular and extra-glandular effects. The glandular effect is mainly mediated by stimulation of the adrenal adenylate cyclase. Consequently the intracellular cAMP concentration rises and this triggers the conversion of cholesterol to pregnenolone (fig. 2.1.). The other glandular effects of ACTH involve accumulation of cholesterol within the adrenals, stimulation of the activity of pregnenolone synthetase (Caron et al, 1975) and other enzymes that are active in converting pregnenolone to other steroids. ACTH already stimulates the adenylate cyclase in the membranes of the adrenal cell of the rat in concentrations of $1.5 \cdot 10^{-8}$M (Ontjes, 1980). Within minutes after ACTH stimulation the cAMP content of the adrenal cells is increased with a maximum after 3 minutes. This rapidly activates one or more protein kinases (Ontjes, 1980), resulting in a very rapid activation of steroidogenesis. Within 3 minutes after the ACTH peak, steroid secretion will rise and wane over a period of 10 minutes or more.

The extra-glandular effects of ACTH involve a rapid activation of tissue lipase, resulting in an increase in plasma non-esterified fatty acid (NEFA) concentration and the induction of a pronounced increase in sulfatase activity in ovarian tissue (Dominguez et al, 1975). The secretion of

ACTH by the anterior pituitary gland is largely governed by the hypothalamus by means of a releasing hormone, the corticotropin releasing factor (CRF). The regulation of CRF and ACTH secretion is controlled by three factors:

- the plasma corticosteroid concentration, which acts at the level of the pituitary. A rise in cortisol level diminishes ACTH secretion.
- a "biological clock". The ACTH secretion follows a diurnal pattern with a maximum just after awakening and a minimum in the evening. The biological clock works through the central nervous system.
- stress (mental and/or physical). This factor also works through the central nervous system.

It depends on the actual situation which factor is most important. In periods of great physical stress, this factor overrules the biological clock, while in prolonged physical exercise the rising cortisol levels are not able to suppress ACTH secretion.

2.3 TRANSPORT OF STEROID HORMONES

In the human blood only a small fraction of the total concentration of unconjugated sex steroids exists in the free form. The major portion is specifically bound with high affinity to plasma proteins such as sex hormone binding globulin (SHBG) and with low affinity to albumin. These proteins serve the following purposes (Traeger, 1977):

- protection of the circulating hormones against inactivation by enzymes in blood and perfused organs. Consequently the half-life time will be lengthened.
- formation of a "buffer-compartment" from which the steroid dissociates relatively easy, if necessary. This buffer is also able to neutralize an acute rise in steroid concentration.
- modulation of the intracellular activity of the steroid by competition between the extracellular carrier (mainly albumin) and the intracellular receptor for the steroid.

Between SHBG and albumin marked differences exist in binding affinity for the various sex steroids (table 2.3).

Table 2.3
Association constants (K_{ass}) of sex hormone binding globulin (SHBG) and albumin for several steroid hormones (according to data from Vermeulen et al, 1973 and Dunn et al, 1981).

Steroid	K_{ass} SHBG ($10^6 M^{-1}$)	K_{ass} albumin ($10^4 M^{-1}$)
Δ4-A	29	2
E_2	680	6
P^2	8,8	6
T	1600	4

An additional difference is that albumin has an almost unlimited steroid-binding capacity due to its high concentration (about 19000 times higher than that of SHBG). Besides, it has multiple binding sites per molecule in contrast to SHBG which has only one binding site per molecule. These differences in binding affinity and capacity of plasma proteins for sex steroids make that in non-pregnant women about 37% of the total E_2 concentration circulates in complex with SHBG, while about 60% circulates in complex with albumin. For T these values are 66% and 30%, respectively. Consequently, only a very small fraction of the total plasma concentration of sex steroids circulates in the free form. Only this fraction is considered to be biologically active (Pearlman et al, 1967; Tavernetti et al, 1967; Kato and Horton, 1968; Vermeulen and Verdonck, 1968; Vermeulen et al, 1973).

The mean percentage of free E_2 and T was reported to be constant throughout the menstrual cycle (Wu et al, 1976). The percentages measured by several investigators range from 1.5 - 2.6% (Hammond et al, 1980; Dunn et al, 1981; Moll et al, 1981) for E_2 and 1-1.9% for T (Hammond et al, 1980; Dunn et al, 1981).

The free percentage of for example E_2 may be elevated either by a decrease in SHBG concentration or an increase in competing steroids or other substances that interact directly or indirectly with SHBG or albumin. Body temperature will also play an important role in the association of sex steroids with plasma proteins since the K_{ass} is dependent on temperature (Vermeulen and Verdonck, 1968; Lata et al, 1980; Vigersky et al, 1979). This will result in a higher percentage of unbound and non-specifically

bound steroids with increased blood temperature. However, Vigersky and co-investigators (1979) showed that the dissociation rates of E_2 and T from plasma were markedly different.

From the work of Lata and associates (1980) it became apparent that the K_{ass} of the steroid binding proteins is insensitive to changes in pH in the physiological range (6.8-7.4).

2.4 SECRETION, PRODUCTION, CONVERSION AND DEGRADATION OF SEX STEROIDS

2.4.1 Introduction

As mentioned in the preceding paragraphs interpretation of the plasma concentration of sex steroids is complicated by the fact that it is derived from several sources i.e. glandular synthesis and peripheral conversion. Besides, these processes are counteracted by the degradation, that occurs in splanchnic and extra-splanchnic tissue. Thus, some concepts (Tait, 1963; Gurpide, 1975) had to be introduced to differentiate between these processes, which all contribute to the actual plasma concentration. These concepts include secretory rate (SR), blood production rate (PR_B) and metabolic clearance rate (MCR), which are defined as follows:

- the secretory rate of a hormone A (SRA) is the amount of A released by an endocrine gland into the circulation per unit time.
- the blood production rate (PR_B) of a hormone A, is the total rate at which A enters de novo into the circulation per unit time (Tait, 1963; Gurpide, 1975). When a hormone is derived exclusively from glandular secretion, the SR and PR_B are the same. When the hormone also originates from peripheral conversion, PR_B will be higher than SR.
- the MCR of a hormone equals the volume of plasma irreversibly cleared from a hormone per unit time. The MCR usually measured is the overall MCR, which can be considered to be the sum of all organ MCR's.

In the next paragraphs the literature about steroid hormone secretion, blood production and conversion will be briefly reviewed.

2.4.2 Secretion Rate (SR), Blood production rate (PR_B) and Metabolic Clearance Rate (MCR) of sex steroids

2.4.2.1 Estradiol.

Secretion Rate (SR)

In women, the major part (95%) of the circulating E_2 in the blood originates from ovarian secretion as revealed by both indirect techniques (isotope dilution technique, Baird and Fraser, 1974) and direct sampling of ovarian venous blood (Abraham and Chamakjian, 1973; Aedo et al, 1980). The SR of E_2 ranges from 0.26 to 1.5 and from 3 to 0,92 µmol/day in the early follicular and the luteal phase of the menstrual cycle, respectively (Tagatz and Gurpide, 1973).

Blood production rate (PR_B)

The PR_B of E_2 almost equals the SR. It was found to be 0.3 µmol/day in the early follicular phase and to increase to 1.63-3.45 µmol/day in the late follicular phase and to decrease somewhat (0.99 µmol/day) in the mid-luteal phase.

The difference between SR and PR_B can be explained by the fact that several tissues, including adipose tissue, skeletal muscle and bone marrow, are able to convert androgens to estrogens. Longcope and co-investigators (1978) calculated that muscle accounts for 25-30% and adipose tissue for 10-15% of the total extra-gonadal aromatization of androgens to estrogens. In addition about 3% of the estrone (E_1) entering the circulation of skeletal muscle is converted to E_2 in this tissue, while adipose tissue converts about 2% of E_1 to E_2 (Longcope et al, 1976).

Metabolic Clearance Rate (MCR)

In women, the overall MCR of E_2 was reported to vary between 1025 and 1350 l.plasma/day (Longcope et al, 1963; Hembree et al, 1969). The spanchnic bed is the major site of metabolism for estrogens, progestins and androgens. However, due to marked differences in affinity for plasma proteins the magnitude of the splanchnic clearance varies considerably from steroid to steroid. Experiments in dogs have shown that E_2 is metabolized for almost 90% in splanchnic tissue (Collins et al, 1970). The extra-

splanchnic metabolism of E_2 mainly occurs in skeletal muscle and adipose tissue. Longcope and co-investigators (1976) have shown that in men both tissues account for about 10% of the overall metabolism of E_2.

2.4.2.2 Progesterone.

Secretion Rate (SR)

During the follicular phase of the menstrual cycle almost all P is derived from adrenal secretion (Traeger, 1977), while in the luteal phase significant amounts of this steroid are secreted by the corpus luteum. The SR by the ovaries is reported to be 3.5 to 7 µmol/day in the follicular phase, and 70-105 µmol/day during the luteal phase (Tagatz and Gurpide, 1973).

Blood Production Rate (PR_B)

In normally cycling women the PR_B of P ranges from in the average 9 µmol/day in the follicular phase to in the average 110 µmol/day in the luteal phase of the menstrual cycle (Tagatz and Gurpide, 1973). The close agreement of PR_B and SR reveals that there is no peripheral conversion of other steroids to P.

Metabolic Clearance Rate (MCR)

The MCR of P was reported to vary between 1800 and 2500 l.plasma/day (Tagatz and Gurpide, 1973; Little et al, 1975). Besides the splanchnic metabolism, which accounts for approximately 40% of the overall metabolism, extensive metabolism occurs in several other organs, including brain and uterus (Little et al, 1975). In experiments with ^{14}C-progesterone it has been shown (Lin et al, 1978) that bovine muscle and adipose tissue are also able to metabolize P in considerable amounts. This suggests that in man muscle and adipose tissue are also involved in P metabolism.

2.4.2.3 Androgens.

Secretion Rate (SR)

In the early follicular phase Δ4-A and DHEA are the major androgen products of the ovaries which are secreted mainly by stromal tissue.

Accumulation of stromal tissue, which normally occurs at mid-cycle, results in an additional rise in SR of Δ4-A and T. (Judd and Yen, 1973; Abraham, 1974; Vermeulen and Verdonck, 1976). In women, the SR of T is not known, but assuming that about 50% of the circulating T arises from peripheral conversion from prehormones, the SR can be calculated to be about 0.35-0.45 μmol/day for both ovaries and adrenals.

The SR of Δ4-A by both ovaries ranges from 2.8 to 5.6 μmol/day, while the SR of DHEA-S by the adrenal cortex is about 18 μmol/day (Gurpide et al, 1963; McDonald et al, 1965; Van de Wiele et al, 1963; Poortman et al, 1973).

Blood Production Rate (PR_B)

The PR_B of Δ4-A and T is relatively constant during the menstrual cycle (10 and 1 μmol/day, respectively). Virtually all (95%) of the plasma Δ4-A concentration arises from glandular secretion (Bardin and Lipsett, 1967; Horton and Tait, 1966; Kirschner and Bardin, 1972; Kirschner and Jacobs, 1971; Poortman, 1974). This means that the PR_B of Δ4-A, which was calculated to be 5.2-6.4 μmol/day (Poortman et al, 1973; Tagatz and Gurpide, 1973), almost equals the SR of both the adrenal cortex and the ovary.

For T the PR_B is derived from adrenal and ovarian secretion (both accounting for approximately 50% of the PR_B) and peripheral conversion mainly from Δ4-A and to a lesser extent from DHEA (Bardin and Lipsett, 1967).

Metabolic Clearance Rate (MCR)

The overall MCR of Δ4-A and T in women is reported to be 2000 and 700 l.plasma/day respectively (Baird et al, 1969; Horton et al, 1966; Poortman et al, 1973). Due to the extremely high affinity for albumin the MCR of DHEA-S is very low. It is estimated to be 10-15 l.plasma/day (Longcope et al, 1972).

Both Δ4-A and T are metabolized extensively outside the splanchnic bed. Longcope and co-investigators (1976) showed that skeletal muscle and adipose tissue contributes to the overall conversion of Δ4-A to T with 10-15% and 5-10%, respectively. Muscle appears to contribute to the overall metabolism of Δ4-A and T by 5-12% and adipose tissue by 2-7%.

In the light of the afore mentioned in the present study the following hormones were measured in blood plasma: E_2, P, LH, FSH, T, Δ4-A, DHEA-S, PRL, ACTH and in addition the free E_2 and T concentration. A, DHEA-S and ACTH were measured to get more insight into the probable involvement of the adrenal cortex as a source of plasma androgens. Besides, we were interested in the relation between exercise plasma PRL and DHEA-S levels, because hyperprolactinemia with or without concomitant increased DHEA-S levels have been associated with secundary amenorrhoea (Vermeulen et al, 1977; David and Drucker, 1979; Fachinetti et al, 1979; Lobo et al, 1980; Lake Polan and Behrman, 1981). It is not known whether the ovarian secretion rate enhanced or the degradation rate of sex steroids is decreased during exercise. Ovarian secretion is stimulated by the gonadotropins (especially LH), hence the measurement of LH and FSH during exercise provides insight into the involvement of the pituitary. In addition, the changes in episodic secretion pattern in gonadotropins and E_2 during and after physical exercise, if any, was investigated.

In human volunteers, measurement of the degradation rate is only possible with indirect techniques such as the isotope dilution technique. Therefore, this technique was used to monitor the degradation rate of E_2 (measured as the MCR) during exercise to provide us more insight into this possible mechanism.

CHAPTER III

MATERIALS AND METHODS

3.1 SUBJECTS

Fourty seven healthy women agreed to participate in five different studies (see below). The nature and intent of the studies were carefully explained to each individual before they gave their written consent. The subjects met the following qualifications:

1. their menstrual cycle had to be normal for at least 1 year prior to the study as revealed from the menses data. A normal menstrual cycle length was considered to be 28±4 days. Ovulation was detected by the basal body temperature (BBT) method and the measurement of plasma P levels at rest. We considered a subject to be in the luteal phase if the plasma P levels at rest exceeded 10 nmol.l^{-1} since Wetzels (1983) showed that ovulation always occurred when this level had been reached.
2. no gynaecological disorders as assessed by a trained gynaecologist.
3. normal liver and renal functions.
4. no use of drugs that could interfere with the menstrual cycle for at least 6 months prior to the study.

The subjects participating in study I and III (n=19) were untrained students. From this group two dropped out because their veins did not allow repeated blood sampling, while a third subject became amenorrhoic for several months. The remainder of the group (n=16) participated in study I. Tabel 3.1 summarizes their physical characteristics and $\dot{V}O_2max$ values. All women of this group were nulliparous. From this group eight subjects (1002, 1008, 1010, 1011, 1012, 1015, 1017 and 1021) were retested after a three month period of endurance training (study III).

Table 3.1
Age, physical characterisics, maximal physical working capacity (MPWC) and maximal oxygen uptake ($\dot{V}O_2max$) of the subjects participating in study I and III when entering the study.

code	age (yr)	Ht (cm)	Wt (kg)	bodyfat (%)	MPWC (Watts)	$\dot{V}O_2max$ ($ml.kg^{-1}.min^{-1}$)
1001	19	171	72	31.2	225	41.7
1002	20	162	60	27.9	230	50.8
1003	22	165	70	32.7	190	37.1
1004	29	168	66	25.0	255	49.7
1005	22	178	72	31.2	190	36.8
1006	20	165	60	26.4	215	49.5
1008	19	164	54	26.9	185	48.7
1010	26	168	58	24.8	232	53.2
1011	20	165	69	30.9	220	43.0
1012	20	168	58	25.5	230	52.2
1013	19	160	52	23.4	190	50.5
1015	19	168	58	25.1	205	47.4
1017	19	165	53	21.7	175	46.9
1018	22	170	56	26.3	193	46.9
1020	32	167	58	25.9	180	38.2
1021	21	170	73	33.3	200	37.7
mean	21.8	167.2	61.1	27.2	209.1	45.6
S.D.	3.6	4.1	7.0	3.3	25.6	5.8

The subjects participating in study II (n=6) were highly trained marathon runners. Their performances varied between 2 hr 54 min and 3 hr 05 min for the marathon. Training age and training volume (i.e. > 6 yrs, 100-140 km/week) were comparable. They participated in study II. Table 3.2 summarizes their physical characteristics and $\dot{V}O_2max$ values. Three out of 6 were multiparous, the other three were nulliparous.

Table 3.2
Age, physical characteristics, maximal physical performance (MPWC) and maximal oxygen uptake ($\dot{V}O_2max$) of the subjects participating in study II.

code	age (yr)	H (cm)	W (kg)	bodyfat (%)	MPWC (km/hr)	$\dot{V}O_2max$ ($ml.kg^{-1}.min^{-1}$)
1302	39	165	58.0	13.4	18.0	57.2
1303	32	167	52.0	12.2	18.0	59.2
1304	39	154	45.2	11.0	18.0	58.0
1305	39	163	56.4	13.6	17.0	52.3
1307	36	168	57.1	14.5	18.0	64.8
1309	31	170	55.2	11.8	18.5	64.1
mean	36.0	164.5	54.0	12.8	17.9	59.3
S.D.	3.4	5.2	4.4	1.2	0.5	4.2

The subjects participating in study IV (n=7) were untrained students. This study was done in cooperation with Dr. A. Bonen (School of Physical Education, Dalhousie University, Halifax, Nova Scotia, Canada). Three Canadian subjects received exactly the same exercise treatment as the dutch girls. The protocol and handling of blood samples were thoroughly discussed and apparantly the same. All radio-immuno-assays were done in Maastricht. Table 3.2 summarizes the physical characteristics and $\dot{V}O_2max$ values of the subjects participating in this study.

Table 3.3
Age, physical characteristics, maximal physical working capacity (MPWC) and maximal oxygen uptake ($\dot{V}O_2max$) of the subjects participating in study IV.

code	age (yr)	H (cm)	W (kg)	bodyfat (%)	MPWC (Watts)	$\dot{V}O_2max$ ($ml.kg^{-1}.min^{-1}$)
1002	20	162	60	27.9	230	50.8
1006	20	165	60	26.4	215	49.5
1010	26	168	58	24.8	232	53.2
1012	20	168	58	25.5	230	52.2
1022	24	165	62.3	24.8	260	57.3
1023	23	166	56.4	25.0	230	53.8
1024	22	164	60.5	26.2	215	46.6
Mean	22.1	165.4	59.3	25.8	230.3	51.9
S.D.	2.3	2.2	2.1	1.1	15.0	3.4

The subjects participating in study Va (n=9) were physical education students. All subjects of this group were nulliparous. Their physical characteristics and $\dot{V}O_2max$ values are given in table 3.4.

Table 3.4
Age, physical characteristics, maximal physical working capacity (MPWC) and maximal oxygen uptake of the subjects participating in study Va.

code	age (yr)	H (cm)	W (kg)	bodyfat (%)	MPWC (Watts)	$\dot{V}O_2max$ ($ml.kg^{-1}.min^{-1}$)
1201	23	178	76.4	33.1	256	46.7
1202	22	170	60.3	30.6	212	44.7
1204	20	178	66.1	33.0	193	46.1
1206	20	158	63.2	28.1	225	53.5
1207	23	168	74.2	27.4	250	50.5
1211	25	167	59.0	24.4	200	44.6
1214	20	165	54.1	24.3	225	53.4
1215	26	163	52.0	22.5	215	46.7
1218	21	173	68.8	31.0	262	49.8
mean	22.2	168.9	63.8	28.3	226.6	48.4
S.D.	22.1	6.3	7.9	3.7	24.6	3.3

The subjects participating in study Vb were physical education students (n=3) and trained runners (n=3). Their physical characteristics and $\dot{V}O_2max$ values are given in table 3.5.

Table 3.5
Age, physical characteristics, maximal working capacity (MPWC) and maximal oxygen uptake ($\dot{V}O_2max$) of the subjects participating in study Vb.

code	age (yr)	H (cm)	W (kg)	bodyfat (%)	MPWC (Watts)	$\dot{V}O_2max$ ($ml.kg^{-1}.min^{-1}$)
1221	21	161	70.3	27.0	210	43.6
1222	21	169	55.0	18.0	210	46.0
1223	20	167	63.2	28.0	200	36.5
1224	22	172	52.7	15.8	240	57.1
1225	20	172	52.5	19.0	240	52.0
1226	20	172	52.5	17.0	250	60.0
mean	20.7	168.8	58.5	21.0	225	49.2
S.D.	0.8	4.4	7.0	5.6	20.7	8.8

Although the characteristics of the subjects participating in study II were different from those of the participants in the other studies, as far as age and parity are concerned, a better selection was not possible because it was very difficult to find female marathon runners, with a training volume of 100 km per week or more, who did not experience any menstrual irregularity (i.e. a variability between the length of the menstrual cycle of less than 8 days).

3.2 EXERCISE PROTOCOLS

3.2.1 General considerations

For evaluation of differences in hormonal and metabolic responses to various types of exercise in trained and untrained women, the exercise protocols used in this investigation had to be comparable, both in intensity and duration. To meet this qualification three criteria had to be fullfilled:

- the type of energy expenditure for muscle contraction had to be similar. Therefore, we have chosen for an exercise intensity below the $\dot{V}O_2max$ level.
- the workloads were selected in such a way that the metabolic response to exercise was expected to be comparable, despite interindividual differences in physical fitness. This criterion could be fullfilled best if the workloads were expressed as a percentage of the workload at which the maximum oxygen uptake ($\dot{V}O_2max$) was attained (Åstrand and Rodahl, 1977; Hollmann and Hettinger, 1981).
- the duration of each workload had to be sufficiently long to achieve a physiological steady-state. This means that it had to be at least 6 min.

In addition it was recognized that the exercise protocols should be comparable with those used in some recent studies in this field (Jurkowski et al, 1978; Bonen et al, 1979). Although this is difficult to achieve completely, a good compromise seemed to be an exercise intensity of at least 60% $\dot{V}O_2max$ and a duration of the workloads of 15 min. Based upon these assumptions the exercise-protocols used in this investigation can be subdivided into protocols to monitor the hormonal response to short-term exercise (>6, < 15 min) and protocols to monitor the hormonal response to prolonged exercise (> 30 min). The influence of prolonged physical exercise on hormonal responses was investigated in study I to IV, the influence of short-term physical exercise on hormonal responses was investigated in study V.

3.2.2 Ergometers

Two types of ergometers were used for several reasons. Although we intended to use the treadmill as an ergometer in all studies, a pilot study with several untrained subjects, using the same protocol as the marathon runners, (i.e. incremental speed changes only) revealed that they were not able to run for a period longer than 5 - 10 min. Therefore, a bicycle ergometer was used in all studies, except for the one in marathon runners. In these athletes the exercise performance was thought to be assessed most adequately on a treadmill because of their high-intensity long distance running for years which in addition is known to result in specific adaptations of the body (Åstrand and Rodahl, 1977; Verstappen et al, 1982). A bicycle ergometer has the following advantages:

- standardization of external workload is easy with a bicycle ergometer.
- the bicycle is a commonly used transport vehicle in The Netherlands. Therefore, most subjects are skilled in bicycling.

The bicycle ergometer (Lode NV, Groningen, The Netherlands) was electrically braked. The external workload was independent of revolution rate in the range from 60-90 revolutions/min.

In study II a motor-driven treadmill (Quinton 18-50, Seattle, Washington, U.S.A) was used.

3.2.3 $\dot{V}O_2$max tests

To estimate the workload to be used in each experiment an incremental bicycle ergometer (all studies except for II) or treadmill (study II) test preceded the experiments. During this test respiratory data were recorded continuously from which the oxygen uptake was calculated.

In study II (marathon runners) an incremental $\dot{V}O_2$max test preceded each experiment (thus twice in a menstrual cycle), because changes in the training program might alter their test performance. In the other studies, where untrained subjects volunteered, only minor differences in maximal physical performance could be expected and hence the $\dot{V}O_2$max test was performed only before the first experiment.

The bicycle ergometer test consisted of a load of 100 Watt for the first 5 min, followed by increments of 50 Watt every 2½ min until the subject was exerted. The maximal physical working capacity (MPWC) from which the workloads for the experimental protocol were estimated, was calculated as follows:

$$MPWC = Wout + t/150 \times \Delta W$$

where, Wout = the last workload (in Watts) which the subject completed for 2½ min, t = the time (in seconds) which the subject could sustain the highest workload and ΔW = the final load increment.

In the treadmill test the runners performed at a speed of 12 km/hr for 10 min, followed by an increase in speed of 2 km/hr every 2 min, until the subject was exerted. The slope was kept at 0°. The workloads to be used during the experimental days were calculated in a similar way as in the bicycle ergometer test. From these tests it appeared that in all cases the

$\dot{V}O_2max$ was attained at the highest workload. Hence in the following sections the intensity of the workloads will be expressed as a percentage of the $\dot{V}O_2max$.

3.2.4 Exercise protocols used in the studies

In study I, III and IV a load of 60% $\dot{V}O_2max$ for 15 min, was followed by an increase of 10% $\dot{V}O_2max$ each 15 min until the subject had to give up. In study II (marathon runners) a workload of 60% $\dot{V}O_2max$ was followed by a run with a workload of 85% $\dot{V}O_2max$ for one hour. In study Va a workload of 70% $\dot{V}O_2max$ for 10 min was followed by a workload of 100% $\dot{V}O_2max$ until the subject was exerted, while in study Vb a workload of 70% $\dot{V}O_2max$ was followed by a recovery period at 25% $\dot{V}O_2max$ for 30 min.
Study I-IV were done in the exercise laboratory (Department of Physiology) of the University of Limburg (Maastricht, The Netherlands), while study V was performed in the exercise laboratory of the Department of Physiology of the University of Utrecht (Utrecht, The Netherlands).

3.3 EXPERIMENTAL PROCEDURES

3.3.1 General procedures

On the experimental days, the subjects came to the laboratory in the morning of the 7th - 10th day of their menstrual cycle. The subjects who participated in study I, II or III also performed in the luteal phase of their menstrual cycle (between the 20th - 25th day). All exercise tests were performed between 8.15 and 10.30 a.m. If the subjects had to exercise twice in a menstrual cycle (as in study I, II and III) both exercise tests were performed at the same time of the day (difference less than 15 min).

After dressing in a sport-suit, a teflon catheter (18-gauge, Quick cath., Travenol Laboratories, Deerfield, Ill., U.S.A.) was inserted into an antecubital vein, which was kept patent by a slowly dripping infusion of saline. After a rest period in a chair of 30 (study I, II, III) or 120 min (study IV and V)) the subjects began to exercise. Blood was collected twice at rest (15 and 2 min prior to the exercise bout).

During exercise several blood samples were collected. In each case blood was obtained without interrupting the exercise. All exercise samples were

corrected for hemoconcentration by measurement of total protein, which was compared with the mean of the two concentrations at rest. The details of the various experiments are described in the following sections.

3.3.2 Study I

The aim of this part of the study was to collect data about hormonal responses to prolonged incremental physical exercise in both the follicular and luteal phase of the menstrual cycle in 16 untrained women. In addition, the influence of the phase of the menstrual cycle on maximal physical performance (MPWC) was evaluated. The physical characteristics of these subjects are given in table 3.1. They reported to the laboratory between 8.15 and 9.30 a.m. after an overnight fast.

After a 30 minutes rest period, the subjects started the bicycle ergometer test at 60% $\dot{V}O_2$max for 15 min. Thereafter the workload was increased by 10% $\dot{V}O_2$max every 15 minutes until the subject was exerted. Blood was collected every 15 min through the indwelling catheter. In these samples the plasma concentration of lactate, glucose, total protein, E_2, P, T, Δ4-A, DHEA-S, PRL, ACTH, LH and FSH were determined. The free E_2 and free T fractions were determined in the plasma samples at rest, and those obtained at the end of the exercise bout.

The whole procedure is depicted schematically in figure 3.1.

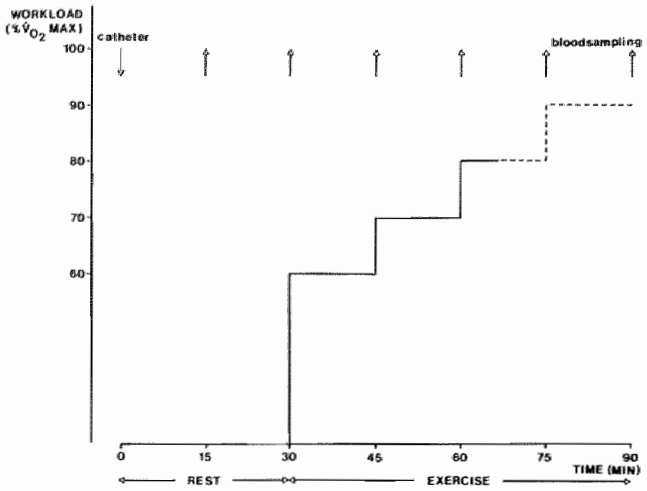


Figure 3.1
Schematic representation of the experimental procedures in study I and III.

3.3.3 Study II

The aim of this part of the study was to collect data about hormonal responses to prolonged physical exercise in highly trained marathon runners both in the follicular and luteal phase of their menstrual cycle. Additionally, the influence of the phase of the menstrual cycle on MPWC was evaluated. The physical characteristics of the subjects participating in this study are given in table 3.2. All subjects reported to the laboratory between 9.00 and 9.30 a.m. after a light breakfast (ca. 350 kcal) taken 2 hrs before the experiment. This was done because in a previous experiment the subjects complained about symptoms of hypoglycemia at the end of a long treadmill run.

After a 30 min rest period the subjects ran on a treadmill for 75 min. The exercise test consisted of a warming-up period of 15 min at 60% $\dot{V}O_2$max, followed by a continuous run for one hour at about 85% $\dot{V}O_2$max. Blood was obtained every 15 min through the indwelling catheter without interrupting the run. In these samples the plasma concentrations of lactate, glucose, total protein, E_2, P, T, Δ4-A, DHEA-S, PRL, ACTH, LH and as well as the FSH, free E_2 and free fractions at rest and after the exercise T were determined.

The whole experimental procedure is depicted schematically in fig. 3.2.

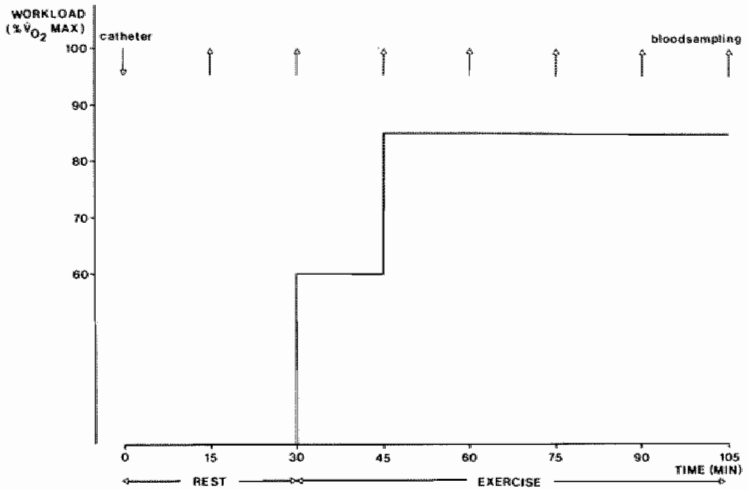


Figure 3.2
Schematic representation of the experimental procedures in study II.

3.3.4 Study III

The aim of this part of the study was to evaluate the influence of a short period (3 month) of endurance training on metabolic and hormonal responses to exercise in previously untrained women. Additionally, the influence of the phase of the menstrual cycle on MPWC was evaluated. For this purpose 8 of the subjects (1002, 1008, 1010, 1011, 10112, 1015, 1017

and 1021), participating in study I, volunteered in this part of the study. The physical characteristics of these subjects are given in table 3.1.

The training program consisted of running and cycling 3-4 times per week. The training volume was increased progressively during a period of 3 months from about 25 min to 50-60 min per training unit. The training consisted of interval and continuous running and/or cycling. The absolute intensity of the exercise was adapted to heart rate, which was not allowed to fall under 160 beats/min. After this training period each subject was retested in the follicular and luteal phase of her menstrual cycle. The absolute workload was reajusted according to the changes in MPWC, the relative workload, however, was the same as in the pre-training tests. The whole experimental procedure was identical to the one in study I.

3.3.5 Study IV

The aim of this part of the study was to collect data about changes in the pulsatile pattern of gonadotropin release before, during and after physical exercise.

For this purpose 7 subjects participated in this study. In the follicular phase (between the 7th and 10th day of their menstrual cycle) they performed the same exercise protocol as described in study I. To monitor changes in the pulsatile pattern of gonadotropins and E_2 the exercise was preceded and followed by a rest period of 2 hours. Blood (about 3 ml) was obtained every 15 min during the whole experimental period. The exercise protocol was indentical to that of study I.

3.3.6 Study Va and Vb

The aim of these parts of the study was to determine if a decreased degradation rate, measured as the metabolic clearance rate, might be responsible for increments in E_2 concentration of the blood. For this purpose 9 subjects participated in study Va, whereas 6 subjects participated in study Vb. Their physical characteristics are given in table 3.4 and table 3.5, respectively.

The MCR of E_2 in each subject was studied in the follicular phase, 7-10 days after the beginning of the menses, using the constant infusion technique (MacDonald et al, 1969) as described by Poortman and colleagues

(1973). The subjects came to the laboratory at 8.15 a.m. and after insertion of the catheter a resting blood sample was obtained. Immediately after the collection of blood, a priming dose of 8 μCi, ^{3}H-labeled estradiol in 10 ml of 5% ethanol in saline was injected into the arm vein. The continuous infusion was started 30 min after the priming dose. The radioactive solution (approx. 24 μCi in 30 ml of 5% ethanol in saline) was infused into the right antecubital vein at a constant rate, using an infusion pump (Braun, Melsingen, Germany).

Another catheter was inserted into an antecubital vein of the opposite arm, which was used for the collection of blood samples. To avoid influences on MCR due to differences in body position (erect vs supine) the subject was seated on the bicycle ergometer without cycling for 20 min before the start of the exercise test (i.e. from 75 min to 95 min after the priming dose). Approximately 135 min after the priming dose the subject started to pedal. Two exercise tests (study Va and Vb) were used:

- in the first test (study Va) 9 subjects (group 4) performed at a load of 70% $\dot{V}O_2$max during 10 min, followed by a load of 100% $\dot{V}O_2$max till exhaustion.
- in the second test (study Vb) 6 subjects (group 5) performed during 10 min a 70% $\dot{V}O_2$max followed by a recovery period of 30 min at a load of 25% $\dot{V}O_2$max.

In both studies blood (10-12 ml) was obtained through the catheter in the left arm vein at the same time intervals as in study IV. The first two samples, obtained 15 min and just before the exercise tests, were taken to establish whether a steady-state level of the tracer was reached. In study Va blood was collected during exercise at the end of the 70 and 100% $\dot{V}O_2$max period, while in study Vb blood was collected each 5 min, and also during the recovery period. The whole experimental procedure for study Vb is depicted in fig. 3.3.

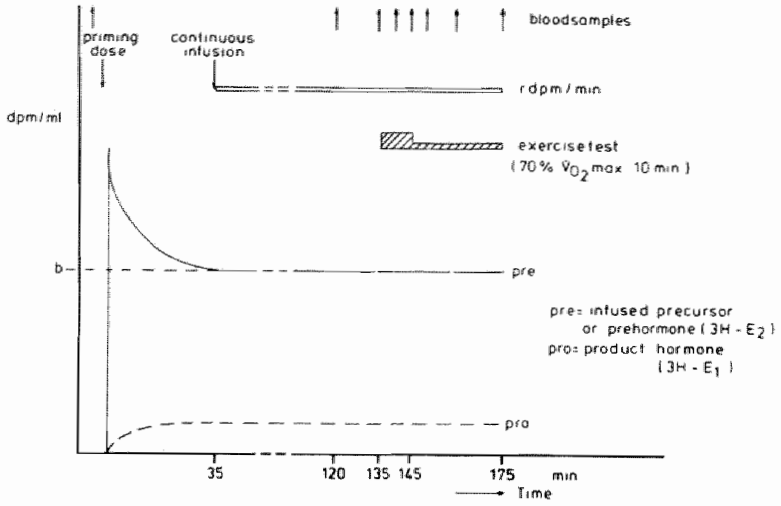


Fig. 3.3 Time schedule and schematic representation of radioactive steroids in blood during the infusion experiment of study Vb.

3.4 ANALYTICAL METHODS

3.4.1 Handling of blood samples

Blood samples (12 ml) were drawn through the indwelling catheter in 10 ml disposable syringes. About 1 ml (study I to IV) was allowed to clot in a glass tube. To accelerate the blood clotting, some glass pearls were present in the tube, which was gently turned for several times and placed in an ice bath. Immediately after the exercise test the blood was centrifuged (2000 g, 10 min, 4° C) and thereafter serum was collected. Two hundred microliters of serum were immediately analyzed for glucose and total protein.

The major part of each blood sample (about 10 ml) was collected in a heparinized glass tube, which was stored in an ice bath. Immediately after

each experiment the blood was centrifuged (2000 g, 10 min, 4°C), the plasma divided in 0.5 ml portions and stored at -20°C for subsequent analysis. All determinations, except those for the clearance studies were done in duplicate.

3.4.2 Glucose and total protein

Serum glucose and total protein were analyzed by centrifugal analysis (Cobas Bio, Hoffmann La Roche, Basel, Switzerland). Glucose was determined by the hexokinase method. The principle is as follows:

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{glucose-6-phosphate} + \text{ADP}.$$

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

The NADPH concentration equals the glucose concentration and was measured spectrophotometrically (340 nm). The intra and inter assay coefficients of variation were calculated to be 1.2% and 3.5%, respectively.

Total protein was determined by the biuret method. The biuret reagent was obtained from Hoffman La Roche nr. 1010083 (K-Na tartrate 200 mmol/L, $CuSO_4$ 120 mmol/L, KJ 100 mmol/L, NaOH 2 mmol). The intra and inter assay coefficients of variation were calculated to be 0.9% and 1.5%, respectively.

3.4.3 Lactate

Lactate in blood plasma was determined with an electro-chemical-enzymatic method as described by Racine (1975). The principle is based on the highly specific oxidation of lactate to pyruvate in the presence of the enzyme cytochrome b_2 and hexacyanoferrate-(III) according to:

$$\text{Lactate} + 2\ Fe(CN)6^{-3} \xrightarrow{\text{cyt.b2}} \text{pyruvate} + 2\ Fe(CN)6^{-4} + 2H^{+}.$$

The hexacyanoferrate-(II) produced in this reaction is reoxydized electrochemically at a P+-electrode:

$$2\ Fe(CN)6^{-4} \rightarrow 2\ Fe(CN)6^{-3} + 2e^{-}.$$

The current measured is related to the lactate concentration in the solution. For this purpose a semi-automatic lactate analyser (lactate analyser 640, Kontron, Zurich, Switserland) was used.
The intra-assay coefficient of variation for low (2 $mmol.l^{-1}$) and high (15 $mmol.l^{-1}$) lactate concentrations was calculated to be 8% and 1.5%, respectively.

3.4.4 Hormone analysis

The hormone analyses were performed in the clinical laboratory of the "Sint Annadal" Hospital, Maastricht. All hormone measurements were done by commercial radioimmuno-assays (RIA), as specified in detail below. In general the following definitions were used:

1. Accuracy
All standards used in the hormone analyses were assayed against Medical Research Council (MRC) standard preparations.

2. Precision
The intra-assay (within) and inter-assay (between) coefficients of variation were determined to estimate the variability of the assays. The intra-assay coefficients of variation (VC_i) were calculated from the duplicates. The between-run coefficients of variation (VC_b) were computed from three separate series of standards with low, medium and high hormone concentrations.

3. Minimal detectable concentration (mdc)
The sensitivity of the assays was expressed as the minimal detectable concentration which is defined as the detectable concentration of at least twice the standard deviation of the zero value.

4. Specificity
The specificity of the assays was expressed as the cross-reactivity, defined as the ratio between the mass of the hormone analyzed and the mass of the respective substance at 50% inhibition of binding of the hormone tracer.
The specifications of the RIA's used in this investigation are partly

summarized in table 3.6, and partly described below.

Estradiol (E_2)

Cross-reactivity.

Substance	(%)
estradiol	100
estrone	2
estriol	2
progesterone and testosterone	<0.1

Precision.

n	$\bar{x}$±SD ($nmol.l^{-1}$)	VC_b (%)
--	----------	----
15	0.34±0.02	7.3
15	1.25±0.12	9.7
15	2.32±0.24	10.3

The intra-assay coefficient of variation was calculated to be 4.1%.

(Luteinizing Hormone) LH

Cross-reactivity

Substance	(%)
LH	100
FSH	1.9
TSH	4.0
HCG	100

Precision.

n	$\bar{x}$±SD ($IU.l^{-1}$)	VC_b (%)
--	---------	----
9	2.5±3.9	15.5
15	26.0±2.5	8.7
15	38.4±2.2	5.7
15	108.0±8.3	7.7

The intra-assay coefficients of variation were calculated to be 5.0%.

Follicle Stimulating Hormone (FSH)

Cross-reactivity

Substance	(%)
FSH	100
LH	0.13
TSH	0.25

Precision.

n	$\bar{x}$±SD ($IU.l^{-1}$)	VC_b (%)
--	-------	---
14	7.9±0.5	6.3
14	14.7±1.14	7.8
14	30.5±2.3	7.8

The intra-assay coefficients of variation were calculated to be 4.3%.

Progesterone (P)

Cross-reactivity

Substance	(%)
Progesterone	100
11-β-hydroxy-Progesterone	75
5-α-dihydroprogesterone	8.8
5-β-dihydroprogesterone	7.1
Pregnenolone, cortisol, etc.	<1.0

Precision.

n	$\bar{x}$±SD ($nmol.l^{-1}$)	VC_b (%)
12	3.15±0.28	8.8
12	12.40±1.09	8.8
12	49.90±3.57	7.2

The intra-run coefficient of variation was calculated to be 4.2%.

Prolactin (PRL)

Cross-reactivity

No detectable reactivity with TSH, FSH, LH, HGG and ACTH.

Precision.

n	$\bar{x}$±SD ($IU.l^{-1}$)	VC_b (%)
18	0.11±0.01	9.2
18	0.45±0.03	6.7
18	0.92±0.05	6.2

The intra-run coefficient of variation was calculated to be 3.7%.

Testosterone (T)

Cross-reactivity

Substance	(%)
Testosterone	100
dihydrotestosterone	22
5-β-androstan-3-α, 17β-diol	3.8
11-Hydroxy-testosterone	3.3
5-α-androstan-3-α, 17β-diol	2.4
5-α-androstan-3-β, 17β-diol	2.7
Androsterone	2.1

Precision.

n	$\bar{x}$±SD ($nmol.l^{-1}$)	VC_b (%)
10	2.5±0.22	8.8
14	5.8±0.37	6.3
10	20.4±1.17	5.7

The intra-run coefficients of variation were calculated to be 4.5%.

Androstenedione (Δ4-A)

Cross-reactivity Substance	(%)
Δ4-androstenedione	100
Androsterone	11.6
Adrenosterone	2.7
Dehydroepiandrosterone	2.1

Precision.

n	$\bar{x}$±SD ($nmol.l^{-1}$)	VC_b
14	1.61±0.23	14.2
14	3.82±0.16	4.2
14	5.78±0.19	3.2

The intra-run coefficient of variation was calculated to be 4.1%.

Dehydroepiandrosterone sulphate (DHEA-S)

Cross-reactivity Substance	(%)
DHEA-S	100
DHEA	78
Δ4-androstenedione	3.3

Precision.

n	$\bar{x}$±SD	VC_b
25	3.10±0.26	8.4
8	4.72±0.58	12.4

The intra-assay coefficient of variation (VC_i) was calculated to be 4.7%.

Adrenocorticotropic hormone (ACTH)

Cross-reactivity.
The only known reactive substances are human and porcine $ACTH^{1-39}$ and human $ACTH^{1-24}$.

Precision.

n	$\bar{x}$±SD ($pmol.l^{-1}$)	VC_b (%)
14	10.7±1.26	11.8
14	52.0±5.2	9.9

The intra-run coefficient of variation (VC_i) was calculated to be 5.0%.

3.4.5 Free fraction of testosterone and estradiol

Separation of free from protein bound testosterone and estradiol in undiluted plasma was done by centrifugal ultrafiltration, using a commercial available filtration system (IUPS-1 with YMT filter, Amicon corp. Danvers, Mass. USA). The method is as follows. Radio-actively labelled estradiol [2,4,6,7-^{3}H (N)] estradiol 17-β sp act 91 Ci.mmol.l^{-1}, [1,2,6,7-^{3}H (N)] testosterone, sp act 93.9 Ci.mmol.l^{-1} and glucose, D-(U-^{14}C) glucose. sp act 257 mCi.mmol^{-1} were obtained from Amersham Int. (UK). Radiochemical purity was checked before use by thin layer chromatography (TLC) (E_2 and T) and paper chromatography (^{14}C-glucose). Working solutions of ^{14}C-glucose were made up in distilled water immediately before use. If necessary, purification was done on sephadex LH-20 chromatography columns using hexane (bp 67^{0}C, analytical grade) and chloroform (analytical grade) as the elution solvents 1-2 days before use.

Methodology

Plasma samples were centrifuged a second time after thawing and any precipitate was discarded. Aliquots of ^{3}H-steroid in ethanol (10^6 dpm) were evaporated in glass tubes under nitrogen and 20x10^{3}dpm ^{14}C glucose in 5 µl of distilled water was added to each tube. Plasma samples (250 microl) were transferred into these tubes, briefly blended on a Vortex mixer, and incubated for 30 min under an atmosphere of 95% O_2: 5% CO_2 at the desired temperature (37 or 39,5°C) and for an additional 30 min at room temperature. Aliquots (200 microl) of the incubations where then pipetted onto the YMT membrane of the MPS-1 system and placed for another 10 min in a prewarmed rotor of an ultracentrifuge (Beckman L8-70, with 60-TI angle rotor). The centrifugation angle was 35°. Centrifugation occurred at 1000 g at the desired temperature (±0.2°C) for 10 min. After centrifugation 50 µl from both the ultrafiltrate and the plasma was pipetted into scintillation vials. Scintillation liquid (3 ml of Picofluor) was added to the vials, which were capped, blended on a vortex mixer and counted in a liquid scintillation spectrophotometer (Packard Tricarb 3390) adjusted for the simultaneous measurement of ^{3}H and ^{14}C. The precision of counting <1.0% (coefficient of variation of counts per min) for ^{3}H and ^{14}C. The intra and inter run coefficient of variation were obtained from repeated (n=10) runs of

series (n=32) of the same plasma and were calculated to be 7.3% and 10%, respectively.

Calculation of the percentage of free steroid

Because ^{14}C-glucose is not bound to plasma proteins or the dialysis membrane, the ratio's between ^{3}H-steroid and ^{14}C-glucose on either side of the dialysis membrane can be used to evaluate the relative concentration of labelled steroids and glucose irrespective of volume. After corrections for background counts and ^{14}C to ^{3}H spill-over in the counting system, the percentage of free steroid could be calculated as follows:

$$\% \text{ free steroid} = \frac{\frac{^{3}\text{H-steroid (dpm)}}{^{14}\text{C glucose(dpm)}}}{\text{ultrafiltrate}} \div \frac{^{3}\text{H-steroid dpm}}{^{14}\text{C-glucose(dpm)}}{\text{plasma}}$$

Metabolic clearance rate studies (Va and Vb)

The MCR of E_2 was calculated by a modification of the constant infusion technique as described by Poortman et al (1973). With this method a tracer amount of ^{3}H-E_2 was infused.
After extensive purification of plasma extracts for estimation of radiochemical homogenity the MCR could be calculated. Correction for procedural losses was done by gas liquid chromatography (GLC). The details of the method are as follows.

Materials

Labelled estradiol, [2, 4, 6, 7-^{3}H(N)] estradiol-17β, [TRK 322, SA] 98Ci.mmol^{-1} (batch 51, study Va), sp act 85 Ci.mmol^{-1} (batch 44, study Vb, subj. 1-3) and sp act 104 Ci.mmol^{-1} (batch 46, study Vb, subj. 4-6) was obtained from The Radiochemical Centre (Amersham, UK). The non-radio-active steroids (E_2 and E_1) were obtained from Makor chemicals, Ramat-Gan, Israel and Organon, Oss, The Netherlands. Purity of labelled steroids was checked by TLC performed on 20x20 cm Merck precoated silica gel GF-254 glass plates.

All solvents for extraction of plasma samples and for chromatography

were of analytical grade and redistilled before use. Gas Liquid Chromatography (GLC) was performed on a Pye gas chromatograph (model 104) with automatic solid injection and a Perkin Elmer gas chromatograph (model P-17) using 1% XE-60 packing and a hydrogen flame ionization detector. Acetylation and saponification of the steroids were done as described by Bush (1961)

Analytical procedures.

The analytical procedures were as follows: 200 µgram E_1 and E_2 were dissolved in 0.2 ml ethanol and the plasma sample added, which was diluted with an equal volume distilled water. Extraction occured with diethylether (3x20 ml), thereafter the ether extract was collected and evaporated to dryness. The residue was dissolved in 1 ml ethanol and after addition of 25 ml toluene a phenolic separation according to Brown (1955) was performed. The water phase (with E_3) was discarded and the organic phase (after the pH was brought at 8.8) was extracted with diethyl-ether (2x30 ml). After washing with 5 ml distilled water the etherphase was collected and evaporated. The residue was dissolved in methanol and subjected to TLC (toluene-methanol 9:1 (v/v).

After localization of the E_1 and E_2 band, the silicagel was collected, inactivated with a drop of distilled water and eluated (3x) with toluene-ethanol (9:1, v/v). The extracts were evaporated to dryness under a stream of nitrogen. The residue was dissolved in 5 drops of pyridine, vortexed, whereafter 10 drops of acetic anhydride were added. The air in the tube was removed by a stream of nitrogen, whereafter it was stoppered. Acetylation of E_1 and E_2 occurred at room temperature in darkness. It was shown that for complete acetylation 18 hrs was sufficient. After acetylation the tubes were evaporated to dryness under a stream of nitrogen, the residue dissolved in some drops of ethanol and again subjected to TLC. As a control 100 µgram of crystalline E_2-diacetate and E_1-acetate were dissolved in ethanol and also subjected to TLC. After detection of the E_1-acetate and E_2-diacetate bands the silicagel was removed, eluated with ethylacetate (3x) and evaporated to dryeness under N_2. The residues then were dissolved in ethylacetate (1 ml) and from this solution 0.4 ml was subjected to LSC and 0.2 ml to GLC. The GLC was done to quantitate procedural losses.

To further evaluate the effectiveness of the procedures a deacetylation

step was performed. For this purpose the remainder of the acetates (0.4 ml) was evaporated to dryeness under N_2. Deacetylation occured in a solution of 0.4 ml NaOH in 70% methanol and an atmosphere of N_2 for 18 hrs at room temperature. Thereafter ether extraction was done (3x2 ml), and the ether extract washed with 2 ml 0.01 HaC and 1 ml distilled water. The organic phase was evaporated to dryness under N_2, the residue dissolved in 0.2 ml ethanol and subjected to TLC.

After elution of the silicagel, containing E_1 and E_2 the elutes were evaporated under N_2, and the residue dissolved in ethylacetate (1 ml) and subjected to LSC and GLC. If after correction for procedural losses the E_1 and E_2 content of the acetylated and deacetylated samples disagreed for more than 12%, they were discarded and the values not used. The whole procedure is schematically depicted in fig. 3.4.

The MCR was calculated according to the formula:

$$MCR = \frac{r}{b} \; ml.min^{-1}$$

where, r = the infusion rate (dpm/min) of the radioactive steroid and b = the level of radio-activity of E_2 (dpm/ml) in plasma after correction for procedural losses.

3.5 DATA ANALYSIS

3.5.1 Criteria for maximal tests

For the evaluation of the influence of the phase of the menstrual cycle on maximal physical working capacity only maximal tests were used. To decide whether a test could be considered as maximal, the following criteria, of which 4 out of 6 had to be fulfilled were used (Kuipers, 1983):

1. a plasma lactate concentration of 8 $mmol.l^{-1}$ or more (Åstrand and Rodahl, 1977; Hollmann and Hettinger, 1981).

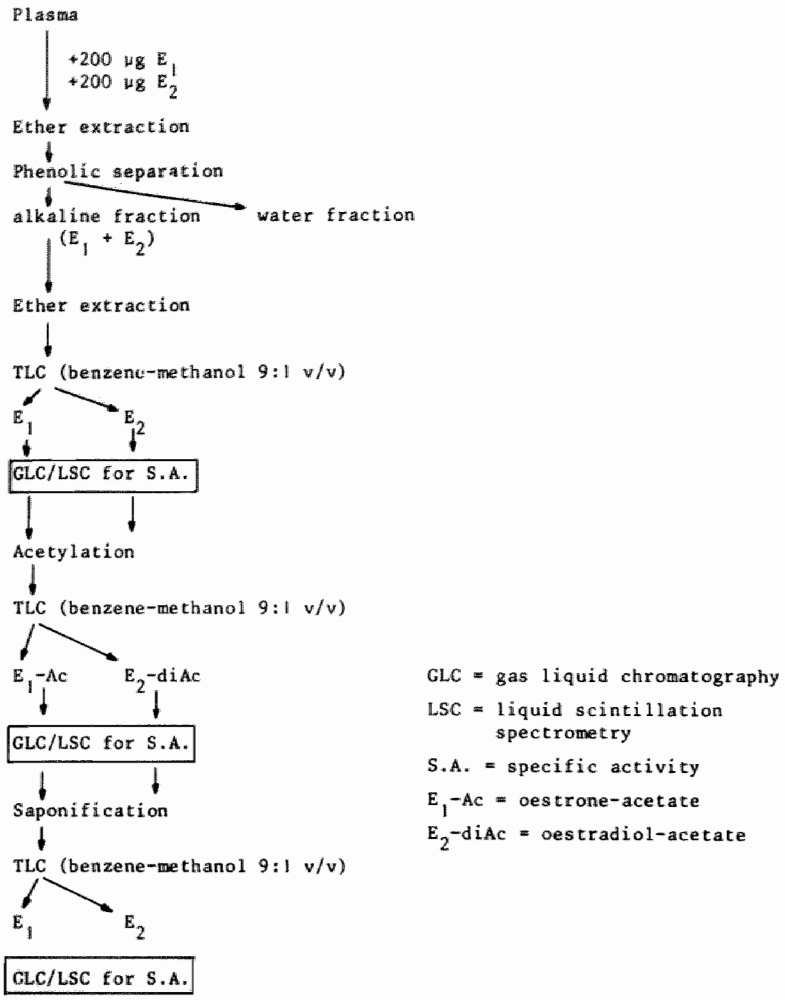


Fig. 3.4 Purification of plasma extracts for estimation of radiochemical homogeneity of estrone and estradiol.

2. a heart rate exceeding 180 beats.min^{-1}.
3. a respiratory equivalent ($\dot{V}_E/\dot{V}O_2$) above 30 (Hollmann and Hettinger, 1981).
4. an R value ($\dot{V}CO_2/\dot{V}O_2$) over 1.00 (Binkhorst, 1963).
5. a respiratory rate of 45 or more
6. the investigator's judgement of the subject's distress.

3.5.2 Statistical analysis

Statistical analysis was done by using the Biomedical Computer Program (BMDP package, Dixon and Brown, 1981). Glucose, lactate and all hormonal data, including the percentual changes, were analyzed with a two-way (exercise versus phase) ANOVA among groups with repeated measures (BMDP-2V). For the hormonal data comparison among groups were also conducted with the relative values. For this purpose, the mean of the two resting samples was aimed to be 100%. The remainder of the values were expressed as the percentage of the mean resting value. Because not all subjects were able to reach the highest (90% $\dot{V}O_2$max) workload, only the first three workloads (60, 70 and 80% $\dot{V}O_2$max) were used to make intergroup comparisons. In addition, comparisons between the absolute values at rest of the untrained (study I) and trained subjects (study II) were also conducted, using the same program. If the hormonal responses to exercise were significant a Wilcoxon signed-rank, test was conducted to evaluate which workload induced significant changes in plasma hormone concentrations compared to the values at rest. For study III both the absolute and relative values during exercise were analyzed with BMDP-2V. For most of the changes in hormone concentration variance stabilizing transformations were used. For the absolute values separate analyses of variance (2-way and 1-way ANOVA) were also conducted (BMDP-7D) for each group. Differences between maximal physical working capacity in the follicular and luteal phase, and between the percentages free E_2 and T before and after exercise (study I and III) were tested on statistical significance with a multiple paired t-test (BMDP-3D).

The data of study IV were analyzed as follows:
For each individual the data were plotted as hormone concentrations versus time. To analyse the frequency and amplitude of secretory periods a hormone pulse was defined as occurring when the hormone concentration of a sample exceeded the previous concentration by at least twice the intra-assay

coefficient of variation. Thus LH, FSH and E_2 pulses were considered when the plasma concentration increased by more than 10.0, 8.6 and 8.2%, respectively. The frequency was determined during the pre- and post-exercise period separately. The pulse amplitude was expressed in $IU.l^{-1}$ for LH and FSH and $nmol.l^{-1}$ for E_2. In addition for each individual the pre- and post exercise periods were compared for each hormone by a multiple paired t-test (BMDP-3D) using the last 8 pre- and the first post-exercise values only. BMDP-3D was also used to compare these periods for the group as a whole. Besides, the general trend of the changes in hormone concentrations before and after exercise were graphical depicted making use of techniques of exploratory data analysis as described by Tukey (1979).

The data of study V were analysed with Mosteller's test for a specified order (Sarris and Wilkening, 1977).

Table 3.6
Specifications, minimal detectable concentration and manufacturers of the RIA's used in the present investigation.

Hormone	Specification RIA	Tracer	MDC	Manufacturer
E_2	Direct, double antibody, solid-phase	^{125}I	0.03 $nmol.l^{-1}$	Eir, Wurlingen, Switserland
P	direct, double antibody, liquid phase	^{125}I	0.28 $nmol.l^{-1}$	Farmos Diagnostica, Turku, Finland
LH	Direct, double antibody, liquid phase	^{125}I	1.1 $IU.l^{-1}$	Farmos Diagnostica, Turku, Finland
FSH	Direct, double antibody, liquid phase	^{125}I	0.8 $IU.l^{-1}$	Farmos Diagnostica, Turku, Finland
T	Extraction, double antibody, solid phase	^{125}I	0.02 $nmol.l^{-1}$	Diagnostic Products, Los Angelos, USA
A	Direct, solid phase (without chromatography) separation of antibody bound A from free A by dextran coated charcoal	^{3}H	0.042 $nmol.l^{-1}$	Bio Merieux, Charbonnieres-les-Bain, France
DHEA-S	Direct, solid phase. Separation of antibody bound DHEA-S from free DHEA-S by dextran coated charcoal	^{3}H	0.05 $\mu mol.l^{-1}$	Bio Merieux, Charbonnieres-les-Bain, France
ACTH	Direct, solid phase, double antibody	^{125}I	4.4 $pmol.l^{-1}$	Immuno Nuclear Corp., Stillwater, USA
PRL	Direct, solid phase, double antibody	^{125}I	0.05 $IU.l^{-1}$	Abbot, North Chicago USA

CHAPTER IV

RESULTS

4.1 MAXIMAL PHYSICAL WORKING CAPACITY (MPWC) IN RELATION TO THE PHASE OF THE MENSTRUAL CYCLE (STUDY I)

The mean MPWC of the untrained subjects participating in study I was calculated to be 164.8±4.2 Watt and 165.5±4.5 Watt in the follicular and luteal phase, respectively. This difference was not statistically significant. All subjects (except for subject 1307) participating in study II were able to complete the 75 min treadmill run in both phases of the menstrual cycle. Subject 1307 performed only for 60 min in the luteal phase of her menstrual cycle.

4.2 METABOLIC AND ENDOCRINE RESPONSES TO PROLONGED PHYSICAL EXERCISE IN UNTRAINED AND TRAINED WOMEN (STUDY I AND II)

The experiments in the follicular phase occurred 7-10 days after the first day of menstruation. Ovulation had not occurred in any of these subjects as indicated by the low P concentrations at rest (< 6 nmol.l^{-1}). Conversely, from the untrained group, 15 out of 16 subjects were also tested in the luteal phase of their menstrual cycle as judged by a rise of 0.3°C in BBT, later on confirmed by the increased concentrations of plasma P (> 11 nmol.l^{-1}) at rest prior to exercise. It appeared that one subject (no. 1020) was tested on the day the LH surge occured instead of during the luteal phase, therefore her data were discarded from further statistical analysis. Two subjects (no. 1003 and 1004) could not fulfil the terms for a maximal physical performance, so we excluded their data from further analysis too. The remainder of the untrained subjects (n=13) and all trained subjects fulfilled all terms for both a maximal test and an apparent ovulation prior to the "luteal phase" experiment.

Considerable care was taken to test each subject under the same experimental conditions. In study I and II, the exercise protocol started between

8.30 and 10.30 a.m. while each subject was retested at the same time (±15 min), thus decreasing scattering of results due to differences in diurnal secretion pattern of some hormones. The experimental design of these studies permitted analyses of the data according to group membership (group), the phase of the menstrual cycle (phase) and the responses during the exercise period (exercise). Consequently, comparisons of these main effects as well as their interaction were performed among the untrained and trained subjects and for both groups seperately. Because we were also interested in the relation between workload and exercise intensity, we tested each plasma hormone value obtained during exercise against the mean of the two values at rest. The plasma concentrations of glucose and lactate are presented as absolute values versus time or workload while the hormonal data are presented as absolute and relative values (X±SEM) versus time or workload. For the latter purpose the mean of the two resting values obtained 15 and 30 min after the insertion of the catheter was taken as 100%. Relative values were used since we were mainly interested in the pattern and magnitude of the hormonal responses to exercise. Besides, this response will be more clear when expressed as relative values, because of the large inter-individual variation in plasma hormone concentrations. The values of both groups had to be expressed in different ways (concentration versus workload or time) because not all subjects of the untrained group were able to sustain the 80% $\dot{V}O_2$max workload for 15 min (mean: 13 min 5 sec and 13 min 23 sec in the follicular and luteal phase, respectively). Therefore it was felt that expressing the hormone, glucose and lactate concentrations
against the workload was more appropriate. However, one has to bear in mind that despite these differences the first 45 min of the exercise period were well comparable between the two groups. Thus, the workloads of 60, 70 and 80% $\dot{V}O_2$max in the untrained group are comparable with the workload, 45, 60 and 75 min after insertion of the catheter in the trained group.

4.2.1 Metabolic responses to exercise

Glucose

In the untrained and trained subjects the glucose concentrations increased significantly ($p<0.001$) during exercise. In the untrained subjects (fig. 4.1) the increments in plasma glucose concentration tended ($p=0.06$)

.o be more pronounced in the luteal phase when compared to the follicular)hase, whereas in the trained subjects (fig. 4.2) no differences could be)bserved between both phases. The responses to exercise in the trained ;ubjects differed significantly from those in the untrained subjects (p<0.001). Whereas the plasma glucose concentration did not change signi-ficantly at lower workloads in the untrained subjects; it increased at the same workload in the trained subjects.

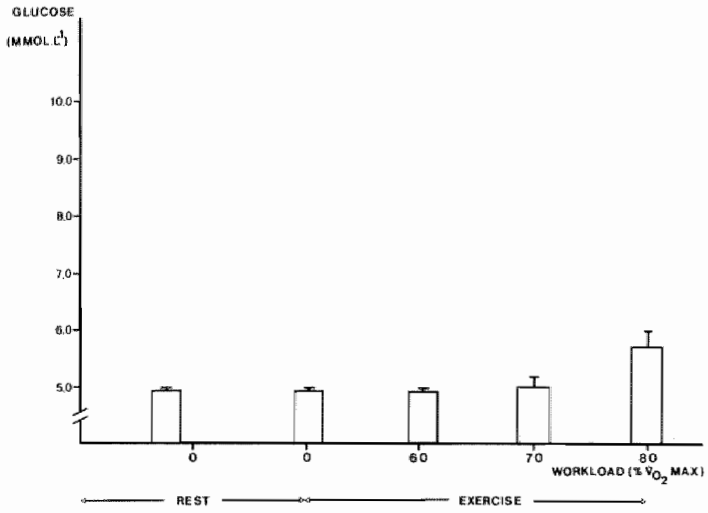


Figure 4.1
Plasma glucose concentration during exercise in untrained women (n=13) in the follicular phase of their menstrual cycle.

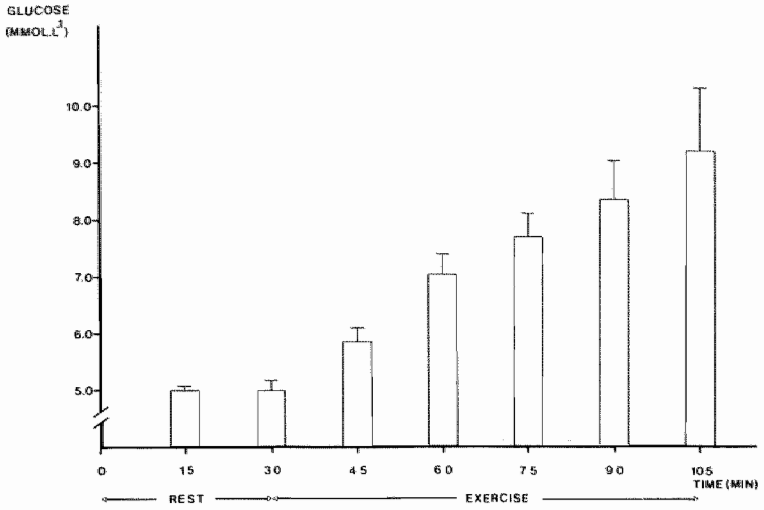


Figure 4.2
Plasma glucose concentration during exercise in trained women (n=6) in the follicular phase of their menstrual cycle.

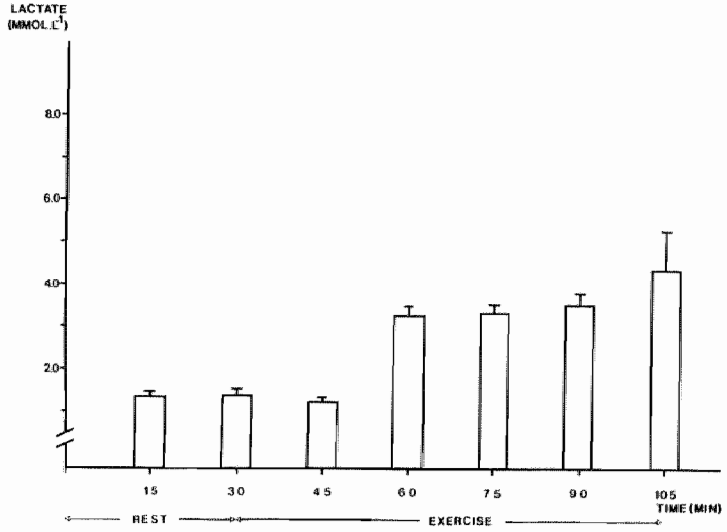


Figure 4.3
Plasma lactate concentration during exercise in trained women (n=6) in the follicular phase of their menstrual cycle.

Lactate

In the untrained and trained subjects the plasma lactate concentrations increased significantly during exercise ($p<0.001$). No statistically significant differences could be observed between the follicular and luteal phases of the menstrual cycle. As could be expected the plasma lactate concentration was significantly lower ($p<0.001$) in the trained (fig. 4.3) than in the untrained subjects (fig. 4.4) at comparable workloads (i.e. 60, 70 and 80% $\dot{V}O_2max$ and exercise times 15, 30 and 45 min. after the start of the exercise).

4.2.2 Endocrine responses to exercise

The basal hormone concentrations (the mean of the two values at rest) of the untrained and trained subjects are depicted in table 4.1.

Estradiol (E_2)

During exercise the absolute E_2 values increased significantly ($p<0.001$) in both groups. In the untrained subjects (fig. 4.5) the plasma E_2 concentrations were higher in the luteal phase ($p<0.001$), while in the trained subjects (fig. 4.6) the plasma E_2 concentrations were more elevated in the follicular phase (table 4.1). In the untrained subjects all workloads were able to induce significant increments in plasma E_2 concentration (fig. 4.5). In the trained subjects similar results were obtained, with the exception of the first exercise value (15 min. after the start of the exercise), which was not statistically different from the values at rest (fig. 4.6). When expressed as relative values (data not shown) no significant effect of the phase of the menstrual cycle could be detected. In both phases the plasma E_2 concentration increased by about 15% at a workload of 60% $\dot{V}O_2max$ and by about 40-50% at a workload of 80% $\dot{V}O_2max$. These increases were comparable in both groups. In the untrained subjects the relative exercise-induced increments in E_2 concentration varied between 0 and 55%, and between 5 and 64% in the follicular and luteal phase, respectively. In the trained subjects the increments ranged from 11 to 108% and 23 to 100% in the follicular and luteal phase, respectively. The differences in response between these groups appeared to be not significant. In the untrained subjects, the free fraction of E_2 at rest was calculated to be 1.55±0.04% and 1.62±0.08% in the follicular and luteal phase, respectively. The values

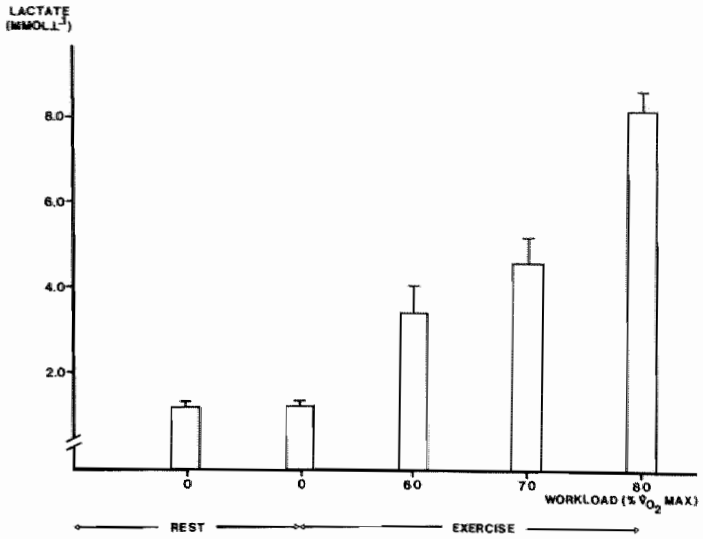


Figure 4.4
Plasma lactate concentration during exercise in untrained women (n=13) in the follicular phase of their menstrual cycle.

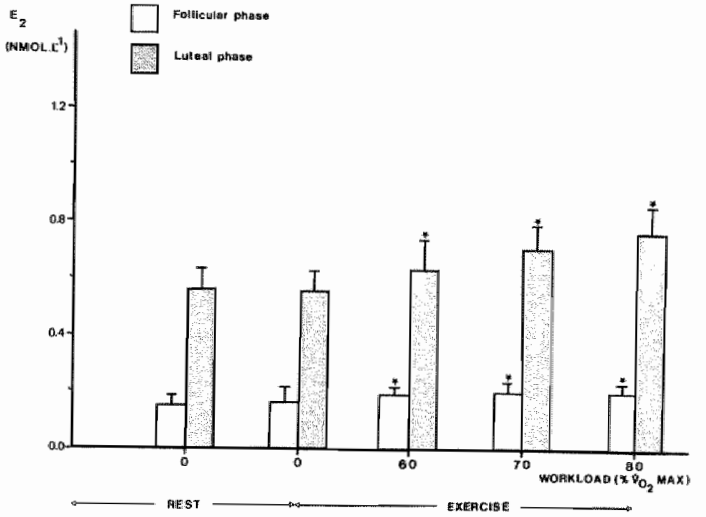


Figure 4.5
Plasma E_2-concentrations during exercise in untrained women (n=13) in the follicular and luteal phases of their menstrual cycle.
** = significantly different ($p<0.05$) from the values at rest.*

Table 4.1
Plasma concentration at rest (mean ±SEM of the two values obtained 15 and 30 min after insertion of the catheter) of E_2, P, LH, FSH, T, Δ4-A, DHEA-S, PRL and ACTH in untrained and trained subjects in the follicular and luteal phases of their menstrual cycle.

HORMONE	UNTRAINED SUBJECTS		TRAINED SUBJECTS	
	PHASE		PHASE	
	FOLLICULAR	LUTEAL	FOLLICULAR	LUTEAL
E_2 ($pmol.l^{-1}$)	0.15±0.03*	0.56±0.07	0.56±0.12*	0.44± 0.03
P ($nmol.l^{-1}$)	5.2 ±0.43	27.3 ±5.19	3.9 ±0.6	39.6 ±11.5
LH ($IU.l^{-1}$)	9.0 ±0.59	11.0 ±1.5*	9.4 ±0.18	5.8 ± 1.1*
FSH ($IU.l^{-1}$)	4.9 ±0.44	3.5 ±0.48	4.3 ±0.5	2.7 ± 0.6
T ($nmol.l^{-1}$)	1.8 ±0.2*	1.9 ±0.2*	1.3 ±0.44*	1.1 ± 0.3*
Δ4-A ($nmol.l^{-1}$)	7.76±0.52*	8.77±0.78*	5.7 ±0.68*	5.5 ± 0.6
DHEA-S ($\mu mol.l^{-1}$)	7.04±0.92*	7.32±0.80*	2.3 ±0.32*	2.36± 0.4*
PRL ($IU.l^{-1}$)	0.34±0.1	0.27±0.02	0.22±0.05	0.21± 0.06
ACTH	11.1 ±1.06*	9.7 ±1.1*	6.1 ±1.2*	5.6 ±1.4*

* denotes a significant difference ($p<0.05$) between the values of the untrained and trained group.

after exercise were 1.52±0.05% and 1.51±0.05% in both phases respectively. The post-exercise values were significantly ($p<0.05$) decreased as compared to the pre-exercise values. In the trained group, the free E_2 fraction at rest was calculated to be 1.22±0.04% and 1.25±0.02% in the follicular and

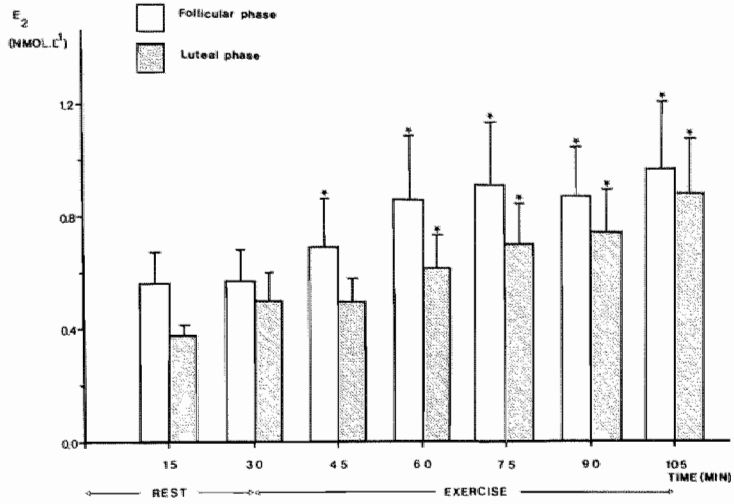


Figure 4.6
Plasma E_2 concentrations during exercise in trained women (n=6) in the follicular and luteal phases of their menstrual cycle.
** = significantly different ($p<0.05$) from the values at rest.*

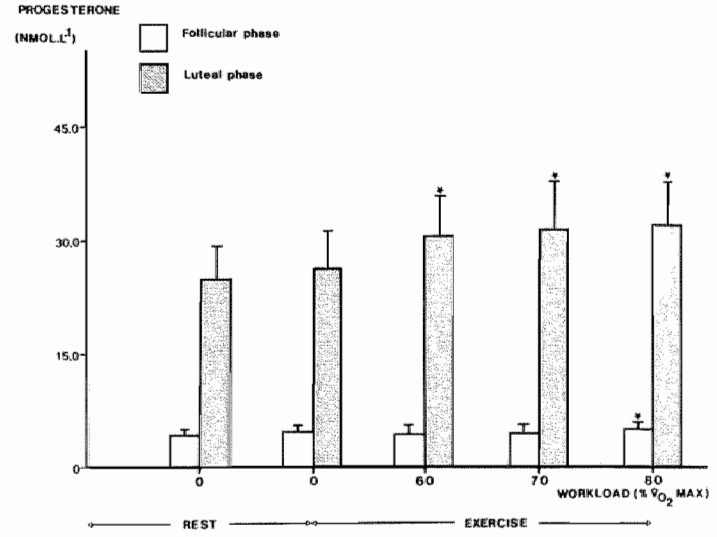


Figure 4.7
Plasma P concentrations during exercise in untrained women (n=13) in the follicular and luteal phases of their menstrual cycle.
** = significantly different ($p<0.05$) from the values at rest.*

luteal phase, respectively. No significant differences could be observed after the exercise. In the latter situation the values were measured to be 1.32±0.05 and 1.24±0.04% in the follicular and luteal phase, respectively. The mean free E_2 fraction at rest in the untrained subjects was significantly ($p<0.01$) higher than the mean E_2 fraction in the trained subjects.

Progesterone (P)

In the untrained and trained subjects the absolute plasma P concentrations increased significantly ($p<0.001$) during exercise in the follicular and luteal phase (Fig. 4.7 and 4.8). In the untrained subjects only the highest workload (80% VO_2max) was able to induce a significant increment in plasma P levels in the follicular phase (fig. 4.7). In the trained subjects, all plasma P values during exercise (except for the first exercise value) were significantly elevated as compared to the values at rest (fig. 4.8).

Exercise in the luteal phase provoked significant increments at all exercise intensities (60, 70 and 80% VO_2max) in the untrained subjets, whereas this was also true for the trained subjects with the exception of the lowest workload (i.e. 15 min. after the start of the exercise). No statistically significant differences in relative values between both phases could be observed in the trained group in the follicular phase as compared to the luteal phase (fig. 4.9). In the untrained subjects the relative increase was greater ($p<0.005$) in the luteal phase (fig. 4.10). No differences in percentual changes in P concentration could be observed between the trained and untrained group at comparable exercise times (15, 30 and 45 min. after the start of the exercise) and workloads (60, 70 and 80% VO_2max).

Luteinizing Hormone (LH).

In the untrained and trained subjects the plasma LH concentration decreased significantly ($p<0.05$) during exercise.In the untrained group only the highest workload (80% VO_2max) was able to induce a significant decrease in plasma LH concentration in the follicular phase, whereas in the luteal phase, the mean plasma concentrations of this hormone were decreased at all workloads (fig. 4.11). In the trained subjects exercise induced significant decrements in the mean plasma LH concentration (with the excep-

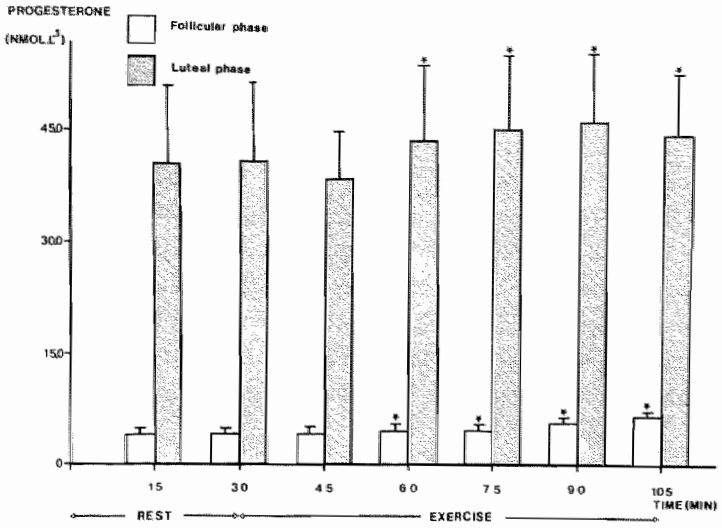


Figure 4.8
Plasma P concentrations during exercise in trained women (n=6) in the follicular and luteal phases of their menstrual cycle.
** = significantly different ($p<0.05$) from the values at rest.*

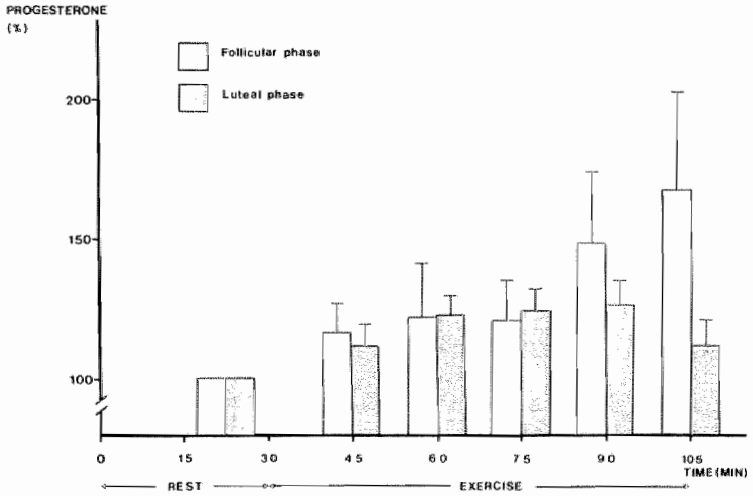


Figure 4.9
Relative changes (percentage of the mean basal value) of the plasma P concentration in trained women (n=6) in the follicular and luteal phases of their menstrual cycle.

tion of the last experimental value of the follicular phase, which tended to increase as compared to the previous samples (fig. 4.12). In the untrained subjects, the absolute mean LH concentration in the blood was significantly higher in the luteal phase ($p<0.005$) (fig 4.11), whereas in the trained subjects the LH values were higher in the follicular phase ($p<0.05$) (figure 4.12). The relative changes revealed a significant linear decrease ($p<0.001$) in plasma LH concentration during exercise in both phases in the untrained group; the decrease being more pronounced ($p<0.005$) in the luteal phase of the menstrual cycle (fig. 4.13). Although the relative decrease in plasma LH concentration was also significant ($p<0.001$) in the trained group the linear trend was not existent and there was no phase effect (fig. 4.14).

Follicle Stimulating Hormone (FSH).

In the untrained and trained subjects the absolute plasma FSH concentration was not significantly affected by exercise. In both groups the absolute FSH concentrations were significantly ($p<0.01$) higher in the follicular phase of the menstrual cycle (table 4.1). In the untrained group there was a significant ($p<0.005$) linear decrease of the relative values during exercise and a significant ($p<0.05$) phase effect. The relative decrease in the luteal phase was less pronounced than in the follicular phase ($p<0.05$) (fig. 4.15). In the trained group no significant differences could be observed in relative FSH values as a function of the exercise period or phase of the menstrual cycle (data not shown). In the untrained subjects the relative changes varied between -33 and 0% and between -44 and 7% in the follicular and luteal phase, respectively. In the trained subjects these values varied between -21 and 74% and -50 and 119% in the follicular and luteal phase, respectively.

Testosterone (T).

In the untrained subjects the mean plasma T concentration at rest was significantly ($p<0.05$) higher than in the trained subjects (table 4.1). In the untrained group the absolute plasma T concentrations were significantly ($p<0.05$) increased above the resting value at workloads exceeding 60% $\dot{V}O_2max$. No significant differences could be detected between the phases of

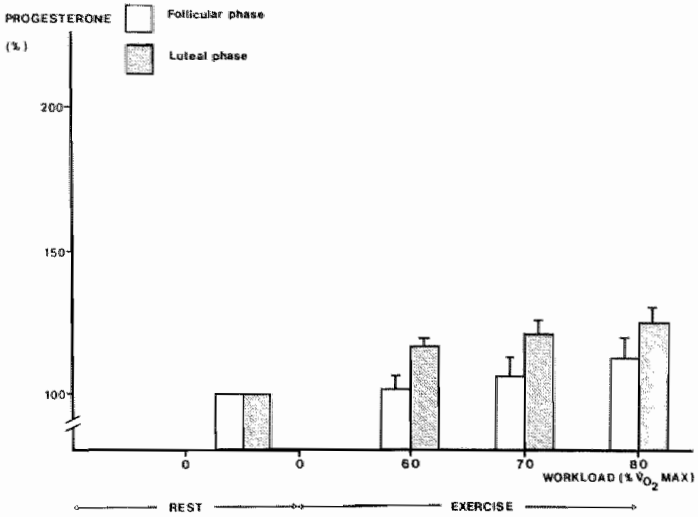


Figure 4.10
Relative changes (percentage of the mean basal value) of the plasma P concentration in untrained women (n=13) in the follicular and luteal phases of their menstrual cycle.

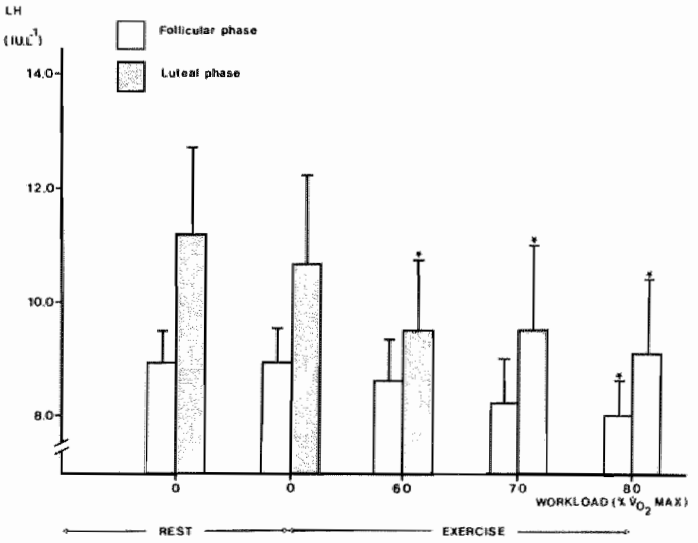


Figure 4.11
Plasma LH concentration during exercise in untrained women (n=13) in the follicular and luteal phases of their menstrual cycle.
** = significantly different ($p<0.05$) from the values at rest.*

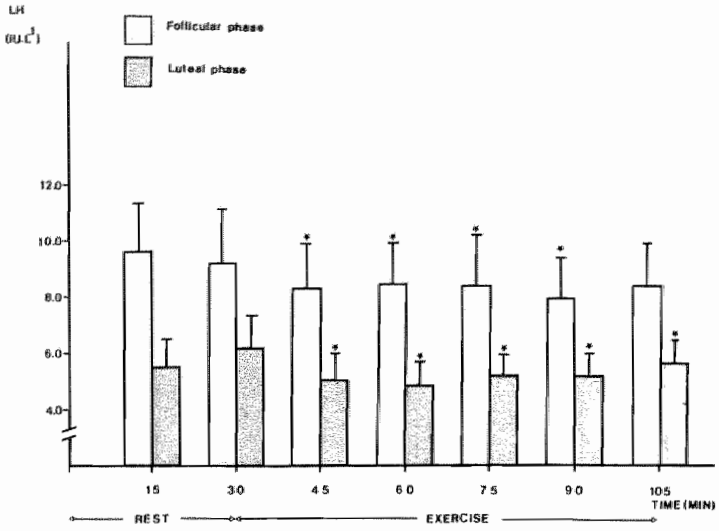


Figure 4.12
Plasma LH concentration during exercise in trained women (n=6) in the follicular and luteal phases of their menstrual cycle.
** = significantly different (p<0.05) from the values at rest.*

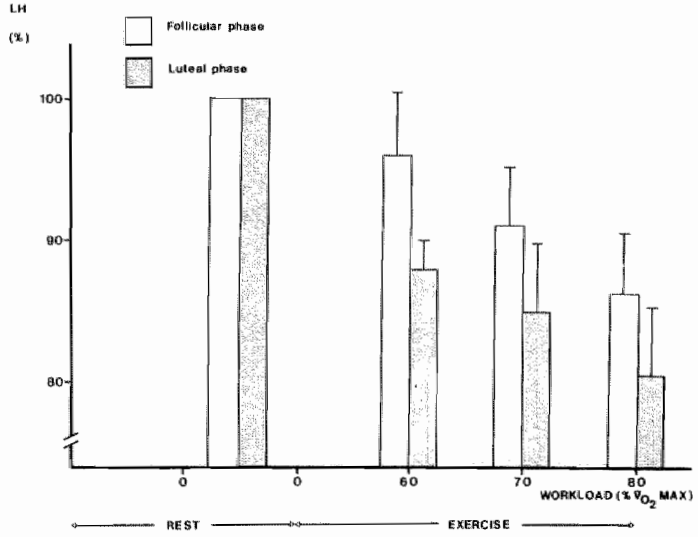


Figure 4.13
Relative changes (percentage of the mean basal value) in plasma LH concentration during exercise in untrained women (n=13) in the follicular and luteal phases of their menstrual cycle.

the menstrual cycle in this group (fig. 4.16). In the trained subjects the absolute plasma T concentration increased significantly ($p<0.01$) during the exercise period (fig. 4.17). This increase was significant at exercise times greater than 15 min. (follicular phase) or 30 min. (luteal phase) after the start. The relative values showed a significant linear increase ($p<0.001$) during exercise in both groups. At comparable workloads (60, 70 and 80% $\dot{V}O_2max$) and exercise times (15, 30 and 45 min after the start of the exercise) the relative T values increased more pronounced ($p<0.05$) in the trained than in the untrained subjects as shown for the follicular phase in fig. 4.18 and 4.19. No significantly different responses between the phases of the menstrual cycle could be detected.

In the untrained group the percentage of free T increased significantly ($p<0.05$) from 2.32±0.11% and 2.33%±0.11% at rest to 2.52±0.09% and 2.57±0.11% at the end of the exercise period in the follicular and luteal phase, respectively. In the trained group the values at rest were calculated to be 2.08±0.07% and 2.02±0.08% in the follicular and luteal phase, respectively. These values increased significantly ($p<0.01$) to 2.26%±0.06 in the follicular and 2.25±0.09% in the luteal phase. In both groups no difference could be observed between the phases.

Androstenedione (Δ4-A).

In the untrained subjects the mean plasma Δ4-A concentrations at rest were significantly ($p<0.01$) higher than those in the trained subjects (table 4.1). In the untrained subjects the mean plasma Δ4-A concentration did not change significantly (fig. 4.20) during exercise. In the trained subjects, however, exercise induced a significant increase ($p<0.001$) (fig. 4.21) in the plasma concentration of this hormone in both phases of the menstrual cycle. These increments occurred at each workload (fig. 4.21). At comparable workloads (60, 70 and 80% $\dot{V}O_2max$) and exercise times (15, 30 and 45 min after the start of the exercise) the relative values showed a significant ($p<0.01$) different response between both groups, the relative increments, being more pronounced in the trained group (fig. 4.22 and 4.23). In the latter group the relative values showed a levelling-off after 60 min of exercise, especially in the follicular phase of the menstrual cycle (fig. 4.23).

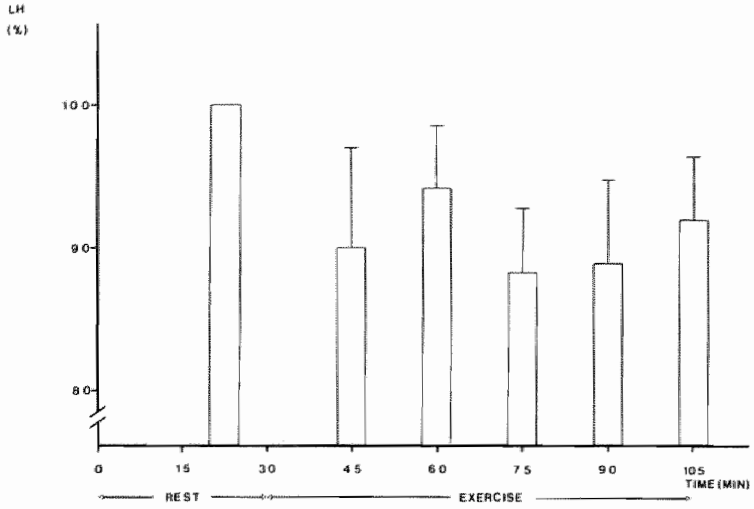


Figure 4.14
Relative changes (percentages of the mean basal values) in plasma LH concentration during exercise in trained women (n=6) in the follicular phase of their menstrual cycle.

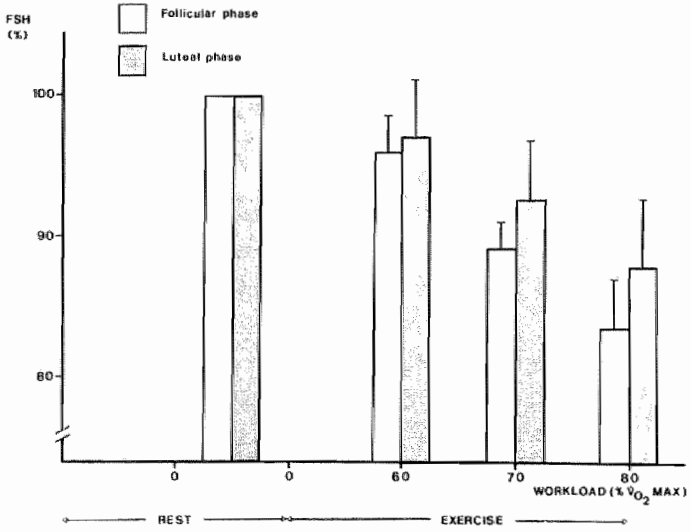


Figure 4.15
Relative changes (percentage of the mean basal value) in plasma FSH concentration during exercise in untrained women (n=13) in the follicular and luteal phases of their menstrual cycle.

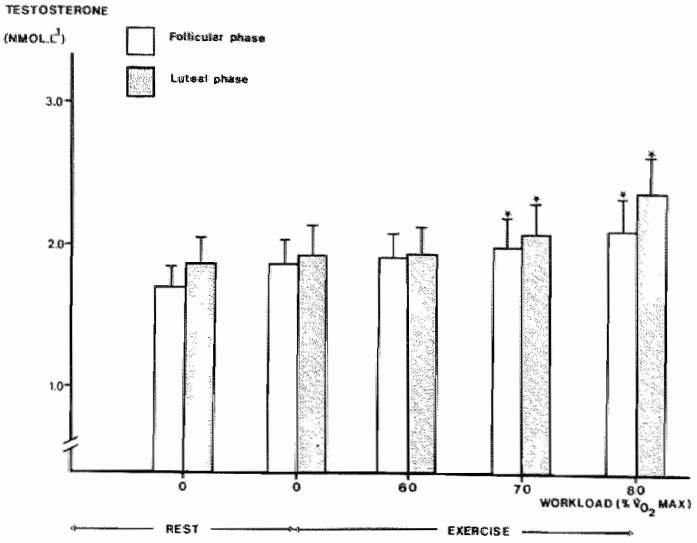


Figure 4.16
Plasma T concentrations ($nmol.l^{-1}$) during exercise in untrained women (n=13) in the follicular and luteal phases of their menstrual cycle.
** = significantly different ($p<0.05$) from the values at rest.*

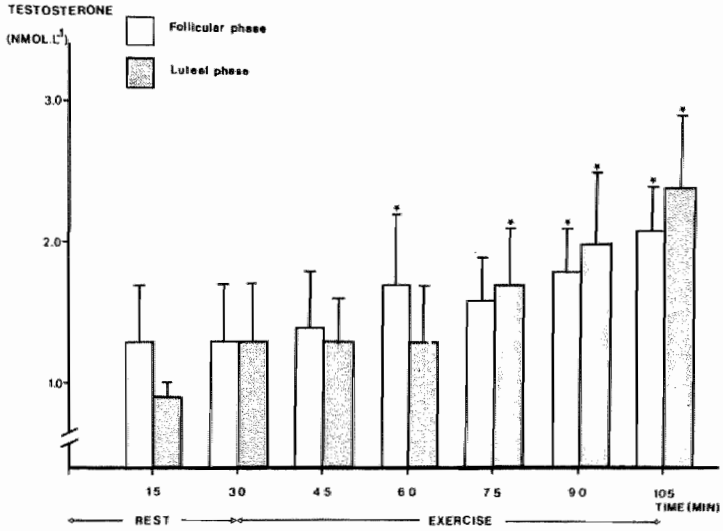


Figure 4.17
Plasma T concentration during exercise in trained women (n=6) in the follicular and luteal phases of their menstrual cycle.
** = significantly different ($p<0.05$) from the values at rest.*

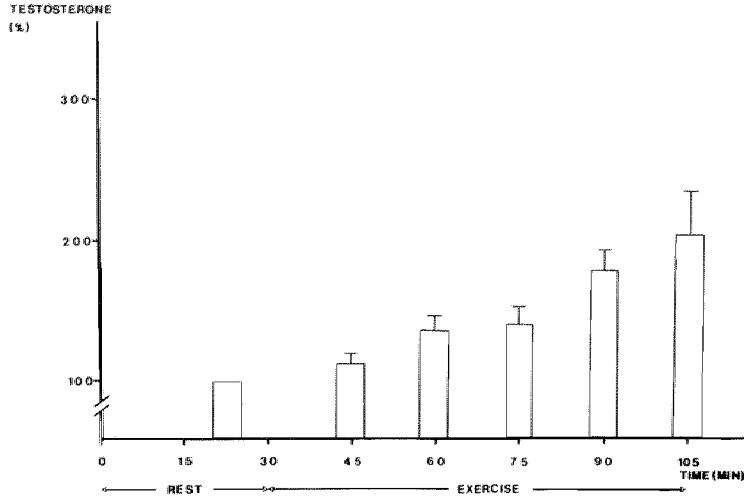


Figure 4.18
Relative changes (percentage of the mean basal value) in plasma T concentration during exercise in trained women (n=6) in the follicular phase of their menstrual cycle.

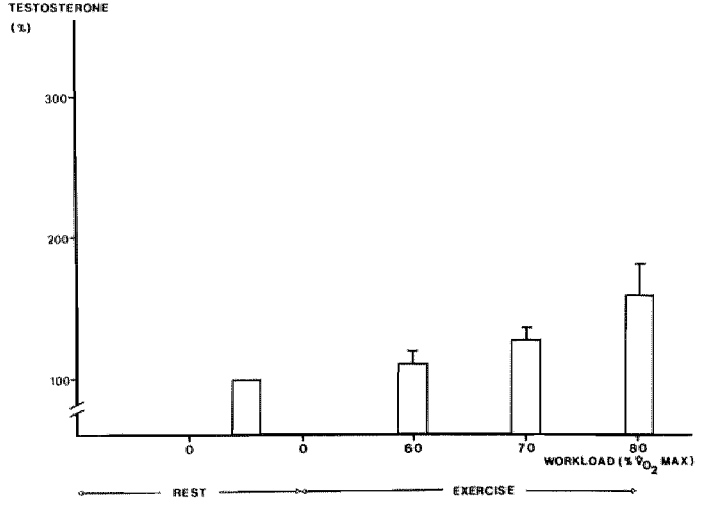


Figure 4.19
Relative changes (percentage of the mean basal value) in plasma T concentration during exercise in untrained women (n=13) in the follicular phase of their menstrual cycle.

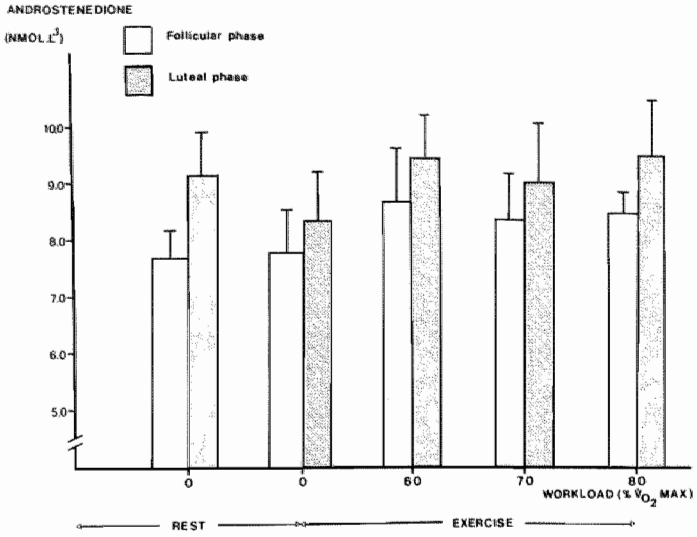


Figure 4.20
Plasma Δ4-A concentration during exercise in untrained women (n=13) in the follicular and luteal phases of their menstrual cycle.

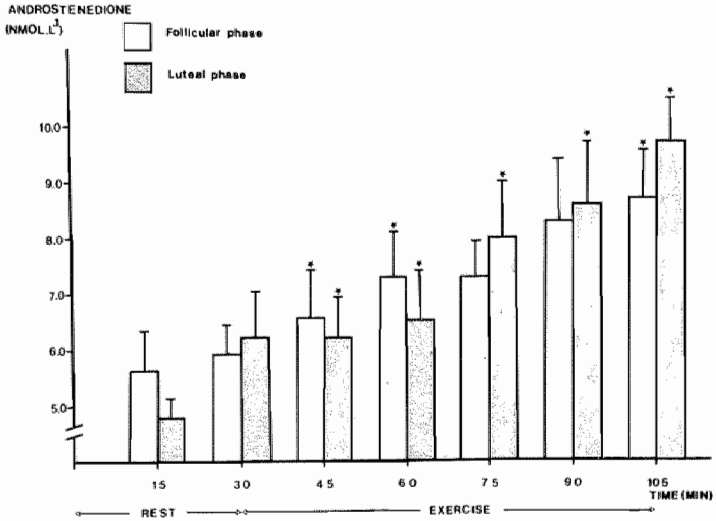


Figure 4.21
Plasma Δ4-A concentration during exercise in trained women (n=6) in the follicular and luteal phases of their menstrual cycle.
** = significantly different (p<0.05) from the values at rest.*

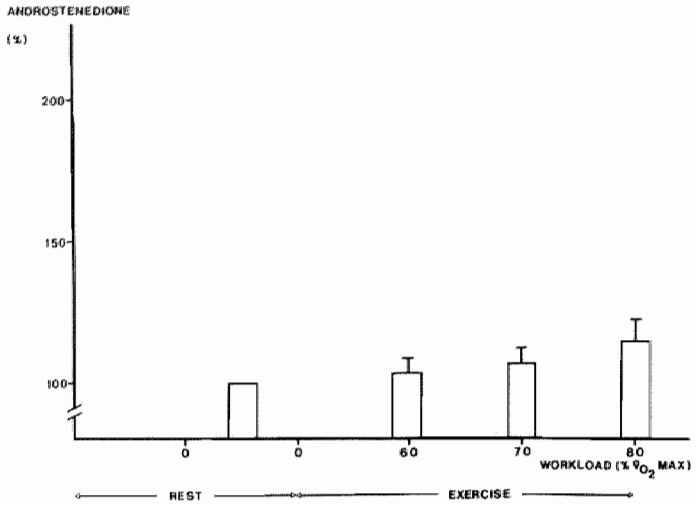


Figure 4.22
Relative changes (percentage of the mean basal value) in plasma Δ 4-A concentration during exercise in untrained women (n=13) in the follicular phase of their menstrual cycle.

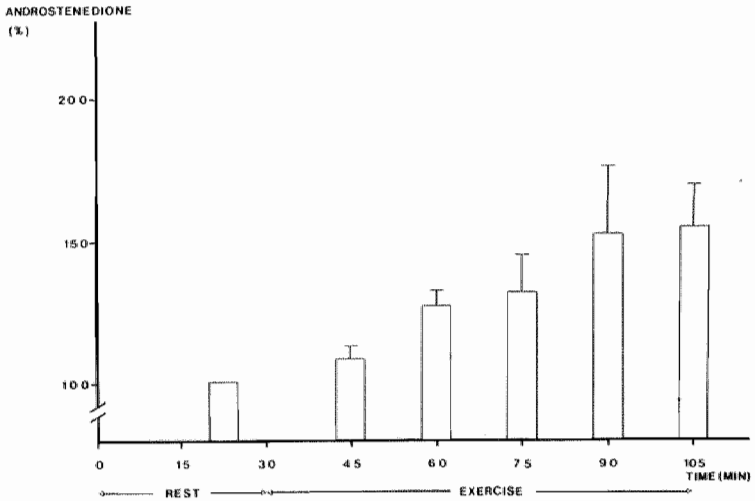


Figure 4.23
Relative changes (percentages of the mean basal value) in plasma Δ4-A concentration during exercise in trained women (n=6) in the follicular phase of their menstrual cycle.

Dehydroepiandrosterone sulfate (DHEA-S).

In the untrained subjects the mean plasma DHEA-S concentrations at rest were significantly ($p<0.05$) higher than those in the trained subjects (table 4.1). In the untrained subjects the absolute DHEA-S concentration was not significantly affected by exercise nor by the phase of the menstrual cycle. In the trained subjects both the absolute and the relative values increased significantly with exercise ($p<0.05$ and <0.001) (fig. 4.24 and 4.25). Physical exercise was able to provoke a statistically significant increase in plasma DHEA-S levels at the lowest workloads (fig. 4.24). In the trained group, the plasma DHEA-S concentration changed relatively more in the follicular phase ($p<0.01$) as compared to the luteal phase, ranging from 46 to 64% and from -6 to 54% in the follicular and luteal phase, respectively (fig. 4.25). In both phases the relative values showed a levelling-off after 30-45 min of exercise.

Prolactin (PRL).

In both groups and in both phases of the menstrual cycle the absolute plasma PRL concentrations increased significantly ($p<0.005$) during exercise (fig. 4.26 and 4.27). However, in the untrained subjects a significant increase ($p<0.05$) could only be observed at the highest workloads (80% $\dot{V}O_2max$) in the follicular phase, whereas the plasma PRL levels decreased significantly ($p<0.05$) at 60% $\dot{V}O_2max$ in this phase. In the luteal phase physical exercise was able to induce significant increases ($p<0.05$) in plasma PRL concentrations at workloads exceeding 60% $\dot{V}O_2max$ in this group (fig. 4.26). In the trained subjects physical exercise was able to induce significant increments in plasma PRL concentrations at all workloads (fig. 4.27). The individual responses could be strikingly different in both groups. In the untrained group the relative values showed an initial decrease at a workload of 60% $\dot{V}O_2max$, followed by a gradual increase at higher workloads (fig. 4.28). At comparable workloads (60, 70 and 80% $\dot{V}O_2max$) and exercise times (15, 30 and 45 min after the start of the exercise) the relative changes in plasma PRL were more pronounced ($p<0.01$) in the trained than in the untrained group (fig. 4.28 and 4.29).

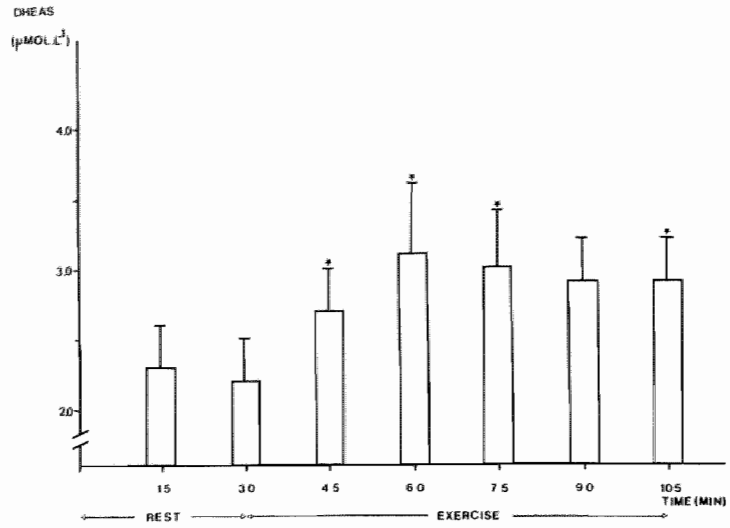


Figure 4.24
Plasma DHEA-S concentration during exercise in trained women (n=6) in the follicular phase of their menstrual cycle.
** = significantly different (p<0.05) from the values at rest.*

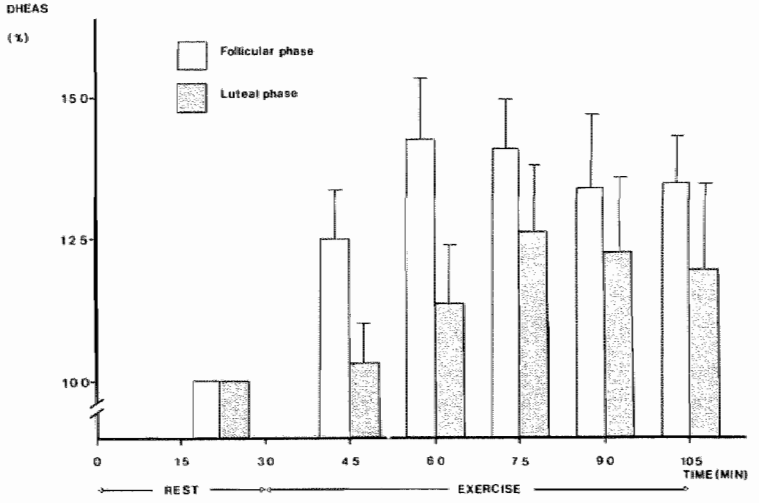


Figure 4.25
Relative changes (percentage of the mean basal value) in plasma DHEA-S concentration during exercise in trained women (n=6) in the follicular and luteal phases of their menstrual cycle.

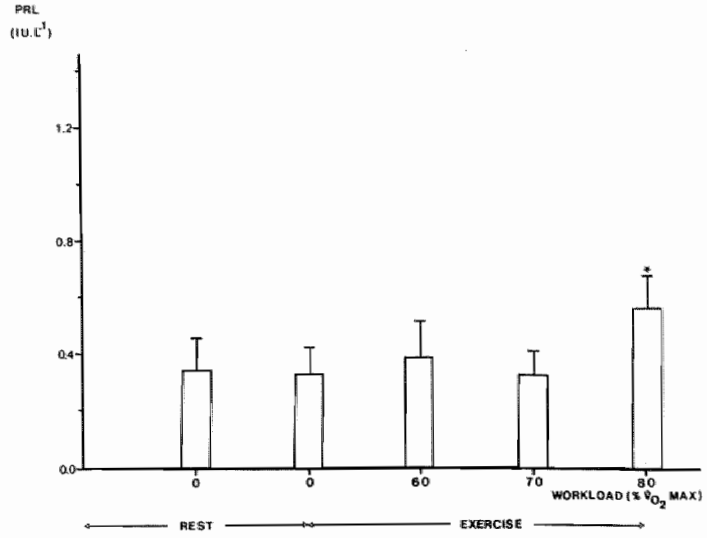


Figure 4.26
Plasma PRL concentration during exercise in untrained women (n=13) in the follicular phase of their menstrual cycle.
** = significantly different (p<0.05) from the values at rest.*

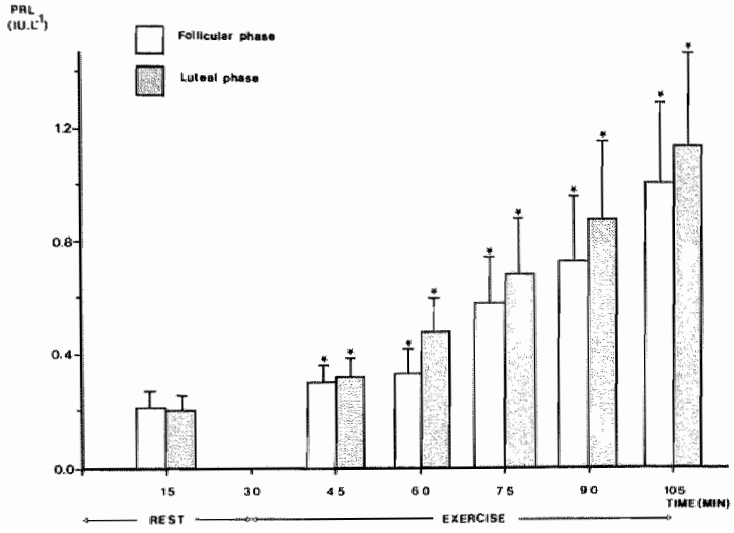


Figure 4.27
Plasma PRL concentration during exercise in trained women (n=6) in the follicular and luteal phases of their menstrual cycle.
** = significantly different (p<0.05) from the values at rest.*

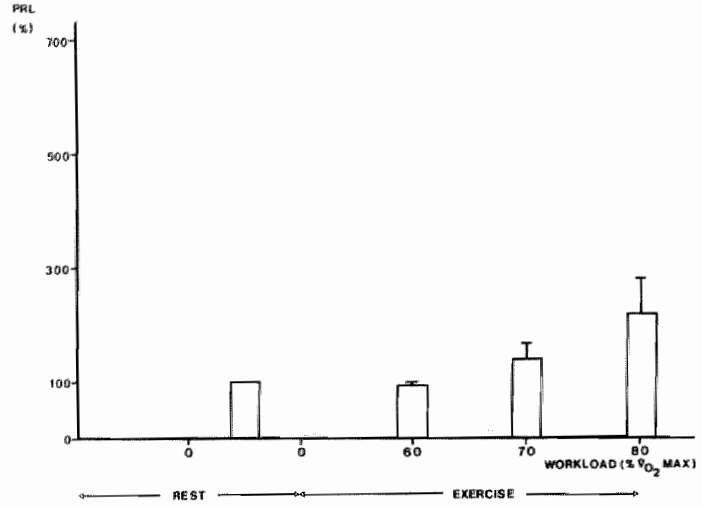


Figure 4.28
Relative changes (percentage of the mean basal value) in plasma PRL concentration during exercise in untrained women (n=13) in the follicular phase of their menstrual cycle.

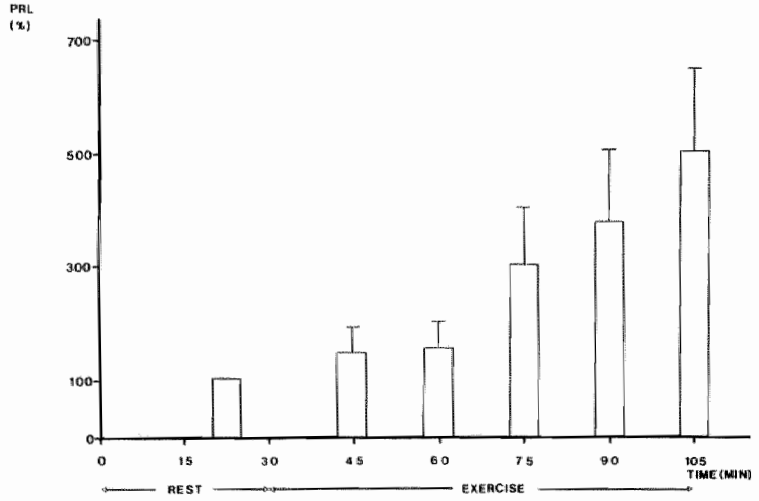


Figure 4.29
Relative changes (percentage of the mean basal value) in plasma PRL concentration during exercise in trained women (n=6) in the follicular phase of their menstrual cycle.

Adrenocorticotropic hormone (ACTH).

In the untrained subjects the mean plasma ACTH concentrations at rest were significantly higher ($p<0.05$) than those in the trained subjects (table 4.1). During exercise the absolute plasma ACTH concentrations showed a significant increase ($p<0.05$) in both groups (fig. 4.30 and 4.31). No statistically significant effect of the phase of the menstrual cycle could be detected. In the untrained group the plasma ACTH concentration increased only significantly at the highest workload (80% $\dot{V}O_2max$)(fig. 4.30). In the trained group the plasma ACTH concentration was significantly elevated after 15 min of exercise (85% $\dot{V}O_2max$)(fig. 4.31). The relative values differed significantly between the groups ($p<0.05$) (fig. 4.32 and 4.33). In the untrained group (fig. 4.32) the plasma ACTH concentrations showed a significant increase only at the highest workloads, whereas in the trained group (fig. 4.33) the concentration was already significantly increased at 60% $\dot{V}O_2max$ (15 min after the start of the exercise).

4.2.3 The influence of a three month training period on hormonal and metabolic responses to exercise (study III)

4.2.3.1 Maximal physical working capacity (MPWC).

In the 8 volunteers, participating in this part of the study, the MPWC increased significantly ($p<0.05$) from 168.1±4.6 to 178.9±5.4 Watts and from 168.8±6.0 to 182.1±5.9 Watts in the follicular and luteal phase, respectively, after a three months endurance training program.
Neither in the pre- nor in the post-training tests the MPWC was significantly different between both phases of the menstrual cycle.

4.2.3.2 Metabolic responses to exercise.

Exercise-induced glucose and lactate responses.

No differences in glucose responses to exercise could be found between the post- and pre-training period. After the training period, blood lactate increased significantly ($p<0.001$) more pronounced as compared to the pre-training period. After training the blood lactate responses appeared to be lower ($p<0.01$) in the luteal than in the follicular phase (fig. 4.34), whereas no such a difference could be observed before the training period.

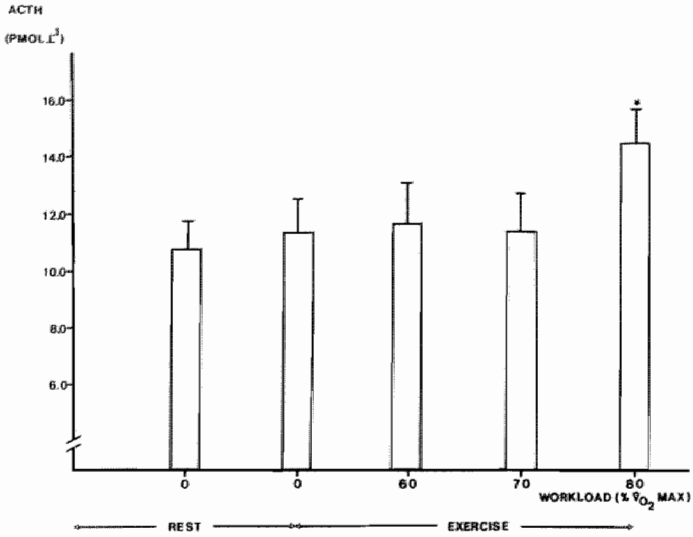


Figure 4.30
Plasma ACTH concentration (pmol.l^{-1}) during exercise in untrained women (n=13) in the follicular phase of their menstrual cycle.
** = significantly different (p<0.05) from the values at rest.*

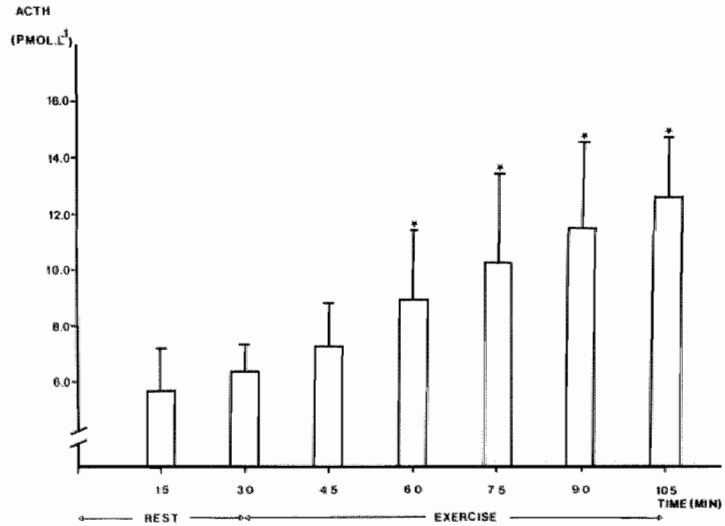


Figure 4.31
Changes in plasma ACTH concentration (pmol.l^{-1}) during exercise in trained women (n=6) in the follicular phase of their menstrual cycle.
** = significantly different (p<0.05) from the values at*

4.2.3.3 Endocrine responses to exercise.

All relative values are depicted in tables 4.2 and 4.3.

Table 4.2
Relative changes (mean ± SEM; the mean basal value was aimed to 100%) in plasma E_2, P, T, Δ4-A, PRL and ACTH during exercise before and after a three month training period in 8 subjects in the follicular phase of their menstrual cycle.

	WORKLOAD (% $\dot{V}O_2max$)		
Hormone	60	70	80
E_2			
Before	120,9±7,6	133,2±8,8	136,4±4,9
After	112,9±5,4	119,0±4,0	131,7±4,8
P			
Before	101,6±5,6	104,1±6,9	118,3±9,3
After	102,9±5,0	110,9±9,1	121,7±9,2
T			
Before	105,9±8,4	110,3±5,9	126,4±4,7
After	124,9±17,4	136,2±19,1	146,3±20,7
Δ4-A			
Before	106,2±6,1	108,5±5,2	121,8±6,5
After	99,2±3,7	107,2±7,5	114,3±7,5
PRL			
Before	94,0±9,2	113,2±25,7	334,8±159,6
After	97,3±7,2	132,3±22,2	233,6±61,8
ACTH			
Before	111,5±7,3	105,0±7,1	145,4±10,7
After	108,9±7,6	113,9±10,8	111,6±39,5

Table 4.3
Relative changes (mean ± SEM; the mean basal value was aimed to 100%) in plasma E_2, P, T, Δ4-A, PRL and ACTH during exercise before and after a three month training period in 8 subjects in the luteal phase of their menstrual cycle.

	WORKLOAD (% $\dot{V}O_2$max)		
Hormone	60	70	80
E_2			
Before	113,0±4,2	128,0±4,8	142,7±4,8
After	109,8±3,0	121,2±3,6	136,0±3,4
P			
Before	110,3±4,8	110,4±5,3	119,6±9,1
After	110,0±3,8	116,6±5,1	121,7±6,5
T			
Before	102,5±19,5	111,3±7,1	128,1±8,4
After	96,0± 3,3	109,6±3,6	129,2±6,3
Δ4-A			
Before	101,8±5,4	103,4±4,7	109,7±5,3
After	100,4±1,6	111,4±4,5	118,4±6,5
PRL			
Before	95,1±2,5	106,7±8,2	219,8±40,8
After	92,2±4,3	152,1±21,8	291,2±58,9
ACTH			
Before	98,4±6,4	101,3±5,7	157,1±25,3
After	91,4±5,4	96,4±6,8	136,8±15,5

Estradiol (E_2)

After training, the mean E_2 levels in the luteal phase were lower ($p<0.05$) as compared to the pre-training tests (fig. 4.35). The mean of the two values at rest were measured to be 0.15±0.03 and 0.58±0.1 nmol.l^{-1} in the pre-training test in the follicular and luteal phase, respectively. After training these values were 0.26±0.1 and 0.33±0.07 nmol.l^{-1} for the follicular and luteal phase, respectively. The post-training absolute plasma E_2 levels increased ($p<0.01$) significantly during exercise both in the follicular and luteal phase. The luteal phase values were higher than the follicular phase values ($p<0.001$).

Testosterone (T)

The mean of the two values at rest was measured to be 1.8±0.18 and 2.2±0.2 nmol.l^{-1} in the pre-training test in the follicular phase and luteal phase, respectively. After training these values were measured to be 1.6±0.2 and 1.7±0.1 nmol.l^{-1} in the follicular and luteal phase, respectively. In the pre-training test physical exercise did not affect ($p=0.08$) the absolute T values. After training, however, the T concentrations increased significantly ($p<0.005$) during exercise (fig. 4.36). No effect of the phase of the menstrual cycle could be detected in this situation, but in the pre-training test the T concentrations in the luteal phase were significantly ($p<0.005$) increased as compared to the follicular phase (fig. 4.36). In the luteal phase the post-training values before and during exercise were significantly lower as compared to the pre-training values (fig. 4.36). No significant differences could be observed between the relative values of the post- and pre-training tests in both phases of the menstrual cycle, although there was a slight ($p=0.07$) indication that the increments in the follicular phase were more pronounced after training (table 4.2 and 4.3).

Androstenedione (Δ4-A)

In the follicular phase the mean plasma Δ4-A concentrations at rest were measured to be 8.22±0.74 nmol.l^{-1} and 8.79±0.7 nmol.l^{-1} in the pre- and post-exercise periods, respectively, whereas in the luteal phase these values were 10.0±0.75 nmol.l^{-1} and 8.98±0.77 nmol.l^{-1}. The absolute Δ4-A levels were not affected by exercise, neither in the pre- nor in the post-

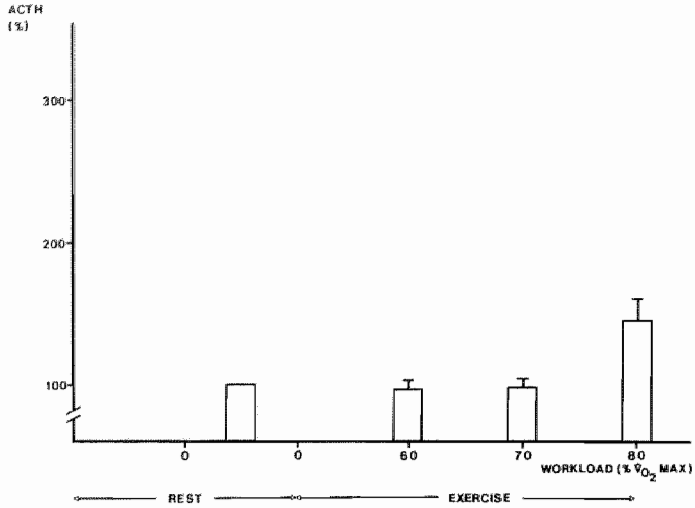


Figure 4.32
Relative changes (percentage of the mean basal value) in plasma ACTH concentration during exercise in untrained women (n=13) in the follicular phase of their menstrual cycle.

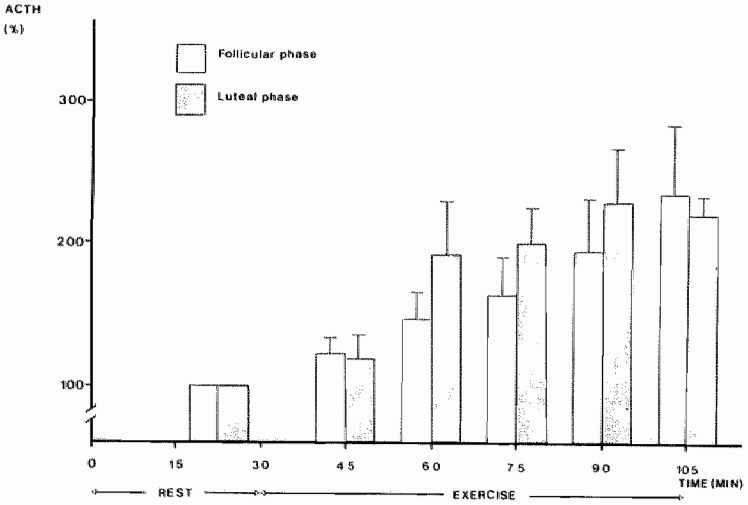


Figure 4.33
Relative changes (percentage of the mean basal value) in plasma ACTH concentration during exercise in trained women (n=6) in the follicular and luteal phases of their menstrual cycle.

training test. The relative values increased significantly ($p<0.001$) both in the pre- and post-training period. No statistically significant differences between the two periods could be observed (table 4.2 and 4.3).

Dehydroepiandrosterone sulfate (DHEA-S)

In the follicular phase, the mean plasma DHEA-S concentrations at rest were measured to be 8.44±0.96 $nmol.l^{-1}$ and 6.02±0.75 $nmol.l^{-1}$ in the pre- and post-exercise periods, respectively, whereas in the luteal phase these values were 8.68±0.68 $\mu mol.l^{-1}$ and 6.71±0.89 $\mu mol.l^{-1}$.
Neither the absolute nor the relative DHEA-S values were significantly affected by exercise both in the pre- and post-training period (data not shown).

Progesterone (P)

In the pre-training test the mean of the two values at rest was measured to be 4.8±0.4 and 22.3±4.9 $nmol.l^{-1}$ in the follicular and luteal phase, respectively. After training these values were 4.0±0.51 and 20.8±0.4 $nmol.l^{-1}$ in the follicular and luteal phase, respectively. In both phases the absolute P values increased significantly ($p<0.001$) during exercise both in the pre- and post-training tests (fig. 4.37). In both cases the absolute values were as expected significantly ($p<0.001$) higher in the luteal phase as compared to the follicular phase (fig. 4.37). The relative increases in plasma P concentrations were not significantly different between both phases and both periods (table 4.2 and 4.3).

Adrenocorticotropic hormone (ACTH)

In the pre-training test, the mean of the two values at rest was measured to be 12.8±1.1 and 10.6±1.5 $pmol.l^{-1}$ in the follicular and luteal phase, respectively. After training these values were 6.1±0.89 and 6.8±1.0 $pmol.l^{-1}$ in the follicular and luteal phase, respectively. The absolute ACTH values increased significantly ($p<0.05$) both in the pre- and post-training tests. The post-training values were significantly ($p<0.001$) lower than the pre-training values (fig. 4.38). No significant differences between the follicular and luteal phase could be observed neither in the pre- nor in the post-training tests.

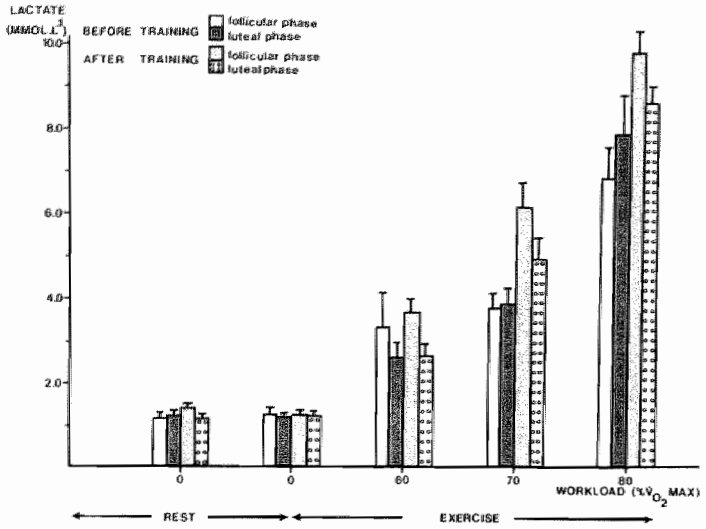


Figure 4.34
Plasma lactate concentration during exercise in women (n=8), before and after a three month endurance training program, in the follicular and luteal phases of their menstrual cycles.

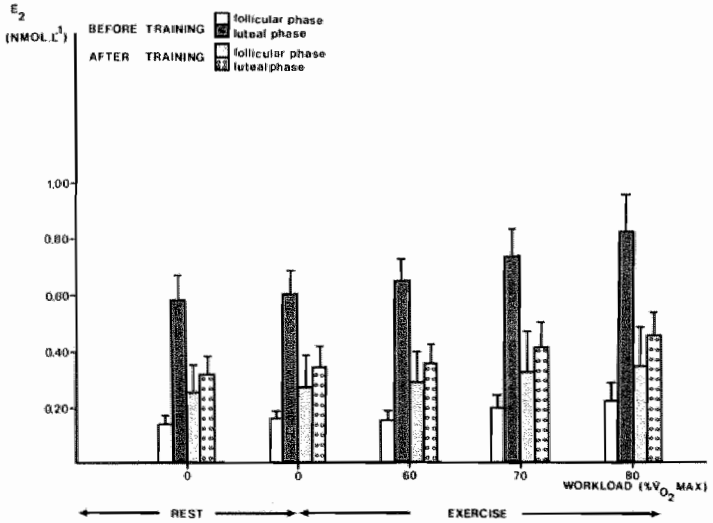


Figure 4.35
Plasma E_2 concentration during exercise in women (n=8), before and after a three month endurance training program, in the follicular and luteal phase of their menstrual cycle.

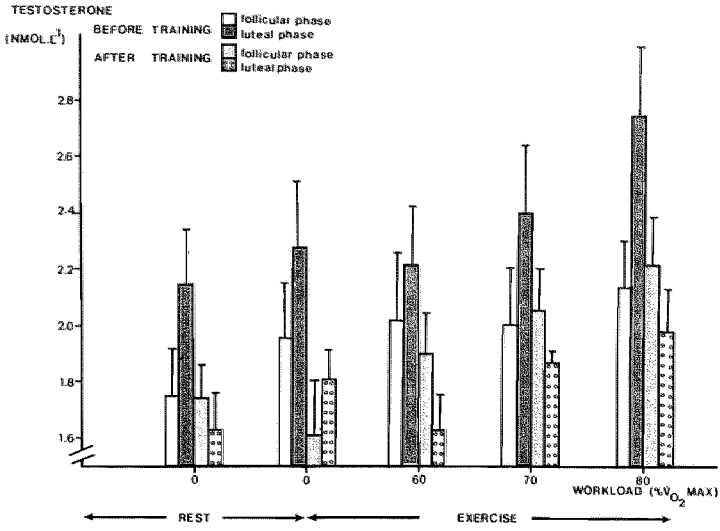


Figure 4.36
Plasma T concentration during exercise in women (n=8), before and after a three month endurance training program, in the follicular and luteal phases of their menstrual cycle.

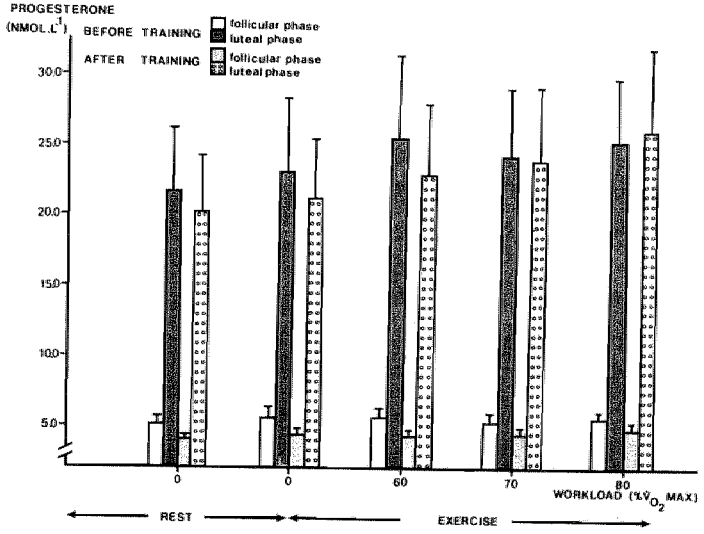


Figure 4.37
Plasma P concentration during exercise in women (n=8) before and after a three month endurance training program in the follicular and luteal phases of their menstrual cycle.

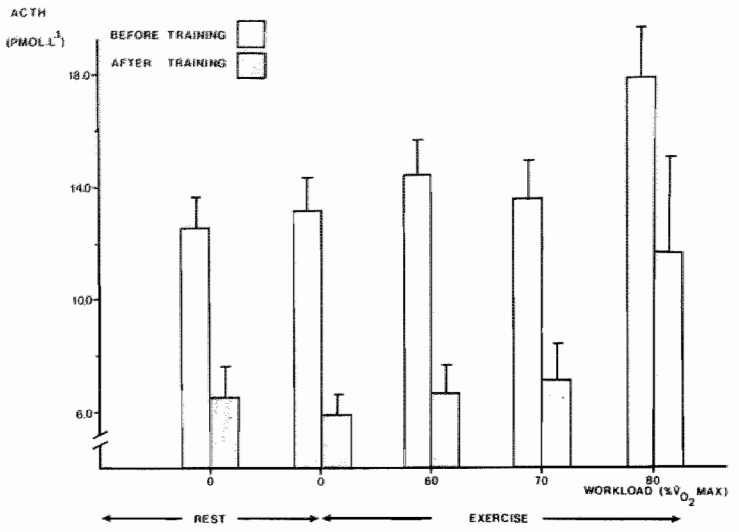


Figure 4.38
Plasma ACTH concentration during exercise in women, before and after a three month endurance training program in the follicular phase of their menstrual cycle.

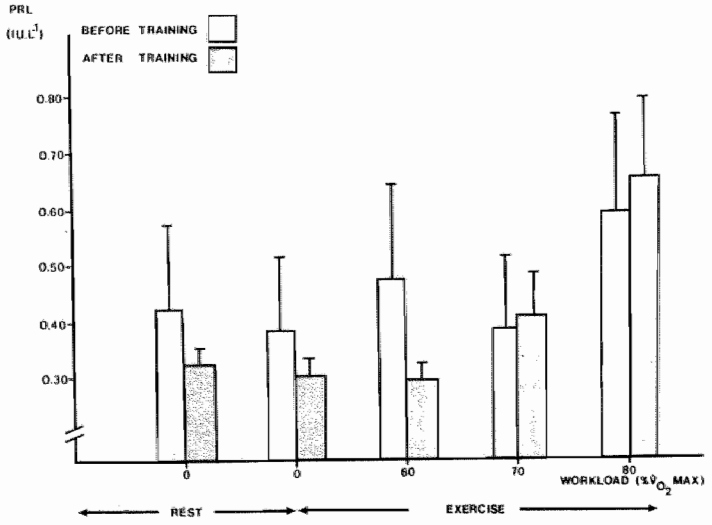


Figure 4.39
Plasma PRL concentration during exercise in women (n=8) before and after a three month endurance training program in the follicular phase of their menstrual cycle.

The relative ACTH responses to exercise in the post-training period were not statistically different from those in the pre-training period (table 4.2 and 4.3).

Prolactin (PRL)

In the follicular phase the mean of the two samples obtained at rest was 0.4±0.02 and 0.32±0.03 $IU.L^{-1}$, in the pre- and post training test, respectively. In the luteal phase these values were 0.29±0.03 and 0.25±0.03 $IU.l^{-1}$. Before and after training the absolute PRL values increased significantly ($p<0.05$) (fig. 4.39). No effect of the phase of the menstrual cycle could be detected, neither in the post- nor in the pre-exercise tests. The relative changes during exercise were not statically different between the pre- and post-training periods (table 4.2 and 4.3).

4.3 GONADOTROPIN AND ESTRADIOL SECRETION PATTERNS BEFORE AND AFTER PROLONGED PHYSICAL EXERCISE (STUDY IV)

The subjects who volunteered in this part of the study were in the follicular phase of their menstrual cycle. The experiments were necessarily performed on different days in the follicular phase, which probably introduced marked differences in plasma E_2 levels. Subjects 1006, 1012 and 1023 were investigated on the 8th day of their menstrual cycle, whereas subjects 1002, 1010, 1022 and 1024 were investigated on the 10th or 11th (1022) day of their cycles. Except for subject 1023, all investigations started between 7.30 and 8.30 a.m. Subject 1023 was necessarily studied between 12.00 a.m. and 17.00 p.m.

The results of this study show an extreme, inter-individual variation in pre- and post-exercise gonadotropin and E_2 levels. Therefore, statistical analyses for the whole group, could easily masque the typical individual responses to exercise, reason why all individual pre- and post-exercise values are given in table A-1 to A-3. Data concerning pulse frequency, pulse amplitude and pulse increment before and after exercise are depicted in tables 4.4 to 4.6. For the purpose of this study a hormone pulse was defined as occurring when the hormone concentration of a sample exceeded the previous concentration by at least twice the intra-assay coefficient of

variation. The pulse amplitude was calculated from nadir to the peak of the pulse, whereas the pulse increment was calculated as the relative (percentual) difference in concentration between peak and nadir of a pulse.
From table 4.4 it can be observed that the mean post-exercise values for the pulse frequency, pulse amplitude and pulse increment of LH were increased as compared to the pre-exercise values. The post-exercise pulse frequency, pulse amplitude and pulse increment of FSH were not different from the pre-exercise values (table 4.5). The post-exercise pulse frequency, pulse amplitude and pulse increment of E_2 seems to be different from the pre-exercise values (table 4.6). In some cases (1010, 1012, 1022) no distinct pulses could be observed after exercise.

The mean pre- and post-exercise LH, FSH and E2 levels are depicted in table 4.7. From this table it can be observed, that in two cases (subjects 1002 and 1010) the mean post-exercise LH values were significantly ($p<0.05$) increased as compared to the pre-exercise values. In two subjects (1012 and 1022) the mean post-exercise FSH values exceeded the pre-exercise values significantly ($p<0.001$), while in one subject (1024) this value was decreased ($p<0.01$). In 6 out of 7 subjects, the mean post-exercise E_2 levels were significantly ($p<0.05$) elevated as compared to the pre-exercise levels. As an example of the changes in gonadotropin and E_2 levels the data of two subjects (no. 1006 and 1012) are depicted in figures 4.40 to 4.43)

Pooling all individual data of the pre- and the post-exercise period showed a significantly ($p<0.05$) increased mean LH level in the post-exercise period as compared to the pre-exercise period. Trend analysis also showed a pre-start increase in plasma LH levels (figure 4.44).

Table 4.4
Pulse frequency (pulses/2 hours), pulse amplitude ($IU.l^{-1}$) and pulse increment (%) of plasma LH before and after physical exercise.

	Before exercise			After exercise		
subject code	pulse frequency (in 2 hrs)	pulse amplitude ($IU.l^{-1}$)	pulse increment (%)	pulse frequency (in 2 hrs)	pulse amplitude ($IU.l^{-1}$)	pulse increment (%)
1002	1	1.7	34.0	2	1.85	26.9
1006	2	0.9	20.6	2.5	1.7	38.2
1010	1	1.7	34.0	1	1.3	20.6
1012	2	1.6	30.0	2	4.2	96.4
1022	2	1.7	23.0	2	2.1	34.1
1023	1	3.6	87.8	2	3.3	86.4
1024	3	3.3	41.7	2	2.6	50.4
$\bar{x}$	1.71	2.08	38.8	1.93	2.43	50.4
SEM	0.265	0.34	7.98	0.158	0.357	10.39

Table 4.5
Pulse frequency (pulses/2 hours), pulse amplitude ($IU.l^{-1}$) and pulse increment (%) of plasma FSH before and after physical exercise.

	Before exercise			After exercise		
subject code	pulse frequency (in 2 hrs)	pulse amplitude ($IU.l^{-1}$)	pulse increment (%)	pulse frequency (in 2 hrs)	pulse amplitude ($IU.l^{-1}$)	pulse increment (%)
1002	2	0.9	25.0	3	1.6	41.4
1006	3	1.4	28.7	1	1.2	21.0
1010	1	1.6	37.2	1	1.7	34.0
1012	2	2.6	39.4	2	0.9	34.6
1022	2	1.2	30.0	2	2.5	28.8
1023	1	1.4	25.0	1	2.0	14.3
1024	1	2.3	54.8	2	1.4	36.6
$\bar{x}$	1.71	1.64	34.3	1.71	1.62	30.1
SEM	0.265	0.213	3.73	0.265	0.181	3.32

Table 4.6
Pulse frequency (pulses/2 hours), pulse amplitude ($nmol.l^{-1}$) and pulse increment (%) of plasma E_2 before and after physical exercise.

	Before exercise			After exercise		
subject code	pulse frequency (in 2 hrs)	pulse amplitude ($nmol.l^{-1}$)	pulse increment (%)	pulse frequency (in 2 hrs)	pulse amplitude ($nmol.l^{-1}$)	pulse increment (%)
1002	1	0.04	50.0	1	0.05	50
1006	1	0.09	69.2	1.5	0.04	16.4
1010	1	0.25	108.7	0	-	-
1012	2	0.07	11.9	0	-	-
1022	0	-	-	0	-	-
1023	1	0.05	100.0	1	0.014	14.0
$\bar{x}$	1	0.09	58.4			
SEM	0.2	0.031	14.6			

Table 4.7
Plasma LH, FSH and E_2 concentration ($\bar{x}$±SEM) before and after exercise. The values represent the mean of the last 8 values before and the mean of the first 8 values after exercise. Significancy is denoted by * if 0.05>p>0.01; **if 0.01>p>0.001; ***if p<0.001.

Subject code	LH ($IU.l^{-1}$)		FSH ($IU.l^{-1}$)		E_2 ($nmol.l^{-1}$)	
	Before	After	Before	After	Before	After
1002	5.5±0.26	6.3±0.25*	4.3±0.14	4.9±0.45	0.07±0.002	0.09±0.006*
1006	4.8±0.21	5.3±0.44	5.6±0.68	5.8±0.18	0.18±0.009	0.21±0.008*
1010	6.0±0.19	6.6±0.17*	5.6±0.22	5.8±0.2	0.33±0.04	0.25±0.01*
1012	6.2±0.25	7.4±1.05	2.3±0.11	3.4±0.22***	0.63±0.012	0.66±0.04
1022	8.1±0.28	8.1±0.54	4.7±0.22	7.6±0.18***	0.13±0.008	0.15±0.005**
1023	5.9±0.4	6.7±0.6	6.7±0.19	6.8±0.29	0.09±0.29	0.12±0.007**
1024	6.6±0.47	6.9±0.48	5.7±0.4	4.3±0.16**	0.31±0.008	0.37±0.02*

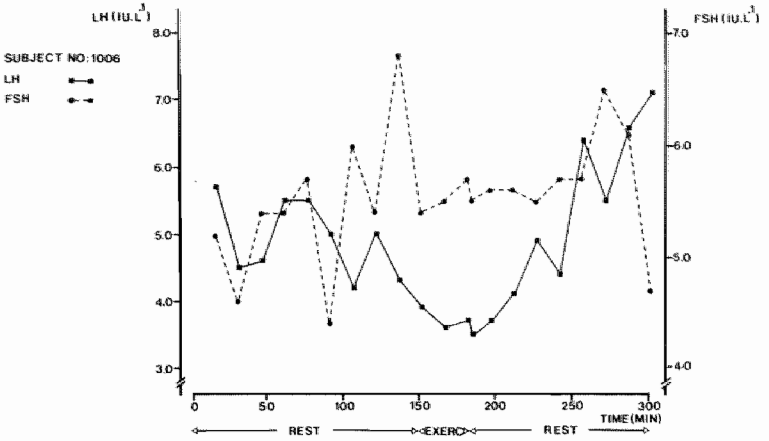


Figure 4.40
Changes in plasma LH and FSH concentration before, during and after physical exercise in subject 1006.

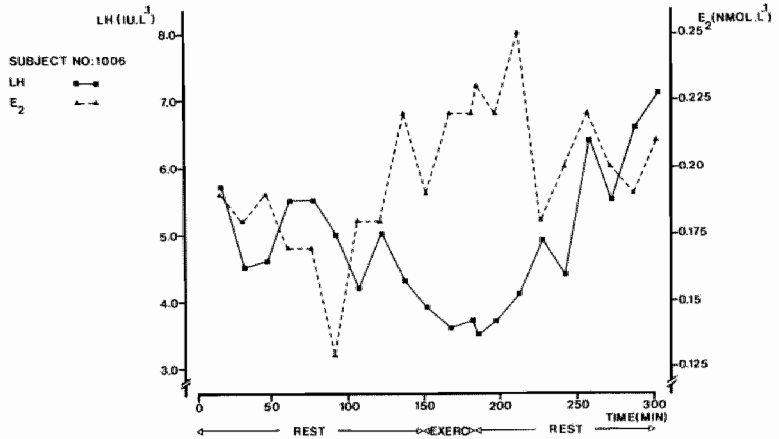


Figure 4.41
Changes in plasma LH and E_2 concentration before, during and after physical exercise in subject 1006.

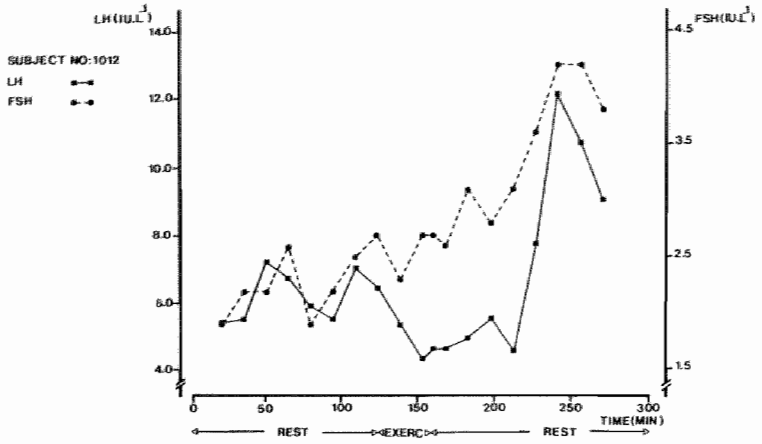


Figure 4.42
Changes in plasma LH and FSH concentration before, during and after physical exercise in subject 1012.

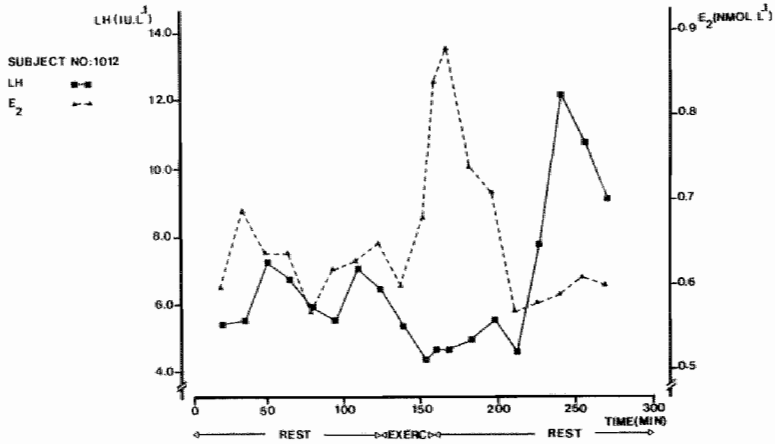


Figure 4.43
Changes in plasma LH and E_2 concentration before, during and after physical exercise in subject 1012.

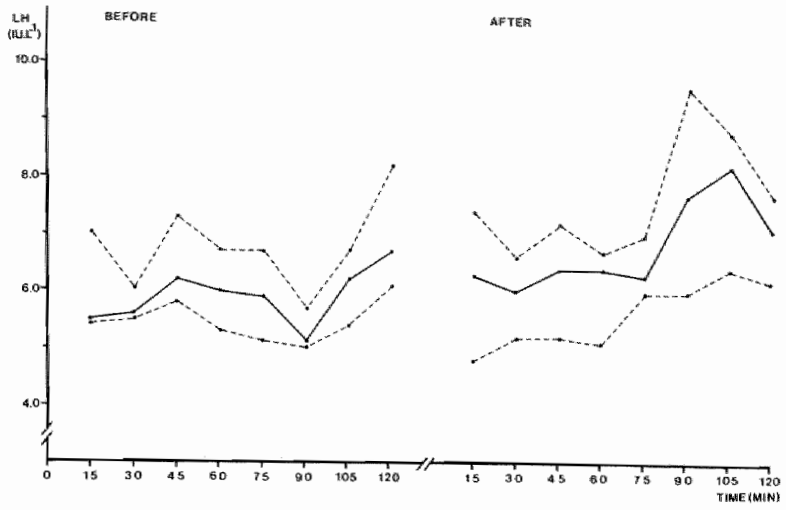


Figure 4.44
Pre- and post-exercise plasma LH concentrations in 7 women in the follicular phase of the menstrual cycle. The solid line represents the median of all LH concentrations, the dotted lines represent the second and third quartiles, respectively.

4.4 THE INFLUENCE OF SHORT-TIME PHYSICAL EXERCISE ON THE METABOLIC CLEARANCE RATE OF ESTRADIOL (STUDY Va AND Vb)

The first two blood samples were drawn 120 and 135 min after the priming dose. These two bloodsamples were used to establish the steady state condition between the infusion rate and the disappearance rate of the tracer. It appeared that in all cases a steady state was reached 120 min after the priming dose. The MCR as calculated from these two samples differed less than 10% with no systematic upward or downward trend. The mean of these two values was used as the resting or basal value of the MCR. The mean basal value for all subjects participating in both studies was calculated to be 1254 l/day (range 669-1765 l/day) or 870.8 $ml.min^{-1}$. In table 4.8 and 4.9 the MCR values (corrected for hemoconcentration) are depicted in relation to the duration and intensity of the exercise. In fig. 4.45 and 4.46 the changes in the MCR of E_2 are presented as a percentage of the basal MCR, which was assumed to be 100%. The data in these figures show that there is a sharp and consistent decrease ($p<0.001$) in the MCR of E_2 for all subjects at 70 and 100% VO_2max. The mean decrease at the end of the workload of 70% VO_2max was 41.3 and 36% for study Va and Vb, respectively. The mean decrease at the end of the 100% VO_2max load (study Vb) was calculated to be 80.0%. During the recovery period at 25% VO_2max (fig. 4.46) the MCR of E_2 remained well below the basal value.

Table 4.8
The metabolic clearance rate of E_2(ml.min^{-1}) during short-term maximal exercise in young women in the follicular phase of their menstrual cycle (study Va).

Work load (%VO_2 max)	Time after priming dose (min)	Subject 1201	1202	1204	1206	1207	1211	1214	1215	1218	Mean ±SEM
Rest	135[a]	986	807	732	1043	871	850	1226	1050	953	946.6 ±47.2
70%	145	1027	747	732	509	316	148	804	411	306	555.6 ±90.3
100%	149.4[b]	167	121	82	339	127	106	207	336	219	189.5 ±29.9

[a]: The basal MCR is calculated as mean from 2 samples, i.e. 120 and 135 min after the priming dose.
[b]: This value represents the mean time at which the subjects had to give up (range 147-150 min after the priming dose).

Table 4.9
The metabolic clearance rate of E_2 (ml.min^{-1}) during submaximal exercise, and in the recovery period in young women (study Vb).

Work load (%VO_2 max)	Time after priming dose (min)	Subject 1221	1222	1223	1224	1225	1226	Mean ±SEM
Rest	135*	509	465	796	1083	858	1056	794.4 ±98.1
70%	140	432	529	976	689	669	433	621.4 ±76.9
"	145	343	383	597	804	450	346	487.3 ±67.8
25%	150	501	440	626	763	405	493	540.0 ±49.8
"	160	322	349	465	902	505	494	506.3 ±77.7
"	175	-	242	519	657	396	524	467.6 ±57.1

* The basal MCR is calculated as the mean from 2 samples i.e. 120 and 135 min after the priming dose.

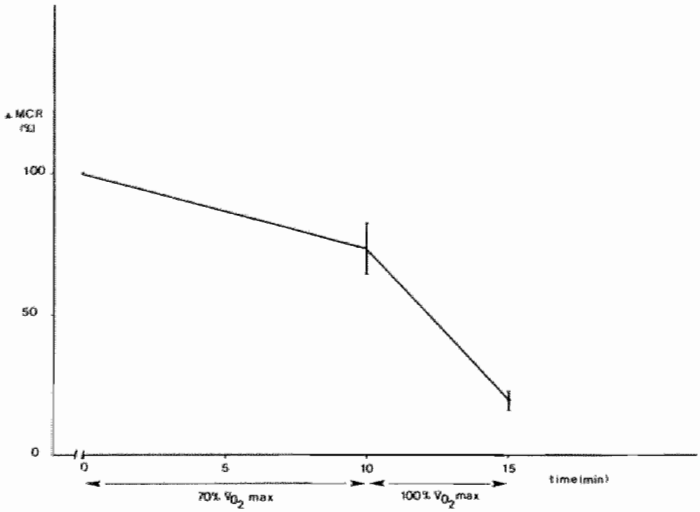


Figure 4.45
Mean relative changes (percentage of the mean basal value) of the MCR E_2 during physical exercise in women (n=9) in the follicular phase of the menstrual cycle.

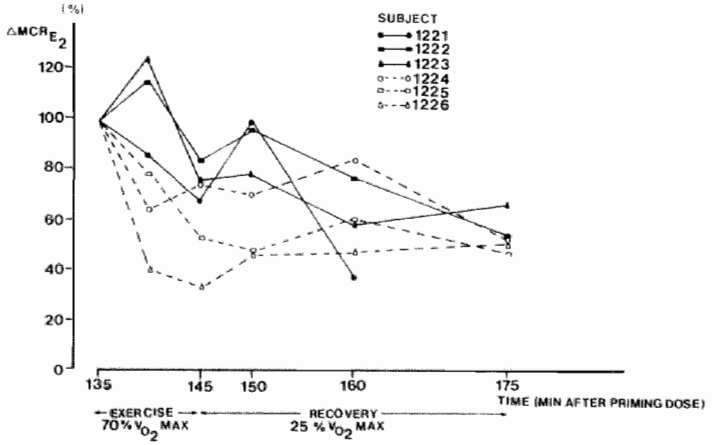


Figure 4.46
Mean relative changes (percentage of the mean basal value) of the MCR E_2 during physical exercise in women (n=6).
Solid lines, changes in untrained subjects; dashed lines, changes in trained ones.

CHAPTER V

DISCUSSION

5.1 PHYSICAL WORKING CAPACITY AND METABOLIC RESPONSES IN RELATION TO THE PHASE OF THE MENSTRUAL CYCLE (STUDIES I AND II)

Maximal physical working capacity.

In the present investigation we were not able to demonstrate a significant effect of the phase of the menstrual cycle on maximal physical performance neither in trained, nor in untrained women. In the trained group, one subject was not able to complete the 75 min treadmill run in the luteal phase. In the other subjects, no differences in physical performance between both phases of the menstrual cycle could be observed.

The lack of influence of the phase of the menstrual cycle on physical performance is in agreement with data of several other investigations (Doolittle and Engebretsen, 1972; Drinkwater, 1973; Verstappen et al, 1981; Kuipers, 1983). However, other investigators showed an enhanced physical performance in either the follicular phase (Millahn and Drecoll, 1960; Klaus and Noack, 1961; Pahlke and Smitka, 1977; Schoene et al, 1981), or the luteal phase of the menstrual cycle (Muller-Limmroth and Lohaus, 1963; Jurkowski et al, 1978). These conflicting results may be explained partly by differences in the experimental protocols and partly by the lack of a correct determination of the phase of the menstrual cycle (by measurement of plasma steroids and/or gonadotropins). Especially the latter is important and therefore the data of the investigations lacking hormonal data have to be interpreted with care (Doolittle and Engebretsen, 1972; Drinkwater, 1973; Millahn and Drecoll, 1960; Klaus and Noack, 1961; Pahlke and Smitka, 1977; Verstappen et al, 1981; Kuipers, 1983). The results of the only two investigations where the phase of the menstrual cycle was correctly determined (Jurkowski et al, 1981; Schoene et al, 1981) render conflicting data. In both studies the exercise experiments were performed on a bicycle ergometer and hence they are comparable in this respect. Jurkowski and co-investigators (1978) found an enhanced maximal physical performance in the luteal phase in 10 untrained women, whereas Schoene and co-investi-

gators (1981) found an increase in the follicular phase in both trained and untrained women.

Glucose and lactate plasma levels during exercise.

During exercise, no differences in lactate response could be detected between both phases of the menstrual cycle in study I and II of the present investigation. This is in agreement with findings of Bonen and co-investigators (in press) and Dalsky (1982), in untrained and trained women during bicycle ergometer and treadmill work. Jurkowski and co-investigators (1978 and 1981), however, have reported a lower lactate level in the luteal phase during progressive bicycle ergometer work (20 min consecutive loads at 33, 66 and 90% $\dot{V}O_2max$).

In the present study, the blood glucose responses to exercise were different in the untrained and trained groups. The blood glucose response to exercise in the untrained group is comparable with the results of previous reports for male subjects in similar experiments (Ahlborg et al, 1977; Bonen et al, 1979; Bonen et al, 1981; Costill et al, 1977; Galbo et al, 1977). The high blood glucose levels, however, found in the trained group is an unexpected finding which has not been observed in males. Although comparisons between trained and untrained women are lacking in the literature, our findings confirm the data of Berg and Keul (1981) who also reported significantly higher plasma glucose values after endurance work (long distance running, cross country skiing) in a group of 38 female athletes as compared to their male counterparts. The underlying mechanism is unknown, but one should bear in mind that steroid hormones are able to influence carbohydrate and fat metabolism (Reinke et al, 1972; Gorski et al, 1976; Chainy et al, 1976; Morrow et al, 1981) both in man and rats. For example Gorski and co-investigators (1976) showed in ovariectomized rats a glycogen sparing effect of E_2 during prolonged exercise. This suggests an enhanced fatty acid oxidation under these circumstances. The higher plasma glucose levels in the trained women as compared to their male counterparts may originate from higher nor-epinephrine levels in the former as reported by Berg and Keul (1981).

However, controlled animal experiments with and without sex hormones administration together with measurements of glucose turn-over rates are needed to get more insight into the mechanisms of these hormones on sub-

strate turn-over.

5.2 ACUTE EFFECTS OF PHYSICAL EXERCISE ON PLASMA LEVELS OF SEX HORMONES AS A FUNCTION OF THE LEVEL OF PHYSICAL FITNESS

In the present investigation serial hormonal concentrations were measured during physical exercise in two groups of women with different daily physical activities to ascertain the typical hormonal responses in the follicular and luteal phases of their menstrual cycles. The hormone concentrations were expressed as absolute and relative (percentual change) values, both as a function of time (trained women) and workload (untrained women). We used relative values because we were primarily interested in the changes relative compared to the resting situation. Besides, they also reduce the large inter-individual variation in hormone concentrations and responses to exercise. Therefore, intergroup comparisons were also made making use of the relative values.

Estradiol (E_2)

During exercise, the absolute E_2 concentration increased significantly in the two groups, both in the follicular and luteal phase of the menstrual cycle. The relative values showed similar increments in the untrained and trained group, with no significant differences between both phases of the menstrual cycle. Our results agree well with those of other investigators as far as the luteal phase is concerned (Jurkowsky et al. 1978; Bonen et al. 1979; Cumming et al. 1981). These investigators did not observe an increase in the follicular phase of the menstrual cycle. The discrepancy between their and our findings is difficult to recover, but it may be caused by a lower sensitivity of their assay. Support for this idea can be found by expressing their data as relative changes, resulting in comparable relative increments in both phases of the cycle as found in the present investigation.

Free estradiol fraction

In the present investigation we have studied the effect of physical exercise and the related increase in body temperature on the free percentage of plasma E_2 and T. In the method we employed, undiluted plasma and

physiological temperatures (37° C and 39.5° C) were used. For practical reasons, we used a fixed post-exercise temperature, although it is well recognized that there could be inter-individual differences in this respect.

In the untrained group the mean free E_2 fraction at rest was measured to be about 1.5% and decreased significantly with exercise. In the trained group the mean free E_2 fraction at rest was measured to be about 1.23%. In this group physical exercise did not affect this value. In both groups no differences in free E_2 values between the phases of the menstrual cycle were found.

Our data show a free percentage of E_2 which is lower as those reported by other investigators (Wu et al. 1976; Hammond et al, 1980; Dunn et al, 1981; Moll and Rosenfield, 1980). Dunn and colleagues (1981) reported mean free E_2 fraction of 1.81% in both phases of the menstrual cycle in 40 women, whereas Wu and co-investigators (1976) found even higher values (2.0-2.6%). The differences between our values and those of these authors may be explained by the different techniques used and the endocrine characteristics of the subjects participating in the present investigation.

The slight, but significant decrease in free plasma E_2 fraction after physical exercise is an unexpected finding. This decrease, however, has to be handled with much reservation. Since the intra-assay coefficient of variation of our method was calculated to be 7.3%, it is obvious that the decrease in free percentage E_2 is well within these limits. Therefore, we consider this finding as a coincidence. The finding that a concomitant rise in total plasma E_2 and body temperature does not necessarily affect the free E_2 fraction may be explained by the observations of Vigersky and co-investigators (1979), who were not able to show any significant E_2 binding to SHBG at 37°C. This means, that albumin accounts for almost all E_2 transport in the blood under physiological conditions. Since the binding capacity of albumin for E_2 is almost unlimited it is unlikely that an exercise induced rise in total plasma concentration can overwhelm it. Hence, the free percentage is left unaffected. At the other hand, the results of other investigations (Wu et al, 1976; Moll and Rosenfield, 1980; Dunn et al, 1981) are not in agreement with the findings of Vigersky and colleagues. They found that SHBG accounted for 35-40% of the E_2 transport in plasma. Nevertheless, we assume that the biological activity of E_2 may

be increased due to the increased dissociation rate of E_2 from albumin (Vermeulen, 1973; Lata et al. 1980) at body temperatures corresponding with prolonged heavy exercise.

Progesterone (P)

The data reported in the present investigation might indicate that most participants did ovulate as judged by the rise of the plasma P concentration above 10 $nmol.l^{-1}$. The low plasma P concentration may indicate that some of the experiments in the luteal phase, which were considered to be done mid-luteal, were possibly conducted in the early luteal phase. However, it is well recognized that the inter-individual variation in plasma P concentration is rather extensive. The unreliability of the BBT for detection of the moment of ovulation (Wetzels et al, 1982) made that in the present investigation, the mid-luteal phase could not be accurately determined.

In the untrained and trained subjects the absolute P concentrations increased significantly with exercise. The relative values showed a significant linear increase with exercise (mean 25.1%, range 10.8 to 74.2%) in the untrained group, while in the trained group, the changes in relative values (mean increase 67%, range 0-192.8%) showed a levelling-off, especially in the luteal phase. No phase effect could be detected in the trained group, while in the untrained group the relative increase in P concentration was more pronounced in the luteal phase. The differences in response between the groups were not significant.

Our results agree well with the findings of other investigators (Jurkowski et al, 1978; Bonen et al, 1979; Bonen et al, in press). They found increments of about 38% (Bonen, 1979) and 40% (Jurkowski, 1978) at comparable workloads (70% $\dot{V}O_2max$) and exercise times (30-40 min), while at 80% $\dot{V}O_2max$ Bonen and co-investigators (in press) found increments of about 71% in the luteal phase and of more than 100% in the follicular phase. A closer examination of the data of Jurkowski and co-investigators (1978) showed that the increase in P concentration levels off at high workloads, which corroborates our findings. The explanation for this finding might be a levelling-off of the MCR, which is considered to reach its minimum values at high workloads (see paragraph 5.3). The almost 200% rise in P concentration, as recorded in some of our subjects, suggests in the first place a

decreased MCR. In the second place also other mechansisms such as an enhanced adrenal secretion, may be involved. However, this hypothesis has to be handled with care. Since the greatest relative increments have been shown in the follicular phase it is obvious that the high variability of the radio-immuno-assay occurring with low plasma P concentrations can easily overestimate the true values. Expressing the absolute values in relative ones may even increase this error considerably.

Gonadotropins

The absence of any change in absolute FSH levels during exercise is in keeping with the findings as reported by others (Bonen et al, 1979; Jurkowski et al, 1978). In both groups significant differences between the phases could be observed at rest. In the trained subjects, the LH and FSH concentrations were lower in the luteal than in the follicular phase, but in untrained subjects this was only true for FSH. The relative plasma LH concentrations showed a linear decrease with exercise in the untrained group, which was calculated to be about 13% and 20% in the follicular and luteal phase, respectively, at a workload of 80% $\dot{V}O_2max$, whereas in the trained group a linear trend was not apparent. At comparable exercise time (45 min) and workloads (85% $\dot{V}O_2max$) the mean decrease was calculated to be 8.2% (range -22.5% to 3.4%). The relative FSH concentration decreased only in the untrained group. The differences in FSH responses between the untrained and trained subjects were not significant ($p=0.06$). In the untrained subjects the relative FSH values were slightly less affected by exercise than those of the relative LH values whereas this was not the case in the trained group. This means, that in both groups the LH/FSH ratio's decreased by about 10-20%. Our results do not confirm the findings in recent investigations (Jurkowski et al, 1978; Bonen et al, 1979; Bonen et al, 1982). Jurkowski and co-investigators (1978) have reported unchanged absolute LH and FSH levels during a progressive exercise protocol (20 min consecutive loads at 33, 66 and 90% $\dot{V}O_2max$) in young women, although the LH concentration tended to be lowered in the luteal phase during the first 35 min of exercise, whereafter it seemed to increase. Their graphs further suggest a divergent response of this hormone between the phases i.e. the plasma concentration tended to increase during the follicular phase. Bonen and co-investigators (1979) did not find any significant effect of a 30 min

bicycle ergometer ride (74% $\dot{V}O_2max$) on the gonadotropin response in young women, although they reported a mean (non-significant) exercise-induced decrease of 7.1% and 2.6% in untrained (n=10) and moderately trained (n=5) young women, respectively. Recently Bonen and co-investigators (in press) found an increase in plasma LH concentration at the end of a progressive treadmill protocol (30 min consecutive loads at 40 and 80% $\dot{V}O_2max$) in 19 women. This increase, however, was not significant for the lower workloads. In additon, their results suggest a slightly different response in fasted, glucose loaded and control subjects. In the fasted subjects the LH concentrations at rest were lower than in the control and glucose loaded group. Their results show that the plasma LH concentration did not change during exercise in the fasted subjects, whereas it seemed to increase in the glucose-loaded group. It might be that the differences between our results and the results of the afore mentioned investigators can be explained by differences in methods. In our study, also the untrained subjects exercised after a overnight fast, and especially in this group gonadotropin levels, were significantly decreased. A pre-exercise increase in plasma LH levels, as observed in study IV (paragraph 4.3) of the present investigation, cannot be excluded. This might cause an overestimation of the resting value. Nevertheless, also in this part of the study it has been shown that most subjects responded to exercise with a decrease of the plasma LH levels during exercise.

Prolactin

Exercise induced a significant linear increase in plasma PRL concentrations in the untrained and trained subjects. This finding partly disagrees with the results of Brisson and co-investigators (1980). They found a significantly increased PRL concentration after a 30 min bicycle ergometer ride (70% $\dot{V}O_2max$), but only in women with a "sport history". This disagreement is probably caused by the specific endocrine characteristics of the subjects participating in this study. This idea is not supported by the findings in study III of the present investigation. In this part of the study eight previously untrained women volunteered in a training program. Both in the untrained and trained state the PRL values were affected by exercise. On the other hand in study I and II we have shown a more pronounced relative increase in plasma PRL concentration in the trained runners

than in the untrained subjects, which confirms the observations of Brisson and colleagues (1980). It is most likely that the enhanced plasma levels of PRL are caused by a greater sensitivity of the anterior pituitary to stimulating agents. This is supported by the observations of Boyden and co-investigators (1982), who showed a significantly increased PRL secretion during physical exercise and thyroid releasing hormone (TRH) stimulation after a training period in a group of previously untrained women. This might be caused by the chronic enhanced plasma E_2 levels during exercise, which sensitize the pituitary to prolactin releasing factors. Hence, it is likely that in trained women the greatly enhanced PRL concentration after physical exercise as reported in the present and other studies (Shangold et al, 1981; Baker et al, 1982) can be attributed to physical conditioning. However, the present study clearly indicates that the inter-individual variance is extremely large. In some untrained subjects post-exercise values exceeding 1.5 $IU.l^{-1}$ or an increase of more than 1250% were found, while in others no changes at all or even a decrease could be observed. Therefore, it is concluded from the present investigation that a "sport history" is not a condition per se to increase PRL secretion during exercise.

An exaggerated response to exercise in untrained women may be a sign of greater sensitivity of the hypothalamic-pituitary system to stress, which might make them more sensitive to menstrual cycle disturbances, especially because PRL is able to inhibit GnRH secretion.

Androgens

- Testosterone (T)

One of the most striking findings in the present investigation is the difference in plasma androgen levels between the untrained and highly trained subjects. Whereas in the untrained group the plasma T concentration was about 1.8 $nmol.l^{-1}$, which was at the upper limit of the normal range, the trained group showed a plasma T concentration of about 1.2 $nmol.l^{-1}$, which is the same as reported for untrained women (see table 2.2).

Our results are not in agreement with those of Dale and co-investigators (1979), who reported high levels of T in female long distance runners as compared to an age matched control group. On the contrary, Carli and co-investigators (1983) found a marked reduction in plasma T levels at

rest, in a group of girl swimmers after a period of competitive swimming training. The discrepancy between our results and those of Dale and colleagues may be due to a different training program or to higher pre-existing androgen levels. There is little doubt, that competitive running training may considered to be stressful. In men it has been shown that physical exertion is associated with a decrease in plasma T levels (Kuoppasalmi et al, 1981; Opstad and Aakvaag, 1982). The reason for this phenomenon is not clear, but may be due to a decreased secretion rate and probably an increase in MCR mediated by the increased free fraction (Vermeulen and Ando, 1979) and elevated ACTH levels which in turn enhance the MCR of T (Pratt and Longcope, 1978). In addition the body temperature after long-lasting physical exercise remains elevated for more than 24 hours (L. Hermanssen, personal communication) which may enhance the MCR at rest.

During exercise the absolute T concentration increased significantly in the trained and in the untrained group when the exercise intensity exceeded 70% $\dot{V}O_2max$. The relative changes were more pronounced in the trained than in the untrained group. In some cases in the trained group the plasma T concentration exceeded the base line value with more than 200%, whereas in the untrained women the highest value reached was 65% above the resting level. This finding has to be handled with some reservations. Especially in the trained runners, we have measured rather low T levels (mean 1.3, range 0.6-3.5 $nmol.l^{-1}$), which implicates a considerable intra-assay coefficient of variation (with a concentration of 2.5 $nmol.l^{-1}$, the variation was measured to be 8.8%). Expressing the results in relative values could double the error. Nevertheless, this cannot fully explain the differences between the trained and untrained subjects.

Only a few investigations deal with androgen responses in women. Keil and co-investigators (1979) reported a 17-230% increase in plasma T concentration after a 75 km run in 13 women of variable age (16-51 years). Schmitt and co-investigators (1981) reported a mean increase in plasma T concentration of 84% in women (mean age 48 yr) after a marathon run. No information was provided in both cases about the relation between the time of the experiment and the phase of the menstrual cycle. Shangold and co-investigators (1981) reported a mean increase in plasma T levels of 21% in the follicular phase and of 54% in the luteal phase in two groups of 3 women after a 30 min run (no indication of the workload). Baker et al

(1982) compared the plasma T levels of 6 women after a marathon race with samples collected 12-24 hrs after a practice run. They found significantly elevated T levels (about 120%) after a marathon run as compared to the control value. An increase in plasma T levels may be caused by a decreased MCR as has been shown by Sutton and co-investigators (1973) in males, but also an enhanced glandular secretion rate and/or conversion rate might be involved. Although the enormous potential of the ovary for synthesizing steroid hormones is not easily overestimated, it is unlikely that it plays an important role especially since the LH levels decrease during exercise (chapter 4.2). It seems more likely that the adrenal cortex is involved, particularly in the trained subjects. This assumption is supported by the finding that both the plasma ACTH and DHEA-S levels are markedly increased in these subjects.

An enhanced peripheral conversion from Δ4-A is also likely to play an important role in the increased plasma T levels, although this has to be proven in future research. The results of the present investigation did not show any effect of the phase of the menstrual cycle upon the plasma T response. This finding is in disagreement with the results of Shangold and co-investigators (1981), who found an enhanced plasma T response in the luteal phase. However, due to the methodological weaknesses of their experiment (different volunteers, n=3, were used in different phases of the menstrual cycle) their findings have to be interpreted with some reservations.

- Free testosterone fraction

The free T fraction at rest was 2.33 and 2.05% (mean of the values in the follicular and luteal phase) in the untrained and trained group, respectively, with no differences between the phases of the menstrual cycle. These values are well above those reported in the literature (Vermeulen, 1973; Hammond et al, 1980; Moll and Rosenfield, 1979) and are close to values reported for hirsute females (Vermeulen, 1973; Moll and Rosenfield, 1979). Vermeulen (1973) and Hammond and co-investigators (1980) reported normal values of about 1%. However, it has to be mentioned that the method of Vermeulen (dialysis, diluted plasma) is clearly different from our method. Although Hammond and co-investigators (1980) also used undiluted plasma with a centrifugal ultrafiltration dialysis method, the number of

subjects (n=5) studied in their investigation is too small to draw conclusions about a normal distribution. Besides, any information about the subjects is lacking in their paper. Moll and Rosenfield (1979) found a mean free T percentage of 1.38% (range 0.86-2.40) in 32 normal women (age 19-36 yrs) using the flow-dialysis technique. Our values also clearly exceed the values of these investigators. We cannot provide an explanation for these differences, but the differences in techniques used or intergroup differences may be considered. Besides, repeated measurements (the free percentage T and E_2 was measured in each subject 4-6 times) revealed a high reproducibility of the individual values, thus excluding methodological errors so far.

Physical exercise produced a significant increase in the mean percentage of free T in the untrained group (8.6 and 10.2%) and in the trained group (8.7% and 11.4%) in the follicular and luteal phase, respectively. These results corroborate the data of Vigersky and co-investigators (1979) and Dunn and co-investigators (1981) who showed that an increase in total T concentration caused a less pronounced increase in free T concentration due to the binding capacity of the plasma for this steriod. In women, no data are available concerning exercise-induced changes in free T. However, Kuoppasalmi (1980) found a rise in 10 male athletes after strenous running in the T/SHGB ratio and concluded that the free T fraction might have increased, which is confirmed by the present investigation in women. As mentioned earlier the enhanced free fraction of T will increase the MCR and interconversion of this hormone (Vermeulen and Ando, 1979). The high percentage of free T may be attributed to a low plasma SHBG concentration. For the trained women the daily physical exercise with its concomitantly enhanced plasma T levels, may be attributed to a decline in SHBG concentration.

- Androstenedione (A)

The findings of the present investigation indicate that in the untrained women the plasma Δ4-A concentration was clearly different from that in trained women. In the untrained group we measured a mean Δ4-A concentration of 7.8 and 8.8. $nmol.l^{-1}$ in the follicular and luteal phase, respectively, whereas these values in the highly trained women were about 5.5 $nmol.l^{-1}$ in both phases. The values of the untrained subjects are slightly above the upper limit of the normal range (see table 2.2) (Judd and Yen, 1973),

whereas the values of the trained group are within the normal range. The high values in the untrained group are certainly not attributable to methodological errors since the plasma of the trained subjects was assayed in the same runs.

The findings in the present study indicate that prolonged physical exercise induced a significant increase in plasma Δ4-A concentrations. The response to exercise was much less variable in the trained than in the untrained group, as judged from the significant increases in both absolute and relative values. In the trained subjects a significant increase in absolute Δ4-A concentrations was observed at all exercise intensities.

Data on Δ4-A responses to physical exercise in women are hardly available. Only Baker and co-investigators (1982) recently reported a significantly increased plasma Δ4-A concentration after a marathon race in six women. From their graphs (no detailed information was given in their paper) we calculated a mean increase of about 60% after the marathon as compared to the control situation. These values are comparable with our findings in the trained women. In men several authors (Brisson et al, 1981; Kuoppasalmi et al, 1981; Pesquies et al, 1981) have reported an increased plasma Δ4-A concentration after physcial exercise of different duration and intensity. Brisson and his colleagues (1981) observed a slight increment in plasma Δ4-A concentration in 8 male basketball players after two bicycle ergometer rides of 30 min (70% and 85% $\dot{V}O_2max$). Kuoppasalmi and co-investigators (1981) found an almost similar increase of the plasma Δ4-A concentration after anaerobic (0.25-2 min) and intense aerobic running (15-230 min) in well trained athletes. Our results agree well with these findings; the increments in plasma Δ4-A showed levelling-off at the end of the treadmill run in the trained subjects in the follicular phase.

- Dehydroepiandrosterone sulfate (DHEA-S)

In the untrained subjects, which were considerably younger than the trained ones, the DHEA-S concentration at rest (about 7.0 $\mu mol.l^{-1}$) was at the upper limit of the normal range which is reported to be 2-9 $\mu mol.l^{-1}$ (Buster and Abraham, 1972; Metcalf, 1976). On the contrary, in the marathon runners the plasma DHEA-S concentration (about 2.3 $\mu mol.l^{-1}$) was measured to be at the lower limits. Although at first glance, a methodological error may be considered, this has to be rejected because the plasma samples of

the trained and untrained subjects were done in the same assay. The discrepancy may likely be explained by the difference in age, since Drucker and David (1980) reported a clear difference between two groups of women within the third and fourth decade of their lives. The data they reported for these two groups agree rather well with our findings. They found a mean plasma DHEA-S level of 6.6 and 4.7 $\mu mol.l^{-1}$ for women of 21-30 and 31-40 years of age, respectively, while we found about 7.5 and 2.5 $\mu mol.l^{-1}$ for the same age categories. In the highly trained runners, but not in the untrained subjects, prolonged exercise induced a 40% increase in plasma DHEA-S concentration. During the first 45 min, the plasma DHEA-S concentration increased linearly with exercise, whereafter a levelling-off was observed. Until now, no detailed information is available about plasma DHEA-S responses to physical exercise in women. To our knowledge, only one investigation (Baker et al, 1982) has been dealing with this subject matter in trained women. These investigators compared the DHEA-S concentration in blood as sampled within 20 min after a marathon race with that in blood samples taken in the control situation (12-24 hrs after a practice run) in 6 women. From their graphs we calculated a mean increase of about 24% which is in the same order of magnitude as the increase observed in the present investigation. After the treadmill run we found mean increments of 35 and 20% in the follicular and luteal phase, respectively. In our study no significant change in plasma DHEA-S concentration could be observed in the untrained women.

- Adrenocorticotropic hormone (ACTH)

In the present investigation we observed an exercise induced increase in plasma ACTH concentration, both in trained and untrained women. At comparable workloads and exercise times, the absolute plasma ACTH concentration was higher in the untrained than in the trained subjects; the relative changes being more pronounced in the trained group. Several investigators (Fraioli et al, 1981; Carr et al, 1981) showed exercise-induced increments in plasma ACTH concentrations using trained or untrained volunteers. These findings are in agreement with the results of the present investigation. However, in the afore mentioned investigations no comparisons have been made between trained and untrained subjects regarding the response of plasma ACTH to physical exercise. This makes it difficult to

test our finding, that exercise of the same relative intensity and duration provokes a more pronounced increase in plasma ACTH concentration in trained women. This finding suggests an increased sensitivity of the pituitary gland or the hypothalamus to physical stress in trained subjects, but further investigations are required to test this hypothesis.

The relatively more pronounced exercise-induced increase in plasma ACTH concentration in trained as compared to untrained women may explain the differences in plasma concentration of adrenal sex hormones between these two groups. In addition, experiments in rats (Frenkl et al, 1975) have shown that administration of ACTH causes a more pronounced increase in plasma sex steroids in the trained as compared to the untrained animals.

5.2.1. Effect of a three month endurance training program on metabolic and hormonal responses to exercise (study III)

5.2.1.1 Maximal physical working capacity and metabolic responses to exercise.

In the present investigation eight subjects from the untrained group volunteered in an endurance training program for three months. The training was initiated directly after the first set of experiments, and continued until the subjects finished the last experiments. The endurance training program consisted of 2-3 times per week running (interval and continuous running) during 25-60 min. In addition the subjects performed one training per week on the bicycle ergometer. The intensity and volume of the training was gradually increased till all subjects were able to run for 45 to 55 min continuously. It has to be mentioned that the intensity and volume of the training had to be very low during the first 1 to 2 months because most of the subjects experienced (minor) injuries of the lower extremities since they were unacquainted to this type of exercise.

Maximal physical working capacity (MPWC)

The MPWC increased by about 6-8% after the training period. Several investigators (Åstrand and Rodahl, 1977; Hollmann and Hettinger, 1981) reported greater increments (about 15%) in MPWC after a three month training period, using different exercise protocols. If we calculate the MPWC from the $\dot{V}O_2max$ test which preceded the prolonged experiment, a 12% increa-

se in MPWC was obtained. However, this test lasted for only 10-15 min, whereas the prolonged experiments had a duration between 40 and 60 min. Nevertheless, we conclude that the increase in physical performance after the three month training period is moderate.

Metabolic responses after training.
Glucose and Lactate

The blood lactate response to exercise was significantly increased after the training period despite the same relative workload. This again indicates the moderate adaptation of the subjects to the endurance training program. Whereas in the pre-training tests no difference in exercise-induced changes in blood lactate concentration could be observed between the luteal and follicular phases, a significant difference between both phases was found in the post-training tests, the luteal phase values being lower as compared to the follicular phase values. This finding is in agreement with the findings in some other studies (Jurkowski et al, 1978; Hall-Jurkowski et al, 1981), but disagrees with the results as obtained in the present investigation (studie I and II) and other investigations (Dalsky et al, 1982; Bonen et al, in press). To our opinion the lower plasma lactate concentration in the luteal phase has to be considered as a coincidence. This idea is supported by recent findings of Kuipers (1983) who showed a variability of blood lactate concentrations of 5% during a series of 20 or more bicycle ergometer rides performed weekly in a group of male and female volunteers. The changes in blood lactate concentration, as found in this part of our study, might be well within these limits. The blood glucose response after training was similar to the one in the pre-training situation. No clear differences between the phases could be detected.

5.2.1.2. Endocrine responses to exercise after a period of endurance training

The results of the present prospective study showed at rest decreased post-training levels of ACTH in both phases of the menstrual cycle, whereas the E_2 and T concentration were lowered in the luteal phase. After the three month training period a significant increase in plasma T levels was observed during exercise, whereas in this period the plasma levels of this hormone rose relatively more during exercise as compared to the pre-trai-

ning period. The relative post-training changes of E_2, P, and PRL were not statistically different from the pre-training values.

Prospective controlled studies dealing with hormonal changes in women are limited. Bonen and co-investigators (1979) found in 10 young women a decrease in baseline FSH values after a training period (3 times/week, 30 min cycling). They could not find an exercise-induced increase in plasma E_2 levels after physical conditioning. Prior and co-investigators (1981) found decreased baseline gonadotropin levels and a decreased sensitivity of the pituitary to GnRH in a pilot-study conducted in 4 young women. However, despite these lower gonadotropin levels no significant differences could be observed in E_2 and P values during exercise and 90, 120 and 180 minutes after the GnRH injections.

Our results probably confirm the data of Bonen and co-investigators. Although we could not find an altered E_2 and P response to exercise, the E_2 levels in the post-training test conducted in the luteal phase were lower as compared to the pre-training tests. However, this finding has to be handled with care, because it is well recognized that the cyclic changes in plasma E_2 levels make comparison between two days in different menstrual cycles hazardous. We could also not confirm the results of some investigators (Brisson et al, 1981; Boyden et al, 1982) concerning the increased plasma PRL response after physical conditioning. Some possible explanations for this discrepancy will be given. Firstly, in our study the volume and intensity of the training program were rather low. Secondly our subjects responded already with an enhanced plasma PRL concentration to exercise before they were submitted to the training program. To our opinion the results of Brisson and co-investigators are at least questionable. Too much information about their subjects is lacking, i.e. no other hormonal data were presented while they also did not describe what a "sport history" means. In the present investigation we have shown that untrained women, and especially those who did not have any experience in athletic or physical activies, are able to respond to physical exercise with a pronounced increase in plasma PRL levels. Nevertheless, the elegant study of Boyden and co-investigators (1982) showed a greater PRL response to physical stress after a training period. Another striking finding in the present investigation is the significant increase of plasma T concentration after the training period. The increase in plasma T is very well explainable by a decrea-

sed MCR. One should know, however, that hepatic blood flow is significantly less decreased, even with the same relative workloads, after physical conditioning (Rowell et al, 1964). Therefore, it is most likely that beside a decreased MCR, an increased adrenal secretion rate is involved, which idea is supported by the relatively greater increase in plasma ACTH as compared to the pre-training period. In conclusion, the results of the present study suggests that the pituitary-adrenal axis respond to physical exercise with greater sensitivity. However, future research is required to estimate this hypothesis at its true value.

5.3 CHANGES IN GONADOTROPIN AND ESTRADIOL SECRETION PATTERNS (study III)

The findings in study III, which have to be considered as preliminary ones, indicate that physical exercise is able to induce marked changes in gonadotropin and E_2 secretion patterns during and after exercise. However, due to the extreme variation in secretion patterns and the relatively small number of subjects, adequate statistical analysis could not be performed. Therefore, simple analyses (multiple paired t-test) were used to evaluate possible general trends.

In general, the pulse amplitude and pulse increment of LH was enhanced after physical exercise. No changes in pulse frequency, pulse amplitude and pulse increment of FSH could be observed after exercise as compared to the pre-exercise period, whereas the pulsatile secretion pattern of E_2 almost disappeared after exercise.

The individual results showed marked differences in response pattern after physical exercise. Both an enhanced mean post-exercise LH and FSH level was observed, causing an increased or decreased LH/FSH ratio.
Despite the fact that it is well recognized that a sampling period of 2 hours is too short to gather appropriate results about gonadotropin secretion patterns, our data agree well with those of other investigators (Backstrom et al, 1982; Dmowski et al, 1983). They found an LH and FSH pulse frequency of about 4-5 per 6 hours. We found a mean pre-exercise LH and FSH pulse frequency of 5.2 in 6 hours. The pre-exercise E_2 pulse frequency as found in the present investigation was in keeping with the results of Lenton and co-investigators (1978). During the post-exercise period the

pulsatile E_2 secretion pattern disappeared almost completely. This might be explained by the disappearance of the normal LH secretion pattern during exercise, which occured in almost all subjects, although the degree of this disappearance was different from subject to subject. The depression of the LH secretion during and directly after exercise may be explained by the greatly enhanced E_2 concentration, which is thought to inhibit gonadotropin secretion at the hypothalamic pituitary level (Knobil, 1980). The different response of the plasma LH and FSH levels to inhibitory factors may be explained by the much lower MCR of FSH (Trager, 1977). The inter-individual variety in gonadotropin and E_2 responses before, during and after exercise as found in the present study may be explained by differences in hypothalamic-pituitary sensitivity to changes in E_2 levels.

Since E_2 exerts both a negative inhibitory effect and a positive stimulatory effect on LH secretion, which is depending on the "estrogenic state" of the subject, an increase as well as a decrease of LH secretion can be expected after a rise in E_2 levels. The two hours after physical exercise we observed in three out of seven subjects an increased LH/FSH ratio, whereas in all subjects a post-exercise rebound in LH levels was observed. Recently, Judd and co-investigators showed a similar effect after withdrawal of a dopamin infusion in fertile women. The LH rebound clearly exceeded the FSH rebound and was most pronounced near mid-cycle. These observations might suggest that after physical exercise the LH/FSH ratio increases. The magnitude of this increase will probably depend on the intensity and duration of the exercise (i.e the inhibitory factors) and the relation between the phase of the menstrual cycle and the moment of the exercise.

In conclusion, the present study revealed a dramatic influence of physical exercise on gonadotropin and E_2 secretion patterns. Although due to the marked inter-individual differences no general conclusions can be drawn, it is tempting to speculate that the observed changes may be attributable to menstrual cycle disturbances if they occur too often (i.e. the training frequency is high). One of the possible mechanisms may be the enhanced LH/FSH ratio after exercise, which may stimulate ovarian steroidogenesis. If, however, the stage of follicular development is not appropriate i.e. the granulosa cell, is not able to aromatize the enhanced supply of androgens, the follicle will become more androgenic and atretic.

5.4 POSSIBLE MECHANISMS OF THE EXERCISE-INDUCED INCREMENTS OF PLASMA SEX HORMONES

5.4.1 The adrenal cortex as a possible source of enhanced plasma androgen levels

In the present investigation serial blood samples were taken during exercise to measure the plasma concentrations of androgens of mixed ovarian and adrenal origin (T and Δ4-A), DHEA-S, which originates exclusively from the adrenal cortex and ACTH. The changes in plasma concentration, if any, of the two latter hormones could provide us more insight into the involvement of the adrenal cortex in the production of androgens during exercise.

The exercise-induced increase in ACTH concentration in trained and untrained subjects indicates that the hypothalamo-pituitary system is activated in this situation. Since the DHEA-S concentration rose in accordance with the extent and intensity of the exercise in trained, but not in untrained women, the synthesis of sex steroids by the adrenal cortex might be enhanced during exercise. Observation of the pattern of the DHEA-S changes in the trained group (fig. 4.25 and 4.26) revealed a levelling-off after an initial linear increase. Two explanations can be given for this phenomenon:

- an increase of the MCR during the later stages of the experiment.
- a decrease in adrenal secretion rate.

Although under resting conditions, the MCR of DHEA-S is reported to be very low (Longcope et al, 1972; Poortman et al, 1973)) this does not necessarily have to be the case during exercise, because the rise in body temperature will probably enhance the dissociation rate from albumin considerably. Consequently the MCR of DHEA-S might be increased. Since Δ4-A, but also T partly arises by peripheral conversion from DHEA and DHEA-S investigation of the relative increases in plasma concentration of these hormones may provide some insight into the relations existing between these hormones. Examination of the relative increments of DHEA-S, Δ4-A, T and P reveals that the pattern is dissimilar. Whereas the relative plasma T values showed an almost linear increase with exercise, the percentage changes in Δ4-A and DHEA-S concentrations showed a non-linear trend, especially the last half our of exercise. However, the Δ4-A curve is shifted to the right

as compared to the DHEA-S curve. This indicates that peripheral conversion from DHEA-S to Δ4-A might be enhanced. The linear increase of the relative plasma T values and the non-linear trend of the Δ4-A changes also suggest an enhanced peripheral conversion from Δ4-A to T, assuming that the hepatic clearance is equally affected (i.e. decreased) for both hormones. This hypothesis is supported by the work of Pratt and Longcope (1978), who reported an increased P_B of Δ4-A after ACTH administration in women. Since the P_B of T is derived from conversion of this hormone to an important part, an increase in P_B of Δ4-A may lead to a concomitant increase in plasma T levels during exercise.

A decrease of the adrenal secretion rate seems unlikely because, during exercise plasma ACTH concentrations continued to rise. A remarkable finding in the present investigation is the difference in plasma androgen content between trained and untrained subjects during exercise at comparable workloads. This suggests an enhanced pituitary-adrenal responsiveness in the trained subjects. Unlike in untrained subjects, the plasma ACTH, Δ4-A and DHEA-S concentrations were already increased at lower workloads. This idea corroborates the data of Frenkl and co-investigators (1975) who showed in rats a greater responsiveness of the hypothalamo-pituitary-adrenal system after physical training. In addition Chandra and co-investigators (1978b) showed higher activities of steroid synthesizing enzymes in the adrenal cortex of trained as compared to untrained rats. Both the relatively higher ACTH levels and the greater capacity of the adrenal cortex to synthesize steroid hormones are responsible for the increased output of these hormones during physical exercise.

5.4.2 Exercise induced changes in the MCR of estradiol

The fifteen volunteers who participated in study Va and Vb were young women with apparently normal menstrual cycles and no evidence of endocrine disorders. Except for the subjects 1225 and 1226 (study Vb), who used oral contraceptives, all subjects were in the follicular phase of their menstrual cycles at the time of the experiment as evidenced by BBT and plasma hormone concentrations. We confirmed that an infusion time of 120 min after a priming dose (one third of the total dose) is sufficient to reach a steady state for the ^3H-E_2 concentration in blood plasma (Tait, 1963; Longcope et al, 1968; Poortman, 1974). Therefore, the mean of the two MCR

values as determined after 120 and 135 min was used as the resting or basal value of the MCR. The mean basal value for all sujects participating in both clearance studies was found to be 1254 l/day (range 669-1765 l/day), or 870.8 $ml.min^{-1}$, which is in agreement with the data reported by other investigators (Longcope et al, 1968; Hembree et al, 1969; Longcope and Tait, 1971; Longcope and Williams, 1974; Baird and Fraser, 1974; Gurpide, 1975).

Physical exercise induced a pronounced and highly significant decrease in the MCR of E_2, especially at the highest workloads. The decrease in MCR is probably caused by a reduction in hepatic blood flow because Rowell and co-investigators (1964) demonstrated that hepatic blood flow and consequently hepatic clearance is inversely related to the physical work intensity. The diminished MCR may explain the exercise induced rise in plasma E_2 concentration as found in the present study and by other investigators (Jurkowski et al, 1978; Bonen et al, 1979; Bonen et al, 1981; Schmitt et al, 1981). However, theoretically it may be possible that other factors such as an enhanced ovarian production and/or peripheral conversion from androgens might also play a role. The results of the present study indicate that an enhanced ovarian E_2 production is not very likely, because the plasma LH concentration decreased significantly. However, peripheral conversion from androgens might be involved. The latter is supported by the work of Pratt and Longcope (1978). They showed a considerable increase in production rate of both Δ4-A and estrogens after ACTH administration in men and women. They also measured greatly enhanced (20-25%) contributions of these hormones to circulating estradiol.

Additional support for the idea that peripheral conversion might play a role is given by the following findings: In male runners the plasma E_2 concentration was found to increase with 30 to 80% after a marathon run (Schmitt et al; unpublished results from our own laboratory). Since in men, plasma E_2 arises almost completely from peripheral conversion of A and T (for example in skeletal muscle) it is tempting to speculate that this mechanism might play an even more important role during exercise, when muscle blood flow is greatly enhanced.

The results as reported in study Vb clearly demonstrate that the MCR of E_2 is still significantly diminished at the end of the recovery period of 30 min at 25% $\dot{V}O_2max$. This observation, however, has to be interpreted with

care. Because of the often reported hemodilution after physical exercise (Åstrand and Rodahl, 1977; Hollmann and Hettinger, 1981) it is possible that the steady state between infusion rate and disappearance rate of the tracer is not reached, in other words the infusion rate may be too low. This means that one of the basic presumptions for the MCR calculation (i.e. the steady-state condition) does not hold. Hemodilution may also partly explain the finding in study III that the E_2 concentration falls rapidly after the exercise-test.

In conclusion, the present clearance experiments showed that even short-term physical exercise is able to induce a marked decrease in the MCR of E_2. This observation is in keeping with the findings of study I, II and III and other investigations showing a mean increase in plasma E_2 concentrations of about 60%. However, based upon the increased plasma Δ4-A levels and evidence from literature it may be that beside the diminished MCR, other factors are responsible for the enhanced plasma E_2 levels.

5.5 GENERAL DISCUSSION

In summary the findings in the present investigation demonstrate that prolonged physical exercise is able to introduce significant increments in absolute plasma E_2, P, T, PRL and ACTH levels in trained and untrained women irrespective of the phase of the menstrual cycle. In the trained runners physical exercise was also able to provoke marked increments in absolute plasma, Δ4-A and DHEA-S levels. When the absolute values were expressed as a percentage of the baseline value, it was shown that also in the untrained women the plasma Δ4-A concentration was increased by exercise.

The LH levels were markedly decreased during exercise, whereas this was much less the case with FSH. In a separate study it was shown that physical exercise provokes marked changes in the post-exercise pulsatile patterns of LH, FSH and E_2 as compared to the pre-exercise period. However, wide inter-individual variations in this clearance pattern was observed.

With respect to the present investigation two questions have still to be answered:

- what causes the exercise-induced changes in plasma-hormone concentration.
- which factors might be responsible for the reported increase in menstrual cycle disturbances in women athletes.

Factors causing exercise-induced changes in plasma hormone levels.

An increase in plasma sex hormone levels might be caused by an enhanced glandular secretion rate, a decreased MCR and enhanced peripheral conversion rate from pre-hormones. From our results it is likely that the secretion rate of the ovary is not affected by exercise. This is deduced from the decreased plasma LH levels. However, it is well recognized that a definite prove for this statement is lacking, because we are not informed of the sensitivity of the theca and granulosa cells for circulating LH under these circumstances. Based upon the investigations of Rowell and co-investigators (1964), however, we assume that the blood flow through this region is greatly reduced.

In study V we were able to show a pronounced decrease of the MCR of E_2 during submaximal and maximal bicyle ergometer work. These results strongly suggest that a decreased MCR also plays a dominant role in the increase of Δ4-A, P and T, because these steroids are cleared in splanchnic tissue to a large extent. For DHEA-S this mechanism is less likely, due to its tight binding to plasma albumin, which causes a very slow turn-over rate. Based upon the increase in plasma DHEA-S and ACTH levels during exercise in the trained runners we assume that the adrenal cortex is involved in the production of androgens during exercise. This increases the pool of precursor hormones for conversion to T and E_2 (Pratt and Longcope, 1978). We assume that the exercising skeletal muscles are heavily involved in this process. This assumption is based upon the observations that the blood flow through this tissue increases 10-20 fold during exercise as compared to the situation at rest. However, this hypothesis has to be proved in future investigations. The exercise-induced increments in plasma PRL levels are partly explained by the enhanced E_2 levels, which have been shown to sensitize the pituitary to prolactin releasing factors (Williams, 1981).

Possible factors responsible for exercise-induced menstrual cycle disturbances.

Menstrual cycle disturbances may originate at various levels. In the first place the disturbance may originate at a level above the hypothalamo-pituitary system. This is clearly substantiated in an investigation of Fries and colleagues (1974) who showed that pronounced psychological stress was more frequent in women with secondary amenorrhea than in their age-matched controls. It is without doubt that competitive running can be considered to be stressfull. Whether a laboratory experiment provokes the same feelings is not easy to prove. In the present investigation the plasma ACTH and PRL levels at rest were not elevated (except for one case). Hence it is unlikely that this mechanism plays a role in the observed exercise-induced decrements of plasma LH.

The second level at which the menstrual cycle disturbances may occur is the hypothalamo-pituitary axis. We have witnessed a marked decrease in plasma LH levels during exercise, which might be caused by an enhanced level of endogenous opiates (Carr et al, 1981), E_2 and PRL. Endogenous opiates diminish the LH pulsatile secretion from the pituitary (Ropert et al, 1980; Mcarthur et al, 1980; Blankstein et al, 1981; Grossman et al, 1982). These authors also showed that the LH response to naloxone is mediated by the serum E_2 levels, the greater response being reached at higher E_2 levels.

Excessive PRL does alter the menstrual cycle probably by suppressing GnRH secretion (Mcneilly, 1980). The PRL secretion during exercise is most likely to be modulated by an increase in plasma E_2 and TRH levels as suggested by the increase in TSH levels (Galbo et al, 1977). Another possibility of suppression of pituitary gonadotropin secretion is suggested by Kalkarni and co-investigators (1977) who showed in ovariectomized rats a significant suppressed FSH response to GnRH-stimulation after T administration. The greatly enhanced plasma T levels as found in the present investigation are likely to induce the same phenomenon. This is suggested by the increased LH/FSH ratio after exercise in 4 out of 7 subjects participating in study III.

The third level at which menstrual cycle disturbances may originate is the ovary. McNatty (1974) suggested that plasma PRL levels above 0.6 $IU.l^{-1}$ should be responsible for disturbances in follicular maturation. In the

present investigation we have shown that physical exercise is able to provoke increments in plasma PRL levels both in trained and untrained women. These sometimes exceeded the critical levels indicated by McNatty by a factor 2 or 3. The menstrual cycle disturbances in women athletes as described in literature (Baker et al, 1981) are likely to be caused by a complex mechanism acting on the hypothalamo-pituitary axis. It will probably depend on the pre-training endocrine status of the individual whether she will experience menstrual cycle disturbances. From our results it becomes apparant that the exercise-induced changes in endocrine milieu will wane for several hours after the exercise before returning to normal. If the exercise frequency and/or duration is too high or too long, a normal follicular development is hampered. Therefore it is tempting to speculate that especially the post-exercise period is very important to study in future research.

CHAPTER VI

6.1 SUMMARY AND CONCLUSIONS.

The hormonal response to standardized physical exercise in trained and untrained women in relation to the follicular and/or luteal phases of their menstrual cycle was the subject of the present investigation. The aim of the study was:

- to evaluate the influence of the phase of the menstrual cycle on maximal physical working capacity (MPWC) and related physiological parameters.
- to collect detailed information about the plasma sex hormone and gonadotropin responses in highly, moderately and untrained women in order to provide some insight into the possible cause of the often described menstrual cycle disturbances in women athletes.
- to differentiate between changes in degradation rate (measured as the metabolic clearance rate) and an enhanced ovarian secretion rate as a possible cause of the exercise-induced increments in sex hormone levels.

The first two objectives were hoped to be solved by serial measurements of plasma hormones of ovarian, mixed ovarian and adrenal, and adrenal origin during exercise. The related gonadotropins, adrenocorticotropic hormone (ACTH) and prolactin (PRL) were also measured.
The third objective was hoped to be solved by measurement of the MCR of E_2 during exercise.

In chapter I, a brief review is given of recent investigations concerning exercise-induced changes in plasma hormone concentrations in women.

In chapter II, the literature about hormonal events, which take place during the normal menstrual cycle is reviewed. This chapter is included to provide the reader with some information about the complex relationship between phase of the menstrual cycle, follicular maturation, sex hormone synthesis as well as blood production and degradation of these hormones. Also included in this chapter is a short review of the literature about adrenal steroidogenesis, especially the androgens, in women.

In chapter III the experimental set-up and the purposes of the different studies are described. Fourty seven healthy women, with normal menstrual cycles, volunteered in 5 separate studies (I-V). The effects of the

phase of the menstrual cycle on MPWC and hormonal responses to exercise were investigated in study I-III. In study I a group of 13 untrained women volunteered. The exercise test consisted of an incremental bicycle ergometer ride (60% $\dot{V}O_2$max for 15 min, followed by an increase of 10% $\dot{V}O_2$max each 15 min until the subject had to give up). In study II 6 highly trained marathon runners volunteered. The exercise test consisted of a treadmill run for 75 min (60% $\dot{V}O_2$max for 15 min, followed by 60 min at 85% $\dot{V}O_2$max). In study III 8 previously untrained women were retested after a three month endurance training program.

In these three studies each subject performed twice in the menstrual cycle; once in the follicular and once in the luteal phase. Each exercise bout started between 8.30 and 9.30 a.m., and was preceded by a 30 min period of rest. Blood sampling occured every 15 min, through an indwelling venous catheter. The first two samples were obtained 15 min (15 min after the insertion of the catheter) and just before the start of the exercise. In each blood sample plasma glucose and lactate were measured to evaluate the metabolic responses to exercise in relation to the phase of the menstrual cycle. Additionally, estradiol-17β (E_2), progesterone (P), testosterone (T), androstenedione (Δ4-A), dehydroepiandrosterone sulfate (DHEA-S), luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin (PRL) and andrenocorticotropic hormone (ACTH) were measured in each plasma sample by radio-immuno-assy (RIA), whereas the free E_2 and T fractions before and after exercise were determined using a centrifugal ultrafiltration technique.

All data were presented as absolute and relative values (hormonal concentration values only) versus time or workload. Glucose, lactate and all hormonal data, including the percentual changes, were analyzed with a two-way ANOVA (exercise versus phase). Intergroup comparisons (study I versus II; study III: the pre- versus post-training values) were conducted with a multi-way ANOVA. Differences in MPWC between the follicular and luteal phases of the menstrual cycle and the pre- and post-exercise values of the free steroid fraction were evaluated for statistical significance with a multiple paired T-test.

The results of these three studies showed:

- no influence of the phase of the menstrual cycle on MPWC.
- no clear influence of the phase of the menstrual cycle on blood lactate and glucose response to exercise.
- a pronounced difference in blood glucose response to exercise between the highly trained marathon runners and the untrained women (study I and II). At all workloads the blood glucose concentration was more elevated in the highly trained group as compared to the untrained group.
- a pronounced exercise-induced increase in absolute plasma E2, P, PRL and ACTH concentration in the follicular and luteal phases of the menstrual cycle in all groups. The relative increments in plasma PRL and ACTH were more pronounced in the highly trained subjects as compared to the untrained subjects (study I and II). In the untrained subjects the workload had to exceed 70% VO_2max to provoke increments in plasma PRL and ACTH concentrations, whereas in the trained subjects also the lowest workloads were able to produce such changes.
- an exercise induced increase in plasma T, Δ4-A and DHEA-S concentration in the highly trained runners (study II). These changes even occurred at the lowest workloads.
- an exercise-induced increase in relative plasma T and Δ4-A , but not in DHEA-S concentration, in the untrained group (study I).
- a lower absolute plasma T, Δ4-A and DHEA-S concentration at rest in the highly trained versus untrained or moderately trained women. The relative increments in plasma T and Δ4-A concentration, however, were more pronounced in the marathon runners as compared to the untrained women.
- a pronounced decrease in relative plasma LH concentration in the untrained (study I) and highly trained (study II) subjects. The relative FSH concentration decreased only in the untrained group.
- an exercise-induced increase in the free T fraction in both the untrained and highly trained group. The free E_2 fraction after exercise was slightly decreased (untrained women, study I) or did not differ significantly (study II) from the pre-exercise values. The mean free E_2 fraction at rest was lower in the trained than in the untrained group.
- a significantly lower absolute plasma ACTH concentration at rest after a three months training period. However, the relative increments during exercise were statistically not different between the pre- and post-training tests (study III).

- physical exercise provoked a significant increase in plasma T concentrations after a three month training program, whereas the plasma concentrations of this hormone were not statistically significant increased in the untrained situation.

In study IV the gonadotropin and E_2 secretion patterns in the two hours after exercise were compared with those in the two hours before exercise in 7 women in the follicular phase of their menstrual cycle. The exercise protocol was identical to that of study I and III. Blood sampling occured every 15 min during the whole period.
Although the responses were strikingly different between the individuals, the results showed a marked influence of physical exercise on the secretion pattern of LH, FSH and E_2. For the whole group a significant post-exercise increase in mean LH levels could be observed which was accompanied by a concomittant rise in pulse frequency, pulse amplitude and pulse increment. In 4 subjects the LH/FSH ratio was increased after exercise, whereas it was decreased in one subject. In the other two subjects this ratio was about the same in the post-exercise period as compared to the pre-exercise period.

The influence of short-term submaximal and maximal bicyle ergometer work on the metabolic clearance rate (MCR) of E_2 was evaluated in study Va and Vb. For this purpose 15 young women volunteered in the follicular phase of their menstrual cycle. In study Va the influence of submaximal and maximal (70% $\dot{V}O_2$max for 10 min followed by 100% $\dot{V}O_2$max till the subject was exerted) bicycle ergometer work on the MCR of E_2 was investigated in 9 physical education students. In study Vb the influence of a 10 min 70% $\dot{V}O_2$max load, followed by a 30 min 25% $\dot{V}O_2$max load on the MCR of E_2 was evaluated in 6 women with different levels of physical fitness.

The results showed an exercise-induced pronounced decrease (up to 80%) in MCR of E_2, which was inversely related to the workload. At the end of the recovery period (study Vb) the MCR of E_2 was still well below the base-line values.

The findings of the present investigation indicate that physical exercise is able to induce marked elevations in plasma E_2, P, Δ4-A and T levels. These elevations may be attributed to a decrease in MCR. Since the plasma LH concentrations decreased with exercise, an enhanced ovarian secretion rate is not likely to contribute to the increments in plasma sex

hormones. However, an enhanced adrenal secretion rate is most likely to be involved, especially in the highly trained subjects, since the plasma DHEA-S concentration rose in accordance with exercise.

The results of the present investigation also showed that physical exercise is able to induce marked elevations of plasma PRL levels in trained and untrained women, although the relative increments were greater in the former group. The dysregulation of the normal pulsatile secretion pattern of the gonadotropins as shown in study IV of the present investigation may originate from the enhanced PRL, E_2, and/or T (especially free T) levels, which may inhibit gonadotropin releasing hormone (GnRH) (by means of PRL) and/or gonadotropin (by means of E_2 and T) secretion patterns.

6.2 SAMENVATTING EN CONCLUSIES

Het is de vrouw slechts sinds enige jaren toegestaan om deel te nemen aan zulke zware sportieve belastingen als marathon lopen en lange afstand ski-loop wedstrijden. Tot die tijd werd (voornamelijk door de man) gedacht, dat zulke belastingen schadelijk zouden zijn voor het vrouwelijk organisme, alhoewel bewijzen voor dit idee ontbraken. Ten gevolge van deze vooroordelen ontbraken tot voor kort gegevens over de specifiek vrouwelijke (hormonale) reacties op fysieke belasting.

De huidige toename in trainingsarbeid en intensiteit van de sportende vrouw veroorzaakte echter een nieuwe, meer wetenschappelijke, belangstelling voor de hormonale veranderingen welke optreden tijdens lichamelijke inspanning. Deze belangstelling werd mede gewekt door de grotere incidentie van stoornissen in de menstruele cyclus bij sportvrouwen vergeleken met niet aan sport deelnemende vrouwen. Tevens kreeg de vraag of de fase van de cyclus van invloed is op het prestatievermogen hernieuwde aandacht.

Door het nog geringe aantal onderzoekingen op dit gebied en de ingewikkelde regelsystemen waaraan de menstruele cyclus onderworpen is, bleek het niet goed mogelijk te zijn een goede hypothese voor de oorzaak van de cyclusstoornissen op te stellen.

Het huidige onderzoek wil dan ook een bijdrage leveren aan een verbetering van onze inzichten in de hormonale veranderingen bij de vrouw tijdens fysieke inspanning in de folliculaire en luteale fase van de menstruele

cyclus. Hierbij werd getracht een antwoord te vinden op de volgende vragen:
- Wat is de invloed van de fase van de menstruele cyclus op het fysieke prestatievermogen;
- Welke mechanismen zijn verantwoordelijk voor de in de literatuur gemelde veranderingen in geslachtshormoonconcentraties tijdens fysieke inspanning.

Aangezien zware lichamelijke inspanning tevens invloed heeft op de periode erna, werd de additionele vraag gesteld of fysieke inspanning een effect heeft op hormoonconcentraties, met name de gonadotropinen (LH en FSH) en oestradiol (E_2), in de twee uur na beeindiging van de arbeid.

Om deze vragen te kunnen beantwoorden werd een groep van 47 vrouwelijke proefpersonen, met een varierende mate van getraindheid, aan een of meerdere inspanningsproeven onderworpen. De vrijwilligsters participeerden in vijf verschillende series experimenten.

De invloed van de fase van de menstruele cyclus op het maximale fysieke prestatievermogen en de hormonale veranderingen t.g.v. fysieke inspanning werd onderzocht in de experimenten I-III. In experiment IV werd de invloed van fysieke belasting op het secretiepatroon van LH, FSH en E_2 gedurende de twee uur volgend op de inspanning vergeleken met het patroon gedurende twee uur voorafgaand aan de belasting.
In experiment V werd de afbraaksnelheid van E_2, uitgedrukt als de metabole klaringssnelheid (metabolic clearance rate = MCR) gemeten tijdens inspanning.

Teneinde de lezer enig inzicht te verschaffen in de processen welke ten grondslag liggen aan de cyclische variaties in plasma geslachtshormoonconcentraties gedurende de menstruele cyclus wordt hiervan een beschrijving gegeven in hoofdstuk 2. Speciale aandacht wordt besteed aan de normale follikel rijping en de daarbij plaats vindende veranderingen in hormoonsecretie, terwijl vervolgens wordt ingegaan op het belang van het pulsatiele secretiepatroon van de gonadotropinen. Tevens wordt in dit hoofdstuk aandacht besteed aan de afbraaksnelheid, conversie en transport van de verschillende geslachtshormonen. Aangezien de bijnier bij de vrouw een belangrijke bijdrage levert aan de plasma androgenenspiegel werd hieraan tenslotte een korte beschrijving gewijd. Dit werd mede gedaan in verband met de cyclusstoornissen ten gevolge van hyperprolactinemie, al of niet gepaard gaande met een verhoogde dehydroepiandrosteronsulfaat (DHEA-S) spiegel.

Naar aanleiding van de in dit hoofdstuk beschreven complexe interacties tussen de verschillende geslachtshormonen van ovariele en adrenale oorsprong alsmede de regulatie van hun synthese door middel van de trope hormonen, werd het noodzakelijk geacht de volgende hormonen voor en tijdens inspanning in het plasma te bepalen: oestradiol (E_2), progesteron (P), luteiniseringshormoon (LH), follikel stimulerend hormoon (FSH), testosteron (T), androstendion (Δ4-A), dehydroepiandrosteronsulfaat (DHEA-S), prolactine (PRL) en adrenocorticotroop hormoon (ACTH).

In hoofdstuk III wordt de samenstelling van de groepen van proefpersonen, de gebruikte methodes en de statistische analyse nader uiteen gezet.

In onderzoek I participeerden 19 vrijwilligers, waarvan er 6 afvielen om uiteenlopende redenen. De belasting bestond uit een opklimmende fietsergometertest (60% VO_2max gedurende 15 min, waarna de belasting iedere 15 min werd verhoogd met 10% VO_2max totdat de proefpersoon moest opgeven).

In onderzoek II participeerden 6 zeer goed getrainde marathonloopsters. De belasting bestond uit een tredmolentest (60% VO_2max, gedurende 15 min, gevolgd door 60 min met een belasting van ±85% VO_2max). De hellingshoek werd op 0° gehouden.

In onderzoek III werden 8 aanvankelijk ongetrainde vrouwen voor en na de periode van drie maanden duurtraining (3-4 keer per week) belast waarbij gebruik gemaakt werd van hetzelfde protocol als in studie I.

Alle proefpersonen welke in deze drie studies participeerden, ondergingen dezelfde belastingstest in de folliculaire en luteale fase van hun menstruele cyclus, respectievelijk 7-10 en 20-26 dagen na de eerste dag van de menstruatie. Elk experiment vond 's ochtends plaats tussen 8.30 en 9.30 uur. De inspanningsproeven werden vooraf gegaan door een rustperiode van 30 min. Bloed werd afgenomen 15 min voor de inspanning en vervolgens elke 15 min (aan het einde van elke belastingsstap).

In het bloedplasma werden het glucose en melkzuur gehalte bepaald, alsmede de calculatie van E_2, P, T, Δ4-A, DHEA-S, LH, FSH, PRL en ACTH. Voorts werden de percentages vrij E_2 en T voor de inspanning vergeleken met die erna. Alle inspanningshormoonwaarden werden gecorrigeerd voor hemoconcentratie door middel van de totaal-eiwit methode. De hormoonwaarden werden weergegeven als absolute en relatieve (procentuele) waarden als functie van tijd en/of belastingsintensiteit.

Veranderingen in relatieve en absolute plasma concentraties van glucose, melkzuur en de verschillende hormonen tijdens inspanning, verschillen in reacties tussen de fasen van de menstruele cyclus, alsmede verschillen tussen de groepen werden getoetst op statistische significantie door middel van een tweeweg variatie analyse voor vergelijking tussen groepen. De verschillen in fysiek prestatievermogen, uitgedrukt in Watts, tussen de fasen van de menstruele cyclus en de veranderingen in vrij E_2 en T werden getoetst met een multipel-gepaarde T-test.

De belangrijkste resultaten van deze onderzoeken kunnen als volgt samengevat worden:

- er was geen aantoonbaar verschil in fysiek prestatievermogen tussen de folliculaire en luteale fase. Tevens was de plasma melkzuur en glucose reactie niet duidelijk verschillend tussen deze twee fasen in de menstruele cyclus.
- de relatieve plasma LH concentraties daalden in alle gevallen tijdens inspanning, terwijl de relative FSH concentratie daalde (onderzoek I, ongetrainden)) of onveranderd bleef (onderzoek II, getrainden).
- de absolute plasma concentraties van E_2, P, PRL en ACTH stegen significant tijdens inspanning. Terwijl de relatieve veranderingen in E_2 en P concentraties tijdens inspanning niet aantoonbaar verschilden tussen de ongetrainde (onderzoek I) en zeer goed getrainde (onderzoek II) groep, bleken de relatieve PRL en ACTH concentraties in de laatste groep meer toe te nemen. Bij de ongetrainden was de toename in plasma PRL en ACTH concentratie slecht significant als de arbeidsintensiteit boven de 70% VO2max kwam.
- de absolute T, Δ4-A en DHEA-S concentratie namen tijdens inspanning alleen toe bij de zeer goed getrainden. Indien de plasmaconcentraties uitgedrukt werden als relatieve veranderingen, bleken de T en Δ4-A concentraties ook in de ongetrainden toe te nemen. Deze toename was reeds significant bij een arbeidsintensiteit van 60% VO_2max.
- de absolute plasma T, Δ4-A en DHEA-S concentraties in rust waren in de ongetrainde groep hoger dan in de zeer goed getrainde groep.
- het vrije percentage T was zowel in de getrainde als in de ongetrainde groep gestegen na inspanning, de vrije E_2 concentratie bleef onveranderd (onderzoek I en II) na inspanning.
- na een relatief korte trainingsperiode (onderzoek III) kon een signifi-

cante stijging van de plasma T concentraties tijdens inspanning geconstateerd worden, terwijl de rustwaarden niet verschilden (folliculaire fase) of na training waren gedaald (luteale fase). De absolute ACTH spiegels in rust en tijdens inspanning waren gedaald na de trainingsperiode.

In onderzoek IV werd het secretiepatroon van LH, FSH en E_2 in de twee uur na fysieke inspanning vergeleken met dat in de twee uur ervoor in 7 ongetrainde vrouwen in de folliculaire fase van hun menstruele cyclus. Het belastingsprotocol was identiek aan dat van onderzoek I.

De resultaten van dit onderzoek, dat opgevat moet worden als een pilotstudie, leverden een zeer gevarieerd beeld op hetgeen de statistische analyse bemoeilijkte. Nochthans bleek dat vooral het LH secretiepatroon na inspanning duidelijk veranderd was; de gemiddelde LH concentratie was in deze periode verhoogd vergeleken met de periode voor inspanning. De LH pulsen waren na inspanning eveneens verhoogd. Dit had tot gevolg dat de LH/FSH ratio bij 4 proefpersonen in de gehele periode na de inspanning verhoogd was ten opzichte van de twee uur ervoor. Bij de andere proefpersonen trad een daling op (1x) ofwel bleven de verhoudingen gelijk.

In onderzoek V werd de MCR van E_2 bepaald teneinde een inzicht te krijgen in een van de mogelijke oorzaken van de door inspanning geinduceerde stijging in steroid hormoon concentaties. Hiertoe werden 15 vrijwilligsters onderzocht in de folliculaire fase van hun menstruele cyclus. Gebruik werd gemaakt van de continue infusie-techniek, waarbij het radioactieve isotoop (^3H-E_2) werd geinfundeerd met behulp van een infusiepomp. De resultaten toonden een daling van de MCR tijdens inspanning, de mate hiervan was omgekeerd evenredig met de intensiteit van de arbeid.

Samenvattend kan gesteld worden, dat de stijging in steroid hormoon concentraties tijdens inspanning in de eerste plaats toegeschreven moet worden aan een daling van de MCR. Alhoewel een toename van ovariele secretie theoretisch tot de mogelijkheden behoort lijkt dit onaannemelijk gezien de daling van de plasma LH concentratie.

Gezien de significante stijging van de plasma DHEA-S concentratie in de zeer goed getrainde groep wordt een toegenomen androgenen secretie van de bijnierschors aannemelijk geacht.

Het gestoorde secretiepatroon van LH en FSH vindt mogelijk zijn oorzaak

in remming van de hypothalaam-hypofysaire as door PRL, E_2 en mogelijk T. De observatie dat in getrainden bij eenzelfde relatieve belastingsintensiteit een relatief grotere toename van PRL en ACTH in plasma wordt gevonden dan in ongetrainden leidt tot de hypothese dat de hypofyse gevoeliger reageert op fysieke stress. Of dit echter zal leiden tot stoornissen in de menstruele cyclus, zal waarschijnlijke mede afhankelijk zijn van het intrinsieke reactiepatroon van de vrouw in kwestie, daar in dit onderzoek is aangetoond dat in een aantal ongetrainde vrouwen de plasma PRL en androgenen spiegels vrijwel even sterk stegen als in de getrainde groep.

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Table A.1
Plasma LH concentrations ($IU.l^{-1}$) in the two hours before and after exercise (study IV).

	Time (min)	Subject code 1002	1006	1010	1012	1022	1023	1024
BEFORE EXERCISE	-105	5.5	4.6	5.5	5.4	7.9	7.1	6.8
	- 90	5.6	5.5	5.0	5.5	9.2	5.6	6.5
	- 75	5.1	5.5	6.2	7.2	8.2	6.2	7.4
	- 60	5.0	5.0	6.7	6.7	7.2	5.6	6.0
	- 45	5.0	4.2	6.3	5.9	7.1	5.2	7.8
	- 30	5.0	5.0	5.9	5.5	7.6	4.1	5.1
	- 15	6.2	4.3	6.4	7.0	8.2	5.9	4.8
	0	6.7	3.9	5.8	6.4	9.2	7.7	8.7
AFTER EXERCISE	15	7.5	3.7	6.3	4.6	7.7	7.3	5.0
	30	6.5	4.1	6.7	4.9	6.0	7.1	5.6
	45	6.8	4.9	7.6	5.5	8.4	4.4	6.4
	60	5.7	4.4	7.0	4.5	6.4	6.4	8.4
	75	6.1	6.4	6.3	7.7	9.9	4.6	5.8
	90	5.2	5.5	6.4	12.1	10.2	9.0	7.7
	105	6.3	6.6	6.3	10.7	8.8	8.7	8.2
	120	6.1	7.1	6.3	9.0	7.3	6.2	8.1

Table A.2
Plasma FSH concentration ($IU.l^{-1}$) in the two hours before and after exercise (study IV).

	Time (min)	Subject code 1002	1006	1010	1012	1022	1023	1024
BEFORE EXERCISE	-105	4.4	5.4	4.7	1.9	4.7	7.4	6.0
	- 90	3.8	5.4	4.7	2.2	4.1	7.0	5.8
	- 75	4.5	5.7	5.3	2.2	4.2	6.6	6.2
	- 60	4.4	4.4	5.3	2.6	4.8	5.6	6.0
	- 45	3.8	6.0	5.6	1.9	4.7	7.0	5.2
	- 30	4.1	5.4	6.3	2.2	4.2	6.5	4.4
	- 15	4.3	6.8	6.0	2.5	4.9	6.8	4.2
	0	5.0	5.4	6.1	2.7	6.0	7.0	7.8
AFTER EXERCISE	15	3.8	5.6	5.4	2.6	8.3	5.8	4.1
	30	3.4	5.6	5.0	3.1	7.3	5.7	4.5
	45	4.5	5.5	6.0	2.8	7.2	6.3	4.8
	60	3.6	5.7	6.3	3.1	8.0	6.8	5.0
	75	5.9	5.7	6.7	3.6	8.1	6.6	3.7
	90	5.2	6.5	6.1	4.2	7.3	7.7	3.9
	105	6.1	6.1	5.4	4.2	7.2	7.6	3.9
	120	6.8	4.7	5.4	3.8	7.0	7.7	4.3

Table A.3
Plasma E_2 concentration ($nmol.l^{-1}$) in the two hours before and after exercise (study IV).

	Time (min)	Subject code 1002	1006	1010	1012	1022	1023	1024
BEFORE EXERCISE	-105	0.08	0.19	0.23	0.6	0.14	0.07	0.33
	- 90	0.06	0.17	0.32	0.69	0.14	0.05	0.29
	- 75	0.07	0.17	0.42	0.64	0.12	0.08	0.32
	- 60	0.07	0.13	0.48	0.64	0.11	0.10	0.27
	- 45	0.07	0.18	0.43	0.57	0.13	0.09	0.30
	- 30	0.08	0.18	0.22	0.62	0.12	0.10	0.30
	- 15	0.08	0.22	0.23	0.63	0.15	0.10	0.33
	0	0.08	0.19	0.30	0.65	0.15	0.10	0.33
AFTER EXERCISE	15	0.10	0.22	0.24	0.88	0.17	0.16	0.57
	30	0.09	0.25	0.28	0.74	0.17	0.14	0.36
	45	0.08	0.18	0.30	0.71	0.16	0.13	0.37
	60	0.05	0.20	0.25	0.57	0.14	0.10	0.35
	75	0.10	0.22	0.23	0.58	0.14	0.10	0.32
	90	0.09	0.20	0.24	0.59	0.14	0.11	0.34
	105	0.10	0.19	0.22	0.61	0.14	0.11	0.32
	120	0.11	0.21	0.24	0.60	0.15	0.10	0.30

NAWOORD

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CURRICULUM VITAE

Hans Keizer werd in 1940 geboren in Medemblik. Na het behalen van het einddiploma HBS-B (1959) werd de diensplicht vervuld. Van 1962 tot en met 1966 volgde hij de opleiding tot Leraar Lichamelijk Opvoeding. In deze functie was hij tot en met 1969 werkzaam in de gemeenten Haarlemmermeer en Leiden. In 1969 ging hij Geneeskunde studeren aan de Rijksuniversiteit te Utrecht, terwijl hij tevens deels part-time, deels full-time werkzaam was als wetenschappelijk ambtenaar aan het fysiologisch laboratorium van dezelfde universiteit. In zijn studieperiode was hij tevens werkzaam als universitair sportleider en bondscoach voor het midden- en lange afstand lopen bij de Koninklijke Nederlandse Atletiek Unie. Van 1975 tot 1980 was hij eveneens als part-time docent fysiologie verbonden aan de Academie voor Lichamelijke Opvoeding te Amsterdam. Na in 1977 het artsexamen te hebben afgelegd, was hij tot februari 1980 als wetenschappelijk medewerker verbonden aan het fysiologisch laboratorium te Utrecht. Vanaf deze laatste datum is hij als wetenschappelijk medewerker, later als hoofdmedewerker, werkzaam bij de Capaciteitsgroep Fysiologie van de Rijksuniversiteit te Limburg.