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A LONGITUDINAL STUDY OF HORMONAL AND SEMEN PROFILES IN MARATHON RUNNERS

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ABBREVIATIONS

Progest	Progesterone
Testos	Testosterone
LH	Luteinizing hormone
FSH	Follicle stimulating hormone
E2	Oestradiol
GnRH	Gonadotrophin releasing hormone
TSH	Thyroid stimulating hormone
MAR	Mixed antibody reaction
hCG	Human chorionic gonadotrophin
BMI	Body mass index
SEM	Standard error of the mean
Std dev	Standard deviation
ng/ml	nanogram per millilitre
nmol/l	nanomol per litre
ml	millilitre
%	percentage
kg	kilogram
kg/m^2	kilogram per meter squared
km/wk	kilometers per week
IgG	immunoglobulin G
SHBG	sex hormone binding globulin
S	significant
NS	not significant

ABSTRACT.

Over the past decade long distance marathon running has become an important recreational activity. There is evidence that males with high levels of physical activity have some impairment of fertility. In order to investigate this further, 24 male marathon runners were studied over a period of a year. Each runner was assessed at regular intervals using hormonal profiles, anthropomorphic indices and semen evaluation. The training time and distance run increased progressively over the first five months of the study as the runners prepared for the Two Oceans marathon.

Analysis of the serum hormonal profiles in this longitudinal study showed that the prolactin level increased when comparing the initial study month with the rest of the year and the progesterone level decreased. However the luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone and estradiol (E2) levels remained unchanged. When the runners were divided into a high and low training group according to the distance run in the preceding week, the only significant difference was the lower mean serum FSH level in the high training group.

A decrease in semen volume was demonstrated as the training time increased. This trend was reversed as the runners' training decreased after the Two Oceans marathon. The percentage of morphologically normal spermatozoa showed an initial significant decrease in the first month of training. However, no significant difference was observed throughout the rest of year. An overall downward trend in semen motility in the first 5 months of the study was shown but this was only significant if the first and fifth study months were compared. The decrease in semen motility coincided with the period of maximum training. Since patients with an adequate sperm count but decreased motility have impaired fertility this finding is of considerable importance. In addition to the decrease in motility, there was a decrease in the percentage of morphologically normal

spermatozoa when the initial month of low physical activity (December) was compared to all of the subsequent months analysed. This, too, is an important finding as the percentage of morphologically normal spermatozoa correlates directly with fertilisation and pregnancy rates. When the results were analysed in the high and low training months there was a significant difference in mean semen count and semen morphology. The mean count was higher in the high training group and this group also had a significantly higher normal morphology. However, there was no significant difference in semen volume and motility in the high and low training groups.

DECLARATION.

I, CARL EDWARD JENSEN hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other University. I empower the university to reproduce, for the purpose of research, either the whole, or any portion of the contents in any manner whatsoever.

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Carl Edward Jensen.

14/11/93

Date.

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INTRODUCTION.

During the last decade, public interest in weight control and general health has led to running becoming an important recreational and health activity. This widespread interest in long distance running has led to observations of altered endocrine physiology in such groups, particularly in women runners. It has been shown that disturbances occur in normal hypothalamic-pituitary-gonadal (H-P-G) function in individuals participating in endurance training and competitive running (Ayers *et al.*, 1985). Evidence suggests that acute exercise and endurance training has a suppressive effect on the H-P-G axis in men and women (McColl *et al.*, 1989). Although there has been considerable investigation into the effects of exertion on reproductive function in women, there is very little information describing the chronic effects of endurance training on H-P-G function in men (Wheeler *et al.*, 1984).

Endurance training in women has been associated with reproductive dysfunction. A prospective study demonstrated that the abrupt imposition of strenuous aerobic exercise combined with caloric restriction can suppress luteal function, ovulation and menstruation in women potentially leading to reproductive dysfunction (Bullen *et al.*, 1985). A cause-effect relationship between exercise and infertility is, however, difficult to demonstrate because of the presence of life-style variables which also may influence the reproductive system (Loucks *et al.*, 1989). Clinical medicine tends to look at fertility, potency or menstruation as an "all-or-none" phenomenon, as normal or abnormal yet subtle and reversible alterations occur outside the "disease" state (Prior 1987). An exercise-induced deficiency of gonadotropin releasing hormone (GnRH) results in amenorrhoea. The prevalence of "athletic amenorrhoea" is variable and has been reported as 1 - 43% compared to 2 - 5% in the general population (Rogol *et al.*, 1992). Reduced pulse frequency of GnRH may be the cause of the persistent anovulation observed in females participating in endurance training (Reame *et al.*, 1985). Although the exact mechanisms causing such alterations in reproductive

function are not known, sustained aerobic exercise effectively stimulates the endogenous opiate system (Colt *et al.*, 1981). Studies in humans indicate that endogenous opioids and exogenous morphinomimetic compounds inhibit, whereas opiate-receptor antagonists acutely stimulate LH and possibly FSH secretion (Cicero *et al.*, 1980). Inhibition in the secretion of GnRH in women is manifested by amenorrhoea - a well defined end-point. This has led to the extensive investigation of women long-distance marathon runners. Acute strenuous exertion also elicits a demonstrable increase in serum prolactin concentrations in women runners (Shangold *et al.*, 1981). Although hyperprolactinaemia can be associated with clinical or biochemical hypogonadism, the role of exercise induced prolactin secretion in reproductive disturbances attributed to strenuous exercise is not defined. Endurance training in women has also been reported to decrease estradiol levels and reduce gonadotropin response to GnRH (Boyden *et al.*, 1983, Prior *et al.*, 1981). These changes may result in a shortened luteal phase, oligomenorrhoea or amenorrhoea (Wall *et al.*, 1983). The diminished luteal function and amenorrhoea appear to be separate end-points in different types of women (Loucks *et al.*, 1989). In contrast the clinical signs of hypogonadism in males are more subtle and often clinically inapparent. The findings among various studies suggest that the threshold for exercise or training induced H-P-G suppression is much higher in men than in women (McColl *et al*, 1989).

In both sexes there are numerous other variables which may affect fertility, including body weight, acute weight loss, emotional distress, physical illness and sleep disruption (Prior, 1987). Some of these variables may also be influenced by exercise *per se*. Given these multiple factors, studies attempting to isolate exercise as the causative agent must control for these variables. Prospective, longitudinal studies with each subject acting as her / his own control best achieve this. However, there are no published prospective studies where exercise is the major variable, and where gonadal and pituitary hormones have been sampled. Whilst many features of the H-P-G axis are common to both males and females, additional complex control mechanisms are involved in woman in order to regulate cyclical reproductive phenomena relating to germ cell production, and the

maintenance of an appropriate micro-environment for fertilisation and development of the zygote, should one be formed (Ur, 1992). Disruption of reproductive function in women athletes may not be directly associated with factors in the athletic life-style itself, but, rather with the degree to which the H-P-G axis is disturbed in the individual woman (Loucks *et al.*, 1989).

Research regarding the effects of exercise training on male reproductive function has primarily focused on the serum hormone modifications with little emphasis on determining the effects on spermatogenesis (Acre *et al.*, 1993). A few studies have documented semen parameter changes in runners but the findings have been inconsistent and often contradictory (Bagatell and Bremner, 1990; Ayers *et al.*, 1985; Griffith *et al.*, 1990). The effects of exercise on spermatogenesis have not, therefore, been clearly established.

Hypothalamic-pituitary-gonadal (H-P-G) axis

The hypothalamus regulates the secretion of pituitary hormones through neurohormonal mechanisms involving hypothalamic-releasing and inhibiting factors. Hypothalamic influence on reproduction is most likely reflected in the pulsatile characteristics of LH, secreted simultaneously with gonadotropin-releasing hormone (GnRH) (Prior, 1987). H-P-G function is subject to physiological regulation by metabolic substrates, hormones and other factors. In females the menstrual cycle depends on the pulsatile release of gonadotropins, which, in turn, are controlled by the frequency and amplitude of GnRH from the hypothalamus. Gonadal hormones, severe caloric deficit and stress, both physiological and psychological, are common factors in the disruption of menstrual cycles and alterations in the physiological release of LH and FSH (Rogol *et al.*, 1992).

The endocrine control of testicular function involves a complex, finely regulated interaction between the central nervous system, in particular the hypothalamus, the anterior pituitary gland and the testes. The testis consists of two structurally and

functionally distinct compartment, each of which is responsible for one of the two major physiological functions of the testes. The seminiferous tubules produce and transport spermatozoa, which determines a man's ability to conceive children (ie fertility). The interstitial or Leydig cells produce and secrete sex steroid hormones, primarily testosterone, that mediates the development and maintenance of primary and secondary sexual characteristics and normal sexual behaviour and potency, as well as playing an important role in the initiation and maintenance of spermatogenesis (Matsumoto and Bremner, 1987). To understand the effects of physical activity on the H-P-G axis, a knowledge of the factors regulating GnRH, gonadotropin release and those involved in controlling the gonads is essential.

Gonadotropin releasing hormones (GnRH).

GnRH is a decapeptide which stimulates the release of both pituitary gonadotropins. The relative amounts of follicle stimulating hormone (FSH) and luteinizing hormone (LH) vary. With low dosages of GnRH however, more LH than FSH is released. Only a single GnRH has, to date been identified and synthesised (Schally *et al.,* 1973). Pulsatile GnRH is necessary for normal function at the pituitary level and is as important in men as in women (Naftolin *et al.,* 1972). Hypothalamic GnRH neurons are regulated by numerous stimulatory and inhibitory neurotransmitters (eg catecholamine, serotonin and amino acids) and neuropeptides (eg opioids) systems. Many of the neuromodulatory systems participate in the regulation of GnRH-secreting neurons by higher neural centres (eg the limbic system and cerebral cortex) and by gonadal steroids. Therefore, the hypothalamic-GnRH neuronal system serves an important integrating function in the regulation of gonadal function. It receives input from higher central nervous system centres and from negative feed-back from the gonads to alter GnRH output. Alterations in GnRH secretion regulate pituitary gonadotropin secretion which, in turn, control gonadal function (Matsumoto and Bremner, 1987). Highly trained male athletes have been reported to have deficient GnRH secretion (MacConnie *et al.,* 1986). Barron *et al* reported major hypothalamic, pituitary and exogenously

stimulated hormone dysfunction in long distance male runners who were "over-trained" (Barron *et al.*, 1985). GnRH is also found within the central nervous system, ovary and testis. The function of GnRH at these sites remains to be defined (Bevan and Scanlon, 1992). It does not, however, appear to have a direct effect on testicular steroidogenesis in humans (Rajfer *et al.*, 1987).

Pituitary gonadotropins.

The substances that directly affect gonadal function, including spermatogenesis, are the anterior pituitary gonadotropins, FSH and LH. Both are glycoproteins, and both are composed of two subunits, termed alpha and beta, linked by non-covalent bonds. The alpha subunit of the two hormones are similar, if not identical, (Shome and Parlow, 1974) and are also common to two other glycoproteins; thyroid-stimulating hormone (TSH) and the placental gonadotropin, human chorionic gonadotropin (hCG). The beta subunits, on the other hand, give each hormone its biological and structural specificity (Hafez, 1976).

In males LH binds to specific, high-affinity membrane receptors on Leydig cells of the testis and stimulates testicular steroidogenesis via a cyclical adenosine mono-phosphate (AMP) mediated process. LH stimulation leads to increased secretion of testosterone, the major steroid product of the testis (Matsumoto and Bremner, 1987). Similarly, in females LH bind to specific receptors in the ovary and together with FSH regulates ovarian function. LH acts on the theca interna cells, regulating their androgen production and LH is also responsible for the destruction of the follicular wall which precedes ovulation.

On the other hand, FSH in males binds to specific receptors on the plasma membrane of Sertoli cells and probable spermatogonia of the seminiferous tubule compartment of the testis. FSH receptor binding results in stimulation of adenylate cyclase activity, increase in intracellular cyclical AMP and protein kinase, and production of a variety of proteins

that may be important in regulating spermatogenesis. FSH is necessary for the initiation of spermatogenesis (Braunstein, 1983). In contrast FSH in females acts primarily on granulosa cells, promoting ovarian follicular maturation.

LH and, to a lesser extent, FSH are secreted into the peripheral circulation by the anterior pituitary gland in a pulsatile fashion (Bardin, 1978). The published evidence concerning the effect of exercise on pulsatile LH release is conflicting. In male runners the basal pulsatile LH release was reported to be similar to normal in runners with a training distance of approximately 80 km per week (Rogol *et al,* 1984), and decreased in runners with a much higher (120 - 200 km) weekly training distance. Both of these groups had normal testosterone levels. (MacConnie *et al,* 1986). In the latter study there was no significant effect of acute exercise on pulsatile LH release although the runners had lower LH pulse frequency and amplitude compared to sedentary men (MacConnie *et al.,* 1986). In a more recent study in females there was no effect on the pulsatile characteristics of LH during the early follicular phase of the menstrual cycle by exercise (Weltman *et al.,* 1990). However, in an earlier study in female runners LH concentrations were depressed during exercise followed by a significantly increased LH pulse amplitude and pulse increment during two hours of recovery, with no corresponding change in FSH. Mean LH and FSH levels were reported to be significantly higher during the two hours of recovery than during the exercise (Keizer *et al,* 1984). These observations have direct implications for designing prospective exercise programmes in which measurements of exercise effect on gonadotropin release are determined.

In men the pulse pattern of LH and FSH is variable between and within subjects and in women the LH pulse pattern depends on the stage of the menstrual cycle. In the follicular phase there is a marked progressive increase in LH pulse frequency which is associated with an increased estrogen secretion from the developing ovarian follicles. Positive estrogenic feed-back ultimately induces the pre-ovulatory LH surge at which time there is a considerable increase in the frequency and size of LH pulses. In contrast,

LH cycle frequency is relatively low during the luteal phase of the menstrual cycle (Bevan and Scanlon, 1992).

Circulating FSH levels are regulated by non-steroidal factors with inhibin being the most clearly identified substance. Inhibin is synthesised predominantly in ovarian granulosa cells in the female and Sertoli cells in the male. Inhibin levels increase during the late follicular phase and the hormone acts synergistically with estradiol to inhibit FSH synthesis and release, although this inhibition is over-ridden at the time of the pre-ovulatory gonadotropin surge (Bevan and Scanlon, 1992). It is not known whether inhibin also exerts a negative feedback control at a hypothalamic locus, but testosterone, and estradiol are also capable of inhibiting the secretion of FSH (Sherins *et al.*, 1982). The role of inhibin in the male is poorly defined and the significance of inhibin in relation to disorders of gonadal function, such as infertility, is currently unknown (Ur, 1992).

Full maturation of spermatozoa appears to require not only FSH but also testosterone (Braunstein, 1983). The control of LH in men operates primarily by negative feedback because normal levels of gonadal steroids inhibit secretion. Other mechanisms that have been implicated in altered gonadotropin secretion in women include loss of estrogenic positive feed-back or, conversely, enhanced sensitivity to estrogenic negative feed-back (Rogol *et al.*, 1992). Both testosterone and estradiol can inhibit LH secretion and testosterone can be converted to estradiol in the brain and pituitary (Santen, 1975). The mechanism of testosterone-mediated gonadotropin inhibition at the pituitary level is not known but possibly a reduction of gonadotropin receptor number or uncoupling of receptors from subsequent biochemical events is the cause (Cumming *et al.*, 1989). Endogenous opiates have a role in the negative feedback actions of androgen and estrogen on pulsatile LH secretion in men (Veldhuis *et al.*, 1984).

Gonadal steroid hormones.

The three steroids of primary importance in male reproductive function are testosterone, dihydrotestosterone and estradiol (Braunstein, 1983). The gonadal steroid hormones are secreted by the interstitial cells and in addition to their role in the development of the secondary sexual characteristics, testosterone in particular, is of fundamental importance in the initiation and maintenance of spermatogenesis (Steinberger *et al.*, 1973). Normal levels of testosterone are necessary for sexual differentiation of male internal and external genitalia during embryogenesis, development and maintenance of secondary sexual characteristics at the time of puberty, maintenance of sexual functioning and behaviour, and negative feed-back regulation of gonadotropin secretion in adults (Matsumoto and Bremner, 1987).

The mechanism by which testosterone affects spermatogenesis and the actual quantity of testosterone necessary in the seminiferous tubules to maintain normal sperm production and fertility are not well defined. Over 95% of the testosterone is secreted by the testicular Leydig cells with the remainder derived from the adrenals (Lee, 1978). Testosterone is a potent androgen which in circulation is mainly bound to SHBG (44%) and albumin (54%). Only the unbound steroid (approximately 2%), and probably the albumin-bound fraction, is biologically active. Estrogens raise SHBG levels whereas androgens tend to lower them (Duignan, 1992).

Androgen deficiency in adult males is characterised by reduced libido and potency, infertility, behavioural changes, weakness and fatigue and loss of androgen-dependent hair. During prolonged physical stress combined with lack of food, metabolism is directed at energy mobilisation from fat and proteins. All catabolic hormones, such as glucocorticoids, increase, whereas anabolic hormones, such as testicular androgens, decrease (Opstad, 1992). Several authors have reported that serum testosterone levels decrease after acute prolonged exercise such as marathon running, but return to normal within 24 hours (Morville *et al.*, 1979; Dessypris *et al.*, 1976; Aakvaag *et al.*, 1978).

There is now evidence that endurance training may have chronic effects on pituitary gonadal function in male distance runners in a manner similar to that described in women. It has been shown that the mean levels of total as well as non-sex hormone-binding globulin-bound and free testosterone levels were significantly lower than controls, although levels remained within the physiological range (Wheeler *et al.*, 1984). These authors suggested that the lower testosterone levels in runners must reflect differences in either clearance or production of the hormone because the non-specifically bound portions were reduced with no significant difference in serum binding. In a more recent article, Wheeler again stated that the lower testosterone levels in exercising men suggest that peripheral mechanisms are responsible and that this decreased testosterone was not related to changes in pulsatile LH release (Wheeler *et al.*, 1991). Opstad, in contrast, also noted a decrease in testosterone during prolonged physical stress, but concluded that this decrease in testicular androgens is mainly regulated through the H-P-G axis (Opstad, 1992).

Dihydrotestosterone and estradiol are derived not only by direct secretion from the testes but also by conversion in peripheral tissues of androgen and estrogen precursors secreted by both the testes and adrenals. Approximately 80% of the circulating concentrations of these two steroids is derived from such peripheral conversion (MacDonald *et al.*, 1979). It has been shown in a study that there is a decrease in all androgen hormones, testicular as well as adrenal, after hours and days of continuos physical exercise (Opstad, 1992). Although testosterone is the major product, 17-hydroxyprogesterone, progesterone and pregnenolone are also secreted by the testes (Hammond *et al.*, 1977). Some pregnenolone may be converted to progesterone which is then hydroxylated before being converted to 17-hydroxyprogesterone and then to androstendione and ultimately testosterone. This pathway is, however, not commonly followed in humans (Lee, 1978). Progesterone is also secreted by the adrenal cortex. Little is known concerning the precise mechanism of release of progesterone in men or of its transport in the blood, although it is probably mostly bound to plasma proteins. It

has a short half-life of approximately 5 minutes in the blood, and is converted to pregnanediol mainly in the liver, and subsequently conjugated with glucuronic acid and excreted in the urine chiefly in the form of glucuronide. The effect of progesterone includes a rise in basal metabolic rate and body temperature, and stimulation of respiration. The biological functions of plasma progesterone and 17-hydroxyprogesterone in the male are not known (Griffin *et al.*, 1992). Estrogen formation in the testis is regulated by gonadotropins and circulating androgens can be aromatised to estrogens in the extraglandular tissues (Sitteri *et al.*, 1973).

Prolactin

Prolactin is necessary for normal manufacture of sex steroids within the testis but hyperprolactinaemia may result in the decreased testosterone production and impaired libido as seen in athletes (Wheeler *et al.*, 1984). Raised prolactin levels due to either psychotropic medications or a pituitary prolactinoma can present with gonadal dysfunction due to suppression of GnRH secretion by prolactin (Wang and Swerdloff, 1992). Prolactin plays an important role in control of the reproductive axis in men and women. In men, decreased steroidogenesis can occur with hypoprolactinaemia via decreased Leydig cell sensitivity to LH, or hyperprolactinaemia probably via a central mechanism (Rubin *et. al.*, 1978). Prolactin potentiates the effect of LH on Leydig cells (Davies *et al.*, 1980).

Stress.

The possibility that stress could lead to reproductive dysfunction has been considered for many years. Both physical and psychological stress has been linked to abnormal H-P-G axis function and can therefore affect spermatogenesis (Acre *et al.*, 1993; Ayers *et al.*, 1985). In man there are a number of modulating factors, both individual and related to social circumstances, that have potent effects on the way that social stress is reflected in endocrine activity (Herbert, 1987). In addition life-events may negatively influence reproductive function by increasing the inhibitory neuro-endocrine

modulation of opioids on the H-P-G axis (Genazzani *et al.*, 1991).

Most studies on stress in human subjects have been of rather short duration, up to a few hours, and frequently associated with the administration of drugs, anaesthesia or surgery. The numerous discrepancies reported in the literature on the response to stress may be related not only to differences in the type of stress, but also to interference by additional factors such as drugs, anaesthesia and possibly the duration of the stress (Aakvaag *et al.*, 1978).

The pituitary-prolactin release represents one area of controversy regarding the stress response. Various studies have yielded conflicting results. Herbert *et al* showed that prolonged social stress imposed by a forth-coming examination resulted in prolactin levels in male medical students showing a small, but significant increase (Herbert, 1987). In contrast, the serum level of prolactin was significantly suppressed during a severe physical combat course in army personnel (Aakvaag *et al.*, 1978). In the latter study it is, of course, not possible to separate the effects of physical from those of emotional stress on the prolactin levels. The underlying mechanism for this reduction in prolactin is not known, but it might be postulated that dopamine could be involved. Dopamine is elevated during stress resulting in a reduction in prolactin levels which may in turn influence testosterone levels (Horowski *et al.*, 1976).

In men, an acute decrease in serum testosterone levels in association with a variety of stressful conditions such as surgery or myocardial infarction has been observed (Cartensen *et al.*, 1972; Wang *et al.*, 1978). The rate of testosterone decline seems to be related to the severity of the surgical stress (Aono *et al.*, 1976). A similar decline in testosterone levels without a concomitant decrease in LH was also observed in patients after vigorous exercise (Cumming *et al.*, 1983). In men, over 95% of circulating testosterone is derived from the Leydig cells of the testis and the adrenal-testicular axis may have biological implications on the reproductive adaptation to stress (Lipsett *et al.*, 1966).

In females it is well recognised that both physical and psychological stress can result in amenorrhoea, but the mechanism by which stress alters GnRH secretion remains unknown (Biller *et al.,* 1990). Other factors, such as the opioid and dopaminergic systems, have been implicated as potential mediators of stress-related amenorrhoea in women (Quigley *et al.,* 1980). These have not been well researched in men.

Nutrition and Energy Balance

Epidemiological observations have pointed to a relationship between nutrition, fat stores and the H-P-G axis. Most exercise studies in men and women show some decrease in relative fat weight (Boyden *et al.,* 1983; Bullen *et al.,* 1985). It is unlikely that a given individual has a absolute weight or percent fat which will alter reproduction. However, change in weight or fat weight is important. Runners who loose weight during training had more significant menstrual cycle alterations than those whose weight remained constant, despite having comparable exercise levels (Bullen *et al.,* 1985). There are no studies published which have correlated weight change with changes in semen parameters, a less well-defined end-point.

Athletic activity requires significant increase in caloric intake usually as carbohydrates (Bullen *et al,* 1985). Nutrition has a major influence on neuro-reproductive function as exercise requires fuel and the careful regulation of energy stores. Weight loss and re-feeding are associated with menstrual cycle and hormonal changes in women (Prior, 1987). Weight or fat loss seems to interact in an additive way with exercise to alter reproduction.

Nutrition most likely affects reproduction by modifying the pulsatile release of GnRH at the hypothalamic level (Prior, 1987). When exercise is added to weight loss or negative energy balance there is an increased likelihood, other factors being equal, of reproductive suppression. Clinical data suggests that these changes are reversible with

either a decrease in training and no weight gain, or with weight gain and no change in training (Prior, 1987).

Spermatogenesis and Semen Evaluation

The testis is primarily under the control of the hypothalamic-pituitary axis. The hormonal milieu necessary for the initiation and maintenance of spermatogenesis in man is poorly understood. It is well established that sperm production requires the stimulatory actions of pituitary gonadotropins and the role of androgens in the initiation of the spermatogenic process and its progression through the meiotic prophase was demonstrated by Steinberger (Steinberger *et al.*, 1970). In a study to evaluate the effect of exercise on sperm production, Ayers showed that, despite significantly depressed gonadal steroid levels, chronic endurance training (40-80 miles/week) was not associated with a detrimental effect on sperm production in most men. Morphologic evaluation revealed no significant abnormalities of spermatogenesis, stress pattern, or abnormal forms (Ayers *et al.*, 1985). However, the exact effects of strenuous exercise on male fertility remains to be precisely defined.

In man sperm production is a biological constant which takes approximately 70 days for the process to progress from spermatogonia to spermatid (Heller and Clermont, 1964). The gonadal steroid hormones are known to be involved in both the initiation and maintenance of spermatogenesis. The rate of spermatogenesis cannot be altered by hormonal suppression or stimulation, other noxious agents such as radiation, or a change in temperature. Once a spermatogonium becomes committed to the process of spermatogenesis, it appears that a period of about 70 days in man is mandatory to complete spermatogenesis (Hafez, 1976).

Spermatogenesis in the adult begins with the spermatogonia and proceeds through a number of simple mitotic divisions that culminate in the formation of a primary spermatocyte. The primary spermatocyte then passes through six substages as a result of

meiosis: preleptotene, leptotene, zygotene, pachytene, diplotene and diakinesis. After these six sub-stages are completed, secondary spermatocytes are formed. The secondary spermatocytes undergo a mitotic division resulting in the formation of two spermatids from each spermatocyte. Finally, spermiogenesis results in the formation of mature spermatozoon from the spermatids (McConnell *et al.*, 1984).

Spermatogenesis is under the control of the central nervous system with the various afferent impulses integrated in the hypophysiotropic area of the hypothalamus (Halasz *et al.*, 1962). This area contains nerve cells and fibres rich in biogenic amines, notably norepinephrine and dopamine. These neurotransmitters lead to the stimulation of other specialised cell collections containing peptidergic neurons. The releasing hormones are then secreted from the nerve terminals in close proximity to the capillary loops that form the primary plexus of the hypophyseal portal system. The specialised monoaminergic and peptidergic neurons are grouped together to form "centres", and each of the anterior pituitary hormones is under the control of one such centre. The precise anatomical localisation of the gonadotropin-controlling centres in the human hypothalamus is unknown, but are likely to be situated in the arcuate nucleus and medial basal hypothalamus (Hafez, 1976). The effect of major athletic competition on hormonal levels, libido and sperm production remains largely unexplored. The general perception has been that strenuous exercise is not associated with a significant decrease in fertility, but this is probably a reflection of a lack of available data rather than scientific facts. Baker has suggested that a decrease in fertility may occur in males who participate in strenuous physical activity (Baker *et al.*, 1984.). In addition anecdotal data have also suggested that libido may be impaired in some runners during periods of intense endurance training (Wheeler *et al.*, 1984). The aetiology of this is not clear. Reduced testosterone levels may play a role but chronic fatigue could also be a significant factor (Wheeler *et al.*, 1984.). More recently a study has shown significant changes in semen characteristics associated with endurance training in the form of running, suggesting that running quantitatively and qualitatively modifies the semen parameters (Arce et al., 1993).

Several hormonal factors appear to influence spermatogenesis in laboratory animals and man. Spermatogenesis is under the control of FSH and LH, whose secretion is regulated by gonadal steroids and inhibin (Knuth *et al.,* 1989.). In adult males LH and FSH regulate both Leydig cell and seminiferous tubule function. The sex steroids manufactured by the Leydig cells interact with neuronal elements in the hypothalamus and gonadotropin-secreting cells in the pituitary to affect particularly LH levels. FSH appears to be regulated by non-steroidal factors from the seminiferous tubules with the most clearly identified substance from this source being inhibin which serves to reduce FSH levels (Wall *et al.,* 1985). The significance of inhibin in relation to disorders of gonadal function, such as infertility is currently unknown. The FSH receptor-hormone formation at the Sertoli cell results in the initiation of the spermatogenesis process (Catt and Pierce, 1978). However, in a series of studies by Matsumoto *et al,* they demonstrated that normal serum levels of FSH are not required for qualitatively normal spermatogenesis in man since sperm counts in the range of 20×10^6 to 50×10^6 were typically found in a variety of hormonal milieus, which included severely suppressed FSH levels. To achieve quantitatively normal levels of sperm production, FSH replacement was required (Matsumoto *et al.,* 1986). This implies that the physiological role of FSH in man is the quantitative stimulation of spermatogenesis.

The process of spermatogenesis has an absolute requirement for androgens. Testosterone in circulation is bound to a specific, high affinity, low capacity protein, sex hormone binding globulin (SHBG), and with low affinity to albumin, which has a large capacity. In the Sertoli cells, testosterone plays an essential role in the facilitation of the spermatogenesis process. The pulsatile release of LH results in some fluctuations of testosterone levels in the circulation. There is also a circadian cycle imposed where large nocturnal elevations in testosterone are observed (Bardin, 1978). Plasma testosterone has been shown to be constant between 8 and 10 am (Kalra and Kalra, 1977). Testosterone is essential for primary spermatocyte to complete meiosis and there is general agreement that testosterone is the physiological feedback inhibitor of LH in

man (Sutton *et al.*, 1973).

Several other non-hormonal influences have a direct effect on semen parameters. Chronic high dose alcohol ingestion is directly toxic to the testis and may affect the testis and pituitary indirectly through liver disease (Boyden and Pamenter, 1983). Heavy cigarette smoking is associated with reduction in sperm quality (Handelsman *et al.*, 1984). Opiates are inhibitory to the H-P-G axis and lead to hypogonadism and infertility.

The period of continence preceding collection of the semen specimen has a remarkable influence on spermatozoan concentration but has little effect on motility or morphology (Freund, 1963). Most laboratories do set a period of continence for the patient, but this varies from laboratory to laboratory. A fixed period of continence preceding the collection of a specimen means that the specimen is no longer a random sampling of the patient's spermatozoan output at his usual frequency of ejaculation. This means that the data are no longer appropriately subject to statistical tests based on random sampling and also that the complex relationship between spermatozoan output in the ejaculate and spermatozoan production by the testes, is further confounded by setting a fixed period of continence. It has been shown that individuals respond quite differently to experimental variations in the frequency of intercourse and duration of continence; some men show very large changes in spermatozoan output, and some quite modest changes (Freund, 1963).

Transitory elevation of testicular temperature have been shown to depress spermatogenesis. In a study by Lazarus and Zorgniotti the critical point at which testicular thermoregulation was disrupted occurred when the oral body temperature reached 37.7^{o}C (Lazarus and Zorgniotti, 1975). In another study by Macleod an elevation of body temperature in man in conjunction with a simultaneous elevation of the environmental temperature resulted in a decrease in the sperm count and changes in the morphology and motility of the sperm. The effect of hyperpyrexia upon

spermatogenesis did not become apparent for nearly 3 weeks and may have lasted for a period of 50 days thereafter (Macleod and Hotchkiss, 1941). In contrast to this McConnell concluded that temperature changes associated with endurance exercise did not affect sperm production rates in humans. However, the results of McConnell and co-workers must be interpreted with caution as the duration of exposure to high temperatures during exercise may not have been long enough to alter spermatogenesis. In this study peak rectal temperature was not obtained until the last 10 minutes of the 45 minute exercise period (McConnell and Sinning, 1984). It should be noted that the scrotum possesses a very efficient capacity of thermoregulation, and that the temperature in the testis is markedly lower than the body temperature. In an article by Politoff a seasonal rhythmicity of spermatogenesis was noted and they postulated this might be due to external, seasonally induced temperature fluctuations, which could also be partially responsible for fluctuations in testicular temperature. Peak spermatogenesis was noted in autumn and winter but peak levels of testosterone and LH were also noted at these times. Prolactin, on the other hand peaked in spring (Politoff *et al.*, 1989). A study of out door workers during a hot summer showed a reduction in sperm counts and motility when compared with winter months (Levine *et al.*, 1990). Such environmental factors are being identified in increasing numbers by careful epidemiological studies with objective measurement of semen parameters.

The role and importance of semen analysis has grown over the past two decades as gynaecologists who treat infertile couples have recognised that evaluation of semen is a logical early step in the overall infertility work-up. Predominantly male factor abnormalities account for at least one third of the infertile couples and male infertility is the most common clinical presentation of testicular dysfunction (Wang and Swerdloff, 1992). The traditional semen parameters monitored in most laboratories including semen count, motility and morphological features cannot clearly distinguish between infertile and fertile men. Despite this, multivariate discriminant analyses using conventional semen parameters showed that sperm concentration and morphology are variables providing the most consistent and valuable information discriminating fertile

and infertile men (Wang and Swerdloff, 1992). Sperm morphology was found to have the least variability within a patient (Sherins *et al.*, 1977), and was the most useful in predicting the success of *in vitro* fertilisation (Kruger *et al.*, 1988). The sperm count plays a crucial role in the definition of infertility. However, other criteria such as motility and rate of forward progression are also integral parts of the semen analysis. Semen analysis at Groote Schuur Hospital is based primarily on rating of the number, motility and morphology of the spermatozoa in the specimen. Biochemical analysis of semen in human infertility have attempted to relate the concentration of single compounds to accessory organ function (eg. fructose levels to seminal vesicle function and zinc levels to prostrate function) (Freund, 1963). Seminal plasma, which makes up more than 95% by volume of ejaculated semen, is an extracellular fluid that is a composite mixture of secretions originating from the accessory organs of reproduction. The secretions of the various glands are specific in chemical composition, and it has been proposed that this specificity be used as an indicator of the functional status of the accessory glands. The measurement of single substances like fructose or zinc in semen has not yielded a reliable clinical indicator of the cause of infertility (Hafez, 1976). In this study no biochemical analysis were performed on the semen samples.

Several additional characteristics of semen are also noted and recorded in different laboratories. These include semen liquefaction, viscosity, colour, spermatozoan agglutination and the presence of extraneous cells. While there have been attempts to link abnormalities in these characteristics with infertility, the cause-and-effect relationship between any of the characteristics and infertility has yet to be proved (Hafez, 1976). These parameters were not, therefore, included in our study. However, gynaecologists treating the infertile male depend to a large extent on the findings of semen analysis in determining the course and the efficacy of treatment.

AIMS OF THE STUDY

This study was undertaken to determine the serum hormonal and semen changes during intense training for marathons. A group of male runners was studied over a year, between December 1989 and November 1990, at 1-3 monthly intervals. Weight, hormone and semen parameters were measured at each visit.

The aims of the study were:

1. To determine the range and means for specific serum hormones and semen parameters longitudinally in a group of runners training for a marathon.

2. To assess whether significant changes occur in weight, BMI, hormone and semen parameters during training for a marathon.

3. To evaluate if changes occur in serum hormone levels and semen parameters during months of low training compared with months of high training.

SUBJECTS AND METHODS

Study population

Twenty four male long-distance runners volunteered to participate in the study. Informed consent was obtained from each subject. The study was approved by the Ethics and Research Committee of the University of Cape Town Medical School.

The mean age of the runners was 38.2 years (range 25-54 years). All participants intended to run the Two Oceans Marathon (56 km) five months after the start of the study and ten the Comrades Marathon (90 km) six weeks later. During the 12 months of the study, the athletes' training consisted of individualised programs which became progressively more intense in preparation for the Two Oceans marathon and then tailed off gradually towards the end of the year. Each runner was responsible for estimating and recording the distance trained in the preceding week.

Study Time and Parameters

The study was started in December 1989 and was completed in November 1990. A baseline assessment of the runners was done in December 1989 which included weight and height. They were then reassessed in January, February, April, May, August and November of 1990.

At each visit the runner was questioned on his general health. In addition the following was recorded:

1. The distance run weekly since the previous visit.
2. The runner's weight.

The following samples were obtained:

1. Ten millilitres of clotted blood
2. A semen sample.

Calculation of the body mass index (BMI).

The BMI of all of the runners was calculated for each visit as follows: BMI = weight (kg)/height (m^2).

Blood sample collection.

Non-fasting blood samples were obtained from the subjects between 7.00 am and 9.00 am. The blood samples were allowed to clot at room temperature and the serum was separated by centrifugation and stored at -20°C until assayed. All the samples from each participant were analysed in the same assay thereby avoiding inter-assay variation.

Determination of FSH, LH, progesterone, testosterone, prolactin and E2 levels.

LH and FSH was measured by immunoradiometric assay (IRMA) using the Serono "Maiaclone" kits. Total testosterone (T.Testo) and progesterone (Prog) were measured with the antibody coated tube radioimmunoassay (RIA) kits from the Diagnostic Products Corporation (DPC). Prolactin (PRL) was measured with the double antibody RIA kit from Pharmacia Diagnostics and E2 with the IRMA coated tube assay from Medgenix.

The limit of detection of the standard curve and the biological sensitivity of the assay for the serum samples are given in Table 1.

The measurements of the samples in this study were performed in duplicate in a single assay for each hormone and the coefficient of variation (CV) of the duplicates in the assays was 3.5% for FSH; 4.1% for LH; 3% for T.Testo; 5.1% for Prog; 3% for PRL and 3.6% for E2. The intra-assay coefficient of variation (% CV) for each assay at low, medium and high levels are given in Table 1.

Standard normal values for serum hormone and gonadotropin levels.

Prolactin	< 15 ng/ml
Progesterone	< 2 nMol/L
LH	1.5 - 9.2 miu/ml
FSH	1 - 14 miu/ml
Testosterone	9.2 - 37 nmol/ml
E2	100 - 500 pMol/L

Table 1
Details of assays

			INTRA-ASSAY C.V.			SENSITIVITY		
Assay	**Type**	**Kit**	**Low**	**Medium**	**High**	**Study**	**STD Curve**	**Samples**
LH (mIU/ml)	IRMA	Serono Maiaclone	at <5 3.7%	at <10 2.7%	at >10 2.8%	4.1%	0.2	0.7
FSH (mIU/ml)	IRMA	Serono Maiaclone	at <5 5.7%	at <10 4.8%	at >10 3.9%	3.6%	1.4	1.8
Prog (nmol/l)	RIA	Diagnostic Prod Corp	at <5 7.8%	at <10 6.1%	at >10 8.3%	5.1%	0.2	1.2
Prolactin (ng/ml)	RIA	Pharmacia Diagnotics	at 9.0 4.4%	at 22 2.3%	at 43 2.8%	3.0%	1.5	5.0
E2 (pmol/l)	IRMA	Medgenix	at 450 4.5%	at 1000 5.9%	at >1000 6.8%	3.6%	20	43
Testos (nmol/l)	RIA	Diagnostic Prod Corp	at 10.6 4.5%		at 27 8.5%	3.0%	0.7	1.7

This illustrates the laboratory details and quality assurance of the techniques used to assay the hormonal profiles in the runners.

Semen Sample collection

Semen analysis was performed on a fresh masturbation sample for physical characteristics including volume, count, motility, morphology and antibodies. A specimen bottle was supplied to each patient to be used at the next collection which avoided the problem of receiving specimens in a variety of used and possibly contaminated containers. The use of these plastic containers had been preceded by careful chemical and biological studies of their effects on spermatozoan motility and survival. No fixed period of continence was specified. Masturbation was the routine method of collection of a specimen. This was either performed at the laboratory or at home and brought directly to the laboratory for analysis. Coitus interruptus was discouraged as this results in the loss of the first (sperm-rich) fraction of the ejaculate,

and the use of a rubber condom may result in a specimen that does not yield a useful motility rating. All samples were examined within six hours from the time of ejaculation.

Semen Examination

Semen Volume

Volume was measured to the nearest tenth of a millilitre in a 10 millilitre graduated test-tube.

Motility and rate of forward progression.

The raw semen was mixed by placing the semen specimen into a shaker for several minutes. A drop of well-mixed semen was placed on a clean glass slide, covered with a cover-slip and left for a few minutes. Five to ten microscope fields for each slide were examined under a magnification of both x10 and x40 objectives for percentage of motile sperm and speed of forward progression. Percent motility was rated from 100% (all spermatozoa motile) to 0% (no spermatozoa motile) and is estimated to the nearest 5% (Mcleod, 1973).

The rate of forward progression was estimated using the arbitrary criteria of Hotchkiss. This estimates the speed of progression and is scored on a scale of 0 to 4 (with a plus or minus sign after the number). Four was designated as the best quality of progression whilst no significant progression observed in a sample was scored as 0. The level 2+ was considered the line of demarcation between acceptable and poor motility (Hotchkiss, 1941).

Spermatozoan Concentration.

Depending on the estimated sperm concentration, a dilution 1/10, 1/20 or 1/100 of the well-mixed sperm was made accordingly and counted using an improved Neubauer hemocytometer.

Spermatozoan Morphology.

Percent normal morphology was rated from the examination of 200 spermatozoa in stained smears. A thin, well spread smear was made in a manner similar to that used in making blood smears. The smears were air-dried and stained according to the method described by Papanicolaou. (Papanicolaou, 1942). There is considerable variation among laboratories in the rating of spermatozoan morphology. There is little agreement on what the words "normal" and "abnormal" mean when they are applied to spermatozoan morphology. The greatest single difficulty in the rating of spermatozoan morphology lies in the disagreement among investigators as to what constitutes a "normal" or "standard" spermatozoan. This disagreement comes from the fact that "normal" human spermatozoa vary considerably from the ideal composite drawings in textbooks and are not uniform in shape or size. Many different concepts of the appearance of a normal human spermatozoon are evident from the literature, and the usual description includes a sketch of the "ideal type" of normal spermatozoon which usually resembles a tennis racket. This model is often accompanied by several drawings of "typical" abnormal cells, which are labelled with the investigator's own set of descriptive terms. In this study, morphology was assessed according to the Tygerberg system (Kruger *et al.*, 1986) which uses strict criteria where "borderline" forms are considered abnormal. Two hundred spermatozoa were assessed and classified into groups. In this laboratory, a spermatozoa is considered normal when the head has a smooth, oval configuration with a well-defined acrosome comprising about 40% to 70% of the sperm head. Also, there must be no neck, midpiece, or tail defects and no cytoplasmic droplets of more than one-half the size of the sperm head.

Mixed antibody reaction (MAR)

The IgG MAR test was performed on all samples by mixing semen with sensitised red-blood cells. To this mixture IgG antiserum is added. The formation of mixed agglutinates between particles and motile spermatozoa indicates the presence of IgG antibodies on the spermatozoa. The reaction is regarded as positive when more than 10% of motile spermatozoa are adherent to the particles.

Standard International normal values for semen parameters

Volume	2.0-6.0 ml.
Motility	> 40%
Count	> 20 million/ml
Morphology	> 40%
Mar test	0-< 10% (negative).

Figures and Tables

The figures were produced using Harvard graphics 3.0 and the tables using Microsoft word for Windows.

Statistical Methods

Statistical analysis were performed on an Apple Machintosh SE computer using the statistics packages Multistat version 1.01 (distributed by Biosoft, 22 Hills road, Cambridge) and Systat version 3.2 (distributed by Systat Inc., 2902 Central street, Evanston. Il60201 ;U.S.A.). A normal distribution of values was assumed, and analyses were performed using paired/unpaired Student "t" tests and Spearman correlation coefficients. A "p" value of <0.05 was considered significant.

RESULTS

Individual Results

The individual data for each of the runners are illustrated in tables 17 - 40. This includes information on the weight, height, body mass index, age, hormonal profile, semen analysis and the distances run per week for each study month during the study period. Each of the runners is represented by his initials for reasons of confidentiality. The key to the tables is given in appendix 1.

Clinical Characteristics

The clinical characteristics of the study group are listed in Table 2. Thirteen of the runners had previously fathered children and one subject had been investigated for infertility and subsequently found to have poor semen parameters. (Subject A.B -Table 21). The ages of the study group ranged from 25 years to 54 years with a mean of 38.2 years. Their heights varied from 163 centimetres to 190 centimetres with a mean height of 176 centimetres.

Table 2
Clinical characteristics of the study group

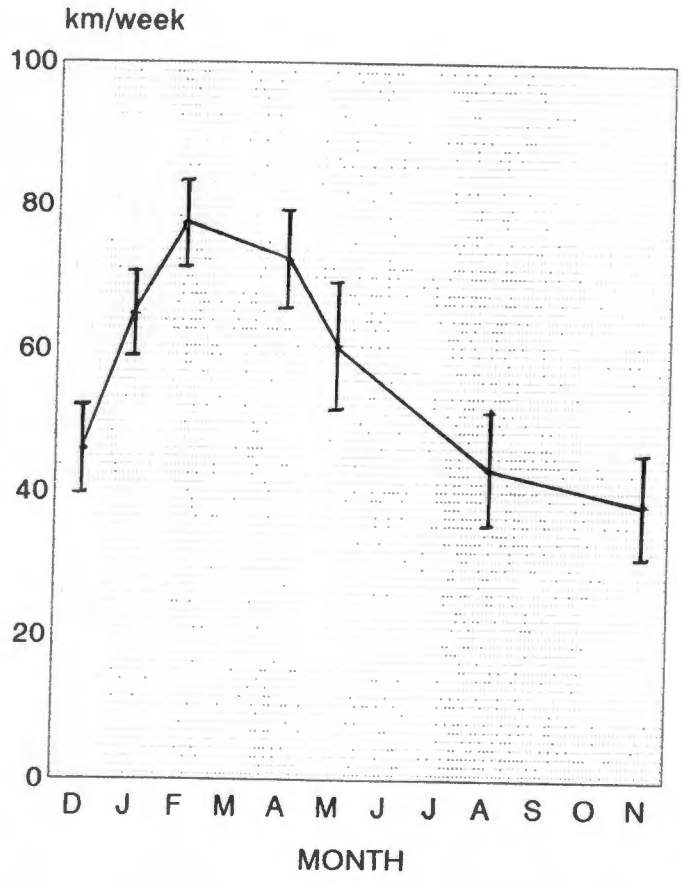
	Minimum	Maximum	Mean (SD)
Age (years)	25	54	38.2 (8.7)
Height (cm)	163	190	176 (6.0)
Weight (kg)	54.8	92.9	74.6 (10)
BMI (kg/m^2)	20.2	30.7	23.7 (2.6)
Low Training Distance / week (km)	0	55	28.7 (18.5)
High Training Distance / week (km)	60	160	84.9 (20.8)

The runners' characteristics including age, height, weight and body mass index. The mean values are illustrated with the standard deviation (SD) indicated in brackets together with the high and low training time distances run.

Training Time

There was a significant increase in training distance between December and January (T=4.347 p<0.001), December and February (T=5.321 p<0.001) and December and April (T=3.874 p=0.001) and also between January and February (T=2.944 p=0.006) (Table 3). In table 3 the T statistic and p values are included for the comparison of training times between the months indicated. Significant differences are shown with the asterix (*). This increase in training distance was in preparation for the marathon which was run in April. (Figure 1). There was no significant difference in training distance when comparing December with April to November. A significant decrease in training was noted after the marathon in April.

Figure 1
Distance run during training



This illustrates the distance run by the study group for each of the study months (mean +/- SEM). Distance run in km/week is shown on the y axis and months are illustrated on the x axis.

Table 3

Comparison of the distance run during each of the study months

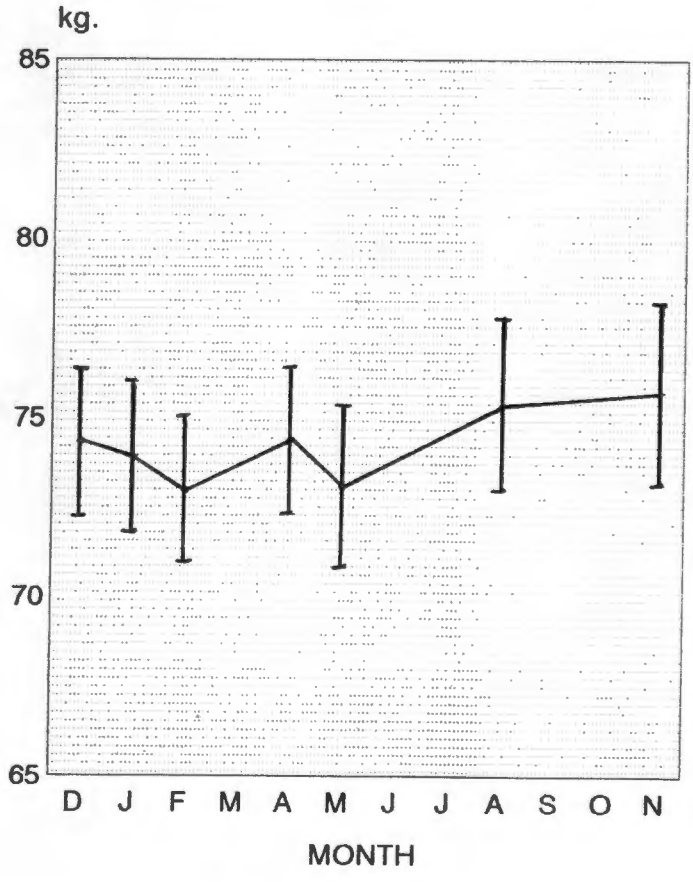
	January	February	April	May	August	November
December (baseline)	T = 4.347 p=<0.001*	T = 5.321 p =<0.001*	T = 3.874 p =<0.001*	T = 1.343 p = 0.195	T = 0.031 p = 0.976	T = 0.830 p = 0.417
January		T = 2.944 p = 0.006*	T = 1.643 p = 0.116	T = 0.909 p = 0.375	T = 3.112 p = 0.006*	T = 3.997 p = 0.001*
February			T = 0.309 p = 0.761	T = 1.701 p = 0.106	T = 4.838 p=<0.001*	T = 8.381 p =<0.001*
April				T = 2.601 p = 0.019*	T = 4.156 p = 0.001*	T = 5.015 p =<0.001*
May					T = 1.174 p = 0.256	T = 1.357 p = 0.194
August						T = 0.559 p = 0.583

The month of December (used as a baseline) is compared with each of the other months studied. The individual months are also compared with each other. The T statistic and p value for each comparison is illustrated. (* = significant)

Anthropomorphic indices

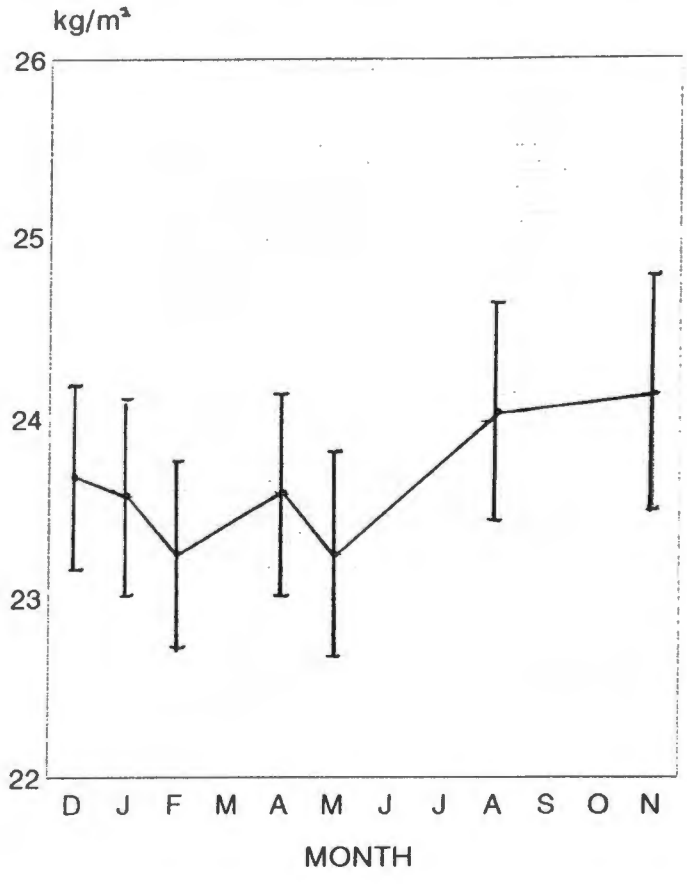
Over the year long study there was a significant change in the runners' weight (Table 4) and body mass index (Table 5). In table 4 the T statistic and p values are included for the comparison of weight between the months indicated. Significant differences are shown with the asterix (*). The same is shown for BMI in table 5. Initially there was a significant decrease in weight between December and January ($T=4.347$ $p<0.001$), December and February ($T=5.321$ $p<0.001$) and December and April ($T=3.874$ $p=0.001$) although the change in the BMI over the same period was not significant (Figure 2 and 3). Thereafter there was a significant increase in both the weight and BMI as the training time decreased from April to the end of the study.

Figure 2
Weight during training



This illustrates the weight in kilograms of the study group for each of the study months (mean +/- SEM). Weight is shown on the y axis and months are illustrated on the x axis.

Figure 3
Body mass index during the study period



This illustrates the body mass index in kg / m^2 of the study group for each of the study months (mean +/- SEM). Body mass index is shown on the y axis and months are illustrated on the x axis.

Table 4
Comparison of the runners' weight during the study period

	January	February	April	May	August	November
December (baseline)	T = 4.437 p=<0.001*	T = 5.321 p=< 0.001*	T = 3.874 p = 0.001*	T = 0.586 p = 0.565	T = 3.048 p = 0.007*	T = 3.811 p = 0.001*
January		T = 1.026 p = 0.317	T = 0.034 p = 0.973	T = 0.023 p = 0.982	T = 3.868 p = 0.001*	T = 4.378 p=< 0.001*
February			T = 1.063 p = 0.301	T = 0.958 p = 0.351	T = 4.908 p=<0.001*	T = 4.805 p=< 0.001*
April				T = 0.393 p = 0.699	T = 4771 p=<0.001	T = 6.026 p=< 0.001*
May					T = 4.304 p = 0.001*	T = 3.923 p = 0.001*
August						T = 1.551 p = 0.138

The month of December (used as a baseline) is compared to each of the other months studied. The individual months are also compared with each other. The T statistic and p value for each comparison is illustrated (* = significant)

Table 5

Comparison of the runners' body mass index during the study period

	January	February	April	May	August	November
December (baseline)	T = 0.923 p = 0.365	T = 2.007 p = 0.058	T = 0.768 p = 0.451	T = 0.739 p = 0.469	T = 3.080 p = 0.006*	T = 3.799 p = 0.001*
January		T = 1.415 p = 0.172	T = 0.037 p = 0.971	T = 0.308 p = 0.762	T = 3.917 p = 0.001*	T = 4.448 p = 0.001*
February			T = 1.666 p = 0.112	T = 1.109 p = 0.282	T = 5.351 p=<0.001*	T = 5.294 p=< 0.001*
April				T = 0.048 p = 0.962	T = 4.554 p=<0.001*	T = 6.022 p=< 0.001*
May					T = 4.965 p=<0.001*	T = 4.392 p=< 0.001*
August						T = 1.238 p = 0.232

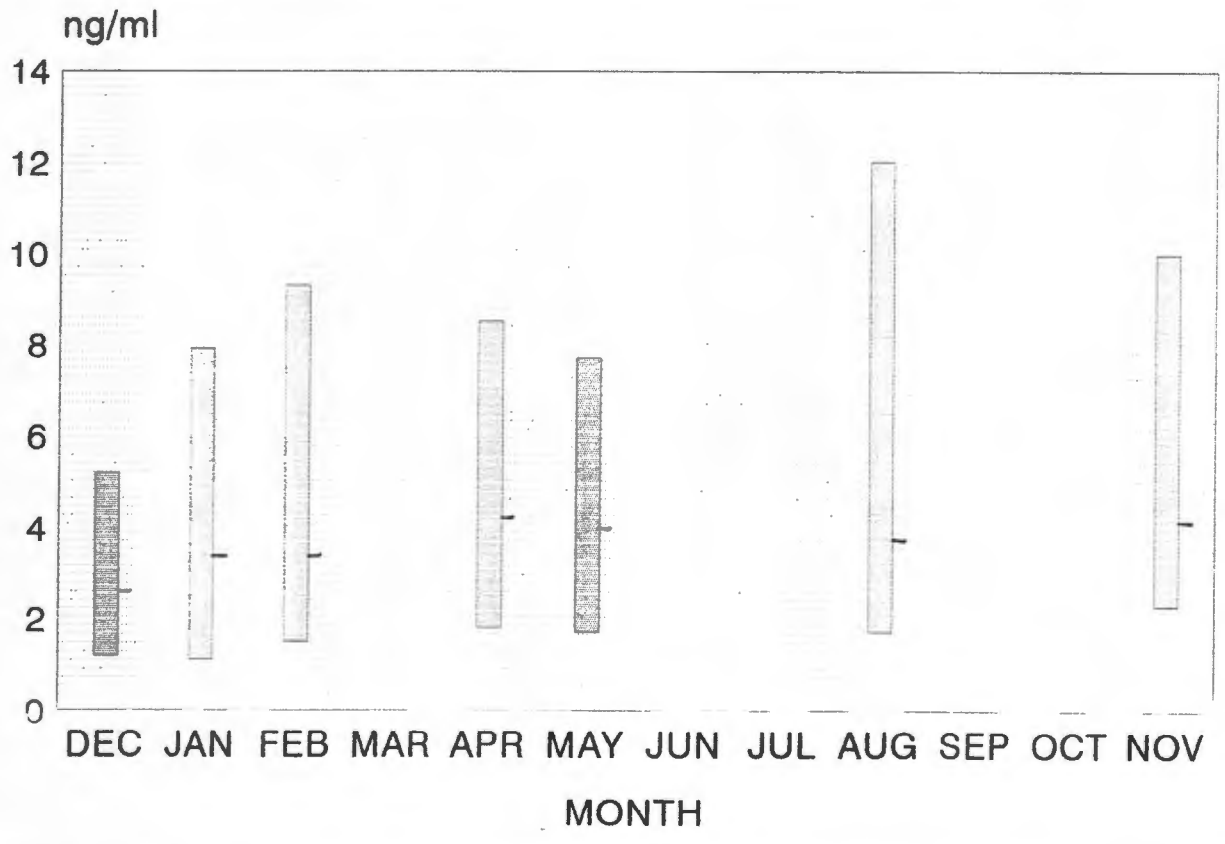
The month of December (used as a baseline) is compared to each of the other months studied. The individual months are also compared with each other. The T statistic and p value for each comparison is illustrated (* = significant)

Hormones

Prolactin

There was a significant increase in prolactin levels when comparing December with all the other months studied (Table 6). In table 6 the T statistic and p values are included for the comparison of mean prolactin levels between the months indicated. Significant differences are shown with the asterix (*). Prolactin levels increased from December to January but then there was no further significant change over the rest of the study period (Figures 4 and 5). Prolactin minimum and maximum levels remained well within the normal range for males with the mean value of each study month consistently in the lower quarter of the normal range.

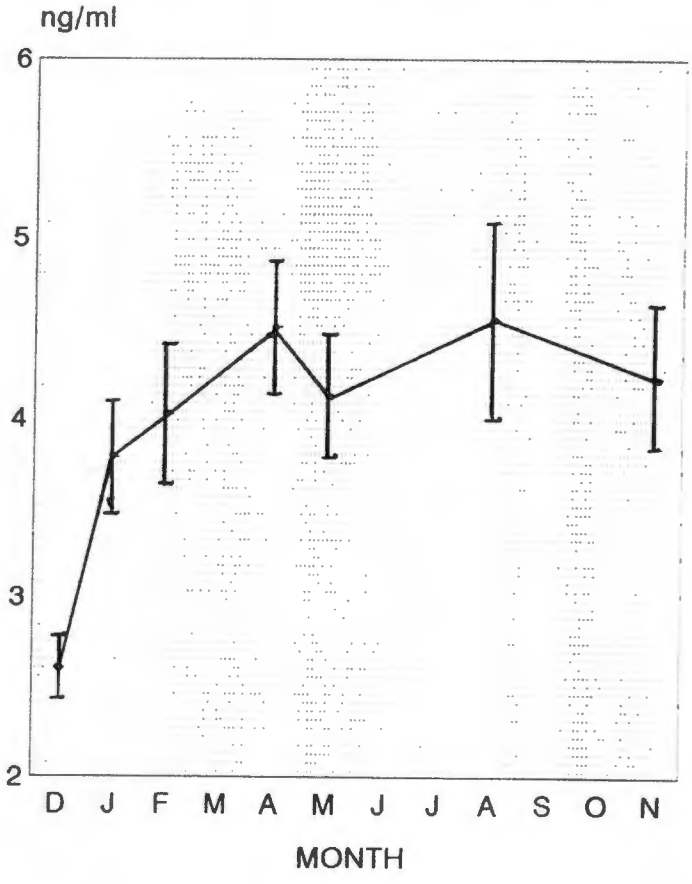
Figure 4
Maximum and minimum prolactin levels during the study period



This illustrates the maximum and minimum prolactin levels (in ng/ml) of the runners for each of the months studied. The median value is indicated with the mark -. The normal range for prolactin in males is < 15 ng/ml. Prolactin levels are shown on the y axis and the months are illustrated on the x axis.

Figure 5

Mean prolactin levels during the study period



This illustrates the mean prolactin level of the study group for each of the study months (mean + / - SEM). Prolactin levels in ng / ml are shown on the y axis and months are illustrated on the x axis.

Table 6

Comparison of prolactin levels of the runners during the study period.

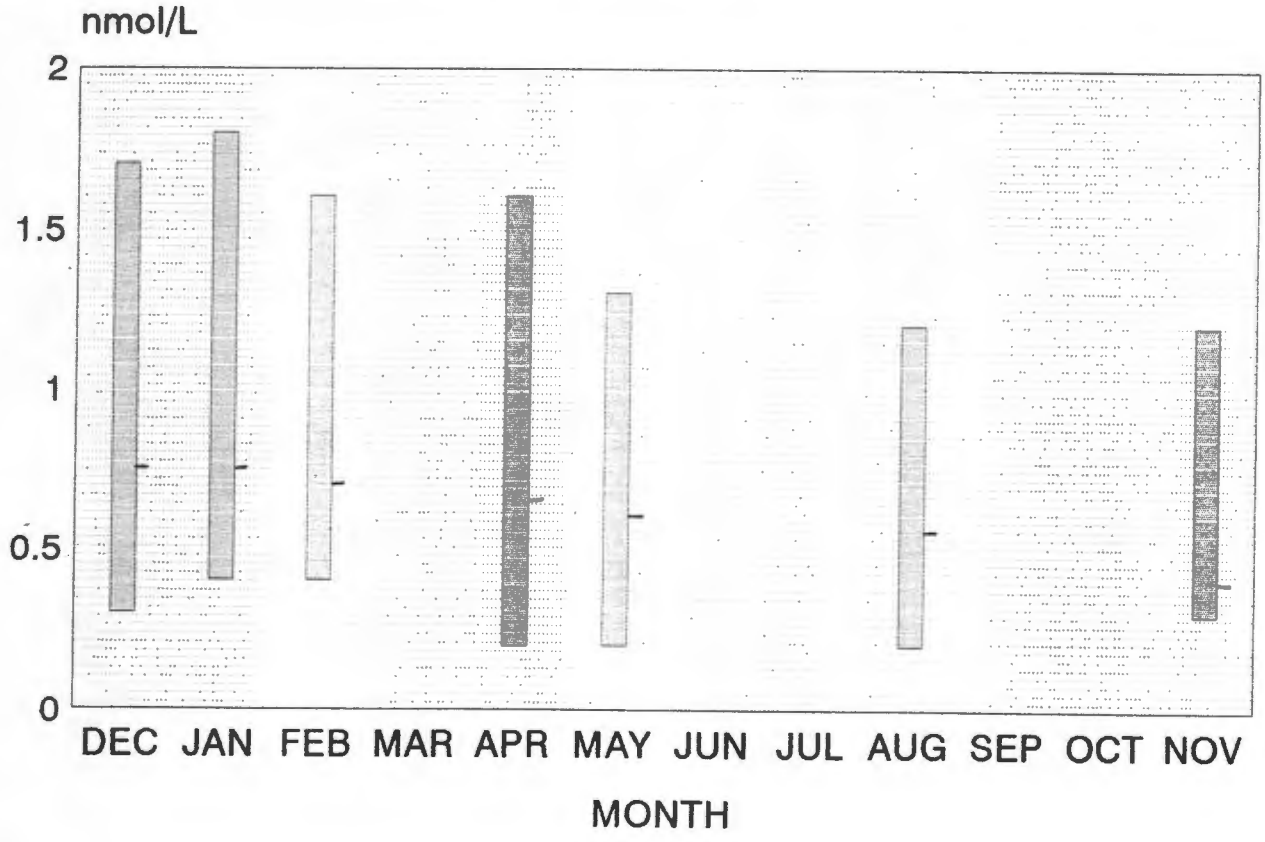
	January	February	April	May	August	November
December (baseline)	T = 3.448 p = 0.002*	T = 3.493 p = 0.002*	T = 5.150 p = < 0.001*	T = 5.017 p = < 0.001*	T = 3.859 p = 0.001*	T = 4.418 p = < 0.001*
January		T = 0.584 p = 0.566	T = 1.770 p = 0.091	T = 1.966 p = 0.063	T = 1.205 p = 0.243	T = 0.922 p = 0.368
February			T = 1.127 p = 0.274	T = 1.853 p = 0.080	T = 0.776 p = 0.447	T = 0.256 p = 0.801
April				T = 0.674 p = 0.509	T = 0.250 p = 0.806	T = 0.400 p = 0.694
May					T = 0.238 p = 0.815	T = 0.144 p = 0.888
August						T = 0.712 p = 0.486

The month of December (used as a baseline) is compared with each of the other months studied. The individual months are also compared with each other. The T statistic and p value for each comparison is shown. (* = significant)

Progesterone

When compared with the baseline concentration (December) progesterone levels fell significantly in May, August and November (Table 7). In table 7 the T statistic and p values are included for the comparison of mean progesterone levels between the months indicated. Significant differences are shown with the asterix (*). Progesterone minimum and maximum levels for each study month remained well within the normal range for males with the mean value of each study month consistently in the lower half of the normal range (Figures 6 and 7).

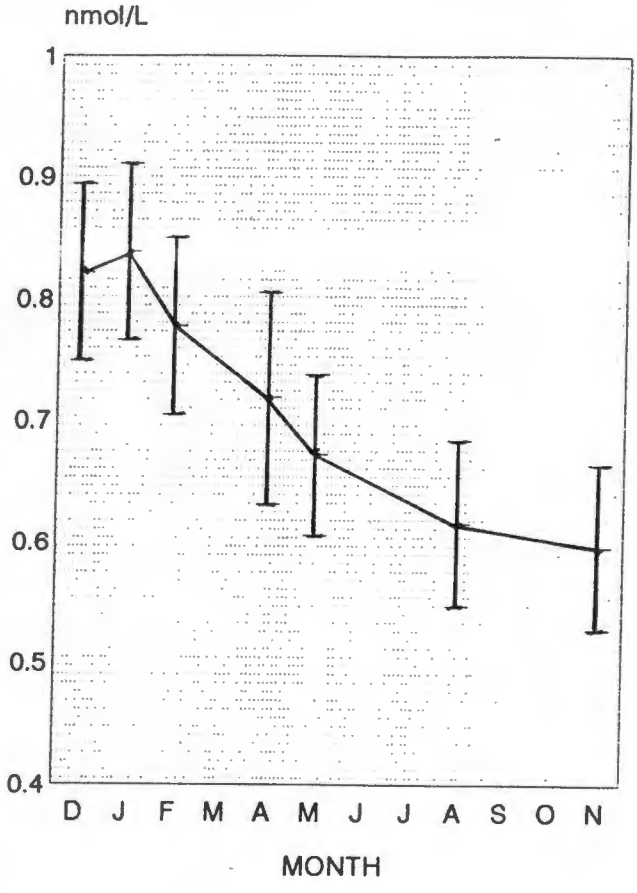
Figure 6
Maximum and minimum progesterone levels during the study period



This illustrates the maximum and minimum progesterone levels (in nmol/l) of the runners for each of the months studied. The median value is indicated with the mark -. The normal range for prolactin in males is < 2 nmol/l. Progesterone levels are shown on the y axis and the months are illustrated on the x axis.

Figure 7

Mean progesterone levels during the study period



This illustrates the mean progesterone level of the study group for each of the study months (mean + / - SEM). Progesterone levels in nmol/l are shown on the y axis and months are illustrated on the x axis.

Table 7

Comparison of progesterone levels of the runners during the study period.

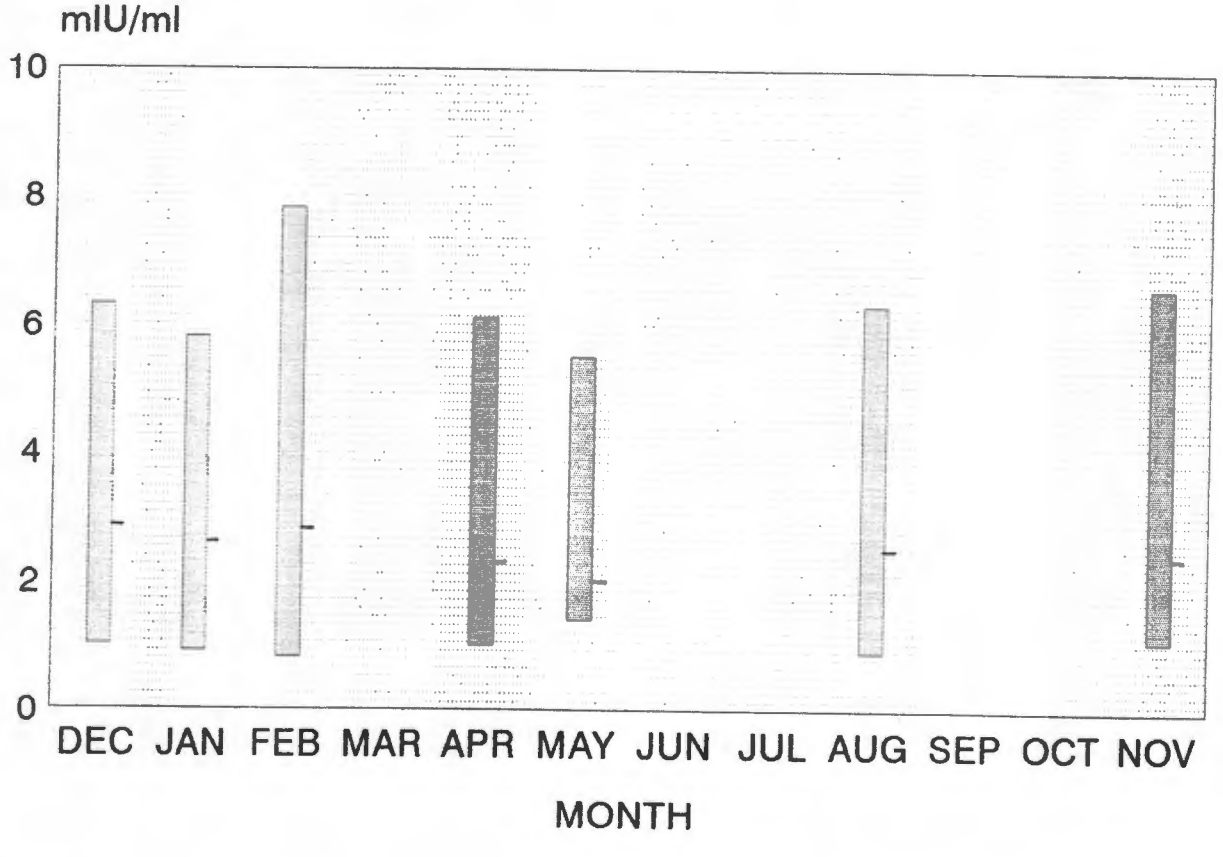
	January	February	April	May	August	November
December (baseline)	T = 0.235 p = 0.816	T = 1.275 p = 0.216	T = 1.433 p = 0.166	T = 2.226 p = 0.038*	T = 3.335 p = 0.003*	T - 2.910 p = 0.009*
January		T = 0.928 p = 0.364	T = 1.469 p = 0.157	T = 2.649 p = 0.015*	T = 3.228 p = 0.004*	T = 3.705 p = 0.002*
February			T = 1.174 p = 0.255	T = 1.673 p = 0.112	T = 2.873 p = 0.010*	T = 2.804 p = 0.012*
April				T = 1.794 p = 0.090	T = 1.492 p = 0.154	T = 1.913 p = 0.073
May					T = 2.129 p = 0.049*	T = 1.057 p = 0.306
August						T = 0.634 p = 0.534

The month of December (used as a baseline) is compared with each of the other months studied. The individual months are also compared with each other. The T statistic and p value for each comparison is shown. (* = significant)

LH

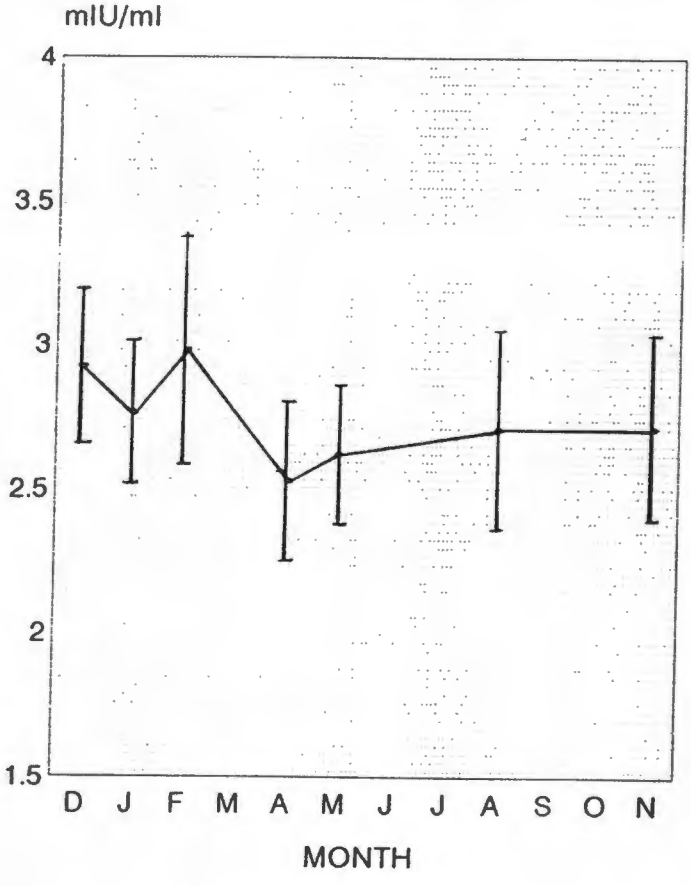
There were no significant changes in LH levels during the period under study (Table 8). In table 8 the T statistic and p values are included for the comparison of mean LH levels between the months indicated. LH minimum and maximum and mean levels for each study month remained within the normal range for males (Figures 8 and 9).

Figure 8
Maximum and minimum LH levels during the study period



This illustates the maximum and minimum LH levels in mIU/ml of the runners for each of the months studied. The median value is indicated with the mark -. The normal range for LH in males is 1.5 - 9.2 mIU/ml. LH levels are shown on the y axis and the months are illustrated on the x axis.

Figure 9
Mean LH levels during the study period



This illustrates the mean LH levels of the study group for each of the study months (mean + / = SEM). LH levels in mIU/ml are shown on the y axis and months are illustrated on the x axis.

Table 8
Comparison of the LH levels of the runners during the study period.

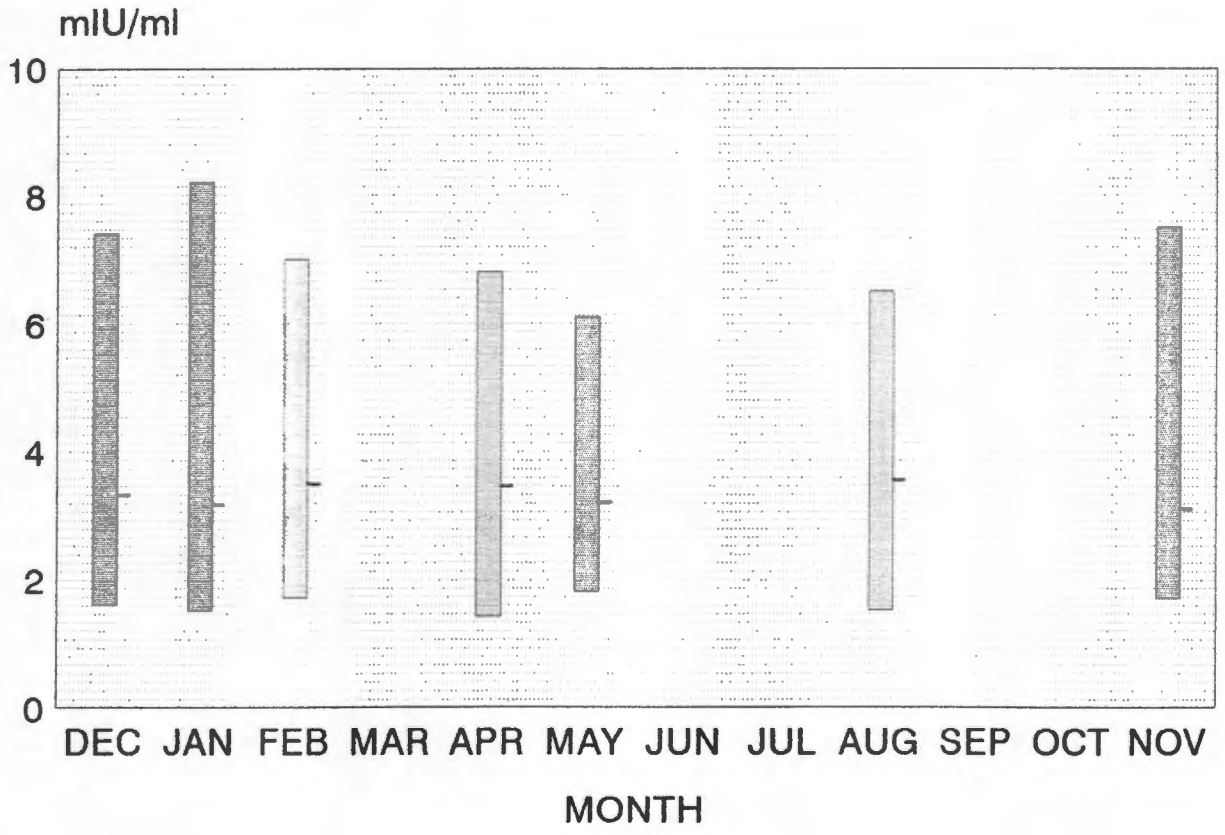
	January	February	April	May	August	November
December (baseline)	T = 0.735 p = 0.470	T = 0.237 p = 0.815	T = 1.461 p = 0.159	T = 0.479 p = 0.637	T = 1.634 p = 0.119	T = 1.168 p = 0.257
January		T = 0.576 p = 0.571	T = 1.166 p = 0.257	T = 0.173 p = 0.865	T = 0.840 p = 0.411	T = 0.150 p = 0.882
February			T = 0.911 p = 0.374	T = 0.524 p = 0.606	T = 1.387 p = 0.182	T = 0.990 p = 0.335
April				T = 1.404 p = 0.177	T = 0.353 p = 0.729	T = 0.055 p = 0.957
May					T = 0.986 p = 0.339	T = 0.223 p = 0.826
August						T = 0.737 p = 0.470

The month of December (used as a baseline) is compared with each of the other months studied. The individual months are also compared with each other. The T statistic and p value for each comparison is shown. No significant differences were noted.

FSH

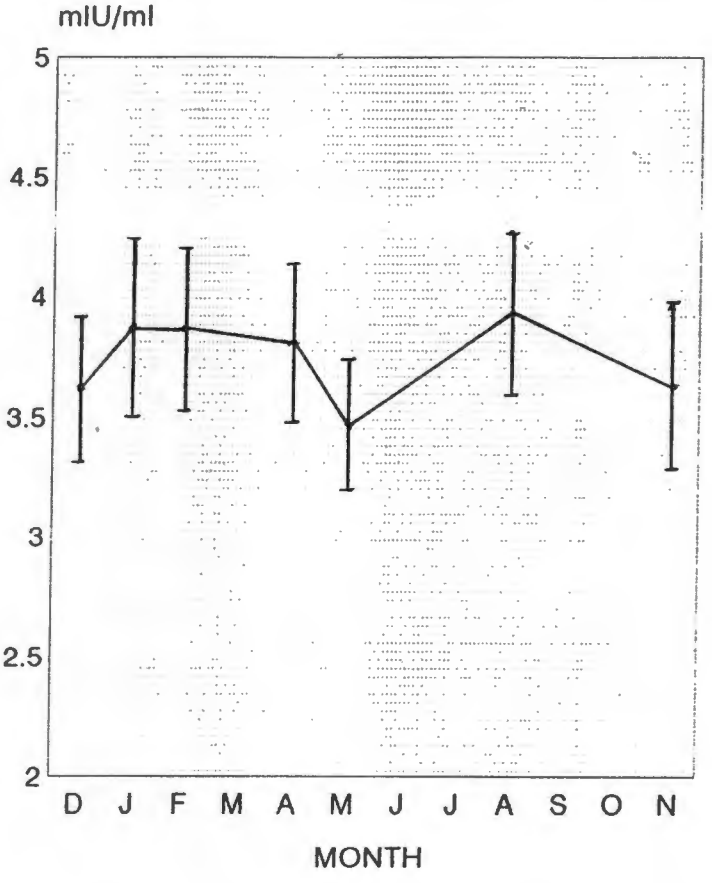
There were no significant changes in FSH levels during the period under study (Table 9). In table 9 the T statistic and p values are included for the comparison of mean FSH levels between the months indicated. FSH minimum and maximum and mean levels for each study month remained well within the normal range for males (Figures 10 and 11).

Figure 10
Maximum and minimum FSH levels during the study period



This illustates the maximum FSH levels (in mIU/ml) of the runners for each of the months studied. The median value is indicated with the mark -. The normal range for FSH in males is 1 - 14 mIU/ml. FSH levels are shown on the y axis and the months are illustrated on the x axis.

Figure 11
Mean FSH levels during the study period



This illustrates the mean FSH levels of the study group for each of the study months (mean + / SEM). FSH levels in mIU/ml are shown on the y axis and months are illustrated on the x axis.

Table 9
Comparison of the FSH levels of the runners during the study period.

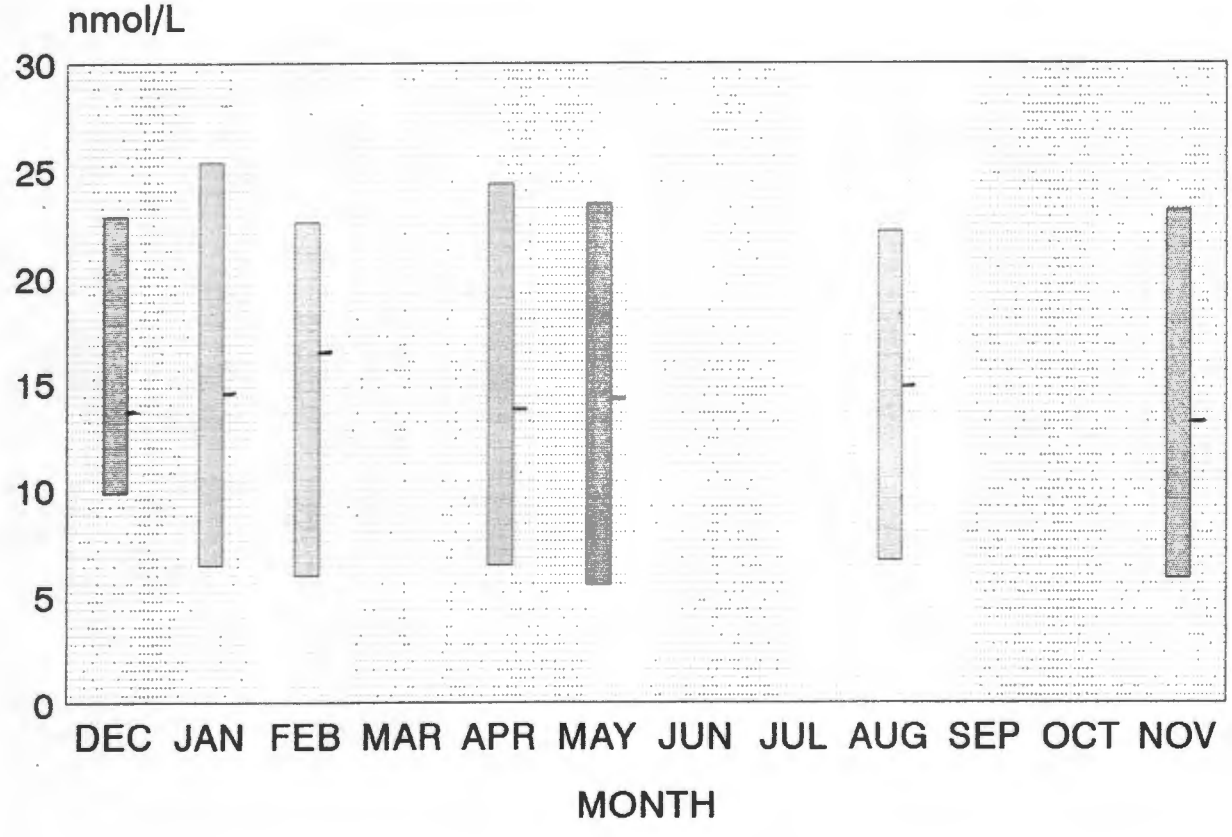
	January	February	April	May	August	November
December (baseline)	T = 1.589 p = 0.126	T = 1.157 p = 0.260	T = 0.445 p = 0.661	T = 0.150 p = 0.882	T = 0.511 p = 0.615	T = 0.834 p = 0.415
January		T = 0.940 p = 0.358	T = 1.061 p = 0.301	T = 1.136 p = 0.269	T = 1.265 p = 0.221	T = 1.845 p = 0.081
February			T = 0.155 p = 0.878	T = 0.496 p = 0.626	T = 0.479 p = 0.638	T = 1.744 p = 0.098
April				T = 0.214 p = 0.833	T = 0.663 p = 0.517	T = 0.791 p = 0.440
May					T = 0.266 p = 0.794	T = 0.076 p = 0.940
August						T = 0.401 p = 0.693

The month of December (used as a baseline) is compared with each of the other months studied. The individual months are also compared with each other. The T statistic and p value for each comparison is shown. No significant differences were noted.

Testosterone

There were no significant changes in testosterone concentrations during the period under study (Table 10). In table 10 the T statistic and p values are included for the comparison of mean testosterone levels between the months indicated. Testosterone minimum levels for each study month excluding December was found to be below the normal range. The mean testosterone level however remained within the normal range throughout the study. The maximum level was well within the normal range for males (Figures 12 and 13)

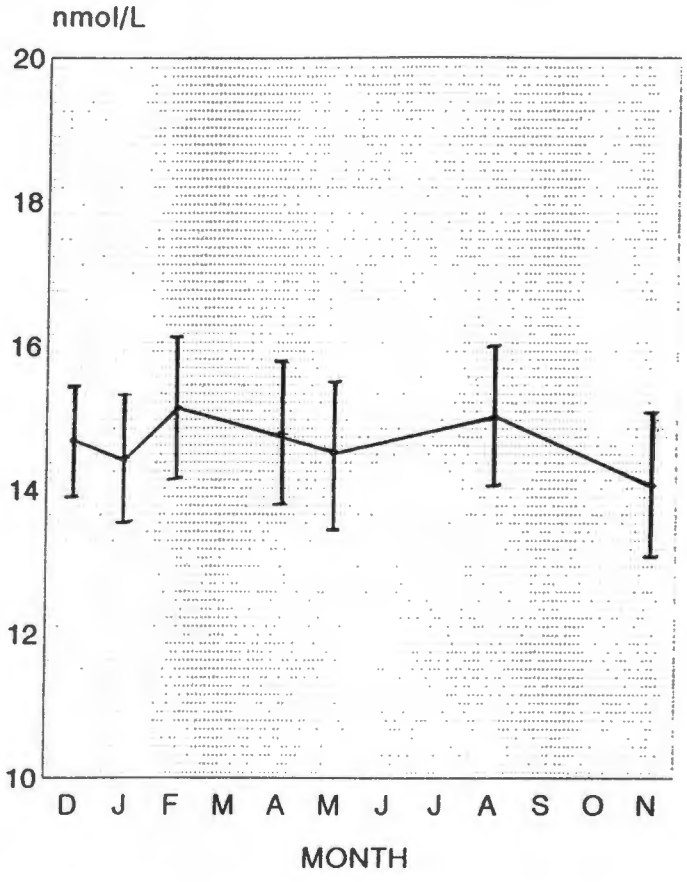
Figure 12
Maximum and minimum testosterone levels during the study period



This illustrates the maximum and minimum testosterone levels (in nmol/l) of the runners for each of the months studied. The median value is indicated with the mark-. The normal range for testosterone in males is 9.2 - 37 nmol/l. Testosterone levels are shown on the y axis and the months are illustrated on the x axis.

Figure 13

Mean testosterone levels during the study period



This illustrates the mean testosterone levels of the study group for each of the study months (mean + / - SEM). Testosterone levels in nmol/l are shown on the y axis and months are illustrated on the x axis.

Table 10

Comparison of the testosterone levels of the runners during the study period.

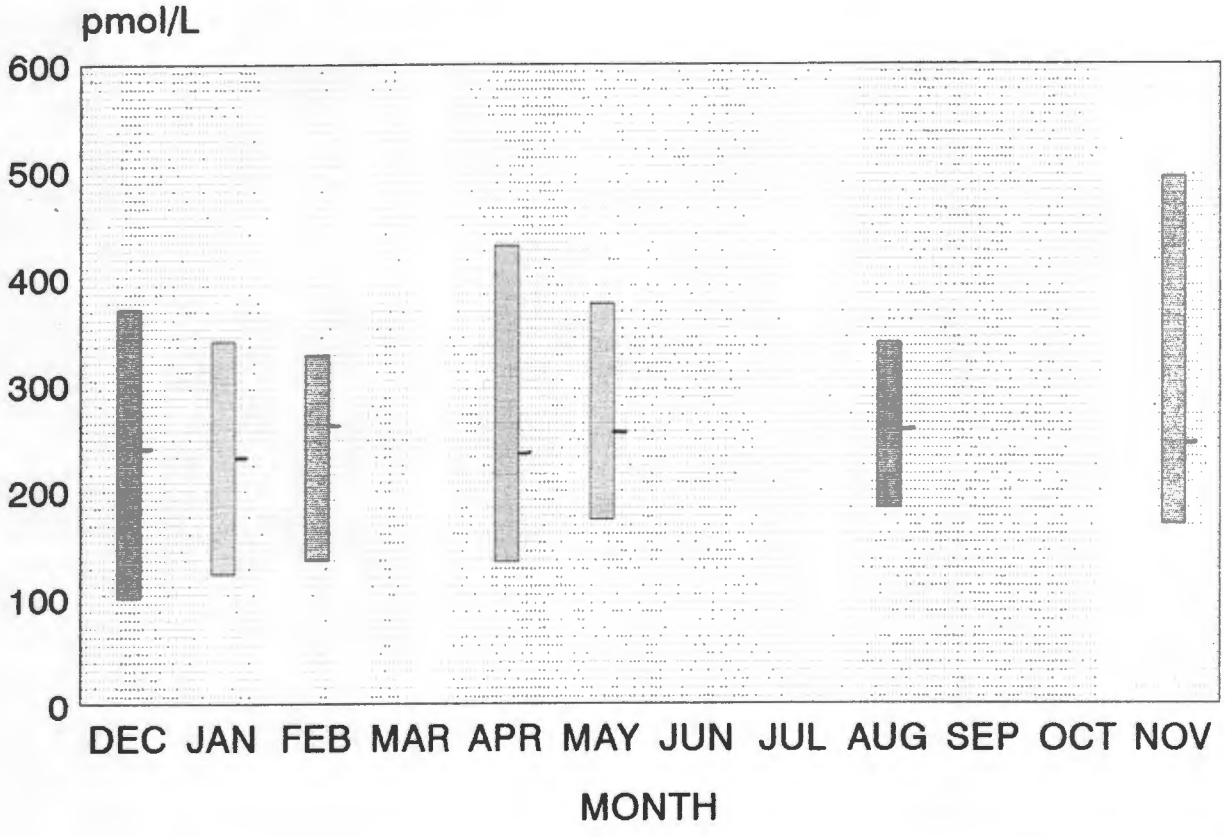
	January	February	April	May	August	November
December (baseline)	T = 0.352 p = 0.728	T = 0.186 p = 0.854	T = 0.449 p = 0.658	T = 0.372 p = 0.714	T = 0.143 p = 0.888	T = 0.255 p = 0.802
January		T = 1.043 p = 0.309	T = 1.023 p = 0.318	T = 0.968 p = 0.344	T = 0.939 p = 0.360	T = 0.029 p = 0.977
February			T = 0.222 p = 0.827	T = 0.267 p = 0.793	T = 0.007 p = 0.994	T = 0.584 p = 0.566
April				T = 0.573 p = 0.573	T = 0.196 p = 0.847	T = 0.906 p = 0.378
May					T = 0.270 p = 0.791	T = 0.044 p = 0.966
August						T = 0.979 p = 0.340

The month of December (used as a baseline) is compared with each of the other months studied. The individual months are also compared with each other. The T statistic and p value for each comparison is shown. No significant differences were noted.

E2

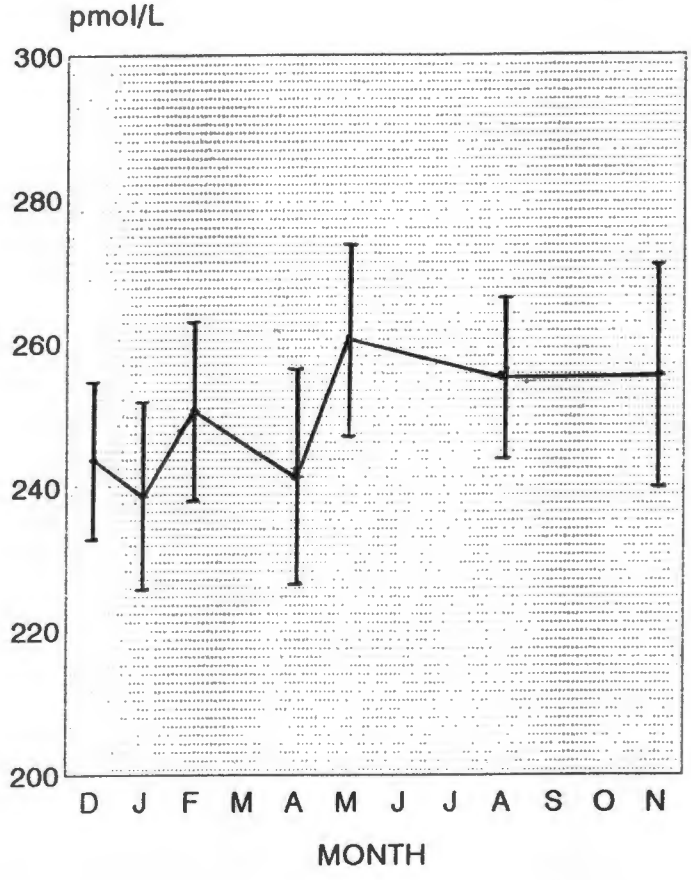
The only significant change in E2 levels was an increase from January to May (T=2.081 p=0.050) (Table 11). However, when comparing the other months under study there were no significant changes. In table 11 the T statistic and p values are included for the comparison of mean E2 levels between the months indicated. Significant differences are shown with the asterix (*). E2 minimum and maximum levels for each study month remained within the normal range for males (Figure 14 and 15).

Figure 14
Maximum and minimum E2 levels during the study period



This illustrates the maximum and minimum E2 levles (in pmol/l) of the runners for each of the months studied. The median value is indicated with the mark -. The normal range for E2 in males is 100 - 500 pmol/l. E2 levels are shown on the y axis and the months are illustrated on the x axis.

Figure 15
Mean E2 levels during the study period



This illustrates the mean E2 levels of the study group for each of the study months (mean + / - SEM). E2 levels in pmol/l are shown on the y axis and months are illustrated on the x axis.

Table 11
Comparison of the mean E2 levels of the runners during the study period.

	January	February	April	May	August	November
December (baseline)	T = 0.448 p = 0.658	T = 0.837 p = 0.412	T = 0.044 p = 0.965	T = 1.942 p = 0.066	T = 1.331 p = 0.199	T = 1.045 p = 0.309
January		T = 1.211 p = 0.239	T = 1.049 p = 0.306	T = 2.081 p = 0.050*	T = 1.737 p = 0.099	T = 1.437 p = 0.167
February			T = 0.302 p = 0.766	T = 1.548 p = 0.139	T = 0.381 p = 0.707	T = 0.362 p = 0.722
April				T = 1.371 p = 0.187	T = 1.615 p = 0.125	T = 1.393 p = 0.182
May					T = 1.097 p = 0.289	T = 0.147 p = 0.885
August						T = 0.058 p = 0.954

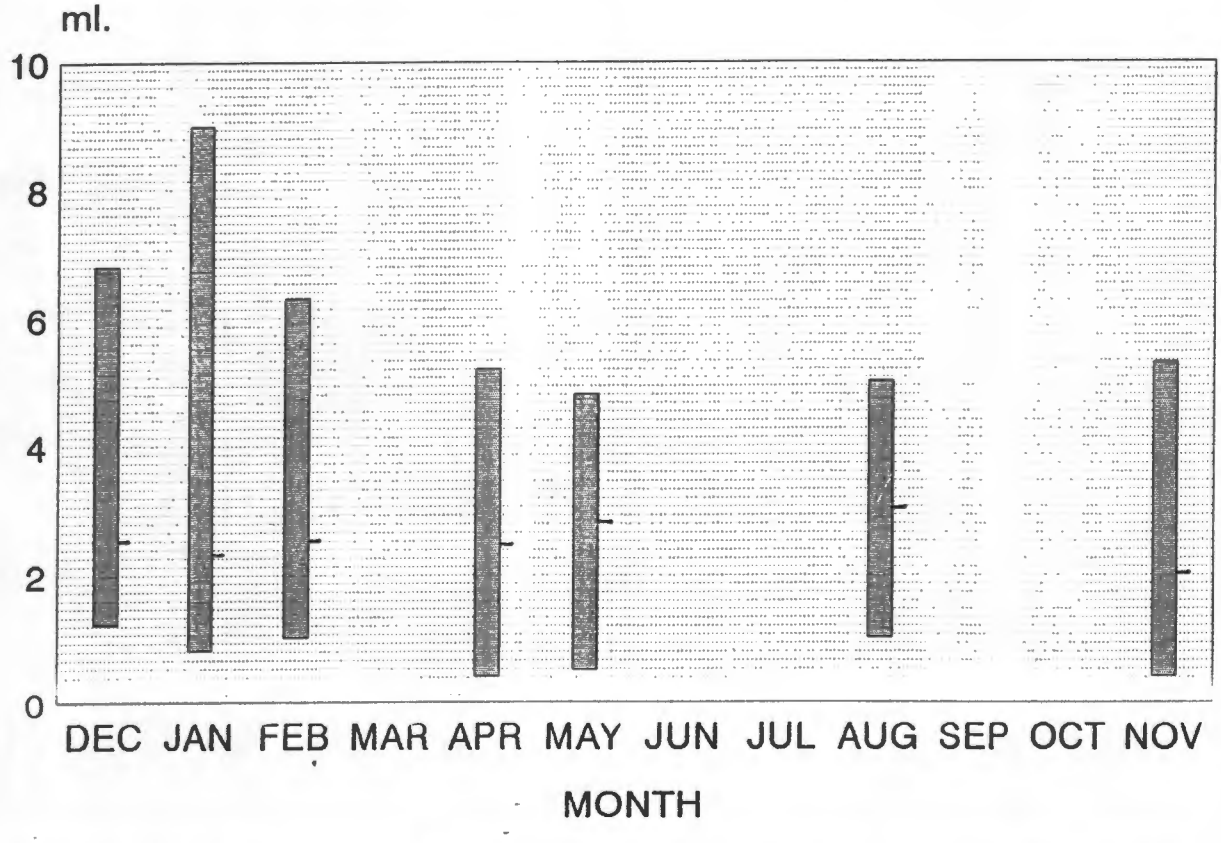
The month of December (used as a baseline) is compared with each of the other months studied. The individual months are also compared with each other. The T statistic and p value for each comparison is shown. (* = significant).

Semen

Semen Volume

The semen volume decreased significantly decrease between months December and April (T=2.144 p=0.044), December and November (T=2.394 p=0.027) and August and November. (T=2.952 p=0.009). A significant increase was noted between April and August (T=2.307 p=0.033) and May and August (T=2.548 p=0.021). All other months studied did not show any significant differences (Table 12). In table 12 the T statistic and p values are included for the comparison of mean semen volume between the months indicated. Significant differences are shown with the asterix (*). The minimum and maximum and mean values are represented in Figures 16 and 17.

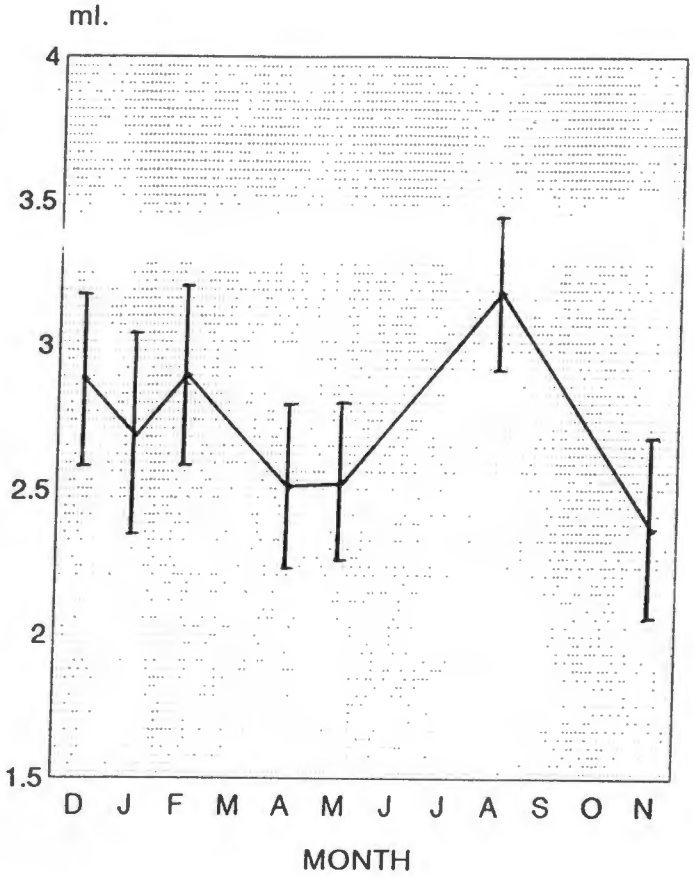
Figure 16
Maximum and minimum semen volume during the study period



This illustrates the maximum and minimum semen volume (in ml) of the runners for each of the months studied. The median value is indicated with the mark -. The normal range for semen volume is 2.0 - 6.0 ml. Semen volume is shown on the y axis and months are illustrated on the x axis.

Figure 17

Mean semen volume during the study period



This illustrates the mean semen volumes of the study group for each of the study months (mean + / - SEM). Semen volume in ml is shown on the y axis and months are illustrated on the x axis.

Table 12

Comparison of the mean semen volumes of the runners during the study period.

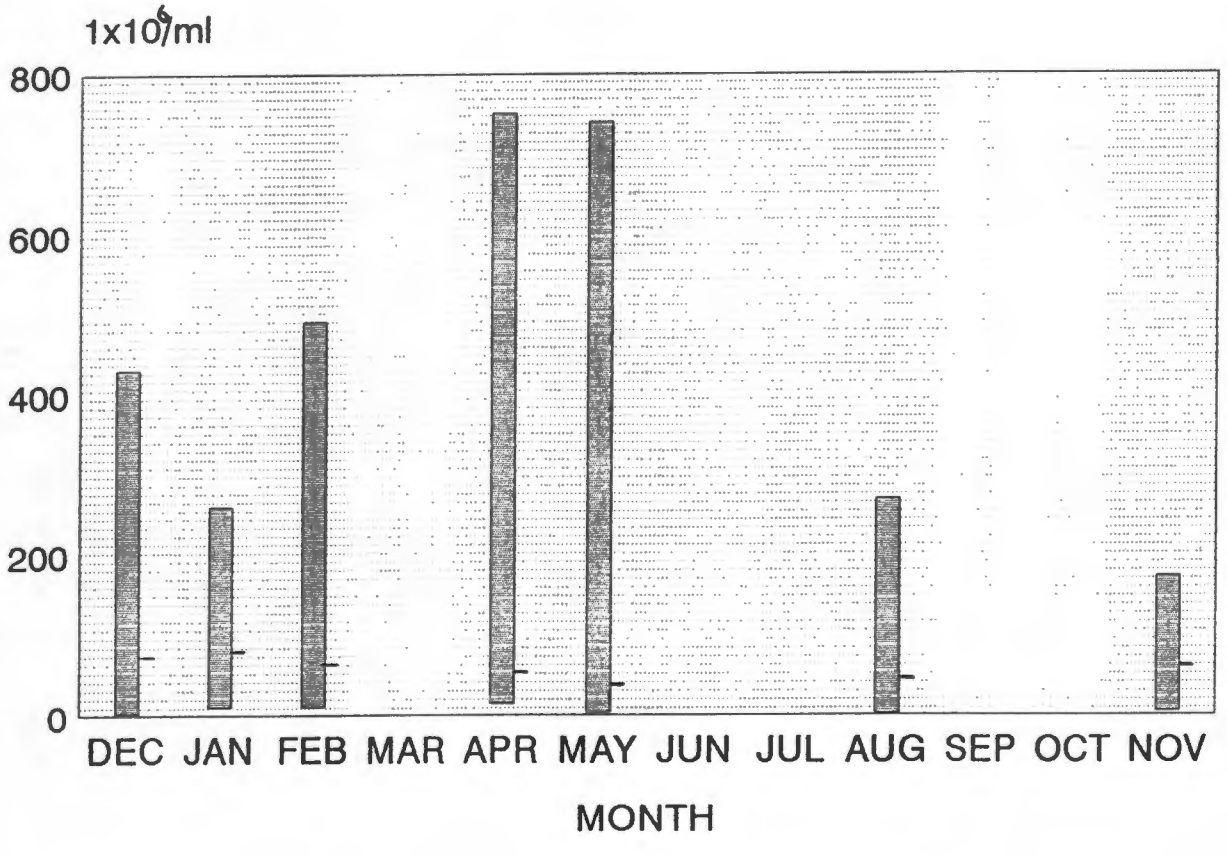
	January	February	April	May	August	November
December (baseline)	T = 1.371 p = 0.184	T = 0.753 p = 0.460	T = 2.144 p = 0.044*	T = 1.120 p = 0.277	T = 0.812 p = 0.427	T = 2.394 p = 0.027*
January		T = 0.512 p = 0.614	T = 0.530 p = 0.601	T = 0.775 p = 0.447	T = 0.917 p = 0.370	T = 1.361 p = 0.189
February			T = 1.018 p = 0.321	T = 1.837 p = 0.083	T = 0.721 p = 0.480	T = 1.696 p = 0.108
April				T = 0.044 p = 0.965	T = 2.307 p = 0.033*	T = 0.619 p = 0.544
May					T = 2.548 p = 0.021*	T = 0.346 p = 0.734
August						T = 2.952 p = 0.009*

The month of December (used as a baseline) is compared with each of the other months studied. The individual months are also compared with each other. The T statistic and p value for each comparison is shown. (* = significant).

Semen Counts

There were no significant changes in the semen count during the period under study (Table 13). In table 13 the T statistic and p values are included for the comparison of mean semen counts between the months indicated. The minimum and maximum and mean values are represented in Figures 18 and 19.

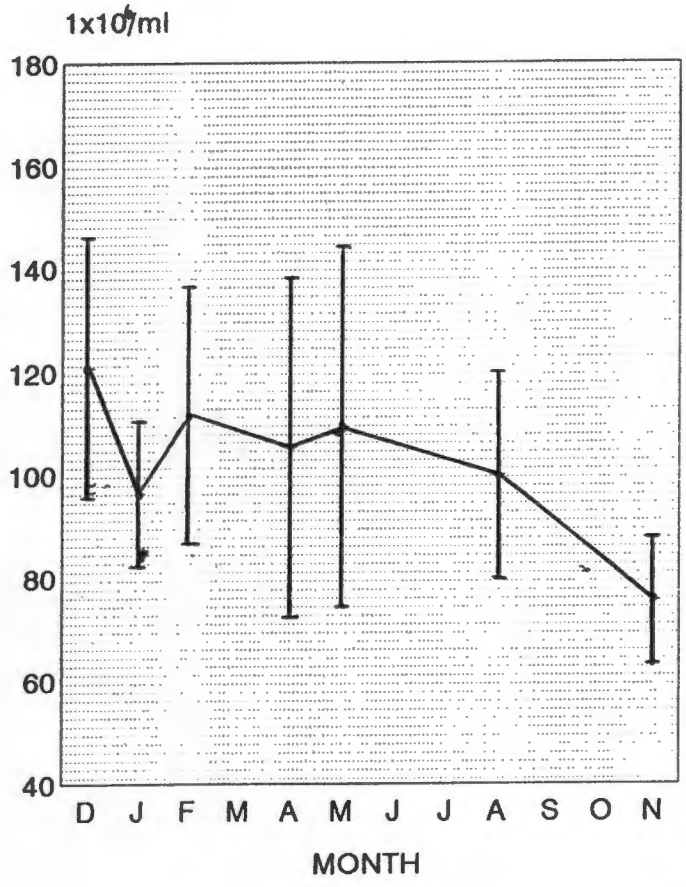
Figure 18
Maximum and minimum semen count during the study period



This illustrates the maximum and minimum semen count (in 1 x 10^6 / ml) of the runners for each of the months studied. The median value is indicated with the mark -. The normal range is for semen count is > 20 x 10^6 / ml. Semen count is shown on the y axis and the months are illusated on the x axis.

Figure 19

Mean semen count during the study period



This illustrates the mean semen count of the study group for each of the study months (mean + / - SEM). Semen count in 1 x 10^6 is shown on the y axis and months are illustrated on the x axis.

Table 37

Comparison of the mean semen count of the runners during the study period.

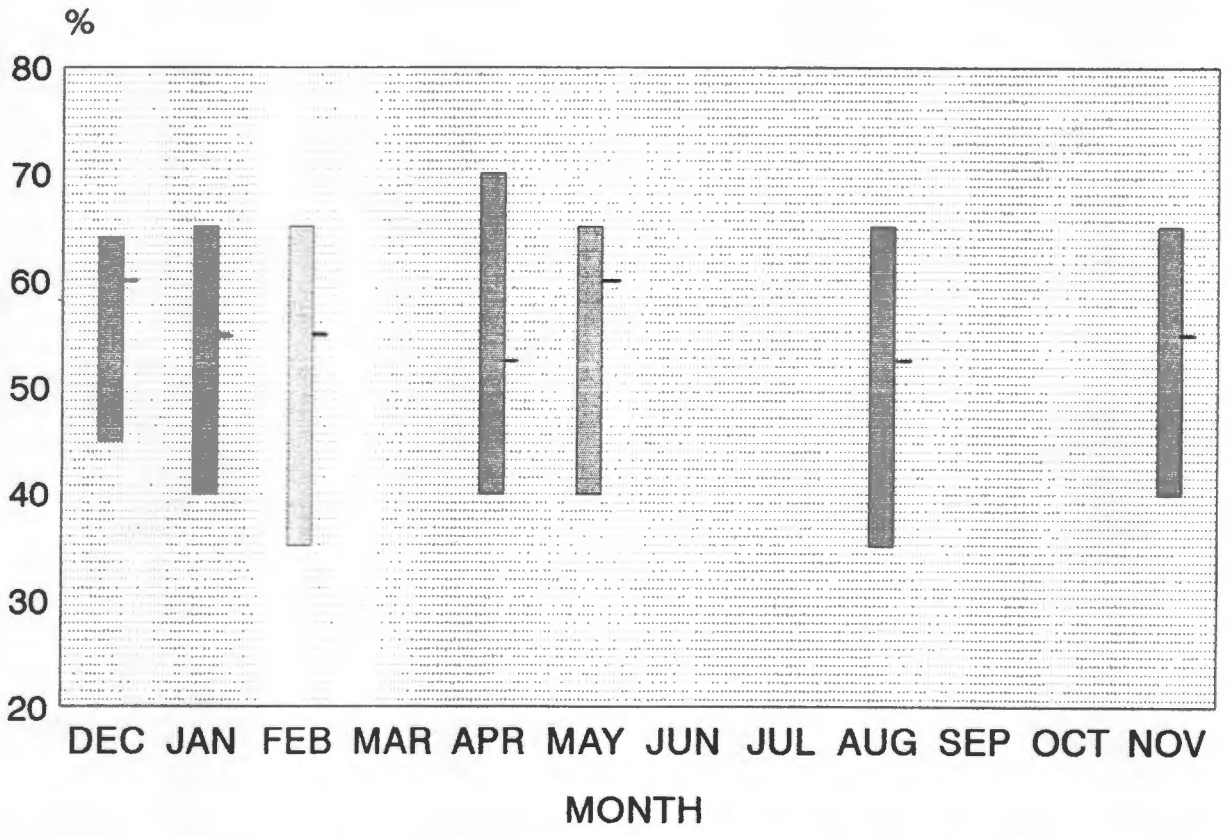
	January	February	April	May	August	November
December (baseline)	T = 1.743 p = 0.095	T = 1.199 p = 0.245	T = 0.649 p = 0.523	T = 1.007 p = 0.327	T = 0.795 p = 0.437	T = 1.572 p = 0.132
January		T = 0.871 p = 0.384	T = 0.556 p = 0.584	T = 0.231 p = 0.820	T = 0.603 p = 0.553	T = 0.904 p = 0.378
February			T = 0.521 p = 0.609	T = 0.100 p = 0.922	T = 0.042 p = 0.967	T = 1.229 p = 0.236
April				T = 0.672 p = 0.510	T = 1.220 p = 0.238	T = 0.500 p = 0.623
May					T = 1.249 p = 0.229	T = 0.040 p = 0.968
August						T = 1.499 p = 0.151

The month of December (used as a baseline) is compared with each of the other months studied. The individual months are also compared with each other. The T statistic and p value for each comparison is shown. No significant differences were noted.

Semen Motility

Semen motility decreased significantly from December to April (T=2.434 p=0.024) and also when comparing May to August (T=2.828 p=0.012). However all other months studied did not show any significant changes (Table 14). In table 14 the T statistic and p values are included for the comparison of mean semen motility between the months indicated. The significant differences are shown with the asterix (*). The minimum and maximum and mean values are represented in Figures 20 and 21.

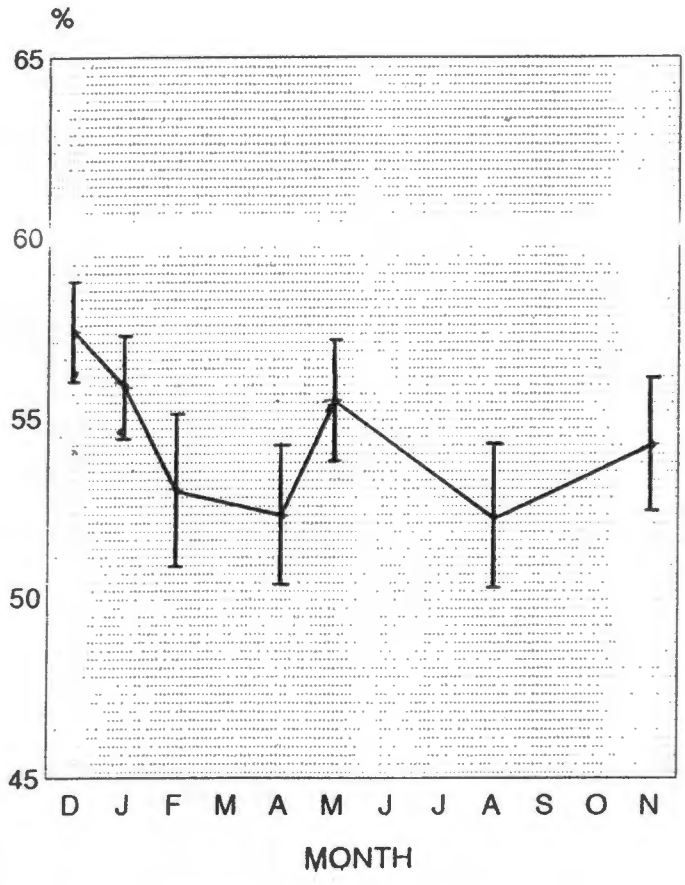
Figure 20
Maximum and minimum semen motility during the study period



This illustrates the maximum and minimum semen motility (expressed as a percentage of motile spermatozoa) of the runners for each of the months studied. The median value is indicated with the mark -. The normal range for semen motility is > 40% motile spermatozoa. Percentage of motile semen is shown on the y axis and the months are illustrated on the x axis.

Figure 21

Mean percentage of semen motility during the study period



This illustrates the mean semen motility of the study group for each of the study months (mean + / - SEM). The percentage of motile sperm is shown on the y axis and months are illustrated on the x axis.

Table 14

Comparison of the mean semen motility of the runners during the study period.

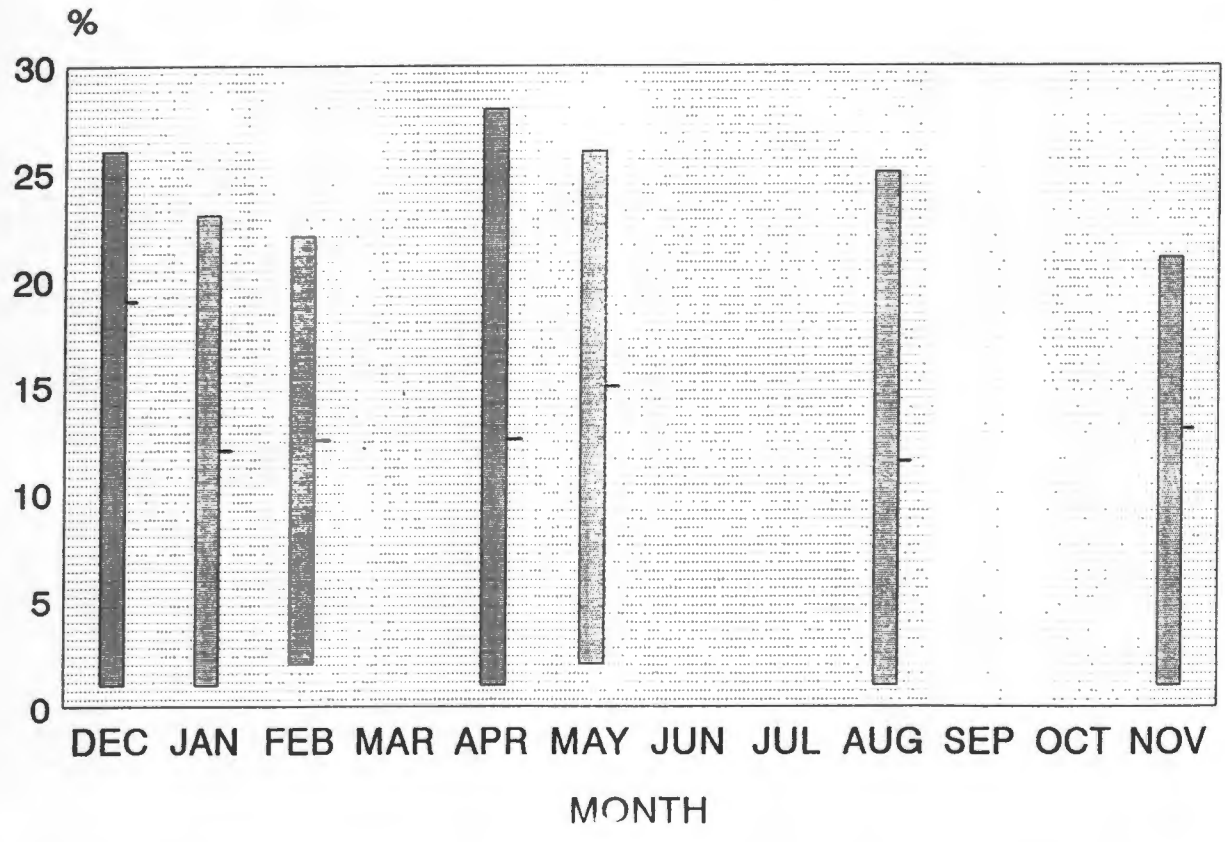
	January	February	April	May	August	November
December (baseline)	T = 0.743 p = 0.466	T = 1.504 p = 0.148	T = 2.434 p = 0.024*	T = 1.116 p = 0.278	T = 1.969 p = 0.064	T = 1.285 p = 0.214
January		T = 1.268 p = 0.219	T = 1.733 p = 0.098	T = 0.165 p = 0.871	T = 1.702 p = 0.105	T = 0.825 p = 0.419
February			T = 0.603 p = 0.554	T = 0.889 p = 0.385	T = 0.288 p = 0.776	T = 0.187 p = 0.854
April				T = 1.511 p = 0.148	T = 0.329 p = 0.746	T = 0.498 p = 0.625
May					T = 2.828 p = 0.012*	T = 1.295 p = 0.214
August						T = 0.800 p = 0.435

The month of December (used as a baseline) is compared with each of the other months studied. The individual months are also compared with each other. The T statistic and p value for each comparison is shown. (* = significant)

Semen Morphology

There was a significant decrease in semen morphology between December (baseline) and January (T=2.796 p=0.011), February (T=2.955 p=0.008), April (T=2.626 p=0.016), May (T=3.202 p=0.005), August (T=2.534 p=0.021) and November (T=3.727 p=0.001). However no other significant differences were demonstrated after the initial fall (Table 15). In table 15 the T statistic and p values are included for the comparison of mean semen morphology between the months indicated. Significant differences are shown with the asterix (*). The minimum and maximum and mean values are represented in Figures 22 and 23.

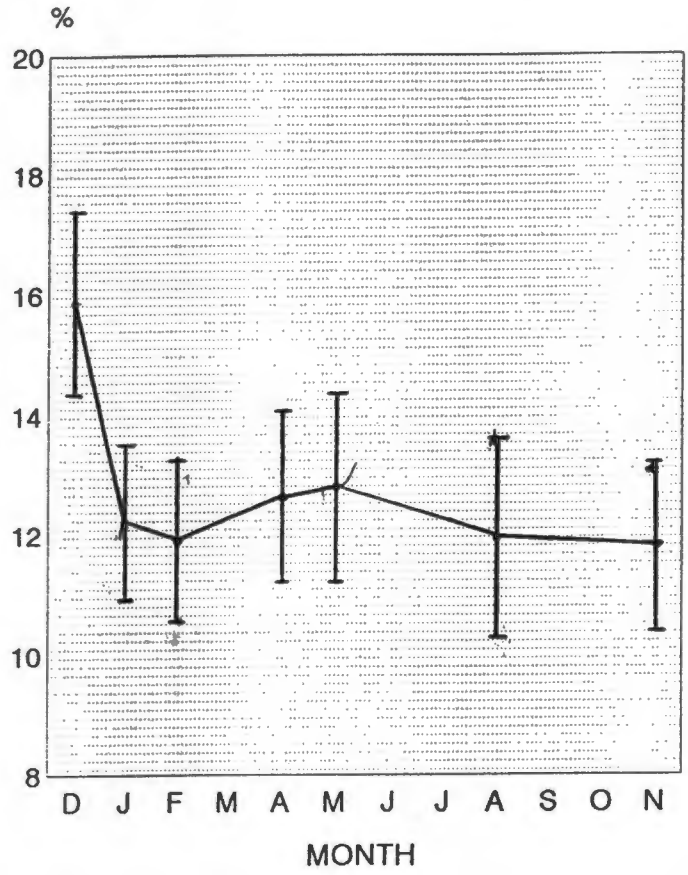
Figure 22
Semen morphology during the study period.



This illustrates the maximum and minimum semen morphology (expressed as a percentage of morphologically normal spermatozoa) of the runners for each of the months studied. The median value is indicated with the mark -. The normal range for semen morphology is > 40% of morphologically normal spermatozoa. The percentage of morphologically normal semen is shown on the y axis and the months are illustrated on the x axis.

Figure 23

Mean percentage of the morphologically normal spermatozoa during the study period



This illustrates the mean of the morphologically normal spermatozoa of the study group for each of the study months (mean + / - SEM). Percentage of morphologically normal spermatozoa is shown on the y axis and the months are illustrated on the x axis.

Table 15

Comparison of the mean of the morphologically normal spermatozoa of the runners during the study period.

	January	February	April	May	August	November
December (baseline)	T = 2.796 p = 0.011*	T = 2.955 p = 0.008*	T = 2.626 p = 0.016*	T = 3.202 p = 0.005*	T = 2.534 p = 0.021*	T = 3.727 p = 0.001*
January		T = 0.309 p = 0.760	T = 0.630 p = 0.536	T = 0.119 p = 0.907	T = 0.799 p = 0.435	T = 0.156 p = 0.878
February			T = 0.911 p = 0.374	T = 0.424 p = 0.677	T = 0.557 p = 0.584	T = 0.342 p = 0.736
April				T = 0.780 p = 0.445	T = 0.765 p = 0.221	T = 0.221 p = 0.828
May					T = 0.428 p = 0.674	T = 0.105 p = 0.917
August						T = 0.090 p = 0.930

The month of December (used as a baseline) is compared with each of the other months studied. The individual months are also compared with each other. The T statistic and p value for each comparison is shown. (* = significant)

Low and High Training.

Results were analysed by dividing the runners each month into two groups according to the distance trained each week. Each individual runner could only be represented in one group and this group could change from month to month according to the distance run in the preceding week. Low training involved running up to 55 kilometres per week and high training included training between 60 and 160 kilometres per week. The mean training distance in the low group was 28.7 (SD 18.5) kilometres and in the high group 84.9 (SD 20.8) kilometres.

Seventy-three results were analysed in the low group and 78 results in the high training group. Runners results who had no training for a particular month were excluded from the analysis. Only one runner had a training distance of 160 kilometres and only on one occasion. When comparing the results between low training time and high training time (Table 16) there was a significant fall in FSH levels, with a mean value of 4.0 mIU/ml for low training and a mean value of 3.5 mIU/ml for high training (T=2.203 p=0.029). There was however no significant differences in the mean values between high and low training for any of the other hormones measured. Mean LH values for high training were 2.6 mIU/ml and for low training 2.9 mIU/ml (T=1.089 p=0.278). Mean E2 values for high training were 243 pmol/l and for low training 254 pmol/l (T=1.200 p=0.232). This also was the case for testosterone, with the mean value for high training being 14.7 nmol/l and for low training 14.5 nmol/l (T=0.282 p=0.779), prolactin, with the mean value for high training being 3.7 ng/ml and low training 4.2 ng/ml (T=1.827 p=0.070) and progesterone, with the mean value for high training being 0.8 nmol/l and for low training 0.7 nmol/l (T=1.374 p=0.172).

The mean semen volume for high training was 2.7 ml and for low training was 2.8 ml. (T=0.626 p=0.532). The mean semen motility for high training was 54% and for low training was 55% (T=0.512 p=0.610). There was however a significant difference in semen count and semen morphology when comparing months of high training with

months of low training. The mean semen count for high training months was 133 million/ml and for low training 71 million/ml (T=3.459 p=0.001). The mean semen morphology during high training was 15% and for low training 11% (T=3.416 p=0.001).

There was a significant difference in weight between months of high training and months of low training. The mean weight for high training was 72.0 kg and for low training 76.6 kg (T=2.923 p=0.004). However BMI did not change significantly. The mean value for high training was 23.2 kg/m^2 and for low training 24.1 kg/m^2 (T=1.969 p=0.051).

Table 16

Comparison of the mean hormonal, semen and anthropomorphic parameters of the group of runners during periods of high and low training.

	High training mean (SD) number = 78	Low training mean (SD) number = 73	T value	p value
Hormones				
FSH (mIU/ml)	3.5 (1.5)	3.0 (1.5)	2.203	0.029*
LH (mIU/ml)	2.6 (1.4)	2.9 (1.4)	1.089	0.278
E2 (pmol/l)	243 (66.2)	254 (54.5)	1.200	0.232
Testosterone (nmol/l)	14.7 (4.3)	14.5 (4.4)	0.282	0.779
Prolactin (ng/ml)	3.7 (1.6)	4.2 (2.0)	1.827	0.070
Progesterone (nmol/l)	0.8 (0.4)	0.7 (0.3)	1.374	0.172
Semen				
Volume (ml)	2.7 (1.4)	2.8 (1.4)	0.626	0.532
Count (x10^6/ml)	133 (142)	71 (65)	3.459	0.001*
Motility (%)	54 (9)	55 (8)	0.512	0.610
Morphology (%)	15 (6)	11 (7)	3.416	0.001*
Weight (kg)	72.0 (10.0)	76.6 (9.0)	2.923	0.004*
BMI (kg/m^2)	23.2 (2.8)	24.1 (2.3)	1.969	0.051

The T statistic and p value for each comparison is shown (* = significant)

DISCUSSION

The purpose of this study was to determine if there were changes in hormone levels and semen parameters in a group of athletes training for marathon running. Although the occurrence of hypogonadotropic hypogonadism in female athletes is a well described entity (Jacobs, 1982; Reame *et al.*, 1985), very limited attention has been paid to the possibility of a similar phenomenon occurring in male athletes. The effects of strenuous physical exercise on the reproductive system in men have been evaluated in uncontrolled situations in the majority of reports often with only single-point measurements of reproductive hormones. In addition, the degree of previous training, as well as the intensity of the short-term exercise, was not standardised (MacConnie *et al.*, 1986).

In this longitudinal study serial serum hormonal and semen analysis was performed over a year long period in a group of marathon runners. The increase in training distance was in preparation for the Two Oceans marathon, a 56 km race, which was run in April. Thereafter there was a gradual decrease in training times (Figure 1). The marathon took place in the fifth month of the study. The runners' weight decreased as training time increased in preparation for the marathon. This was obviously paralleled by a decrease in the body mass index, which remained however, within the normal range. Whilst it has been shown that females who have weight loss during training have more significant menstrual cycle alterations (Bullen *et al,* 1985), the correlation of weight changes with semen parameters and reproductive dysfunction in males has not been well defined.

In this study the effect of long distance running on the hypothalamic-pituitary-gonadal axis was studied. Other investigators have studied the effect of exercise on the pituitary-adrenocortical system, but this only involved the determination of a single blood cortisol level in response to exercise (Jurimae *et al.*, 1989). The increased blood cortisol level observed by Jurimae reflects an increase in adrenocortical activity, but as only a

single measurement was done in response to strenuous exercise the long-term effect of this remains to be defined. Highly trained male athletes have been reported to have deficient GnRH secretion (MacConnie *et al.*, 1986), and also the term "over-training" has been related to the hypothalamic dysfunction provoked by exercise (Barron *et al.*, 1985). Exercise induced infertility seems to be less common in men than in women (Ayers *et al.*, 1985), with findings suggesting that the threshold for exercise or training induced H-P-G axis suppression is much higher in men than in women.

A number of biological rhythms are known to exist in man (Smolensky, 1980). There is the one-hourly rhythm of pituitary response to GnRH secretion, or the circadian (24 hour) rhythm of the excretion of steroid hormones such as oestrogen, progesterone and testosterone (Politoff *et al.*, 1989). All specimens were taken between 7.00 am and 9.00 am to minimise possible variations introduced by diurnal rhythms.

Evaluation of the hormonal profile in our group of 24 marathon runners showed that although there was a significant initial increase in the prolactin levels and fall in progesterone, both of these remained within the normal range for males. Prolactin levels showed an increase when comparing baseline samples taken in December prior to the start of strenuous training to those of the other months studied. The effect of strenuous training on prolactin levels in males remains to be well defined. In contrast to our study, Aakvaag reported a suppression of prolactin during a five day combat course involving army personnel (Aakvaag *et al.*, 1978). These findings were confirmed in a study by Wheeler who demonstrated a reduction in prolactin levels in a group of male distance runners, although the levels remained (as in our study) within the normal range (Wheeler *et al.*, 1984). The study by Wheeler involved the determination of a single prolactin level in contrast to our study which was a longitudinal study performed over a period of a year. However, in a recent study Hackney *et al* suggested that endurance-trained subjects had normal resting prolactin, but following the injection of a dopamine antagonist had higher prolactin levels than a sedentary group (Hackney *et al.*, 1990). The significance of the initial increase in prolactin levels in our group of runners is

uncertain and requires further investigation. The physiological significance of prolactin in the male is currently uncertain. However, an increase in prolactin may be significant as prolactin may influence testicular function (Aakvaag *et al.*, 1978). There are prolactin receptors in the adult human testis and it has been suggested that prolactin may also be involved in regulating testicular sex steroid production (Rubin *et al.*, 1978). Prolactin is necessary for normal manufacture of sex steroids within the testis but when present in an increased quantity may result in decreased testosterone production and impaired libido (Wheeler *et al.*, 1984). However it is difficult to attribute the decreased libido anecdotally reported in some runners during intense training to changes in testosterone only, as chronic fatigue may also be important. However, the effect of prolactin on the H-P-G axis is also central and this may be important in endurance-trained athletes as training could, via endogenous opioids result in an increased response of the lactotropic cells of the pituitary. Other possibilities include a decreased short-loop prolactin-dopamine feed-back or suppressed prolactin - prolactin inhibition (Hackney *et al,* 1990). Alterations in circulating prolactin alone cannot explain the serum testosterone changes seen in runners (Cumming *et al.*, 1989). As prolactin levels in this study were within the normal range for adult males it would seem unlikely the alterations in the semen parameters of the runners were due to these changes.

The median testosterone levels in this group of runners were at the lower end of the normal range, a finding consistent with those of McColl and co-workers (McColl *et al.*, 1989). However, several studies have shown a decrease in serum testosterone levels in male athletes (Ayers *et al,* 1985; Hackney *et al,* 1990). The fall in serum testosterone levels may result from decreased production rates, decreased binding or increased clearance. There is scant evidence of decreased binding (Wheeler *et al.*, 1984). The clearance of testosterone is through both hepatic and extrahepatic mechanisms. There are no studies demonstrating long term increases in hepatic or extrahepatic clearance of testosterone in endurance-trained or other athletes. Wheeler *et al,* in a more recent article, stated that the lower testosterone levels in exercising men are probably the result

of peripheral mechanisms such as an increase in tissue testosterone utilisation without triggering any changes in gonadotropin release (Wheeler *et al.*, 1991). Ayers confirmed the observation of decreased serum testosterone levels in the majority of male subjects undergoing endurance training, but despite these depressed gonadal steroid levels, this was not associated with a decrease in fertility potential (Ayers *et al.*, 1985). Despite the alterations in gonadal steroids, 18 of the 20 subjects they studied had no evidence of a concomitant effect on sperm production. Furthermore, they found no correlation between testosterone and sperm counts and suggested either that male spermatogenesis and steroidogenesis have separate control mechanisms, or that this "down-setting" of testosterone steroid production has little effect on the testicular sperm production of endurance athletes (Ayers *et al.*, 1985). What is clear from their study is that significant physical exercise and chronic endurance training alone are not associated with a detrimental effect on sperm production in most men. While testosterone was significantly decreased in their subjects, most did not demonstrate the profound disturbance in reproductive function seen in similarly trained female athletes.

Wheeler provided evidence that vigorous physical exercise in men may have chronic effects on H-P-G axis function similar to those changes seen in female endurance athletes (Wheeler *et al.*, 1984). The results of our study confirmed their observations of a decreased serum testosterone level with the mean value in the lower range of normal for males. However there was no further significant fall in testosterone levels as the training progressed. Therefore although spermatogenesis is an androgen dependent biological function, it is unlikely that the lower testosterone levels, which did not change specifically over the study period, found in our runners produced any significant clinical impact on the semen parameters.

The lack of significant changes in LH and FSH levels observed in our study, a finding consistent with the results of Wheeler, implies that impaired gonadotropin release is not responsible for the lower testosterone levels (Wheeler *et al.*, 1984). This finding does not exclude more subtle alterations in GnRH-pituitary function. Luteinizing hormone is

generally accepted as the pituitary gonadotropin controlling testosterone secretion (Sutton *et al.*, 1973). These findings support the lack of association between androgens and luteinizing hormone observed by other workers (Cartensen *et al.*, 1972; Rogol *et al.*, 1984). Exercise does not seem to affect mean serum LH levels (Wall *et al.*, 1985). This finding by Wall and co-workers was confirmed in our study where no significant changes in LH values over the year long period. As the serum levels of LH and FSH remained within the normal range for males and did not change significantly over the study period, they do not appear to play a part in the altered semen parameters in our runners.

The estradiol levels remained constant throughout the study period. Female athletes with hypothalamic amenorrhoea have low serum levels of estrogen (Reame *et al.*, 1985). In female runners with normal menstrual cycles, episodes of intense exercise have been associated with elevated plasma concentrations of estradiol (Jurkowski *et al.*, 1978). In a study by Ayers *et al* mean estradiol concentrations did not differ from control values. The mean estradiol concentration was, however, deceptive, because in 75% of the runners the mean values were markedly lower than the control range (Ayers *et al.*, 1985). In another study of male marathon runners each subject had normal serum concentrations of estradiol (Rogol *et al.*, 1984). In our study the estradiol values remained within the normal range for males throughout the year long follow-up. Estrogen formation in the testes is regulated by gonadotropins and as the LH and FSH remained unchanged during the study it seems logical that the estradiol levels should remain constant and therefore unlikely to cause the alterations in the semen parameters in the study group.

There is very little information available on progesterone levels in male athletes. In men, progesterone is formed as an intermediate link in the biosynthesis of other steroid hormones. Although the function of progesterone in the male is not known the Leydig cells in the testes secrete small quantities and the adrenal gland is also capable of producing small amounts. A study by Jurimae showed a considerable increase in

progesterone levels after long triathlons. Comparison with this study is not valid however as Jurimae sampled the triathletes on one occasion only (immediately after the event) (Jurimae *et al.,* 1989). In contrast this study has looked at serial samples over a year. Although the levels fell significantly they remained within the normal range for males. It could be postulated that if the progesterone levels decrease this could lead to the lower testosterone levels seen in runners and indicate a decrease in production and not a change in clearance as proposed by Wheeler (Wheeler *et al.,* 1984). More evidence is needed. However, as the progesterone levels remained within the normal range for males throughout the study, it seems unlikely that the alterations in the semen parameters in our runners has any relationship to progesterone levels.

The effect of major athletic competition on hormonal levels, libido and sperm production remains largely unexplored. The general perception has been that strenuous exercise is not associated with a significant decrease in fertility, but this is probably a reflection of a lack of available data rather than scientific facts. Baker has suggested that a decrease in fertility may occur in males who participate in strenuous physical activity (Baker *et al.,* 1984.). In addition anecdotal data have also suggested that libido may be impaired in some runners during periods of intense endurance training (Wheeler *et al.,* 1984). The aetiology of this is not clear. Reduced testosterone levels may play a role but chronic fatigue could also be a significant factor (Wheeler *et al.,* 1984). As libido is a very subjective condition and, in addition to this, is may be influenced by factors other than exercise, it was not analysed as a variable in this study.

The period of continence preceding collection of the semen specimen has a remarkable influence on spermatozoan concentration but has little effect on motility or morphology (Freund, 1963). In this study no fixed period of continence was specified. A fixed period of continence preceding the collection of a specimen means that the specimen is no longer a random sampling of the patient's spermatozoan output at his usual frequency of ejaculation. It has been shown that individuals respond quite differently to experimental variations in the frequency of intercourse and duration of continence;

some men show very large changes in spermatozoan output, and some quite modest changes (Freund, 1963).

The age of the specimen at the time of examination, that is the time elapsed from collection at home to examination in the laboratory, is of great importance, since it has been demonstrated that at room temperature (20-25^{o}C) the percent motility and forward progression of spermatozoa declines by half in the first seven hours after collection (Freund, 1963). In this study the semen sample was either collected in the laboratory or immediately prior to attending the regular follow-up visit. All were examined within six hours of collection. This optimisation of semen sample collection enabled us to minimise the chance of erroneous results due to a delay in specimen processing.

A specimen bottle was supplied to each runner to be used at the next semen collection. By supplying the bottle it was possible to avoid the problem of receiving specimens in a variety of used and possibly contaminated containers like drug, food or cosmetic bottles. The plastic containers used in this study were those routinely used to obtain donor semen samples at the infertility clinic at Groote Schuur Hospital. It had been previously demonstrated that these containers had no effect on spermatozoan motility and survival and therefore suitable for the analysis of semen samples.

Precise semen analysis, mainly comprising count, motility and morphology analysis, remain the most important investigation for determining fertility potential in the male. It is well known that semen values must be carefully interpreted since wide fluctuations of semen parameters as well as limitations and inaccuracies of the various methods and the investigators bias the results. In this study all semen samples were analysed by the same experienced laboratory technologist to limit these factors.

In this study the semen volume was noted to decrease when comparing December to April and November. However there was a significant increase in semen volume

between April and August. This change was inversely proportional to the training time. The mean volume was never below 2 ml in any specific month of study and this is reassuring as authorities in the field of semen parameters agree that a fall in volume below 1 ml in total may influence conception rates (Macleod, 1973; Baker, 1984). Baker defined a low semen volume as <2.0 ml and this correlated positively with poor pregnancy rates in their artificial insemination by donor program when compared with a group with semen volume >2.0 ml (Baker, 1984). It is clear that although the semen volume changed during the study period these changes would probably not compromise fertility potential.

No difference in the mean semen count was demonstrated over this year long study and this finding was consistent with the findings of Bagatell who studied a group of male marathon runners for 12 weeks. They suggested that long term, strenuous exercise does not exert major effects on reproductive function in men. They found no difference in mean sperm counts between exercising and sedentary men (Bagatell and Bremner, 1990). Although the sperm count per millilitre is not of paramount importance, it is the parameter according to which patients' semen is classified, and remains the parameter which can be explained easily to patients to offer them a relative understanding of fertility prognosis (Van Zyl, 1980). Macleod considers that true oligospermia is represented in counts under 20 million/ml and at that level, provided the sperm motility is good, the chance of conception is reasonably good, and also states that conceptions occurring at higher sperm count levels are not in proportion to the rise in sperm count (Macleod, 1973) In another study Ayers *et al* found that despite alterations in gonadal steroids, 18 of the 20 men had no evidence of a concomitant effect upon sperm production. There was also no correlation between testosterone levels and sperm count, these authors suggested either that male gametogenesis and steroidogenesis have separate control mechanisms, or that the "down-setting" of testicular steroid production has little effect on testicular sperm production of endurance athletes (Ayers *et al.*, 1985). In a more recent study however, Griffith *et al* noted a decrease in semen count (although not significant) in a group of athletes undergoing exhaustive endurance

training for two weeks (Griffith *et al.*, 1990). The findings in our study that the highest mean semen count was found during December do not correlate with the findings of Levine *et al* who noted a reduction in semen counts in summer when compared with winter (Levine *et al.*, 1990). However, the lowest mean semen count in this study was found in November. Whether this change is due to exercise or seasonal variations is not clear and needs further investigation.

In contrast to the study by Bagatell who found no consistent abnormality of motility in their runners' semen samples (Bagatell and Bremner, 1990) in our study a significant decrease in sperm motility was noted between December and April, and May and August. Acre *et al* confirmed a significantly reduced sperm motility in a group of endurance trained runners when compared to sedentary controls (Acre *et al.*, 1993). As the sperm motility decreased significantly between December and May, the increase in training time might have had some influence on these changes, other factors being equal. Motility is of paramount importance, since patients with an adequate sperm count per ml but impaired motility and those with a relatively poor count per ml but exceptionally good sperm motility deserve careful evaluation to determine fertility prognosis (Van Zyl, 1980).

The evaluation of sperm morphology is a controversial issue. Normal morphology evaluated by strict criteria is a valuable predictor of fertility (Kruger *et al.*, 1988). Semen morphology decreased significantly from baseline when compared with all other months studied. The mean normal sperm morphology was consistently less than 14% which is in contrast to the study by Bagatell who found no abnormalities of morphology in their runners semen samples (Bagatell and Bremner, 1990). This is a significant finding as there is excellent correlation with the percentage normal sperm morphologic features and fertilisation and pregnancy rates. In a study by Kruger the group of patients with <14% normal morphologic features had significantly lower fertilisation and pregnancy rates (Kruger *et al.*, 1986). These findings are not insignificant as the decrease in sperm morphology may compromise future fertility. However, whether

these alterations are a result of the endurance training needs further investigation as the sperm morphology should have returned to December mean levels as the training decreased. This did not occur however, but the time taken for normal spermatogenesis of seventy days might be a significant factor.

When comparing the results between months of low training and high training the only significant difference in the hormonal parameters was found with FSH levels. The mean FSH in months of high training was 3.49 nmol/l and in months of low training was 4.03 nmol/l ($T = 2.203$: $p = 0.029$). Although not strictly comparable, this does contrast to the findings of Bagatell who found no difference in mean FSH levels between a matched group of marathon runners and healthy, lean controls (Bagatell *et al.,* 1990). Circulating FSH levels are regulated by non-steroidal factors from the seminiferous tubules with inhibin being the most clearly identified substance from this source which reduces serum FSH levels at the pituitary. It is not known whether inhibin also exerts a negative feed-back control at a hypothalamic locus, but testosterone, and estradiol are also capable of inhibiting secretion of FSH (Sherins *et al.,* 1982). However, there was no significant difference in testosterone or estradiol levels in the two groups so some other mechanism must influence the suppression of FSH mean levels in the high training group. Clearly more detailed endocrinological studies are necessary as the effect of exercise on hormonal changes is immediate while the semen changes might be delayed for several months. In addition in this study a confounding problem was the ability of a runner to move from the low training group to high training group within a two month period. It is not therefore possible to compare hormonal changes and alterations in semen parameters within a single month.

The effect of major athletic competition on sperm production remains largely unexplored and the hormonal regulation of spermatogenesis in man is poorly understood. FSH is believed to be required for spermatid maturation (spermiogenesis) and during the initiation of spermatogenesis at the time of puberty. However the role of FSH in the maintenance of sperm production in adults is unclear. Matsumoto *et al* have

shown that stimulation of sperm production can occur despite undetectable serum FSH levels and concluded therefore that normal levels of FSH are not an absolute requirement for re-initiating spermatogenesis (Matsumoto *et al,* 1986). In this study when the months of high training were compared with the months of low training significant differences in semen counts and semen morphology were noted. The mean semen count was higher in the group in the high training months and the mean normal morphology was also higher in this group when compared with the group in low training months. However, there were no differences in semen volume and semen motility between the two groups. It must be remembered that the process of spermatogenesis takes a period of approximately seventy days to be completed. Therefore by analysing semen in a specific month hormonal influences that have taken place over the preceding two months must be taken into consideration. The rate of spermatogenesis cannot be altered by hormonal suppression or stimulation, other toxic agents such as radiation or a change in temperature.

Spermatogenesis is under control of FSH and LH, whose secretion is regulated by gonadal steroids and inhibin (Knuth *et al.,* 1989). Although the mean serum FSH levels in both high and low training groups were within the normal range for males, the mean was significantly lower in the high training group. When looking at the changes in spermatogenesis seen in low months, it must be remembered that these might actually be the influence of high training in the previous two or three months. High training months preceded by low training will probably have no effect on sperm with the seventy day cycle of maturation. In the study by Matsumoto *et al* on patients with prolonged severely suppressed FSH levels, all eight subjects had significant but not complete suppression of spermatogenesis although this was highly variable between the subjects (Matsumoto *et al,* 1986). Testosterone is essential for primary spermatocytes to complete meiosis and there is general agreement that testosterone is the physiological feed-back inhibitor of LH in man (Sutton *et al.,* 1973). The differences in semen parameters in the high and low training groups are probably multifactoral with hormonal influences, stress and possibly temperature variations playing the most

critical roles. The findings of this study are confusing as it does not make sense that heavy training should give better semen parameters than months of low training. However, the "better" semen parameters seen in the high training group may have been the result of fewer ejaculations during the high training months due to a decrease in libido that has been anecdotally reported in some runners during intense training. It is known that the frequency of ejaculation can influence semen parameters.

When comparing the mean weight in the high and low training groups there was a significant lower weight in the months of high training. This can be expected as the runners would probably at their leanest and in peak physical condition in preparation for the marathon during the period of high training. Weight loss interacts in an additive way with exercise to alter fertility potential. However, the months of peak physical condition will only have a possible effect on semen parameters in two to three months due to the constant rate of spermatogenesis. The lower body weight would not be expected to influence the runners fertility immediately.

Although the effect of strenuous exercise on fertility in men remains to be well defined, a study involving an artificial insemination programme showed that partners of donors who participated in strenuous exercise with low semen volumes had significantly lower pregnancy rates than partners of men with normal activity and a low semen volume. When semen volume was normal there was no reduction in fertility (Wall *et al.*, 1985). Despite significant physical exercise (40 - 80 miles/week) and chronic endurance training in Ayers' study there was no associated detrimental effect on sperm production in most men (Ayers *et al.*, 1985).

A cause-effect relationship between fertility and endurance training is difficult to demonstrate because of the presence of life-style variables which may also influence the reproductive system. The hormonal milieu necessary for the initiation and maintenance of spermatogenesis in man is poorly understood. It remains to be clarified whether exercise related elevation of prolactin (which remain, however, within normal limits),

as demonstrated in this study, will be accompanied by a decrease in fertility. It may be argued that these alterations are subclinical in nature and do not warrant fertility concerns.

Further investigations are necessary to determine if testicular function is temporarily or permanently compromised by long periods of endurance training, similar to menstrual changes in female athletes, and what effect this has on fertility. Reduction in physical exercise in female athletes or an optimal diet is often enough to restore normal menstrual cycles. It needs to be determined by further studies if similar measures would improve semen parameters.

This study has demonstrated, as in a recent study (Acre *et al.*, 1993), that endurance training can modify hormonal profiles and semen parameters quantitatively and qualitatively in long distance runners. However, the pathophysiological mechanisms explaining the alterations in spermatogenesis are unclear but it is almost certainly multifactoral in nature and could include increased scrotal temperature, inadequate dietary intake for energy expenditure and nutritional deficiencies and not only due to endurance training. At present it is unclear whether the hormonal changes and alteration in semen parameters as defined in this study will cause a reduction in fertility potential and further studies in the future should address this issue.

CONCLUSION

This study demonstrated changes in both the hormonal profile and semen parameters in a cohort of male marathon runners. An important difference between this study and many of the previous studies performed was that this was a prospective, longitudinal study of a relatively large group of runners over a period of a year. In addition the runners were monitored regularly whilst they were preparing for a marathon.

Prolactin levels were noted to increase, a finding consistent with the study of Shangold, who noted increased prolactin concentrations in women runners after acute strenuous exertion (Shangold *et al.*, 1981). Although the progesterone levels fell significantly during the study, they remained with the normal range for males, as did all other hormonal and gonadotropin assays.

Precise semen analysis, mainly comprising volume, count, motility and morphology, remains the most important investigation of fertility potential in male marathon runners. In this study the mean semen volume decreased from December to April, but then increased again between April and August. The semen count remained unchanged throughout the study, but both the mean semen motility and mean normal morphology decreased between the beginning of the study and April, the month of maximum training.

At present it is unclear whether the laboratory changes observed in this study will result in the clinical expression of reduced fertility and further studies are required in this fascinating field of reproductive medicine.

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APPENDIX 1

Key to raw data tables

N/A Not available

Neg Negative

Pos Positive

Weight in kilogrames

BMI in kilogrames/meter squared

FSH in ng/ml

LH in ng/ml

E2 in nmol/l

Testosterone in ng/ml

Prolactin in ng/ml

Progesterone in nmol/l

Semen volume in mls.

Semen count in million/ml.

Motility in percentage

Morphology in percentage

Dist/week in kilometers/week

Patient Data

Table 17: Runner JA
Age: 41 years; Height 183cm

	December	January	February	April	May	August	November
Weight	83.5	82.5	83.5	82.0	84.5	84.5	84.5
BMI	24.9	24.6	24.9	24.9	24.5	25.2	25.2
Hormone							
FSH	3.3	3.9	3.9	3.6	3.5	3.7	3.6
LH	1.3	3.7	3.0	2.6	1.9	1.5	1.1
E2	235	279	327	275	313	282	244
Testosterone	13.3	16.6	15.1	13.8	13.1	17.6	13.0
Prolactin	3.9	3.1	2.0	1.8	2.9	4.0	3.1
Progesterone	0.7	0.4	0.5	0.4	0.5	0.3	0.4
Semen							
Volume	2.0	2.5	2.9	2.4	2.5	2.7	1.2
Count	30.0	31.0	44.0	32.0	34.0	37.0	35.0
Motility	65	60	40	60	60	55	55
Morphology	5	9	4	4	4	6	7
MAR	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Distance run per week	0	40	50	60	15	80	35

Table 18: Runner BC
Age: 46 years; Height 178cm

	December	January	February	April	May	August	November
Weight	72.0	71.5	69.8	71.0	71.0	N/A	N/A
BMI	22.7	22.6	22.0	22.4	22.4	N/A	N/A
Hormone							
FSH	3.1	3.1	2.9	2.9	3.0	N/A	N/A
LH	1.2	1.8	1.2	1.0	1.8	N/A	N/A
E2	279	334	256	429	375	N/A	N/A
Testosterone	13.2	13.6	14.0	13.4	14.8	N/A	N/A
Prolactin	2.2	2.0	2.5	3.1	3.3	N/A	N/A
Progesterone	0.9	0.8	0.7	0.4	0.5	N/A	N/A
Semen							
Volume	2.0	1.2	1.0	1.8	0.5	N/A	N/A
Count	425	260	490	750	740	N/A	N/A
Motility	60	55	40	40	50	N/A	N/A
Morphology	15	12	20	19	17	N/A	N/A
MAR	Neg	Neg	Neg	Neg	Neg	N/A	N/A
Distance run per week	60	75	70	70	60	N/A	N/A

Table 19: Runner HH
Age: 50 years; Height 173cm

	December	January	February	April	May	August	November
Weight	74.5	76.5	73.5	74.5	74.0	75.0	75.5
BMI	24.9	25.6	24.6	24.9	24.7	25.0	25.3
Hormone							
FSH	4.1	3.8	3.9	3.7	4.2	3.7	3.4
LH	3.2	2.6	2.3	2.5	2.6	2.6	3.1
E2	230	183	211	174	186	196	234
Testosterone	10.4	14.1	13.8	13.8	14.3	13.3	14.7
Prolactin	2.9	3.1	2.4	3.3	3.0	3.0	3.0
Progesterone	0.6	0.5	0.5	0.7	0.5	0.5	1.0
Semen							
Volume	3.3	4.0	5.7	4.8	3.5	4.0	4.0
Count	210	202	200	175	206	159	111
Motility	65	55	45	40	60	65	55
Morphology	21	20	20	14	16	13	16
MAR	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Distance run per week	60	60	80	100	110	55	55

Table 20: Runner BJ
Age: 31 years; Height 185cm

	December	January	February	April	May	August	November
Weight	87.2	85.5	82.5	82.5	84.0	89.5	90.5
BMI	25.5	25.1	24.1	24.1	24.6	26.2	26.5
Hormone							
FSH	3.6	4.2	3.9	4.3	3.8	4.0	3.1
LH	3.2	4.2	3.0	3.5	3.8	3.0	1.4
E2	284	245	279	272	247	243	242
Testosterone	16.0	15.0	19.4	22.9	23.4	20.3	13.2
Prolactin	3.4	2.6	5.2	4.8	6.6	6.1	5.4
Progesterone	1.2	0.9	0.7	1.0	0.7	0.6	0.4
Semen							
Volume	1.5	1.5	1.0	1.5	0.5	1.0	2.4
Count	93	45	19	70	4	18	103
Motility	55	40	35	45	40	35	60
Morphology	22	9	7	12	8	13	11
MAR	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Distance run per week	40	45	80	60	50	10	30

Table 21: Runner AB
Age: 46 years; Height 179cm

	December	January	February	April	May	August	November
Weight	82.0	78.0	80.0	81.0	N/A	83.0	86.5
BMI	25.6	24.4	25.0	25.3	N/A	25.0	27
Hormone							
FSH	5.0	5.5	5.9	5.5	N/A	5.7	5.1
LH	6.3	5.2	6.7	6.1	N/A	6.3	6.6
E2	325	334	315	394	N/A	339	363
Testosterone	20.1	25.3	22.5	24.3	N/A	22.1	18.6
Prolactin	2.9	5.8	5.3	8.5	N/A	12.0	10.0
Progesterone	0.8	0.9	0.7	0.6	N/A	1.0	0.5
Semen							
Volume	1.8	0.8	2.0	0.7	N/A	1.5	1.3
Count	20	1	52	35	N/A	46	47
Motility	45	65	55	50	N/A	50	45
Morphology	1	1	2	1	N/A	1	1
MAR	Neg	Neg	Neg	Neg	N/A	Neg	Neg
Distance run per week	40	30	40	20	N/A	10	0

Table 22: Runner RK
Age: 29 years; Height 175cm

	December	January	February	April	May	August	November
Weight	73.5	73.5	72.0	73.5	73.5	N/A	N/A
BMI	24.0	24.0	23.5	24.0	24.0	N/A	N/A
Hormone							
FSH	2.5	2.2	2.2	2.4	2.3	N/A	N/A
LH	3.8	3.0	1.4	1.6	1.9	N/A	N/A
E2	237	198	219	238	275	N/A	N/A
Testosterone	17.8	17.2	18.2	18.5	17.3	N/A	N/A
Prolactin	2.6	5.5	3.4	3.8	4.6	N/A	N/A
Progesterone	0.5	1.1	0.5	0.9	1.0	N/A	N/A
Semen							
Volume	2.3	1.4	1.8	1.8	3.8	N/A	N/A
Count	60	87	43	47	25	N/A	N/A
Motility	65	55	60	60	45	N/A	N/A
Morphology	26	22	20	28	25	N/A	N/A
MAR	Neg	Neg	Neg	Neg	Neg	N/A	N/A
Distance run per week	30	80	90	100	80	N/A	N/A

Table 23: Runner MK

Age: 32 years; Height 178cm

	December	January	February	April	May	August	November
Weight	70.0	69.0	68.8	N/A	70.0	70.0	70.0
BMI	22.1	21.8	21.7	N/A	22.1	22.1	22.1
Hormone							
FSH	2.5	3.1	3.2	N/A	3.1	3.2	2.5
LH	3.7	3.7	3.1	N/A	2.7	3.7	2.8
E2	284	340	328	N/A	352	311	493
Testosterone	13.9	16.8	18.5	N/A	20.6	19.1	23.1
Prolactin	2.2	2.8	2.6	N/A	2.4	3.3	2.9
Progesterone	0.9	1.0	0.7	N/A	0.8	0.7	0.8
Semen							
Volume	1.6	2.2	2.5	N/A	1.8	2.0	2.0
Count	135	151	142	N/A	121	45	145
Motility	60	55	60	N/A	65	60	65
Morphology	22	8	12	N/A	13	8	17
MAR	Neg	Neg	Neg	N/A	Neg	Neg	Neg
Distance run per week	50	100	160	N/A	0	100	110

Table 24: Runner RL

Age: 36 years; Height 174cm

	December	January	February	April	May	August	November
Weight	67.9	65.1	68.5	68.0	66.5	69.2	68.0
BMI	22.4	21.5	22.6	22.4	21.9	22.8	22.4
Hormone							
FSH	6.0	6.8	6.6	6.4	5.0	6.0	5.8
LH	5.0	5.8	7.8	4.1	4.5	5.6	5.3
E2	227	170	173	191	221	247	261
Testosterone	16.0	19.5	16.5	15.8	16.7	15.4	19.7
Prolactin	2.8	5.1	5.3	6.7	4.6	5.1	6.3
Progesterone	0.5	0.5	0.5	0.3	0.2	0.3	0.4
Semen							
Volume	1.4	2.3	2.0	1.8	2.0	1.8	0.8
Count	34	34	39	38	30	36	26
Motility	60	65	60	55	60	65	45
Morphology	20	19	14	13	15	16	21
MAR	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Distance run per week	30	100	115	125	50	75	75

Table 25: Runner IM
Age: 39 years; Height 171cm

	December	January	February	April	May	August	November
Weight	58.0	57.0	58.5	58.0	59.6	60.5	61.0
BMI	19.9	19.5	20.0	19.9	20.4	20.7	20.9
Hormone							
FSH	1.6	1.5	1.7	1.4	1.8	1.5	1.7
LH	1.7	1.0	0.8	1.1	1.7	0.9	1.3
E2	163	169	135	172	187	184	168
Testosterone	13.6	6.4	6.8	10.3	13.7	11.0	15.7
Prolactin	3.4	2.9	2.6	3.1	4.7	7.6	4.4
Progesterone	0.6	0.6	0.5	0.5	0.6	0.9	0.4
Semen							
Volume	3.2	2.0	3.8	3.2	2.8	3.3	2.4
Count	56	108	110	136	91	66	73
Motility	55	55	60	55	55	55	65
Morphology	22	21	22	21	19	19	17
MAR	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Distance run per week	110	100	100	110	125	100	85

Table 26: Runner CM
Age: 28 years; Height 163cm

	December	January	February	April	May	August	November
Weight	54.5	54.5	54.5	N/A	54.5	56.5	54.5
BMI	20.5	20.5	20.5	N/A	20.5	21.2	20.5
Hormone							
FSH	1.8	2.2	2.2	N/A	2.2	2.7	2.6
LH	2.2	1.7	4.1	N/A	1.9	2.1	3.8
E2	243	307	275	N/A	311	266	206
Testosterone	22.8	20.5	17.0	N/A	21.6	21.4	22.0
Prolactin	1.2	1.1	1.5	N/A	2.2	1.7	2.3
Progesterone	1.0	1.1	1.0	N/A	1.2	0.8	1.0
Semen							
Volume	N/A	4.5	5.0	N/A	3.0	3.3	N/A
Count	N/A	150	243	N/A	150	205	N/A
Motility	N/A	50	35	N/A	50	40	N/A
Morphology	N/A	15	11	N/A	10	22	N/A
MAR	N/A	Neg	Neg	N/A	Neg	Neg	N/A
Distance run per week	90	100	90	N/A	120	110	80

Table 27: Runner AP
Age: 25 years; Height 177cm

	December	January	February	April	May	August	November
Weight	79.5	83.5	83.5	85.5	83.0	89.0	89.0
BMI	25.4	26.7	26.7	27.3	26.5	28.4	28.4
Hormone							
FSH	2.7	2.6	2.5	2.8	2.6	2.2	2.4
LH	3.3	3.4	1.7	2.4	2.0	3.5	4.3
E2	370	279	296	302	333	315	301
Testosterone	13.1	8.9	7.9	9.2	8.0	10.6	10.4
Prolactin	1.9	1.9	3.1	4.1	3.7	4.4	4.3
Progesterone	0.8	0.7	0.4	0.2	0.4	0.2	0.4
Semen							
Volume	2.3	2.2	1.9	1.5	1.5	3.0	1.0
Count	220	154	97	124	166	156	63
Motility	60	65	55	60	65	55	55
Morphology	7	12	14	10	9	12	10
MAR	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Distance run per week	40	60	50	70	90	20	0

Table 28: Runner MP
Age: 53 years; Height 180.5cm

	December	January	February	April	May	August	November
Weight	72.5	71.0	71.8	71.0	70.5	73.5	72.0
BMI	22.4	21.9	21.2	21.9	21.8	22.7	22.2
Hormone							
FSH	6.0	5.3	5.5	5.7	6.1	5.6	6.2
LH	1.8	1.9	1.4	2.2	1.6	1.4	1.3
E2	229	247	199	197	175	208	237
Testosterone	17.0	8.9	5.9	6.4	7.2	6.6	6.3
Prolactin	1.2	4.0	2.2	2.0	3.9	2.5	2.5
Progesterone	0.7	0.7	0.9	1.0	0.6	0.3	0.5
Semen							
Volume	3.0	2.8	2.8	2.6	2.8	2.5	1.2
Count	430	172	138	146	146	168	44
Motility	50	50	60	40	45	50	50
Morphology	20	16	19	22	26	24	12
MAR	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Distance run per week	80	65	110	110	35	0	55

Table 29: Runner GR
Age: 31 years; Height 179cm

	December	January	February	April	May	August	November
Weight	75.4	73.5	74.0	73.6	75.0	75.0	N/A
BMI	23.7	23.0	23.1	23.0	23.4	23.4	N/A
Hormone							
FSH	4.8	8.2	5.3	5.9	5.8	6.5	N/A
LH	4.5	4.2	4.4	3.4	5.5	5.3	N/A
E2	239	122	296	211	316	276	N/A
Testosterone	20.6	19.4	19.4	19.2	17.4	15.9	N/A
Prolactin	5.2	3.8	5.1	7.1	7.7	7.2	N/A
Progesterone	1.7	0.9	1.5	1.2	1.1	0.8	N/A
Semen							
Volume	3.8	2.6	3.3	3.4	3.2	3.0	N/A
Count	45	11	33	39	10	7	N/A
Motility	50	50	45	50	50	50	N/A
Morphology	15	N/A	4	7	2	3	N/A
MAR	Neg	Neg	Neg	Neg	Neg	Neg	N/A
Distance run per week	60	80	100	110	110	40	N/A

Table 30: Runner FS
Age: 35 years; Height 163cm

	December	January	February	April	May	August	November
Weight	53.5	55.0	55.5	54.0	56.0	57.5	57.0
BMI	20.1	20.7	20.9	20/3	21.1	21.6	21.4
Hormone							
FSH	3.5	3.2	2.6	2.8	3.2	2.8	3.1
LH	2.0	1.6	1.3	1.4	1.7	1.5	1.7
E2	237	223	259	226	223	211	221
Testosterone	11.3	13.8	16.6	9.3	16.1	14.2	17.5
Prolactin	2.6	4.7	6.4	4.8	7.1	6.5	4.8
Progesterone	0.5	0.8	0.4	0.4	0.5	0.4	0.4
Semen							
Volume	2.8	3.2	1.7	2.5	2.0	3.3	2.8
Count	132	74	40	57	65	185	130
Motility	65	50	65	60	65	50	50
Morphology	23	4	14	10	15	15	15
MAR	Neg	Neg	Pos	Pos	Pos	Pos	Pos
Distance run per week	80	100	80	30	0	30	50

Table 31: Runner RS
Age: 37 years; Height 178cm

	December	January	February	April	May	August	November
Weight	72.0	70.5	N/A	70.5	73.5	N/A	71.5
BMI	22.7	22.2	N/A	22.2	23.2	N/A	22.6
Hormone							
FSH	2.0	2.2	N/A	1.9	2.1	N/A	1.9
LH	2.3	2.3	N/A	1.5	2.0	N/A	2.4
E2	227	193	N/A	211	257	N/A	268
Testosterone	9.8	11.7	N/A	11.6	9.6	N/A	12.9
Prolactin	2.4	3.0	N/A	5.0	5.0	N/A	4.9
Progesterone	0.9	1.2	N/A	1.1	0.9	N/A	1.2
Semen							
Volume	1.2	0.8	N/A	0.4	0.8	N/A	0.4
Count	73	107	N/A	25	25	N/A	26
Motility	65	65	N/A	50	65	N/A	50
Morphology	22	23	N/A	14	23	N/A	18
MAR	Neg	Neg	N/A	Neg	Neg	N/A	N/A
Distance run per week	80	100	N/A	100	40	N/A	0

Table 32: Runner HS
Age: 38 years; Height 178cm

	December	January	February	April	May	August	November
Weight	65.7	66.0	65.0	65.0	63.0	64.8	67.0
BMI	20.7	20.8	20.5	20.5	19.9	20.4	21.1
Hormone							
FSH	3.4	2.7	3.3	3.4	3.4	3.1	3.3
LH	2.9	2.7	3.5	2.8	3.4	1.5	1.3
E2	239	234	263	244	237	283	211
Testosterone	10.7	10.7	17.5	17.8	5.5	9.2	5.8
Prolactin	2.1	5.0	4.3	4.0	3.7	2.9	2.7
Progesterone	1.4	0.7	1.3	1.1	0.9	0.9	0.4
Semen							
Volume	4.6	4.0	4.5	2.4	4.8	4.8	5.3
Count	140	188	56	17	18	1	28
Motility	45	45	55	40	50	N/A	45
Morphology	16	11	7	12	4	N/A	6
MAR	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Distance run per week	0	0	45	50	60	0	0

Table 33: Runner GS
Age: 32 years; Height 180cm

	December	January	February	April	May	August	November
Weight	69.0	69.0	67.5	66.5	67.0	69.0	68.5
BMI	21.3	21.3	20.8	20.5	20.7	21.3	21.1
Hormone							
FSH	3.3	3.6	3.4	3.2	3.5	3.4	3.1
LH	3.0	2.4	2.8	4.1	2.9	3.1	3.3
E2	192	199	192	240	207	223	249
Testosterone	18.9	15.1	14.2	14.2	14.2	12.5	13.9
Prolactin	2.7	5.1	3.9	4.8	4.2	3.1	5.6
Progesterone	1.3	1.8	1.6	1.6	1.3	1.0	1.1
Semen							
Volume	3.3	2.3	2.2	2.8	1.5	3.0	3.5
Count	290	152	330	170	230	260	164
Motility	60	55	55	70	60	60	55
Morphology	16	10	13	16	15	21	20
MAR	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Distance run per week	60	75	70	65	50	40	40

Table 34: Runner DT
Age: 30 years; Height 176cm

	December	January	February	April	May	August	November
Weight	76.5	77.0	75.0	76.0	N/A	78.0	77.5
BMI	24.8	24.9	24.3	24.6	N/A	25.2	25.1
Hormone							
FSH	3.9	4.0	3.7	3.5	N/A	4.6	3.7
LH	3.8	2.7	2.4	1.3	N/A	2.4	1.9
E2	248	212	266	230	N/A	334	246
Testosterone	16.2	15.0	16.2	13.7	N/A	18.0	11.8
Prolactin	2.8	7.9	9.3	7.8	N/A	5.0	3.9
Progesterone	1.4	1.6	1.1	1.5	N/A	1.2	1.0
Semen							
Volume	4.3	2.2	2.0	3.0	N/A	5.0	2.0
Count	137	118	126	155	N/A	185	103
Motility	60	55	40	55	N/A	65	60
Morphology	23	13	18	13	N/A	25	16
MAR	Neg	Neg	Neg	Neg	N/A	Neg	Neg
Distance run per week	30	40	70	10	N/A	40	30

Table 35: Runner PvdL
Age: 40 years; Height 174cm

	December	January	February	April	May	August	November
Weight	92.5	93.3	92.0	92.5	91.7	93.5	94.5
BMI	30.5	30.8	30.4	30.5	30.3	30.9	31.2
Hormone							
FSH	3.1	2.9	3.6	4.1	2.8	3.1	3.1
LH	2.0	2.4	2.8	4.3	3.4	2.7	2.4
E2	100	133	153	144	212	206	188
Testosterone	10.9	11.5	11.5	10.2	12.5	13.8	9.6
Prolactin	2.4	3.4	3.6	2.9	1.7	2.0	2.8
Progesterone	0.5	0.7	1.0	0.7	0.7	0.9	0.4
Semen							
Volume	1.2	1.0	1.8	0.5	1.5	2.8	1.5
Count	96	44	119	103	100	270	172
Motility	60	60	50	40	60	40	40
Morphology	20	17	16	21	15	8	14
MAR	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Distance run per week	40	70	80	80	100	70	25

Table 36: Runner BvZ
Age: 47 years; Height 190cm

	December	January	February	April	May	August	November
Weight	83.0	84.5	84.0	82.0	81.7	82.2	85.0
BMI	23.0	23.4	23.3	22.7	22.6	22.8	23.5
Hormone							
FSH	3.2	2.9	3.0	3.0	3.3	2.9	2.9
LH	2.8	0.8	0.9	1.6	1.4	1.0	3.3
E2	293	279	327	275	313	282	244
Testosterone	12.5	10.4	11.2	12.0	12.3	13.4	12.8
Prolactin	1.9	4.8	5.2	4.3	4.7	5.0	4.5
Progesterone	0.4	0.6	0.7	0.4	0.5	0.5	0.7
Semen							
Volume	2.0	1.8	2.5	2.2	2.8	2.8	1.5
Count	32	52	72	86	38	26	62
Motility	50	65	65	45	60	55	60
Morphology	11	9	7	10	3	2	3
MAR	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Distance run per week	40	60	50	65	65	40	40

Table 37: Runner JvZ
Age: 46 years; Height 174cm

	December	January	February	April	May	August	November
Weight	69.5	68.0	69.5	69.5	N/A	72.0	72.5
BMI	22.9	22.4	22.9	22.9	N/A	23.8	23.9
Hormone							
FSH	5.9	6.2	6.5	6.8	N/A	6.5	5.5
LH	2.4	2.7	3.0	3.3	N/A	2.7	1.8
E2	280	230	312	133	N/A	295	261
Testosterone	19.6	16.9	21.2	16.2	N/A	17.7	13.1
Prolactin	1.9	4.8	5.6	4.5	N/A	3.1	4.4
Progesterone	0.6	1.2	0.7	0.2	N/A	0.4	0.3
Semen							
Volume	5.3	3.8	2.8	3.0	N/A	5.0	3.0
Count	3	14	11	14	N/A	12	5
Motility	60	50	65	60	N/A	60	65
Morphology	4	3	5	2	N/A	5	2
MAR	Neg	Neg	Neg	Neg	N/A	Neg	Neg
Distance run per week	0	30	50	55	N/A	10	50

Table 38: Runner GW
Age: 25 years; Height 186cm

	December	January	February	April	May	August	November
Weight	82.4	79.2	78.6	83.3	82.5	83.5	85.0
BMI	23.8	22.9	22.7	24.1	23.8	24.1	24.6
Hormone							
FSH	2.1	2.6	2.2	2.3	2.4	2.3	2.2
LH	2.0	2.6	2.2	1.6	3.7	2.3	2.8
E2	173	286	211	222	254	216	221
Testosterone	10.3	14.1	19.1	20.1	18.7	18.8	14.7
Prolactin	3.2	3.4	3.3	5.4	4.3	2.8	2.7
Progesterone	0.8	0.5	0.7	0.7	0.6	0.3	0.3
Semen							
Volume	6.8	9.0	6.3	5.2	4.5	5.0	4.8
Count	49	73	37	31	39	18	15
Motility	60	65	60	65	60	45	65
Morphology	9	14	10	14	8	6	9
MAR	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Distance run per week	20	70	60	50	20	20	10

Table 39: Runner GWa
Age: 46 years; Height 174.5cm

	December	January	February	April	May	August	November
Weight	79.0	80.5	78.0	81.0	80.0	81.0	84.0
BMI	26.1	26.6	25.7	26.7	26.4	26.7	27.7
Hormone							
FSH	7.4	7.6	7.0	5.6	5.6	5.1	7.5
LH	4.8	1.7	5.7	1.6	1.6	1.1	2.4
E2	246	197	218	245	172	183	249
Testosterone	10.3	9.5	10.1	13.4	9.6	9.4	12.7
Prolactin	2.6	2.4	3.6	3.8	2.2	3.5	4.0
Progesterone	0.7	0.5	0.5	0.3	0.3	0.3	0.3
Semen							
Volume	4.0	4.0	4.2	5.2	4.3	5.0	4.3
Count	11	10	15	15	23	11	7.6
Motility	60	60	60	50	45	40	40
Morphology	6	7	3	6	7	9	4
MAR	Pos	Pos	Pos	Pos	Pos	Pos	Pos
Distance run per week	0	15	60	80	30	15	0

Table 40: Runner DW
Age: 54 years; Height 178cm

	December	January	February	April	May	August	November
Weight	89.0	89.0	N/A	N/A	N/A	N/A	N/A
BMI	28.2	28.2	N/A	N/A	N/A	N/A	N/A
Hormone							
FSH	2.4	2.6	N/A	2.6	3.1	N/A	N/A
LH	1.0	2.1	N/A	1.6	3.0	N/A	N/A
E2	266	333	N/A	282	302	N/A	N/A
Testosterone	13.7	15.2	N/A	18.9	17.2	N/A	N/A
Prolactin	1.9	2.5	N/A	3.5	4.0	N/A	N/A
Progesterone	0.3	0.4	N/A	0.6	0.3	N/A	N/A
Semen							
Volume	2.5	2.5	N/A	2.5	3.0	2.0	2.0
Count	57	64	N/A	52	34	188	155
Motility	45	50	N/A	60	55	50	60
Morphology	19	6	N/A	9	15	11	17
MAR	Neg	Neg	N/A	Neg	Neg	Neg	Neg
Distance run per week	60	60	N/A	N/A	N/A	N/A	N/A