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Annual patterns of luteinizing hormone, follicle stimulating hormone, testosterone and inhibin in normal men

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Reproductive functions in most animals demonstrate seasonal fluctuations that allow young to be born at a time of the year favourable for their survival. Whether there is a seasonal change in the human reproductive system is unclear. In the present study, we measured serum concentrations of luteinizing hormone, follicle stimulating hormone, testosterone and inhibin in the same 16 normal men sampled monthly for 1 year. A statistically significant increase in all four measured hormones was found in June, with a nadir in August. Our findings suggest that a circannual rhythm of gonadotrophins and testicular hormones exists in normal men. The mechanism leading to this rhythm and the importance of the rhythm in human biology are unknown.

Key words: circannual/hormones/reproductive function

Introduction

In most mammals, reproductive function is affected by season, an effect that has probably evolved to allow young to be born at a time of the year when food and climatic conditions are favourable for their survival (Lincoln, 1989). Such seasonal variations are mediated through the hypothalamus under the influence of a variety of external factors. The environmental photoperiod seems to play a major role in the synchronization of these cycles in most species (Lincoln, 1989).

Although reproduction in humans is not obviously seasonal, evidence for circannual rhythms of the human reproductive function has been presented, including observations on serum hormones, testicular and ovarian function, ovum and zygote quality and endometrium receptivity. Indirect evaluations of the human reproductive function through the measurements of births and sexual behaviour have also been published (Udry and Morris, 1967; Parkes, 1968; Smolensky, 1980; Martikainen *et al.*, 1985; Kauppila *et al.*, 1987; Reinberg *et al.*, 1988; Dabbs, 1990; Levine *et al.*, 1990; Broder *et al.*, 1991). An interesting association between geographic latitude and the acrophase in births has been reported (Smolensky, 1980). Further evidence of a circannual rhythm of the human sexual function comes from records on frequency of sexual intercourse, reports of rapes, sexual activity, sexually transmitted disease and the sale of contraceptives (Udry and Morris, 1967; Smolenski *et al.*, 1981). Taken together, these data have suggested the presence of a physiological rhythm of reproductive function. However, analyses of these variations throughout the year are difficult to evaluate because of the confounding influence of social, religious and cultural influences.

In most studies, investigation of testicular function, as measured by semen analysis, has shown a slight reduction in seminal parameters in the summer (Tjoa *et al.*, 1982; Mortimer *et al.*, 1983; Levine *et al.*, 1988, 1990; Politoff *et al.*, 1989; Saint Pol *et al.*, 1989; Spira and Ducot, 1989; Broder *et al.*, 1991). Whether this reported summer decrease in sperm production is due to changes in the concentrations of pituitary hormones controlling the testes, or is the result of the effect of temperature on testicular function, is not clear. Previous data on reproductive hormone concentrations throughout the year are scanty and often inconsistent (Smals *et al.*, 1976; Reinberg and Lagoguey, 1978; Martikainen *et al.*, 1985; Dabbs, 1990). Inhibin concentrations throughout the year have never been reported. In this study, we measured monthly serum concentrations of pituitary gonadotrophins and testicular hormones in the same 16 normal men for an entire year. Our results provide direct evidence that a circannual rhythm of production of luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone and inhibin exists in normal men.

Materials and methods

Subjects

A total of 16 men (age range 19–42 years, mean 29 years) were studied. Our subjects were defined as normal, based on medical history, physical examination, blood chemistry and urinalysis, and normal blood concentrations of LH, FSH, testosterone and inhibin. In particular, there was no history of reproductive problems. The men were within normal limits of height and weight for their age according to life insurance tables (Metropolitan Insurance Company, 1959). They were not smokers or alcohol abusers, and they were taking no medications.

Experimental protocols

Monthly blood samples were drawn from each man throughout a 12 month period for LH, FSH, testosterone and inhibin measurements. Each man started the study at a different time of the year. Samples were collected in the afternoon and sampling time was kept constant throughout the study period. Eight men performed the study in the year 1980–1981 and eight in the year 1986–1987. Samples clotted at room temperature, were centrifuged and the serum was separated,

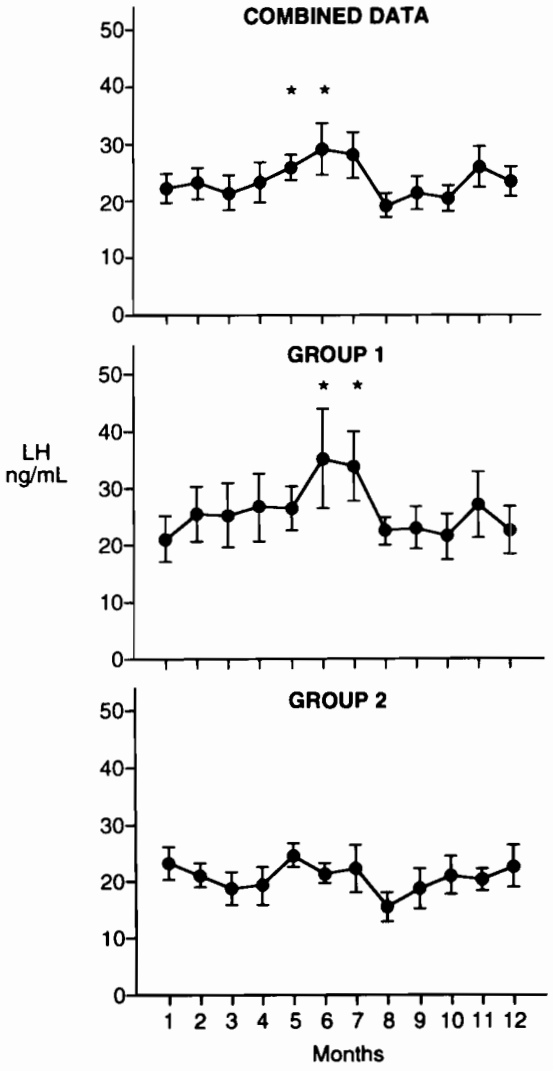

Figure 1. Mean (±SEM) serum concentrations of luteinizing hormone in each of the two groups of men (middle and lower panels) and in the two groups combined (upper panel) throughout 1 year. * = significantly different from the other months of the year ($P < 0.05$).

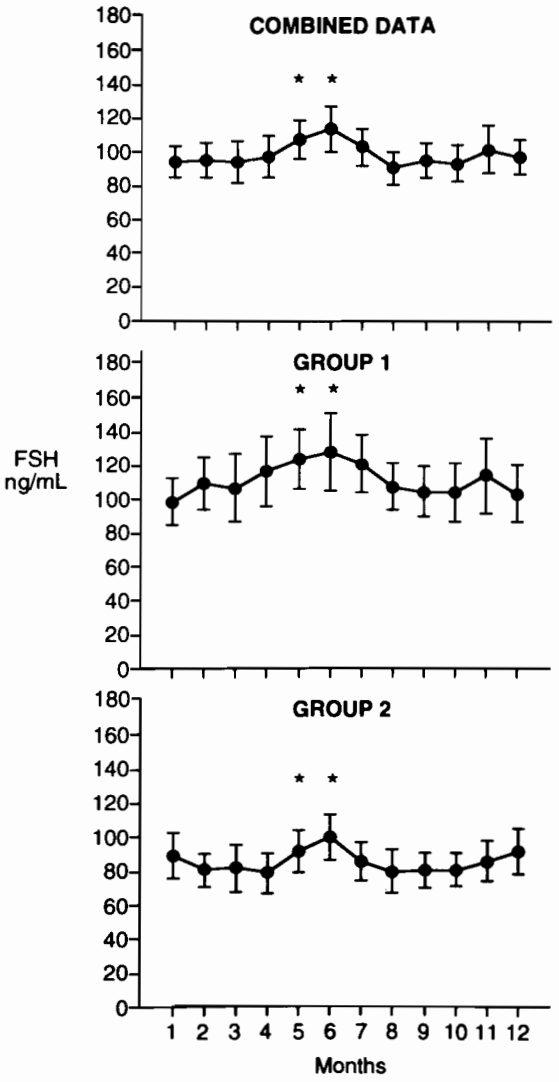

Figure 2. Mean (±SEM) serum concentrations of follicle stimulating hormone in each of the two groups of men (middle and lower panels) and in the two groups combined (upper panel) throughout 1 year. * = significantly different from the other months of the year ($P < 0.05$).

frozen and stored at –20°C until the assays were performed. All the samples were run in the same assay for all the subjects.

Hormone assays

All samples were analysed in duplicate by radioimmunoassay for inhibin, testosterone, LH and FSH. The radioimmunoassays for FSH, LH and testosterone have been described previously (Matsumoto *et al.*, 1983). Stability of these hormones in serum has been proved (Henderson *et al.*, 1988; McLachlan *et al.*, 1988; Bolelli *et al.*, 1995). All samples from each man were assessed in a single assay. Serum inhibin concentration was measured in duplicate as previously described (McLachlan *et al.*, 1988) in an heterologous double antibody radioimmunoassay using purified 31 kDa bovine follicular fluid inhibin as a tracer and antigen to generate antiserum. A serum pool from women undergoing ovarian stimulation for in-vitro fertilization was used as a standard. The sensitivity of this assay was 117 IU/l, with an inter-assay variability of 10.4%. The intra-assay variability was 3.3% in the middle range of the male quality control serum. Transforming growth factor β, bovine activin-A and bovine Müllerian inhibitory substance had <1% cross-reactivity. An inhibin precursor (termed pro-αc) strongly cross-reacts with this radioimmunoassay (Robertson *et al.*, 1989). However, whether pro-αc is present in human peripheral blood has not been determined.

Statistics

All the data were log-transformed to obtain a normal distribution before statistical analysis was performed. The two data sets, each for eight men in different years, were pooled and the monthly mean of the hormonal values of all 16 men was determined over a period of 12 months. Analysis of variance with repeated measures was used, followed by Duncan's multiple range test to determine hormone concentration differences between the months.

Results

Mean (±SEM) LH, FSH, testosterone and inhibin concentrations in each of the two groups of subjects separately and combined are shown in Figures 1–4. No significant difference in the hormonal pattern was detected between the two groups.

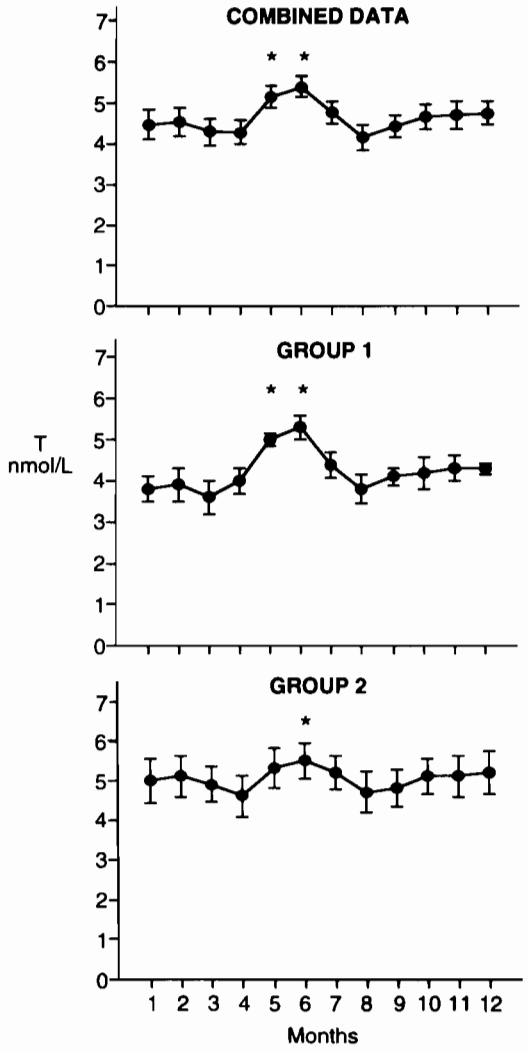

Figure 3. Mean (±SEM) serum concentrations of testosterone in each of the two groups of men (middle and lower panels) and in the two groups combined (upper panel) throughout 1 year. * = significantly different from the other months of the year ($P < 0.05$).

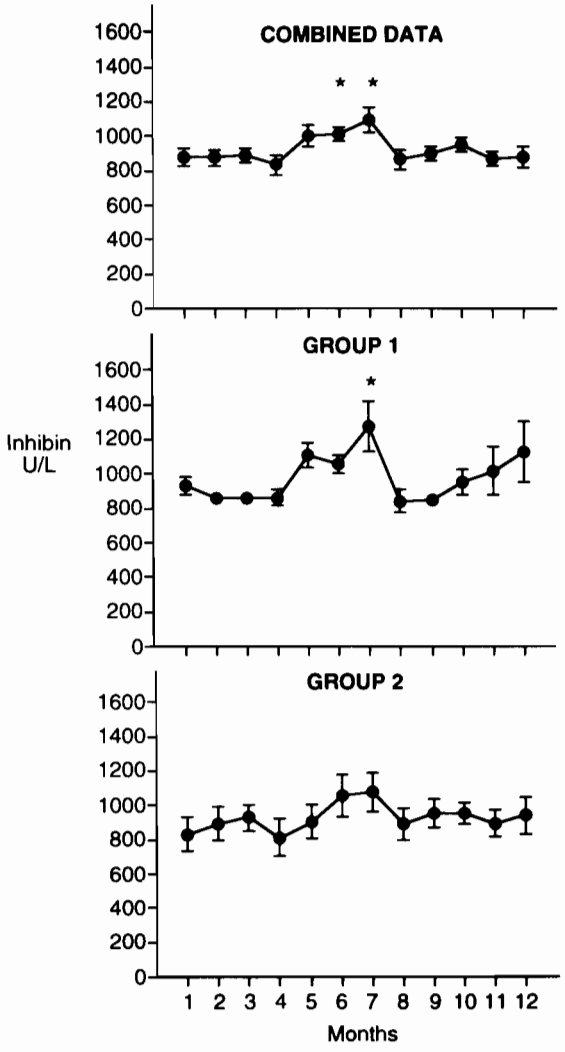

Figure 4. Mean (±SEM) serum concentrations of inhibin in each of the two groups of men (middle and lower panels) and in the two groups combined (upper panel) throughout 1 year. * = significantly different from the other months of the year ($P < 0.05$).

LH concentrations were significantly higher in June and July than in the other months in group 1 and in May and June when the two groups were combined (Figure 1). FSH concentrations were significantly increased in May and June compared to the other months of the year when the single groups were analysed separately and when they were combined (Figure 2). Testosterone concentrations in group 1 were significantly higher in May and June compared to the other months and in June in group 2 (Figure 3). May and June testosterone concentrations were significantly higher than those of the other months when the two groups were combined. Inhibin concentrations were significantly increased in July in group 1 and in June and July when both groups were combined (Figure 4).

Discussion

These data imply that pituitary and testicular hormones undergo circannual variations in men. Serum concentrations of LH, FSH, testosterone and inhibin all increased at the same time of year (June), suggesting that a circannual rhythm, presumably depending on the central nervous system, may cause the increase of the pituitary hormones and, consequently, of the testicular hormones.

Although reproduction in humans is not considered to be seasonal, various hormones, such as growth hormone and prolactin, undergo variation throughout the year in men (Martikainen *et al.*, 1985; Abbaticchio *et al.*, 1986; Malarkey *et al.*, 1991). The physiological significance and the biological mechanism(s) of these variations are not clear. Our results provide strong evidence that a circannual rhythm of reproductive hormones is also present in men. The absence of a difference among the two sets of samples drawn in different years suggests that this is a cyclic phenomenon that recurs every year. The increase of reproductive hormones during the spring might be driven directly by the hypothalamus under the influence of some unknown endogenous or exogenous factor. Alternatively, one of these factors might act directly at the pituitary level, affecting the sensitivity of the gland to gonadal

steroids or to gonadotrophin-releasing hormone. The gonadal products (inhibin and testosterone) begin to increase in May, probably under the stimulation of increasing gonadotrophin concentrations; testosterone peaks in June and inhibin reaches the highest concentration in July.

Our work is in agreement with a previous study performed in Finland (Martikainen *et al.*, 1985) that reported increasing FSH and LH concentrations in May. In this study, testosterone concentrations showed a slight, though not statistically significant increase in July. However, different and inconsistent results have been reported by other authors. The geographical location where the studies were performed may account for some differences. The collection of an inadequate number of samples (in particular, the assessment of only one sample from each man), as well as differences in the screening of the subjects, are probably the most relevant factors that may account for the inconsistencies in these studies. Analysing the archival data from 4462 military veterans, Dabbs (1990) found a seasonal peak of testosterone in the early winter, with a shift from November to January in older subjects. Reinberg *et al.* (1988) measured serum hormone concentrations in 207 subjects participating in a pre-vasectomy study. He found a peak of plasma LH, testosterone and oestradiol in the fall, while FSH peaked in the summer. In this study, it was not stated whether the subjects were normal, and their ages were not fully described. In neither of these studies were the same men studied throughout the year; the studies are therefore difficult to interpret, due to the numerous and uncontrollable variables that can affect those data. Touitou *et al.* (1983) found higher LH concentrations in late spring, and peak values of FSH in October; however, he performed blood samples in seven young men in only four different months throughout the year.

The biological mechanism(s) underlying seasonal hormonal rhythms is not understood. In animals, the length of daylight is believed to play a primary role in regulating reproductive rhythms, probably acting through a variation in melatonin secretion from the pineal gland (Wickings and Nieschlag, 1980; Tamarkin *et al.*, 1985; Clarke and Horton, 1989; Reiter, 1991). Whether the length of daylight affects human reproductive function and whether the pineal gland, through the secretion of melatonin, is the interface between the two systems, is not clear. Melatonin modifies its circadian rhythm in response to the variation of the day-length (Wehr, 1991), and a significant circannual melatonin rhythm, characterized by increasing concentrations in winter, has been reported (Martikainen *et al.*, 1985; Kivela *et al.*, 1988). Impaired ovarian function in winter has been reported in the arctic regions, where there is a great difference in the length of daylight between summer and winter (Kauppila *et al.*, 1987). Therefore, changes in the day-length, modifying the duration of nocturnal melatonin secretion, might act as stimulatory or inhibitory signals for the reproductive system. However, humans are usually exposed to a fixed photoperiod by virtue of artificial light. Therefore, it is unclear whether seasonal changes of the photoperiod can still affect human melatonin secretion and hence the human reproductive system.

In animals, seasonal variations of reproductive function result from endogenous as well as environmental factors. These rhythms are probably important for the survival of the species, allowing young to be born at the most favourable time of year for their survival. Whether circannual rhythms play a similar role in humans is unknown. It may be speculated that the presence of slight seasonal variations in human reproductive hormones can be considered a vestigial physiological adjustment to environmental conditions that might have been important for the development and survival of the human species (Dobzhansky, 1955; Smolensky, 1980). These hormonal changes throughout the year must be taken into account when designing studies involving measurements of reproductive hormones.

In conclusion, circannual variations of the reproductive function in animals are considered adaptive phenomena related to environmental conditions. Our data show the existence of a seasonal rhythm of reproductive hormones concentrations in men throughout the year. The mechanisms leading to this rhythm and the importance of the rhythm in human biology are unknown.

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