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Sykes, Jean Crane

PROGESTERONE RESPONSE OF FEMALES PARTICIPATING IN A PROGRESSIVE RUNNING PROGRAM

The University of North Carolina at Greensboro PH.D. 1986

PROGESTERONE RESPONSE OF FEMALES PARTICIPATING IN A PROGRESSIVE RUNNING PROGRAM

by

Jean Crane Sykes

A Dissertation Submitted to
The Faculty of the Graduate School at
The University of North Carolina at Greensboro
in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Greensboro
1986

Approved by

Dissertation Adviser

APPROVAL PAGE

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ABSTRACT

SYKES, JEAN CRANE, Ph.D. Progesterone response of females participating in a progressive running program. (1986). Directed by Terry L. Bazzarre, Ph.D. 167 pp.

To assess plasma progesterone (P) response to progressive exercise, 17 female recreational runners participated in a study spanning 7 menstrual cycles. Control subjects (C) (n=8) maintained their usual physical activity at baseline levels (14-19 mi/week), while experimental subjects (E) increased their average mileage from 20 to 32 mi/week. Blood samples drawn every other day during the 1st and 7th menstrual cycles, beginning on Day 10 of each cycle, were analyzed for luteinizing hormone and P. Activity and diet records for Days 3-10 of the 1st, 3rd, 5th, and 7th menstrual cycle provided estimates of energy balance. Coinciding with these records were 24-hr urine samples, which were analyzed for 3methylhistidine (3-MH). Subjects consumed a lacto-ovo vegetarian diet required for measurement of 3-MH. Among E subjects, the median number of days in which P concentration was greater than 6.0 ng/ml declined from 5.0 days at the 1st menstrual cycle to 0.0 days by the 7th cycle ($p<.05$). A significant decrease in the P-vs-time area under the curve was observed among C subjects ($p<.05$). There were no statistically significant differences between C and E with respect to the degree of change in P concentrations, suggesting that the progressive exercise of the E subjects was not responsible for alterations in P. All subjects

maintained relatively constant body weight and fat levels. No evidence of energy deficit or protein inadequacy was apparent from dietary analyses, estimates of energy expenditure, or from values of urinary 3-MH excretion. Results of the study suggest that some unidentified factor (possibly dietary) influenced menstrual function among these female runners. A more rapid progression of exercise, as well as a more in-depth exploration of dietary variables is recommended for future research.

ACKNOWLEDGEMENTS

The allocation of research resources by the following persons is gratefully acknowledged:

°Dr. Alan Rogol (University of Virginia School of Medicine)

°Dr. Johannes Veldhuis (University of Virginia School of Medicine)

°Dr. Terry Bazzarre (University of North Carolina at Greensboro Department of Nutrition)

°Dr. Lynis Dohm (East Carolina University School of Medicine)

Contribution to financial support of the research project was also provided by Sigma Xi Scientific Research Society. Additional acknowledgements I wish to make include the following.

°Shih-Min Wu and Ed Tapscott, for sharing their expertise in laboratory procedures, and for brightening my long hours spent in the lab.

°Alan Rogol, for his help in research design; and Al and his staff, for many hours of hormone analyses.

°Terry Bazzarre, for his assistance throughout the research project, and for his editorial assistance in the preparation of the dissertation.

°Dissertation Committee members Betsy Schiller, Diane Spitler, and Karen Graves, and former Committee member Mike Liebman, for their generosity and willingness to help.

°Coinvestigators Bill Landis, Henry Trollinger, and Bonnie Irvin, for all of their time, energy, and efforts contributed to the study.

°Marje Martin, for her generous provision of statistical consultation and her active interest in the study from beginning to end.

°My husband, Wayne Sykes, for his patience, and for his constant support and encouragement.

°Above all, I wish to thank the runners who participated in the study, for their enthusiasm, hard work, and sacrifices.

* * * * * * * * * *

This dissertation is dedicated to my parents, Ruth and Frank Crane, who taught me the importance of setting high standards, and of completing a task begun.

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CHAPTER I

INTRODUCTION

Women have become increasingly active in sports and exercise activities in recent years. Since the passage of Title IX of the Education Amendments Act of 1972 (Bodnar & Bodnar, 1980), there have been more opportunities for women to participate in sports. There has been a growing sense of societal acceptance of female athleticism, as well as greater recognition of the physical and athletic abilities of women. Encouraging women to become more physically active has been an emerging interest of society in preventive health care.

As a result of the increased participation of women in sports and exercise activities, greater attention has been focused on the relationship between physical activity and female reproductive function. Investigations of exercise-related menstrual irregularities and athletic amenorrhea have led to a better understanding of the phenomenon; however, the etiological mechanisms remain obscure.

The initial objective of the present study was to test the assumption that reproductive hormone function is affected by a progressively vigorous running program over a period spanning 7 menstrual cycles. Fulfilling that assumption, the hypotheses to be tested were as follows.

Among females showing evidence of menstrual dysfunction secondary to exercise training (as indicated by alterations in reproductive hormone concentrations):

1. There is a greater energy drain (ratio of energy expenditure to energy intake) than among eumenorrheic females participating in a similar exercise program.

2. There is a higher prevalence of negative protein balance than in eumenorrheic females in a similar program.

3. There is a negative correlation between energy drain and hypothalamic and pituitary function, as indicated by urinary luteinizing hormone.

CHAPTER II

REVIEW OF LITERATURE

To help clarify the distinction between normal menstrual status and that of oligo- and amenorrheic athletes, the following review of literature was developed. This review includes an overview of menstrual physiology as well as a description of the criteria that have been used to define eumenorrhea, oligomenorrhea, and amenorrhea. Following this background information is a description of the hormonal characteristics reported to be associated with exercise-related menstrual disturbances.

Various estimates of the prevalence of exercise-associated menstrual irregularities are summarized, and the potential ramifications of oligomenorrhea and amenorrhea are discussed. Finally, the mechanisms that have been proposed to explain the development of exercise-related menstrual irregularities are reviewed.

Overview of Menstrual Physiology

The menstrual cycle consists of two phases: follicular and luteal. The follicular phase begins on the first day of menstruation (Day 1 of the cycle) and extends until ovulation. The follicular phase is so named because of the presence of the ovarian follicle, which is the structure containing the ovum. (Numerous follicles respond to hormonal stimulation for growth and development, but

usually only one follicle survives to ovulate, the "dominant" follicle that matures most rapidly.) After expulsion of the egg at ovulation, the remains of the ovarian follicle is called a corpus luteum; hence the name "luteal" for the second phase of the cycle. The luteal phase begins at ovulation and lasts until Day 1 of the next menstrual cycle.

There are distinct hormonal patterns associated with each phase of the menstrual cycle. The pituitary hormones luteinizing hormone (LH) and follicle-stimulating hormone (FSH) play an essential role in the development of menstrual events. These two glycoproteins are regulated by gonadotropin-releasing hormone (GnRH), a decapeptide that is produced and secreted by the hypothalamus.

During the early follicular phase, the primary functions of FSH are to promote follicular maturation, and to cause estrogen synthesis (Shangold, 1984). The estrogen precursors are androgens originating in the theca cells of the follicle, production of which is regulated by LH. The androgens migrate to the granulosa cells where FSH causes aromatization (McNatty, Makris, DeGrazia, Osathanondh, & Ryan, 1979). The rising estrogen concentration resulting from aromatization within the granulosa cells fosters the development of FSH receptor sites on the granulosa cells. Circulating levels of estrogen modulate GnRH and gonadotropin production critical to follicular development (Speroff, Glass, & Kase, 1983, chap. 3).

The estrogen concentration of the dominant follicle (probably the follicle that is most mature) continues to rise. Locally, the high estrogen concentration further promotes the development of

FSH receptors. Thus, despite a fall in circulating FSH, the concentration of the hormone remains high within the follicle. The decrease in plasma FSH levels is attributable to the high estrogen levels. With fewer FSH receptor sites, the less-developed follicles begin to degenerate, leaving the dominant follicle to thrive in the estrogen-rich microenvironment it is able to sustain for itself (Speroff et al., 1983, chap. 3).

There is a pronounced rise in estrogen concentrations just prior to ovulation, which initiates the midcycle LH surge. This abrupt elevation of LH triggers an increase in follicular progesterone production, which is evident as a small but significant rise in circulating progesterone levels 12-24 hours before ovulation (Moghissi, Syner, & Evans, 1972). The progesterone surge is believed to initiate a less precipitous surge in FSH (Shaw, Butt, & London, 1975), the significance of which is poorly understood. The high LH concentrations late in the follicular phase stimulate ovulation, the rupture of the follicle and expulsion of the ovum.

The remains of the follicle undergo histologic changes, forming the corpus luteum. The corpus luteum is capable of secreting large quantities of both estrogen and progesterone, which peak about 8 or 9 days postovulation. Estrogen's primary function is to cause proliferation of the endometrium, the lining of the uterus. Progesterone helps to further prepare the endometrium for implantation of a fertilized ovum. The lifespan of the corpus luteum is dependent on its capacity for steroid synthesis, which, in turn, is dependent upon

tonic LH secretion (Speroff et al., 1983, p. 92). Gonadotropin release is inhibited by the high concentrations of estrogen and progesterone during the luteal phase (Shangold, 1984). In the absence of fertilization, the corpus luteum begins to degenerate 9-11 days after ovulation, the mechanism of which is unknown. (If the ovum is fertilized, human chorionic gonadotropin maintains steroidogenesis until placental steroidogenesis is established in the 9th or 10th week of gestation.) With the decline of the corpus luteum, estrogen and progesterone levels fall. Progesterone withdrawal results in the degeneration of the endometrium, resulting in menstruation. Gonadotropin levels, especially FSH, then start to rise, initiating another menstrual cycle.

Definitions of Normal Menstruation and of Oligo- and Amenorrhea

When menarche has not occurred by the age of 16, primary amenorrhea is diagnosed, whereas cessation of menstrual cycles in women who have previously menstruated represents secondary amenorrhea. There is a departure from consensus beyond this level of defining amenorrhea. Indeed, there has not always been agreement on what conditions represent normal (eumenorrheic) or irregular (oligomenorrheic) menstrual status. A eumenorrheic pattern has been described by Loucks and Horvath (1985) as menstrual cycles occurring at consistent intervals ranging from 25-38 days, with regularity more important than the absolute length of the intervals. Similarly, Loucks and Horvath point out that a 39-90-day interval used to define oligomenorrhea is an arbitrary limitation; that a key characteristic of oligomenorrhea is

inconsistency of menstrual cycles. In other words, by these definitions, a woman whose menstrual cycles recur at consistent intervals of 40 days would be classified as eumenorrheic.

Absence of menstruation for 6 months or more is accepted by some authorities as constituting amenorrhea (Frisch et al., 1981; A.D. Rogol, personal communication, December 15, 1985; Speroff et al., 1983, p. 142). Ninety or more days with no menses is also frequently used as a defining criterion of amenorrhea (Bachmann & Kemmann, 1982; Petterson, Fries, & Nillius, 1973; Sanborn, Martin, & Wagner, 1982; Yen, 1978).

Amenorrhea has been classified according to diagnostic procedures (Ross, Vande Wiele, & Frantz, 1981; Speroff et al., 1983, chap. 5). For the purpose of this paper, however, a more useful system of classification is the pathophysiological model provided by Yen (1978). Yen categorizes amenorrhea by underlying cause: (a) anatomic cause, (b) ovarian failure, and (c) chronic anovulation. Examples of anatomic causes include endometrial adhesions and abnormal differentiation of the genital tract. Ovarian failure includes gonadal dysgenesis and "gonadotropin-resistant ovaries" (probably due to insufficient ovarian gonadotropin receptor sites). These amenorrheic women typically exhibit hypergonadotropinism.

Chronic anovulation syndrome, the most common type of amenorrhea, is the category in which athletic amenorrhea belongs (Loucks & Horvath, 1985). In women with this disorder, the administration of estrogen followed by progesterone for the appropriate number of days produces

withdrawal bleeding, suggesting that the ovaries are at least minimally functional (Speroff, et al., 1983, chap. 5). The mechanism of the amenorrhea involves either the hypothalamic-pituitary axis or disturbances in the peripheral metabolism of steroid hormones (Yen, 1978). Yen further categorizes chronic anovulation syndrome according to the site of the defect:

1. Hypothalamic chronic anovulation: Characterized by abnormal GnRH release due to a defect in the CNS-hypothalamic unit.

2. Pituitary chronic anovulation: Disturbance in gonadotropes resulting from either impairment of the synthesis of the gonadotropin α-β subunit or from abnormalities in gonadotropin receptor sites.

3. Inappropriate feedback: (a) abnormal sex steroid levels due to steroid therapy or to androgen or estrogen-producing tumors, (b) excessive extraglandular estrogen or androgen production, and (C) abnormal buffering system (alterations in sex-hormone binding globulins or in interconversions between steroids).

4. Other endocrine or metabolic dysfunctions: (a) Excessive cortisol and/or androgen production (Cushing's disease and syndrome), (b) hyper- and hypothyroidism, (c) prolactin or growth hormone excess, and (d) malnutrition.

Prevalence of Athletic Amenorrhea

Reports of the prevalence of athletic amenorrhea have varied widely, depending on the population surveyed, and upon the definition of amenorrhea used by investigators. In a study of 128 college track and field or cross-country runners, the frequency of amenorrhea

(defined as 3 or fewer cycles per year) was 6% among women who ran less than 10 miles per week, and as high as 43% among runners who trained more than 60 miles per week (Feicht, Johnson, Martin, Sparkes, & Wagner, 1978). Using the defining criterion of at least 3 months with no menses, Calabrese et al. (1983) reported that 44% of 34 classical ballet dancers were amenorrheic. Sanborn et al. (1982) observed a higher prevalence of amenorrhea among 237 runners (25.7%) than among swimmers (12.3%) or cyclists (12.1%). Other studies of athletes in general, and of runners, have reported prevalence rates ranging from 3.4 to 12.1% (Carlberg, Buckman, Peake, & Riedesel, 1983b; Lutter & Cushman, 1982; Schwartz et al., 1981; Speroff & Redwine, 1980). The lower prevalence of 3.4% reported by Lutter and Cushman is a reflection of their stringent definition of amenorrhea of at least 12 months duration with no menses.

Investigations of the prevalence of secondary amenorrhea in the general population have yielded more consistent results of approximately 3% (2.6-3.3%) (Bachmann & Kemmann, 1982; Carlberg et al., 1983b; Petterson et al., 1973). These researchers all defined amenorrhea as a minimum of 3 months with no menses.

Although the lack of menstruation can be determined by survey questionnaire, the prevalence of menstrual irregularities is virtually impossible to accurately assess by questionnaire. A number of investigators have reported evidence of a shortened luteal phase among athletes whose menstrual cycles appear otherwise normal (Bonen, Belcastro, Ling, & Simpson, 1981; Jacobson & Bonen, 1981; Jacobson, Wilkinson,

& Bonen, 1983; Prior, Cameron, Yuen, & Thomas, 1982; Shangold, Freeman, Thyson, & Gatz, 1979). Such information would go undetected by a survey or interview, as it can only be ascertained via evaluating athletes' hormone profiles (Bonen & Keizer, 1984). Consequently, prevalence rates documented by survey methodology are likely to be an underrepresentation of the degree of menstrual irregularities among athletes. Basal body temperature is sometimes used as an indicator of ovulation (Collett, Wertenberger, & Fiske, 1954; Frisch et al., 1981; Prior et al., 1982), but even these data can appear normal despite abnormal luteal function (Ross et al., 1981).

Relevance of the Study of Athletic Amenorrhea

The immediate effects of amenorrhea and chronic anovulation on fertility are obvious. If for no other concern than fertility, athletic amenorrhea warrants investigation. The long-term effects are less clear. The first published evidence of a potential harmful effect directly attributable to athletic amenorrhea was reported by Cann, Martin, and Genant (1982). They studied 25 amenorrheic women, 6 of whom were runners with diagnosed hypothalamic amenorrhea. The remainder of subjects were nonrunners with amenorrhea due either to ovarian failure or to hyperprolactinemia secondary to prolactinomas. Bone mass of all of the amenorrheic women, including runners, was 28% less than that of 45 age-matched controls, suggesting that women with secondary amenorrhea have an increased risk of developing osteoporosis. More recent studies have confirmed these early findings in athletes (Cann, Martin, Genant, & Jaffe, 1984; Drinkwater et al., 1984;

Lindberg et al., 1984; Linnell, Stager, Blue, Oyster, & Robertshaw, 1984; Marcus et al., 1985).

Reversibility of athletic amenorrhea has been difficult to study, since most athletes are not receptive to the suggestion that they cease or moderate their current training regimen or that they gain weight, two recommendations generally believed to be associated with resumption of menses ("Impact of exercise," 1982; Speroff et al., 1983, p. 177). Reversibility of amenorrhea as well as reversion back to an amenorrheic state has been observed in ballet dancers. Abraham, Beumont, Fraser, and Llewelyn-Jones (1982) categorized 29 ballet students as (a) eumenorrheic (menstrual cycle interval of 25-34 days), (b) irregular (interval of 14-24 or 35-46 days), (c) oligomenorrheic (interval of 7-11 weeks), or (d) amenorrheic (no menses for 3 or more months). Each subject's menstrual pattern was followed for one year. Improvement in menstrual status was defined as a change of at least one category toward regular menstruation. During periods of 4 or more weeks of decreased physical activity (vacation or injury), 19 out of 21 dancers showed improvement in menstrual status.

Warren's study of 13-15-year-old ballet dancers (1980) revealed similar findings. The progression of pubertal development increased, and the prevalence of amenorrhea decreased with reduced physical activity of 2 months or more. Dancers have reported more frequent menstruation during vacations or when not dancing than during periods of heavy physical activity (Abraham et al., 1982; Calabrese et al., 1983).

Although athletic amenorrhea may be reversible, it is not known if reproductive function is permanently affected, or if there is a period of time beyond which an amenorrheic state cannot be reversed to restore reproductive function. Stager, Ritchie-Flanagan, and Robertshaw (1984) detected no relationship between the length of time of amenorrhea and the time required to regain normal periodicity in their survey of former college runners. No women reported more than 6 months as the time for normal periodicity to return after training cessation, and the mean time was 1.7 months.

O'Herlihy (1982) described two amenorrheic runners who did not respond to 5 days of clomiphene citrate administration. Ovulation was achieved in both runners, however, using half the dosage of the previously ineffective drug, administered within 6 and 8 weeks of running cessation; one woman conceived during that first ovulatory cycle.

Hormone Evaluation in Oligo- and Amenorrheic Athletes

There have been conflicting reports of hormone profiles among athletes with menstrual irregularities. Many of the discrepancies reported in the literature may be attributed to poor research design or implementation, as well as the lack of standard defining criteria for oligo- and amenorrhea. The following section is a review of endocrine research relative to the study of athletic amenorrhea. A summary of basal endocrine results of studies of amenorrheic athletes is included in Table I.

Luteinizing Hormone. Comparisons of basal LH concentrations among amenorrheic and normally menstruating athletes have yielded

Table 1
Comparisons of Endocrine Profiles of Amenorrheic Runners with Eumenorrheic Runners or Nonathletes*†

Reference	Sample and Comment		LH, mIU/ml	FSH, mIU/ml	E_2, pg/ml	P, ng/ml	Other
Baker et al. (1981)	-14 cyclic (EUM) -9 amenorrheic (AM) -(3 of 9 became AM during study) -1 sample/cycle -Cycle phase of sample not reported	EUM AM	9.7±1.0 6.5±1.0 ($p<.05$)	8.0±0.6 7.6±0.8 (NS)	58.0±8.0 20.0±6.0 ($p<.005$)		-NS differences for: DHEA, DHEAS, A, T, PRL
McArthur et al. (1980)	-6 cyclic nonathletes (NA) -3 amenorrheic (AM) -Serial sampling for 4 hr (15-min interval)	NA AM	2.1±0.3 0.6±0.3	3.8±0.7 2.7±0.2			
Richards et al. (1985)	-5 cyclic nonathletes (NA) -7 eumenorrheic (EUM) -8 amenorrheic (AM) -Midfollicular sampling: 1/hr for 24 hr -EUM ran 39 MPW -AM ran 35 MPW	NA EUM AM	1.7±0.5 3.3±3.8 1.5±0.6 (NS)	10.8±1.7 8.7±2.1 9.2±1.2 (NS)	105.4±46.3 142.7±147.2 97.4±23.5 (NS)		-NS differences for PRL, T, TSH -T_4: NA 9.5±1.8* EUM 8.8±2.1 AM 7.3±1.2* *($p<.05$)
Schwartz et al. (1981)	-20 cyclic nonathletes (NA) -25 eumenorrheic (5-30 MPW) (Middle distance) -41 eumenorrheic (>30 MPW) (Long distance) -12 amenorrheic (AM) -1 early follicular sample -Exact hormone values not reported		↑ in AM vs. distance runners or NA ($p<.05$)	NS difference			-NS differences for: DHEAS, A, T, E_1, E_2 -E_1:E_2 ratio: ↑ in runners vs. NA ($p<.01$) -TSH: ↓ in AM vs. all others ($p<.05$)
Veldhuis et al. (1985)	-10 cyclic nonathletes (NA) -9 amenorrheic (AM) -Early follicular serial sampling: 24 hr (20-min intervals)	NA AM	8.0±1.4 6.6±0.5		80.9±11.0 52.3±7.5	<1.0 <1.0 (NA)	-Normal values for T and thyroid function in AM

*All subjects were runners except where noted (designated NA for nonathlete)
†Abbreviations: DHEA, Dehydroepiandrosterone; DHEAS, DHEA sulfate; A, Androstenedione; T, Testosterone; PRL, Prolactin; TSH, Thyroid-stimulating hormone; T_4, Thyroxine; E_1, Estrone; E_2, Estradiol; NS, Statistically nonsignificant; MPW, Miles per week

conflicting results. Reduced basal concentrations of LH have been reported for amenorrheic runners (Baker, Mathur, Kirk, & Williamson, 1981; McArthur et al., 1980), whereas similar concentrations have been reported by other investigators (Richards, Chang, Bossetti, Malarkey, & Kim, 1985; Wakat et al., 1982). Schwartz et al. (1981) reported elevated basal LH concentrations in amenorrheic runners as compared to eumenorrheic nonathletes during the early follicular phase of their menstrual cycle ($p<.05$). Differences in results may be attributable to the pulsatile nature of LH secretion. Analysis of a single sample has a predictable accuracy rate of only 38% (Santen & Bardin, 1973).

The pulsatile pattern of LH release may be even more important than the mean LH concentration in regulating reproductive function (Loucks, 1985). Reduced frequency and amplitude of LH pulsations have been reported in amenorrheic runners (Veldhuis et al., 1985) and in eumenorrheic runners (Cumming, Vickovic, Wall, & Fluker, 1985). Veldhuis et al. (1984) demonstrated that in 6 normal men, venous sampling at 4-minute intervals disclosed 3.6-fold more LH pulses than could be detected with sampling intervals of 20 minutes. Thus, frequent serial sampling is necessary to provide a comprehensive and accurate assessment of basal LH levels. Intervals of 20 minutes have been suggested as a minimum interval length for serial sampling in order to assess the secretory pattern of LH (Reame, Sauder, Kelch, & Marshall, 1984).

Follicle Stimulating Hormone. Basal FSH concentrations have consistently been reported as similar to eumenorrheic athletes during the

early-to-midfollicular phase (Richards et al., 1985; Schwartz et al., 1981). Baker et al. (1981) reported no significant difference between the basal FSH levels of amenorrheic and eumenorrheic runners, but did not specify the cycle phase in which sampling occurred.

Progesterone. Veldhuis et al. (1985) reported basal P values of both amenorrheic athletes and eumenorrheic nonathletes(early follicular phase) as less than 1.0 ng/ml. No other investigators have reported comparisons of amenorrheic vs. cyclic athletes or nonathletes.

Shangold et al. (1979) observed lower midluteal phase plasma progesterone concentration during training (8.2±0.85 ng/ml) than during nontraining menstrual cycles (23.7±2.9 ng/ml) in 1 cyclic runner ($p < .001$). Dale, Gerlach, Martin, & Alexander (1979) also detected lower mean progesterone values among 19 runners than among 9 nonrunning athletes and 13 sedentary controls, although the authors did not report the hormone values or level of statistical significance. Other investigators have reported lower plasma P levels associated with exercise training (Bonen et al., 1981; Jacobson & Bonen, 1981; Jacobson et al., 1983). Ellison and Lager (1985) evaluated salivary P concentrations among cyclic runners who ran an average of 12.5 miles per week (MPW), and sedentary controls during the luteal phase. The runners' mean salivary P concentration was 194±24 pmol/l, whereas the P concentration for the controls was 287±30 pmol/l ($p < .05$). The authors concluded that even modest exercise is associated with a reduction in luteal phase P levels in the absence of obvious clinical evidence of menstrual abnormality, and without excessive thinness.

Estrogen. Estradiol levels of 20.0±6.0 pg/ml among amenorrheic runners were significantly lower ($p<.005$) than the estradiol levels of eumenorrheic runners reported as 58.0±8.0 pg/ml (cycle phase not specified) (Baker et al., 1981). Other comparisons between amenorrheic and menstruating runners have revealed no differences when controls are sampled during the early-to-midfollicular phase (Richards et al., 1985; Schwartz et al., 1981; Veldhuis et al., 1985).

Schwartz et al. (1981) measured estrone in runners as well, and observed that the mean ratio of serum estrone to estradiol was significantly higher ($p<.01$) among both amenorrheic and normally menstruating runners than among nonrunning controls. Cumming et al. (1981) observed a significantly higher level of estrone ($p<.01$) among runners vs. untrained women in response to acute maximal exercise.

Estradiol, the predominant and most active estrogen, normally constitutes about 95% of the total circulating estrogen (Yen, 1978). Under normal conditions, the estrone:estradiol ratio is less than 1.0. An elevated ratio may be indicative of increased extraglandular conversion of androgens (peripheral androgen conversion is a significant source of estrone); or the lack of ovarian follicle maturation, which results in diminished ovarian estradiol secretion (Yen, 1978). Accordingly, Schwartz et al. (1981) suggested that the runners' increased estrone : estradiol ratio observed was indicative of an alteration in peripheral steroid metabolism, and that among the amenorrheic runners, the elevated ratios represented the absence of significant ovarian follicular activity. Altered steroid metabolism among

the runners may be attributed to alterations in the rate of androstenedione production or clearance, or to abnormal conversion enzyme activity (Loucks & Horvath, 1985).

Androgens. Elevated androgen levels may influence menstrual function through hypothalamic feedback or through a relative decrease in peripherally produced estrogen (Baker, 1981). Few researchers have evaluated androgen levels in female athletes. Dale, Gerlach, Martin, & Alexander (1979) observed higher serum testosterone levels among 19 runners than among 22 nonrunners (exact values not reported), but the levels were still within the normal female range. In addition, the sampling protocol (i.e., resting or nonresting state) was not described. No significant differences in serum testosterone levels were reported between cyclic nonrunners, and amenorrheic and cyclic runners (Baker et al., 1981; McArthur et al., 1980; Richards et al., 1985; Schwartz et al., 1981; Veldhuis et al., 1985).

In a prospective study of progressive exercise over several months, baseline testosterone levels did not increase with increasing distance run (Boyden, Pamenter, Stanforth, Rotkis, & Wilmore, 1983). Other androgens have been evaluated in a limited number of studies. No differences were observed between amenorrheic runners and eumenorrheic runners in androstenedione, dehydroepiandrosterone (DHEA), or in DHEA sulfate (DHEAS) (Baker et al., 1981; Schwartz et al., 1981). On the basis of currently available data, there is little evidence of any abnormal androgen synthesis or clearance among amenorrheic athletes.

Prolactin. The physiological role for prolactin in follicular maturation and corpus luteum function is poorly understood; yet hyperprolactinemia is a recognized clinical syndrome associated with anovulation (Speroff et al., 1983, chap. 6). Both exercise and stress stimulate prolactin secretion (Reichlin, 1981, pp. 616-617). Normal basal prolactin concentrations have been reported for ballet dancers and runners with menstrual irregularities (Baker et al., 1981; Cohen et al., 1982; Richards et al., 1985). However, a progressive increase in the prolactin response to thyroid-stimulating releasing hormone (TRH) associated with increasingly weekly running mileage from 15 MPW to 64 MPW has been observed ($p<.04$) (Boyden, Pamenter, Grosso, Stanforth, Rotkis & Wilmore, 1982). There was no correlation between TRH-stimulated prolactin response and body composition. Hence, Boyden et al. concluded that exercise per se was probably responsible for the augmented prolactin response. The mechanism by which this would occur is unknown. Bullen et al. (1984) observed significant increases in prolactin levels in response to 60 minutes of graded exercise among healthy young women ($p<.05$). The authors theorized that intermittent exercise-induced prolactin elevations could contribute to menstrual disorders, in light of the recognized association between hyperprolactinemia and menstrual dysfunction. Bazzarre and Royster (1984) reported continued rises in prolactin among male marathon runners during a 2-hour submaximal (70% VO_2 max) treadmill run, in contrast to growth hormone (GH), which peaked at 20 minutes and returned to baseline levels thereafter. They postulated that exercise-induced release

of GH and prolactin are controlled by different hypothalamic regulatory mechanisms. Furthermore, they cited other reports in the literature that suggest that prolactin may function in the regulation of carbohydrate energy substrate availability during exercise stress. Perhaps an exercise-associated energy drain provides an additional stimulus for prolactin release due to increased need for glucose.

Opioid Hormones. Endogenous opioids may function in the hypothalamic regulation of LH secretion from the pituitary (Ellingboe, Veldhuis, Mendelson, Kuehnle, & Mello, 1982), and in the mechanisms governing several other pituitary hormones (Meites, 1980). Administration of naloxone, an opiate receptor antagonist, was reported to increase the amplitude of LH pulsations in hypothalamic amenorrheic women (Quigley, Sheehan, Casper, & Yen, 1980), and in 1 of 3 amenorrheic runners (McArthur et al., 1980). These findings suggest that endogenous opiates such as β-endorphin, may diminish LH pulsatility. Some studies have demonstrated a rise in plasma β-endorphin and in β-lipotropin in response to acute exercise, and that exercise training augments this response (Bullen et al., 1984; Carr et al., 1981). More research is required to quantify these exercise-induced changes. There is much concern, however, that peripheral opioid concentrations do not accurately reflect brain concentrations of the opioids.

Evidence that both exercise and eating elevate β-endorphins suggests that changes in energy substrate availability may alter the release of pituitary hormones. Walker, and Bazzarre (1985) cited a report in the literature (Givens, Wiedmann, Andersen, & Kitabchi, 1980)

noting elevated fasting β-endorphins in obese, hirsute women with menstrual dysfunction. In their study, however, they observed no alterations in fasting β-endorphin levels among obese females with no known menstrual dysfunction. Furthermore, in the study by Walker and Bazzarre, β-endorphins decreased significantly following a 12-week weight reduction program that emphasized physical activity and caloric restriction.

Thyroid Hormones. Alterations in sex hormone metabolism have been documented in disorders of thyroid function (Fishman, Hellman, Zumoff, & Gallagher, 1962; Gordon, Southren, Tochimoto, Rand, & Olivo, 1969), and are often associated with menstrual dysfunction (Burrow, 1978). Normal thyroid function of oligo- and amenorrheic athletes has been demonstrated in most studies in which thyroid hormones have been evaluated (Cohen et al., 1982; McArthur et al., 1980; Veldhuis et al., 1985; Wakat et al., 1982; Warren, 1980). However, Richards et al. (1985) reported significantly lower basal thyroxine (T_4) levels among amenorrheic runners (7.3$\pm$1.2 mcg/dl) than sedentary controls (9.5$\pm$1.8 mcg/dl) ($p<.05$), whereas there was no significant difference in the T_4 levels of eumenorrheic runners (8.8$\pm$2.1 mcg/dl) and the controls. Schwartz et al. (1981) also observed evidence of thyroid dysfunction among amenorrheic runners. Basal levels of thyroid-stimulating hormone (TSH) were reduced among amenorrheic runners as compared to cyclic nonathletes or runners (exact values not reported; $p<.05$).

Boyden, Pamenter, Stanforth, Rotkis, and Wilmore (1982) evaluated thyroid function in women undergoing a prolonged endurance

training program. The investigators documented decreases in triiodothyronine (T_3) and reverse T_3 ($p<.03$) as well as accentuated TSH response to TRH stimulation ($p<.01$). The underlying mechanism and the physiological importance of this mild thyroidal impairment accompanying exercise training is not known. The direct relevance to menstrual function remains unclear. In another report of the same study, Boyden et al. (1983) documented that amenorrhea did not develop in any of the 19 subjects who completed the 14-month study. However, basal plasma estradiol concentrations decreased from 70.6±13.9 pg/ml at baseline to 33.6 pg/ml ($p=.03$) following an increase in weekly mileage of 50 MPW.

Gonadotropin Releasing Hormone. Administration of exogenous gonadotropin releasing hormone (GnRH) has produced normal or exaggerated LH responses among amenorrheic runners as compared to eumenorrheic controls (McArthur et al., 1980; Veldhuis et al., 1985; Wakat et al., 1982). Such a response suggests that the pituitary is capable of synthesizing and storing LH, but that release of the gonadotropin is inhibited due to inadequate GnRH stimulation. Heightened sensitivity of the pituitary to exogenous GnRH may be indicative of a chronic low exposure to GnRH.

Summary of Endocrine Review. Luteinizing hormone and FSH concentrations may be low or normal for early follicular phase values in the amenorrheic athlete, and LH is likely to exhibit a decreased pulse frequency. Luteal phase estrogen and progesterone may be reduced or within normal range. Androgen concentrations are in the normal

female range. Thyroid function may be normal or somewhat diminished as indicated by reduced levels of TSH. Basal prolactin levels can be expected to be normal.

The ovaries and the pituitary of the amenorrheic athlete appear to be capable of normal function, although stimulation of the pituitary-ovarian axis may be impaired at the level of the hypothalamus. Evidence of pituitary hyperresponsiveness to exogenous GnRH suggests that hypothalamic GnRH release may be impaired.

Further endocrine research is needed to gain an accurate representation of the hormone profile that is characteristic of athletic amenorrhea. It is important to note as well, that circulating hormone concentrations cannot be directly equated with function. For example, changes in hormone turnover rates or in receptor sites or affinity may account for alterations in circulating hormone concentrations despite normal function of the hormone system.

Proposed Mechanisms of Athletic Amenorrhea

Early investigators of athletic amenorrhea attempted to gain some understanding of the etiology through cross-sectional studies designed to determine the usual characteristics of oligo- and amenorrheic athletes. Cause and effect cannot be ascertained from cross-sectional research, although exploratory data analysis of this nature has been useful in the development of hypotheses and experimental research designs. Many of the cross-sectional studies, as well as the few prospective studies that have been conducted, have provided conflicting results. Nevertheless, some consistent patterns have emerged, contributing to the generation of hypotheses regarding mechanisms involved.

Factors such as physical or emotional stress, reproductive maturity, age of onset of exercise training, body composition, and diet have been suggested to be of etiological importance. For the purposes of this report, the discussion of proposed mechanisms will be limited to mechanisms associated with body composition and diet.

Body Composition. Although widely publicized in the popular media, an association between low body weight or fat and menstrual disorders has not been consistently documented in the scientific literature. In 1974, Frisch and McArthur postulated that a critical weight for height, corresponding to a critical percentage of body fat, must be attained for the initiation of, as well as for the maintenance of, menstruation. Their conclusions have since been criticized for a number of reasons, including the statistical management of the data (Trussel, 1980) and methodology (Loucks, 1983). In addition, it is important to note that the amenorrheic subjects studied by Frisch and McArthur (n=17) were not athletes. The appropriateness of inferences to amenorrheic athletes from these data is, therefore, questionable.

Several studies have failed to confirm a relationship between low body fat levels and the incidence of amenorrhea (Abraham et al., 1982; Calabrese et al., 1983; Feicht et al., 1978; Stager et al., 1984; Wakat, et al., 1982), further diminishing support for the theory that the reduction of body fat to some critical level causes the cessation of menses. Although a number of studies have revealed a greater tendency toward amenorrhea among leaner athletes (Carlberg et al., 1983a, 1983b; Dale, Gerlach, & Wilhite, 1979; Lutter & Cushman, 1982; Schwartz

et al., 1981), Frisch's hypothesis that a minimum level of body fat is required for normal menstrual function may be too simplistic. In most athletes, a low percentage of body fat is primarily a function of the energy expenditure associated with training. As pointed out by Bonen and Keizer (1984), amenorrhea cannot be attributed to leanness alone in an athletic population without also examining daily exercise, which is itself known to be a strong stimulant of the endocrine system.

In her study of ballet dancers, Warren (1980) observed changes in menstrual function that occurred in relation to exercise levels, which were independent of body weight changes. Evidence of the effect of exercise on menstrual function (and on pubertal development) was stronger among the leaner dancers than among dancers with higher estimated body fat. (Body fat was estimated from height and weight using the equation of Mellits and Cheek, 1970.) Sanborn et al. (1982), relying on retrospective survey data, reported that the prevalence of amenorrhea increased with weekly mileage among 237 runners, but no such relationship was observed among 197 swimmers or 33 cyclists. Noting that the runners reported lower weights for heights than the other athletes, the investigators then categorized the runners into 3 weight groups, and found that the exercise-amenorrhea relationship was stronger in leaner runners. These results suggest an interplay between body fat and exercise levels as possibly being an important etiological factor.

Loss of body weight has been associated with menstrual dysfunction among nonathletes (Graham, Grimes, & Gambrell, 1979; Knuth, Hull, & Jacobs, 1977). This relationship has also been documented for

athletes (Bullen et al., 1985; Lutter & Cushman, 1982; Speroff & Redwine, 1980). Few investigations have addressed the issue specifically, though, and a causal relationship has not yet been demonstrated.

In a study of 24 amenorrheic women, Wentz (1980) concluded that the loss of fat is more important than absolute body weight in the etiology of amenorrhea. In contrast to the theory that absolute weight is a determinant of menstrual function, she noted that a wide range of body weights was associated with the onset of amenorrhea. All of the amenorrheic women except for 3 runners, however, had lost a substantial amount of weight. Wentz speculated that the runners' body composition had changed with the training despite no apparent weight changes. She has insisted that weight measurements alone are insufficient to assess the effect of fat loss on menstrual function. Decreasing levels of body fat with no change in weight have occurred in women participating in an endurance running program (Boyden, Pamenter, Stanforth, et al., 1982). Wentz maintains that a shift in body composition from fat toward muscle, with or without weight loss, may affect gonadal hormone production.

Adipose tissue serves as a site for the aromatization of androgen precursors to estrogens (Speroff et al., 1983, chap. 1). The loss of body fat may reduce a significant amount of available tissue for extra-gonadal estrogen production, thereby altering estrogen feedback influences on GnRH (Rebar & Cumming, 1981; Wentz, 1980). Steroid conversions also occur in muscle tissue (Longcope, Pratt, Schneider, & Fineberg, 1978). Refuting the theory that body fat levels are critical

to menstrual function, Bonen and Keizer (1984) proposed that increased muscle mass may represent a compensatory source of estrogen in very lean athletes.

There is evidence that estrogen metabolic pathways vary according to body weight and fat levels. Fishman, Boyar, and Hellman (1975) compared urinary metabolites of labeled estrogen between 7 women with anorexia nervosa, 4 obese women (75% over normal body weight), and 8 controls. In the obese women compared to the anorectic women, there was a greater formation of the estrogen metabolic byproduct estriol ($p < .01$), suggesting greater estrogen synthesis and breakdown. Urinary levels of the catecholestrogen 2-hydroxyestrone were markedly increased in the anorectic women ($p < .01$), and decreased in the obese ($p < .02$), as compared to controls. The estrogenic activity of 2-hydroxyestrone is only about 0.01% that of estradiol (Gordon et al., 1964), and may actually function as an antiestrogen due to its greater affinity for uterine cytosol receptors (Martucci & Fishman, 1976). It is tempting to infer from the endocrine conditions associated with obesity and with anorexia nervosa that an increasing estrogenicity is associated with increased body fat. Fishman (1980) has pointed out, however, that the increased peripheral aromatization observed in obese women does not decrease with weight loss. Furthermore, there has been no documentation of a reversal of the 2-hydroxylation deficit among obese women in response to weight loss.

Diet. Few studies of athletic amenorrhea have included dietary assessment. A number of hypotheses related to dietary factors have been proposed, however.

The results of two studies have suggested a possible association between hypercarotenemia and menstrual dysfunction (Page, 1971; Kemman, Pasquale, & Skaf, 1983). Ten anovulatory women with elevated serum carotene levels were studied by Kemman et al. There was no evidence of hyperprolactinemia or thyroid dysfunction among the subjects. Diets of all of the women were characterized by a high intake of raw vegetables and a lack of red meat. These patients were counseled to reduce their intake of carotene-rich foods and to increase their protein consumption. In the 2 patients who did not alter their diets, elevated serum carotene levels as well as amenorrhea persisted. The other patients menstruated subsequent to diet modification and a reduction of serum carotene levels.

In the study by Kemman et al. (1983), dietary assessments were based on interviews. The authors failed to evaluate (or to report) relevant dietary information such as energy or protein intake. Nor was there any indication that other lifestyle factors were investigated following the completion of initial interviews and questionnaires. Information regarding stress and exercise levels, for example, would be useful. In addition, it is important to note that one patient was a professional ballet dancer, and that one patient had lost more than 4.5 kg of body weight prior to the onset of her menstrual dysfunction. In these two women, therefore, exercise or weight loss may have contributed to the onset of amenorrhea.

Frumar, Meldrum, & Judd (1979) reported that carotenemia was present in 6 patients with hypogonadotropic amenorrhea, which was not associated with an excessive intake of carotene-rich foods.

Frumar et al. proposed that the hypercarotenemia was attributable to weight-induced catabolic changes including lipid mobilization. It has been suggested that carotene exerts a peripheral antiestrogenic effect (Romney et al., 1981, p. 894), but there is little evidence to support this contention.

Richards et al. (1985) evaluated serum carotene levels of 7 eumenorrheic and 8 anovulatory runners, and 5 sedentary controls. Serum carotene levels were similar in the three groups, and only 1 amenorrheic runner exhibited a serum carotene level above the upper limit of normal (274 mcg/dl vs. 250 mcg/dl). The authors concluded that hypercarotenemia is not associated with menstrual dysfunction among long-distance runners.

One nutritional challenge for the endurance athlete is to maintain an energy intake that is sufficient to compensate for high energy demands. Reflecting this high energy expenditure, the mean percentage of body fat among competitive female distance runners has been reported to be approximately 15% (Butts, 1982; Wilmore & Brown, 1974), and as low as 8-10% among nationally ranked women runners (Costill, 1979, p. 11). This is well below the normal value for nonathletic adult females, which has been estimated to be approximately 25% (Durnin & Rahaman, 1967; Wilmore & Behnke, 1970).

Warren (1983) has proposed that while amenorrheic athletes do not appear to be malnourished, the energy drain they experience may resemble that of undernutrition as seen in anorexia, a syndrome in which the incidence of amenorrhea is high. Thus, she purports that a

caloric deficiency may exist relative to the energy demands of physical training.

Among adolescent ballet dancers, progression of sexual development and the onset of menstruation was associated with forced exercise cessation of at least 2 months (Warren, 1980). In the same study, 11 out of the 13 dancers became amenorrheic again upon resumption of exercise. Several studies have provided evidence of a positive association between exercise levels and the incidence of menstrual irregularities (Abraham et al., 1982; Bullen et al., 1985; Carlberg et al., 1983b; Lutter & Cushman, 1982). In a number of surveys, athletes have reported a perceived association between their exercise level and menstrual patterns (Calabrese et al., 1983; Dale, Gerlach, Martin, & Alexander, 1979; Schwartz et al., 1981; Wakat et al., 1982; Webb & Proctor, 1983).

Warren has suggested that the amenorrhea associated with exercise or with weight loss may represent a physiological adaptation to large metabolic demands, and that it may serve as a natural form of fertility control. She cites evidence of such a phenomenon among females of the !Kung bushmen of South Africa, who have exhibited low plasma estradiol, progesterone, and testosterone levels, suggestive of suppression of ovulation (van der Walt, Wilmsen, & Jenkins, 1978). Van der Walt et al. believed that the caloric-deficient diets of the !Kung women during the hot dry season may be associated with the apparent ovarian suppression. The birth rate of the !Kung peaks at exactly 9 months from the time of the year when their body weight is at maximum. Van der Walt et al. hypothesize that a mechanism exists whereby ovarian

suppression in response to undernutrition serves to limit conception to times of high nutrition.

Winterer, Cutler, and Loriaux (1984) support the theory that menstrual function is affected by caloric balance. They propose that when caloric availability is reduced beyond a critical level, GnRH release is impaired. Winterer et al. suggest that energy drain may contribute to delayed menarche as well as to secondary amenorrhea.

Although few investigators of athletic amenorrhea have assessed energy intake, none have evaluated energy balance. The amenorrheic runners studied by Schwartz et al. (1981) consumed 1800 kcal per day, yet were running about 40 miles per week, as well as participating in other routine exercises. The oligo- and amenorrheic dancers studied by Calabrese et al. (1983) reported an even lower mean caloric intake. The dancers' intake of 1360 kcal/day is 71.6% of the U.S. Recommended Dietary Allowance (RDA) (National Research Council, 1980). The investigators speculated that progressive weight loss did not occur in the dancers because of the high incidence of food binging (70%) reported. It is also possible, however, that a physiological adaptation to a low energy intake results in increased metabolic efficiency, i.e., a reduced basal metabolic rate (Sukhatme & Margen, 1982).

In contrast to the energy drain theory, the caloric intake of amenorrheic runners (1730$\pm$83 kcal/day was similar to that of eumenorrheic runners (1795$\pm$140 kcal/day) (Berning, Sanborn, Brooks, & Wagner, 1985). All of the subjects ran more than 40 miles per week and participated in other vigorous athletic activities. Although the runners' actual energy expenditure was not quantified, the authors

noted that the mean intake of the runners did not appear to be adequate to maintain such a level of energy expenditure. Comprehensive dietary analysis together with reliable estimates of energy expenditure are needed to address the relevance of energy drain to the development of menstrual dysfunction.

The dancers studied by Calabrese et al. (1983) reported a mean protein consumption that met 99% of the RDA (National Research Council, 1980). However, their mean protein intake of 47.4 g/day may be inadequate as a consequence of their deficient energy intake (1360 kcal/day) (Inoue, Yoshiaki, & Yoshiaki, 1973). The amenorrheic runners studied by Schwartz et al. (1981) consumed a lower percentage of kcal as protein than cyclic runners or nonathletic controls ($p<.01$). In light of the runners' weekly mileage (40 MPW) and other exercise activities, a daily energy intake of 1800 kcal would appear to be insufficient to support energy demands. The adequacy of the energy intake (and therefore, of protein) was not addressed by the authors, however.

Lean body mass serves as an energy source during periods of caloric insufficiency (Van Itallie & Yang, 1978). Moreover, there is increasing evidence that protein may contribute significantly to energy metabolism during exercise (Evans, Fisher, Hoerr, & Young, 1983), and that aerobic exercise training may increase protein requirements (Hoerr, Young, & Evans, 1982). Amino acid deficiency (primary, or secondary to energy insufficiency) may lead to impaired neurotransmitter synthesis (Fernstrom, 1981), which may affect LH release from pituitary cells as well as GnRH release from the hypothalamus (Wentz,

Jones, & Sapp, 1976). Unfortunately, no studies have investigated protein balance among amenorrheic athletes.

Goldin et al. (1982) evaluated the dietary intake and estrogen status of 10 vegetarian and 10 nonvegetarian women. The vegetarians, who had a greater fecal weight ($p=.005$), also had a greater fecal estrogen excretion ($p<.03$) than the omniverous women. Among the vegetarians, plasma estrone and estradiol were negatively correlated with fecal estrogen excretion ($p=.005$).

The high fiber content of the vegetarian diet (28 g/day vs. 12 g/day in the omniverous diet), leading to a greater fecal bulk, may be responsible for the increased fecal excretion of estrogen and a concomitant decrease in plasma estrogens. Goldin et al. (1982) offer an alternative hypothesis, however, based on the observation of reduced activity of β-glucuronidase activity in the feces of the vegetarians ($p<.05$). β-glucuronidase is an intestinal enzyme that is responsible for deconjugating biliary estrogen conjugates, which is necessary for estrogen reabsorption. Thus, it appears that some factor of the vegetarian diet reduced β-glucuronidase activity, thereby contributing to increased estrogen excretion.

Dietary fiber has been associated with a reduction of β-glucuronidase activity, and dietary fat and beef have been associated with increased activity of the enzyme in rats (Goldin & Gorbach, 1976). It is not possible to determine from the study by Goldin et al. (1982) which dietary factor is responsible for the elevated estrogen excretion. In addition to their greater fiber consumption ($p<.001$),

the vegetarians also consumed less fat than the omnivores (34 g/1000 kcal vs. 44 g/1000 kcal; $p<.05$).

Howie and Schultz (1985) investigated the diets and plasma hormone levels of male vegetarian Seventh-Day Adventists (SDA), nonvegetarian SDA, and non-SDA nonvegetarians. Vegetarian SDA consumed significantly more crude and dietary fiber than did nonvegetarian SDA ($p<.05$) and non-SDA nonvegetarians ($p<.025$). The vegetarians also exhibited a lower plasma testosterone level ($p<.02$) and estradiol-17β level ($p<.05$) than non-SDA nonvegetarians. Dietary intake of total fat and fat/1000 kcal was similar between the three groups. Regression analysis revealed a statistically significant inverse relationship between plasma estradiol-17β level and crude fiber intake among the subjects ($p<.001$).

Brooks, Sanborn, Albrecht, and Wagner (1984) analyzed the diets of 15 amenorrheic and 15 eumenorrheic runners who were similar in age, menarcheal age, exercise level, and percentage of body fat. They reported that 82% of the amenorrheic runners were vegetarians, whereas only 13% of the cyclic runners were vegetarians. Protein, energy, and fiber intakes were similar between the two groups. Fat consumption was the only dietary variable that differed significantly; the eumenorrheic runners consumed a mean of 98 g of fat per day, and the mean intake of the amenorrheic runners was 68 g/day ($p<.05$). The investigators suggested that a lower intake of certain minerals such as zinc or iron, which may accompany a vegetarian diet, may be of etiological importance. The low fat content of a vegetarian diet may affect the availability of

hormonal precursors as well, especially in combination with a negative energy balance.

Based on the review of the literature, the following points have been established.

1. Menstrual irregularities appear to be more prevalent among leaner athletes, although several reports have indicated no difference in the body composition of eumenorrheic and amenorrheic athletes.

2. The caloric intake of some amenorrheic athletes appears to be insufficient to meet predicted energy demands of their training.

3. A number of amenorrheic athletes consume vegetarian diets.

4. No prospective exercise studies have included a comprehensive dietary component.

The present investigation was designed in an attempt to answer some of the dietary questions related to athletic amenorrhea. Specifically addressed was the question of whether a negative protein and/or energy balance is related to the development of exercise-associated menstrual irregularities.

CHAPTER III

METHODS

Subjects

Twenty-one apparently eumenorrheic recreational runners, ages 18-40, were recruited for the study. Recruitment fliers (Appendix A) were distri buted in the monthly newsletter of a local running club, and at running races in the Greensboro, NC area. Newspaper and radio public service announcements were also utilized, as were notices posted at the University of North Carolina at Greensboro (UNCG), local colleges, health food stores, sporting goods stores, and fitness centers in Greensboro.

At recruitment, subjects ran 10-25 miles per week. They consumed no steroid drugs or unusual diets for at least 6 months prior to study participation. Volunteers were screened from participation for any of the following reasons: history of metabolic or pituitary disorders, hypertension, more than 10% under or 20% overweight for height, and regular use of tobacco or marijuana.

The study was approved by the Human Subjects Review Committee of UNCG. Before signing a consent form (Appendix B), subjects were fully informed of the study procedures. The potential risks and benefits of the project were described to all participants.

Questionnaires regarding personal and medical histories were completed by the subjects at the beginning of the study (Appendix C). The prestudy questionnaire included questions regarding athletic

history; menstrual history; tobacco, drug, and alcohol use; diet; and changes in body weight since beginning running.

Each subject selected the group in which she would participate; experimental (E) or control (C). Control subjects each maintained a running program that was relatively consistent with their prestudy training (i.e., the weekly distance run and the intensity of the workouts were held constant). The E group received coaching that was designed to increase running mileage and speed as tolerated by the subjects. The coaching was provided by an experienced competitive runner (J. C. S.). Two high-intensity workouts per week were usually included in the training schedules. The high-intensity workouts (speed-training) included an anaerobic component, and were sometimes performed at the track. Most subjects had no previous exposure to this type of training.

Experimental Design

For the entire study period, which spanned 7 menstrual cycles, running was the only significant source of physical activity for the subjects. All runners kept an exercise log, which was reviewed regularly by the E group coach and project coordinator, as a means of monitoring study and coaching compliance.

Subjects from both groups consumed a lacto-ovo vegetarian diet for the duration of the study because the measurement of urinary 3-methylhistidine requires a flesh-free diet. Information on how

to plan a well-balanced vegetarian diet was provided to subjects, who were encouraged to contact any of the project investigators if there were specific questions or concerns about the diet.

During the experimental period there were 19 workshops held that were designed to educate subjects about running, diet, and other aspects of health. In addition, the workshops provided a vehicle for communication to and between subjects. The workshops were designed to motivate subjects to comply with the study protocol. Lacto-ovo vegetarian meals along with printed recipes were offered as healthy examples of this meal pattern.

The study consisted of four data collection periods designated as Test Periods I through IV (TP I-IV), corresponding to the 1st, 3rd, 5th, and 7th menstrual cycles of each subject. During each test period, which began on Day 3 of each subject's menstrual cycle (Day 1 = onset of bleeding), the following data were collected: a 7-day diet record, a 7-day activity record, seven 24-hour urine samples, body weight, and body fat estimations by hydrostatic weighing and skinfold measurements. Height was measured at TP I.

From the tenth day of each subject's menstrual cycle until the next onset of menstruation, blood samples were obtained every other day during TP I and IV only. Maximal oxygen uptake and resting metabolic rate were also measured during the first and last test periods. A data-collection summary appears in Table 2.

Table 2
Data collection

Test period	Tests and data collection
Prestudy	Personal, medical, athletic, and menstrual histories 3-day food record[a] Two 7-ml fasting blood samples[a]
I (Baseline; first menstrual cycle)	Height, weight, skinfold thicknesses,[b] and hydrostatic weight Resting metabolic rate Maximal oxygen uptake For each of Days 3-10 of menstrual cycle:[c] 24-hour food record, 24-hour activity record, and 24-hour urine collection
I-A	From Day 10 of menstrual cycle until the onset of the next cycle: Two 10-ml blood samples, every other day
II and III (Third and fifth menstrual cycle)	Weight, skinfold thicknesses,[b] hydrostatic weight 24-hour food record, 24-hour activity record, and 24-hour urine collection (Days 3-10 of cycle) Two 7-ml fasting blood samples (Day 10 of cycle)[a]
IV (Seventh menstrual cycle)	Identical to Test Period I, but with following differences: Height measurement omitted Two 7-ml fasting blood samples (Day 10 of cycle)[a]
IV-A	See Test Period I-A

[a]Data collected for separate study
[b]Skinfold thicknesses measured at 4 sites: biceps, triceps, subscapular, and suprailiac
[c]Day 1 = first day of menstruation

Collection of Dietary Data

Subjects were given detailed instructions on how to keep a daily food record in household measures (Appendix D), and were encouraged to include product labels and recipes for unusual foods in their food records. The subjects collected a 7-day food record at each test period. Nutrient intakes were calculated using the Nutranal nutritional analysis program (S.N. Services, Denver, CO). Analyses of the records included the following: kilocalories (kcal); protein, carbohydrate, fat, absolute quantities and percentage of kcal; saturated and polyunsaturated fatty acids; cholesterol; dietary fiber; iron; and zinc. Protein intake was also determined as g protein per kg of body weight, and per 1000 kcal.

Activity Records and Running Mileage

Activity records (Appendix E) were completed for 7 days of each test period according to the method of Bouchard et al. (1983). For recording purposes, each day was divided into ninety-six 15-minute intervals. Subjects entered a number ranging from 1-9 for each 15-minute period, corresponding to the predominant level of physical activity for that time period. The nine activity categories were explained to the subjects, and they were given written guidelines. Energy expenditure was calculated using the estimated energy costs of the Physical Activity Levels 1-9 developed by Bouchard et al.

All subjects were given a running logbook (Appendix F) in which they recorded for each workout, the date, the number of miles run, total running time, and exercise heart rates when measured.

Additional space was provided to record any other relevant information about the workout, i.e., how the run felt that day, any pain or injury problems, weather conditions, or shoes worn.

The usual weekly mileage (MPW) recorded for each subject at TP I was ascertained by prestudy interview, and confirmed by questionnaire upon entry to the study. Values for weekly running mileage for subsequent test periods represent the average weekly mileage of the preceding four weeks, as recorded in the subjects' logbooks.

Anthropometric Measurements

Each test period included measurements of weight (in kg), hydrostatic weight, and skinfold thicknesses. Height and weight were measured with a beam balance scale with an attached stadiometer. Hydrostatic weighing was performed according to the procedure of Sinning (1975, chap. 7). Body density and fat was estimated using the equation of Brožek, Grande, Anderson, and Keys (1963). Residual lung volume used in the body density equation was estimated (Wilmore, 1969).

Skinfold thicknesses, measured on the right side of the body, included the following sites: biceps, triceps, suprailiac, and subscapular. All measurements were performed using a Lange skinfold caliper by the same individual to eliminate inter-examiner variation. The sum of skinfolds was used to estimate body fat according to the equation developed by Durnin and Womersly (1974).

Measurement of Work Capacity and Resting Metabolic Rate

Maximal oxygen consumption (VO_2 max) was measured at pre- and poststudy. Subjects performed a maximal exercise stress test following the Bruce protocol (Bruce, Kusumi, and Hosmer, 1973). Expired air samples were analyzed with Beckman O_2 and CO_2 analyzers calibrated with gases of known concentrations. Attainment of VO_2 max was determined on the basis of the following criteria: treadmill duration, heart rate, respiratory exchange ratio, and leveling off of VO_2. Measurements of VO_2 max were intended to confirm that (a) E subjects had complied with the exercise program, and (b) C subjects had not increased the quantity or quality of their training.

Resting metabolic rate (RMR) was assessed pre- and poststudy in an effort to detect physiological adaptation to an energy deficit should it occur in any of the subjects. Subjects reported to the UNCG Human Performance Laboratory immediately after arising in the morning in a 12-hour postabsorptive state. They then rested in a reclining position for 20-30 min, after which 5-min breath samples were collected for RMR estimation via indirect calorimetry (McArdle, Katch, and Katch, 1981, chap. 9).

Urine Samples

Twenty-four-hour urine samples collected for each entire 7-day test period were begun following the first void of the first day of the test period and included the first void of the next day. Samples were collected in containers into which 10 ml of N HCl had previously been added. Following each 24-hour period subjects measured

and recorded total urine volume using either 500- or 1000-ml graduated cylinders. A 100-ml aliquot was then frozen. At the end of each test period, the seven frozen samples were brought to the laboratory where they were stored at -20°C until preparation for hormone assay and 3-methylhistidine determination.

Determination of Urinary 3-Methylhistidine and Creatinine

Urinary 3-methylhistidine (3-MH) was measured according to the high-pressure liquid chromatography method of Wassner, Schlitzer, and Li (1980), which involves sample deproteinization and precolumn derivatization. Sodium borate was added to the deproteinized samples to increase the pH to approximately 12, which is necessary for the formation of fluorescamine derivatives. An acetonitrile-fluorescamine solution was then added, after which the sample pH was reduced to about 2 with concentrated perchloric acid, and samples were heated in a water bath at 70°C for one hour. Before loading on the column, samples were neutralized with 0.5 morpholinopropanesulfonic acid in 3 M NaOH.

Only two of the 24-hour urine samples per subject per test period were analyzed for 3-MH. In an effort to control for the potential effects of exercise on 3-MH excretion (Dohm, Williams, Kasperek, & van Rij, 1982), samples to be analyzed were chosen from a day following an exercise day and from a day following a nonrunning day. Wilcoxon signed-ranks tests of the difference between the exercise- and the nonexercise-day excretion of 3-MH revealed no significant

differences between the two samples. The two 3-MH values from each test period were then averaged to represent the respective test period values.

Urinary creatinine was determined by the Jaffe reaction (1886) as modified by Chasson, Grady, and Stanley (1961), utilizing the Technicon Autoanalyzer II™. This method is based on the reaction of saturated picric acid with creatinine in the presence of alkali.

Determination of Urinary Luteinizing Hormone

Urine samples were prepared for luteinizing hormone (LH) assay in the following way. Frozen aliquots of urine were thawed and centrifuged, and the pH adjusted to 4.5 with concentrated HCl. To 15 ml of urine, 4 drops of 30% bovine serum albumin and 30 ml of acetone were added. Samples were then vortexed and placed in the freezer at approximately -20°C for 30 to 45 minutes. Subsequent centrifugation yielded a pellet which was then lyophilized to dryness and stored at room temperature until assays were performed.

Reagents were supplied by the hormone distribution program of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (NIADDK) (Bethesda, MD). The LH concentrations were measured in the laboratory of Alan D. Rogol, M.D., Ph.D., Departments of Pediatrics and Pharmacology, University of Virginia (UVA) School of Medicine (Charlottesville, VA). The double antibody radioimmunoassay (RIA) used for the measurement of urinary LH was a modification of the technique of Odell, Ross, and Rayford (1966). All samples were run in duplicate, and at two different dilutions. Urinary extract, first

antibody, and ^{125}I-iodo-LH were incubated for 3 days at 4°C. The antibody-bound iodo-LH was then precipitated with second antibody. The precipitate was collected by centrifugation and the supernatant solution was aspirated. The remaining pellets were counted for gamma radiation.

Collection of Blood Samples

Most blood samples were drawn by coinvestigators of the project, with alternative blood-drawing arrangements made when necessitated by subjects' travel or proximity to the university. Peripheral venous samples to be used for plasma progesterone analyses were collected in Vacutainers™ containing EDTA (Fisher Scientific, Raleigh, NC), while samples for serum luteinizing hormone analysis were collected in Vacutainers™ with no anticoagulant.

Blood was centrifuged at 2500 rpm for 15-20 min, after anti-coagulant-free Vacutainers™ had remained at room temperature 30-90 min to allow clotting. Plasma or serum was then decanted and samples were frozen at -20°C. Frozen samples were packed in dry ice and transported to the University of Virginia (Pediatric Endocrinology, UVA School of Medicine, Charlottesville, VA) where they were stored frozen until hormone analyses were performed.

Determination of Serum Leutinizing Hormone and Plasma Progesterone

Duplicate samples of serum LH were measured using the modified method of Odell et al., (1966), as previously described.

Measurements of plasma progesterone (P) were also carried out in duplicate, employing the technique of Thorneycroft and Stone (1972). All LH assays were performed in the laboratory of Dr. A. Rogol, and P was measured in the laboratory of Johannes D. Veldhuis, M.D., Department of Internal Medicine, UVA School of Medicine.

Interpretation of Hormone Data

Five distinct variables were derived from the hormone data. These variables are (a) the number of days between the LH peak and the P peak; (b) the number of days in the test period in which the subject's plasma P concentration was 6.0 ng/ml or greater; (c) the number of days plasma P concentration was 4.0 to 5.9 ng/ml; (d) the number of days plasma P concentration was less than 4.0 ng/ml; (e) the area under the curve for P vs. time.

Each subject's hormone concentrations were plotted against time, serum LH and plasma P both occupying the same graph (Appendix G). The number of days between the LH surge and P peak was counted as evidence of the length of the luteal phase. For three subjects these TP I values were unavailable, due to a nondetectable P peak (Subjects 3 and 23) or to missing data (Subject 8).

The classifications of P concentration were chosen to represent three ranges indicative of luteal adequacy (6.0 ng/ml or greater), inadequate luteal phase (< 4.0 ng/ml), and a midrange (4.0 - 5.9 ng/ml) (A. D. Rogol, personal communication, October 8, 1985). The area under the P-vs.-time-curve was determined by summation of the P concentrations for Days 10 through the end of the subject's cycle.

Since blood was drawn only every other day during test period cycles, and because of the difficulties in coordinating blood-drawing with subjects' schedules, actual measured values of hormone concentrations are not available for all days in the test period. When a scheduled blood-drawing was missed, subjects came for blood-drawing on the following day, whenever possible. All missing blood samples were handled in the following ways:

1. Hormone concentrations of a nonblood-drawing day falling between two days on which blood sampling occurred were estimated to be the average value between the days preceding and following that day. For example, if the P concentration was determined to be 0.9 ng/ml on Day 16, and 3.1 ng/ml on Day 18 of a subject's cycle, then Day-17 P concentration would be estimated to be 2.0 ng/ml. In the event that two consecutive days of samples were missing, an estimate for each day was obtained from the graph of the plotted known values.

2. If a blood sample was missing at Day 10 or at the end of a test period, then the 10th day or last day of the cycle was presumed to correspond with what appeared to be "baseline" concentrations for P. Estimated concentrations of missing values directly following Day 10 or immediately preceding the last day of the cycle were based on the averages falling between known concentrations and the presumed "baseline" values. (Blood samples for some subjects were drawn on Day 1 of the cycle following the test period. In these cases, the nontest period-progesterone value was used to estimate the P concentration of the last day of the test period.) The following are

subjects for whom blood samples are unavailable for the beginning or end of the scheduled blood-drawing days for a given test period. Raw hormone data for each subject, indicating actual values measured and those estimated, as well as graphic displays of the data are presented in Appendix G.

Subject	Days of Cycle for which Blood Samples are Missing
02	10, TP I
03	29-33, TP I
04	25-28, TP I
06	29-30, TP IV
08	28-36, TP I
09	33-34, TP I
11	10-12, TP I; 26-28, TP IV
15	25-26, TP I
21	10, 24-26, TP I
22	10, TP I

In summary, there were one or more missing blood samples for nine of the subjects at Test Period I. At Test Period IV, there were 2 and 3 missing blood samples for two subjects, respectively.

Workshops

Workshops were conducted weekly at the beginning of the study, and approximately semiweekly by the end of the study. Project coinvestigators and guest speakers addressed a variety of topics, i.e., vegetarianism, nutrition and exercise performance, race strategies, stretching, injury prevention, body composition evaluation, and safety. One coinvestigator prepared vegetarian meals at each workshop, and provided printed recipes of all foods prepared. A brief discussion of the meal was included at each session for clarification of food preparation and of the nutritional contribution of each item.

A UNCG women runners' club was established in conjunction with the study, on the basis of a perceived demand for an organization that could provide camaraderie, information, and support to local women runners. The investigators believed that nonsubjects could be helpful to study participants in terms of motivation and compliance. Membership for nonsubjects included coaching (individualized training schedules) from the project coordinator/coach in addition to the regularly scheduled workshops, which served as club meetings for the group that became known as the "Women Runners Research Club." Membership fees from nonsubjects financed the meals provided at the workshops.

Statistical Analyses

All of the statistical analyses were performed using the SAS statistical computer package. Variables were evaluated for compliance with the assumptions required of subsequent statistical tests. Nonparametric analyses were employed because of the distributional characteristics (nonnormal, limited ranges) of the variables and small sample size.

In order to evaluate the magnitude of change from TP I to TP IV for each group, variables representing change over time were created. The Wilcoxon signed-ranks test was used to assess the magnitude of change within C and E. If the null hypothesis (no change) is true, then the probability of observing a positive difference of a given magnitude is equal to the probability of observing a negative difference of the same magnitude. Sufficiently small values for the sums of the signed ranks (positive or negative, separately), therefore, justifies rejection of the null hypothesis.

To test differences between C and E, as opposed to within each group, the Wilcoxon two-sample test was used. This analysis is similar to the signed-ranks test, requiring ranking of the data for subsequent analysis. Observations from each group are combined and ranked from the smallest to the largest value. The sums of the ranks for each population are then compared. One assumes that the sums will be close in value if the two population distributions are similar.

For all variables, the median is reported in lieu of the mean. The median is a better representation of the population's central tendencies due to skewness of many of the variable distributions. In addition, reporting median values is consistent with the use of the median in the signed-ranks test.

In addition to the previously described procedures, the Day 10 blood samples from each test period were analyzed for hemoglobin, hematocrit, serum ferritin, serum cholesterol, and plasma zinc. These assays were carried out for separate reports of the present study, although the results of the measurements are included in the present report for descriptive purposes. Hematocrit values were obtained via microcentrifugation. Hemoglobin was analyzed using the cyanmethomoglobin method (Interdepartmental Committee on Nutrition for National Defense, 1963). Serum ferritin was measured using a solid-phase enzyme immunoassay kit from Abbot Laboratories (North Chicago, IL). A description of serum cholesterol and plasma zinc assays are presented in Appendix H.

CHAPTER IV

RESULTS

Nineteen of the 22 original subjects completed the study; one subject moved away from the area, and two subjects found the study to be incompatible with their lifestyles at that time. The data from one E subject were judged to be unreliable by the investigators, and have, therefore, been excluded from all analyses. The data of one C subject were also omitted because of the subject's lack of compliance to the study protocol. Finally, the TP IV endocrine data from one C subject have been omitted from analysis because she became pregnant in the last test period. Other data from this subject are included in statistical anaylses, however. The final numbers of subjects evaluated were 8 and 9 for C and E groups, respectively.

Urinary LH values were intended to be used to define cycle days as well as to determine integrated LH secretion. Marked variability of the results remains unexplained, however, and has precluded the usefulness of the data. The values are, therefore, not included in the data analysis.

Results of the resting metabolic rate (RMR) measurements were determined to be nonrepresentative of resting metabolism. In spite of repeated measures on separate days in cases where the validity of initial values was questionable, values for some subjects were unusually high. The $\underline{r}$ values for some subjects were also elevated above normal resting values. Travel from home to the Human Performance Laboratory,

as well as the subjects' emotional state, apparently exerted a marked effect upon the measurement of RMR. Hence, this variable has been omitted from statistical analysis.

Baseline Comparisons

Wilcoxon two-sample tests were used to evaluate baseline (TP I) equality between C and E. Descriptve statistics of selected characteristics of C and E subjects are provided in Table 3. There were no statistically significant differences between C and E subjects with respect to age, height, weight, sum of skinfolds, percent body fat as determined by hydrostatic weighing, maximal oxygen uptake, and body weight lost since beginning running. The difference between C and E in total energy expenditure, per day, and per kg body weight, was not statistically significant (Table 3). The median expenditure for C subjects was 2505 kcal/day and 44.9 kcal/kg. The median expenditure for E was 2886 kcal per day and 47.8 kcal/kg. The accuracy of the method used to estimate energy expenditure is believed to be somewhat diminished in a highly active population (C. Bouchard, personal communication, October 10, 1983), and is probably not appropriate for a precise determination of energy balance (Bouchard et al., 1983). The magnitude and direction of error is assumed to be constant throughout the four test periods. For this reason, the values are used for comparison between and within these two groups, but are not considered valid for comparison to other populations and cannot necessarily be interpreted as absolute values.

Table 3
Baseline Characteristics of Subjects

Variable	C (n=8) Median	(Range)	E (n=9) Median	(Range)
Age, yr.	33.5	(21-40)	31.6	(21-36)
Height, cm	166.0	(161-175)	167.0	(160-170)
Weight, kg	54.9	(48.5-71.4)	58.9	(52.8-71.9)
SSF, mm[a]	49.5	(30-93)	47.0	(30-82)
% Body Fat[b]	26.2	(20.5-34.0)	22.4	(15.6-29.4)
$\dot{V}O_2$ Max, $ml \cdot kg \cdot min^{-1}$	43.5	(34-54)	43.0	(34-56)
Lb lost since beginning running	0.0	(0-12)	0.0	(0-13)
Average Kcal expenditure[c] per day	2505	(1935-3042)	2886	(2525-3247)
per kg	44.9	(39.2-52.5)	47.8	(40.9-51.8)

[a]Sum of 4 skinfold sites; triceps, biceps, subscapular, and suprailiac

[b]As determined by body density estimation by hydrostatic weighing, using the formula of Brožek (1963)

[c]Estimated according to the method of Bouchard et al. (1983)

Menstrual history of the subjects is provided in Table 4. Similar values were observed between C and E for the age of menarche, history of dysmenorrhea, and number of pregnancies. Two C and four E subjects reported some degree of menstrual irregularity prior to the year immediately preceding their participation in the study, although these differences in frequency were not statistically significant. Three C and one E had experienced some change (not described) in their menstrual cycles since beginning running (again, a nonsignificant difference). Menstrual characteristics at TP I are provided in Table 5. The median length of menstrual cycles among C was significantly longer than that of E; 29.5 vs. 26.0 days (ranges 26-36 and 24-33 days; $p<.05$). The median number of days in which plasma P concentration was below 4.0 ng/ml among C subjects (15.0) was greater than that of E subjects (9.0) ($p<.05$). These baseline differences cause concern for comparability between the two groups. However, no other differences between C and E were observed in plasma P concentrations as categorized in Table 5, nor in luteal phase length as indicated by the number of days from the surge of serum LH to the plasma P peak (Table 5).

Running history differed in one respect between C and E (Table 6). The reported number of weeks per year that subjects ran was greater among C than E ($p<.01$). This difference can be attributed to three E subjects, one of whom reported running 36 weeks per year. This measure is a misleading indicator of physical activity for this

Table 4
Menstrual History

Variable	C (n=8)	E (n=9)
No. of subjects with history of dysmenorrhea	3	3
No. of subjects with history of dysfunction	2	4
No. of subjects reporting change in cycle since beginning running	3	1
Median age at menarche, yr	13.0 (range 12-14)	13.0 (range 11-16)
Median no. of pregnancies	0.5 (range 0-2)	0.0 (range 0-2)

Table 5
Menstrual Characteristics at the Onset of the Study

Variable	C (n=8) Median	(Range)	E (n=9) Median	(Range)
Usual no. of flow days	5.0	(3-8)	5.0	(2-7)
No. of days from LH peak to P peak	6.0	(4-8)	8.0	(4-10)
P conc > 6.0 ng/ml[a]	1.0	(0-7)	5.0	(0-11)
P conc of 4.0-5.9 ng/ml[a]	2.0	(0-4)	1.0	(0-4)
P conc < 4.0 ng/ml[a]*	15.0	(10-2)	9.0	(6-24)
Area under curve, P vs. time	53.0	(28.4-1.1)	57.0	(7.9-166.4)
TP 1 cycle length, days*	29.5	(26-3)	26.0	(24-33)

[a]Values provided represent number of days in which plasma P concentrations were in the range indicated.

*Significant difference between C and E ($p<.05$)

Table 6
Running History

Variable	C (n=8) Median	(Range	E (n=9) Median	(Range)
Age began running, yr	30.0	(18-40)	22.0	(17-36)
Time since beginning running, months	36.0	(4-68)	12.0	(3-156)
Min/day run	37.5	(30-50)	40.0	(30-60)
Day/week run	5.0	(4-6)	6.0	(3-6)
Week/year run*	52.0	(50-52)	50.0	(30-52)
Miles/week run	18.5	(15.22)	20.0	(10-23)
Hours/year run	159.7	(125-195)	150.0	(60-300)

*Significant difference between C & E ($p<.01$)

subject, however, because during most of the 16 nonrunning weeks she participates in downhill skiing several times a week. The values reported by subjects 21 and 22, 30 and 44 weeks per year, respectively, are likely to be valid representations of their running histories. To further evaluate the usual amount of time spent running, a variable derived from the number of minutes per day, days per week, and weeks per year spent running was computed. These three variables were multiplied together, and the product divided by 60 minutes to yield the total number of hours run per year. The median value for C was not significantly different from that of E (159.7 vs. 150.0 hours per year, respectively).

Time per day and days per week spent running were similar between C and E. There were no significant differences in the length of time since subjects had first started running, nor in the age at which subjects began running, but ranges were large for both variables. Both groups ran a similar number of miles per week (MPW) at the beginning of the study. The median MPW for C was 18.5 MPW (range 1522), and for E, 20.0 MPW (range 10-23).

Baseline dietary variables are presented in Table 7. The protein percentage of total kcal was greater among C than E at TP I (13.4% vs. 11.0%) ($p<.05$). Accordingly, the protein intake per 1000 kcal was also significantly greater among C than E at TP I (33.4 g/1000 kcal vs. 27.4 g/1000 kcal; $p<.05$). The difference in protein intake expressed per kg of body weight was not statistically

Table 7
Baseline Dietary Variables

Variable	C (n=8) Median	(Range)	E (n=9) Median	(Range)
Kcal intake per day	1683	(1392-2361	1816	(741-2769)
per kg	31.2	(19.5-48.7)	32.7	(12.8-43.8)
Protein g/day	56.6	(49.9-90.8)	44.2	(26.6-92.0)
% of kcal*	13.4	(10.0-16.8)	11.0	(8.1-14.3)
g/kg	1.00	(0.78-1.87)	0.78	(0.46-1.46)
g/1000 kcal*	33.4	(25.0-41.9)	27.4	(20.3-35.9)
Carbohydrate g/day	215.0	(172-317)	240.0	(126-382)
% of kcal	44.2	(31.9-88.2)	49.5	(39.2-77.3)
Fat, g/day	70.4	(41.3-85.1)	62.4	(17.1-121.7)
% of kcal	34.4	(24.0-40.3)	32.5	(20.8-39.6)
Saturated fat, g	26.0	(13.3-35.2)	23.3	(4.7-57.5)
Polyunsaturated fat, g	38.4	(22.0-48.8)	35.0	(8.4-59.0)
Cholesterol, mg	275.0	(77-536)	255.0	(34-546)
Fiber, g	6.1	(3.4-8.4)	6.1	(4.1-8.4)
Iron, mg	12.8	(6.9-23.5)	10.6	(5.8-19.8)
Zinc, mg	6.8	(4.9-14.7)	8.4	(4.0-15.8)

*Significant difference between C and E ($p<.05$)

different, however. No other significant differences were observed in dietary variables between the two groups.

No between-group differences were observed in any biochemical values measured at TP I, including hemoglobin, hematocrit, serum ferritin, serum cholesterol, plasma zinc, and urinary 3-MH/creatinine excretion (Table 8). Considerable controversy exists as to the normal range of 3-MH excretion, and few studies have included female subjects. The values reported in the current study, however, are consistent with reports of previous investigations (Frisch et al., 1984; Munro, 1978).

To summarize, the baseline comparisons between C and E confirmed equality on most variables. Statistically significant differences were observed, however, for the following variables: (a) the usual length of the menstrual cycle (Table 5), (b) the number of days in the TP I cycle in which plasma P concentration was less than 4.0 ng/ml (Table 5), (c) weeks run per year (Table 6), and (d) protein consumption expressed as percent of total kcal and as g/kg body weight (Table 7).

Menstrual Characteristics at Test Periods I and IV

Changes in menstrual characteristics including P values are presented in Tables 9 and 10. As confirmed by the Wilcoxon signed-ranks test, the length of the luteal phase did not change for C or E subjects between TP I and TP IV. There was no change in the median length of the menstrual cycle for either group, and the usual number of flow days remained unchanged as well.

Table 8
Baseline Biochemical Comparisons

Variable	C (n=8) Median	(Range)	E (n=9) Median	(Range)
Hemoglobin, g/dl	13.4	(11.0-14.8)	13.4	(12.8-14.5)
Hematocrit, %	40.5	(37.5-45.0)	41.0	(39.5-45.0)
Serum ferritin, ng/ml	24.0	(1-71)	27.0	(3-79)
Serum cholesterol, mg/dl	173.5	(132-253)	185.0	(158-231)
Plasma zinc, mcg/dl	85.0	(78-110)	92.0	(84-108)
Urinary 3-MH/creatinine, nmol/mg	133.8	(68.5-236.7)	133.7	(82.3-219.1)

Table 9
Menstrual Characteristics of Control Subjects,
Test Period I vs. IV[a]

Variable	I Median	IV Median	Change
Usual no. of flow days	5.0	5.0	0.0
No. of days from LH peak to P peak	6.0	7.0	0.0
P conc > 6.0 ng/ml[b]	1.0	0.0	0.0
P conc of 4.0-5.9 ng/ml[b]	2.0	0.0	-1.0
P conc < 4.0 ng/ml[b]	15.0	19.0	+2.0
Area under curve, P vs. time	53.0	25.3	-16.6*
Cycle length, days	29.5	28.0	-4.0

[a]TP I n=8; TP IV n=7

[b]Values provided represent number of days in which plasma P concentrations were in the range indicated

*Significant decrease from TP I to IV (p<.05)

Table 10
Menstrual Characteristics of Experimental Subjects, Test Period I vs. IV[a]

Variable	I Median	IV Median	Change
Usual no. of flow days	5.0	5.0	0.0
No. of days from LH peak to P peak	8.0	6.0	0.0
P conc > 6.0 ng/ml[b]	5.0	0.0	-5.0*
P conc of 4.0-5.9 ng/ml[b]	1.0	0.0	-1.0
P conc < 4.0 ng/ml[b]	9.0	16.0	+6.0
Area under curve, P vs time	57.0	21.2	-22.6
Cycle length, days	26.0	27.0	+1.0

[a] $n=9$

[b] Values provided represent number of days in which plasma P concentrations were in the range indicated

*Significant decrease from TP I to IV ($p<.05$)

Among E subjects there was a statistically significant decrease in the number of days in which P concentrations were greater than 6.0 ng/ml ($p<.05$). The E median number of days of high P concentrations decreased from 5.0 days in TP I to 0.0 days in the last test period.

Among the C subjects, the area under the P-vs.-time curve decreased significantly ($p<.05$) from TP I to IV (53.0 units to 26.7 units). The P area under the curve also decreased among the E subjects (57.0 units to 21.2 units), but the change was not statistically significant, probably due to the wide variability of observations.

A Wilcoxon two-sample test was performed to compare the degree of change of P variables between the two groups. The results confirmed that the magnitude of change among E did not differ significantly from that of C for any of the P variables; area under the P-vs-time curve; number of days in which P concentration was greater than 6.0 ng/ml, 4.0-5.9 ng/ml, or less than 4.0 ng/ml; and the number of days between the LH surge and the P peak.

Anthropometric Measures and Variables Related to Exercise, Test Period I to Test Period IV

Anthropometric measures as well as exercise-related variables are presented in Tables 11 and 12. Experimental subjects ran a greater number of MPW than C at TP II, III, and IV; however,

Table 11
Median Values of Anthropometric and Physical Activity Measures for Control Subjects, Test Period I through IV

Variable	I Median	II Median	III Median	IV Median
Miles run per week	18.5	14.5	13.5	17.0**
Energy expenditure[a] per day	2505	2579*	2723	2621**
per kg	44.9	44.1*	48.8	47.1
Weight, kg	54.9	54.9	55.7	55.7
SSF, mm[b]	49.5	51.5	53.0	49.5
% Body fat[c]	26.2	25.1	25.6	27.5
$\dot{V}O_2$ max, $ml \cdot kg \cdot min^{-1}$	43.5	---	---	41.5

[a]Estimated according to the method of Bouchard et al. (1983)

[b]Sum of 4 skinfold sites; triceps, biceps, subscapular, and suprailiac

[c]As determined by body density estimation by hydrostatic weighing, using the formula of Brožek (1963)

*Statistically significant difference from E group ($p<.05$)

**Statistically significant difference from E group ($p\leq.01$)

Table 12
Median Values of Anthropometric and Physical Activity Measures for Experimental Subjects, Test Period I through IV

Variable	I Median	II Median	III Median	IV Median
Miles run per week	20.0	20.0	29.0	32.0**†
Energy expenditure[a] per day	2886	2928*	2897	2997**
per kg	47.8	49.0*	49.4	48.7†
Weight, kg	58.9	59.0	60.8	59.1
SSF, mm[b]	47.0	44.0	45.0	46.0
% Body fat[c]	22.4	21.9	23.8	23.5
$\dot{V}O_2$ max, $ml \cdot kg \cdot min^{-1}$	43.0	---	---	45.0

[a]Estimated according to the method of Bouchard et al. (1983)

[b]Sum of 4 skinfold sites; triceps, biceps, subscapular, and suprailiac

[c]As determined by body density estimation by hydrostatic weighing, using the formula of Brožek (1963)

*Statistically significant difference from C group ($p<.05$)

**Statistically significant difference from C group ($p\leq.01$)

†Statistically significant difference from TP I ($p<.05$)

††Statistically significant difference from TP I ($p<.01$)

statistical significance of this difference was not achieved until the last test period (32 MPW vs. 17 MPW; $p<.01$). By TP III and IV, the E subjects were running 45% and 60% more MPW, respectively, than at baseline. The variability in MPW among E rose progressively as their mileage increased. The large variability was primarily attributable to injuries, which necessitated a slower rate of increase in mileage among some subjects than others.

The difference between C and E in estimated daily energy expenditure, expressed per kg of body weight, was statistically significant only at TP II (44.1 kcal/kg for C and 44.9 kcal/kg for E; $p<.05$). The total daily energy expenditure, however, was significantly greater among E than C at TP II (2928 kcal/day vs. 2579 kcal/day) and IV (2997 kcal/day vs. 2621 kcal/day; $p<.05$ and .01, respectively). Control subjects reported their greatest estimated energy expenditure during TP III (2723 kcal/day), although their running mileage (13.5 MPW) was lower during that test period than in any other test period. Among E, the difference between the estimated total energy expenditure at TP I (2847 kcal/day) and at TP IV (2997 kcal/day) was not statistically significant. There was a significant increase in caloric expenditure per kg of body weight among E from TP I to IV; 47.8 kcal/kg at TP I to 48.7 kcal/kg at TP IV ($p<.05$).

Measures of skinfold thicknesses, body density via hydrostatic weighing, and body weight indicated that body weight and fat levels remained relatively constant for both C and E throughout the study.

There were no statistically significant differences between the two groups on any of the anthropometric measures at any test period. The median $\dot{V}O_2$ max achieved by C at TP IV decreased slightly from that of TP I (43.5 ml/kg to 41.5 ml/kg). The E median $\dot{V}O_2$ max increased marginally from 43.0 ml/kg at TP I to 45.0 ml/kg at the end of the study. Neither change was statistically significant.

Dietary Intake, Test Period I to Test Period IV

The median values of dietary variables are provided in Tables 13 and 14. Out of all of the dietary variables evaluated, C and E differed significantly only on protein intake. At TP I, the proportion of kcal derived from protein among C was 13.4%, while that of E was 11.0% ($p<.05$). Protein intake expressed as g/1000 kcal was greater among C than E at TP I (33.4 g/1000 kcal vs. 27.4 g/1000 kcal; $p<.05$). The change from TP I to IV in protein intake was significantly different between C and E ($p<.01$). While E subjects consumed 2.5 g more protein per 1000 kcal, the protein intake of C decreased by a median of 2.4 g per 1000 kcal. The difference in protein intake expressed per kg of body weight, or as total daily intake, was not statistically significant, however, at any test period.

The total daily kcal intake of E subjects appeared to increase slightly from TP I to IV (1816 kcal/day to 1918 kcal/day), but was not statistically significant. The kcal intake per kg of body weight among E remained constant at 32.7 kcal/kg. The median kcal intake of C also increased by 100 kcal per day, from 1683 kcal/day

Table 13
Median Values of Dietary Intake of Control Subjects
Test Period I through IV

Variable	I Median	II Median	III Median	IV Median
Kcal intake per day	1683	1547	1638	1783
per kg	31.2	27.7	30.8	33.7
Protein g/day	56.6	48.9	51.9	55.7
% of kcal*	13.4*	13.1	12.8	13.0
g/kg	1.00	0.87	0.88	0.94
g/1000 kcal*	33.4*	32.8	31.9	32.4
Carbohydrate g/day	215.0	207.0	219.0	238.0
% of kcal	44.2	42.6	45.4	49.0
Fat, g/day	70.4	56.0	51.7	64.4
% of kcal	34.4	34.0	29.6	32.3
Saturated fat, g	26.0	19.6	17.0	22.2
Polyunsaturated fat, g	38.4	29.4	31.4	35.8
Cholesterol, mg	275.0	230.0	221.5	202.0
Fiber, g	6.1	4.7	4.7	6.4
Iron, mg	12.8	10.0	10.6	10.1
Zinc, mg	6.8	6.2	8.5	8.3

*Significant difference from E group ($p<.05$)

Table 14
Median Values of Dietary Intake of Experimental Subjects, Test Period I through IV

Variable	I Median	II Median	III Median	IV Median
Kcal intake per day	1816	1569	1753	1918
per kg	32.7	29.2	29.4	32.7
Protein g/day	44.2	49.2	55.9	51.3
% of kcal*	11.0*	11.2	11.7	12.3
g/kg	0.78	0.90	1.00	0.95
g/1000 kcal*	27.4*	28.0	29.4	30.8
Carbohydrate g/day	240.0	23.5	237.0	233.0
% of kcal	49.5	44.8	51.8	49.3
Fat, g/day	62.4	55.3	67.9	72.7
% of kcal	32.5	31.9	34.8	36.0
Saturated fat, g	23.3	21.1	25.3	26.6
Polyunsaturated fat, g	35.0	28.7	38.8	39.9
Cholesterol, mg	255.0	231.0	284.0	279.0
Fiber, g	6.1	5.9	6.9	7.1
Iron, mg	10.6	12.5	12.0	13.3
Zinc, mg	8.4	8.6	9.2	8.2

*Significant difference from C group ($p<.05$)

at TP I to 1783 kcal/day at TP IV. The median kcal/kg intake for C was 31.2 kcal/kg at TP I and 33.7 kcal/kg at TP IV. Neither measures of energy intake at TP IV were significantly different from TP I among C subjects.

Although the estimated energy expenditure exceeds the kcal intake at every test period, the increase in energy intake reported for both C and E (although statistically nonsignificant), parallels the increase in the estimated energy expenditure from TP I to IV. The increment for each group is about 100 kcal per day. The median values of C and E subjects for all other dietary variables were similar between TP I and IV.

In spite of large difference in the medians, serum ferritin levels were not significantly greater among C than E at any test period, probably because of the large variability in each group (Tables 15 and 16). Serum ferritin decreased significantly among E subjects from 27.0 ng/ml at TP I to 5.0 ng/ml at TP IV ($p<.01$).

Excretion of 3-MH per mg of creatinine was significantly greater among C than E at TP II (241.1 nmol/mg vs. 147.1 nmol/mg; $p<.05$). There were no other significant differences in any biochemical variables at any test period.

Table 15
Median Biochemical Values for Control Subjects,
Test Period I through IV

Variable	I Median	II Median	III Median	IV Median
Hemoglobin, g/dl	13.4	13.6	13.3	13.5
Hematocrit, %	40.5	41.0	42.0	42.3
Serum ferritin, ng/ml	24.0	15.0	16.5	21.5
Serum cholesterol, mg/dl	173.5	171.5	174.0	181.0
Plasma zinc, mcg/dl	85.0	85.0	96.5	91.0
Urinary 3-MH/creatinine, nmol/mg	133.8	242.1	137.0	141.8

*Significant difference from E group ($p \leq .05$)

Table 16
Median Biochemical Values for Experimental Subjects, Test Period I through IV

Variable	I Median	II Median	III Median	IV Median
Hemoglobin, g/dl	13.4	12.9	12.6	12.5
Hematocrit, %	41.0	40.0	40.5	39.5
Serum ferritin, ng/ml	27.0	9.5	9.0	5.0
Serum cholesterol, mg/dl	185.0	170.0	184.0	175.0
Plasma zinc, mcg/dl	92.0	90.0	95.0	90.0
Urinary 3-MH/creatinine, nmol/mg	133.7	147.1*	166.0	141.1

*Significant difference from C group ($p<.05$)
†Significant difference from TP I ($p<.01$)

CHAPTER V

DISCUSSION

The present investigation was implemented to test hypotheses that were based upon the assumption that a program of progressive exercise will result in alterations in reproductive hormone function in female recreational runners. Although changes in plasma P concentrations were observed among subjects participating in the progressive running program, the hypotheses can not be specifically addressed due to the changes in progesterone concentrations also observed among C subjects, which were of a similar magnitude and direction. The changes that occurred in the plasma P concentrations among E subjects, therefore, can not be attributed to their increase in exercise quantity or intensity.

Menstrual Characteristics

At TP I, the length of the menstrual cycle was significantly longer among C than E. In addition, the number of days in which P concentrations were below 4.0 ng/ml was greater among C than E. The latter difference may be a reflection of the longer cycles; nevertheless, these baseline difference raise the question of comparability between the two groups with respect to menstrual function. The non-normal distribution of the sample and the small sample size precluded the use of Chi square or regression analysis to control for baseline differences. A limited type of covariate analysis was attempted,

however, by testing for significant differences between computed change variables (TP I to TP IV) for all of the P-related variables. By comparing the degree of change from TP I to IV within C to that of E, one controls for the starting point of each observation.

Another concern regarding the baseline endocrine data is the low P values observed for 3 of the subjects. One C subject (15) and 2 E subjects (3 and 23) had no measured P concentrations in the mid- or high-P range (4.0 ng/ml or greater) at TP I. Thus, these subjects appear to have had compromised luteal function at the onset of the study. The subjects demonstrating low P concentrations at TP I are 3 of the 4 subjects whose P area change scores increased from TP I to IV, in contrast to the decreases exhibited by all of the other subjects. Removing the data of subjects 3, 15, and 23 from statistical analyses did not alter the outcome of statistical tests between the two groups because the resulting small sample size severely limits the power of statistical tests.

The median number of flow days and the length of the menstrual cycle remained unchanged among C and E subjects. This lack of change was anticipated; no changes of these two variables associated with exercise training have been reported in the literature. The lack of a shortening of the luteal phase among E is inconsistent with cross-sectional findings that have been reported for exercising vs. inactive women (Bonen et al., 1981; Ellison & Lager, 1985; Frisch et al., 1984; Jacobson & Bonen, 1981; Jacobson et al., 1983; Prior et al., 1982; Shangold et al., 1979).

Analysis of the individual data for changes in the length of the luteal phase reveals no clear trend among the subjects as a group (Table 17). There was considerable variability among the subjects with respect to changes that occurred in the length of time between the ovulatory LH surge and the P peak.

The number of days in which P concentrations exceeded 6.0 ng/ml (Hi-P) in the menstrual cycles of E subjects decreased significantly from TP I to TP IV. The difference between the change experienced by E subjects and that of the C subjects was not statistically significant, however. The changes in Hi-P days among C subjects were -6, -2, 0, 0, 0, 0, and +3 days, with a median of 0.0 days. The observed changes in Hi-P days among E were -11, -8, -6, -5, -5, -3, 0, 0, and +4 days, with a median of -5 days. There is an obvious trend of decreasing Hi-P days among E, with little evidence any trend among C. The lack of a statistically significant difference between the two groups may be explained on the basis of the small sample size and a high degree of variability among the E subjects. Standard deviations for the Hi-P change scores were 2.8 and 4.6 days for C and E, respectively.

The decrease from TP I to IV in the area under the curve for P vs. time (P area) was statistically significant among C ($p<.05$), but not among E ($p=.097$). Six of the 9 E subjects experienced a decrease in P area ranging from a decrease of 13 units to 158 units, while the P area of 3 E subjects rose from TP I to IV, increments of 1, 7, and 30 units. The decreases in the P area from TP I to IV among 6 C

Table 17
Change in Weekly Running Mileage and Number of Days between LH Surge and Progesterone Peak, Test Period I to Test Period IV

Subject	MPW[a]	Days[b]
2	+60%	-4
21	+90%	-2
16	+17%	-1
11	-29%	0
15	+5%	0
17	+6%	0
7	+85%	0
22	+21%	0
4	-6%	+1
1	+100%	+1
9	-27%	+2
18	+45%	+2
6	+19%	+4

[a]MPW represents the percentage change in miles per week run from Test Period I to Test Period IV

[b]Days represents the change in the number of days from the LH surge to the progesterone peak in the menstrual cycle of Test Period I to that of Test Period IV

subjects ranged from 14 to 44 units, and the P area increased 13 units in 1 C subject. The tremendous variability in the P area changes from TP I to IV within the E group (standard deviations were 19.2 and 57.0 units for C and E, respectively) again contributed to the lack of a statistically significant difference between the two groups. As previously mentioned, 3 of the 4 subjects whose P area increased from TP I to IV exhibited inadequate luteal function at the beginning of the study.

An important consideration with respect to comparisons of circulating hormone levels between and among individuals is the degree to which circulating levels can be assumed to represent system function. Absolute values of circulating hormones provide no evidence of hormone turnover or of changes that may have occurred, for example, in the number of or the sensitivity of receptor sites. Thus, the absence of changes in circulating hormone levels does not rule out the possibility that changes in hormone turnover have occurred at either the site of hormone release or the target tissue.

Running Mileage

The difference between C and E in weekly running mileage was not statistically significant until the last test period, when the median distance run was 17 MPW and 32 MPW for C and E, respectively. The investigators' anticipated increase in running mileage among the E subjects was a minimum of 40 MPW by TP IV, achieving at least 30-35 MPW by TP III. The median TP III mileage among E was 29 MPW, but with a relatively large degree of variability (SD=7.7 MPW) due

primarily to injuries among the runners. A more rapid increase in running mileage may have resulted in greater changes in the menstrual function variables measured.

The protocol of the prospective study by Boyden et al. (1983) required the runners to increase their weekly mileage from 15 to 63 MPW over 14 to 15 months, an average increment of less than 1 MPW. The authors reported measures of only 2 reproductive hormones: Basal testosterone concentrations remained unchanged in response to the progressive increase in running, and basal estradiol levels were reduced. The only clinical evidence of menstrual irregularities observed was in a diminution of blood loss due to both decreased volume of flow and to fewer flow days. Bullen et al. (1985) reported clinical or hormonal abnormalities of menstrual function in 31 of 32 women participating in an 8-week program of progressive running. The runners increased their running distance from 20 to 50 MPW in 5 weeks, an increment of 7.5 MPW, and maintained 50 MPW through the 8th week. Perhaps a more rapid progression such as that used by Bullen et al. is more likely to induce changes in menstrual function. In the present study, the E subjects' weekly mileage averaged an increase of only 0.5 MPW over approximately 7 months.

The gradual progression of exercise training may result in the development of an adaptive mechanism to protect normal menstrual function. One observation of the present study supports this hypothesis. Omitting the subjects for whom plasma P concentrations indicated possible impairment of luteal function, Subject 22 was the only subject whose P area increased from TP I to IV. She was also unique

in that the length of time she had been running before participation in the study was greater than any of the other subjects. Subject 22 had been running for 156 months (13 years) prior to the beginning of the study, whereas running experience among other participants ranged from 3 months to 84 months. Only 2 of these subjects reported running for more than 60 months (5 years). Elimination of the data of Subject 22 in the E group, results in a statistically significant decrease between the P area in TP I and that of TP IV ($p<.05$). No definitive conclusions can be drawn from such an analysis; however, this observation does lend support to the hypothesis that the physiological adaptations to exercise may include a mechanism by which reproductive function is protected when exercise progression is gradual.

Energy Drain

The changes observed among C subjects are difficult to explain, given their relatively constant exercise levels for the duration of the study. By subjective evaluation, there were no apparent changes among C subjects with respect to stress levels, health, or general lifestyles across the four test periods. Although a negative energy balance among C subjects appears to be unlikely, the possibility of its occurrence needs to be addressed. The median energy expenditure of both groups was greater at TP IV than at TP I; however, the difference was statistically significant only for E ($p<.05$), whose running mileage also increased significantly ($p<.01$) (Table 18). During TP III, C reported their greatest energy expenditure, but their lowest MPW. This apparent discrepancy can probably be explained by

Table 18
Median Values of Energy Intake & Expenditure, and Miles Run per Week, Test Periods I through IV

Control Subjects	I	II	III	IV
Kcal intake				
per day	1683	1547	1638	1783
per kg	31.2	27.7	30.8	33.7
Kcal expenditure[a]				
per day	2505	2579	2723	2621
per kg	44.9	44.1	48.8	47.1
Miles run/week	18.5	14.5	13.5	17.0
"Energy deficit"[b]				
per day	-822	-1032	-1085	-838
per kg	-13.7	-16.4	-18.0	-13.4
Experimental Subjects				
Kcal intake				
per day	1816	1569	1753	1918
per kg	32.7	29.2	29.4	32.7
Kcal expenditure[a]				
per day	2886	2928	2897	2997
per kg	47.8	49.0	49.4	48.7
Miles run/week	20.0	20.0	29.0	32.0
"Energy deficit"[b]				
per day	-1070	-1359	-1144	-1079
per kg	-15.1	-19.8	-20.0	-16.0

[a]As estimated using the method of Bouchard et al. (1983)

[b]The difference between energy intake and estimated energy expenditure has been calculated for relative comparisons only. The estimated energy expenditure is not necessarily representative of actual energy expenditure; hence, a true energy balance cannot be ascertained from the data.

the time of the year of TP III; for most subjects, TP III coincided with the the time of the year of TP III; for most subjects, TP III coincided with the beginning of spring. It is feasible that nonrunning energy expenditure did increase as a result of more outdoor activities such as walking, bicycling, yardwork, and tennis.

No significant changes in measures of body weight, skinfold thickness, or in body density as estimated by hydrostatic weighing were observed among C or E. A reduction of basal metabolic rate in response to caloric restriction has been documented in animals and in humans (Apfelbaum, Bostsarron, & Lacatis, 1971; Hill, Fried, & DiGirolamo, 1984; Miller & Parsonage, 1975), thereby making possible the maintenance of a constant body weight and fat level in spite of an energy intake that seems incompatible with energy expenditure. Energy intakes that seem insufficient to meet energy demands have been reported for runners and dancers (Berning et al., 1975; Calabrese et al., 1983). The estimated energy expenditures of C and E in the present study indicate large energy deficits for both groups; yet, the instrument that was used to estimate energy expenditure (Bouchard et al., 1983) has not been validated in a highly active population, and can not be considered to provide a precise estimate of caloric expenditure. (Reliability of the instrument within this sample appears to be good as judged by the fact that median changes of estimated energy expenditure paralleled median changes in caloric intake.) The measurement of RMR was intended to be helpful in addressing the question of metabolic adaptation. Unfortunately, RMR data provided invalid measurements of resting metabolism among these women.

To test the possibility of an effect of energy balance on variables relating to menstrual function, an energy ratio was computed for each subject (ratio of energy intake to energy expenditure). No significant correlations (Kendall Tau) were detected between any of the P-related variables (absolute values or change scores) and the energy ratio.

An indirect measurement of caloric insufficiency is urinary 3-MH, a natural marker of skeletal muscle metabolism (Lukaski & Mendez, 1980). This amino acid is found almost exclusively in skeletal muscle. During catabolism, 3-MH is not oxidizable (Long et al., 1975), nor is it reincorporated into protein (Young, Alexis, Baliga, & Munro, 1970). Measurement of urinary 3-MH provided no evidence of accelerated breakdown of skeletal muscle tissue among runners in the present study. Values were consistent with those previously reported for women (Frisch et al., 1984; Munro, 1978). Tests of correlation revealed a significant association between 3-MH excretion and MPW among E subjects at TP I ($p<.05$). The association was considered to be artifactual, however, because there were no other significant correlations between MPW and 3-MH excretion at any test period in either group. Thus, the available data do not support the possibility of an energy deficit among C or E subjects.

The 3-MH excretion among C deserves mention. At TP II, the C group excreted 242.1 nmol 3-MH/mg creatinine, a substantial increase over values of TP I (133.8 nmol/mg), TP III (137.0 nmol/mg), and TP IV (141.8 nmol/mg). The 3-MH excretion was significantly

greater among C than E (147.1 nmol/mg) at TP II ($p<.05$). Although an increased urinary 3-MH excretion has been reported in response to intensive exercise training (Frisch et al., 1984), the TP II increase in 3-MH excretion among C can not be explained by running mileage; C subjects reported their lowest weekly mileage during TP II (Table 18). Control subjects also reported their lowest kcal intake during TP II, but this was not the test period with the greatest apparent discrepancy between caloric intake and expenditure (Table 18). Furthermore, tests of correlation revealed no consistent associations with energy intake or expenditure. The high 3-MH excretion by C subjects at TP II remains unexplained.

Fiber Intake

The increased fecal bulk resulting from a high-fiber vegetarian diet has been proposed as a possible contributing factor to the development of menstrual dysfunction (Slavin et al., 1984). In the present study, the highest median intakes of dietary fiber reported by C and E subjects respectively, were 6.4 g/day and 7.1 g/day, both at TP IV. This level is well below the daily intake of 28 g of dietary fiber that was associated with altered estrogen metabolism among vegetarian women (Goldin et al., 1982), and below the dietary fiber intake of 12 g/day among vegetarian men who exhibited alterations in testosterone and estrogen metabolism (Howie & Shultz, 1985). There is little information available regarding the fiber intake of the U. S. population. In one study of 200 students, dietary fiber intake

averaged 15.4 g/day (Marlett & Bokram, 1981), more than twice the amount of fiber consumed by subjects in the present study. Dietary fiber intake among C and E subjects apparently did not increase concomitant with their change to a vegetarian diet.

Fat Intake

The median daily fat consumption among C ranged from 51.7 g/day to 70.4 g/day, comprising 29.6% to 34.4% of total kcal. Among the E subjects, the median fat intake ranged from 55.3 g/day to 72.7 g/day, corresponding to a percentage of total kcal of 31.9% to 36%. This level of dietary fat is below the mean fat intake reported for young women in the U. S. (90-100 g/day; 40% of total kcal) (Goor et al., 1985). it is not known if the fat consumption among the C and E subjects declined significantly while participating in the study from their customary prestudy level of intake; however, the nonsignificant changes in serum cholesterol levels suggest that the fat intake of C and E subjects did not change quantitatively or qualitatively from their prestudy diets. The relatively small difference between the fat intake among the runners and that of average American women, would not seem to be a likely cause of the changes in P concentrations observed.

Protein Intake

Protein consumption exceed the RDA of 0.8 g protein/kg body weight (National Research Council, 1980) for all test periods except TP I, during which the protein intake among E subjects was 0.78

g/kg. While this level of dietary protein appears to be adequate, protein balance can not be ascertained from food records alone, especially in the absence of energy balance determinations, and without characterizing and quantifying the protein quality of the diet. The relatively constant levels of 3-MH excretion indicate that skeletal muscle metabolism was not accelerated, suggesting that protein nutriture was sufficient.

The question of whether there is some component of a vegetarian diet that affects reproductive function remains to be answered. Alterations in P concentrations were observed among the subjects; however, the data do not support the hypothesis that such changes are attributable to exercise. Based on the dietary variables available for analysis, no dietary element can be implicated as a contributor to the measured P effects. Nevertheless, the role of diet in the development of menstrual disorders can not be discounted as a potential causative factor.

Summary

No significant changes were observed among C or E subjects with respect to body weight, skinfold thickness, body density, or maximal oxygen uptake from TP I to TP IV. The E runners increased their energy expenditure (47.8 kcal/kg to 48.7 kcal/kg) and running mileage (20 MPW to 32 MPW) significantly. The serum ferritin levels of E subjects declined markedly from 27.0 ng/ml TP I to 5.0 ng/ml at TP IV.

The diets of the two groups differed only in protein intake at TP I, E subjects consuming slightly less protein than C subjects (27.4 g/1000 kcal vs. 33.4 g/1000 kcal). At TP I, significant differences between C and E were observed for the length of the menstrual cycle and for the number of days in which plasma P concentrations were less than 4.0 ng/ml. In an effort to control for these baseline differences in subsequent comparisons between the two groups, TP-I-to-TP-IV change scores were computed.

Among E subjects there was a significant decrease in the number of days in which plasma P concentrations were greater than 6.0 ng/ml, whereas C subjects experienced a significant decrease in the area under the curve for P vs. time. No significant correlations were observed between any of the P-related variables and weekly running mileage or energy expenditure across the four test periods. Nor was there a signifcant decrease in luteal phase length among E, as observed among females participating in other studies of progressive exercise. None of the changes in P variables was significantly different between C and E, suggesting that the alterations observed in P levels were not due to exercise, but rather to some unidentified factor common to both C and E subjects.

It is likely that the etiology of athletic amenorrhea is multifactorial. The possibility exists that individual variability accounts for much of the contrasting responses to exercise among women. Analagous to the recent recognition that there are hyper- and

hyporesponders to dietary cholesterol in terms of a serum cholesterol effect (Oh & Miller, 1985), perhaps there are some women whose response to exercise results in impairment of the reproductive system, whereas other women are unaffected. It is feasible also, that an adaptive mechanism to protect against menstrual dysfunction is operative in some women and not in others. Following are some suggestions for further research based upon the outcome of the present study and on the questions raised as a result of these findings.

Suggestions for Further Research

Possibly the most important addition to the protocol of the present study would be a prestudy control period of 2-6 months. During this time, records would be collected on customary exercise practices and dietary intakes. Menstrual records during the control period would provide evidence of normal menstrual patterns. Hormonal evaluation should be included as well. Only subjects demonstrating unambiguously normal menstrual function would be accepted to participate in the study.

A valuable characteristic of the present study was the use of a free-living population. This is desirable to minimize stress and lifestyle-change effects on menstrual function. In one study of the effects of progressive running on menstrual function, the subjects were moved to a summer camp for the duration of the study (Bullen et al., 1985). The study protocol has been criticized because of the

possibility that the menstrual abnormalities observed were related to the disruption of the subjects' customary living patterns (Metcalf, 1985).

An obvious advantage of the summer camp environment is that the subjects can more readily devote time and energy to exercise, free from family, work, school, or community-related responsibilities. This is an important consideration, and may warrant the dislocation of the subjects to such an environment if exercise demands are severe. To induce an observable menstrual response to exercise, it is probably desirable to intensify the exercise level much more rapidly than the progression of the present study. If subjects are moved from their normal habitat to participate in the study, a control group who maintain their customary residence should be incorporated to the study.

In contrast to the two prospective studies of exercise-related menstrual dysfunction previously reported (Boyden et al., 1983; Bullen et al., 1985), the present study included a control group who did not increase their running. If the sample size were adequate, 2 experimental and 2 types of control groups would allow for the separate evaluation of the effects of diet and the effects of exercise on menstrual function. For example, 1 of the 2 groups whose training mileage remained constant would maintain a normal nonvegetarian eating pattern, and 1 group would change to a vegetarian diet. There would also be a vegetarian and a nonvegetarian group participating in a program of progressive running.

A large sample size would provide greater statistical power for within-group and between-group comparisons. A sample size of 100 would be ideal, and could probably be achieved through coordinated efforts between investigators at various geographical localities. A project of such proportion would require extensive personnel, an aspect of the present study that severely limited the data collection capabilities of the investigators as well as the management of the subjects, i.e., coaching and motivating the runners.

Daily blood samples rather than every other day, which were precluded in the present study due to limited resources, would greatly enhance the value of the hormone data. The use of salivary P concentrations (Ellison & Lager, 1985) should be explored as a means of simplifying data collections.

The issue of energy drain can not be adequately addressed without improved methods of estimating, or simplified methods of measuring energy expenditure. The use of devices such as a portable accelerometer (Montoye et al., 1983) should be explored. In addition, the accurate evaluation of resting or basal metabolic rate would be useful.

Few studies of exercise-related dysfunction have included a dietary component. The effects of protein quality, nitrogen balance, and energy balance on menstrual function need to be investigated. Fat levels of the diet as well as the quality of the fat have not yet been assessed in relation to exercise-associated menstrual dysfunction.

Mineral nutriture, i.e., iron, as demonstrated in the present study, is affected by exercise, and also warrants further investigation. In short, there is a strong need for nutrition research in the search for a better understanding of athletic amenorrhea. Nutrition researchers must work together with endocrinologists and exercise physiologists in the development of research designs and in the analysis and interpretation of dietary evaluation.

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APPENDIX A

RECRUITMENT MATERIALS

WOMEN RUNNERS

NEEDED TO PARTICIPATE IN 6-MONTH TRAINING STUDY

LEARN MORE ABOUT YOUR OWN :

- FITNESS LEVEL
- CALORIC INTAKE
- HORMONE RESPONSE TO EXERCISE
- % BODY FAT
- ENERGY EXPENDITURE

THE STUDY HAS 2 COMPONENTS:

(1) FOR WOMEN WHO WANT TO INCREASE THEIR CURRENT LEVEL OF TRAINING, &

(2) FOR THOSE WHO WANT TO MAINTAIN THEIR CURRENT LEVEL OF RUNNING.

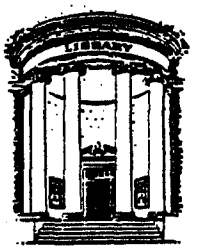
THE OBJECTIVES OF THE STUDY ARE TO (1) DETERMINE HORMONE RESPONSES TO EXERCISE TRAINING & (2) INVESTIGATE DIETARY PATTERNS AMONG WOMEN RUNNERS. SUBJECTS WILL EITHER BE PROGRESSIVELY TRAINED OVER A 6-MONTH PERIOD TO INCREASE RUNNING DISTANCE & INTENSITY OR THEY WILL MAINTAIN A CONSTANT LEVEL OF EXERCISE THROUGHOUT THE 6-MONTH PERIOD.

SUBJECTS SHOULD BE

- FEMALES, AGE 18-35
- CURRENTLY RUNNING 15-25 MILES/WEEK FOR AT LEAST 6 MONTHS PRIOR TO THE STUDY
- NONSMOKERS, NONUSERS OF ORAL CONTRACEPTIVES
- FREE OF METABOLIC DISEASE

THE STUDY IS SCHEDULED TO BEGIN IN NOVEMBER, 1983. FOR MORE INFORMATION, CONTACT JEANNIE CRANE OR DR. TERRY BAZZARRE IN THE UNC-G NUTRITION DEPT. ANY WEEKDAY BEFORE 4 PM (379-5332), OR PHONE 275-4220.

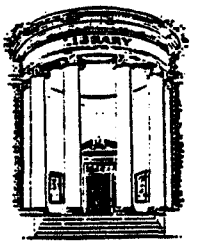
THE UNIVERSITY OF NORTH CAROLINA
AT GREENSBORO



School of Home Economics
Department of Food-Nutrition-Food Service Management
(919) 379-5332; 5313

THE UNIVERSITY OF NORTH CAROLINA
AT GREENSBORO

School of Home Economics



WOMEN RUNNERS NEEDED

TO PARTICIPATE IN A 6-MONTH TRAINING STUDY

IF YOU'RE A FEMALE, AGE 18-35, CURRENTLY RUNNING 15-25 MILES PER WEEK, AND WOULD LIKE TO IMPROVE YOUR RUNNING AND FITNESS LEVEL, HERE'S YOUR CHANCE: I'M RECRUITING SUBJECTS FOR A STUDY IN WHICH WE WILL INVESTIGATE DIETARY PATTERNS AS WELL AS HORMONE RESPONSES TO EXERCISE TRAINING. YOU WILL BE GIVEN A STANDARD TRAINING PROGRAM INDIVIDUALIZED IN ACCORDANCE WITH THE COMBINED RECOMMENDATIONS OF AN EXERCISE PHYSIOLOGIST AND A MEMBER OF THE RACING SOUTH COMPETITIVE TEAM (GUESS WHO). MIKE CALDWELL, COACH OF WORLD-CLASS MARATHONER BENJI DURDEN, HAS ALSO AGREED TO PROVIDE CONSULTATION.

IN ADDITION TO THE REQUIREMENTS STATED ABOVE, SUBJECTS MUST BE NONSMOKERS, NONUSERS OF ORAL CONTRACEPTIVES, AND FREE OF METABOLIC DISEASE. THE STUDY IS SCHEDULED TO BEGIN IN NOVEMBER. FOR MORE INFORMATION CONTACT ME OR DR. TERRY BAZZARRE AT THE NUTRITION DEPARTMENT AT UNC-G (379-5332) OR CALL 275-4220.

THANKS,

Jeannie Crane

JEANNIE M. CRANE, RD, MPH
PHD CANDIDATE, UNC-G
(RSCT)

THE UNIVERSITY OF NORTH CAROLINA
AT GREENSBORO

School of Home Economics
Department of Food-Nutrition-Food Service Management
(919) 379-5332; 5313

October 21, 1983

PUBLIC SERVICE ANNOUNCEMENT

WOMEN RUNNERS are needed to participate in a 6-month study being conducted at the University of North Carolina at Greensboro. Runners must be 18-35, nonusers of oral contraceptives, and currently running 15-25 miles per week. For more information, contact Dr. Terry Bazzarre or Jeannie Crane at 379-5332.

RELEASE DATE: IMMEDIATE END DATE: NOVEMBER 28,1983

CONTACT: JEANNIE CRANE OR TERRY BAZZARRE ; 379-5332 or 275-4220 (evening)
UNIVERSITY OF NORTH CAROLINA-GREENSBORO
DEPARTMENT OF NUTRITION
GREENSBORO, NC 27412

TELEPHONE SCREENING QUESTIONNAIRE

NAME _____ DATE _____

ADDRESS _____

_____

PHONE (H)_____

(W)_____

(+) (−)

YES NO 1. Do you live in the Greensboro area?

YES NO 2. Are you between the ages of 18 & 35? What is your birthdate? ___/___/___ (m d y)

NO YES 3. Do you smoke tobacco?

NO YES 4. Are you currently taking OCA or have you, within the past 6 months?
- Any other medications? (If yes:) What? _____

5. Have you had or do you now have any of the following illnesses or disorders?

NO YES Diabetes

NO YES Heart disease

NO YES Hypertension

NO YES Pituitary disorders

NO YES Menstrual irregularities

YES NO 6. Has your menstrual period been quite regular within the last 6 months?
How often do you usually get your period? _____

YES NO 7. Are you currently running between 15 & 25 miles per week?
How much do you usually run per week? _____
Describe - generally - your workouts during the past 6 months: _____
_____
What other exercise do you do now, or have you done in the past 6 months on a regular basis? _____

NO YES 8. Have you been on any special diet within the past 6 months?
e.g., veget ___ hi-pro ___ other_____ (specify)
wt loss ___ low-cho ___

YES NO 9. Would you be willing to run as your ONLY form of exercise for 6 months?

YES NO 10. Would you be willing to stop eating meat, fish, & poultry for 6 months?

APPENDIX B

LETTER OF CONSENT

INFORMED CONSENT

I agree to participate in the study entitled "Nutritional Status and Reproductive Hormone Response of Females Participating in a 6-Month Exercise Program." I realize that my participation in the study will be required through 7 of my menstrual cycles, which is anticipated to be 6½ to 7 months. The research is being conducted by Dr. Terry Bazzarre, Dr. Michael Liebman, and Jeannie Crane. Other investigators include Henry Trollinger, Bonita Irvin, and Vangie Foshee.

I understand that the purposes of the study are to determine (1) dietary patterns among women runners consuming a lacto-ovo vegetarian diet; and (2) plasma lipid and mineral responses, as well as reproductive hormone responses to exercise training.

Prior to the 6-month training program I will provide 2 fasting blood samples of 15 ml each and a 3-day food record. I realize that a fasting blood sample will also be required on one day of my 3rd, 5th, and 7th menstrual period during the study.

At both the beginning and end of the 6-month training period I will cooperate in the following procedures: (1) a 15-ml blood drawing every other day between the 10th day and last day of my menstrual cycle; and (2) a treadmill test of maximal oxygen uptake. I understand that I will be required to provide information regarding my menstrual, medical, athletic, and dietary history. In addition, I will be asked to complete psychological questionnaires.

I realize that the study involves 4 test periods of 7 days each, during which I will provide 7, 24-hour urine samples and 7, 24-hour records of food intake and activity. During the 4 test periods I will also cooperate in measurements of percent body fat, height, and of resting metabolic rate. The latter requires that on 2 separate days I report to the Human Performance Laboratory at UNC-G immediately after arising in the morning, after a 14-hour fast.

I understand that I will be required to abstain from flesh foods (meat, fish, poultry) from at least 3 days pre-study until completion of the study, and that I will be counseled by a registered dietitian with regard to menu design for a lacto-ovo vegetarian diet.

I understand that I may experience discomfort at times, associated with physical exertion, although the training program I follow will be modified according to my individual needs, and will be carefully monitored by the investigators so as to reduce risk of injury. Potential risks involved in the study include venipuncture (i.e., bruising, infection, air emboli) and injury due to running. I realize that all precautions have been taken to minimize these risks (e.g., use of only highly trained personnel and established protocols), and that I can voluntarily withdraw from the study at any time of my choosing without incurring prejudice from the investigators.

The benefits I may gain from the study include evaluation and improvement of my diet, fitness level, running performance, and body composition. I can also expect to learn about exercise training principles; physiological response to exercise; nutrition; and my own blood lipid and selected mineral levels. Another potential benefit is group support and motivation to carry out my exercise program.

I understand that Dr.Bazzarre, Dr. Liebman, Jeannie Crane, or any member of the investigative staff will be available to answer questions that I may have regarding the study. They may be reached at the UNC-G Nutrition Department on weekdays at 379-5332. All of my immediate questions have been answered.

_____ participant signature _____ date

_____ witness signature _____ date

APPENDIX C

PRESTUDY AND POSTSTUDY QUESTIONNAIRES

NAME_____

PERSONAL DATA

ADDRESS_____

1. Date: ___/___/___ (m d y)

_____

2. Birthdate: ___/___/___ (m d y)

PHONE (H)_____ (W)_____

3. Education: Place a check beside the highest level of education completed.

___Highschool	___Batchelor's (+)	___JD
___Highschool (+)	___Master's	___MD or DDS
___Batchelor's	___Master's (+)	___PhD

4. Occupation: Please check the appropriate space.

___Teacher/Educator/Student	___Attorney/Scientist/Engineer
___Secretary	___Physician/Nurse/Health Professional
___Administrator/Executive	___Artist
___Journalist/Editor	___Marketing/Sales/Advertising
___Homemaker	___Other_____ (please specify)

ATHLETIC HISTORY

5. Check the corresponding column indicating the frequency with which you participate in any of the following sports. (If no participation, leave blank.)

	4-7 days/wk	1-3 days/wk	occasionally	(mi./wk)
1)Running	_____	_____	_____	_____
2)Swimming	_____	_____	_____	_____
3)Bicycling	_____	_____	_____	_____
4)Dance	_____	_____	_____	
5)Tennis/Squash/Racquetball	_____	_____	_____	
6)Walking	_____	_____	_____	_____
7)Basketball/Volleyball	_____	_____	_____	
8)Field Hockey/Soccer	_____	_____	_____	
9)Skating/Skiing	_____	_____	_____	
10)Weight training	_____	_____	_____	
11)Other:_____ (specify)	_____	_____	_____	

Please write your sports in order of amount of participation (# 1, most active, etc.). Write in the box the number of the sport as listed on the previous page, as well as the name of the sport.

6. Sport #1 ☐ _____ (name of sport)

Min./day in training _____
Days/wk _____
Wk/yr _____
Age at onset of training _____

Years in training _____
No. of breaks in training/yr _____
Avg. duration of breaks _____ weeks
No. of competitive events in 1983 _____
in 1982 _____

7. Sport #2 ☐ _____ (name of sport)

Min. /day in training _____
Days/wk _____
Wk/yr _____
Age at onset of training _____

Years in training _____
No. of breaks in training/yr _____
Avg. duration of breaks _____ weeks
No. of competitive events in 1983 _____
in 1982 _____

Racing History:

8. No. of 10-km races completed in 1983: _____; in 1982: _____

9. No. of other races completed:

1983		1982	
Distance	No. Completed	Distance	No. Completed
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

10. Personal best times achieved:

Distance	Time	Date Run
10 km (6.2 mi)	____	_____
_____	____	_____
_____	____	_____

MENSTRUAL HISTORY

11. At what age did you have your first menstrual period? _____years

12. Do you have any history of menstrual dysfunction or irregularities? _____yes _____no
If yes, explain: _____

_____

_____

13. Do you now have regular menstrual periods? _____yes _____no
If yes:

14. How frequently? _____

15. How many flow days? _____

16. Usual course: _____painless
(check one) _____with cramps
_____varies; explain: _____

_____

17. Date of onset of last menstrual period: ___/___/___
m d y

18. Has there been a change in your menstrual cycle since you started running?
_____yes _____no
If yes, what? _____

_____
Does this pattern change if you stop running or run irregularly? _____yes _____no
If yes, how? _____
Does this pattern resume when you begin running again? _____yes _____no
If yes, how long does it take?

19. No. of pregnancies: _____
No. before starting training _____
No. after starting training _____

20. Are you taking birth control pills? _____yes _____no
If yes, when did you start? _____

21. If you are a former BC pill user, when did you take them?
___/___ until ___/___ ; ___/___ until ___/___
mo yr mo yr mo yr mo yr

MEDICAL HISTORY

22. Do you have or have you had any of the following diseases?

___Coronary heart disease
___Hypertension
___Diabetes
___Hyperprolactinemia
___Amenorrhea
___Hypothalamic-pituitary disorder
___Hyperlipidemia
___Anemia

Please indicate your smoking , drug, and alcohol status:

23. Tobacco use: _____never; _____stopped (___years ago); ___yes, ___packs/day

24. Alcohol use: _____never; _____yes: _____beers/wk
_____wine/wk
_____liquor/wk

25. Marijuana use: _____never use it; _____yes: ___once/wk or more
___1-3 times/mo
___fewer than once/mo

26. Are you on any medications? _____yes _____no

If yes:	Name	Dosage and frequency

27. Do you take vitamin or mineral supplements? _____yes ____no

If yes:	What kind	When started	Frequency taken

28. Do you follow a particular diet? _____yes _____no

If yes: _____vegetarian
_____low calorie
_____low fat
_____low carbohydrate
___high protein
___high carbohydrate
___low sodium
___other; please specify:_____

29. Do you take any other dietary supplements? _____yes ____no

If yes, please specify: _____

30. Have you lost weight since beginning training? _____yes _____no

If yes: How much? _____

When? _____

In how long a period of time? _____

31. Has your diet changed since you started training? _____yes _____no

If yes, when? _____

In what way? _____

_____

_____

32. Indicate your normal consumption during the past 6 months of the following:

	< 1/wk	1-2 X wk	3-5 X wk	6-10 X wk	> 10/wk
Red meat (beef, pork, lamb)...					
Poultry					
Fish					
Milk; Whole					
Low-fat					
Skim					
Buttermilk					
Yogurt; Whole-milk					
Low-fat					
Icecream					
Eggs					
Cheese					

Specify type(s):

_____

_____

_____

WOMEN RUNNERS RESEARCH
POST-STUDY QUESTIONNAIRE

NAME _____

ATHLETIC HISTORY

1. Check the corresponding column indicating the frequency with which you partcipated in the following sports during your involvement in the Women Runners Study. (If no participation, leave blank.)

	4-7 days/wk	1-3 days/wk	occasionally	(mi./wk)
1) Running				
2) Swimming				
3) Bicycling				
4) Dance				
5) Tennis/Squash/Racquetball				
6) Walking				
7) Basketball/Volleyball				
8) Field Hockey/Soccer				
9) Skating/Skiing				
10) Weight training				
11) Other: _____ (specify)				

2. Please list any races and corresponding times that you completed since the beginning of your participation in the Women Runners Study. Please star (*) any PRs.

Distance	Time	Date Run	Distance	Time	Date Run

MENSTRUAL HISTORY

3. Since your participation in the Women Runners Study, what has been the usual number of flow days of your menstrual cycle? _____

4. Usual course: (check one)
   _____ painless
   _____ cramps
   _____ varies; explain: _____

5. Has there been any change in your menstrual cycle since the beginning of the study?
If yes, what? _____

_____

Please indicate your smoking, drug, and alcohol status:

6. Tobacco use: _____ never; _____ stopped (____ years ago); _____ yes, ____pks/dy

7. Alcohol use: _____ never; _____ yes: _____ beers/wk
_____ wine/wk
_____ liquor/wk

8. Marijuana use: _____ never; _____ yes: _____ once/wk or more
_____ 1-3 times/mo
_____ fewer than once/mo

9. Have you been on any medications since the beginning of the study? _____yes _____no

If yes:	Name	Dosage & frequency	Dates started/stopped
			/
			/
			/

10. Do you take any vitamin or mineral supplements? _____ yes _____ no

If yes:	what kind	when started	frequency taken	dosage (if known)

11. Do you take any other dietary supplements? _____ yes _____ no
If yes, please specify type of supplement, quantity, frequency, and when started:

_____

_____

12. In the 6 months prior to the beginning of the study, did you experience any changes in body weight? _____ yes _____ no

If so: lost _____lb./gained _____lb.
when: _____ to _____
(date) (date)

lost _____lb./gained _____ lb
when: _____ to _____
(date) (date)

13. Indicate your normal consumption of the following foods since the beginning of your participation in the Women Runners Study:

	1/wk	1-2 x wk	3-5 x wk	6-10 x wk	10/wk
Red meat (beef, pork, lamb)..					
Poultry					
Fish					
Milk: Whole					
Low-fat					
Skim					
Buttermilk					
Yogurt: Whole milk					
Low-fat					
Ice cream					
Eggs					
Cheese					

Specify type(s):

_____

_____

_____

APPENDIX D

INSTRUCTIONS FOR THE 24-HOUR FOOD RECORD

INSTRUCTIONS FOR A FOOD RECORD

These instructions will help you in recording the food that you eat. Please read these instructions carefully. If you have any questions, please feel free to call the Department of Foods and Nutrition at the University of North Carolina at Greensboro, 379-5332 or 379-5313.

1. Measure each item using household measurements (cup, ½ cup, 1/3 cup 1/4 cup, tablespoon, teaspoon, 3/4 teaspoon, 1/2 teaspoon, 1/4 teaspoon, etc.). Abbreviations can be used.
   C=cup
   T= Tablespoon
   t= teaspoon

2. For items that are not measured such as fresh fruit or eggs, write the number of items eaten and whether they are large or small.
Examples:
   1 large egg
   1 small banana
   3 lumps sugar
Additional information about the sizeof the food such as diameter, length, width, thickness or ounces is helpful.
Examples:
   3" carrot
   1 Brownie with walnuts, wedge 2" by 2" by 1" high
   1 - 12 oz. can Coke
   Pizza with mushrooms, green pepper and cheese - 1/8 of 12" pizza
   1 apple - 2 1/2 " diameter
3. If you do not eat all the food you have served on your plate, try to measure the amount of each item you did not eat and subtract that amount from what was served.
Example: If you are served a peanut butter sandwich with two slices of bread and 2 T peanut butter and you eat 3/4 of the sandwich, you have eaten 1 1/2 slices of bread and 1 1/2 T of peanut butter.
4. Describe the food eaten as exactly as possible
   a. For meat fish, poultry and eggs specify the cut or type of meat or fish (chuck or bass), whether you ate or trimmed off the fat, whether you ate the skin (poultry), percent fat (hamburger) and whether the fish was oil pack or water pack (tuna).
   b. For breads, cereal, cakes, cookies, etc. state whether the food is made from white flour or whole grain and whether the food was homemade or bought. Specify the brand names whenever possible.
   c. For margarine state the brand name,whether the margarine comes as a stick, soft or liquid, whether it is diet or regular and whether it is whipped or not.
   d. For oils and shortening state the brand name, the major oil(s) (if known) and whether the fat is solid fat or oil.
   e. For salad dressings specify whether it is homemade, commercial or restaurant, the type of oil or brand name, creamy or clear, and additional ingredients such as cheese or bacon bites.
   f. For dairy products indicate the percent fat, the brand name or relative price and whether it is a true dairy product or a nondairy product.
   g. For bakery items state whether they are homemade, restaurant or commercial, the brand, the principal fat, toppings or frostings, yeast or cake and type of grain. For pies indicate whether it is a single or double crust.
   h. For sauces and gravies indicate the type of fat, the meat fat and what kind of milk added.
   i. For recipes and mixed dishes indicate whether it is homemade, commercial or restaurant, the brand name, cooking method and all ingredients used. Submit a recipe if possible.
   j. For fruits and juices indicate whether they are fresh, frozen, canned, cooked or dried; and whether the food is sweetened or unsweetened.
   k. For vegetables state whether they are cooked or raw, the kind of fat added if any, sauces added, the method cooked and any seasonings added. Specify how mashed potatoes were prepared.

l. For soups speecify whether they are homemade or canned, cream soups or clear soups and the water or kind of milk or stock added.

m. For beverages and cereals, indicate whether they are sweetened or unsweetened, diet-sweetened or unsweetened, the brand, decaffeinated (coffee or tea), and whether it is a cola or noncola.

n. For crackers, snacks, candy bars indicate the brand, weight , type or size.

If you are in doubt as to whether the information is needed or not, include the information.

5. Record accompaniments such as gravey, sauces, salad dressings, mayonaise, ketchup,mustard, seasonings, garnishes, etc. separately. Do not forget to record sugar, lemon, cream, non - dairy creamers or flavorings that you may add to drinks such as coffee, teas or milk. If you drink any liquid other than water, measure the amount in a liquid (pyrex) measuring cup. Or if you use the same cup or glass often, measure the amount of liquid the glass or cup will hold and fill to the samelevel with the beverage.

6. Describe how the food was prepared, including any additional fat, sugar or condiments that may be added.

Example:

sauteed in butter
basted with garlic butter
boiled in water
deep fat fried in peanut oil
simmered in wine

7. Do not forget to record snacks. If you eat a snack away from home carry the wrapping home with you as a reminder.

APPENDIX E

ACTIVITY RECORDS

ACTIVITY RECORD

DAY NO. ____

NAME _____
last first

DATE ___/___/___
m d y

SUBJECT ID _____

Write in the space provided the categorical value which corresponds best to the dominant activity of each 15-minute period. Please consult the activity card to establish proper coding. In case of doubt, make a note and raise the problem during the interview.

Summary		
1=	2=	3=
4=	5=	6=
7=	8=	9=

Hour \ Minute	0-15	16-30	31-45	46-60
0				
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				

Table of activities, energy costs, and corresponding categorical values*

Categorical value	Examples of activities	Energy cost in mets from various studies		Median energy cost used	
		Minimum	Maximum	METS	kcal/kg/15 min
1	Sleeping Resting in bed	1.0		1.0	0.26
2	Sitting: eating, listening, writing, etc.	1.0	2.0	1.5	0.38
3	Light activity standing: washing, shaving, combing, cooking, etc.	2.0	3.0	2.3	0.57
4	Slow walk (<4 km/hr), driving, to dress, to shower, etc.	2.0	4.0	2.8	0.69
5	Light manual work: floor sweeping, window washing, driving a truck, painting, waiting on tables, nursing chores, several house chores, electrician, barman, walking at 4 to 6 km/hr	2.3	5.0	3.3	0.84
6	Leisure activities and sports in a recreational environment: baseball golf, volleyball, canoeing or rowing, archery, bowling, cycling (<10 km/hr), table tennis, etc.	3.0	8.0	4.8	1.2
7	Manual work at moderate pace: mining, carpentry, house building, lumbering and wood cutting, snow shoveling, loading and unloading goods, etc.	4.0	8.0	5.6	1.4
8	Leisure and sports activities of higher intensity (not competitive): canoeing (5 to 8 km/hr), bicycling (>15 km/hr), dancing, skiing, badminton, gymnastic, swimming, tennis, horse riding, walking (>6 km/hr), etc.	5.0	11	6.0	1.5
9	Intense manual work, high intensity sport activities or sport competition: tree cutting, carrying heavy loads, jogging and running (>9 km/hr), racquetball, badminton, swimming, tennis, cross country skiing (>8 km/hr), hiking and mountain climbing, etc.	6.0	~15	7.8	2.0

*from Bouchard et al., 1983

APPENDIX F

SAMPLE TRAINING SCHEDULE AND RUNNING LOGBOOKS

TRAINING SCHEDULE TERMINOLOGY

M.....Moderate pace.
Q.....Quick pace (hard effort).
E.....Easy pace; Very comfortable pace for you; This is the same as "jog."
Surge.....A surge is a Q pace thrown in during a continuous run for a set distance or time.
Fartlek (Fk)...("Speedplay"): A combination of running speeds (very slow to very fast); Done as a continuous run with the distances of the different speeds also varying.
Striders.....80-85% of maximal effort. Striders are usually 50-100 m long & are frequently part of the w. up for a track workout or a race, & are sometimes practiced at the end of a workout. Striders help to loosen & to literally "warm up" muscles. Also useful for building leg speed.
R.....REST, REST, REST! This means NO RUNNING. No, not even an E run!
W. up.....Warm up. For a continuous run, w. up constitutes running (jogging) at a comfortable pace for you. A w. up for a speed or track workout may be quite different & dependent on the type of session to follow.
Cool down.....E pace run.
Ladder.....Progressively increasing (or decreasing) distances or times, e.g., 200, 400, 600, 800 m on the track, or surges of 1, 2, 3, & 4 minutes, & back down the ladder; 4, 3, 2, 1.
HR.....Heart (pulse) rate.
Accelerate.....Progressively increasing speed over the entire distance specified, e.g., to accelerate over 1 mile means that your pace by 1 mile is faster than at ½ mile, which is much faster than your pace was at ¼ mile.
Decelerate.....(I think you can figure this one out!)
S.O.L.....(Speed of Light): All-out or race pace.

NAME _____

Week (Date)	Workout S	M	T	W	Th	F	S
6 (3/11-3/17)	12	6	7(1)	R	8(2)	4	8(3)
7 (3/18-3/24)	11(4)	5	8(5)	R	7	7(6)	6
8 (3/25-3/31)	12	6	7(1)	R	7	7(5)	8

Comments:

(1) 2 mi w.up; 1 mi Q; 1 mi E; 1 mi Q; 1 mi M; 2 mi E.

(2) M pace except for 10 x 100-M striders anywhere between miles 5 & 7.

(3) 2 mi w.up; 4 x 100-M striders; Q 3-mi; 2 3/4 mi. cooldown.

(4) If sore/fatigued from Sat. run, you may want to switch Mon. & Sun. workout. (One of these days needs to be a 9-miler, though.)

(5) Hill surge run: to include 6 hills 100-400 M long.

(6) 2-mi w.up; Q 2 mi; E ½ mi; Q ½ mi; 2 mi cooldown

(7)

NOTE:

DATE 6-10

DISTANCE 7mi

TIME

HEART RATES (and at which points taken during run) 28 ( )

REMARKS (shoes, course, pain, etc.)

really mad - I accidentally turned my watch off at 1.5 mi + didn't realize it until 3 mi. Had myself psyched out of doing 7 + was going to do 5 but told myself not to psych out but do 7 and I did + just mentally good Physically - the sun my (L) achillies really tender + stiff, like w.a my knee.

DATE 6-11

DISTANCE 0

TIME

HEART RATES (and at which points taken during run)

REMARKS (shoes, workout, weather, etc.)

had somewhat of an aching lower back following.

DATE Thurs Je-19

DISTANCE ≈ 5½ Track

TIME See Below

HEART RATES (and at which points taken during run) See below

REMARKS (shoes, course, pain, etc.)

1½ m w.u.

1 mile 7:39 HR 29

440 Jog

1 mile 7:47

440 Jog

¼ mile 1:48

220 jog

¼ mile 1:43 HR 28

220 j

6x 440 STRIDES

DATE Fri Je-20

DISTANCE 8 miles (+-)

TIME 1hr 12 min

HEART RATES (and at which points taken during run) 26 (8 miles)

REMARKS (shoes, workout, weather, etc.)

O.K.

7-2: Rest Day

DATE 7-1

DISTANCE 8.0

TIME 1:25:14

HEART RATES 78 (end)
(and at which points taken during run) ____ (____)
____ (____)

REMARKS (shoes, course, pain, etc.) I can't believe it, but I did make it to 8 - felt real tired, but I kept going & pushing myself - I didn't even care about my time - just wanted to finish

DATE 7-3

DISTANCE 6.0

TIME 55.10

HEART RATES 78 (end)
(and at which points taken during run) ____ (____)
____ (____)

REMARKS (shoes, workout, weather, etc.) felt a lot better than running 8 - I'd like to cut the time down though

DATE 5/28

DISTANCE 11 mi.

TIME 1:40 min

HEART RATES ____ (____)
(and at which points taken during run) ____ (____)
♡ 27 (____)

REMARKS (shoes, course, pain, etc.) Ran over to country park & around battleground area. Weather starting out very humid but then cooled down. (7pm) Felt fine. Had to stop x 3 to get water

DATE 5/29

DISTANCE 6' → 5

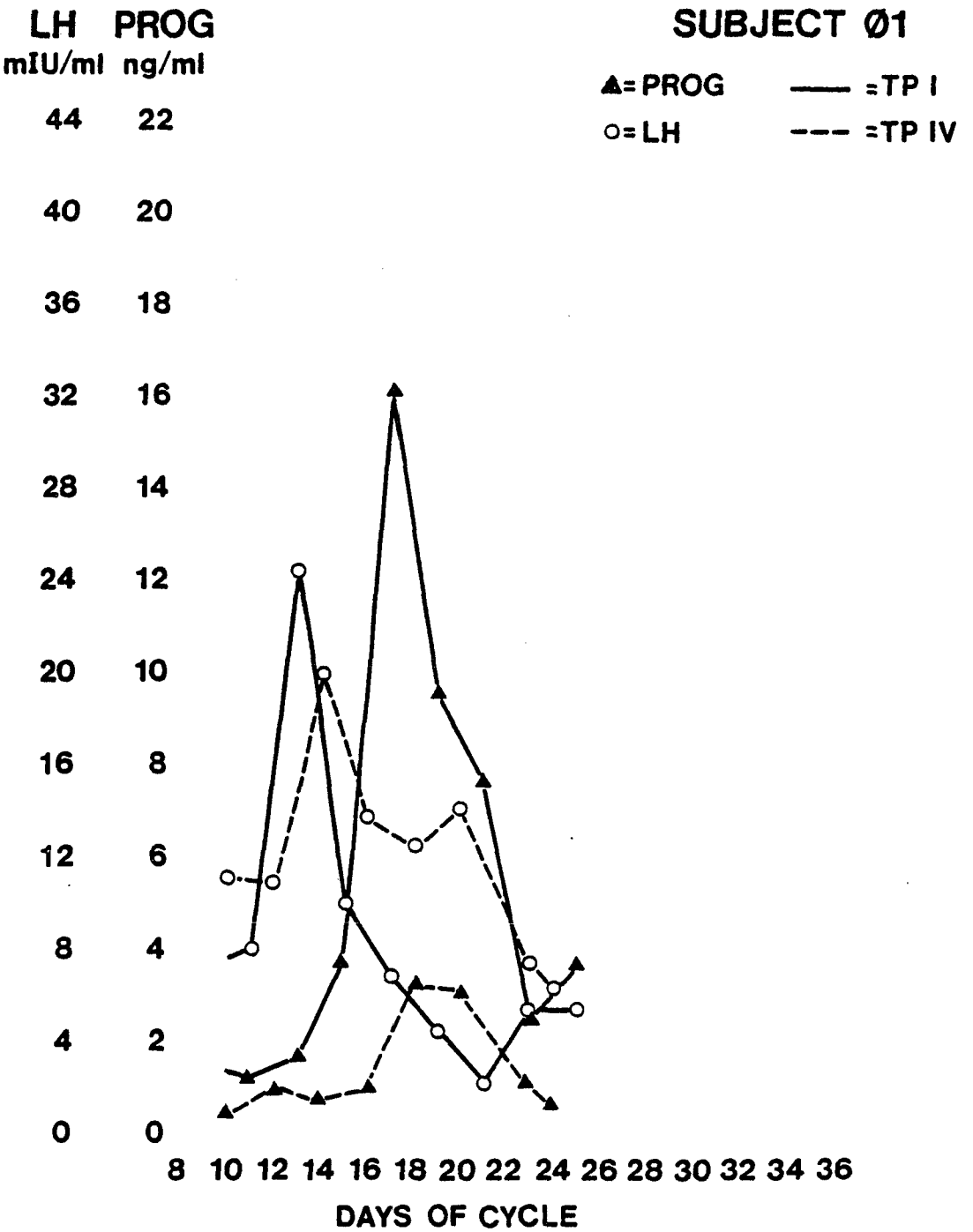
TIME 44 min

HEART RATES ____ (____)
(and at which points taken during run) ____ (____)
____ (____)

REMARKS (shoes, workout, weather, etc.) Did not exactly run 6. Ran about 5 mi. First 2 w/u then quick 1 mi., 1 & 1/2 then I got so tired I stopped. Weather very humid.

APPENDIX G

INDIVIDUAL HORMONE DATA

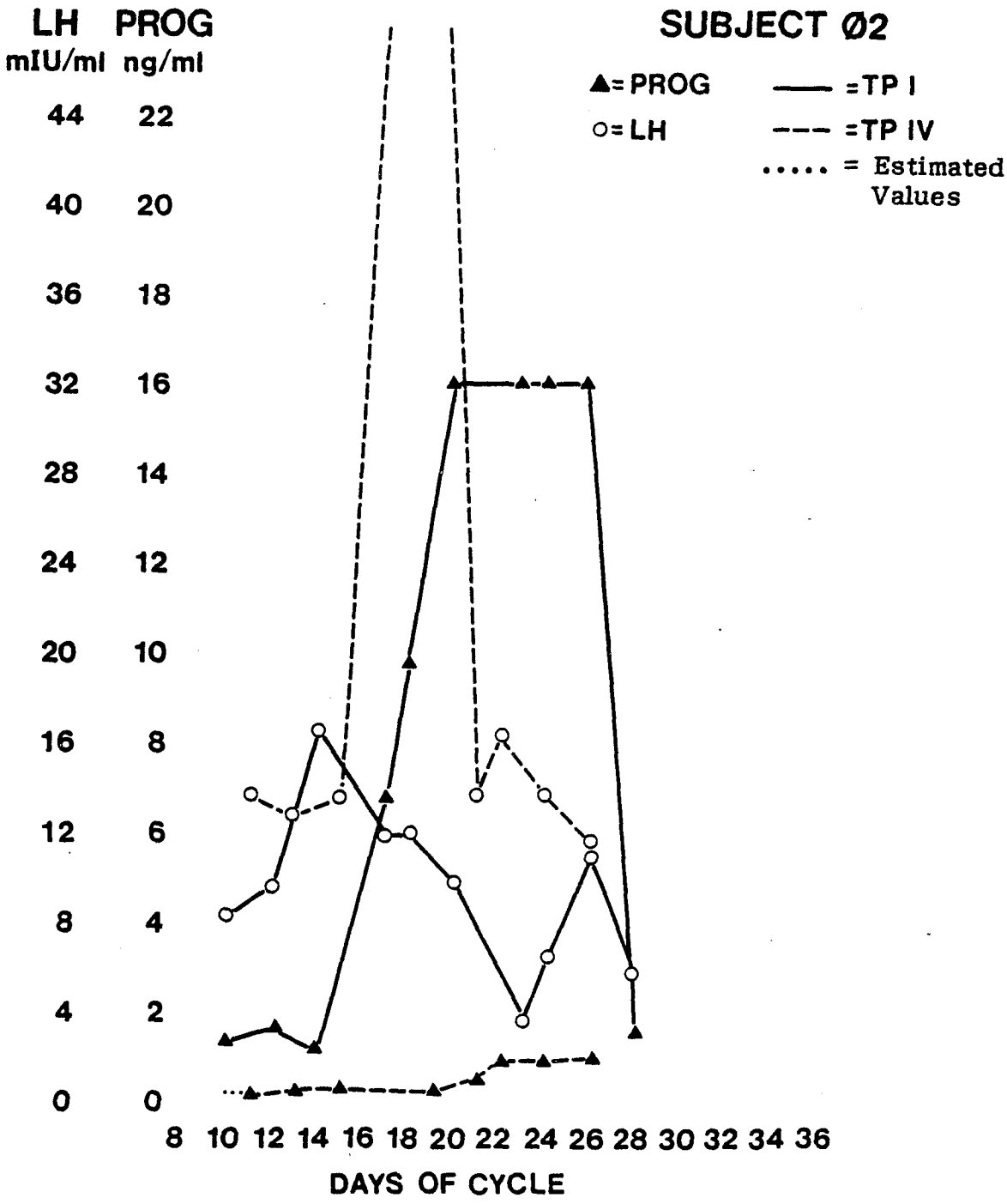


Serum LH Plasma Progesterone, Test Periods I and IV

Serum Luteinizing Hormone (LH) and Plasma Progesterone (P)[a]

		Test Period I		Test Period IV	
Subject	Cycle Day	LH mIU/ml	P ng/ml	LH mIU/ml	P ng/ml
1	9	7.2	1.2		
	10		(1.2)	10.9	0.3
	11	7.8	1.1		(0.6)
	12		(1.3)	10.6	0.9
	13	24.2	1.5		(0.8)
	14		(2.6)	19.7	0.6
	15	9.7	3.6		(0.8)
	16		(9.8)	13.5	0.9
	17	6.6	16.0		(2.0)
	18		(12.7)	12.2	3.1
	19	4.2	9.4		(3.1)
	20		(8.5)	13.9	3.0
	21	2.0	7.5		(2.3)
	22		(4.9)		(1.6)
	23	5.2	2.3	7.2	0.9
	24		(2.9)	6.0	0.5
	25	5.3	3.5		
	26				
	27				
	28				
	29				
	30				

[a]Values in parentheses represent estimates based on measured values of preceding and subsequent days

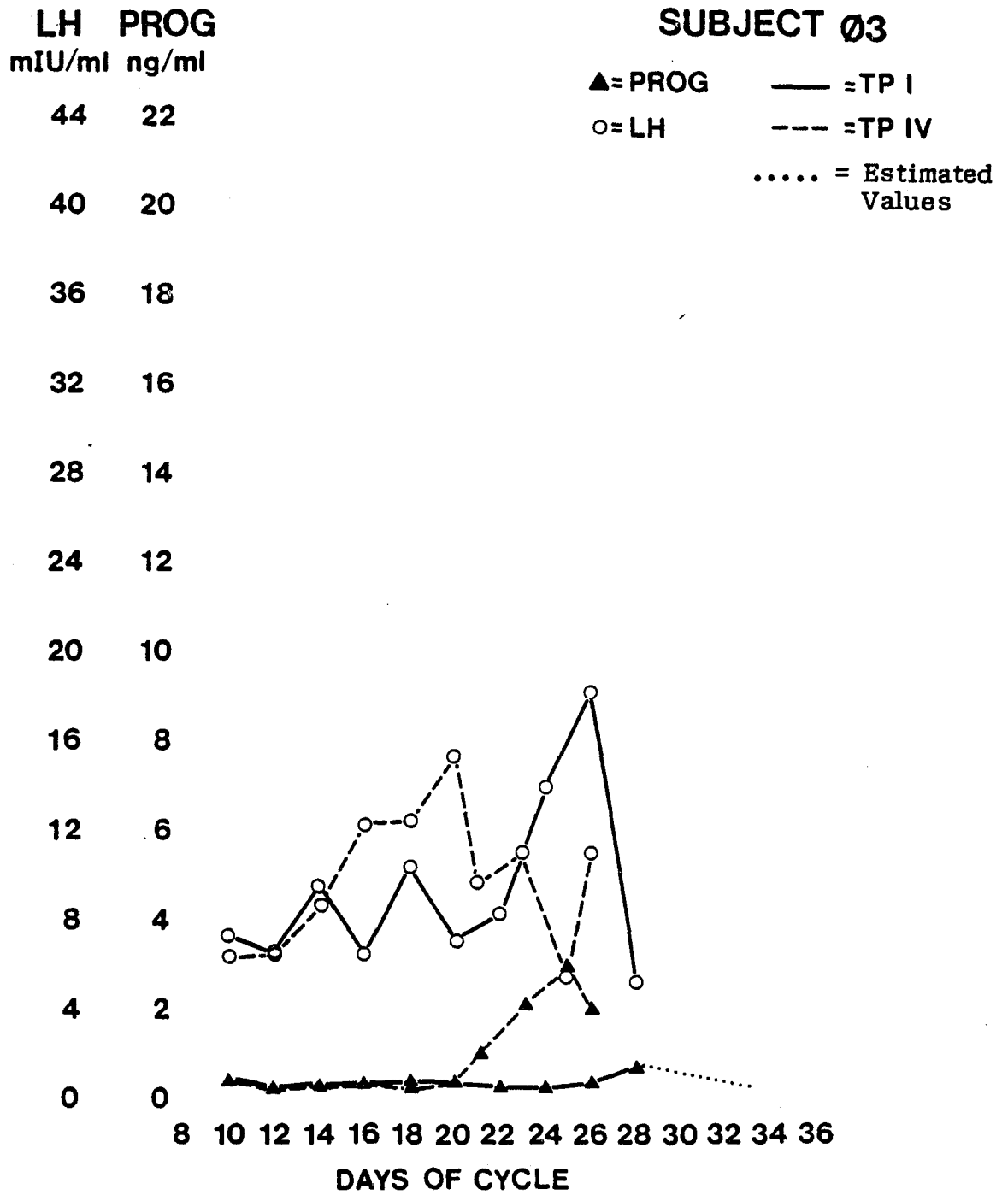


Serum LH Plasma Progesterone, Test Periods I and IV

Serum Luteinizing Hormone (LH) and Plasma Progesterone (P)[a]

		Test Period I		Test Period IV	
Subject	Cycle Day	LH mIU/ml	P ng/ml	LH mIU/ml	P ng/ml
2	10	8.4	1.4		(0.2)
	11		(1.6)	13.7	0.2
	12	9.6	1.7		(0.3)
	13		(1.4)	12.8	0.3
	14	16.6	1.1		(0.3)
	15		(3.0)	13.5	0.3
	16		(4.9)		(0.3)
	17	11.9	6.7		(0.3)
	18	11.9	9.6		(0.2)
	19		(12.8)	80.7	0.2
	20	9.8	16.0		(0.4)
	21		(16.0)	13.6	0.5
	22		(16.0)	16.3	0.9
	23	3.6	16.0		(0.9)
	24	6.4	16.0	13.7	0.9
	25		(16.0)		(1.0)
	26	10.8	16.0	11.5	1.0
	27		(8.8)		
	28	5.7	1.5		
	29				
	30				

[a]Values in parentheses represent estimates based on measured values of preceding and subsequent days

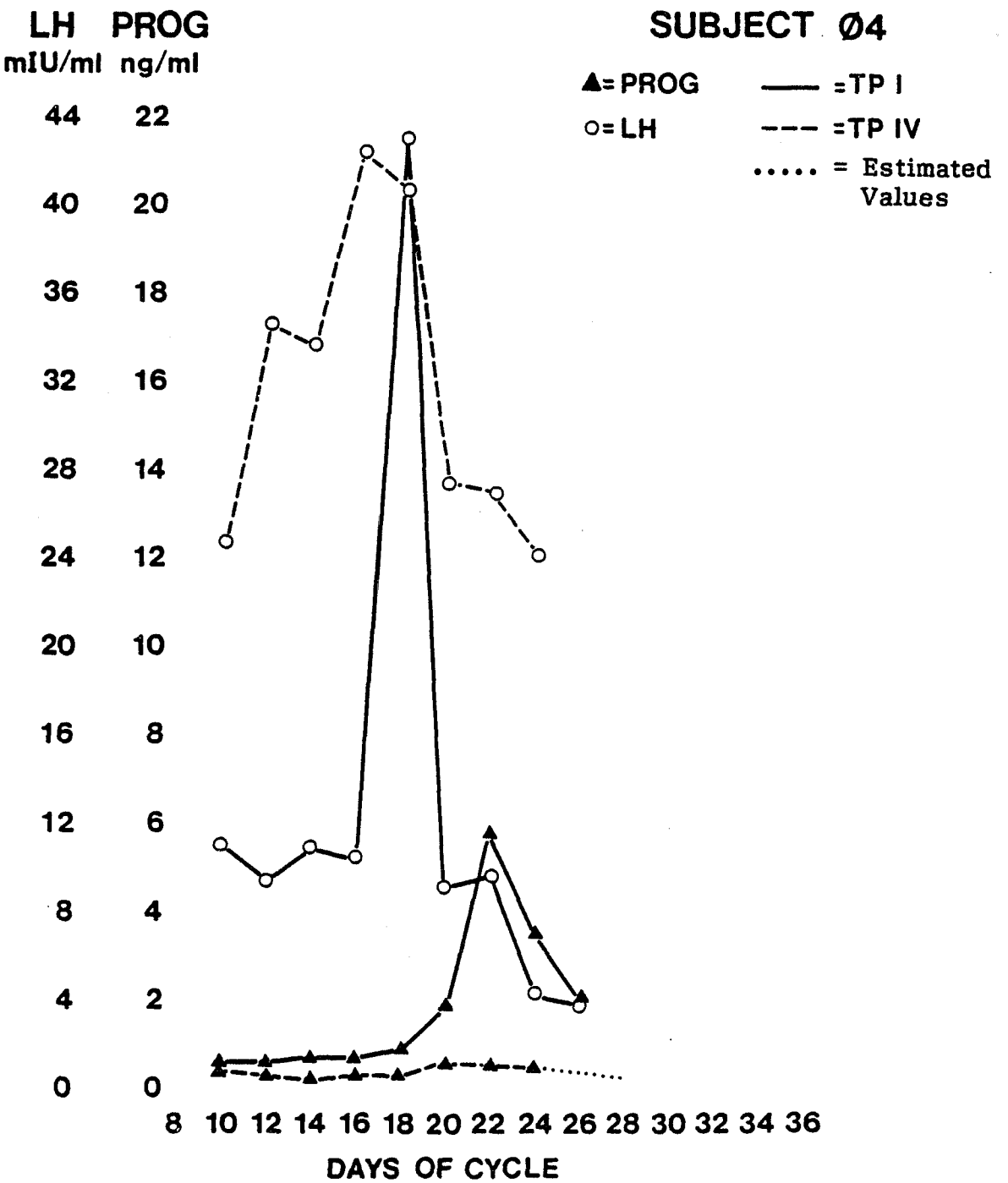


Serum LH Plasma Progesterone, Test Periods I and IV

Serum Luteinizing Hormone (LH) and Plasma Progesterone (P)[a]

		Test Period I		Test Period IV	
Subject	Cycle Day	LH mIU/ml	P ng/ml	LH mIU/ml	P ng/ml
3	10	7.1	0.4	6.2	0.4
	11		(0.3)		(0.3)
	12	6.5	0.2	6.4	0.2
	13		(0.3)		(0.3)
	14	9.4	0.3	8.6	0.3
	15		(0.3)		(0.3)
	16	6.4	0.3	12.2	0.3
	17		(0.3)		(0.3)
	18	10.2	0.3	12.3	0.2
	19		(0.3)		(0.3)
	20	7.0	0.3	15.3	0.3
	21		(0.3)	9.5	0.9
	22	8.1	0.2		(1.5)
	23		(0.2)	10.9	2.0
	24	13.8	0.2		(2.5)
	25		(0.3)	5.3	2.9
	26	18.0	0.3	10.8	1.9
	27		(0.5)		
	28	5.1	0.7		
	29		(0.6)		
	30		(0.5)		
	31		(0.4)		
	32		(0.3)		
	33		(0.2)		

[a]Values in parentheses represent estimates based on measured values of preceding and subsequent days

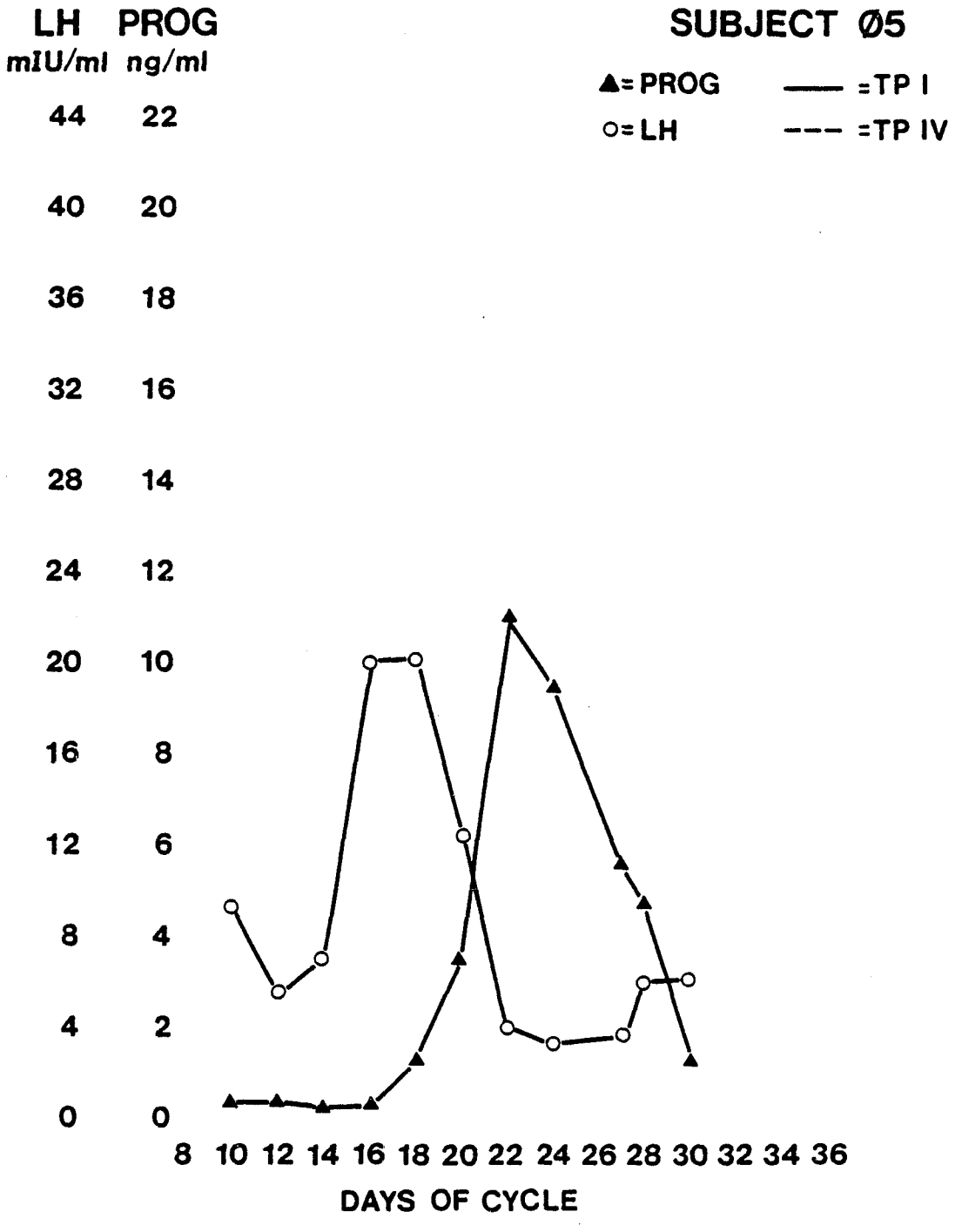


Serum LH Plasma Progesterone, Test Periods I and IV

Serum Luteinizing Hormone (LH) and Plasma Progesterone (P)[a]

		Test Period I		Test Period IV	
Subject	Cycle Day	LH mIU/ml	P ng/ml	LH mIU/ml	P ng/ml
4	10	11.0	0.6	24.9	0.4
	11		(0.6)		(0.4)
	12	8.5	0.6	34.7	0.3
	13		(0.7)		(0.3)
	14	10.8	0.7	33.7	0.2
	15		(0.7)		(0.3)
	16	10.5	0.7	42.5	0.3
	17		(0.8)		(0.3)
	18	43.1	0.9	40.9	0.3
	19		(1.4)		(0.5)
	20	9.1	1.8	27.5	0.6
	21		(3.8)		(0.6)
	22	9.7	5.8	27.1	0.6
	23		(4.7)		(0.6)
	24	4.3	3.5	24.2	0.5
	25		(2.8)		(0.4)
	26	3.9	2.0		(0.4)
	27				(0.3)
	28				(0.2)
	29				
	30				

[a]Values in parentheses represent estimates based on measured values of preceding and subsequent days



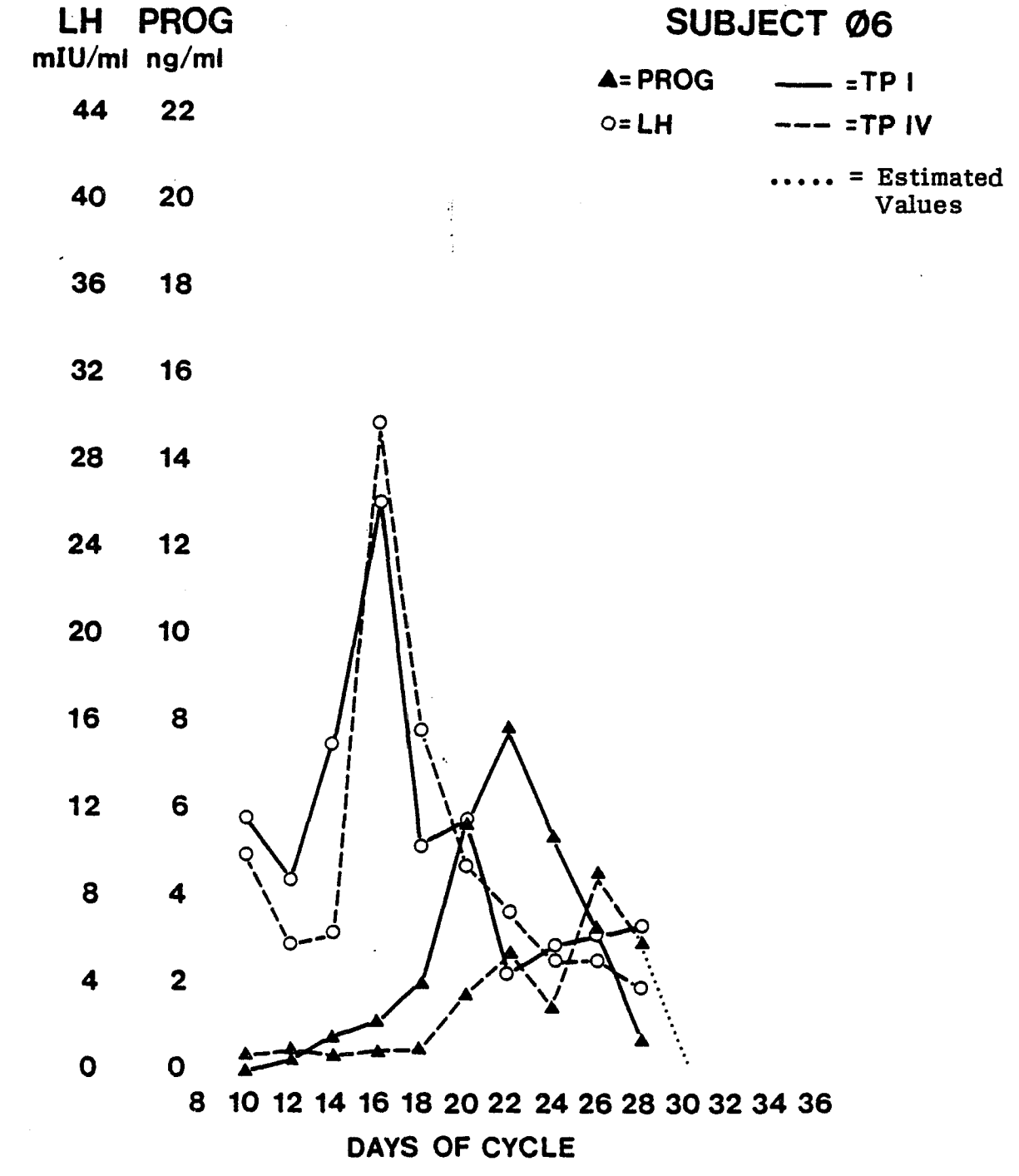
Serum LH Plasma Progesterone, Test Periods I and IV

Serum Luteinizing Hormone (LH) and Plasma Progesterone (P)[a]

		Test Period I		Test Period IV[b]	
Subject	Cycle Day	LH mIU/ml	P ng/ml	LH mIU/ml	P ng/ml
5	10	9.4	0.4		
	11		(0.4)		
	12	5.6	0.4		
	13		(0.4)		
	14	7.1	0.3		
	15		(0.3)		
	16	20.0	0.3		
	17		(0.8)		
	18	20.3	1.3		
	19		(2.4)		
	20	12.5	3.5		
	21		(7.3)		
	22	4.1	11.0		
	23		(10.3)		
	24	3.3	9.5		
	25		(8.2)		
	26		(6.8)		
	27	3.6	5.5		
	28	6.0	4.8		
	29		(3.1)		
	30	6.1	1.3		

[a]Values in parentheses represent estimates based on measured values of preceding and subsequent days

[b]Subject became pregnant in Test Period IV; data not reported

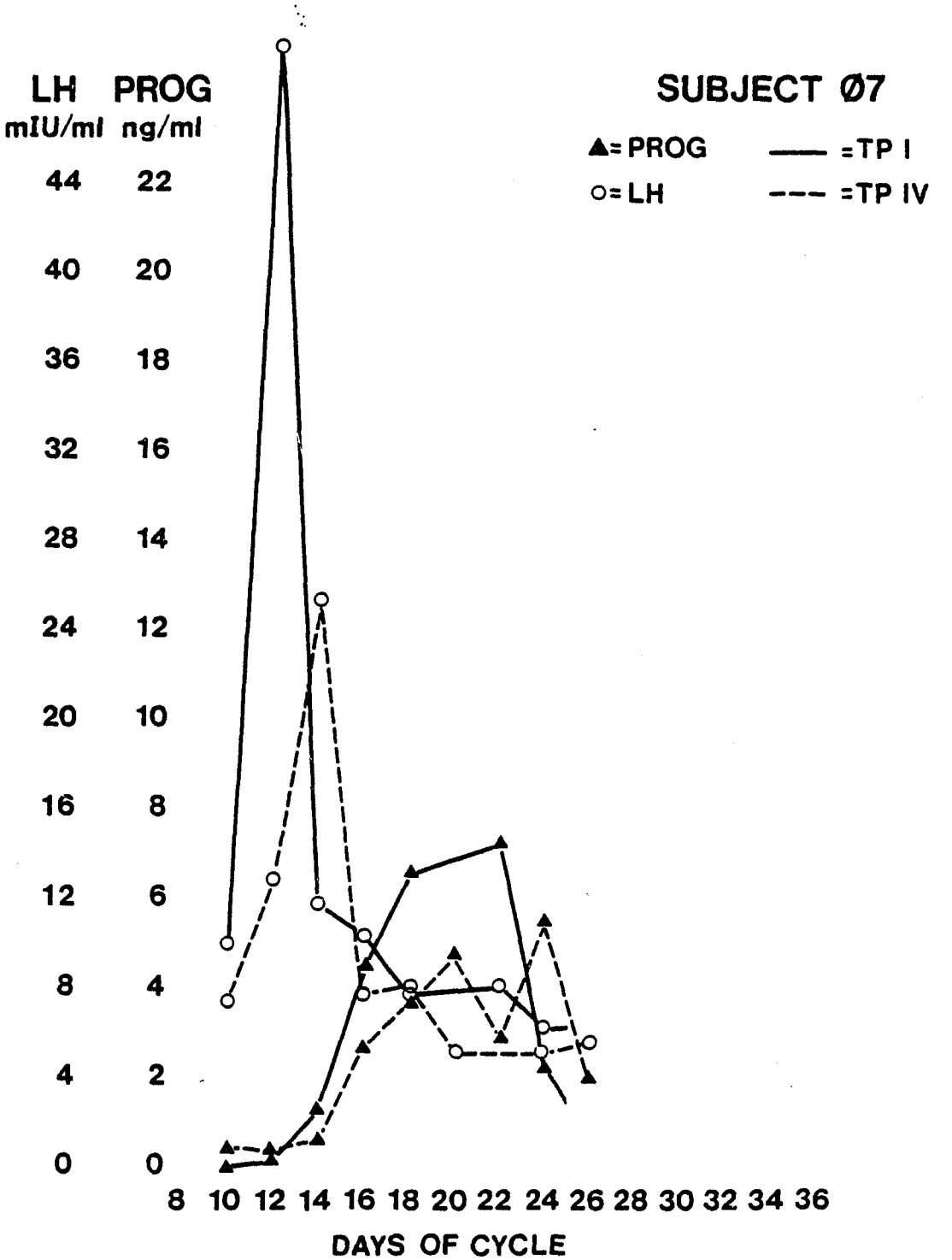


Serum LH Plasma Progesterone, Test Periods I and IV

Serum Luteinizing Hormone (LH) and Plasma Progesterone (P)[a]

		Test Period I		Test Period IV	
Subject	Cycle Day	LH mIU/ml	P ng/ml	LH mIU/ml	P ng/ml
6	10	11.5	0.0	9.8	0.4
	11		(0.1)		(0.4)
	12	8.7	0.2	5.8	0.4
	13		(0.5)		(0.4)
	14	14.9	0.7	6.1	0.3
	15		(0.9)		(0.4)
	16	30.1	1.1	29.7	0.4
	17		(1.5)		(0.4)
	18	10.2	1.9	15.5	0.4
	19		(3.8)		(1.1)
	20	11.3	5.6	9.4	1.7
	21		(6.7)		(2.2)
	22	4.4	7.8	7.2	2.6
	23		(6.6)		(2.0)
	24	5.6	5.3	4.9	1.4
	25		(4.2)		(2.9)
	26	6.0	3.1	4.9	(4.4)
	27		(1.9)		(3.7)
	28	6.5	0.6	3.7	(2.9)
	29				(1.6)
	30				(0.2)

[a]Values in parentheses represent estimates based on measured values of preceding and subsequent days



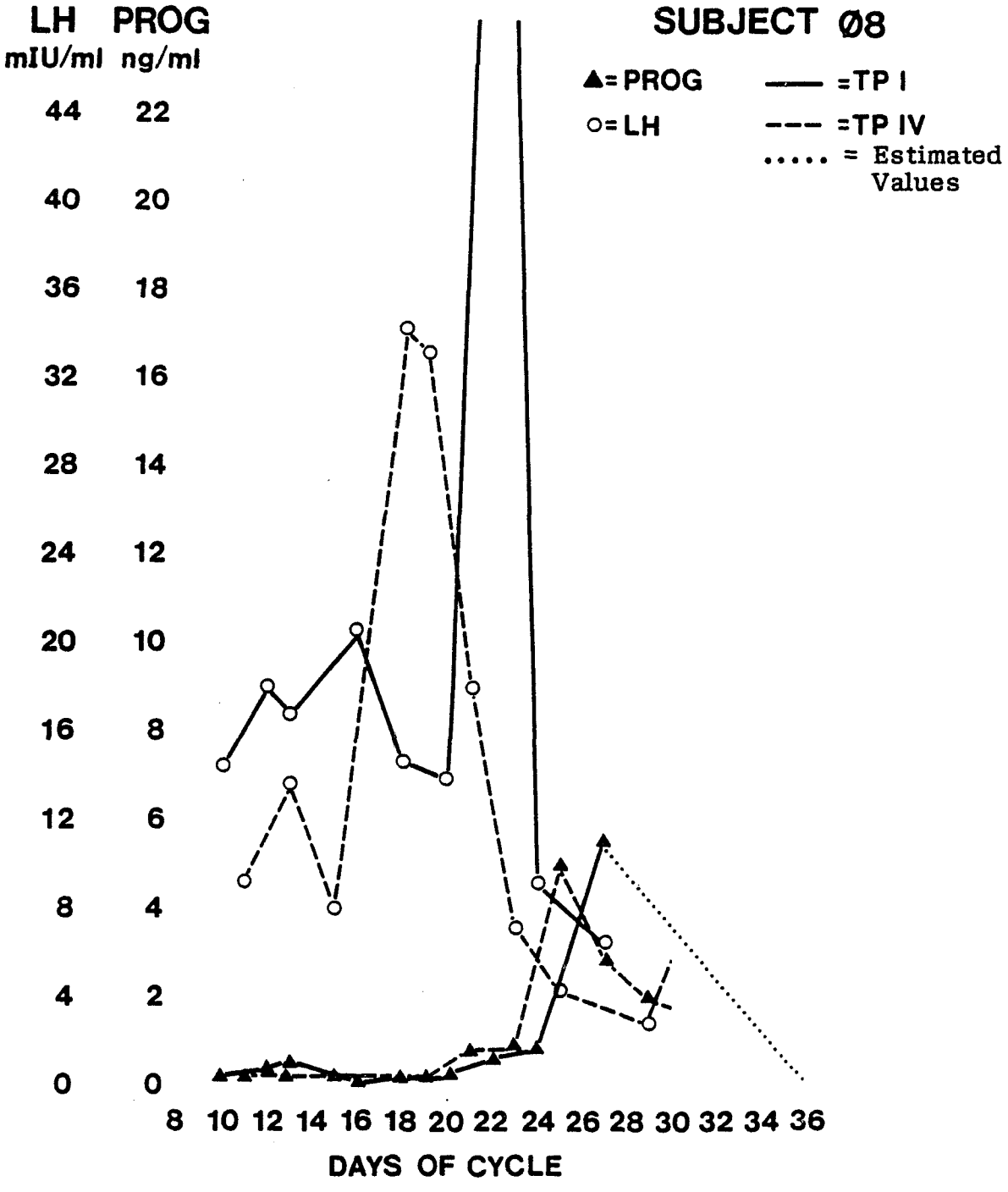
Serum LH Plasma Progesterone, Test Periods I and IV

Serum Luteinizing Hormone (LH) and Plasma Progesterone (P)[a]

		Test Period I		Test Period IV	
Subject	Cycle Day	LH mIU/ml	P ng/ml	LH mIU/ml	P ng/ml
7	10	9.9	0.0	7.2	0.4
	11		(0.1)		(0.4)
	12	50.9	0.1	12.6	0.3
	13		(0.7)		(0.5)
	14	11.7	1.2	25.4	0.6
	15		(2.8)		(1.7)
	16	10.3	4.4	7.7	2.7
	17		(5.5)		(3.2)
	18	7.7	6.5	8.0	3.6
	19		(6.7)		(4.2)
	20		(6.9)	5.0	4.7
	21		(7.0)		(3.8)
	22	8.0	7.2		2.8
	23		(4.7)		(4.2)
	24	6.1	2.2	5.0	5.5
	25		(1.1)		(3.7)
	26[b]	6.3	0.1	5.5	1.9
	27				
	28				
	29				
	30				

[a]Values in parentheses represent estimates based on measured values of preceding and subsequent days

[b]Onset of next menstrual cycle, Test Period I



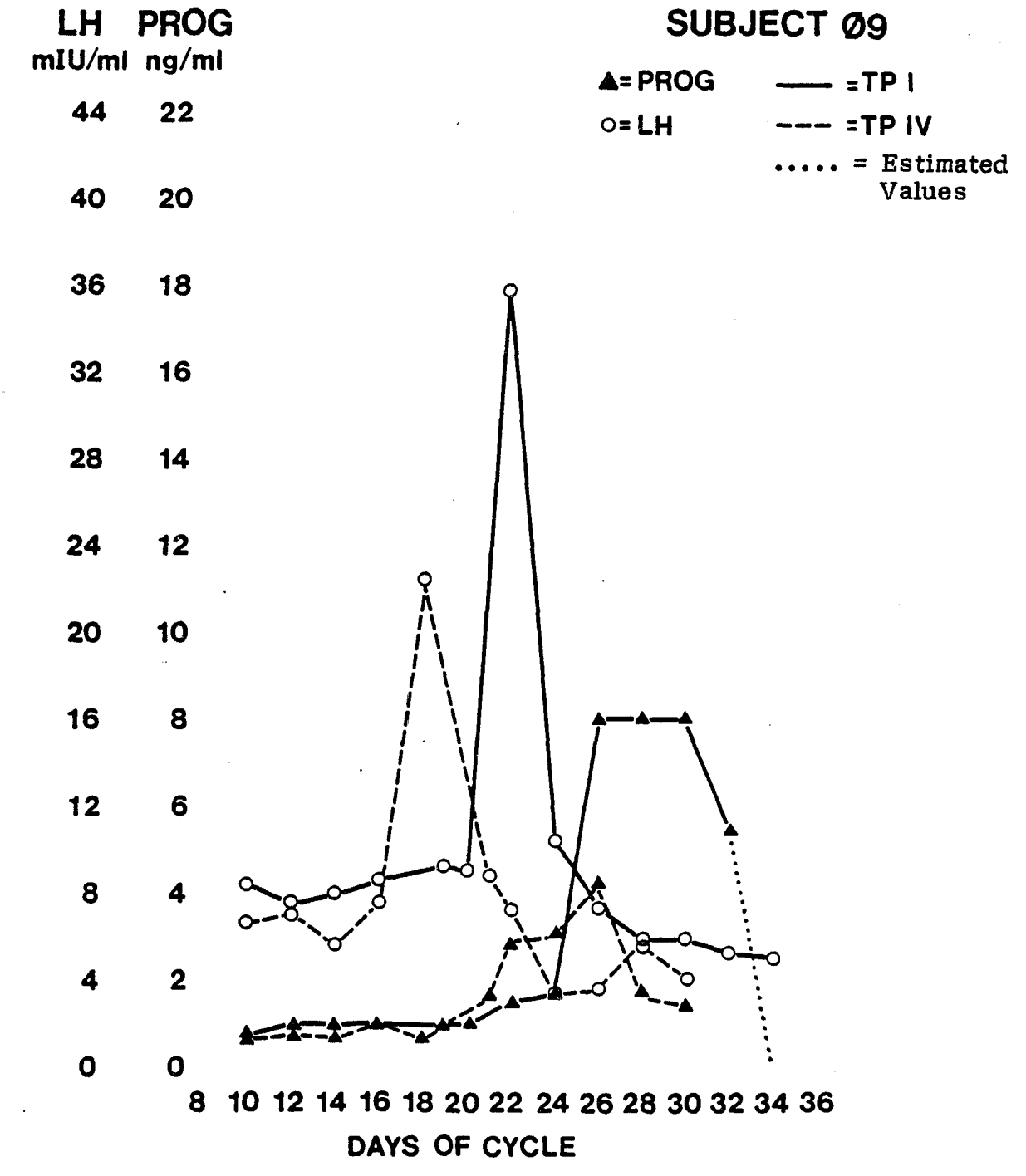
Serum LH Plasma Progesterone, Test Periods I and IV

Serum Luteinizing Hormone (LH) and Plasma Progesterone (P)[a]

		Test Period I		Test Period IV	
Subject	Cycle Day	LH mIU/ml	P ng/ml	LH mIU/ml	P ng/ml
8	10	14.3	0.2		(0.2)
	11		(0.3)	9.2	0.2
	12	17.8	0.3		(0.2)
	13	16.7	0.5	13.5	0.2
	14		(0.4)		(0.2)
	15		(0.2)	7.9	0.2
	16	20.4	0.1		(0.2)
	17		(0.2)		(0.2)
	18	14.6	0.2	34.0	0.2
	19		(0.2)	33.2	0.2
	20	13.7	0.2		(0.5)
	21		(0.4)	17.8	0.8
	22	74.4	0.6		(0.8)
	23		(0.7)	7.1	0.8
	24	9.0	0.8		(2.9)
	25		(2.4)		4.9
	26		(4.0)	4.2	(3.9)
	27	6.3	5.5		2.8
	28		(4.9)		(2.4)
	29		(4.3)	2.7	1.9
	30		(3.7)		(1.7)
	31[b]		(3.1)	8.4	1.5
	32		(2.5)		
	33		(1.9)		
	34		(1.3)		
	35		(0.7)		
	36		(0.2)		

[a]Values in parentheses represent estimates based on measured values of preceding and subsequent days

[b]Onset of next menstrual cycle, Test Period IV

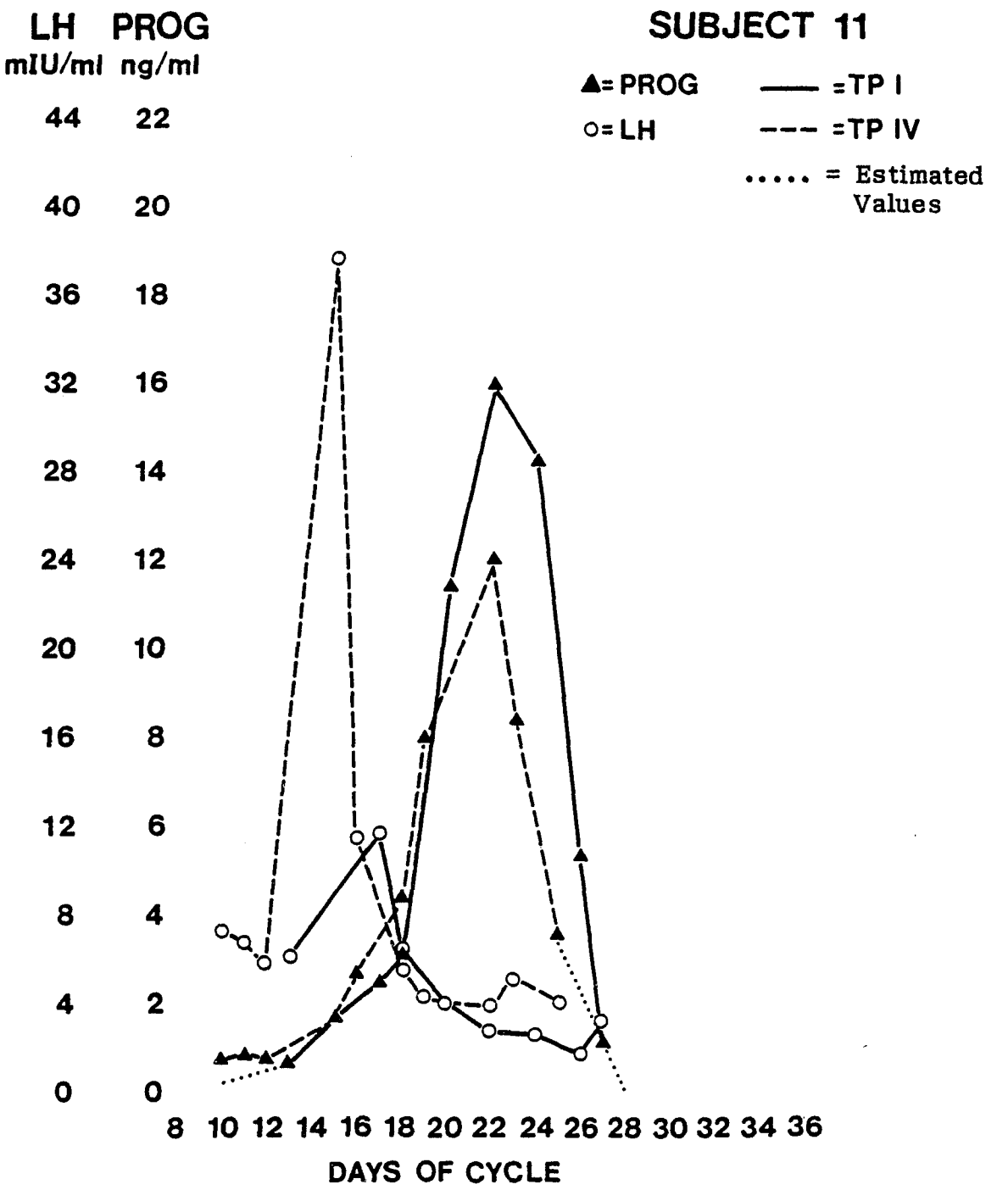


Serum LH Plasma Progesterone, Test Periods I and IV

Serum Luteinizing Hormone (LH) and Plasma Progesterone (P)[a]

		Test Period I		Test Period IV	
Subject	Cycle Day	LH mIU/ml	P ng/ml	LH mIU/ml	P ng/ml
9	10	8.5	0.8	6.7	0.7
	11		(0.9)		(0.8)
	12	7.6	1.0	7.2	0.8
	13		(1.0)		(0.8)
	14	8.0	1.0	5.7	0.7
	15		(1.1)		(0.9)
	16	8.6	1.1	7.6	1.1
	17		(1.1)		(0.9)
	18		(1.0)	22.5	0.7
	19	9.3	1.0		(1.0)
	20	9.0	1.0		(1.3)
	21		(1.3)	8.9	1.6
	22	35.8	1.5	7.2	2.9
	23		(2.4)		(3.0)
	24	10.4	3.2	3.4	3.0
	25		(5.6)		(3.6)
	26	7.3	8.0	3.6	4.2
	27		(8.0)		(2.9)
	28	5.9	8.0	5.6	1.6
	29		(8.0)		(1.5)
	30	5.9	8.0	4.1	1.4
	31		6.7		
	32	5.3	5.4		
	33		(2.8)		
	34	5.0	(0.2)		

[a]Values in parentheses represent estimates based on measured values of preceding and subsequent days

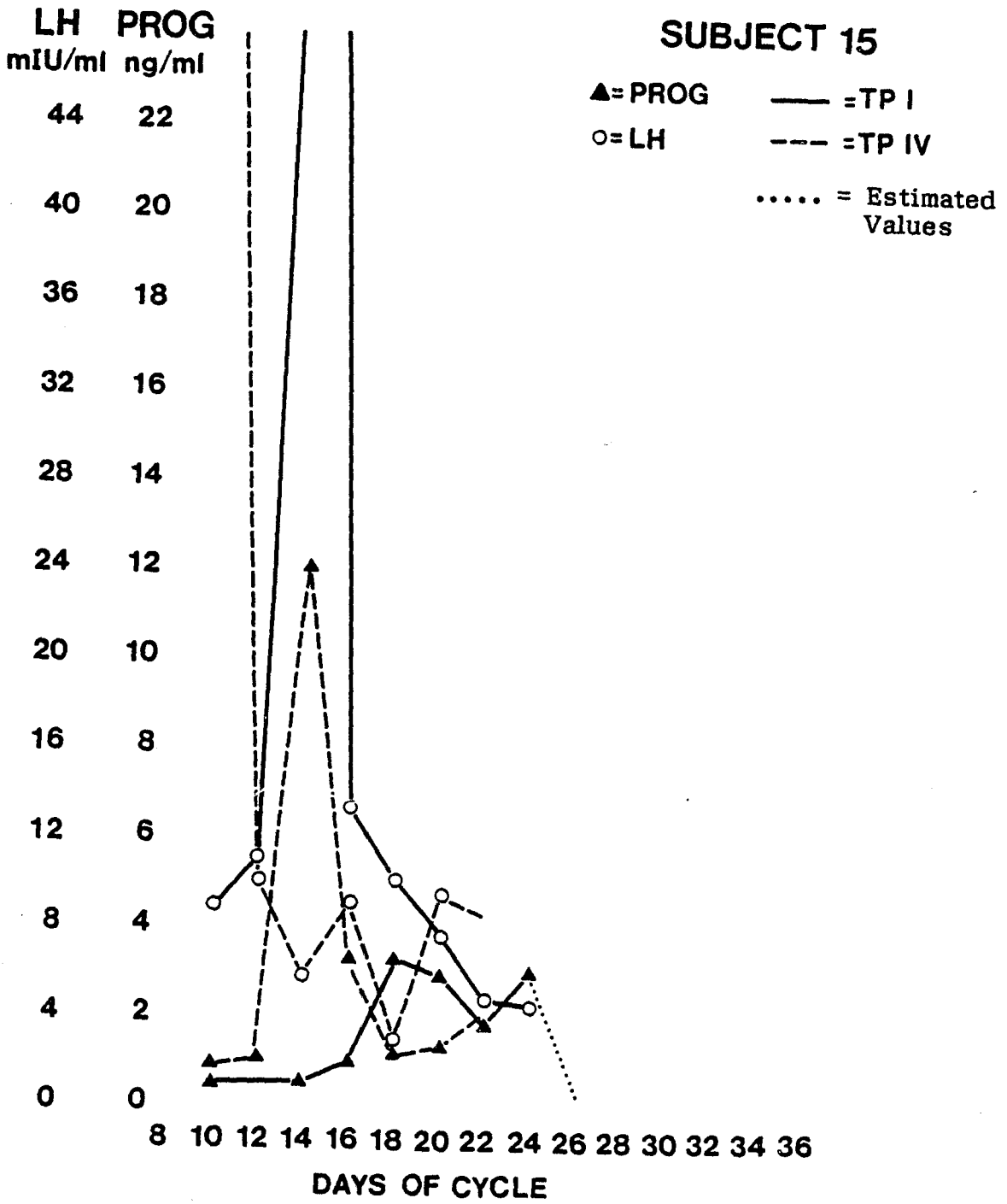


Serum LH Plasma Progesterone, Test Periods I and IV

Serum Luteinizing Hormone (LH) and Plasma Progesterone (P)[a]

		Test Period I		Test Period IV	
Subject	Cycle Day	LH mIU/ml	P ng/ml	LH mIU/ml	P ng/ml
11	10		(0.2)	7.3	0.8
	11		(0.3)	6.8	0.9
	12		(0.5)	5.8	0.8
	13	6.2	0.7		(1.1)
	14		(1.2)		(1.4)
	15		(1.6)	37.7	1.7
	16		(2.1)	11.5	2.7
	17	11.8	2.5		(3.6)
	18	6.6	3.1	5.6	4.4
	19		(7.3)	4.3	8.0
	20	4.1	11.4		(9.3)
	21		(13.7)		(10.7)
	22	2.8	16.0	3.9	12.0
	23		(15.2)	5.2	8.5
	24	2.7	14.3		(6.0)
	25		(9.8)	4.1	3.5
	26	1.8	5.3		(2.4)
	27	3.3	1.2		(1.3)
	28				(0.2)
	29				
	30				

[a]Values in parentheses represent estimates based on measured values of preceding and subsequent days



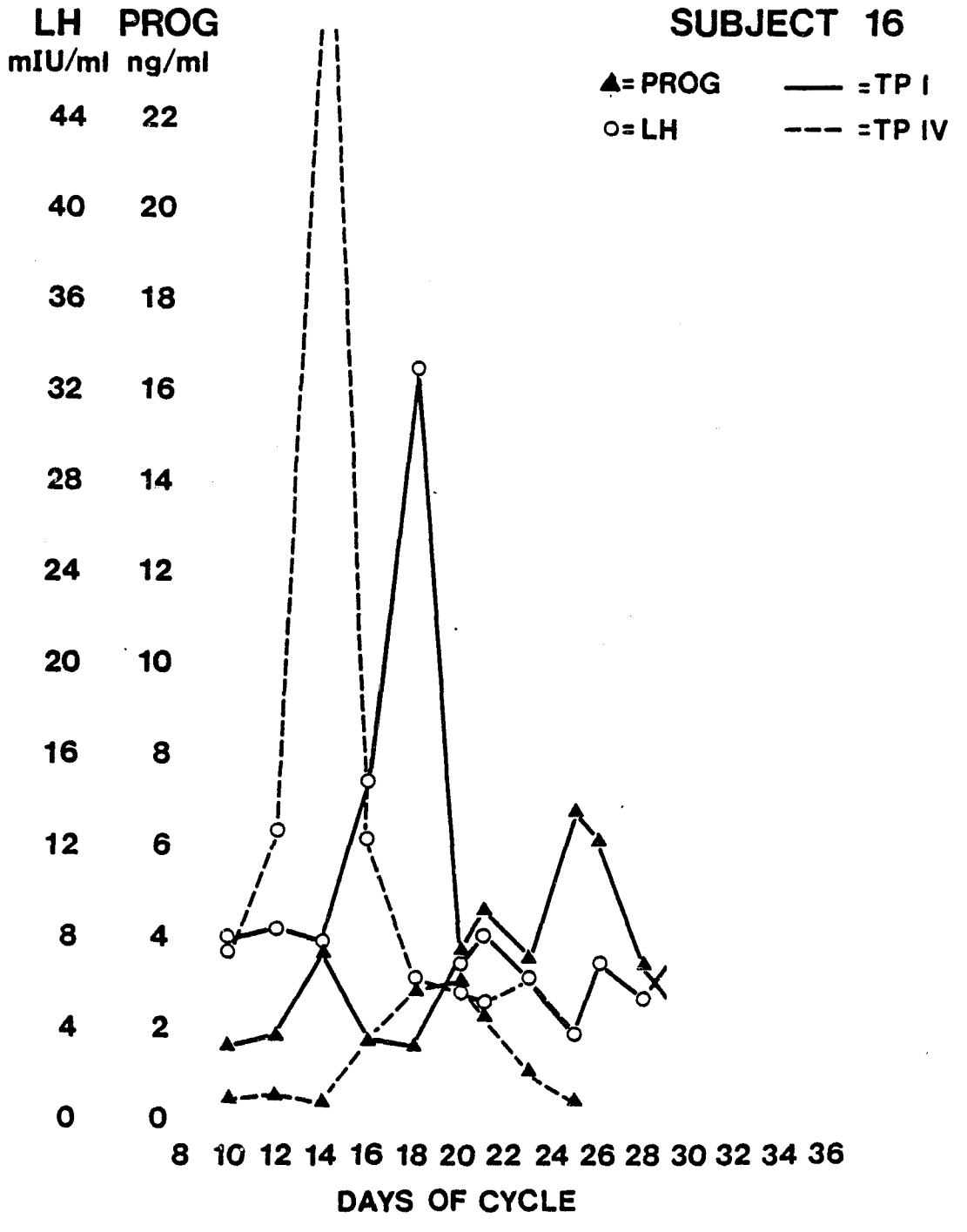
Serum LH Plasma Progesterone, Test Periods I and IV

Serum Luteinizing Hormone (LH) and Plasma Progesterone (P)[a]

		Test Period I		Test Period IV	
Subject	Cycle Day	LH mIU/ml	P ng/ml	LH mIU/ml	P ng/ml
15	10	8.9	0.5	62.0	0.9
	11		(0.5)		(1.0)
	12	11.0	(0.5)	10.0	1.0
	13		(0.5)		(6.5)
	14	78.4	0.5	5.7	12.0
	15		(0.7)		(7.6)
	16	13.2	0.9	9.0	3.2
	17		(2.1)		(2.2)
	18	10.0	3.3	2.9	1.1
	19		(3.1)		(1.2)
	20	7.5	2.9	9.3	1.3
	21		(2.4)		(1.7)
	22	4.7	1.8		(2.0)
	23[b]		(2.4)	8.0	2.4
	24	4.4	3.0		
	25		(1.5)	9.6	
	26		(0.2)		
	27				
	28				
	29				
	30				

[a]Values in parentheses represent estimates based on measured values of preceding and subsequent days

[b]Onset of next menstrual cycle, Test Period IV



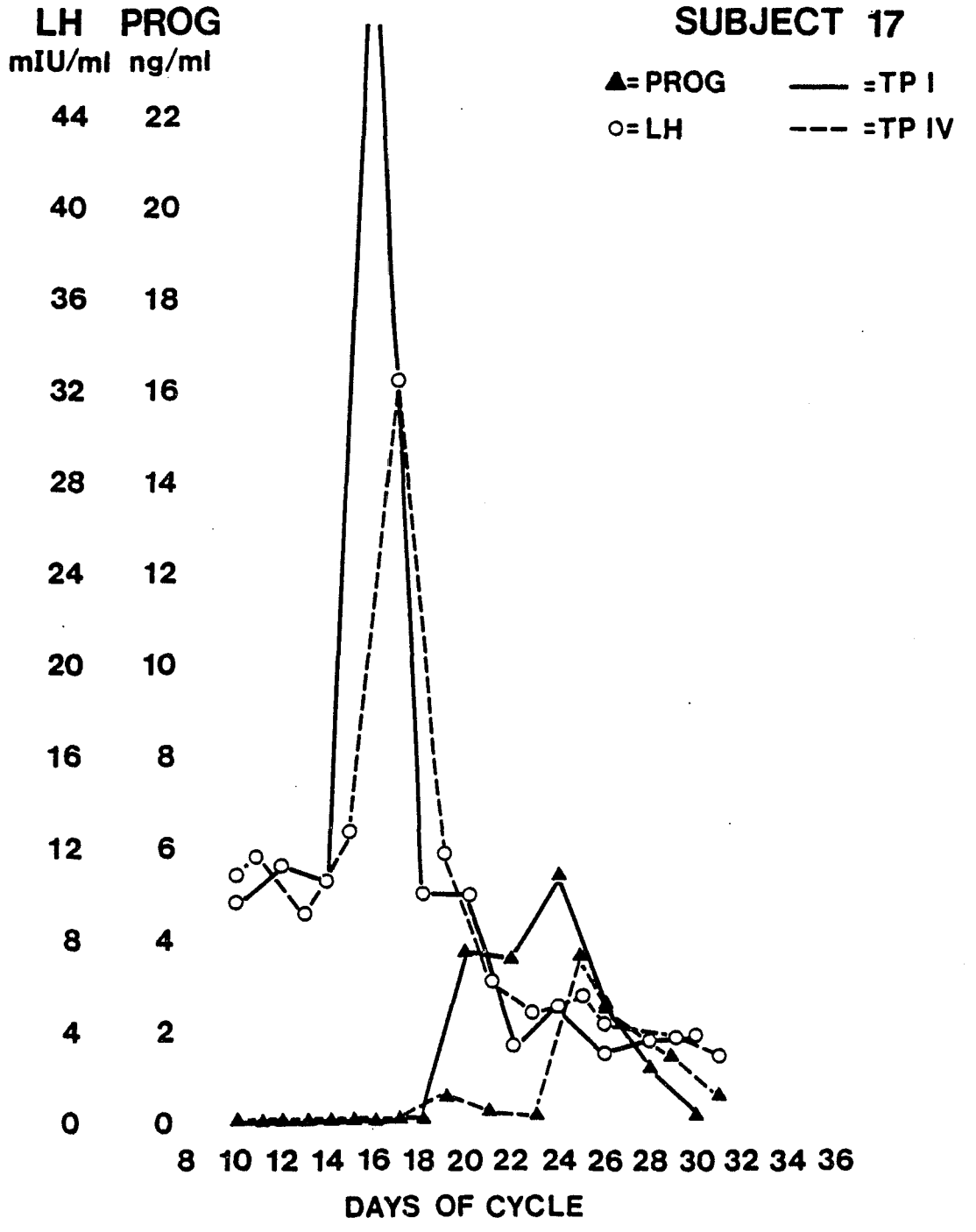
Serum LH Plasma Progesterone, Test Periods I and IV

Serum Luteinizing Hormone (LH) and Plasma Progesterone (P)[a]

		Test Period I		Test Period IV	
Subject	Cycle Day	LH mIU/ml	P ng/ml	LH mIU/ml	P ng/ml
16	10	8.0	1.7	7.4	0.5
	11		(1.8)		(0.6)
	12	8.4	2.9	12.7	0.6
	13		(2.3)		(0.5)
	14	7.9	3.7	55.7	0.4
	15		(2.8)		(1.1)
	16	14.8	1.8	12.4	1.8
	17		(1.7)		(2.4)
	18	33.0	1.6	6.2	2.9
	19		(2.7)		(3.0)
	20	6.8	3.7	5.6	3.0
	21	8.1	4.6	5.2	2.3
	22		(4.1)		(1.7)
	23	6.3	3.5	6.2	1.0
	24		(5.1)		(0.7)
	25	3.8	6.7	3.9	0.4
	26	6.8	6.1		
	27		(4.7)		
	28	5.4	3.3		
	29		(2.6)		
	30[b]	8.2	1.9		

[a]Values in parentheses represent estimates based on measured values of preceding and subsequent days

[b]Onset of next menstrual cycle, Test Period I

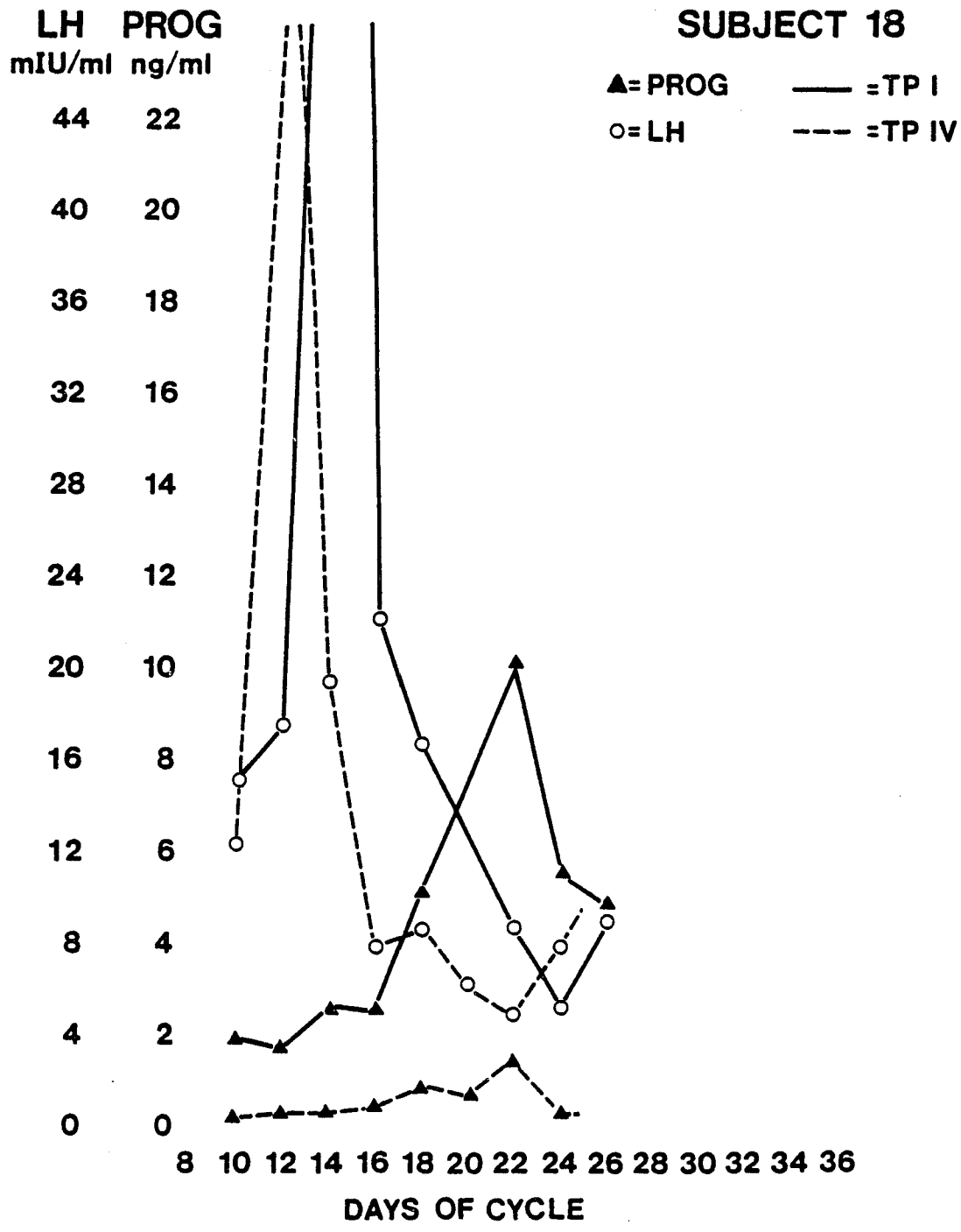


Serum LH Plasma Progesterone, Test Periods I and IV

Serum Luteinizing Hormone (LH) and Plasma Progesterone (P)[a]

		Test Period I		Test Period IV	
Subject	Cycle Day	LH mIU/ml	P ng/ml	LH mIU/ml	P ng/ml
17	10	9.7	0.2	10.9	0.2
	11		(0.2)	11.9	0.2
	12	11.3	0.2		(0.2)
	13		(0.2)	9.2	0.2
	14	10.7	0.2		(0.2)
	15		(0.2)	12.8	0.2
	16	53.6	0.2		(0.2)
	17		(0.2)	32.5	0.2
	18	10.1	0.2		(0.5)
	19		(2.0)	11.9	0.7
	20	10.0	3.8		(0.5)
	21		(3.7)	6.3	0.3
	22	3.4	3.6		(0.3)
	23		(4.5)	4.9	2.0
	24	5.0	5.4		(2.0)
	25		(4.0)	5.6	3.7
	26	3.1	2.6	4.4	2.5
	27		(1.9)		(2.2)
	28	3.7	1.2		(1.9)
	29		(0.7)	3.8	1.5
	30	3.8	0.2		(1.1)
	31[b]			3.0	0.6

[a]Values in parentheses represent estimates based on measured values of preceding and subsequent days



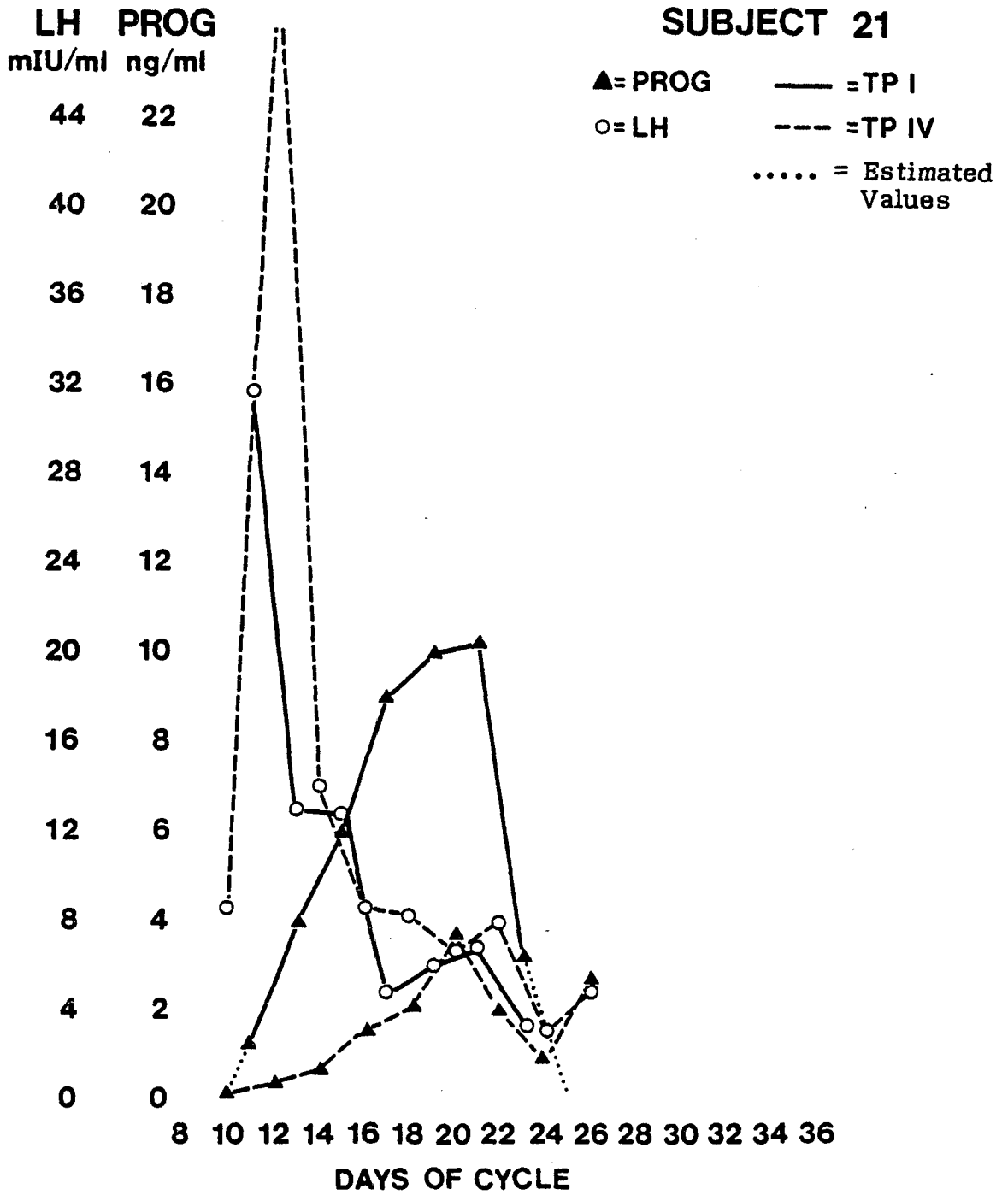
Serum LH Plasma Progesterone, Test Periods I and IV

Serum Luteinizing Hormone (LH) and Plasma Progesterone (P)[a]

		Test Period I		Test Period IV	
Subject	Cycle Day	LH mIU/ml	P ng/ml	LH mIU/ml	P ng/ml
18	10	15.0	1.9	12.2	0.2
	11		(1.8)		(0.3)
	12	17.4	1.7	54.3	0.3
	13		(2.2)		(0.3)
	14	97.6	2.6	19.3	0.3
	15		(2.6)		(0.4)
	16	22.1	2.5	7.8	0.4
	17		(3.8)		(0.7)
	18	16.7	5.1	8.6	0.9
	19		(6.4)		(0.8)
	20		(7.6)	6.1	0.7
	21		(8.9)		(1.1)
	22	8.6	10.1	4.9	1.5
	23		(7.8)		(0.9)
	24	5.3	5.5	7.9	0.3
	25		(5.2)		(0.3)
	26[b]	8.8	4.8	11.3	0.3
	27				
	28				
	29				
	30				

[a]Values in parentheses represent estimates based on measured values of preceding and subsequent days

[b]Onset of next menstrual cycle, Test Period IV

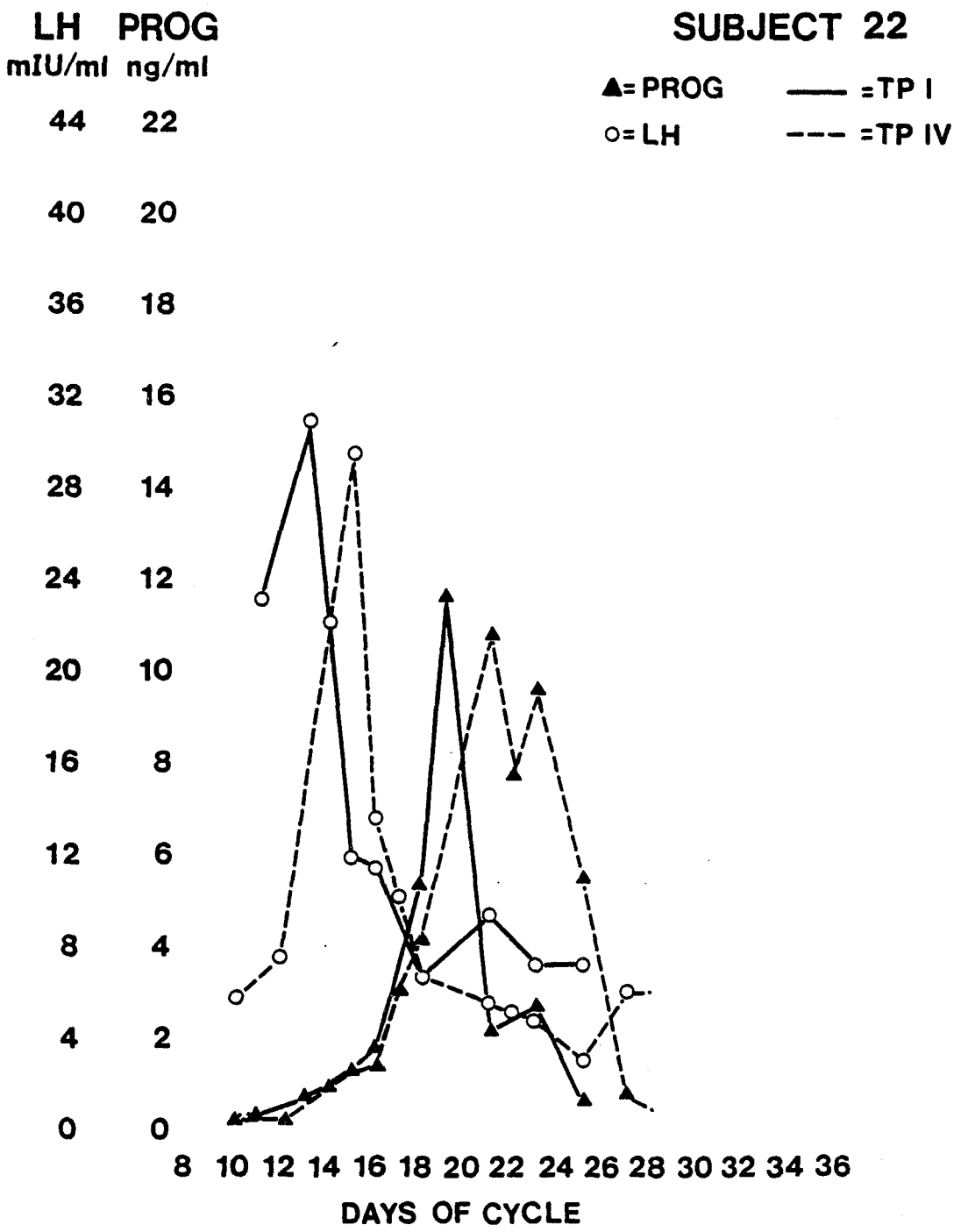


Serum LH Plasma Progesterone, Test Periods I and IV

Serum Luteinizing Hormone (LH) and Plasma Progesterone (P)[a]

		Test Period I		Test Period IV	
Subject	Cycle Day	LH mIU/ml	P ng/ml	LH mIU/ml	P ng/ml
21	10		(0.2)	8.7	0.2
	11	31.7	1.3		(0.3)
	12		(2.6)	52.3	0.4
	13	13.0	4.0		(0.6)
	14		(5.0)	14.0	0.7
	15	12.8	6.0		(1.2)
	16		(7.5)	8.6	1.6
	17	4.8	9.0		(1.9)
	18		(9.5)	8.3	2.1
	19	6.1	10.0		(2.9)
	20		(10.1)	6.6	3.7
	21	6.8	10.2		(2.9)
	22		(6.7)	8.0	2.0
	23	3.4	3.2		1.5
	24		(1.8)	3.0	0.9
	25		(0.2)		(1.3)
	26			5.0	2.7
	27				
	28				
	29				
	30				

[a]Values in parentheses represent estimates based on measured values of preceding and subsequent days



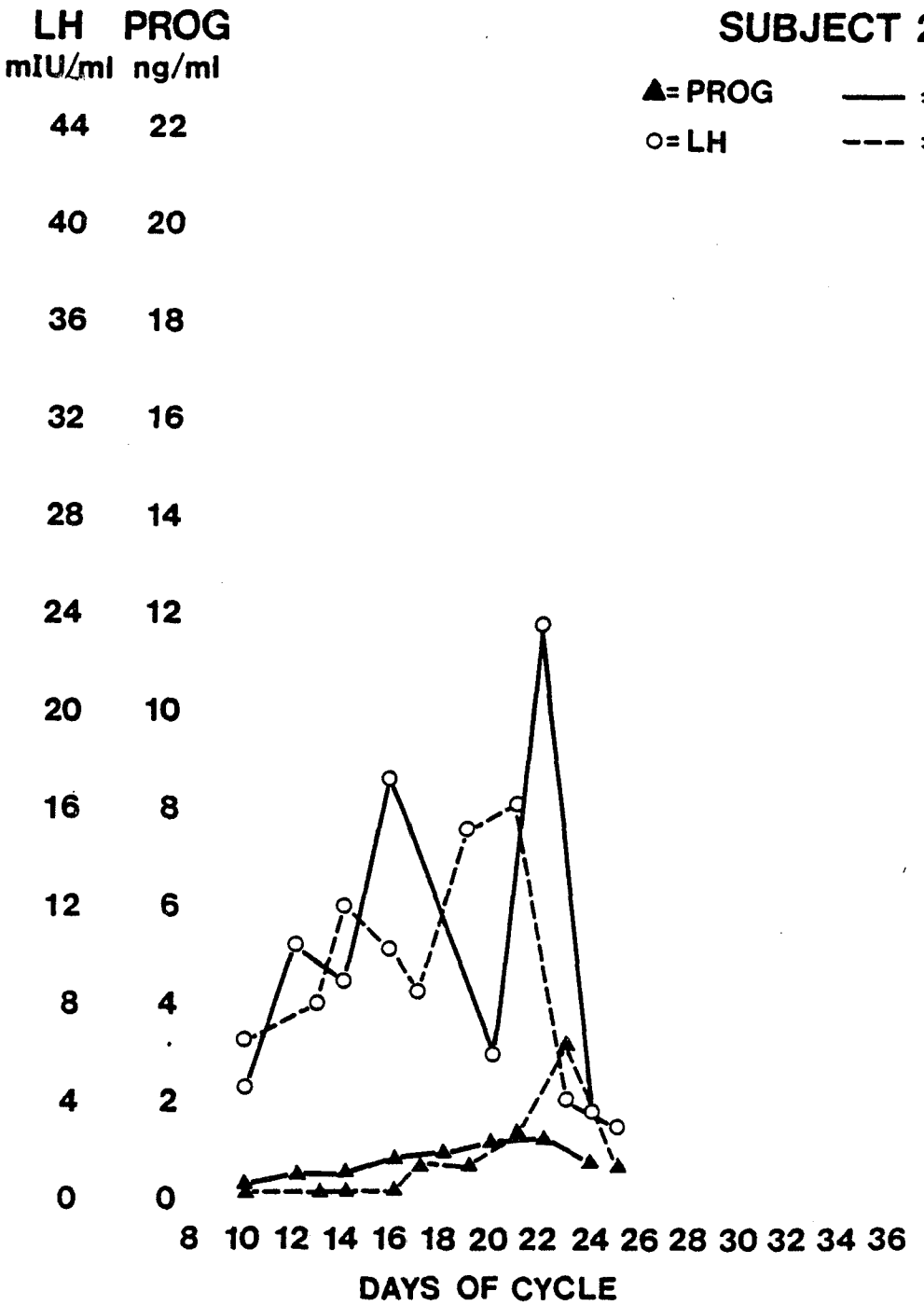
Serum LH Plasma Progesterone, Test Periods I and IV

Serum Luteinizing Hormone (LH) and Plasma Progesterone (P)[a]

		Test Period I		Test Period IV	
Subject	Cycle Day	LH mIU/ml	P ng/ml	LH mIU/ml	P ng/ml
22	10		(0.2)	5.7	0.2
	11	23.0	0.3		(0.2)
	12		(0.5)	7.4	0.2
	13	30.9	0.6		(0.6)
	14		(0.9)	22.0	0.9
	15	11.8	1.2	29.5	1.2
	16	11.3	1.7	13.6	1.4
	17		(3.5)	10.1	3.0
	18	5.5	5.3	6.7	4.1
	19		11.6		(6.4)
	20		(6.9)		(8.5)
	21	9.3	2.1	5.5	10.8
	22		(2.4)	5.1	7.7
	23	7.1	2.7	4.7	9.6
	24		(1.7)		(7.6)
	25	7.1	0.6	3.0	5.5
	26				(3.1)
	27			5.9	0.7
	28				(0.5)
	29[b]			6.1	0.2
	30				

[a]Values in parentheses represent estimates based on measured values of preceding and subsequent days

[b]Onset of next menstrual cycle, Test Period IV



Serum LH Plasma Progesterone, Test Periods I and IV

Serum Luteinizing Hormone (LH) and Plasma Progesterone (P)[a]

		Test Period I		Test Period IV	
Subject	Cycle Day	LH mIU/ml	P ng/ml	LH mIU/ml	P ng/ml
23	10	4.7	0.4	6.6	0.2
	11		(0.5)		(0.2)
	12	10.4	0.6		(0.2)
	13		(0.6)	8.1	0.2
	14	9.0	0.6	12.1	0.2
	15		(0.8)		(0.2)
	16	17.3	0.9	10.3	0.2
	17		(1.0)	8.6	0.8
	18		1.0		(0.8)
	19		(1.1)	15.2	0.7
	20	6.0	1.2		(1.0)
	21		(1.3)	16.3	1.3
	22	23.6	1.3		(2.3)
	23		(1.0)	4.0	3.2
	24	3.6	0.7		(1.9)
	25			3.0	0.6
	26				
	27				
	28				
	29				
	30				

[a]Values in parentheses represent estimates based on measured values of preceding and subsequent days

APPENDIX H

PROCEDURES FOR DETERMINATION OF SERUM CHOLESTEROL AND PLASMA ZINC

HDL AND TOTAL CHOLESTEROL PROCEDURE*

1. To centrifuge tube add:
   0.4 ml of serum
   0.05 ml HDL Precipitating Reagent, Stock No. 350-3

   Mix well

2. Centrifuge (2000 x g) for 5-10 minutes to obtain clear supernatant.

3. Label 3 or more cuvets in duplicate: BLANK, STANDARD, TEST 1, etc.

4. To BLANK add: 0.05 ml water
   To STANDARD add: 0.05 ml Cholesterol Aqueous Standard, Stock No. 350-50
   To TEST add: 0.05 ml Supernatant (HDL fraction) from Step 2

   For determination of total cholesterol include an additional set of test samples (in duplicate) containing 0.05 ml aliquots of serum, and follow the rest of HDL-C procedure

5. To each add: 3.0 ml Cholesterol Reagent. Cover cuvet with Parafilm and mix well.

6. Incubate the cuvets at 37°C for 10-15 minutes.

7. Read Absorbance of STANDARD and TEST vs. BLANK as reference at 625 nm (±15 nm). Complete readings within 30 minutes.

8. Calculate HDL Cholesterol values as follows:

$$\text{Serum HDL Cholesterol (mg/dl)} = \frac{A_{TEST}}{A_{STANDARD}} \times 56$$

*Serum cholesterol was assayed using a Bausch and Lamb Spectronic 2000 spectrophotometer (Fisher Scientific, Raleigh, NC) with the Liebermann-Buchard color cholesterol reagent purchased from Stanbio-Laboratory, Inc. (San Antonio, TX).

PLASMA ZINC STANDARD PREPARATION

1. Prepare six standard solutions of 0.0, 0.1, 0.2, 0.3, 0.4, and 0.5 parts per million (ppm) zinc in dilute sulfuric acid.

2. Prepare 9.0 liter of a 5.0% glycerol stock solution in an acid-washed 1.0-liter volumetric flask.

3. Prepare a 10-ppm zinc working solution in a 100-ml volumetric flask by adding 1.0 ml of 1000-ppm zinc standard to 99-ml of 8% H_2SO_4 solution.

4. From this 10-ppm working solution prepare the concentrations in separate 100-ml volumetric flasks:

   a) 0.1 ppm - add 1.0 ml of working solution to 99 ml of H_2SO_4 solution.

   b) 0.2 ppm - add 2.0 ml of working solution to 98 ml of H_2SO_4 solution.

   c) 0.3 ppm - add 3.0 ml of working solution to 97 ml of H_2SO_4 solution.

   d) 0.4 ppm - add 4.0 ml of working solution to 96 ml of H_2SO_4 solution.

   e) 0.5 ppm - add 5.0 ml of working solution to 95 ml of H_2SO_4 solution.

   f) 0 ppm - bring to volume 100 ml of H_2SO_4 solution.

Reference:

Butrimovitz, G. P., & Purdy, W. C. (1977). The determination of zinc in blood plasma by atomic absorption spectometry. Analytica Chimica Acta, 94, 63-71.