Pediat. Res. 6: 18–25 (1972)

Follicle-stimulating hormone
gonadotropin
luteinizing hormone
menarche
ovulation

Excretion of Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH) in Urine by Pubertal Girls

ALLEN HAYES AND ANN JOHANSON[46]

Department of Pediatrics, University of Virginia School of Medicine, Charlottesville, Virginia, USA

Extract

Daily urinary excretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in one premenarchial and four postmenarchial girls was determined over a 3-month collection period. Samples were collected overnight and a 24-hr excretion was extrapolated. Gonadotropins were acetone-precipitated and assessed by using the Second International Reference Preparation of Human Menopausal Gonadotropin (2nd IRP-HMG).

Excretion of LH and FSH in the premenarchial girl followed no constant pattern, but average levels were comparable to postmenarchial and adult levels for FSH and early postmenarchial levels for LH. During the 3-month observation period, two simultaneous spikes of LH to 55 international units (IU)/24 hr and FSH to 60 IU/24 hr, occurring 6 days apart, were observed. These were of the magnitude of low normal adult midcycle peak values.

In all postmenarchial girls, simultaneous midcycle surges of FSH to as high as 70 IU/24 hr and LH to 43–155 IU/24 hr were observed in all cycles. During the earliest postmenarchial cycles, peak levels were less than those observed during cycles of girls who were postmenarchial for a longer period of time. Some characteristics of adult cycles, such as follicular phase FSH elevations and higher follicular than luteal phase levels of both gonadotropins, were observed during cycles occurring only 1–4 months after menarche. Other characteristics of adult cycles, such as late luteal phase FSH rises, were seen only in the girl who was 3 years postmenarchial.

Speculation

Premenarchial and early postmenarchial girls have gonadotropin surges similar to adult midcycle ovulatory peaks. They presage evolution of the adult gonadotropin pattern in the subsequent postmenarchial years.

Introduction

Much that has been written about the physiologic events that occur in girls at puberty has been speculative. Novak [22] in 1930 stated that he considered it highly probable that anovulatory menstrual cycles occurred in many girls for a variable time after menarche. This view has often been repeated.

Detection of ovulation has been assessed by many means [31], most of them fallible to some extent. Because of its simplicity, basal body temperature (BBT)

recording has provided most of the information regarding the question of ovulation at menarche. Cooperman [7] reported maturation to the typical adult biphasic, presumably ovulatory, BBT pattern from the monophasic "anovulatory" patterns in two postmenarchial girls. Benjamin [3] reported an "ovulatory" rise in BBT in two girls whom he observed before their initial menstrual period. Doring [11] found that, during 60% of the menstrual cycles of 42 girls 12–14 years old, there was no biphasic BBT pattern. These observations were interpreted to suggest that most early menstrual periods were anovulatory, but also that ovulation may occur before menarche.

Midcycle gonadotropin peaks in normal menstruating women have been well documented [1, 6, 15, 29, 37]. Their relation to ovulation was observed by Yussman and Taymor [37], although ovulation was not constantly found after a rise in gonadotropin. With sensitive radioimmunoassays, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) have also been measured in prepubertal and pubertal children [2, 12, 16, 24, 25, 28, 30]. Gonadotropin levels in urine, assessed by radioimmunoassay, correlate with bioassay measurements [1, 2, 13, 32]. These studies have yielded cross-sectional data on the rises that occur at puberty and how they compare with clinical evaluation of sexual development [2, 16, 25].

In an attempt to observe the gonadotropin secretion pattern of pubertal girls as a function in maturation of sexual development, we measured daily excretion of FSH and LH for a 3-month period in five pubertal girls.

Materials and Methods

Subjects

Five normal pubertal school girls were selected for study. Criteria for their inclusion were: (*a*) close proximity to the menarche, (*b*) height and weight between 10th and 90th percentiles [21], (*c*) normal endocrine history and physical examination, and (*d*) willingness to participate. Their ages ranged from 12 to 15 years. Historic information was obtained regarding onset and pattern of pubertal development, onset of menarche, and dates of menses during the study.

Samples

A specimen of first-voided urine was collected each morning from each of the subjects who recorded the period of time since the last voiding. Random specimens were not obtained. Samples were kept for a maximum of 48 hr at room temperature before their handling in the laboratory. (There is no loss of immunoassayable gonadotropin in this period of time [1].) Eleven normal adults collected 24-hr urine samples, keeping separate the first-voided urine collected in the morning.

Extraction

Follicle-stimulating hormone and luteinizing hormone were precipitated from urine aliquots with acetone and were resuspended in the buffer used in the assay (0.14 M NaCl, 0.01 M Na_2PO_4, pH 7.4) [2]. Recoveries of immunoassayable gonadotropins following this procedure approach 100% [1].

Assay

LH. A purified human pituitary LH fraction, LER 822-2 [38], with an LH potency of 4598 international units (IU)/mg [Second International Reference Preparation Human Menopausal Gonadotropin (2nd IRP-HMG)] was iodinated with ^{131}I by the method of Midgley [19]. Specific activities were 387–458 $\mu Ci/\mu g$. Rabbit antiserum developed against electrophoretically homogeneous human chorionic gonadotropin (HCG) was used to complex the ^{131}I LH and either standard or unknown amounts of LH [39]. Cross-reaction occurs between LH and HCG [18, 35]; there is no cross-reaction with FSH [19]. Antiserum against rabbit gamma globulin was developed in sheep and used to precipitate the complexed LH and anti-HCG [4].

FSH. For iodination with ^{125}I, a purified human pituitary FSH fraction LER 869-2, with FSH potency of 2782 IU/mg 2nd IRP-HMG was used [38]. The method of Midgley [20] was used. Specific activities were 74–198 $\mu Ci/\mu g$. Rabbit antiserum developed against electrophoretically homogeneous FSH was used to complex the FSH [40]. This antiserum was previously adsorbed with HCG [5]. Antiserum to rabbit gamma globulin developed in sheep was used to precipitate the complexed FSH and anti-FSH.

Standards. Second International Reference Preparation of Human Menopausal Gonadotropin (2nd IRP-HMG), with an assigned biologic activity of 40 IU of FSH and 40 IU of LH, was used as a standard [41].

Assay technique. Midgley's techniques [19, 20] for radioimmunoassay of LH and FSH were utilized.

Counting and calculations. A Picker automatic γ counter with a 2-inch crystal was used to count precipitates. For analysis and evaluation of the limits of sensitivity, logit plots were used [14]. Standard values were assayed in triplicate. Each sample was assayed in dupli-

cate at each of two dilutions. Statistical quality control was maintained as outlined by the criteria of Rodbard *et al.* [27]. Parallelism with the standard curve was obtained with serial dilutions of urine extract. Interassay variation for LH was ±0.85 mIU/ml SD at a level of 8.6 mIU/ml, and for FSH it was ±1.2 mIU/ml SD at a level of 12.5 mIU/ml.

Results

Test of Extrapolation

Eleven first-voided morning specimens of urine obtained from normal adult volunteers were assayed for gonadotropin content. The excretion rate during the recorded overnight interval was used to calculate the presumed 24-hr excretion. The first-voided morning urine was then added to the remainder of the 24-hr specimen and the *actual* 24-hr excretion was assayed. These two sets of results are shown in Table I. Coefficients of correlation were determined and their significance is given in Table I [9]. Although a coefficient of correlation between 0.7 and 0.8 does not indicate complete dependence of variables, the high degree of significance indicates that the extrapolation technique does yield a valid estimation of 24-hr excretion of urinary gonadotropin.

Urinary Excretion Patterns of Gonadotropin

Figures 1–5 show the patterns of gonadotropin excretion in each of the five girls. Figure 1 shows a 20-day period during the 3-month observation of the premenarchial girl (*TM*) selected to show the two occurrences of peak levels. At the start of the study, she was $12\frac{7}{12}$ years old, *stage 3* of sexual development [34], and was presumed to be approximately 1 year from the menarche [8, 17, 34]. During the 3-month period, two simultaneous LH and FSH peaks occurred. These were of the magnitude of normal adult midcycle peaks [1], although LH was in the very low range of normal and one FSH level could be considered higher than normal.

Table I. Comparison of observed with extrapolated values for FSH and LH

Subject	Follicle-stimulating hormone		Luteinizing hormone	
	24-hr assayed value	24-hr extrapolated value	24-hr assayed value	24-hr extrapolated value
1	9.7	11.1	23.2	27.9
2	8.2	6.6	15.4	15.6
3	9.3	9.8	18.1	21.6
4	7.0	6.0	16.4	14.8
5	7.4	6.4	16.7	11.7
6	9.5	7.1	23.1	20.4
7	7.3	6.3	20.8	19.8
8	5.9	6.3	15.4	18.0
9	10.5	8.4	27.7	23.6
10	12.4	9.8	22.9	24.7
11	11.0	11.7	18.6	16.8
	$r = 0.78$		$r = 0.72$	
	$P < 0.005$ (2 tail)		$P < 0.02$ (2 tail)	

Figures 2–5 are composite plots of three consecutive cycles showing the range of values for FSH and LH on each day, relative to the midcyclic peak day (*day 0*). The cycles are centered on the peak LH value, which in all cases corresponds to a peak in FSH.

Figure 2 depicts the pattern of a $14\frac{10}{12}$-year-old girl (*MM*), who began to menstruate 1 month before the start of the study. The midcycle peak levels are within the normal range for adult levels, although again LH is in the very low normal range. There is a slight early follicular phase rise in FSH.

The levels of gonadotropins in the urine of two girls who were 1 year postmenarchial are shown in Figures 3 and 4. In one $14\frac{6}{12}$-year-old subject, *EC* (Fig. 4), an early follicular phase FSH elevation was observed. In Figure 3 (*subject GW,* 14 years old) a distinct follicular phase peak is not seen. Follicular phase levels are higher than luteal phase levels, without any clearly distinctive pattern other than a prolonged decline from the midcycle peak. Higher levels in follicular phases and peaks for both FSH and LH are seen in Figure 4 than are seen in Figures 2 and 3. The levels in Figure 4 closely resemble those observed in the girl who was 3 years postmenarchial (Fig. 5) and those observed in adults.

Figure 5 (*subject MS,* $14\frac{4}{12}$ years old) represents the urinary gonadotropin pattern in a girl 3 years postmenarchial. A distinct follicular phase rise in FSH is noted. There is a luteal rise in FSH occurring 2 days before the onset of menses. As in the previous figures, the midcycle LH peak shows a prolonged fall.

Quantitative Comparisons

Lengths of luteal and follicular phases for each of the menstruating girls are shown in Table II. Two of the cycles of *MM* (1–4 months postmenarchial) and all of the cycles of *GW* (1 year postmenarchial) had short luteal phases (less than 11 days). Mean values for FSH and LH during follicular and luteal phases for each girl are also shown in Table II. The two observed peaks in the premenarchial subject (*TM*) were excluded from calculation, as were the midcyclic peak

values for LH and FSH in each cycle of the other four girls. Because in all four menstruating subjects there was a prolonged fall in LH after the midcyclic peak, the calculation of mean luteal LH values was made by eliminating the values from the first 5 days after the midcyclic peak. For comparison, similar calculations

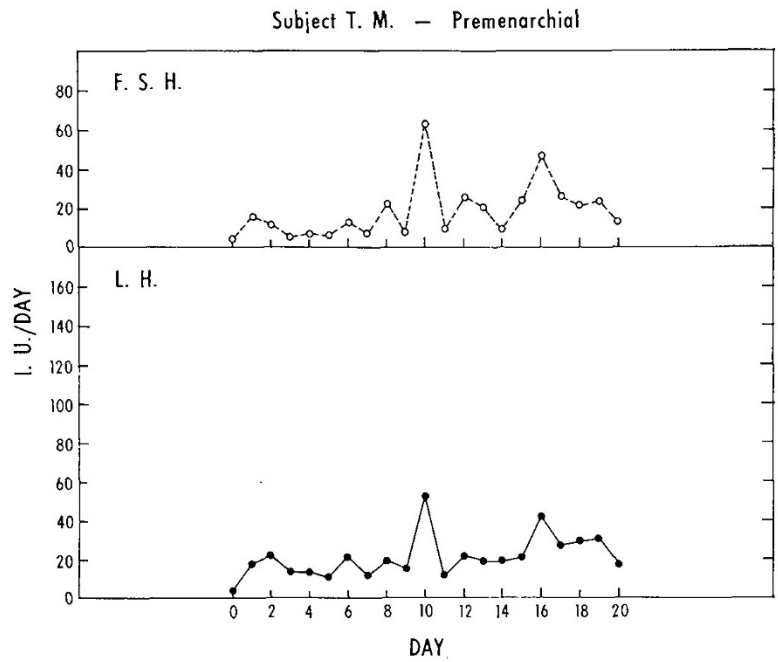

Fig. 1. Selected 20-day period of daily FSH and LH excretion showing the two peak values which occurred during a 3-month observation of a premenarchial girl. International units per day obtained with Second International Reference Preparation of Human Menopausal Gonadotropin.

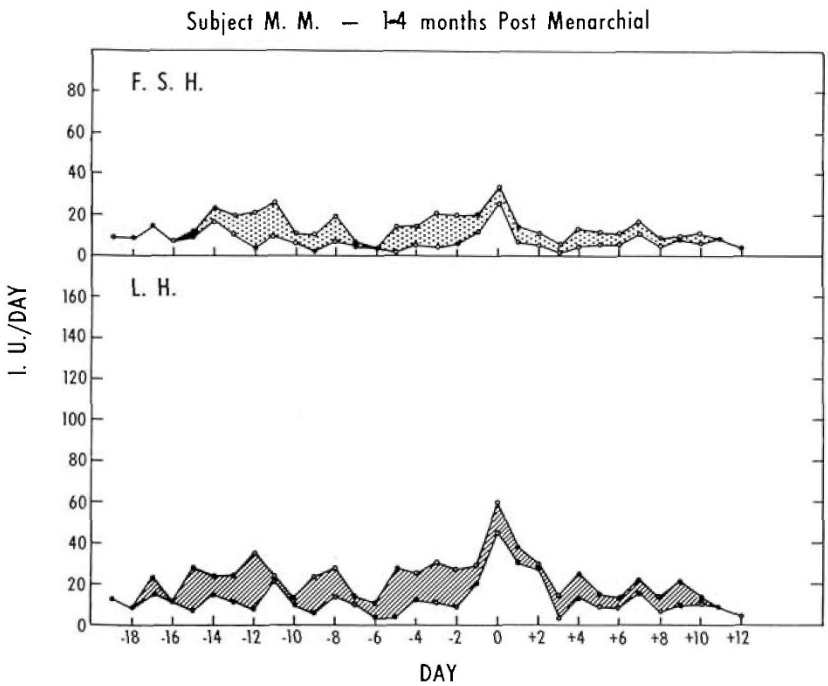

Fig. 2. Composite plot of urinary FSH and LH excretion, showing range of values each day during three menstrual cycles of a girl 1–4 months postmenarchial. Days are numbered from the simultaneous midcycle peaks (*day 0*) of FSH and LH.

were made including those 5 days and are shown in parentheses.

In each of the four menstruating subjects, all mean luteal phase FSH and LH values were found to be significantly lower than follicular phase values when the calculations for LH excluded the first 5 luteal days. This was not the case when data from the first 5 luteal days were included.

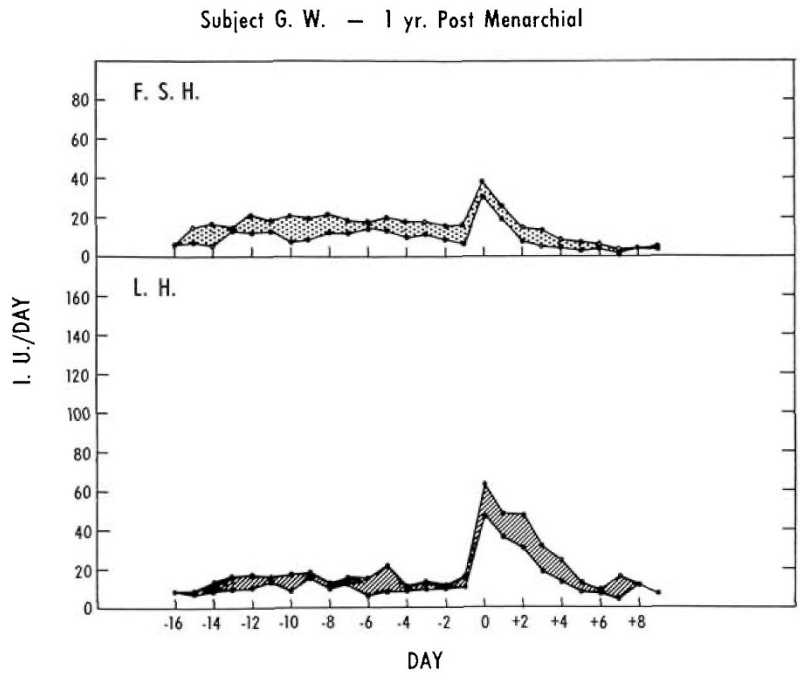

Fig. 3. Urinary FSH and LH during three menstrual cycles of a girl 1 year postmenarchial.

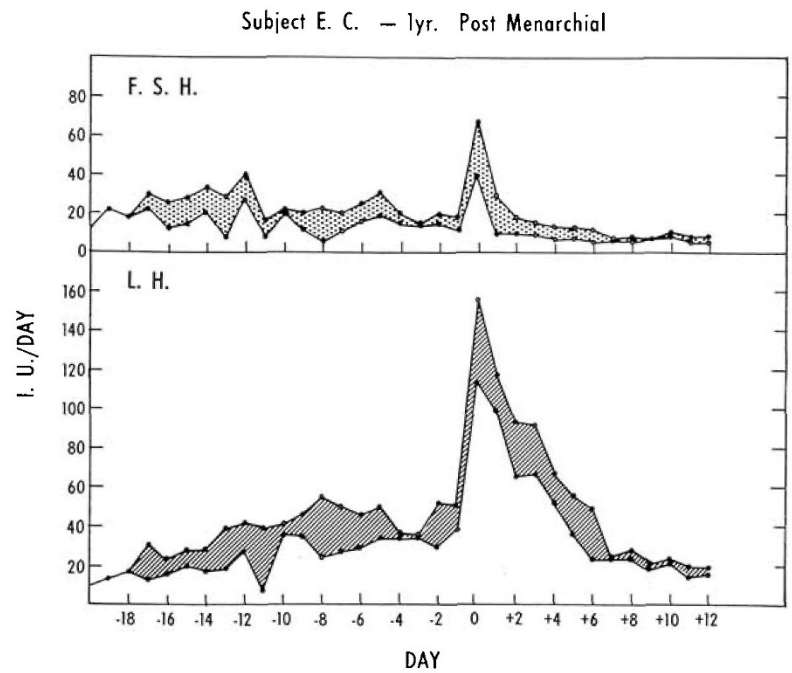

Fig. 4. Urinary FSH and LH during three menstrual cycles of a girl 1 year postmenarchial.

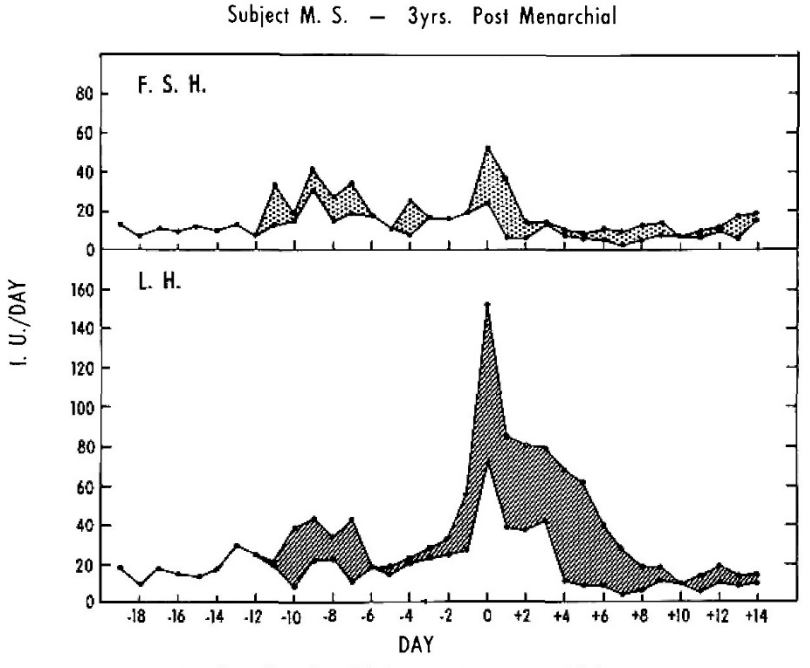

Fig. 5. Urinary FSH and LH during three menstrual cycles of a girl 3 years postmenarchial.

Table II. Follicular and luteal phase FSH and LH levels over a 3-month period[1]

Subject	FSH levels, IU/24 hr			LH levels, IU/24 hr			Follicular phase, days			Luteal phase, days		
	Follicular	Luteal	*P*	Follicular	Luteal	*P*	*Cycle 1*	*Cycle 2*	*Cycle 3*	*Cycle 1*	*Cycle 2*	*Cycle 3*
TM	14.9 ± 7.0 SD			15.5 ± 7.1 SD								
MM	11.3 ± 6.4 SD	8.3 ± 4.5	< 0.025	17.3 ± 8.8	11.9 ± 5.9 (15.9 ± 9.0)	< 0.02	18	17	19	10	12	9
GW	14.2 ± 4.4	10.0 ± 7.0	< 0.01	11.9 ± 3.2	8.8 ± 3.4 (22.2 ± 14.3)	< 0.02	16	15	14	6	9	9
EC	19.2 ± 7.0	10.5 ± 5.1	< 0.001	31.4 ± 11.7	21.5 ± 4.1 (49.9 ± 34.7)	< 0.001	20	17	16	12	12	12
MS	17.3 ± 8.5	11.2 ± 7.4	< 0.005	22.6 ± 11.4	12.7 ± 5.4 (28.6 ± 30.5)	< 0.001		25	15	15	14	12

[1] Values represent average ± SD. Luteal phase LH levels exclude 5 days after midcyclic peaks; values including those days appear in parentheses.

Discussion

Inasmuch as it has been only within the last few years that assays sensitive enough to measure gonadotropins in the prepubertal and pubertal years have been used, many of the hormonal events occurring during those periods are currently being unraveled. In that puberty is a process ending in the ability to reproduce [10], ovulation sometime during that period in the female is tacitly implied. Yussman and Taymor [37] have shown that midcycle peak levels of gonadotropins in serum of adult women correlate with ovulation as documented by recovery of the corpus luteum. Their data indicate that mean LH and FSH levels in serum show significant elevations 24 hr before ovulation.

Previous studies [16, 23, 24, 28] have shown the correlation between FSH and LH values in serum of boys and girls and their stage of sexual development. It has further been demonstrated that the rise in urinary excretion of FSH and LH, as measured by the techniques employed in this work, correlates favorably with the progressing stage of sexual development in males [2, 25]. In this study an attempt has been made to uncover the patterns of gonadotropin excretion in relation to the menarche.

A significant observation in this study was the finding of occasional simultaneous FSH and LH spikes in a premenarchial subject. The values were quantitatively as great as adult midcyclic peaks [1], although LH was in the very low normal adult range.

Intermittent spurts of gonadotropin excretion in

prepubertal children have recently been observed by Rifkind *et al.* [26]. Our data demonstrate the occurrence of such spikes in the late premenarchial years. Because direct visualization of the ovary or subsequent pregnancy is necessary for the unequivocal demonstration of ovulation, it is impossible to say whether such peaks in the pubertal female are ovulatory or anovulatory. One may conjecture that these premenarchial spikes serve as primary stimuli to the previously unresponsive ovaries and precede the gonadotropin surges that subsequently result in ovulation.

Similarity in urinary FSH and LH levels in the premenarchial girl and the early postmenarchial girls was found. This is in accord with the similarity in LH values in serum for these two groups [36]. Such a finding indicates that there is a time lag from the attainment of adult level gonadotropins to the achievement of normal adult urinary gonadotropin excretion patterns, as observed in Figure 5, perhaps correlating with fertility.

In all 4 postmenarchial girls, regular midcyclic peaks were noted to occur in 12 of 12 cycles studied. Whether these were ovulatory peaks remains unknown. Other corroborative evidence, such as BBT or pregnanediol levels, was not obtained in this study. One of the major differences among these cycles was the length of the luteal phase.

In the six cycles of *MM* and *GW* (1 month and 1 year postmenarchial, respectively), there were five with luteal phases of 10 days or less. These girls also differed from *EC* and *MS* (1 and 3 years postmenarchial, respectively) in having midcycle LH peak levels which were less than half those of *EC* and *MS*. *MS*, 3 years postmenarchial, had cycles which resembled those of mature adult women in every way. The association of short luteal phase with infertility has frequently been made, and recently was related to decreased FSH/LH ratios in adult women [33]. Our data certainly do not show this association when midcyclic peak level ratios are compared. In fact, the reverse is true.

In the four postmenarchial girls, urinary gonadotropin excretion patterns were consistent with those previously reported for adult women as determined by immunoassay [1, 6, 15]. There was similar comparability when bioassay was used [29]. The late luteal rise in FSH reported in adult women by Cargille *et al.* [6], however, was noted only in the 3-year postmenarchial subject. Early follicular FSH elevations were seen in three of four postmenarchial subjects, and prolongations in the fall of LH following the midcyclic surge were seen in all four subjects. A previous report [1] also noted this prolongation in the urine of adult women. Further studies on metabolic and renal clearance of gonadotropins must be done to establish the significance of this finding.

Summary

Immunoassays for urinary FSH and LH excretion over a 3-month period in five pubertal girls have led to the following conclusions: (*a*) simultaneous FSH and LH peaks can be seen before the menarche; (*b*) menstrual periods, even in the early (1–12-month) postmenarchial girl, are preceded by a simultaneous midcyclic surge in both FSH and LH; (*c*) after the menarche, gonadotropin excretion patterns show qualitative and quantitative changes resembling adult levels; and (*d*) there would seem to be a continuum in the development of the adult pattern of gonadotropin excretion.

References and Notes

1. BAGHDASSARIAN, A., FISHER, S., GUYDA, H., JOHANSON, A., FOLEY, T., PENNY, R., AND BLIZZARD, R. M.: Immunoreactive luteinizing hormone (LH) in acetone precipitates and kaolin extracts of human urine. Amer. J. Obstet. Gynecol., *108:* 1178 (1970).
2. BAGHDASSARIAN, A., GUYDA, H., JOHANSON, A., MIGEON, C. J., AND BLIZZARD, R. M.: Urinary excretion of radioimmunoassayable luteinizing hormone (LH) in normal male children and adults, according to age and stage of sexual development. J. Clin. Endocrinol. Metab., *30:* 428 (1970).
3. BENJAMIN, F.: Basal body temperature recordings in gynecology and obstetrics. J. Obstet. Gynaecol. Brit. Commonw., *67:* 177 (1960).
4. BURR, I. M., GRANT, D. B., SIZONENKO, P. C., KAPLAN, S. L., AND GRUMBACH, M. M.: Some critical factors in double antibody radioimmunoassay systems utilizing sheep anti-rabbit precipitating sera for measurement of human serum LH, FSH, and HGH. J. Clin. Endocrinol. Metab., *29:* 948 (1969).
5. CARGILLE, C. M., AND RODBARD, D.: Methodological studies of a 24 hour radioimmunoassay for human follicle stimulating hormone (Abstract). Endocrine Society, Program of 49th Meeting, Bal Harbour, Florida, June 15–17, 1967, p. 111.
6. CARGILLE, C. M., ROSS, G. T., AND YOSHIMI, T.: Daily variations in plasma follicle stimulating hormone, luteinizing hormone and progesterone in the normal menstrual cycle. J. Clin. Endocrinol. Metab., *29:* 12 (1969).
7. COOPERMAN, N. R.: The relationship of adolescent menstruation to body temperature and sterility. Amer. J. Obstet. Gynecol., *57:* 701 (1949).
8. DEWHURST, C. J.: Variations in physical signs in pubertal girls. J. Obstet. Gynaecol. Brit. Commonw., *76:* 831 (1969).
9. DIEM, K., ed.: Documenta Geigy: Scientific Tables, ed. 6, p. 178. (Geigy Pharmaceuticals, Ardsley, N. Y., 1962).
10. DONOVAN, B. T., AND VAN DER WERFF TEN BOSCH, J. J.: Physiology of Puberty, p. 1. (Edward Arnold, London, 1965).

11. Doring, G. K.: Uber die relative sterilitat in den jahren nach der menarche. Geburtsh. Frauenheilk., *23:* 30 (1963).
12. Faiman, C., and Ryan, R. J.: Radioimmunoassay for human follicle stimulating hormone. J. Clin. Endocrinol. Metab., *27:* 444 (1967).
13. Faiman, C., Ryan, R. J., and Albert, A.: FSH and LH activity in urinary extracts: a comparison of radioimmunoassay and bioassay methods. J. Clin. Endocrinol. Metab., *28:* 1076 (1968).
14. Finney, D. J.: Statistical Methods in Biological Assay, ed. 2. (Charles Griffin, London, 1964).
15. Goebelsmann, U., Midgley, A. R., Jr., and Jaffe, B.: Regulation of human gonadotropins. VII. Daily individual estrogens, pregnanediol and serum luteinizing and follicle stimulating hormones during the menstrual cycle. J. Clin. Endocrinol. Metab., *29:* 1222 (1969).
16. Johanson, A. J., Guyda, H, Light C., Migeon, C. J., and Blizzard, R. M.: Serum luteinizing hormone by radioimmunoassay in normal children. J. Pediat., *74:* 416 (1969).
17. Marshall, W. A., and Tanner, J. M.: Variations in pattern of pubertal changes in girls. Arch. Dis. Childhood, *44:* 291 (1969).
18. Midgley, A. R., Jr.: Immunofluorescent localization of human pituitary luteinizing hormone. Exp. Cell Res., *32:* 606 (1963).
19. Midgley, A. R., Jr.: Radioimmunoassay: a method for human chorionic gonadotropin and human luteinizing hormone. Endocrinology, *79:* 10 (1966).
20. Midgley, A. R., Jr.: Radioimmunoassay for human follicle stimulating hormone. J. Clin. Endocrinol. Metab., *27:* 295 (1967).
21. Nelson, W. E., Vaughn, V. C., and McKay, R. J.: Textbook of Pediatrics, ed. 9, p. 45. (Saunders, Philadelphia, 1969).
22. Novak, E.: Recent advances in the physiology of menstruation. Can menstruation occur without ovulation? J. Amer. Med. Ass., *94:* 833 (1930).
23. Penny, R., Baghdassarian, A., Guyda, H. J., Johanson, A., and Blizzard, R. M.: Correlation of serum follicle stimulating hormone (FSH) and luteinizing hormone (LH) as measured by radioimmunoassay in disorders of sexual development. J. Clin. Invest, *49:* 1847 (1970).
24. Raiti, S., Johanson, A., Light, C., Migeon, C. J., and Blizzard, R. M.: Measurement of immunologically reactive follicle stimulating hormone in serum of normal male children and adults. Metabolism, *18:* 234 (1969).
25. Raiti, S., Light, C., and Blizzard, R. M.: Urinary follicle-stimulating hormone excretion in boys and adult males as measured by radioimmunoassay. J. Clin. Endocrinol. Metab., *29:* 884 (1969).
26. Rifkind, A. B., Kulin, H. E., Rayford, P. L., Cargille, C. M., and Ross, G. T.: 24-hour urinary luteinizing hormone (LH) and follicle stimulating hormone (FSH) excretion in normal children. J. Clin. Endocrinol. Metab., *31:* 517 (1970).
27. Rodbard, D., Rayford, P. L., Cooper, J. A., and Ross, G. T.: Statistical quality control of radioimmunoassays. J. Clin. Endocrinol. Metab., *28:* 1412 (1968).
28. Root, A. W., Moshang, T., Jr., Bongiovanni, A. M., and Eberlein, W. R.: Concentrations of plasma luteinizing hormone in infants, children, and adolescents with normal and abnormal gonadal function. Pediat. Res., *4:* 175 (1970).
29. Rosemberg, E., and Keller, P. J.: Studies on the urinary excretion of follicle stimulating and luteinizing hormone activity during the menstrual cycle. J. Clin. Endocrinol. Metab., *25:* 1262 (1965).
30. Schalch, D. S., Parlow, A. F., Boon, R. C., and Reichlin, S.: Measurement of human luteinizing hormone in plasma by radioimmunoassay. J. Clin. Invest., *47:* 665 (1968).
31. Speck, G.: The determination of the time of ovulation. Obstet. Gynecol. Survey, *14:* 798 (1959).
32. Stevens, V. C.: Comparison of FSH and LH patterns in plasma, urine and urinary extracts during the menstrual cycle. J. Clin. Endocrinol. Metab., *29:* 904 (1969).
33. Strott, C. A., Cargille, C. M., Ross, G. T., and Lipsett, M. B.: The short luteal phase. J. Clin. Endocrinol. Metab., *30:* 246 (1970).
34. Tanner, J. M.: Growth at Adolescence, p. 29, p. 114. (Blackwell, Oxford, 1962).
35. Wide, L., Roos, P., and Gemzell, C.: Immunological determination of human pituitary luteinizing hormone (LH). Acta Endocrinol., *37:* 445 (1961).
36. Yen, S. S. C., Vicic, W. J., and Kearchner, D. V.: Gonadotropin levels in puberty. I. Serum luteinizing hormone. J. Clin. Endocrinol. Metab., *29:* 382 (1969).
37. Yussman, M. A., and Taymor, M. L.: Serum levels of follicle stimulating hormones and luteinizing hormone and of plasma progesterone related to ovulation by corpus luteum biopsy. J. Clin. Endocrinol. Metab., *30:* 396 (1970).
38. Prepared by Dr. Leo Reichert and provided by the National Pituitary Agency and the National Institute of Arthritis and Metabolic Disease.
39. Prepared by Dr. Rees Midgley.
40. Prepared by Dr. Rees Midgley and provided by the National Pituitary Agency..
41. Provided by Dr. D. R. Bangham, Medical Research Council, Mill Hill, London, England.
42. Mrs. Edith Vann contributed technical assistance. Statistical advice was provided by Dr. Phillip Allen. Mrs. Sarah Hamlin provided secretarial assistance.
43. We are indebted to the staff of Children and Youth Project 613 for their invaluable assistance.
44. Mr. Hayes was a second year medical student fulfilling requirements for the degree of Doctor of Medicine during this study.
45. Supported in part by Grant no. RR-304 from the General Clinical Research Centers Program of the Division of Research Resources, National Institutes of Health.
46. Requests for reprints should be addressed to: A. J. Johanson, M.D., Department of Pediatrics, University of Virginia Medical School, Charlottesville, Va. 22901 (USA).
47. Accepted for publication March 29, 1971.

 Printed in U.S.A.