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Bone age
development
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gonadal dysgenesis
hypopituitarism
luteinizing hormone
menstruation
sexual maturation

Hormonal Changes in Puberty
II. Correlation of Serum Luteinizing Hormone and Follicle Stimulating Hormone With Stages of Puberty and Bone Age in Normal Girls

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Extract

The concentration of serum luteinizing hormone (LH) and follicle stimulating hormone (FSH) was determined using a double antibody radioimmunoassay in 129 normal girls. Of this group, 48 were prepubertal (P_1) and 81 girls were at varying stages of pubertal development from P_2 to P_5. The mean serum LH concentration was similar in the prepubertal (P_1) and nonmenstruating girls (P_{2-4}) (3.8–4.2 ng/ml) (table I, fig. 1). A difference was observed in the serum FSH levels that increased steadily from 1.70 ng/ml at P_1 to 3.4 ng/ml at stage P_3 of puberty. No further change in the concentration of serum FSH was seen in girls from P_3 to P_4. The LH/FSH ratio was lower in girls at stage P_2 than in the prepubertal girls.

In the menstruating girls (P_5), mean concentration of serum LH was within the same range (5.1–4.6 ng/ml) during the three phases of the menstrual cycle. Changes in the concentration of serum FSH was observed in menstruating girls: phase I (days 1–12), 3.99 ng/ml; phase II (days 13–18), 3.59 ng/ml; and phase III (days 19–30), 2.51 ng/ml.

The relation of the serum LH and FSH levels and LH/FSH ratio to chronologic age was studied (table III). The concentration of LH was not significantly different in girls of 8–12 years. Serum FSH increased from 9 to 13 years. The LH/FSH ratio decreased at 9 years of age with no further change from 10 to 15 years. In addition, serum LH and FSH levels and LH/FSH ratio were compared with bone age (table IV). Serum LH was slightly but significantly less in girls with a bone age of 8 years than in those with a bone age of 6 years. A significant increase in FSH and LH levels was observed in girls with a bone age of 12 years compared with those with a bone age of 8 years.

In 14 children with multiple pituitary hormone deficiencies, the serum LH concentration was 2.2 ng/ml and the serum FSH concentration was 1.5 ng/ml. In 12 patients with XO gonadal dysgenesis of pubertal age, the mean concentration of serum LH was 11.2 ng/ml and mean concentration of serum FSH was 54.5 ng/ml. In two patients with XO gonadal dysgenesis, aged 6.5 and 9 years, the serum LH values were 2.5 and 4.5 ng/ml, and the serum FSH values were 1.5 and 2.75 ng/ml, respectively.

The serum values described are of relative not absolute significance. Although the results indicate that small amounts of gonadotropins are secreted prior to the onset of signs of puberty, it is important to emphasize the necessity of better defining the lower limits of sensitivity of the assays for serum samples.

Speculation

The detection of serum FSH and LH in prepubertal children suggests that the hypothalamic-pituitary gonadal axis is at a low level of activity and already operative at this stage of development. The precise time of initiation of cyclical secretion of LH and FSH (the FSH and LH ovulatory surge) in pubertal girls and its relation to maturational changes in the ovary is not known but it seems likely that the increase in estrogen secretion by the ovary as puberty advances acts as a positive feedback signal.

Introduction

Changes in pituitary gonadotropin and gonadal secretions associated with the initiation of puberty have been the subject of considerable investigation. Quantitative data correlating the secretion of these hormones with the physiologic changes of puberty, however, are either not available or scanty in nature. Early reports in which bioassay techniques were utilized for the assay of urinary gonadotropins [4, 10, 16, 20, 25], although inconclusive, provided some evidence for the secretion of gonadotropins in prepubertal children.

Recently, the advent of sensitive radioimmunoassays has enabled the estimation of gonadotropins in the biological fluids of children [2, 17, 21, 34, 35, 38]. The accompanying report [6] describes a double antibody radioimmunoassay technique for the estimations of serum luteinizing hormone (LH) and gives values for serum gonadotropins in normal boys. In the present report, the radioimmunoassay method used for the estimation of follicle stimulating hormone (FSH) is described and measurement of serum LH and FSH levels in 129 normal girls are correlated with chronological and bone age and stages of pubertal development. For comparison, serum gonadotropin levels were evaluated in 14 children with hypopituitary dwarfism and in another 14 with gonadal dysgenesis.

Materials and Methods

Subjects

One-hundred and twenty-nine normal girls, ranging in age from 5 to 16 years, were examined during a routine pediatric multiphasic screening evaluation at the Kaiser-Permanente Foundation Outpatient Clinic. The stage of pubertal development of each patient was evaluated clinically. The assessment was based on skin texture, presence of acne and body odor, the amount and texture of pubic and axillary hair, and development of breasts, nipples, areolae, mons, labia, and vaginal mucosa.

Sexual maturity was scored for five stages (P_1–P_5), utilizing a classification modified from TANNER [55] and YOUNG *et al.* [57]: P_1, no clinical signs of puberty; P_2, budding of breasts, a few pubic or axillary hairs, or both, minimal change in the vaginal mucosa, and early development of the labia majora and minora; P_3, increased fullness of breasts, projection of areola and nipples, presence of Montgomery follicles, a small amount of pubic and axillary hair, moderate enlargement of labia majora and minora, dullness of the vaginal mucosa; P_4, breasts and external genitals well developed, moderate to abundant pubic and axillary hair, no menarche; and P_5, postmenarchial girls; the date of menarche and phase (phase I, days 1–12; phase II, days 13–18; phase III, days 19–30) of the last menstrual period were recorded.

Fourteen patients, ranging in age from 5 to 21 years, with multiple pituitary hormone deficiencies and 14 others with the syndrome of XO gonadal dysgenesis, examined in the University of California, San Francisco Medical Center Pediatric Endocrine Clinic, were used for comparison. The presence of multiple hormone deficiencies was assessed by procedures described previously [23] and the diagnosis of XO gonadal dysgenesis was confirmed in each case by chromosomal analysis.

Bone age was evaluated from films of the wrist and hand, using the standards of GREULICH and PYLE [26]. These procedures were a routine part of the multiphasic screening evaluation. Informed consent was obtained from all subjects in this study.

Radioimmunoassay for FSH

Human pituitary FSH LER-869-2 [58] was used as the standard and for iodination, the FSH activity of which is 2,782 IU/mg 2nd IRP-HMG [59] by bioassay. The biological activity of HLH LER-960 [58] is 923 IU/mg 2nd IRP-HMG [59] of LH activity. Human thyroid stimulating hormone (TSH), with a potency of 10–20 IU/mg was obtained through the courtesy of Dr. R. BATES and Dr. J. G. PIERCE. Rabbit antisera to human FSH, absorbed with 500 IU of HCG/ml [60] was used for the radioimmunoassay of FSH. Antiserum to human chorionic gonadotropin (HCG) was prepared as described [6]. Sheep anti-rabbit gamma globulin serum was raised, using rabbit gamma globulin (Pentex Fraction II) in complete Freund's adjuvant. The diluent was 0.01 M phosphate buffer with 0.15 M sodium chloride (PBS) containing 1% bovine serum albumin (BSA) and 0.01 M EDTA, pH 7.8.

Iodination of the FSH was performed using a modification [30] of the method of GREENWOOD *et al.* [24].

Two micrograms of FSH LER-869-2 were iodinated with 1 mCi of carrier-free ^{131}I [61]. To this was added 0.01 ml of chloramine T (352 mg/100 ml) and 0.040 ml of sodium metabisulfite (240 mg/100 ml) used sequentially to catalyze and to stop the reaction; 0.025 ml of fresh frozen human serum was added at the end of the procedure. The procedure for the iodination of LH is described in a companion paper [6].

The iodination mixture containing free ^{131}I and labeled FSH was applied to a 1-cm by 15-cm column of Sephadex G-75, previously equilibrated with PBS and BSA, and 0.5-ml eluates were collected in 0.1 ml of 5% BSA-PBS solution. Aliquots were counted for the determination of radioactivity peaks.

Iodination 'damage' in eluates, comprising the first radioactivity peak after passage through Sephadex G-75, was evaluated by chromatoelectrophoresis of 0.1-ml aliquots on Chromomedia DE 20 (DEAE) paper strips (at 4° in 0.01 M phosphate buffer, pH 7.8, at 450 v for 150 min). Some aliquots of the labeled FSH were subjected to electrophoresis on acrylamide gel slabs (5 cm by 12 cm) at 4° for 2 h at 30 mA in 0.011 M boric acid, 0.0032 M disodium EDTA, and 0.077 M tris (hydroxymethyl) amino methane-1,3-propanediol (Tris) buffer at pH 8.9 (BET) [35]. Gel sections were counted; the section containing the radioactive peak was eluted by electrodialysis at 110 v at 4° for 2 h in BET. The degree of binding of the ^{131}I-FSH obtained after Sephadex separation to anti-FSH serum and its proportionate displacement from anti-FSH by unlabeled FSH did not differ significantly from that of the ^{131}I-FSH obtained by gel electrophoresis and subsequent dialysis.

To determine the efficiency of iodination, an aliquot of the original iodination mixture was diluted in PBS, 0.1 ml of which was mixed with 0.02 ml of normal human serum (NHS) and bromophenol blue and applied to Whatman no. 3 MC paper strips for electrophoresis at room temperature in a chromatoelectrophoretic chamber, using 0.75 M barbital buffer, pH 8.6, at 450 v for 90 min. Radioactivity peaks were analyzed, using an automatic scanner with integrator. The efficiency of iodination ranged from 35 to 75% and the specific activity of the labeled FSH from 175 to 374 μCi/μg. Labeled FSH kept frozen for 3 weeks had the same binding characteristics and identical elution patterns on both Sephadex G-75 and G-200 columns as the freshly labeled material.

In each assay sample, tubes were arranged as follows: *a)* total count tubes, to which were added 0.1 ml of labeled FSH (containing 10,000 cpm or 0.025–0.05 ng as ^{131}I-FSH); *b)* tracer control tubes, containing 0.1 ml ^{131}I-FSH in buffer BSA-PBS plus similar control tubes to which was added 0.02–0.2 ml of the serum sample to be tested; *c)* tracer and antibody tubes, containing ^{131}I-FSH and FSH antiserum (0.1 ml of 1:8,000); *d)* standard curve tubes, containing known amounts of unlabeled hormone (from 0.1 to 5.0 ng) with ^{131}I-FSH and antibody; and *e)* unknown sample tubes in which 0.02–0.2 ml of the sample of human serum was added to the tracer FSH and the antibody.

Recovery of unlabeled FSH in this system was determined by adding known amounts of LER-869-2 to NHS and to serial dilutions of postmenopausal serum. The final volume was adjusted to 1 ml in all tubes by the addition of buffer. At the end of the first incubation of 5 days at 4°, 0.05 ml of normal rabbit serum (1:50) and 0.05 ml of sheep anti-rabbit gamma globulin were added to all but the total count tubes. To the tubes containing the standards, 0.2 ml of NHS was added and the total volume of human serum in the remaining tubes adjusted to 0.2 ml. After 24 h, the tubes were centrifuged, the supernatant was discarded and the precipitate counted. Background and control tube counts were subtracted from the average of the duplicates. The 'corrected' counts in the precipitates were expressed as a percentage of the counts in the total count tubes.

In this system, anti-FSH antiserum (1:80,000 dilution) bound 30–40% of tracer after incubation for 5 days. The rabbit antiserum did not cross-react with either HLH LER-960 or with HCG. Human TSH displaced ^{131}I-FSH from the antibody only when it exceeded 50 ng (500–1,000 μU). The FSH LER-869-2 had an immunological activity equivalent to 1,770 IU/mg of 2nd IRP-HMG (mean of four determinations).

As previously reported [5], human serum enhances the degree of precipitation of ^{131}I-FSH in the presence or absence of EDTA. Dose-response curves of postmenopausal serum were identical to those of the pituitary standard only when equal concentrations of human serum were present in all tubes throughout the second incubation.

The sensitivity of the assay was estimated to be 0.2 ng/sample (1 ng/ml of serum). Duplicate differences were less than 2% of the total radioactivity added. Recoveries were 90–112% (mean 97%). Reproducibility was 82–109% (mean value of six assays of postmenopausal serum was 27.4±2.39 SD).

Radioimmunoassay for LH

The LH assay was performed in a similar manner [6]. Human LH LER-960, which by immunoassay had a potency of 2,200–2,400 IU/mg 2nd IRP-HMG, was used as the standard and for iodination. The sensitivity of the assay allowed estimation of 0.1 ng of LH/sample (mean value of six assays of postmenopausal serum was 20 ng/ml±1.5 SD).

Statistical analyses were performed by Student's *t* test or Wilcoxon rank test, or both.

Results

Of the 129 normal girls, 48 presented without clinical signs of puberty (P_1), age range: 5–12 years; 28 showed early signs of puberty (P_2), age range: 7.25–12.5 years; 17 were in stage P_3 of pubertal development, age range: 8.25–13 years; 7 were in stage P_4, age range: 11–13 years; and 29 were in stage P_5, mean age of menarche for this group was 12.5 (range: 10–13 years).

Serum LH and FSH concentrations and LH/FSH ratios for each stage of pubertal development to P_4 are indicated in figure 1 and table I. In girls in stages P_1–P_4, the mean LH concentrations were not significantly different from group to group. In contrast, serum FSH levels rose steadily through puberty, from P_1 to P_2 and from P_2 to P_3. The mean serum concentration of FSH of the girls in stages P_3 and P_4 did not differ significantly. A significant fall in the LH/FSH ratio was observed between P_1 and P_2 without any significant change between stages P_2 and P_4.

The mean serum LH concentrations of menstruating girls (table II) in the three phases were comparable: the range of LH values was 5.13–4.6 ng/ml. The mean serum FSH level for girls in phase I was 3.99 ng/ml, in phase II, 3.59 ng/ml, and in phase III, 2.51 ng/ml. The range of FSH levels throughout the three phases was 1–6 ng/ml.

Serum LH and FSH concentrations and the LH/FSH ratios are shown in relation to chronological age in table III. The serum gonadotropin concentration

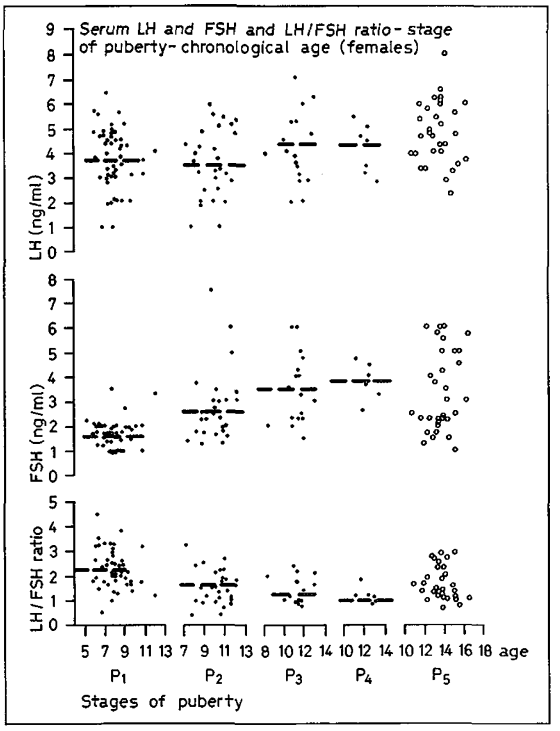

Fig. 1. Comparison of LH (ng/ml), FSH (ng/ml), and LH/FSH ratio in normal girls prepuberty (P_1) and at different stages of puberty (P_2–P_5). The mean for each group except P_5 is indicated by the dashed lines.

Table I. Mean concentrations of LH and FSH in serum and LH/FSH ratio at different stages of pubertal development in 100 nonmenstruating girls

	Stages of puberty	P_1	P_2	P_3	P_4
	N[1]	48	28	17	7
LH	Mean conc, ng/ml	3.81	3.45	4.33	4.20
	SD[2]	1.23	1.39	1.36	0.93
	SEM[3]	0.18	0.26	0.33	0.35
FSH	Mean conc, ng/ml	1.70	2.56	3.40	3.80
	SD	0.47	1.07	1.22	0.67
	SEM	0.06	0.20	0.29	0.26
	P[4]	<— (<0.01) —>			
	P		<— (<0.05) —>		
LH/FSH ratio	$\bar{x}$[5]	2.30	1.53	1.38	1.30
	SD	0.81	0.71	0.51	0.36
	SEM	0.12	0.13	0.12	0.14
	P	<— (<0.001) —>			

[1] N = number of girls in each group.
[2] SD = standard deviation.
[3] SEM = standard error of the mean.
[4] P = probability estimation; the arrows delineate the groups compared.
[5] $\bar{x}$ = mean.

Table II. Concentrations of LH and FSH in serum and LH/FSH ratio in 29 menstruating girls (P_5)

No.	Phase I[1]				Phase II[1]				Phase III[1]			
	Day	LH	FSH	LH/FSH	Day	LH	FSH	LH/FSH	Day	LH	FSH	LH/FSH
1	1	4.0	2.5	1.6	14	5.7	5.0	1.14	19	4.1	1.7	2.41
2	1	4.4	6.0	0.73	14	8.1	3.0	2.7	20	6.0	5.75	1.04
3	2	6.7	3.5	1.91	14	6.2	6.0	1.03	22	5.8	2.25	2.58
4	4	6.1	2.2	2.8	16	3.3	2.5	1.32	23	2.9	1.0	2.9
5	4	6.0	5.0	1.2	16	5.2	2.3	2.26	24	5.5	2.25	2.5
6	4	4.7	3.75	1.25	17	6.2	5.75	1.08	25	7.6	5.5	1.38
7	4	6.0	6.0	1.0	17	4.9	4.0	1.25	26	3.4	1.3	2.62
8	5	5.4	2.25	2.4	17	4.0	1.5	2.67	26	4.9	2.1	2.3
9	9	3.6	4.5	0.8	18	4.1	2.25	1.82	26	2.4	1.5	1.6
10	11	4.4	4.25	1.03					26	3.4	1.75	1.93
$\bar{x}$, ng/ml[2]		5.13	3.99	1.47		5.3	3.59	1.70		4.6	2.51	2.13
SD[3]		0.99	1.35	0.66		1.37	1.56	0.65		1.55	1.60	0.58
SEM[4]		0.31	0.43	0.21		0.45	0.52	0.21		0.49	0.51	0.18

[1] The cycle has been divided into three phases: phase I, 1–12 days; phase II, 13–18 days; phase III, 19–30 days.
[2] $\bar{x}$ = mean concentration.
[3] SD = standard deviation.
[4] SEM = standard error of the mean.

Table III. Mean concentrations of LH and FSH in serum and LH/FSH ratio at different chronological ages in 100 girls

	Chronological age, yr	5	6	7	8	9	10[1]	11[1]	12[1]	13[1]
	N[2]	1	7	8	22	14	12	19	14	3
LH	Mean conc, ng/ml	5.0	4.87	3.7	3.39	3.67	3.36	3.88	4.07	4.65
	SD[3]		1.11	1.34	1.26	1.16	0.98	1.64	1.09	
	SEM[4]		0.42	0.47	0.27	0.31	0.28	0.38	0.29	
	P[5]		<— (<0.05) —>							
FSH	Mean conc, ng/ml	2.25	1.85	1.66	1.70	1.79	2.54	3.07	3.58	3.10
	SD		0.28	0.38	0.6	0.51	0.84	1.40	0.98	
	SEM		0.11	0.14	0.13	0.14	0.24	0.32	0.26	
	P					<(<0.025)>				
	P					<—(<0.005)—>				
LH/FSH ratio	$\bar{x}$[6]	2.2	2.76	2.22	2.09	2.21	1.41	1.55	1.20	1.53
	SD		0.94	0.83	0.75	0.93	0.44	0.66	0.33	
	SEM		0.36	0.29	0.16	0.25	0.13	0.15	0.09	
	P					<(<0.05)>				
	P					<— (<0.05) —>				
	P					<—— (<0.01) ——>				

[1] Nonmenstruating girls.
[2] N = number of girls.
[3] SD = standard deviation.
[4] SEM = standard error of the mean.
[5] *P* = probability estimation; the arrows delineate the groups compared.
[6] $\bar{x}$ = mean.

of menstruating girls was omitted in the values given for age 10–13 years. The fall in the serum LH concentrations observed between the ages of 6–8 years was not associated with any significant change in serum FSH levels or in LH/FSH ratio. Concentrations of LH did not change significantly from age 8 to 12 years, although there was a tendency for a rise from the age of 10 to 13 years. The rise of serum FSH from 9 to 13 years preceded any increase in LH by 1 year. The LH/FSH ratio fell from 9 to 10 years of age and remained constant from 10 years on.

Serum LH and FSH concentrations and LH/FSH ratios are shown relative to bone age in table IV. There was a decrease in LH levels between 6 and 8 years ($P < 0.05$). The mean serum LH concentration for girls with a bone age of 9 years was lower than in those with a bone age of 12 years ($P < 0.05$); during this period there was a significant increase in the mean FSH concentration. The LH/FSH ratio fell between the bone ages of 10 and 11 years ($P < 0.05$) then remained constant from 11 to 12 years, reflecting the rise in LH concentrations which occurred in association with the continued rise in FSH concentrations.

The serum LH values obtained in a normal adult woman during two menstrual cycles ranged from 6.0 to 6.6 ng/ml during the follicular phase, and from 5.5 to 7.6 ng/ml in the luteal phase; midcycle peaks were 19.7 and 28.2 ng/ml. The corresponding values for FSH were 1.75–3.25 and 2.0–2.5 ng/ml, with peaks of 5.5 and 7.6 ng/ml.

The mean serum concentrations in the 14 children with multiple pituitary hormone deficiencies were LH, 2.2 ng/ml (±1.11 SD, ±0.24 SEM) and FSH, 1.53 ng/ml (±0.66 SD, ±0.14 SEM). Of the patients with XO gonadal dysgenesis, the mean serum LH concentration in 12 of pubertal age was 11.2 ng/ml (±1.2 SEM) and the FSH concentration was 54.5 ng/ml (±8.6 SEM, range: 18.5–105); in two patients aged 6.5 and 9 years, serum LH values were 2.5 and 4.5 ng/ml; FSH values were 1.5 and 2.75 ng/ml, respectively.

Discussion

The use of a radioimmunoassay technique with sensitivity, specificity, and reproducibility has permitted an assessment of changes in serum gonadotropin levels during puberty in normal girls. Nevertheless, the limitations of quantifying serum FSH and LH by radioimmunoassay, and of cross-sectional design in contrast

Table IV. Concentrations of LH and FSH in serum and LH/FSH ratio at different bone ages in 100 girls

	Bone age, yr	4	5	6	7	8	9	10[1]	11[1]	12[1]	13[1]
	N[2]	2	3	5	8	17	17	14	15	16	3
LH	Mean conc, ng/ml	5.3	4.7	4.66	3.8	3.3	3.4	3.84	3.59	4.36	4.33
	SD[3]			0.6	1.13	1.19	1.19	1.14	1.46	1.11	
	SEM[4]			0.27	0.40	0.30	0.29	0.30	0.38	0.28	
	P			<— (<0.05) —>			<— (<0.05) —>				
FSH	Mean conc, ng/ml	2.0	1.95	1.8	1.64	1.58	1.8	2.23	2.96	3.95	3.33
	SD			0.43	0.39	0.62	0.37	0.76	0.93	1.36	
	SEM			0.19	0.14	0.16	0.09	0.20	0.20	0.34	
	P						<(<0.01)>				
	P							<(<0.01)>			
	P								<(<0.05)>		
LH/FSH ratio	$\bar{X}$[6]	2.74	2.44	2.79	2.36	2.22	1.98	1.79	1.24	1.34	1.31
	SD			0.98	0.55	0.94	0.74	0.57	0.58	0.44	
	SEM			0.44	0.19	0.23	0.18	0.15	0.15	0.11	
	P				<——— (<0.005) ———			>			
	P							<(<0.05)>			
	P							<— (0.05) —>			

[1] Nonmenstruating girls.
[2] N = number of girls.
[3] SD = standard deviation.
[4] SEM = standard error of the mean.
[5] *P* = probability estimation; the arrows delineate the groups compared.
[6] $\bar{X}$ = mean.

to longitudinal data, are important to appreciate [6].

The observed difference between the biological activity of the pituitary FSH standard LER-869-2 (116 U/mg NIH FSH S-1, i.e., 3,000 IU/mg 2nd IRP-HMG) and the immunological equivalence (1,770 IU/mg 2nd IRP-HMG) is consistent with the previously described discrepancies betweeen biological and immunological potency when using a urinary reference standard [1, 13, 19]. These discrepancies, together with the use of different gonadotropin standards by different groups, limit the number of direct quantitative comparisons that can be made. Indirect comparisons, however, may be made by the use of appropriate conversion factors [1].

The mean serum LH concentration in prepubertal girls observed in this study was equivalent to that reported by ODELL *et al.* [39] and SAXENA *et al.* [49], but was higher than that observed by SCHALCH *et al.* [52] and ROOT *et al.* [45]. Serum FSH concentrations are in accord with those of FAIMAN and RYAN [17] and SCHALCH *et al.* [50] but less than those reported by MIDGLEY [35] and SAXENA *et al.* [49].

In those girls in whom menses had commenced, the LH values observed during phases I and III were comparable to those observed by most investigators in follicular and luteal phases of normal adult women [9, 18, 22, 39, 46, 50] but were higher than those reported by SCHALCH *et al.* [51]. Serum FSH concentrations in phases I and III were comparable to those previously reported [18, 22, 46, 51] but lower than those observed by some investigators [37, 49]. Similar with findings in adult women, the mean concentration of serum FSH was higher in phase I ('follicular' phase) than in phase III ('luteal' phase).

No ovulatory LH peak was recorded in the girls in group P_5; several high values of serum FSH were observed in all three menstrual phases. These values were in the same range as the ovulatory peak values of FSH obtained in the normal women and comparable to reported ovulatory peak values [18, 22, 46], but lower than those reported by SAXENA *et al.* [49] and SCHALCH *et al.* [51]. These high FSH values did not always coincide with the estimated time of 'ovulation'. The apparent absence of ovulatory LH peaks in the presence of temporally random FSH peaks in this group may be a consequence of random sampling or, when considered in the light of the wide range of LH and FSH values, may reflect the occurrence of anovulatory cycling [11, 33]. Although the present data suggest differences in the pattern of FSH and LH secretion in early menarchal girls and mature women, longitudinal sampling of a group of menarchal girls is necessary to assess the significance of this finding.

In our study, there was a general tendency for serum LH and FSH levels to rise with increases in chronological and bone age and with progression of puberty. These findings are consistent with previously reported increases in urinary gonadotropin levels with age, as determined by bioassay [4, 20, 32, 44] and with the elevation of gonadotropin content and concentration in pituitary glands of 10- to 26-day-old female rats [27, 31].

The temporal sequence of changes in serum gonadotropin concentration and in secondary sexual characteristics, when matched against chronological age, was similar to the pattern obtained when matched against bone age. As anticipated [10, 55], however, there seemed to be better correlation of these variables with bone age. This is supported by the observation that, despite the similarity between the mean bone age of the girls in stage P_2 of puberty and the mean chronological age, the range in the bone age was less.

The possibility that some cycling of LH levels occurred in stages P_2–P_4 is suggested by the wide scatter of LH levels observed during these stages of development. Though this may be a reflection of individual variation and the cross-sectional design of the study, some support for the existence of premenarchial ovarian cyclic activity is provided by the nature of the urinary estrogen excretion pattern in prepubertal girls 1.5 years prior to the onset of menses [36].

The sequential changes in serum gonadotropins may be summarized as follows: There appears to be a fall in serum LH concentration (age 8 years), 2.5 years prior to the onset of puberty. One year later, there is a rise in serum FSH levels which continues to the age of 12 years. During this period, the serum LH levels may fluctuate but do not achieve a significant increase in mean levels until the age of 11 years, at which time both FSH and LH levels are in the adult range.

When the changes in serum gonadotropin concentrations with maturation in the girls are compared with those observed in boys [6], the initial sequence of events is remarkably similar though the changes in boys lag approximately 1 year behind those in girls. In contrast to the girls, however, there is a significant rise in mean LH levels at 10 years of age, associated with a slight increase in testicular size and an increase in urinary 17-ketosteroid excretion [15]. This increase in serum LH levels in boys persists to the age of 16 years, by which time there is a tendency for the concentration of FSH to fall.

The patients with multiple pituitary hormone deficiencies had lower serum LH concentrations than normal prepubertal girls. These values were in good agreement with the reports of SAXENA *et al.* [49] and ROOT *et al.* [45]. The mean serum FSH concentrations in children with idiopathic hypopituitarism were not significantly different from those in prepubertal girls but were lower than those observed by SAXENA *et al.*

[49] and comparable to those of FAIMAN and RYAN [17]. The similarity in serum gonadotropin values between prepubertal and hypopituitary subjects again raises the question of defining the sensitivity of the assay for serum samples [6].

Our patients of pubertal age with gonadal dysgenesis showed high levels of serum LH and FSH [22, 45, 46]. The low levels of serum LH and FSH found in two prepubertal cases of gonadal dysgenesis were similar to the findings of SCHALCH *et al.* [51] for LH, but were in contrast to the reports of ODELL *et al.* [39] and ROOT *et al.* [45].

The detection of gonadotropins in the serum of prepubertal children is consistent with the presence of gonadotropic activity in the urine [20, 32, 44] and in the pituitary glands of children [3, 47, 56] and of immature animals [28, 29, 31]. This finding, and the changes in gonadotropin secretion during puberty described in this report, does not shed light on the mechanism responsible for the initiation of puberty. The observations, however, are consistent with current hypotheses [8, 12, 14, 41–43, 53], adduced from studies in the rat, that there is a decrease in sensitivity of the hypothalamic 'gonadostat' to the inhibitory effects of gonadal secretions [7, 41–43, 48, 53], and LH [40] prior to the appearance of secondary sexual characteristics.

Additionally, the present data do not clarify the mechanism by which a sex difference in the secretion of gonadotropins is effected. Gonadotropin secretion in women is cyclical in character and is characterized by a midcycle (ovulatory) surge in LH and FSH secretion [9, 34, 35]; in the mature male, the secretion of FSH and LH [35, 37, 39, 49, 52] is relatively constant and does not exhibit this cyclical pattern. In the rat, testosterone administration prior to puberty induces a male pattern of gonadotropin secretion and suppresses the inherent cyclical secretion of the female [42, 54]. Clinical evidence, however, does not support the primary role of testosterone in this process in the human organism.

Summary

The serum concentration of LH and FSH were determined in each of 129 normal girls (5–16 years) using a double-antibody radioimmunoassay method. The girls were grouped according to their stage of pubertal development, chronological, and bone ages. Concentrations of FSH and LH were detected in serum before the appearance of signs of puberty. An increase in serum FSH was found as the signs of puberty advanced. The changes in serum LH between the prepubertal and pubertal nonmenstruating girls were less striking.

In the menstruating girls, serum LH was not significantly different during the first half and second half of the cycle, whereas the concentration of serum FSH was higher in the first half of the cycle. The use of cross-sectional data may have masked midcycle peaks for FSH and LH.

The serum LH was 2.2 ng/ml and serum FSH was 1.5 ng/ml in 14 children with multiple pituitary hormone deficiencies; these values were utilized to establish the lower limit of sensitivity of the assay. In 12 patients with XO gonadal dysgenesis of pubertal age, the mean serum LH was 11.2 ng/ml and mean serum FSH was 54.5 ng/ml. The serum LH and serum FSH in two patients with XO gonadal dysgenesis, aged 6.5 and 9 years, was comparable to that observed in normal prepubertal girls.

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