Hormonal Changes in Puberty
I. Correlation of Serum Luteinizing Hormone and Follicle Stimulating Hormone with Stages of Puberty, Testicular Size, and Bone Age in Normal Boys

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Extract

The concentration of luteinizing hormone (LH) and follicle stimulating hormone (FSH) in serum was determined using a double antibody radioimmunoassay method in each of 106 normal boys aged 5–16 years. The boys were grouped according to the stage of puberty (P_1-P_5) using the classification of Tanner.

The serum LH and FSH, LH/FSH ratio, and testicular volume index (TVI) for boys prepuberty (P_1) and at different stages of puberty (P_2-P_5) are shown in figure 1 and table I. The mean value of LH in prepubertal boys was 4.2 ng/ml, which steadily rose to a concentration of 5.3 ng/ml at P_{4-5}. The differences between the values for LH in early puberty (P_2) and at a more advanced stage of puberty (P_{4-5}) were statistically significant. The mean value for serum FSH in the prepubertal boy was 1.4 ng/ml. Serum FSH rose steadily through the early stages of puberty (P_1-P_3) with no further rise from P_3 to P_{4-5}. These changes were reflected in the LH/FSH ratio which fell between P_1 and P_2, but increased between P_2 and P_4. Testicular size (TVI) showed a gradual increase from P_1 through P_{4-5}.

A comparison of serum LH and FSH, LH/FSH ratio, and TVI with chronological age is indicated in table II. A slight increase in the mean levels of serum LH and FSH was observed from age 9 to age 12 with a continued rise to 15 years and older. Serum LH seemed to rise at an earlier age than serum FSH. The TVI showed a definite increase from chronologic age 8 through 11. A pronounced change in testicular size from age 11 through 15 was observed during the period of sharp rise in serum FSH.

The correlation of bone age with concentrations of LH and FSH in serum, LH/FSH ratio, and TVI is shown in table III. A significant rise in LH was seen in boys with a bone age of 9–10 years, with a further increase at a bone age of 13–15 years and older. Serum FSH showed a steady increment from a bone age of 11 to 14 years. The most pronounced increase in TVI was seen at a bone age of 11 through 15 years.

The relation of concentrations of LH and FSH in serum and LH/FSH ratio to the TVI is presented in table IV. Mean LH and FSH showed a gradual rise from a TVI of < 2 to 10.1–12. Serum LH rose and serum FSH fell, however, with the increase in TVI from 10.1–12 to 12.1–16.

In four patients with chromatin-positive seminiferous tubular dysgenesis (Klinefelter's syndrome), the mean serum LH concentration was 11.3 ng/ml and the mean serum FSH concentration was 21.6 ng/ml.

The mean serum LH in 14 patients with multiple pituitary hormone deficiencies was 2.1 ng/ml and the mean serum FSH was 1.5 ng/ml. The small differences in the concentration of LH and FSH in prepubertal boys compared with that observed in children with hypopituitarism stress the need for further definition of the sensitivity of the assay for serum samples. The serum values reported are of relative not absolute significance.

Speculation

The detection of serum FSH and LH prior to puberty, and the increase in serum FSH and LH coincident with advancing puberty suggests *1)* that the hypothalamic-pituitary-testicular axis is functional before puberty, and *2)* that the onset of puberty may be associated with a change in sensitivity of the hypothalamus to feedback by testicular steroids.

Introduction

Until recently, determination of gonadotropins in human biological fluids was limited to the use of bioassay procedures of variable specificity, precision, and sensitivity. Even with improvements in methodology [1, 10, 34], few estimates of plasma gonadotropins by bioassay are available, and only in subjects with high plasma concentrations or utilizing large plasma pools [2, 23, 28]. The development of methods for isolation and purification of human pituitary luteinizing hormone (LH) and follicle stimulating hormone (FSH) [36, 37, 41, 43] and the discovery of highly sensitive radioimmunoassay methods for quantifying polypeptide hormones [6] has led to the adaptation of radioimmunoassay techniques for the determination of LH and FSH in serum and urine [4, 12, 13, 24, 29, 30, 33, 42, 44, 50]. Only fragmentary data, however, are available on the values for serum gonadotropins in prepubertal and pubertal children [12, 20, 33, 35, 39, 42, 44].

In this report, serum FSH and LH were measured by radioimmunoassay in 106 normal boys, aged 5–16 years, in an attempt to define the changes in gonadotropin secretion that occur with sexual maturation. The values for serum FSH and LH were correlated with chronologic and bone age, testicular size, and with clinical assessment of sexual maturation. For purposes of comparison with the values obtained in normal subjects, sera from 14 hypopituitary patients and 4 patients with chromatin-positive seminiferous tubular dysgenesis syndrome were assayed for both LH and FSH.

Materials and Methods

Subjects

One-hundred and six normal boys, aged 5–16 years, who attended the Kaiser Foundation Outpatient Clinic for a routine annual multiphasic screening examination were the subjects of this study; they were examined for signs of pubertal development. This was assessed by skin texture, presence of acne, body odor, the amount, texture, and distribution of facial, axillary, and pubic hair, voice change, and penile and testicular size.

Testicular size was determined on all but one patient by measuring the length and width of each testis. An assessment of testicular volume was made by multiplying the testicular length (centimeter) by breadth, adding the products from each side and dividing by two; this value was termed the 'testicular volume index' (TVI). The boys were assigned to one of five grades of sexual development, based on a modification of the criteria of TANNER [48]: P_1, prepubertal, infantile in development; P_2, pubertal, with evidence of slightly increased testicular size and minimal scrotal or pubic hair; P_3, pubertal, with further increase in testicular and phallic size, more pubic and some axillary or facial hair, or both; P_4, pubertal, with moderate pubic, axillary, and facial hair, voice change, acne, or adult body odor, or both; and P_5, adult body habitus, hair distribution, and genitalia.

Bone age was determined on all but three patients from radiographs of the wrist, using the standards of GREULICH and PYLE [17].

Blood was drawn from all patients for the measurement of serum LH and FSH. In addition, sera from 14 children, from 5 to 21 years, all with proven multiple pituitary hormone deficiencies [15], and 4 patients, aged 15–20 years, with chromatin-positive seminiferous tubular dysgenesis syndrome (confirmed by karyotype analysis) were assayed for gonadotropin levels. These procedures were a routine part of the multiphasic screening evaluation. Informed consent was obtained from all subjects in this study.

Radioimmunoassay of LH

Luteinizing hormone was assayed by a double antibody [32] radioimmunoassay technique. Antisera to human chorionic gonadotropin (HCG), 10,000 IU/vial [52], were raised in white New Zealand rabbits by subcutaneous injection of 1,000 IU emulsified with complete Freund's adjuvant every 10–14 days. When a suitable titer of anti-HCG was obtained booster

injections, also of 1,000 IU, were given at 2- to 4-week intervals. Antisera that bound approximately 40% of labeled tracer LH, while allowing displacement of tracer by small quantities of unlabeled LH, were selected for use in the assay. Antisera were absorbed with a kaolin extract of urine from a hypopituitary infant and with human FSH, 100 ng/ml of 1:100 antisera [53]. This concentration of FSH did not displace labeled LH from the absorbed anti-HCG when added to the assay system. Human thyroid stimulating hormone (TSH), however, displaced labeled LH from the anti-HCG in the assay system. This displacement could be accounted for by a small contamination of the TSH by LH, and minor cross-reaction [8]. The binding characteristics of the antiserum to HCG were similar to those of an antiserum to HCG distributed by the National Pituitary Agency [54].

A purified preparation of human LH, LER-960 [55], was used as a standard and for iodination. This preparation had a potency by bioassay of 923 IU/mg 2nd IRP [56], 1 ng of which by immunoassay was the equivalent of 2.2–2.4 mIU 2nd IRP.

Luteinizing hormone was iodinated by a modification [21] of the method of HUNTER and GREENWOOD [19]. Five micrograms of LH were added to 0.025 ml of 0.5 M phosphate buffer, pH 7.5, followed by 2 mCi of 'carrier-free' ^{131}I-Na [57] of high specific activity (300 mCi/ml); then, in rapid order, 0.02 ml of chloramine T (24 mg/10 ml), 0.08 ml of sodium metabisulfite (35.2 mg/10 ml), and 0.05 ml of human serum were added. The efficiency of the iodination procedure was monitored by chromatoelectrophoresis of an aliquot of the iodination mixture on Whatman no. 3 MC in phosphate buffer, pH 7.8 at 4° (the labeled protein remains at the origin and the free ^{131}I moves toward the anode). The mean efficiency for 12 iodinations was 70%±5 (SD). Labeled hormone was separated from free iodide by passing the iodination mixture through a 10-cm by 1-cm G-75 Sephadex column equilibrated with 0.01 M phosphate buffer, pH 7.8, and bovine serum albumin. Aliquots of 0.5 ml were collected and peak counts were obtained between the 6th and 8th tubes (protein peak) and 13th and 14th tubes (iodide peak). Aliquots of the protein peak were checked for 'damage' by paper chromatoelectrophoresis on Whatman no. 3 MC in 0.01 M phosphate buffer, pH 7.8 at 4°. 'Damage' estimated by this procedure was consistently less than 10%. The aliquot containing the least damaged ^{131}I-LH was used in the assay procedure. The specific activity of the ^{131}I-LH varied from 200 to 300 µCi/µg. When ^{131}I-LH from the first Sephadex peak was passed through a 10-cm by 1-cm G-75 column and a 30-cm by 1-cm G-200 column immediately after iodination, and again after 5 and 10 days storage at —20°, single peaks of radioactivity were ob-

tained (suggesting that significant deiodination or polymerization had not occurred).

Each assay was performed within 2 days of iodination. Assays were run in two stages: 1) ^{131}I-LH (10,000 counts equivalent to 0.0125–0.025 ng LH) was incubated with unlabeled LH, 0.05–5.0 ng (standards) or 0.1 ml of serum and 0.1 ml of 1:20,000 rabbit anti-HCG. The diluent, 0.01 M phosphate –0.15 M saline buffer, containing 0.01 M EDTA and 1% BSA, corrected to pH 7.8, was added to make a total volume of 1 ml. Control tubes were set up with each assay, with omission of unlabeled hormone in one set and anti-HCG serum and unlabeled hormone in another. No nonspecific binding of labeled hormone was observed in any assay. Control tubes were also set up for each serum sample omitting the first antibody; 2) After an incubation period of 5 days, sheep anti-rabbit gamma globulin, 0.05 ml, and 1:50 normal rabbit serum (NRS), 0.05 ml, were added to all tubes, including controls and standards. Normal human serum (NHS), 0.1 ml, was added to the standards and to standard control tubes and 0.1 ml buffer to the remainder. The final volume in all tubes was 1.2 ml.

Normal human serum was added to standards to produce identical binding conditions in standards and samples. Previously reported data [7] from this laboratory indicated that NHS augmented the degree of precipitation in some sheep anti-rabbit gamma globulin systems, an effect independent of the presence of EDTA, heparin, NRS, or of the buffer used. The LH present in the NHS, added to the precipitating (second) antibody, did not produce displacement of the tracer from the first antibody.

Under the above assay conditions, the binding of ^{131}I-LH in the absence of unlabeled hormone ranged from 35–45% with significant displacement of ^{131}I-LH by 0.1 ng of unlabeled hormone. Difference between duplicates over the range of 20–45% binding was 2%; the mean value for six assays of a postmenopausal serum was 20 ng/ml±1.5 (SD) (LER-960). The mean recovery of unlabeled hormone added to the assay system in the presence of serum was 104%±9 (SD).

Radioimmunoassay of FSH

The FSH assay was conducted in a similar manner and full details are given in the accompanying paper [46]. Human FSH, LER-869-2 [55] (1 ng, was equivalent to 1.79 mIU, 2nd IRP by immunoassay; 2,782 IU/mg 2nd IRP by bioassay) was used for iodination and standards. Rabbit antiserum to human FSH [58] (Midgley D33-7) and sheep anti-rabbit gamma globulin were utilized. The sensitivity of this assay allowed estimation of 0.2 ng of FSH; 0.2-ml aliquots of serum were assayed when maximum sensitivity was required. Mean difference between duplicates was 2%, and the

mean value of postmenopausal serum pool was 27.4 ng/ml$\pm$2.4 (SD) over six assays; mean recovery of unsabeled FSH when added to systems containing human lerum was 97% (range: 90–112).

Standards and antisera for both assays were stored in small aliquots at —20°. There was no deterioration of standards over a 6-month period under these conditions or any alteration in the binding characteristics of the antisera [7].

Statistical analyses of the data were made by the Wilcoxon rank test and confirmed by Student's t test when applicable.

Results

The number of boys in each stage of puberty and the values obtained for concentrations of LH and FSH in serum, the LH/FSH ratio, and testicular volume index (TVI) in each stage of pubertal development are shown in table I and figure 1. The mean chronological age and mean bone age for boys in stage P_2 was $11^6/_{12}$ years (range: $9–14^3/_{12}$ chronological age; $8^5/_{12}–13$ years bone age). The LH levels rose steadily through the three stages of puberty from a prepubertal mean value of 4.2 ng/ml. The differences between values for

P_1 and P_3, and P_2 and P_{4-5} were statistically significant. The mean prepubertal serum FSH concentration of 1.4 ng/ml rose steadily through the first two stages of puberty. Increases from P_1 to P_2, and P_2 to P_3 were *statistically significant. Unlike the LH levels, FSH* levels did not continue to rise from P_3 to P_{4-5}. The LH/FSH ratio fell from a prepubertal mean of 3.3 to a mean of 1.9 in P_3, corresponding to the rise in FSH levels. From P_3 to P_{4-5} the ratio tended to rise, reflecting a continued increase in serum LH concentration without a corresponding increase in FSH concentration. Testicular size increased steadily from P_1' to P_{4-5}.

The serum LH and FSH concentrations, LH/FSH ratio, and TVI are shown in relation to chronological age in table II. There was some fluctuation in the mean levels of LH and FSH from age 5 to 9, and a slight but statistically significant increase from age 9 to 12, with a continuing rise to 15 years and older. Mean serum FSH concentrations rose from 9 to 11 years. There was a demonstrable rise in both LH and FSH serum concentrations prior to the mean age at which the earliest clinical signs of puberty could be recognized. There was suggestive evidence of an increase in the concentration of serum LH prior to that of serum FSH.

Table I. Mean concentrations of LH and FSH in serum, LH/FSH ratio, and testicular volume index at different stages of puberty in 106 boys

	Stages of puberty N[1]	P_1 58	P_2 21	P_3 14	P_{4-5} 13
LH	Mean conc, ng/ml	4.19	4.42	4.82	5.32
	SD[2]	0.78	0.51	0.91	0.67
	SEM[3]	0.10	0.11	0.24	0.19
	P[4]	<——— (<0.05) ———>			
	P		<——— (<0.01) ———>		
FSH	Mean conc, ng/ml	1.36	1.93	2.93	2.69
	SD	0.37	0.53	1.14	1.13
	SEM	0.05	0.12	0.31	0.31
	P	<—— (<0.001) ——>			
	P		<—— (<0.025) ——>		
LH/FSH ratio	Mean	3.26	2.43	1.94	2.29
	SD	0.86	0.61	0.91	0.93
	SEM	0.11	0.13	0.24	0.25
	P	<—— (<0.001) ——>			
Testicular volume	Mean	2.77[5]	4.80	8.99	13.16
index	SD	0.62	1.71	2.12	2.09
	SEM	0.08	0.37	0.57	0.58
	P	<—— (<0.001) ——>		<—— (<0.05) ——>	

[1] N = number of boys 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] Testes were not measured in one boy.

The mean LH/FSH ratio decreased between 8 and 13 years of age, followed by a rise over the next 2-3 years, which is attributable to a proportionately greater increase in the LH values. The TVI increased from age 8 through 11 with a sharp increase in size from chronological age 11 through age 15, coinciding with the major rise in serum FSH.

The serum LH and FSH concentrations, LH/FSH ratio, and TVI are compared with bone age in table III. The mean serum LH concentration for boys with bone age of 9 years ($8^7/_{12}$–$9^6/_{12}$) was less than that for the groups with bone ages of 8 or of 10 years. There

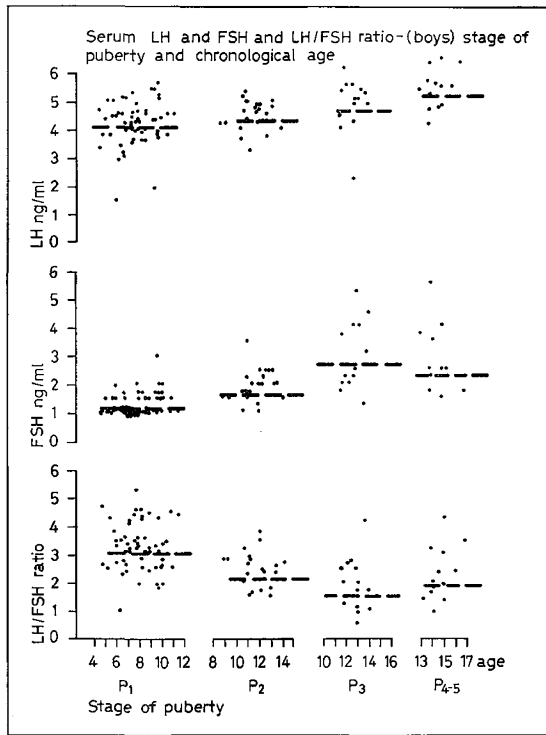Serum LH and FSH and LH/FSH ratio-(boys) stage of puberty and chronological age

Fig. 1. Comparison of LH (ng/ml), FSH (ng/ml), and LH/FSH ratio in 106 normal boys prepuberty (P_1) and at different stages of puberty (P_2–P_5). The mean for each group is indicated by the dashed lines.

[1] The ages of patients in each age group range from 5 months less to 6 months greater than the indicated age.
[2] N = number of boys.
[3] SD = standard deviation.
[4] SEM = standard error of the mean.
[5] P = probability estimation; the arrows delineate the groups compared.
[6] Significant fall in values.
[7] Testes were not measured in one boy.

Table II. Mean concentrations of LH and FSH in serum, LH/FSH ratio, and testicular volume index at different chronological ages in 106 boys

Chronological age, yr[1]	5	6	7	8	9	10	11	12	13	14	15–16
N[2]	4	6	11	15	9	12	11	11	11	9	7
LH											
Mean conc, ng/ml	4.03	4.02	3.97	4.27	4.08	4.42	4.44	4.8	4.55	4.99	5.46
SD[3]	0.53	1.20	0.76	0.45	0.33	1.0	0.7	0.58	0.90	0.66	0.63
SEM[4]	0.27	0.49	0.23	0.11	0.11	0.29	0.21	0.17	0.27	0.22	0.24
P[5]			⟵(<0.01)⟶							$<(<0.05)$⟶	
FSH											
Mean conc, ng/ml	1.18	1.40	1.27	1.24	1.30	1.67	1.77	2.09	3.11	2.86	2.25
SD	0.21	0.28	0.20	0.32	0.20	0.51	0.64	0.64	0.68	1.34	0.80
SEM	0.11	0.11	0.06	0.08	0.07	0.18	0.19	0.19	0.21	0.45	0.30
P				⟵($<0.025^6$)⟶		⟵(<0.005)⟶	⟵($<0.001^6$)⟶		$<(<0.01)$⟶		
LH/FSH ratio											
Mean	3.53	2.95	3.15	3.63	3.20	2.76	2.76	2.46	1.68	2.24	2.70
SD	0.97	0.99	0.61	0.89	0.56	0.72	0.93	0.68	0.61	0.71	0.92
SEM	0.49	0.40	0.18	0.23	0.19	0.21	0.28	0.21	0.18	0.24	0.35
P					⟵($<0.025^6$)⟶			$<(<0.01)$⟶	⟵($<0.05^6$)⟶		
Testicular volume index											
Mean	2.01	2.50	2.65	2.70^7	3.18	2.74	3.4	7.22	8.39	10.17	14.21
SD	0.05	0.34	0.58	0.50	0.71	0.57	0.67	2.21	2.67	3.01	1.55
SEM	0.025	0.14	0.17	0.13	0.24	0.16	0.20	0.67	0.81	1.00	0.59
P					⟵(<0.025)⟶		⟵(<0.001)⟶		⟵(<0.001)⟶		

Table III. Mean concentrations of LH and FSH in serum, LH/FSH ratio, and testicular volume index at different bone ages in 103 boys

	Bone age, yr[1]	5	6	7	8	9	10	11	12	13	14	15–16
	N[2]	8	10	10	10	7	12	10	9	16	4	7
LH	Mean conc, ng/ml	4.12	3.97	4.27	4.25	3.67	4.64	4.65	4.57	4.59	4.9	5.46
	SD[3]	0.77	1.00	0.73	0.42	0.78	0.58	0.53	0.85	0.95	0.78	0.49
	SEM[4]	0.27	0.32	0.23	0.13	0.29	0.17	0.17	0.28	0.23	0.39	0.19
	P[5]				$< (<0.01^6) >$							
	P					$< (<0.01) >$					$<\!\!-\!\!- (<0.05) -\!\!-\!\!>$	
FSH	Mean conc, ng/ml	1.25	1.29	1.37	1.35	1.31	1.64	1.5	1.97	3.08	3.09	2.46
	SD	0.18	0.27	0.59	0.32	0.36	0.64	0.27	0.28	1.22	0.75	0.93
	SEM	0.06	0.08	0.19	0.10	0.14	0.18	0.08	0.09	0.31	0.36	0.35
	P						$<\!\!-\!\!- (<0.01) -\!\!-\!\!>$					
	P								$< (<0.01) >$			
LH/FSH ratio	Mean	3.36	3.21	3.39	3.30	2.95	2.99	3.19	2.35	1.75	1.83	2.67
	SD	0.89	1.02	0.84	0.76	0.93	0.72	0.68	0.28	0.86	0.15	0.98
	SEM	0.31	0.32	0.27	0.24	0.35	0.21	0.22	0.09	0.22	0.08	0.37
	P							$<\!\!-\!\!- (<0.01^6) -\!\!-\!\!>$			$< (<0.05) >$	
Testicular volume index	Mean	2.3	2.93	2.71[7]	3.08	2.64	3.15	3.54	6.31	8.23	13.5	12.88
	SD	0.38	0.54	0.48	0.59	0.47	1.02	0.94	1.89	2.70	1.54	2.42
	SEM	0.13	0.17	0.16	0.19	0.18	0.29	0.30	0.63	0.66	0.77	0.91
	P	$<\!\!-\!\!- (<0.05) -\!\!-\!\!>$				$<\!\!-\!\!- (<0.05) -\!\!-\!\!>$			$<\!\!-\!\!- (<0.01) -\!\!-\!\!>$			
	P								$<\!\!-\!\!- (<0.01) -\!\!-\!\!>$			

[1] The ages of patients in each age group range from 5 months less to 6 months greater than the indicated age.
[2] N = number of boys.
[3] SD = standard deviation.
[4] SEM = standard error of the mean.
[5] P = probability estimation; the arrows delineate the groups compared.
[6] Significant fall in values.
[7] Testes were not measured in one boy.

was a significant rise in LH in boys with a bone age of 9–10 years, with a plateau to bone age 13, and a further rise from bone age 13 to 15 years and older. The mean serum FSH concentration rose gradually from a bone age of 11 to 14 years. Both LH and FSH concentrations in serum rose at a lower bone age than the mean bone age of the boys who exhibited early signs of puberty (P_2) on examination.

The LH/FSH ratio fell from bone age 11 to 13 years, then rose between 14 and 15 years of age. The TVI rose slightly from bone age 9 through 11, with a major increase from 11 through 15 years.

Serum LH and FSH concentrations and the LH/FSH ratios are tabulated in relation to the TVI in table IV. Mean LH values increased gradually from a TVI <2 to 12.1–16. Values for FSH also increased gradually with changing TVI; however, unlike LH they fell as the TVI increased from 10.1–12 to 12.1–16. The LH/FSH ratio fell from a TVI of <2 to 10.1–12, then rose at the last stage from 10.1–12.0 to 12.1–16.

Values in Hypopituitarism and in Primary Gonadal Failure

The mean serum LH concentration in 22 determinations in the 14 patients with multiple pituitary hormone deficiencies was 2.1 ng/ml (sd 1.11; sem 0.24), and the mean serum FSH concentration was 1.53 ng/ml (sd 0.66; sem 0.14). The difference in LH values for the hypopituitary patients and the normal prepubertal boys (P_1) was of borderline statistical signifi-

cance ($P < 0.05$). There were no detectable differences in FSH values between these two groups. The mean serum LH concentration in the four patients with chromatin-positive seminiferous tubular dysgenesis (Klinefelter's syndrome) was 11.25 ng/ml (sd 2.89; sem 1.44) and the mean serum FSH concentration was 21.6 ng/ml (sd 4.35; sem 2.2).

Discussion

The serum values reported are of relative and not absolute significance; unresolved problems in the assay of serum FSH and LH impose limitations on the interpretation of data. Such factors as the purity and stability of the pituitary FSH and LH standard, possible differences in the immunochemical reactivity of serum and pituitary gonadotropins, the variance for low serum values, the 'floor' of the assay for samples of serum, the effects of different serum LH/FSH ratios, and the specificity of currently available anti-human FSH and LH sera have not been completely evaluated. These constraints are of special importance in interpreting the significance of prepubertal serum gonadotropin values. Further, the design of this study is cross-sectional.

Some of the factors affecting the double antibody radioimmunoassay technique for the assay of serum LH and FSH have been reported previously and the

Table IV. Mean concentrations of LH and FSH in serum and LH/FSH ratio at different stages of testicular development in 105 boys

	Testicular volume index	<2	2.1–4	4.1–6	6.1–8	8.1–10	10.1–12	12.1–16
	N[1]	10	55	11	5	6	12	6
LH	Mean conc, ng/ml	4.41	4.14	4.80	4.72	4.55	5.05	5.33
	sd[2]	0.63	0.73	0.38	0.44	1.18	0.72	0.75
	sem[3]	0.20	0.11	0.11	0.20	0.48	0.22	0.31
	P		<—(<0.025)—>		<————— (<0.025) —————>			
FSH	Mean conc, ng/ml	1.30	1.44	2.20	2.55	2.46	3.25	1.96
	sd	0.29	0.41	0.86	1.00	1.05	1.23	0.39
	sem	0.09	0.06	0.26	0.45	0.43	0.37	0.16
	P		<—(<0.025)—>				<—(<0.025[5])—>	
LH/FSH ratio	Mean	3.56	3.04	2.42	2.07	2.29	1.76	2.86
	sd	1.01	0.82	0.73	0.61	1.13	0.67	0.85
	sem	0.32	0.11	0.24	0.27	0.46	0.20	0.35
	P	<————(<0.01[5])————>					<—(<0.05)—>	

[1] N = number of boys.
[2] sd = standard deviation.
[3] sem = standard error of the mean.
[4] P = probability estimation; the arrows delineate the groups compared.
[5] Significant fall in values.

importance of correcting for the enhancement of pre-
cipitation due to human serum has been stressed [7].
No cross-reaction occurred between LH and FSH in
either assay system. Though it has been demonstrated
previously that antisera to HCG bind labeled TSH,
the degree of cross-reaction is insufficient to interfere
with the assay of LH in normal human serum [8].

In these systems, 0.1 ng of purified LH or 0.2 ng of
FSH in buffer can be measured; however, the sensitiv-
ity of the radioimmunoassay systems for the measure-
ment of serum LH and FSH may be different. This
problem, of special importance in the precise quantifi-
cation of low serum values, requires further study. The
lack of differences in serum FSH concentrations be-
tween hypopituitary dwarfs and prepubertal boys,
and the relatively small but significant differences in
the concentrations of serum LH, further stress the
need for defining the sensitivity of the assay for serum
samples.

The standard LH preparation used in these studies
had the immunologic equivalent of 2.2–2.4 mIU 2nd
IRP for 1 ng of LER-960. The levels reported are
similar to those described in prepubertal children by
ODELL et al. [33], SAXENA et al. [42], and ROOT et al.
[39] though higher than those obtained by JOHANSON
et al. [20] and SCHALCH et al. [44]. The values of FSH
reported here for prepubertal boys (1.4 ng of LER-
869-2 is equivalent to 2.5 mIU 2nd IRP-HMG) are
slightly lower than those reported by SAXENA et al. [42]
and RAITI et al. [35], but similar to those of SCHALCH
et al. [44] and FAIMAN and RYAN [12]. In the present
study, a significant rise has been documented in the
concentration of both serum LH and FSH from pre-
puberty through the first stages of puberty, the in-
crease in serum FSH correlating closely with the mark-
ed increase in testicular size observed during this
period.

The change in gonadotropin concentration with
increase in chronological and bone age is of particular
interest. In general, there was good correlation be-
tween the changes associated with increase in bone
age and with chronological age. It has been suggested
[11, 48] that bone age is more physiologically signifi-
cant and, therefore, the better correlate. When evaluat-
ing changes in serum gonadotropin concentration with
bone age, it was observed that LH levels began to rise
by 10 years then plateaued until age 13 (table III).
These changes were associated with an initial slight
increase in testicular growth. They preceded the first
significant rise in FSH levels, which occurred at 12
years and were associated with a rapid increase in
testicular size. The physiologic significance of the ob-
served drop in LH concentration at the bone age of
9 is questionable since a similar change was not detect-
ed at a comparable chronological age.

There was a general tendency for serum LH and
FSH levels to increase with bone and chronological
age—from the age of 10 to 11 years for LH and 12 years
for FSH. The age at which an increase in the concen-
tration of serum LH was observed was slightly earlier
than the mean age ($11^6/_{12}$ years) of the group considered
to be in the first stage of puberty. The increase observed
in serum LH and FSH levels with maturation are in
accord with those reported for human [5, 40, 51] and
animal [18, 25, 45] pituitary gonadotropin content,
and for excretion in human urine determined by bio-
assays [38]. The magnitude of the change in urinary
LH before and after the onset of puberty, however,
was considerably greater in the study of RIFKIND et al.
[38] than for the serum values in the present study.

The progressive increase in testicular size from a
chronological and bone age of 11 years was com-
parable to the values reported previously [11, 49].
The initial increase in testicular size correlates with
the initial increase in serum LH and FSH values
(table IV). The secondary and more marked increase
in testicular size noted from chronological age 12 years
($11^7/_{12}–12^6/_{12}$) onward correlates well with the greatest
interval increase in FSH concentration. This, together
with previous findings that an appreciable increase in
mitosis in the seminiferous tubules occurs at about
11 years of age [47] and that FSH stimulates semini-
ferous tubule development [14] and testicular size
[14, 16], suggests that the increase in FSH accompanies
the phase of rapid testicular growth. The observed in-
crease in LH concentration at this stage may facilitate
the action of FSH in producing an increase in testicular
size, particularly in view of the enhanced effect of
FSH on testicular size in the presence of LH under
experimental conditions [27]. This slight increase in
LH levels from bone age 9 to 10 years may also stimu-
late an initial [3, 9] small rise in testosterone secretion.

The greater rise in LH concentration from bone age
of 13 to 15 years and older, with a continued rapid in-
crease in both testicular size and FSH concentration,
corresponds temporally with the major increase in
17-ketosteroid [11] and testosterone excretion [3, 9].
A similar pattern of growth of the ventral prostate has
been observed in immature rats [22].

During the late phase of rapid testicular develop-
ment, levels of LH continue to rise while levels of FSH
fall, suggesting that maintenance of testicular size and
function requires less FSH than the maturation process.
Further, the enhanced LH and testosterone levels at
this time contribute to the continued development of
spermatogenesis, the initiation of which may require
a greater increase in the secretion of FSH [26].

The wide range in LH and FSH serum concentra-
tions during all stages of puberty and through all
chronological and bone ages suggests either marked

individual variation in gonadotropin levels or, alternately, some fluctuation in gonadotropin release. Although the role of adrenal androgens in male puberty is not completely understood, the findings in the present study suggest that the first rise in serum LH coincides with a sharp increase in adrenal androgen secretion [11]. Whether LH has a significant indirect or direct effect on the alteration of adrenocortical function at puberty, however, remains to be determined [31].

The high gonadotropin levels in the patients with chromatin-positive seminiferous tubular dysgenesis syndrome were consistent with the serum LH and FSH concentrations in other studies [39, 42].

The present data provide information concerning the sequential events that take place at puberty in the male. Further interpretation of these findings are presented in the accompanying paper [46].

Summary

The concentrations of LH and FSH in serum were determined in each of 106 normal boys (5–16 years of age) using a double antibody radioimmunoassay method. The boys were grouped according to their stage of pubertal development (P_1 through P_5), chronological and bone ages, and testicular size. The mean LH concentration in the prepubertal boys was 4.2 ng/ml, rising steadily with maturation (P_5) to 5.3 ng/ml; the corresponding FSH value in prepubertal boys was 1.4 ng/ml with a similar rise in early puberty to 2.9 ng/ml (P_3), with a tendency to plateau prior to the attainment of complete maturation (P_5).

When the gonadotropin concentrations were plotted against chronological and bone age the mean LH concentration rose from 4.1 ng/ml at age 9 to a mean concentration of 5.5 ng/ml at 15–16 years. Serum FSH levels rose steadily from age 9 (1.3 ng/ml) to the age of 13–14 years (3.1 ng/ml) then appeared to decrease slightly.

The mean serum LH and FSH values in 14 hypopituitary children were 2.1 and 1.5 ng/ml, respectively, and in 4 patients with chromatin-positive seminiferous tubule dysgenesis, 11.3 and 21.6 ng/ml.

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