Pituitary-Gonadal Relations in Male Children and Adolescents

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Extract

Data are provided on levels of circulating follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone in healthy male children and adolescents, and these levels are correlated with the stage of sexual maturation, bone maturation, whole body ^{40}K content, and the 24-hr excretion of creatinine, estrogens, 17-hydroxy-corticosteroids, total 17-ketosteroids, and fractionated 11-deoxy-17-ketosteroids (see Tables I and II).

Before age 6 there was no significant change with age in testis size or hormone concentrations. From age 6–10 years there was a gradual rise in testis size (Fig. 1), serum FSH (Fig. 4) and serum LH (Fig. 3), but no change in plasma testosterone (Fig. 2), which remained below 40 ng/100 ml. From age 11–17 years there was a doubling of mean testis length, continued rise in serum FSH, and a steeper rise in LH; these changes were accompanied by a 20-fold increase in plasma testosterone concentration, the appearance of male secondary sexual characteristics (Figure 5), more rapid bone maturation and whole body ^{40}K accretion, and enhanced excretion of estrogens and 11-deoxy-17-ketosteroids. Mean levels of FSH in the adult range (>10 $\mu g/100$ ml) were achieved by age 8, and levels reached a plateau by age 15 (Fig. 6). Adult LH levels (>2.5 $\mu g/100$ ml) were reached by age 13 and a plateau was observed after 17 years. Plasma testosterone also ceased to rise after age 17.

The data suggest that the testis may undergo progressive gonadotropin-mediated maturation in late childhood. Testosterone secretion by the Leydig cells at puberty may result from a further rise in LH levels.

Speculation

Pituitary secretion of gonadotropins, particularly FSH, in male children is a gradual phenomenon, antedating by several years the release of testosterone by the testis. This gradual rise may represent a progressive reduction in hypothalamic sensitivity to feedback by testicular steroids other than testosterone. In late childhood, rising FSH and LH levels may induce increased activity of the enzymes responsible for both synthesis and catabolism of testosterone. At puberty, an LH-mediated reduction in reductase activity might then result in testosterone accumulation and secretion.

Introduction

The present availability of sensitive and specific isotope displacement assays for circulating levels of go- nadotropins and steroid hormones in man permits a reevaluation of the pituitary and Leydig cell changes which initiate and maintain normal male sexual devel-

opment. Recent reports have demonstrated that concentrations of testosterone in plasma [2, 13, 14, 39, 50] and urine [15, 22, 29, 49] of boys are considerably lower than those found in adolescent and adult males. Furthermore, plasma testosterone concentrations appear not only to be low but also unchanging during childhood. With the initiation of somatic adolescence, there is a steep rise in testosterone levels, closely paralleling the observed pubertal changes [13]. Several authors have demonstrated measurable gonadotropin levels in serum [5, 18, 26, 53] and in urine [11, 24, 35, 43] of prepubertal males, but the relation of these levels to testosterone secretion has not been clearly eludicated.

Some authors [3, 43] have reported gradually rising excretion of luteinizing hormone (LH) in urine of males during childhood, with a more abrupt 2-fold increase at puberty. Buckler and Clayton [4] showed low LH excretion levels from age 3 months to 6 years, after which gradually rising levels were seen, with a further marked increase at puberty. Raiti et al. [35] found a progressive increase in follicle-stimulating hormone (FSH) excretion after age 5 years, with a further more abrupt pubertal rise. It appears, therefore, that excretion of gonadotropins in urine is rising in boys long before testosterone-mediated signs of puberty become apparent.

Excretion studies discount possible changes in the renal clearance of gonadotropins with age, and serum concentrations may provide a more valid reflection of secretion rates, particularly at low levels [3]. Various authors have used radioimmunoassay techniques to measure FSH and LH concentrations in serum of male children and adolescents [5, 21, 26, 31, 34, 36, 38, 40–42, 53, 54]. Although the absolute values from these studies are not strictly comparable because of different standards and antisera used, most authors agree that circulating gonadotropin levels are higher in pubertal than in prepubertal males, with, however, some overlap between the groups. Lee et al. [26] and Yen et al. [53, 54] suggest that serum FSH and LH levels do not change until puberty, at which time there is a 2- to 3-fold rise in the concentration of each. On the other hand, Johanson et al. [18] and Raiti et al. [34] suggest that levels begin to rise after age 9, with a steeper rise at puberty. Most authors agree that serum gonadotropin levels continue to rise throughout the various clinical stages of puberty, but only Wieland et al. (50) have correlated gonadotropin concentrations with levels of circulating testoterone. There is therefore some disagreement in the literature regarding the tim-

ing of the pituitary phenomena associated with puberty.

It is the purpose of this report to provide data on levels of circulating FSH, LH, and testosterone in healthy male children and adolescents, and to correlate these data with commonly used physical, radiologic, and laboratory parameters of growth and sexual development. The data support the concept that pituitary gonadotropin release increases several years before puberty. This finding raises various questions about the nature of the feedback systems operant in childhood and the mechanisms involved in initiating adolescence.

Materials and Methods

Subjects

The 253 subjects in the study included: 60 school student volunteers taking part in a longitudinal growth-research program; 21 university students, 41 residents of a training school for mildly retarded children; and 132 patients of the Children's Hospital. All were judged to be free of endocrine disease by history and physical examination. As there was no significant difference in the age-specific means for any variable among the four groups, the data have been pooled. Informed written consent was obtained from all subjects (when possible) as well as from their parents or guardians, or both.

Each subject's pubertal development was assessed from phallic length, mean testicular length, and the amount and distribution of pubic and axillary hair. Pubertal stages were assigned as: P_1: prepubertal in all respects, with longest testis diameter less than 2.4 cm; P_2: early testicular enlargement (diameter 2.4–3.2 cm), sometimes associated with sparse hair at the base of the penis; P_3: testis diameter 3.3–4.0 cm, obvious pubic hair, beginning phallic enlargement and possibly early but sparse axillary hair; P_4: testis diameter 4.1–4.5 cm, pubic hair of adult amount, moderate axillary hair; P_5: testis diameter greater than 4.5 cm and adult secondary sexual characteristics.

In the school-age subjects an estimate of lean body mass was obtained with the whole body ^{40}K counting facilities of the Whiteshell Nuclear Research Establishment. Each subject was counted in a lead, copper, and steel-lined room, for 3 10-min periods, with an 11.5-inch diameter, 4-inch thick sodium iodide crystal located 9.5 inches above the chest, hips, and thighs, respectively. The output from the photomultiplier tubes was recorded by a multichannel pulse-height analyzer

and the counts in the ^{40}K photo peak were integrated by computer. Because of conflicting data on the effects of body size and age on counting geometry and self-absorption, and the paucity of accurate information on lean body potassium concentration in children of different ages, we have presented the ^{40}K data as raw cpm. (Preliminary investigations with this scintillation counter suggest that counting efficiency is not constant with changing body size. However, if one assumes constant efficiency and lean body K concentration of 68.1 mEq/kg [12], an approximate lean body mass in kilograms can be obtained by dividing the ^{40}K cpm by 2.9.)

Skeletal maturation was determined in each subject from x-rays of both hands and wrists, using the standards of Tanner et al. [47].

Blood was drawn from all subjects between 10 AM and 1 PM on the examination day, and serum and plasma aliquots were frozen at −20° until analyzed. In addition, 78 subjects provided a 24-hr urine sample, which was also stored at −20° until analyzed.

Laboratory Methods

Serum LH and FSH levels were measured in duplicate by the double antibody radioimmunoassay techniques of Faiman and Ryan [9, 10], in sample volumes of 0.2 ml serum for adults and 0.4 ml for children. A crude human pituitary gonadotropin preparation, LER-907 [55], was used as the standard in both assays. (The FSH and LH data in this report (micrograms LER-907 per 100 ml) may be converted to milli-international units 2nd IRP-HMG per milliliter by multiplying the FSH value by 0.5 and the LH value by 4.5.) The minimum levels detectable in a 0.4-ml sample averaged 3.1 and 0.8 μg LER-907/100 ml for FSH and LH, respectively. Samples reading below the limit of sensitivity for LH were assigned a value of 0.8 μg/100 ml. The coefficient of variance for duplicate determinations at low serum concentrations at the time of the study were ± 6.3% for LH and ± 4.9% for FSH. A low value control serum pool was repetitively assayed over the course of 1 year; the mean values (± SD) were 2.9 ± 0.5 for LH and 11.0 ± 1.6 for FSH.

Plasma testosterone concentration was measured in duplicate by the competitive protein-binding technique of Winter and Grant [52] in a sample volume of 0.25 ml for adults and 2.5 ml for children. The sensitivity of the assay was 0.15 ng, corresponding to the upper 95% confidence limit for testosterone-free samples. In this study, samples reading below 0.15 ng in 2.5 ml plasma have been assigned an arbitrary value of

6 ng/100 ml. The coefficient of variation, determined from the duplicate samples in this report, was 6.3%.

The 24-hr excretions of creatinine, total 17-ketosteroids (17-KS) [32], and 17-hydroxycorticosteroids (17-OHCS) [46] were determined by standard methods. Individual 11-deoxy-17-ketosteroids were measured by the gas-liquid chromatographic method of Jungmann et al. [20]. Total urinary estrogens were determined by the method of Eechaute and Demeester [7], as modified by van Baelen et al. [48].

Results

The clinical and laboratory data for each 2-year age period are shown in Table I and the hormone results for each developmental stage are summarized in Table II. Plasma testosterone was not measurable (< 6 ng%) in 5 children, all below 11 years of age. Follicle-stimulating hormone was measurable in all subjects. Luteinizing hormone was below the level of sensitivity (0.8 μg/100 ml) in four subjects, all below age 6.

The Preadolescent Years

Below age 10 all of the boys were clinically prepubertal (stage P_1). Mean phallic size increased gradually throughout childhood. Testis size (Fig. 1) changed little before age 6; from age 6 to 10 years there was a gradual increase in mean testis length ($r = 0.48$, $P < 0.001$). There was no significant change in plasma testosterone concentration (Fig. 2) during childhood ($r = 0.18$, $P > 0.1$), with all values below 40 ng/100 ml. Serum LH (Fig. 3) and FSH (Fig. 4) concentrations did not change before age 6; however, there was a gradual but significant rise in both between 6 and 10 years ($r = 0.55$ and 0.53, respectively; $P < 0.001$). This pattern closely resembled that seen for testis growth in late childhood.

Puberty

The earliest recognizable sign of puberty was more rapid testicular enlargement which usually preceded any signs of androgen effect. This has been identified as stage P_2; the mean chronologic age at this stage was 11.8 years (range 9.6–15.1) and the mean bone age was 11.9 years (range 10.5–13.5). Boys in this stage showed significantly higher FSH, LH, and testosterone levels than did the prepubertal subjects. Between age 10–17 years there was an approximately 20-fold increase in plasma testosterone. This was accompanied by more rapid phallic growth and the appearance of pubic and axillary hair (Fig. 5). The anabolic action of testoster-

Table I. Circulating gonadotropin and testosterone levels, urinary steroid excretion, bone maturation and whole body ^{40}K content in 253 males in relation to age and sexual development

Clinical data								24-hr urine content							Serum		Plasma
Age, yr	No.	Puberty stage[1]	Testis diameter, cm	Penis length, cm	Bone maturation score [47]	^{40}K, cpm	^{40}K cpm/kg	Creatinine, g	Total 17-KS,[2] mg	Andro-sterone, mg	Etiochol-anolone, mg	DHA,[3] mg	Estrogens, μg	17-OHCS,[4] mg	FSH[5]	LH[6]	Testosterone, ng/100 ml
															μg LER-907/100 ml		
0.2–2.0	11	P₁	1.4±0.4⁷	2.7±0.5	8±7										7.0±2.4	1.2±0.4	13±10
2.1–4.0	7	P₁	1.2±0.4	3.3±0.4	53±16										7.5±1.4	1.3±0.6	11±4
4.1–6.0	13	P₁	1.5±0.6	3.9±0.9	127±31										7.8±2.5	1.5±0.5	20±9
6.1–8.0	21	P₁	1.8±0.3	4.2±0.8	199±55	59.9±6.3	2.7±0.1	0.49±0.08	0.9±0.4	0.07±0.05	0.07±0.05	0.02±0.01	2.2±0.5	2.8±1.2	10.0±1.5	1.7±0.5	14±7
8.1–10.0	25	P₁	2.0±0.5	4.9±1.0	321±69	74.1±6.9	2.5±0.3	0.50±0.10	1.2±0.6	0.09±0.08	0.09±0.08	0.02±0.01	2.5±0.8	3.3±1.1	10.3±2.8	1.9±0.4	21±12
10.1–12.0	53	P₂	2.7±0.7	5.2±1.3	494±137	92.7±11.7	2.3±0.4	0.85±0.19	2.5±1.1	0.35±0.22	0.35±0.22	0.07±0.05	5.2±1.7	4.7±1.6	12.6±3.6	2.0±0.5	41±46
12.1–14.0	51	P₂	3.4±0.8	6.2±2.0	628±150	110.0±20.2	2.6±0.4	1.01±0.16	3.3±1.3	0.54±0.32	0.54±0.32	0.20±0.20	5.9±3.4	4.8±1.3	13.6±3.1	2.5±0.8	131±172
14.1–16.0	26	P₃	4.1±1.0	8.6±2.4	811±155	155.8±27.7	2.7±0.5	1.31±0.34	5.2±2.0	1.80±0.31	1.80±0.31	1.03±0.30	16.2±2.7	5.1±2.7	15.8±4.1	3.1±0.7	328±211
16.1–18.0	10	P₄	5.0±0.5	9.9±1.7	967±28	189.2±19.1	2.9±0.4								15.7±3.5	4.1±1.1	532±191
18.1–20.0	6	P₅	5.0±0.3	11.0±1.1	992	189.6±19.4	2.8±0.3								20.2±6.1	3.6±0.6	564±157
20.1–25.0	30	P₅	5.2±0.6	12.4±1.6	1000	190.1±20.0	2.8±0.3								17.0±3.9	4.2±1.0	605±194

[1] This represents the stage of puberty most prevalent in each age group.
[2] 17-KS: 17-ketosteroids.
[3] DHA: dehydroepiandrosterone.
[4] 17-OHCS: 17-hydroxycorticosteroids.
[5] FSH: follicle-stimulating hormone.
LH: luteinizing hormone.
Mean ± standard deviation.

Table II. Circulating testosterone, FSH[1] and LH[2] concentrations in males at different stages of puberty

Stage	No.	Plasma testosterone concentration (ng%)	Serum FSH concentration (μg LER-907/100 ml)	Serum LH concentration (μg LER-907/100 ml)
P_1	94	17 ± 1[3]	9.7 ± 0.4	1.7 ± 0.1
		$P < 0.001$[4]	$P < 0.005$	$P < 0.001$
P_2	56	97 ± 5	12.8 ± 0.3	2.2 ± 0.1
		$P < 0.001$	$P > 0.10$	$P = 0.10$
P_3	30	121 ± 17	13.5 ± 0.7	2.5 ± 0.2
		$P < 0.001$	$P < 0.005$	$P = 0.10$
P_4	20	403 ± 42	17.2 ± 1.0	3.0 ± 0.2
		$P < 0.001$	$P > 0.10$	$P = 0.001$
P_5	53	578 ± 27	16.5 ± 0.6	4.0 ± 0.2

[1] FSH : follicle-stimulating hormone.
[2] LH : luteinizing-hormone.
[3] Mean ± standard error of the mean.
[4] Significance of difference between means determined by Student's t test.
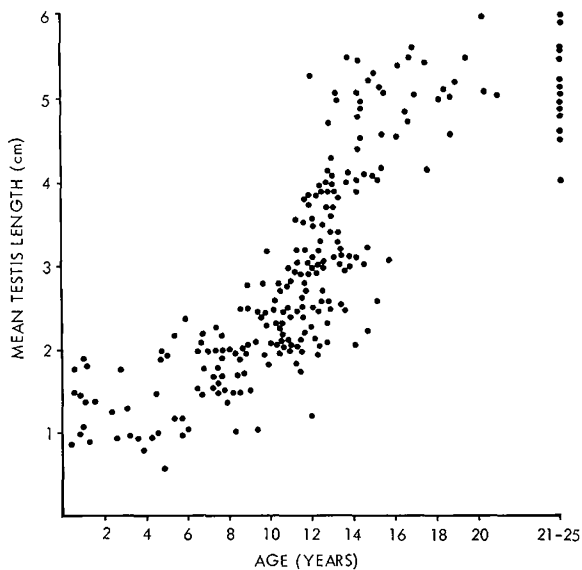
Fig. 1. Testis size (mean of the longest diameters of both testes) at different ages.

one was reflected in the rising creatinine excretion and wholebody ^{40}K content after age 12. As expected, there was a striking rise in urinary estrogens and 17-keto-steroids at this time. Plasma testosterone levels showed a significant correlation with both urinary estrogen ($r = 0.75$, $P < 0.001$) and 17-ketosteroid ($r = 0.63$, $P < 0.001$) content. Urinary 17-hydroxycorticosteroids in-

creased gradually in each age group, with no noticeable spurt at puberty.

Serum FSH concentrations continued to rise during this period, but the slope was not significantly greater than that seen during the preadolescent years ($t = 1.15$, $P > 0.1$). On the other hand, there was a significant increment in the slope of the serum LH rise after age 12 ($t = 4.76$, $P < 0.001$).

The Young Adult

Mean serum FSH and LH levels ceased to rise after about age 16; similarly, there was no further change in mean testis size or in plasma testosterone concentration after this age. Clinically, most of the subjects in the 16–25-year-old age group were adult (*stage* P_5). Bone maturation was almost complete by age 17, and there was no further increase in mean whole body ^{40}K content, showing that the period of rapid bone and muscle growth was completed.

The mean adult serum FSH level was approximately 2 times the mean prepubertal level, while the mean adult LH concentration was 3 times that in children. Although there was a significant correlation between plasma testosterone and both serum LH ($r = 0.77$, $P < 0.001$) and FSH ($r = 0.57$, $P < 0.001$) when all age groups were considered together, there was no correlation between testosterone and either gonadotropin in the adult subjects.

Discussion

The changing level of plasma testosterone with age parallels the pattern of testosterone glucuronide excretion in males [15, 22], and presumably is a reflection of gonadal testosterone production. The low levels seen in the prepubertal boys are not different from those found in girls, or in hypopituitary or castrate children [51], and would appear to arise from a source other than the testis. This is in keeping with the absence of characteristic functioning Leydig cells in the prepubertal human testis [1, 6, 28, 44]. It should be noted, however, that minimal increments with time at these low testosterone levels might by obscured in a cross-sectional study such as this. At puberty there is a relatively rapid rise in plasma testosterone which coincides with, and is undoubtedly responsible for, the appearance of male secondary sexual characteristics, an adolescent growth spurt, and progressive epiphyseal maturation with eventual fusion. Coincident with this there
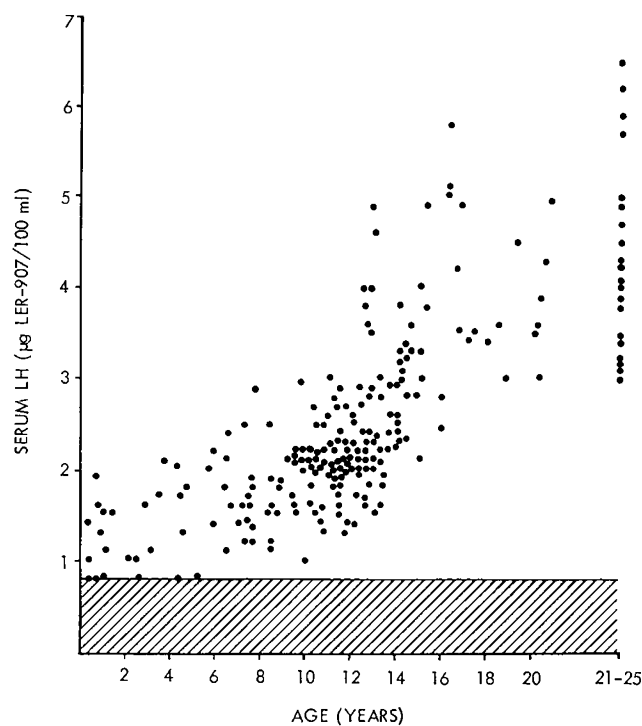
Fig. 3. Serum luteinizing hormone (LH) concentrations in males at different ages. The shaded area represents the limit of sensitivity of the radioimmunoassay.

is a rise in estrogen and 11-deoxy-17-ketosteroid excretion.

Since testicular testosterone production is thought to be gonadotropin-, and primarily LH-dependent, a similar pattern in serum gonadotropin levels might be expected, with pituitary secretion of FSH and LH being initiated at about age 11. Instead, beginning around age 6–8 years, there is a gradual rise in mean FSH and LH concentration. The FSH appears to rise gradually from age 6 to age 15, with no apparent pubertal spurt. Mean serum LH levels begin to rise about age 8, with a more abrupt increase after age 12. This finding, that LH levels begin to rise several years before plasma testosterone, suggests either that testosterone secretion is related to the attainment of a threshold LH concentration (approximately 2.2 $\mu g/$ 100 ml) or that the testis at puberty becomes progressively more sensitive to a slight increment in LH stimulation.

The pattern of rising gonadotropin levels in late childhood resembles that for testis growth (Fig. 1). During this time, although typical Leydig cells are absent, the testis is not a static organ; progressive changes are seen in the seminiferous tubules, as the sex
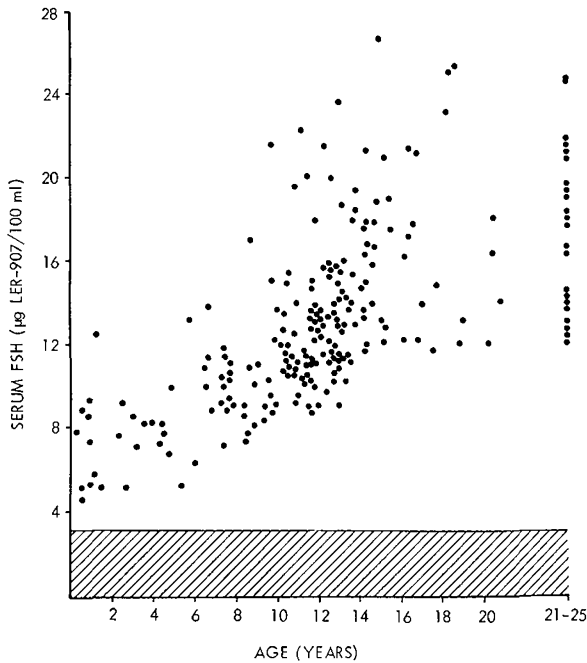

Fig. 2. Plasma testosterone concentrations in males at different ages. The shaded area represents the limit of sensitivity of the assay.

Fig. 4. Serum follicle-stimulating hormone (FSH) concentrations in males at different ages. The shaded area represents the limit of sensitivity of the radioimmunoassay.
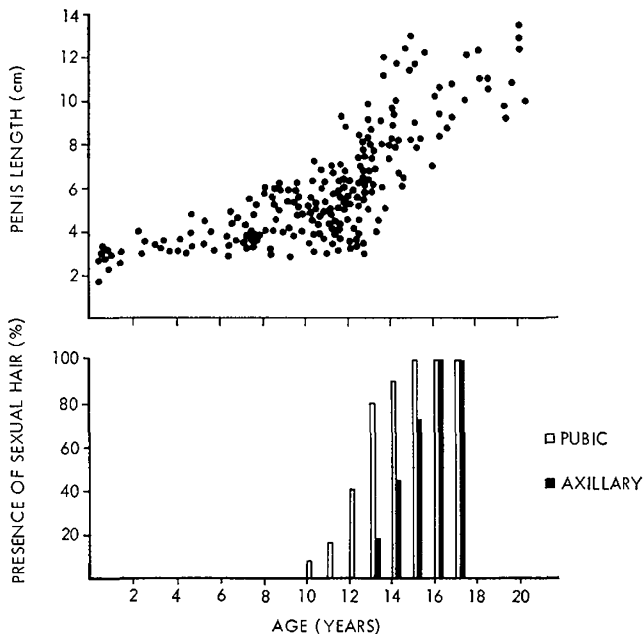

Fig. 5. Lax penis length in males at various ages (*top*); the percentage of subjects showing pubic or axillary hair, or both, at these ages (*bottom*).

cords grow and acquire a lumen and the germ cells mature to the spermatogonial stage [1, 6, 27, 44]. It is possible that these changes are gonadotropin-mediated

since the testis in hypogonadotropic hypogonadism shows small lumenless tubules, and only primitive germ cells [16]. Similar maturational changes may be proceeding in the Leydig cell precursors which render them progressively more responsive to LH stimulation.

These studies have not explored the role of adrenal androgen secretion in male adolescence directly. However, it is known [37, 39] that plasma levels of dehydroepiandrosterone sulfate rise progressively after age 7, a pattern resembling that seen in serum gonadotropin concentrations. This may suggest that adrenal androgen production, at least in late childhood, could be gonadotropin-dependent.

In Figure 6, a schematic summary of the changes in circulating gonadotropins in relation to adolescent development is shown. It can be seen that, by age 10, the mean FSH concentration has entered the adult range. After age 12 there is a relatively greater rise in LH levels, with the adult range being reached around age 15. This pattern supports a model in which FSH plays a role primarily in testis growth and tubular development, with Leydig cell function being primarily LH-dependent. No information is available from these data regarding the roles of FSH, LH, and testosterone
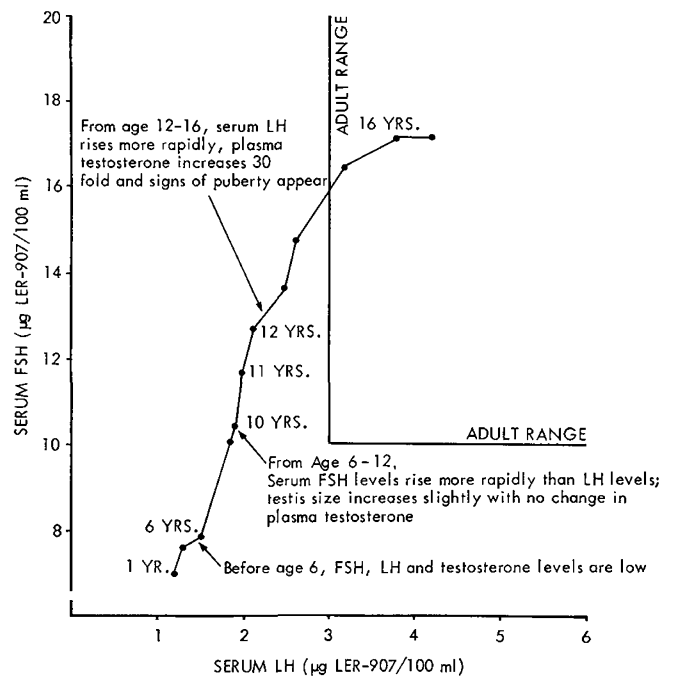

Fig. 6. A schematic representation of the relationship between mean serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) concentrations in males at various ages. The sequential phenomena of puberty are shown.

in the final maturation of spermatozoa, nor their feed-back interrelations [19].

This pattern of pituitary-gonadal development in man parallels in some respects that seen in other species. In the rat, testis growth begins at 20 days of age, possibly as a result of FSH stimulation [33]. Signs of testosterone effect appear at 45–50 days [17, 30]. Studies of pituitary gonadotropin content show that FSH content rises early, reaching adult levels by day 33, while LH levels peak between days 43 and 47 [23, 25]. By day 33, coincident with the FSH rise, the activities of the testicular enzymes related to testosterone biosynthesis reach the adult range, but testosterone secretion does not occur [17] because of a corresponding rise in the activity of testicular reductases [45]. Around 45 days, as LH levels peak, there is a rapid fall in reductase activity and testosterone secretion begins. There is also evidence that in the canine testis the synergistic effects of FSH and LH are required for androgen production [8].

Summary

Circulating levels of FSH, LH, and testosterone were measured in 253 healthy males aged 3 months–25 years. These data were correlated with the stage of pubertal development, lean body mass (by whole body ^{40}K counting), bone maturation (from a wrist x-ray), and 24-hr excretion of creatinine, estrogens, 17-hydroxycorticosteroids, total 17-ketosteroids, and fractionated 11-deoxy-17-ketosteroids in urine. The data show that mean serum FSH and LH levels begin to rise at around age 6–8 years, signaled by slight testicular enlargement, but no rise in plasma testosterone. The onset of somatic puberty is accompanied by a steeper increase in serum LH, and a 20-fold rise in plasma testosterone. Circulating LH and testosterone continue to rise until about age 17, while serum FSH reaches a plateau somewhat earlier.

The data suggest that the testis in late childhood may undergo progressive maturation under the influence of gradually rising gonadotropin levels. The relatively abrupt initiation of gonadal testosterone secretion at puberty appears to follow the achievement of a critical threshold concentration of circulating LH.

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