Serum Inhibin B in Healthy Pubertal and Adolescent Boys: Relation to Age, Stage of Puberty, and Follicle-Stimulating Hormone, Luteinizing Hormone, Testosterone, and Estradiol Levels*

ANNA-MARIA ANDERSSON, ANDERS JUUL, JØRGEN H. PETERSEN,
JØRN MÜLLER, NIGEL P. GROOME, AND NIELS E. SKAKKEBÆK

Department of Growth and Reproduction, Rigshospitalet (A.-M.A., A.J., J.M., N.E.S.), and the Department of Biostatistics, Panum Institute (J.H.P.), Copenhagen, Denmark; and the School of Biological and Molecular Sciences, Oxford Brookes University (N.P.G), Oxford, United Kingdom

ABSTRACT

Inhibin B levels were measured in serum from 400 healthy Danish prepubertal, pubertal, and adolescent males, aged 6–20 yr, in a cross-sectional study using a recently developed immunoassay that is specific for inhibin B, the physiologically important inhibin form in men. In addition, serum levels of FSH, LH, testosterone, and estradiol levels were measured. Serum levels of inhibin B, FSH, LH, testosterone, and estradiol all increased significantly between stages I and II of puberty. From stage II of puberty the inhibin B level was relatively constant, whereas the FSH level continued to increase between stages II and III. From stage III of puberty the FSH level was also relatively constant, although there was a nonsignificant trend of slightly decreased FSH levels at pubertal stage V compared to stage IV. The levels of serum LH, testosterone, and estradiol increased progressively throughout puberty. In prepubertal boys younger than 9 yr, there were no correlation between inhibin B and the other three hormones. In prepubertal boys older than 9 yr, a significant positive correlation was observed between inhibin B and FSH, LH, and testosterone. However, at this pubertal stage, each hormone correlated strongly with age, and when the effect of age was taken into account, only the partial correlation between inhibin B and LH/testosterone remained statistically significant. At stage II of puberty, the positive partial correlation between inhibin B and LH/testosterone was still present. At stage III of puberty, an negative partial correlation be-

tween inhibin B and FSH, LH, and estradiol was present, whereas no correlation between inhibin B and testosterone could be observed from stage III onward. The negative correlation between inhibin B and FSH persisted from stage III of puberty onward, whereas the correlation between inhibin B and LH and between inhibin B and estradiol was nonsignificant at stages IV and V of puberty.

In conclusion, in boys, serum inhibin B levels increase early in puberty; by pubertal stage II the adult level of inhibin B has been reached. The correlation of inhibin B to FSH, LH, and testosterone changes during pubertal development. Early puberty is characterized by a positive correlation between inhibin B and LH/testosterone, but no correlation to FSH. Late puberty (from stage III) is characterized by a negative correlation between inhibin B and FSH (which is maintained in adult men), a diminishing negative correlation between inhibin B and LH, and no correlation between inhibin B and testosterone, suggesting that developmental and maturational processes in the hypothalamic-pituitary-gonadal axis take place, leading to the establishment of the closed loop feedback regulation system operating in adult men. The positive correlation between inhibin B and LH/testosterone at the time when serum inhibin B levels rise early in puberty suggests that Leydig cell factors may play an important role in the maturation and stimulation of Sertoli cells in the beginning of pubertal development. (*J Clin Endocrinol Metab* **82:** 3976–3981, 1997)

T HE ONSET of male puberty is associated with alterations in the circulating concentrations of gonadotropins and gonadal hormones, including a rise in the levels of the gonadal glycoprotein inhibin (1, 2). Several circulating inhibin forms exist, some of which are believed to be biologically inactive (3). In its biologically active form, inhibin is a dimer consisting of an α-subunit linked to either a βA-subunit (inhibin A) or a βB-subunit (inhibin B) (4). Inhibin is believed to regulate FSH secretion by a closed loop negative feedback system, as indicated by *in vitro* (5, 6) and *in vivo*

(6–9) studies in animals, including primates (10). In man, however, it has only recently been possible to demonstrate a negative relationship between circulating immunoreactive inhibin and FSH in normal adult males (11). Earlier immunoassays for inhibin suffered from cross-reaction with inactive monomeric precursor forms present in plasma, lack of sensitivity, or lack of discrimination between inhibin A and B (12). By employing newly developed inhibin assays that are specific for the bioactive inhibin forms and discriminate between inhibin A and inhibin B, it has been shown that inhibin B, which is supposedly produced by the Sertoli cells of the testis, is the principal circulating inhibin form in men (11, 13). Previous studies of inhibin during puberty, in which the earlier unspecific assays were used, failed to demonstrate a correlation between inhibin and FSH in prepubertal and pubertal boys (1, 14). However, this might be due to the unspecificity of the assays employed. We present here what we believe is the first study of inhibin throughout normal male puberty determined by a new specific assay for bioac-

tive inhibin B. The levels of circulating inhibin B during normal male puberty in relation to age, pubertal stage, and FSH, LH, testosterone, and estradiol serum levels were evaluated in a cross-sectional study of 400 healthy prepubertal, pubertal, and adolescent males in the age range 6–20 yr.

Subjects and Methods

Subjects

6–20 yr. Serum was obtained from 400 healthy school boys who were part of a previously described study group (15). None had acute or chronic disease, and none was taking any medication. The pubertal developmental stage was recorded according to Tanner (16) by the same 2 physicians. Testicular volume was measured using a Prader orchidometer. If the sizes of the 2 testes were not identical, the larger was chosen to determine testicular volume. A testicular volume greater than 3 mL was taken as a definite sign of the onset of puberty. All participants and their parents (6–18 yr) gave their informed consent.

Adult control group. In addition, serum was obtained from an adult control group, aged 21–57 yr (n = 55), for comparison of serum hormone levels. This group consisted of teachers at the participating schools and hospital staff.

The study was in accordance with the Helsinki II Declaration and was approved by the local ethical committee of Copenhagen, Denmark (approval V200.1996/90).

Methods

Blood samples were drawn from an antecubital vein between 0800–1300 h and centrifuged. Serum was stored at −20 C until analysis. Samples were stored for up to 4 yr before analysis. Serum inhibin B was measured in duplicate in a double antibody enzyme immunometric assay using a monoclonal antibody raised against the inhibin βB-subunit in combination with a labeled antibody raised against the inhibin α-subunit, as previously described (17). The detection limit was 18 pg/mL, and the intra- and interassay coefficients of variation were 15% and 18%, respectively.

Serum FSH and LH were measured by time-resolved immunofluorometric assay (DELFIA, Wallac, Turku, Finland), with detection limits of 0.06 and 0.05 U/L, respectively. Intra- and interassay coefficients of variation were both below 8% in the FSH and LH assays. Testosterone and estradiol were measured by RIA [Coat-a-Count, Diagnostic Products Corp. (Los Angeles, CA) and ImmunoDiagnostic Systems (Boldon, UK), respectively]. The detection limit for testosterone measurements was 0.23 nmol/L, and the intra- and interassay coefficients of variation were both less than 10%. In the estradiol assay, the detection limit was 18 pmol/L, the intraassay coefficient of variation was less than 8%, and the interassay coefficient of variation was less than 13%.

Statistics

The reproductive hormone measurements within each pubertal stage were subjected to a cubic root transformation to correct for the marked right skewness and to obtain approximate normality. The reference curves for the mean hormone levels were obtained by transforming the data to approximate normality by a cubic root transformation, followed by smoothing the data using local linear regression (18). Similarly, the 2.5, 16, 84, and 97.5 percentiles corresponding to −2, −1, 1, and 2 SD were obtained from smooth variance estimates, followed by back-transformation. Using the procedure LIFEREG in the statistical package SAS (SAS Institute, Cary, NC) for the smoothing allowed taking into account the left-censoring, *i.e.* the unmeasurable hormone levels (19).

Median and 5th and 95th percentiles within each pubertal stage were calculated on nontransformed data in the statistical package SPSS (20). Likewise, the differences in hormone levels between the different pubertal stages were tested with a Mann-Whitney U test using the statistical package SPSS.

Pearson correlations were calculated between inhibin B and FSH, LH, testosterone, and estradiol. A Tobit analysis (21) was used that allowed for correcting the influences of age on hormone levels as well as taking into account the unmeasurable levels of FSH, LH, testosterone, and estradiol, especially in the younger children. The analysis was based on the procedure LIFEREG in the statistical package SAS (19).

Results

Serum inhibin B, FSH, LH, testosterone, and estradiol levels in relation to age and stage of puberty

The individual serum inhibin B, FSH, LH, testosterone, and estradiol levels are shown in relation to age in Fig. 1, in which the mean ± 1 SD and the mean ± 2 SD also are indicated. The median and 5th and 95th percentiles of the results grouped according to stage of puberty are listed in Table 1 and are plotted without percentiles in Fig. 2. The serum levels of all five measured hormones increased significantly between stages I and II of puberty. From stage II of puberty the inhibin B level was relatively constant, whereas the FSH level continued to increase between stages II and III. From stage III of puberty the FSH level was also relatively constant, although there was a nonsignificant ($P = 0.06$) trend of slightly decreased FSH levels at pubertal stage V compared to stage IV. The levels of serum LH, testosterone, and estradiol increased progressively throughout puberty.

Correlation of inhibin B to gonadotropins, testosterone, and estradiol at different stages of puberty

The correlation of serum inhibin B to FSH, LH, testosterone, and estradiol levels according to stage of puberty is shown in Table 2. At the onset of puberty, hormonal changes are likely to precede physical changes. Thus, among the boys who were classified as prepubertal (pubertal stage I) based on physical examination, the pituitary-gonadal axis may be activated in some individuals, although this activation has not yet resulted in physical signs of puberty. For this reason we divided pubertal stage I into two subgroups: group IA, consisting of boys younger than 9 yr and presumably true prepubertal, and group IB, consisting of boys 9 yr or older, some of whom may have had an activated pituitary-gonadal hormonal axis. In pubertal stage IA, there was no correlation between inhibin B and the other four hormones. In pubertal stage IB, a significant positive correlation was observed between inhibin B and FSH, testosterone, and LH. However, at this pubertal stage each hormone correlated strongly with age, and when the effect of age was taken into account, only the partial correlation between inhibin B and LH/testosterone remained statistically significant. At stage II of puberty, the positive partial correlation (*i.e.* the correlation after adjustment for the influence of age) of inhibin B to LH and testosterone was still present. At stage III of puberty, a negative partial correlation between inhibin B and FSH, LH, and estradiol was present, whereas no correlation between inhibin B and testosterone could be observed from stage III onward. The negative correlation between inhibin B and FSH persisted from stage III of puberty onward, whereas the correlations between inhibin B and LH and between inhibin B and estradiol decreased and became nonsignificant through stages IV and V of puberty. No significant correlation between inhibin B and estradiol was observed during pubertal development, except at pubertal stage III, where a negative correlation was found.
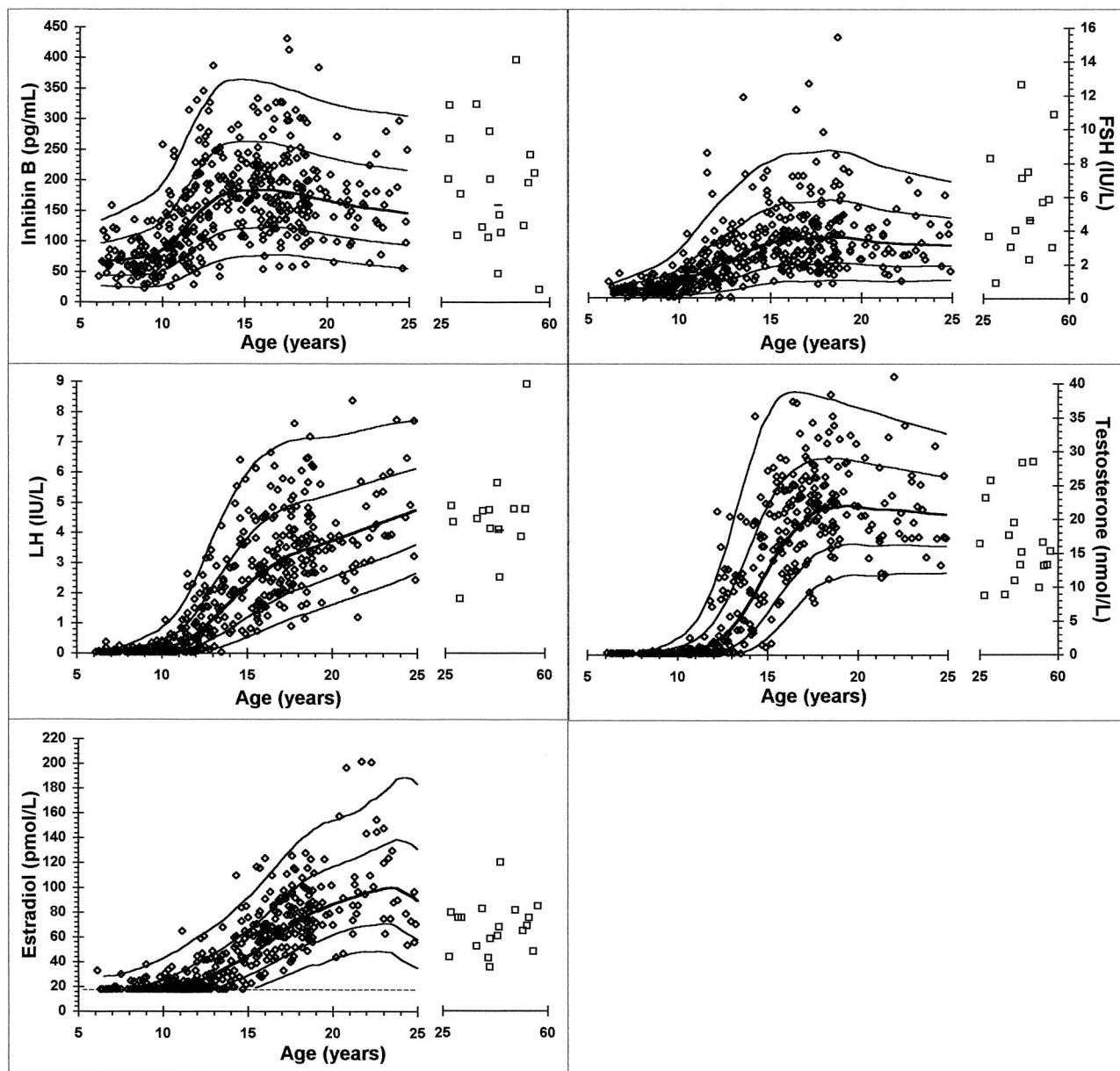
FIG. 1. Individual serum concentrations of inhibin B, FSH, LH, testosterone, and estradiol plotted as a function of age. The mean ± 1 SD and the mean ± 2 SD are indicated by *solid lines*. The levels in adult men are included for comparison. The *dotted line* represents the detection limit in the assay for estradiol measurements.

Discussion

In our cross-sectional study of 400 healthy boys, inhibin B was significantly increased in pubertal stage II compared to the prepubertal (stage I) level. At pubertal stage II, serum inhibin B levels were comparable to adult levels and did not change during the rest of the pubertal development. The finding of a very abrupt rise in inhibin B early in puberty in boys is in contrast to previous studies of inhibin during male puberty in humans (1) and primates (22), in which inhibin was observed to increase progressively through pubertal development. However, in these previous studies the so-called Monash inhibin assay was used. In contrast to the specific inhibin B assay used by us, the Monash assay is now

known to cross-react with the inactive free α-subunit and pro-αC monomeric precursor form (12). The fractions of these inactive inhibin forms in serum during male puberty are unknown. However, it cannot be excluded that the discrepancy between the progressive rise in inhibin through puberty observed using the Monash assay compared to the abrupt early puberty rise in inhibin B observed using the inhibin B assay is due to a combination of differences in assay specificity and developmental changes in the fraction of inactive monomeric inhibin forms in serum.

To our knowledge there is only one other study of inhibin B during puberty in which the specific inhibin B assay has been used (2). In this study seven boys were followed lon-

TABLE 1. Median, 90% prediction interval (5–95 percentiles), and number of measurements of age, testicular volume, serum inhibin B, FSH, LH, testosterone, and estradiol at each stage of puberty

	Stage of puberty				
	I	II	III	IV	V
Age (yr)	9.7	12.6	13.6	15.7	17.3
5–95 percentiles	6.7–12.4	10.6–15.5	11.7–15.8	12.8–18.0	14.3–19.4
n	156	47	27	31	131
Testicular volume (mL)	1.0	6.0[a]	10.0[a]	15.0[a]	20.0[b]
5–95 percentiles	1.0–3.0	2.0–11.6	5.4–15.0	10.0–23.8	12.0–25.0
n	156	47	27	31	129
Inhibin B (pg/mL)	78	195[a]	163	188	187
5–95 percentiles	35–182	62–338	78–323	67–304	95–323
n	156	47	27	31	131
FSH (U/L)	0.85	1.95[a]	3.50[a]	3.61	3.10
5–95 percentiles	0.25–2.55	0.07–4.39	0.94–9.68	1.98–6.88	1.38–7.52
n	154	47	27	31	128
LH (U/L)	0.08	0.88[a]	2.03[a]	2.89[b]	3.40[c]
5–95 percentiles	<0.05–0.99	0.11–2.97	0.51–5.42	1.11–5.89	1.53–6.33
n	154	47	26	31	129
Testosterone (nmol/L)	<0.2	1.9[a]	8.4[a]	17.2[a]	21.0[b]
5–95 percentiles	<0.2–0.9	<0.2–13.4	0.9–21.2	7.7–26.5	11.3–32.3
n	155	47	27	31	130
Estradiol (pmol/L)	<18	21[b]	36[b]	59[a]	71[c]
5–95 percentiles	<18–34	<18–45	<18–85	29–102	44–117
n	154	46	25	31	129

Significance is indicated when the median is statistically significant different from the median of the previous stage of puberty (by Mann-Whitney U test; not shown for age).

[a] $P < 0.0001$.
[b] $P < 0.001$.
[c] $P < 0.05$.

gitudinally through pubertal stages I-III. However, in only two boys were serum samples at both stages II and III obtained. One of these boys showed an increase in inhibin B from stage I to II as well as from stage II to III, whereas the other boy showed an increase in inhibin B from stage I to II and no further increase in inhibin B from stage II to III. Thus, due to the limited material it is impossible to make any conclusions about the general pattern of the rise in inhibin B through early puberty from this small longitudinal study. Serum inhibin B levels have been shown to reflect Sertoli cell function in adult men (13), and FSH/LH replacement therapy in gonadotropin-suppressed normal men results in comparable qualitative changes in serum inhibin levels and sperm counts (23). In this respect, it is interesting that the finding that mature levels of inhibin B are achieved early in puberty coincides with the observation that spermarche also is achieved early in puberty and may occur when the testes have grown only slightly and with little or no pubic hair development (24).

The correlation of inhibin B to FSH, LH, and testosterone changed during pubertal development. The positive correlation of inhibin B to testosterone levels observed early in puberty is in agreement with previous studies (1, 2, 25). We observed a similar positive correlation with LH early in puberty as also described by Orlando *et al.* (25). Interestingly, as previously observed (1, 2, 14), no correlation between inhibin B and FSH was found in prepuberty and early puberty after accounting for the developmental trend indexed by age, suggesting that factors other than FSH are responsible for the stimulation of inhibin B production and secretion

by Sertoli cells at this developmental stage. *In vitro* studies of immature and adult rat Sertoli cell cultures have shown that FSH is a strong stimulator of inhibin production, whereas LH (26, 27) and testosterone (26, 28) have no effect or a suppressive effect on inhibin production. In contrast, previous studies of gonadotropin therapy in normal men (29), gonadotropin-suppressed normal men (23), and men with hypogonadotropic hypogonadism (30) show that both FSH and LH stimulate inhibin production. Likewise, FSH replacement alone is not sufficient to restore spermatogenesis in gonadotropin-deficient rats, suggesting that additional factors are necessary for optimal Sertoli cell function (31). It has been speculated that the mechanism of the LH effect on inhibin production may be indirect, through the action of testosterone or other Leydig cell factors either directly on the Sertoli cells or via the peritubular cells (23). However, destruction of Leydig cells leading to testosterone depletion results in increased inhibin levels in adult rats (32). Nevertheless, if the action of LH stimulation of inhibin production is through factors released by other interstitial cells or peritubular cells, the discrepancy between the effect of LH in *in vivo* studies and that in *in vitro* studies using purified Sertoli cells might be explained. The positive correlation between inhibin B and LH (and testosterone) and the lack of correlation to FSH early in puberty might suggest that LH (via testosterone) plays an important role in the stimulation of inhibin B production in early human puberty. This is supported by the fact that although serum FSH levels are markedly increased at pubertal stage III compared to those at stage II, the serum inhibin B level does not increase beyond pu-

bertal stage II. Support for an important role of LH in Sertoli cell maturation and initiation of spermatogenesis is found in the rare cases of gonadotropin-independent male precocious puberty, which in many cases are caused by a constitutive active mutated LH receptor (33, 34). In these cases pubertal or adult levels of serum testosterone are found, whereas gonadotropin levels are very low. Testicular biopsies from boys with gonadotropin-independent precocity have shown various degrees of seminiferous tubular development, including mature Sertoli cells and germ cells of all stages of spermatogenesis, and in some cases, the testes were indistinguishable from those of normal adult men (35), although there seemed to be some disorganization of the maturation of spermatides (36). Also in prepubertal boys with Leydig cell tumors, maturation of the seminiferous epithelium is observed in tubules located adjacent to the testosterone-producing tumor (37, 38). Likewise, testicular biopsies from prepubertal boys with hCG-producing choriocarcinoma may show maturation of both Leydig and Sertoli cells as well as adult type of spermatogonia (37). In adult rat testis, inhibin secretion has been shown to be stimulated by the presence of late spermatids (39). As high testicular levels of testosterone seem to facilitate spermatogenesis in the absence of FSH in these pathological conditions, the positive correlation between inhibin B and LH (and testosterone) early in puberty may be due to an effect of testosterone on the initiation of spermatogenesis and, via the presence of late spermatides, the stimulation of inhibin B secretion by Sertoli cells.

It is, however, noteworthy that the level of inhibin B in prepubertal boys is well above the undetectable levels seen in castrated adult men, showing that basal inhibin B production takes place in the prepubertal testis, although both gonadotropins and testosterone are suppressed.

Around midpuberty (stage III), a negative correlation between inhibin B and FSH (and to a lesser extent LH) is apparent. This negative correlation between inhibin B and FSH persists into adulthood, suggesting that a negative feedback regulation between the Sertoli cells and the pituitary is established around midpuberty, thereby confirming data indicating that inhibin plays a major role in the *in vivo* regulation of FSH secretion in man (11, 13, 40, 41) and primates (10, 42).

The negative correlation observed between inhibin B and estradiol at pubertal stage III may be purely accidental. On the other hand, it occurs at a time when the transition from a positive correlation between inhibin B and LH/testosterone to a negative correlation between inhibin B and FSH seems to take place. Thus, it is tempting to speculate that the correlation between inhibin B and estradiol may be indirect and a reflection of the maturational changes in the control and sensitivity of the hypothalamic-pituitary-gonadal axis that take place in midpuberty, in which estradiol is likely to play a role.

We conclude that serum inhibin B levels increase early in puberty in boys, and adult levels are reached by pubertal stage II. The changes in the correlation of inhibin B to FSH, LH, and testosterone during pubertal development suggest that developmental and maturational processes in the hypothalamic-pituitary-gonadal axis take place, leading to establishment of the closed loop feedback regulation system operating in adult men.
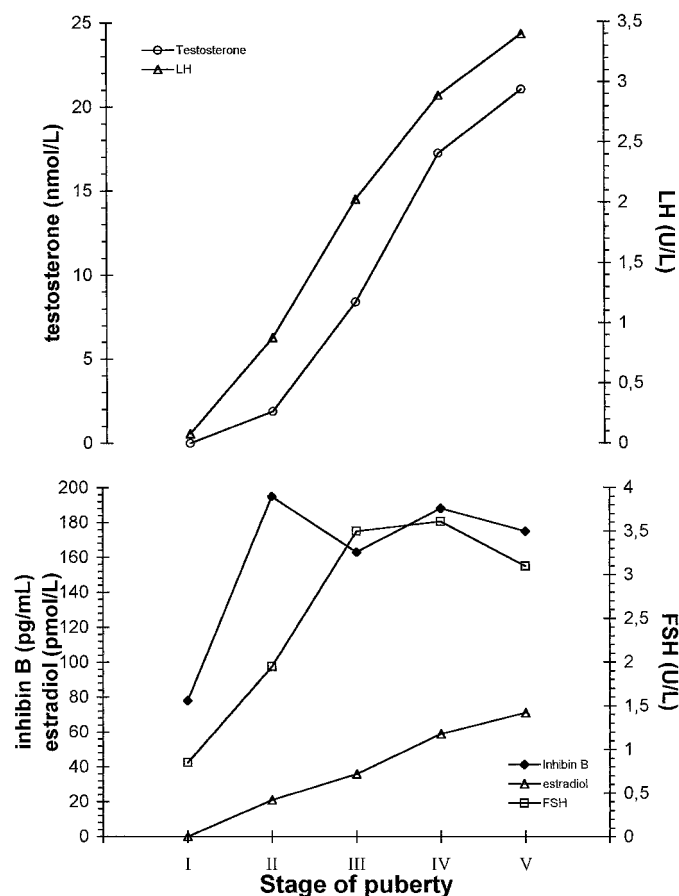

FIG. 2. Median of serum inhibin B, FSH, LH, testosterone, and estradiol plotted as a function of the stage of puberty.

TABLE 2. Correlation of inhibin B to FSH, LH, testosterone, and estradiol within each stage of puberty

Inhibin B	FSH	LH	Testosterone	Estradiol
Stage IA	0.01 (0.93)	0.04 (0.80)	−0.07 (0.59)	−0.11 (0.53)
Stage IB	0.09 (0.41)	0.51 (0.0001)	0.21 (0.035)	0.05 (0.70)
Stage II	0.08 (0.57)	0.51 (0.0001)	0.42 (0.002)	0.25 (0.14)
Stage III	−0.70 (0.0001)	−0.45 (0.005)	−0.24 (0.18)	−0.41 (0.03)
Stage IV	−0.50 (0.0017)	−0.19 (0.31)	0.16 (0.38)	0.08 (0.66)
Stage V	−0.56 (0.0001)	−0.16 (0.07)	0.11 (0.21)	0.06 (0.52)

Values are partial Pearson correlations adjusted for age (with two-sided *P* values). Pubertal stage I is divided into boys younger than 9 yr (IA) and boys 9 yr or older (IB).

References

1. **Burger HG, McLachlan RI, Bangah M, et al.** 1988 Serum inhibin concentrations rise throughout normal male and female puberty. J Clin Endocrinol Metab. 67:689–694.
2. **Crofton PM, Illingworth PJ, Groome NP, et al.** 1997 Changes in dimeric inhibin A and B during normal early puberty in boys and girls. Clin Endocrinol (Oxf). 46:109–114.
3. **Robertson DM, Sullivan J, Watson M, Cahir N.** 1995 Inhibin forms in human plasma. J Endocrinol. 144:261–269.
4. **De Kretser DM, McFarlane JR.** 1996 Inhibin in the male. J Androl. 17:179–182.
5. **Kitahara S, Kotsuji F, Keeping HS, Oshima H, Troen P, Winters SJ.** 1991 Interrelationship between the actions of testosterone and primate sertoli cell inhibin in the control of gonadotropin secretion by cultured pituitary cells. Endocrinology. 128:710–716.
6. **Rivier C, Corrigan A, Vale W.** 1991 Effect of recombinant human inhibin on gonadotropin secretion by the male rat. Endocrinology. 129:2155–2159.
7. **Robertson DM, Prisk M, McMaster JW, Irby DC, Findlay JK, De Kretser DM.** 1991 Serum FSH-suppressing activity of human recombinant inhibin A in male and female rats. J Reprod Fertil. 91:321–328.
8. **Carroll RS, Kowash PM, Lofgren JA, Schwall RH, Chin WW.** 1991 *In vivo* regulation of FSH synthesis by inhibin and activin. Endocrinology. 129:3299–3304.
9. **Wreford NG, O'Connor AE, De Kretser DM.** 1994 Gonadotropin-suppressing activity of human recombinant inhibin in the male rat is age dependent. Biol Reprod. 50:1066–1071.
10. **Medhamurthy R, Culler MD, Gay VL, Negro-Vilar A, Plant TM.** 1991 Evidence that inhibin plays a major role in the regulation of follicle-stimulating hormone secretion in the fully adult male rhesus monkey (*Macaca mulatta*). Endocrinology. 129:389–395.
11. **Illingworth PJ, Groome NP, Bryd W, et al.** 1996 Inhibin-B: a likely candidate for the physiologically important form of inhibin in man. J Clin Endocrinol Metab. 81:1321–1325.
12. **Robertson D, Burger HG, Sullivan J, et al.** 1996 Biological and immunological characterization of inhibin forms in human plasma. J Clin Endocrinol Metab. 81:669–676.
13. **Anawalt BD, Bebb RA, Matsumoto AM, et al.** 1996 Serum inhibin B levels reflect sertoli cell function in normal men and men with testicular dysfunction. J Clin Endocrinol Metab. 81:3341–3345.
14. **Manasco PK, Umbach DM, Muly SM, et al.** 1995 Ontogeny of gonadotropin, testosterone, and inhibin secretion in normal boys through puberty based on overnight serial sampling. J Clin Endocrinol Metab. 80:2046–2052.
15. **Juul A, Bang P, Hertel NT, et al.** 1994 Serum insulin-like growth factor-1 in 1030 healthy children, adolescents and adults; relation to age, sex, stage of puberty, testicular size and body mass index. J Clin Endocrinol Metab. 78:744–752.
16. **Tanner JM, Whitehouse RH.** 1976 Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child. 51:170–179.
17. **Groome NP, Illingworth PJ, O'Brien M, et al.** 1996 Measurement of dimeric inhibin B throughout the human menstrual cycle. J Clin Endocrinol Metab. 81:1401–1405.
18. **Venables WN, Ripley BD.** 1994 Modern applied statistics with S-Plus. New York: Springer-Verlag.
19. **SAS Institute.** 1990 SAS/STAT user's guide: statistics, 4th ed. Cary: SAS Institute.
20. **Norusis MJ.** 1992 SPSS/PC$^+$: base system user's guide, 5th ed. Chicago: SPSS.
21. **Tobin J.** 1958 Estimation of relationships for limited dependent variables. Econometrika. 26:24–36.
22. **Marson J, Fraser HM, Wickings EJ, Cooper RW, Jouannet P, Meuris S.** 1993 Puberty in the male chimpanzee: time-related variations in circulating inhibin. Biol Reprod. 48:490–494.
23. **McLachlan RI, Matsumoto AM, Burger HG, De Kretser DM, Bremner WJ.** 1988 Relative roles of follicle-stimulating hormone and luteinizing hormone in the control of inhibin secretion in normal men. J Clin Invest. 82:880–884.
24. **Nielsen CT, Skakkebæk NE, Richardson DW, et al.** 1986 Onset of the release of spermatozoa (spermarche) in boys in relation to age, testicular growth, pubic hair, and height. J Clin Endocrinol Metab. 62:532–535.
25. **Orlando C, Santoro S, Calabro C, Vannelli GB, Forti G.** 1992 Spermatic and peripheral venous plasma concentrations of immunoreactive inhibin in prepubertal boys with undescended testis and in pubertal boys with varicocele. Acta Endocrinol (Copenh). 127:385–391.
26. **Simpson BJB, Hedger MP, De Kretser DM.** 1992 Characterisation of adult Sertoli cell cultures from cryptorchid rats: inhibin secretion in response to follicle-stimulating hormone stimulation. Mol Cell Endocrinol. 87:167–177.
27. **Bicsak TA, Vale W, Vaughan J, Tucker EM, Cappel S, Hsueh AJ.** 1987 Hormonal regulation of inhibin production by cultured Sertoli cells. Mol Cell Endocrinol. 49:211–217.
28. **Morris PL, Vale WW, Cappel S, Bardin CW.** 1988 Inhibin production by primary Sertoli cell-enriched cultures: regulation by follicle-stimulating hormone, androgens, and epidermal growth factor. Endocrinology. 122:717–725.
29. **Comhaire FH, Rombauts L, Vereecken A, Verhoeven G.** 1995 Inhibin and steroid responses to testicular stimulation in normal men. Hum Reprod. 10:1740–1744.
30. **McLachlan RI, Finkel DM, Bremner J, Snyder PJ.** 1990 Serum inhibin concentrations before and during gonadotropin treatment in men with hypogonadotropic hypogonadism: physiological and clinical implications. J Clin Endocrinol Metab. 70:1414–1419.
31. **McLachlan RI, Wreford NG, De Kretser DM, Robertson DM.** 1995 The effects of recombinant follicle-stimulating hormone on the restoration of spermatogenesis in the gonadotropin-releasing hormone-immunized adult rat. Endocrinology. 136:4035–4043.
32. **Sharpe RM, Swanston IA, Cooper I, Tsonis CG, McNeilly AS.** 1988 Factors affecting the secretion of immunoactive inhibin into testicular interstitial fluid in rats. J Endocrinol. 119:315–326.
33. **Kosugi S, Van Dop C, Geffner ME, et al.** 1995 Characterization of heterogenous mutations causing constitutive activation of the luteinizing hormone receptor in familial male precocious puberty. Hum Mol Genet. 4:183–188.
34. **Latronico AC, Anasti J, Arnhold IJ, et al.** 1995 A novel mutation of the luteinizing hormone receptor gene causing male gonadotropin-independent precocious puberty. J Clin Endocrinol Metab. 80:2490–2494.
35. **Wierman ME, Beardsworth DE, Mansfield MJ, et al.** 1985 Puberty without gonadotropins: a unique mechanism of sexual development. N Engl J Med. 312:65–72.
36. **Gondos B, Egli CA, Rosenthal SM, Grumbach MM.** 1985 Testicular changes in gonadotropin-independent familial male sexual precocity. Familial testotoxicosis. Arch Pathol Lab Med. 109:990–995.
37. **Chemes HE, Pasqualini T, Rivarola MA, Bergadá C.** 1982 Is testosterone involved in the initiation of spermatogenesis in humans? A clinicopathological presentation and physiological considerations in four patients with Leydig cell tumours of the testis or secondary Leydig cell hyperplasia. Int J Androl. 5:229–245.
38. **Kula K, Romer TE, Wlodarczyk WP.** 1980 Somatic and germinal cells' interrelationship in the course of seminiferous tuble maturation in man. Arch Androl. 4:9–16.
39. **Allenby G, Foster PMD, Sharpe RM.** 1991 Evidence that secretion of immunoactive inhibin by seminiferous tubules from the adult rat testis is regulated by specific germ cell types: correlation between *in vivo* and *in vitro* studies. Endocrinology. 128:467–476.
40. **Plymate SR, Paulsen CA, McLachlan RI.** 1992 Relationship of serum inhibin levels to serum follicle stimulating hormone and sperm production in normal men and men with varicoceles. J Clin Endocrinol Metab. 74:859–864.
41. **Seminara SB, Boepple PA, Nachtigall LB, et al.** 1996 Inhibin B in males with gonadotropin-releasing hormone (GnRH) deficiency: changes in serum concentration after short term physiologic GnRH replacement–a clinical research center study. J Clin Endocrinol Metab. 81:3692–3696.
42. **Majumdar SS, Mikuma N, Ishwad PC, et al.** 1995 Replacement with recombinant human inhibin immediately after orchidectomy in the hypophysiotropically clamped male rhesus monkey (*Macaca mulatta*) maintains follicle-stimulating hormone (FSH) secretion and FSHb messenger ribonucleic acid levels at precastration values. Endocrinology. 136:1969–1977.