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*

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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 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 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)

THE 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 B 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 the method 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 against the inhibin β 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 (Baldon, 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 pg/mL, 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.
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-
Serum inhibin B in boys

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

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

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).

<sup>a</sup> P < 0.0001.
<sup>b</sup> P < 0.001.
<sup>c</sup> P < 0.05.

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 vitro 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-
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.

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

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

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).
We acknowledge the skilful technical assistance of Kirsten Jørgensen and Stine Ehlers Jessen.

References