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Inhibin B, Follicle Stimulating Hormone, Luteinizing Hormone and Testosterone during Childhood and Puberty in Males: Changes in Serum Concentrations in Relation to Age and Stage of Puberty

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Summary

Inhibin B is a gonadal dimeric polypeptide hormone that regulates synthesis and secretion of follicle stimulating hormone (FSH) in a negative feedback loop. The aim of the present study was to determine changes in serum inhibin B, gonadotropins and testosterone concentrations during childhood and puberty in males. We studied the relationship between circulating inhibin B, gonadotropins and testosterone in serum of healthy boys during the first two years of life and then in pubertal development. Using a recently developed two-side enzyme-linked immunosorbent assay (ELISA), inhibin B levels were measured in the serum of 78 healthy boys divided into eleven age groups from birth to the end of pubertal development. In addition, serum levels of gonadotropins and testosterone were measured. Serum inhibin B, gonadotropins and testosterone increased during the first months of postnatal life. A peak in serum inhibin B and gonadotropins concentrations was observed around 3-4 months of age. There was a significant positive correlation between serum inhibin B and gonadotropins and testosterone levels during the first 2 years of life. After this early increase, serum inhibin B, gonadotropins and testosterone levels decreased significantly and remained low until puberty followed by an increase beginning with the onset of puberty. Serum levels of inhibin B reached a peak at stage G3 of puberty. Around midpuberty, inhibin B lost its positive correlation with luteinizing hormone (LH) and testosterone from early puberty, and developed a strong negative correlation with FSH, which persisted into adulthood. We conclude that inhibin B plays a key role in the regulation of the hypothalamic-pituitary-gonadal hormonal axis during male childhood and pubertal development. Inhibin B is a direct marker of the presence and function of Sertoli cells and appears to reflect testicular function in boys.

Key words

Inhibin B • Male • Childhood • Puberty

Introduction

The gonads are usually considered quiescent organs in infancy and childhood until puberty. The reproductive axis is dependent upon endocrine and paracrine acting hormones to regulate follicle maturation and ovulation in females and to maintain sperm production in males. It is well recognized that the hypothalamic-pituitary-gonadal hormonal axis is transiently activated during the first few postnatal months of human life in gender-specific pattern; levels of gonadotropins and sex hormones are elevated (Burger *et al.* 1991, Andersson *et al.* 1998a, Bergada *et al.* 1999). Inhibin is a gonadally derived protein that plays an important role in feedback hormone regulation between the gonads and the pituitary gland. The existence of a non-steroidal regulator of follicle stimulating hormone (FSH) secretion was demonstrated by McCullagh (1932), who named it "the water soluble principle in the testis", that was able to suppress the development of castration cells in the pituitary gland. Inhibin was defined by its endocrine activity in suppressing FSH synthesis and secretion (Burger and Igarashi 1988). Inhibin was isolated from bovine and porcine follicular fluid and the molecular characteristics of this protein were determined (Ling *et al.* 1985, Robertsson *et al.* 1985). In its biologically active form, inhibin is a dimeric glycoprotein composed of an α subunit covalently linked by disulphide bridges either to β_A subunit (inhibin A) or to β_B subunit (inhibin B) (Burger 1993). These protein subunits have a structural similarity to transforming growth factor β_1 (TGF β_1) (Kingsley 1994). The synthesis and secretion of biologically active inhibin A and B are confined to the reproductive system – ovary, testis, placenta and fetoplacental unit (Meunier *et al.* 1988). It has been described that only inhibin B is present in the circulation of men (Burger and Robertson 1997). Men do not appear to secrete inhibin A into the circulation. Sertoli cells are the major source of inhibin B. Inhibin correlates inversely with FSH in adult men. It is widely believed that inhibin B is the physiological endocrine regulator of FSH in men (Hayes *et al.* 1998). The functional significance of temporary activation of hypothalamic-pituitary-gonadal hormonal axis during childhood is still not clear.

The aim of present study was to evaluate secretion of the biologically active inhibin B, FSH, luteinizing hormone (LH) and testosterone and their changes during male childhood and puberty. We investigated the relationships between inhibin B and FSH, LH and testosterone in relation to age and stage of puberty in healthy boys. This study was designed to determine whether inhibin B is correlated with the pubertal development in boys. The correlation would suggest that inhibin B could contribute to the changes in FSH, LH and testosterone that occur in childhood and puberty.

Methods

Changes of inhibin B, gonadotropin and testosterone secretion were studied in serum obtained from 78 boys. Serum inhibin B, gonadotropins and testosterone were measured from the day of birth to the final genital stage of puberty. The boys were healthy and they all followed a normal growth pattern and pubertal development. The pubertal developmental stage was recorded according to Tanner (1975) (Table 1). The study was performed in accordance with Helsinki Declaration.

Table 1. Tanner stages of puberty

G1	Preadolescent. Testes, scrotum and penis are about the same size and proportion as in early childhood.
G2	Enlargement of scrotum and testes. Skin of scrotum reddens and changes in texture. Little or no enlargement of penis at this time.
G3	Enlargement of penis which occurs at first mainly in length. Further growth of testes and scrotum.
G4	Increased size of penis with growth in breadth and development of glans. Testes and scrotum larger; scrotal skin darkened.
G5	Genitalia are adult in size and shape.

Venous blood samples were taken from all the boys between 9 and 11 a.m. After clotting, the serum was separated by centrifugation and stored at –70 °C prior to analysis. Serum inhibin B was measured in duplicate by two-side enzyme-linked immunosorbent assay (ELISA), (Oxford Bio-Innovation Inhibin-B Immunoassay Kit, Serotec Ltd, UK). Aliquots (100 µl) of standards or patient samples were added to tubes with 50 µl 6 % SDS (detergent) and then incubated (pre-treated) at 100 °C in a boiling water bath for 3 min. After cooling, 100 µl assay diluent of inhibin B was added to each tube. This is followed by 50 µl 6 % hydrogen peroxide. The tubes were then incubated for 30 min at a room temperature.

This pretreatment of the samples modifies, by oxidation, methionine residues in the epitope for the capture antibody, thereby improving the affinity of the reaction, and enhances the specificity and sensitivity of the ELISA. It also allows analysis of hemolyzed samples. An 80 µlaliquot of each treated sample was added to the wells of the microtiter plate. The wells were covered and then incubated overnight at room temperature. The wells were washed by filling to the top with inhibin washing buffer, then decanted or aspirated thoroughly. This was repeated three times. Following this, 50 µl inhibin detection antibody was added to each well. The plate was covered, sealed and then incubated for 3 h at room temperature. The plate was washed eight times with inhibin washing buffer as above. The wells were then washed three times, each time the inhibin buffer was allowed to stand for 15 min. After washing 50 µl substrate solution was added to each well. Following this, the plate was sealed and incubated at room temperature for 1 h. After incubation, 50 µl amplifier solution was added, and then the plate was gently agitated at room temperature and 50 µl stop solution was added to each well to stop the color reaction. The optical density was measured at 490 nm (Photometer MRX Murex). The sensitivity of the assays was 15 pg/ml. The intra- and interassay coefficients of variation were below 7 %.

Table 2. Distribution of serum inhibin B, gonadotropins and testosterone levels in boys.

Group Number of boys (Age)	**Inhibin B (pg/ml)**	**FSH (UI/l)**	**LH (UI/l)**	**Testosterone (nmol/l)**
A *n = 6*	84.4±13.2	0.56±0.07	0.17±0.05	1.38±0.16
(1-7 days)	(49.2-133.7)	(0.37-0.84)	(0.12-0.34)	(0.45-1.97)
B *n = 5*	146.4±28.6	0.67±0.14	0.19±0.04	0.65±0.12
(1 week-2 months)	(60.8-240.1)	(0.42-1.36)	(<0.10-0.36)	(0.18-1.03)
C *n = 6*	313.9±11.1	1.83±0.14	0.89±0.21	1.02±0.23
(3-4 months)	(270.2-348.7)	(1.48-2.46)	(0.32-1.62)	(0.31-1.62)
D *n = 5*	198.0±13.4	0.79±0.24	0.19±0.05	0.07±0.02
(5-11 months)	(149.3-240.9)	(0.15-1.50)	(<0.10-0.39)	(<0.02-0.13)
E *n = 5*	101.4±9.3	0.45±0.07	0.11±0.008	0.03±0.007
(1-2 years)	(67.8-128.4)	(0.21-0.66)	(<0.10-0.15)	(<0.02-0.06)
F *n = 5*	64.9±7.5	0.25±0.07	0.14±0.02	0.04±0.008
(3-4 years)	(52.4-93.0)	(0.06-0.42)	(<0.10-0.21)	(<0.02-0.07)
G *n = 5*	43.4±8.1	0.31±0.10	0.17±0.04	0.15±0.04
(5 years-puberty onset)	(21.6-64.4)	(<0.05-0.55)	(<0.10-0.33)	(0.06-0.24)
H *n = 11*	61.5±6.8	0.42±0.04	0.21±0.04	0.27±0.05
(Tanner stage G1)	(35.4-99.6)	(0.20-0.58)	(<0.10-0.44)	(0.07-0.55)
I *n = 8*	129.8±11.8	0.85±0.18	0.61±0.18	0.97±0.16
(Tanner stage G2)	(86.9-194.5)	(0.21-1.48)	(0.12-1.43)	(0.44-1.85)
J *n = 12*	174.5±9.3	1.46±0.20	1.23±0.17	3.73±0.58
(Tanner stage G3)	(132.5-230.5)	(0.49-2.63)	(0.45-2.39)	(1.52-7.21)
K *n = 10*	152.0±9.0	2.30±0.27	3.17±0.43	12.74±0.96
(Tanner stages G4 and G5)	(101.8-189.2)	(0.93-3.65)	(1.05-5.54)	(8.9-18.1)

Data are given as mean ± S.E.M. and ranges (min and max).

FSH, LH and testosterone were measured in serum samples. Serum FSH and LH levels were determined by chemiluminescent microparticle immunoassay (CMIA) technology (Architect System, Abbott Laboratories, USA). The detection limit was 0.05 IU/l for FSH and 0.1 IU/l for LH. Serum testosterone levels were measured by radioimmunoassay (RIA) Reagent Kit (Immunotech, Reagent Kit, France) with a sensitivity 0.02 nmol/l.

All values were expressed as means ± S.E.M. Analysis of group means was performed using ANOVA analysis. Correlation coefficients were determined by regression. In all cases, the $p<0.05$ value was considered significant.

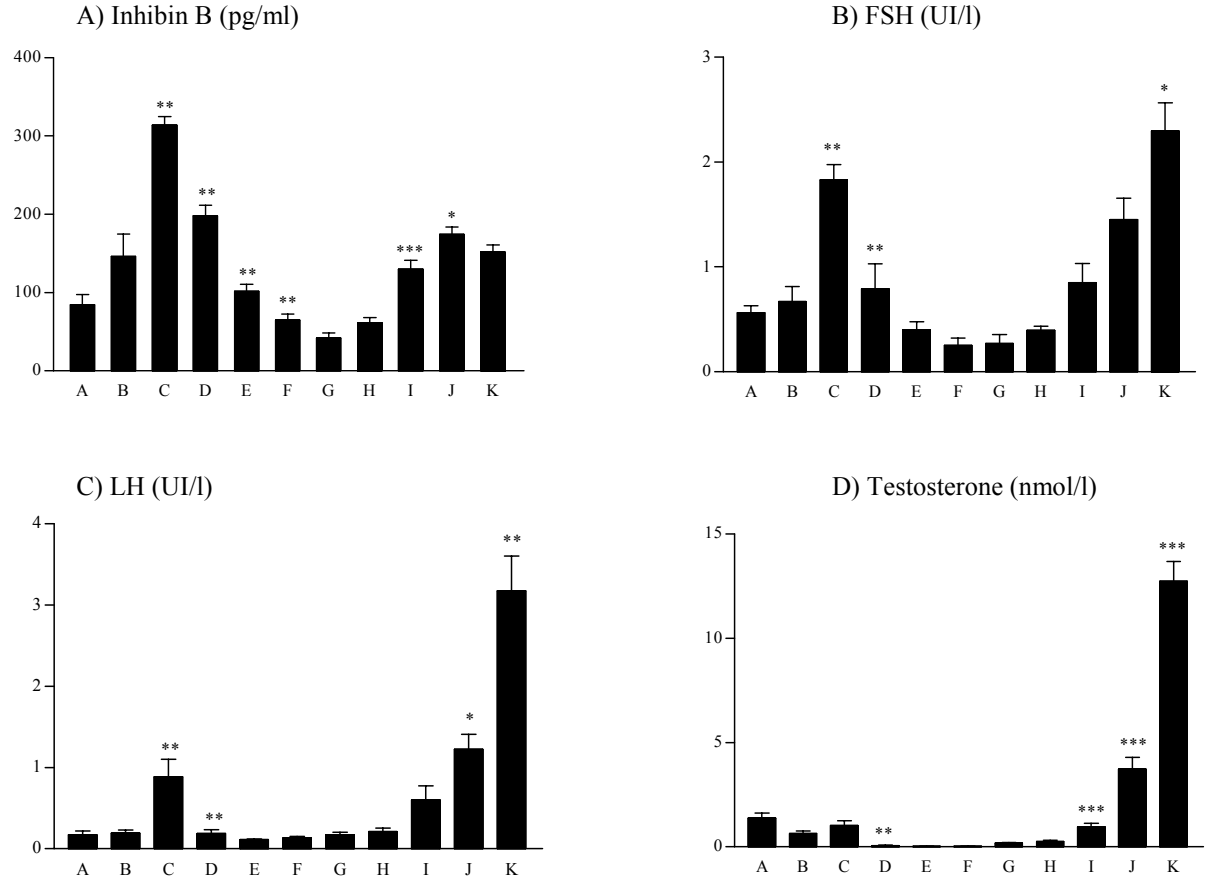

Fig. 1. *Individual serum inhibin B (Fig. 1A), FSH (Fig. 1B), LH (Fig. 1C) and testosterone (Fig. 1D) concentrations (mean ± S.E.M.) in boys during childhood and across the pubertal development. Age categories: A (n = 6), 1-7 days; B (n = 5), 1 week-2 months; C (n = 6), 3-4 months; D (n = 5), 5-11 months; E (n = 5), 1-2 years; F (n = 5), 3-4 years; G (n = 5), 5 years to the onset of puberty, H (n = 11), Tanner stage G1; I (n = 8), Tanner stage G2; J (n = 12), Tanner stage G3; K (n = 10), Tanner stage G4 and G5. Asterisks above the bars indicate statistically significant differences of means with respect to previous age categories (by Mann-Whitney U test): *, p<0.05; **, p<0.01; ***, p<0.001.*

Results

Serum inhibin B, FSH, LH and testosterone concentrations

The mean and range of serum inhibin B, gonadotropins and testosterone levels are summarized in Table 2. The age-related changes in serum inhibin B, gonadotropins and testosterone concentrations during male childhood and puberty are shown in Figure 1. Following birth, inhibin B and gonadotropins started to increase significantly during the first months, with the highest peak of inhibin B (313.9±11.1 pg/ml), FSH (1.83±0.14 IU/l) and LH (0.89±0.21 IU/l) levels found at 3 to 4 months of age. A rise in testosterone levels was observed during the first months of postnatal life. After this early increase, serum gonadotropins and testosterone levels decreased rapidly to barely detectable levels during the first year and remained low until puberty. Serum inhibin B concentrations decreased gradually over a period from 4 months to two years of age. In contrast to testosterone, inhibin B remained well measurable during childhood. At the onset of puberty, the serum levels of all four measured hormones started to rise with similar rates of pubertal progression. Serum inhibin B concentrations increased significantly between pubertal stage G1 and G3 of puberty, reached a peak in stage G3 of puberty (174.5±9.3 pg/ml), and decreased non-significantly thereafter. From stage G3 of puberty, inhibin B levels were relatively constant and remained at similar concentrations in stage G4 and G5 of puberty. Mean

serum testosterone concentrations increased progressively throughout puberty ($p<0.001$). Serum levels of FSH and LH increased with advancing pubertal stage. From G3 to G4-5 of puberty, mean serum FSH and LH levels changed significantly ($p<0.05$ and $p<0.01$, respectively).

Correlation of inhibin B to gonadotropins and testosterone

Correlation coefficients between serum inhibin B and FSH, LH and testosterone are shown in Table 3. There was a significant positive correlation between serum inhibin B and LH ($r = 0.65$, $p<0.001$), FSH ($r = 0.83$, $p<0.001$) and testosterone ($r = 0.58$, $p<0.001$) levels during the first two years of life. In the pubertal stage G1, there was a significant positive correlation between inhibin B and LH. In the pubertal stage G2, strong positive correlations between inhibin B and LH or testosterone were observed. At stages G1 and G2 of puberty, no significant correlation between inhibin B and FSH was found. From stage G3 of puberty onward, serum inhibin B concentrations correlated negatively with FSH (G3: $r = –0.51$, $p<0.05$; G4: $r = –0.57$, $p<0.05$), but no significant correlation was found between inhibin B and LH and testosterone.

Table 3. Pearson correlation coefficients between serum inhibin B levels, gonadotropins and testosterone.

Age	Inhibin B vs. FSH	Inhibin B vs. LH	Inhibin B vs. Testosterone
0-2 years	0.83 ***	0.65 ***	0.58 ***
G1	0.18	0.55 *	0.50
G2	–0.30	0.62 *	0.64 *
G3	–0.51 *	0.17	0.25
G4-G5	–0.57 *	–0.11	0.19

*$p<0.05$; ** $p<0.01$; *** $p<0.001$.*

Discussion

The present study was undertaken to investigate the changes occurring in serum inhibin B, gonadotropins and testosterone concentrations during childhood and pubertal development in healthy boys. Changes in inhibin B concentrations were correlated with gonadotropins and testosterone. We found high levels of inhibin B, FSH, LH and testosterone during the first months of postnatal life, when the hypothalamic-pituitary-gonadal hormone axis is transiently activated (Forest *et al.* 1973, Burger *et al.* 1991, Andersson *et al.* 1998a, Bergada *et al.* 1999). Peak levels of inhibin B and gonadotropins were observed around 3-4 months of age. After this peak, the elevated levels of inhibin B persisted for much longer time than increased levels of FSH, LH, and testosterone. Serum inhibin B concentrations decreased more gradually and remained measurable during childhood.

Serum levels of gonadotropins and testosterone rapidly declined to normal very low childhood levels within 5-12 months after birth. We found that an increment of inhibin B concentrations was in a close positive correlation with FSH, LH and testosterone during the first 2 years of life. A higher correlation between immunoreactive inhibin and LH or FSH was found during the first two years of life (Burger *et al.* 1991). In contrast, no correlation between inhibin B and FSH was found by Andersson *et al.* (1998a) and by Bergada *et al.* (1999). These data suggest that gonadotropins and testosterone may be directly or indirectly involved in the regulation of inhibin B production from Sertoli cells and Leydig cells. Early stimulation of the testis with gonadotropins is important for the Sertoli cell, germ cell and Leydig cell development and proliferation (Andersson *et al.* 1998b, Main *et al.* 2000). In humans the first year of postnatal life is associated with an increase in the total number of Sertoli cells (Cortes *et al.* 1987). The increase of inhibin B during postnatal period may be related to the active proliferation of the Sertoli cells. Dynamic changes in Leydig cell and germ cell number occur in the postnatal testis (Müller and Skakkebaek 1984, Codesal *et al.* 1990). The total number of germ cells and Leydig cells increases until three months of age, followed by a rapid decrease. There is a transition of the gonocytes in the testis during this time. The Leydig cell development stimulated by the

elevated LH levels is responsible for the transient elevated testosterone levels. It has been suggested that the total number of Sertoli cells is important for spermatogenic potential later in life.

The hypothalamic-pituitary-gonadal hormone axis is reactivated at the onset of puberty (Lee *et al*. 1974). We found that inhibin B increased with pubertal progression, reached a peak in stage G3 of puberty. We also observed a rise in serum gonadotropins and testosterone concentrations during pubertal development. The correlation of inhibin B to FSH, LH and testosterone changed during puberty in agreement with previous studies (Crofton *et al.* 1997, Raivio *et al.* 1998). In early puberty, serum inhibin B concentrations have a positive association with testosterone and LH but little relationship to FSH. It might suggest that LH (*via* testosterone) plays an important role in the second period of postnatal Sertoli cell proliferation and in the stimulation of inhibin B production in early puberty. In contrast, around midpuberty (G3), inhibin B lost its positive correlation with LH and testosterone but developed a strong negative correlation with FSH, which persisted into adulthood. Inhibin B is the physiological regulator of FSH levels in men and reflects Sertoli cell number and the maturational state achieved by the sperm in males (Ramaswamy *et al.* 1999).

In conclusion, the postnatal and pubertal gonadotropin peak associated with increased inhibin B levels, is important for Sertoli cell, Leydig cell, and germ cell proliferation and maturation. Inhibin B plays a key role in the regulation of the hypothalamic-pituitary-gonadal hormonal axis during male childhood and pubertal development. Inhibin B is a direct marker of the presence and function of Sertoli cells. Further information about the hypothalamic-pituitary-gonadal hormone axis regulation and determination of serum inhibin B appears to be important in the evaluation of gonadal disorders in children.

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Reprint requests

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