LONG-TERM TREATMENT OF CENTRAL PRECOCIOUS PUBERTY WITH A LONG-ACTING ANALOGUE OF LUTEINIZING HORMONE–RELEASING HORMONE

Effects on Somatic Growth and Skeletal Maturation

M. JOAN MANSFIELD, M.D., DONNA E. BEARDSWORTH, M.A., JACQUELYN S. LOUGHLIN, M.D., JOHN D. CRAWFORD, M.D., HANS H. BODE, M.D., JEAN RIVIER, PH.D., WYLIE VALE, PH.D., DAVID C. KUSHNER, M.D., JOHN F. CRIGLER, JR., M.D., AND WILLIAM F. CROWLEY, JR., M.D.

Abstract The gonadotropin-releasing hormone–like agonist D-Trp^6-Pro^9-NEt-LHRH (LHRH$_a$) has been shown to induce a reversible short-term suppression of gonadotropins and gonadal steroids in patients with central precocious puberty. Since accelerated statural growth and bone maturation are clinical features of precocity not well controlled by conventional therapies, we examined the effects of prolonged LHRH$_a$ therapy for 18 consecutive months on growth and skeletal maturation in nine girls with neurogenic or idiopathic precocious puberty. Suppression of gonadotropin pulsations and gonadal steroids was maintained in all subjects. Growth velocity fell from a mean rate (±S.E.M.) of 9.35±0.64 cm per year during the 19 months before treatment to 4.58±0.60 cm per year during treatment ($P<0.001$). Bone age advanced a mean of 9.4±2.3 months during treatment. These changes resulted in a mean increase of 3.3 cm in predicted height ($P<0.01$).

Complete suppression of the pituitary–gonadal axis can be maintained by LHRH$_a$ therapy, resulting in slowing of excessively rapid growth and skeletal maturation and in increased predicted adult height in girls with precocious puberty. (N Engl J Med 1983; 309:1286-90.)

IN the child with precocious puberty in the absence of a correctable anatomic lesion, the goal of therapy is suppression of gonadal function in order to arrest or reverse secondary sexual development, decrease the linear growth rate to a normal prepubertal velocity, and slow skeletal maturation to prevent short stature caused by premature epiphyseal fusion. To date, no therapeutic approach has achieved all these objectives. Treatment with progestins and antiandrogens has controlled breast development and menstruation in the majority of female subjects but not in all of them[1-7]; antiandrogens have met with similar limited success in male subjects.[2-4,6,7] However, neither of these treatments has been unequivocally successful in slowing growth rates or controlling bone maturation.[1,3,5,6,8-11] In addition, the side effects of these agents, such as adrenal suppression secondary to their glucocorticoid-like action and induction of cytogenetic defects, have limited their usefulness.[6,8,10,12-16] Thus, a new treatment approach offering a more complete and selective suppression of the neuroendocrine–gonadal axis would be welcome.

In previous short-term studies, we have shown that a potent agonist of the gonadotropin-releasing hormone, D-Trp^6-Pro^9-NEt-LHRH (LHRH$_a$), can decrease the responsiveness of the pituitary to luteinizing hormone–releasing hormone (LHRH) and thereby reversibly suppress pubertal gonadotropin and sex-steroid levels over an eight-week period in patients with idiopathic precocious puberty.[17,18] We now report the effects of LHRH$_a$ on secondary sexual development, statural growth, skeletal maturation, and predicted adult height in nine girls with central (idiopathic or neurogenic) precocious puberty who were treated continuously for 18 months.

From the Vincent Memorial Research Laboratories and the Departments of Gynecology, Medicine, Radiology, and Children's Services, Massachusetts General Hospital; the Division of Adolescent and Young Adult Medicine and Endocrinology, Department of Medicine, Children's Hospital; and the Departments of Gynecology, Medicine, Radiology, and Pediatrics, Harvard Medical School, Boston. Address reprint requests to Dr. Crowley at Vincent Memorial Research Laboratories, Massachusetts General Hospital, Boston, MA 02114.

Supported in part by grants (RR-1066 and RR-00128) from the National Institutes of Health.

Presented in part at the Society for Pediatric Research Meetings, Washington, D.C., May 11, 1982.

METHODS

Patients

In all nine patients the diagnosis of central precocity was made after ovarian, adrenal, or clinically active central-nervous-system disease was excluded and serum pituitary gonadotropins in the pubertal range were documented. Six girls (Patients 1 through 6) had idiopathic precocious puberty; three others (Patients 7 through 9) had had central-nervous-system lesions (cerebellar astrocytoma treated by surgical removal and subtotal radiation, congenital toxoplasmosis, and perinatal asphyxia) before the onset of their precocious sexual development (neurogenic precocity).

The radiologic and endocrinologic criteria for inclusion in the study have been described previously.[17,18] All nine patients were less than nine years of age before treatment, and three were postmenarcheal. Sexual maturation was evidenced by Tanner Stages II to IV of sexual development.[19] Accelerated linear growth had occurred in seven of eight patients whose pretreatment growth velocities were known, and bone ages were advanced a mean of 4.0±0.6 years beyond chronologic ages. Patient 4, whose growth rate was not above that expected for her prepubertal growth centile and chronologic age, had a bone age of 14 years and had passed her peak growth velocity when LHRH$_a$ therapy was initiated.[20] Two patients (Patients 5 and 6) had received treatment for sexual precocity before LHRH$_a$ therapy: Patient 5 had received a three-month course of medroxyprogesterone acetate followed by five months of treatment with cyproterone acetate, which was discontinued just before our study; Patient 6 had been treated for 15 months with intramuscular medroxyprogesterone acetate, which was terminated eight weeks before LHRH$_a$ therapy. Treatment with these two agents had been stopped because of continued rapid skeletal growth and maturation.

Protocol

Informed consent was obtained from one or both parents before each patient was enrolled in the study. The patients were examined at the Clinical Research Center of the Massachusetts General Hospital before and after 3, 6, 12, and 18 months of treatment. The protocol employed has been described previously.[17,18] In brief, after a detailed pretreatment evaluation, the LHRH analogue D-Trp^6-Pro^9-NEt-LHRH (LHRH$_a$) was administered daily by subcutaneous injections in doses of 4 to 8 μg per kilogram of body weight; the dose was adjusted until estradiol concentrations and maturation indexes were completely suppressed to prepubertal levels. Vaginal smears for calculation of maturation-index scores were obtained

before treatment and biweekly during therapy. Pubertal development was documented photographically and evaluated clinically according to the system of staging developed by Tanner.[19] Height was measured with a Harpenden stadiometer by the same examiner at each admission, and radiographs of the left hand and wrist were obtained for assessment of bone age before treatment and after 6, 12, and 18 months of therapy. On every admission, blood samples for determination of levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were obtained at 20-minute intervals over four hours during the night (10 p.m. to 2 a.m.) and during the day (10 a.m. to 2 p.m.) in order to study gonadotropin pulsations. The daytime study period was followed by an LHRH test (2.5 μg of the natural-sequence peptide per kilogram, given subcutaneously). Estradiol and dehydroepiandrosterone sulfate levels were assayed in pools made up from the samples drawn for the four-hour gonadotropin-pulsation studies.

Laboratory Methods and Statistical Analysis

Vaginal-maturation-index scores were derived by Meisels' method of assigning superficial cells a score of 1, intermediate cells 0.5, and parabasal cells 0 and summing the values for 100 cells.[21] Growth velocity was estimated by least-squares rectilinear regression analysis of all values for a patient's height as measured during the 17 to 21 months before therapy and the 17 to 22 months during therapy. Pretreatment and post-treatment growth velocities were compared by analysis of variance. Bone age was determined according to the standards of Greulich and Pyle[22] by an experienced pediatric radiologist (D.C.K.), who had no information concerning the date of onset of precocity or the duration of therapy. All films were evaluated in chronologic sequence. Adult height was predicted with the tables of Bayley and Pinneau.[23]

Serum gonadotropin, estradiol, and dehydroepiandrosterone sulfate levels were assayed by previously described methods.[24,25] Gonadotropin pulsations were identified according to the criteria of Santen and Bardin.[26] Response to the LHRH stimulation test was calculated by subtracting the base-line gonadotropin value from the peak value measured during the three hours after LHRH administration. Pretreatment gonadotropin levels, estrogen levels, and maturation-index scores were compared with post-treatment values by analysis of variance, and pretreatment predicted adult height was compared with post-treatment height by the Wilcoxon matched-pairs signed-ranks test for nonparametric data. All data are expressed as means ±S.E.M.

Monitoring for untoward effects of the medication was accomplished by careful review of the history, repeated physical examinations, blood samples for determination of routine hepatic, hematologic, and renal studies, and ultrasonic evaluation of the gonads and adrenals during each hospital admission during and after $LHRH_a$ therapy. Serum was tested for LHRH and $LHRH_a$ antibodies before treatment and after 10 to 12 months of therapy in all patients (Sandow J: personal communication).

Results

Sexual Development

The involution of secondary sexual characteristics previously reported after short-term therapy[17,18] was maintained throughout the 18 months of treatment. Menstruation ceased in all menstruating subjects. The amount of breast tissue decreased or stabilized in all patients, and breast development regressed by a full Tanner stage in two patients (both had been in Tanner Stage III or less before treatment). Pubic hair diminished in two patients whose dehydroepiandrosterone sulfate levels remained preadrenarcheal (i.e., below 60 μg per deciliter), but increased by a full Tanner stage in two of the five patients who had had levels above 60 μg per deciliter before treatment. $LHRH_a$ therapy did not appear to interfere with the progression of established adrenarche; dehydroepiandrosterone sulfate levels rose in four of five patients who had had levels above 60 μg per deciliter before therapy.

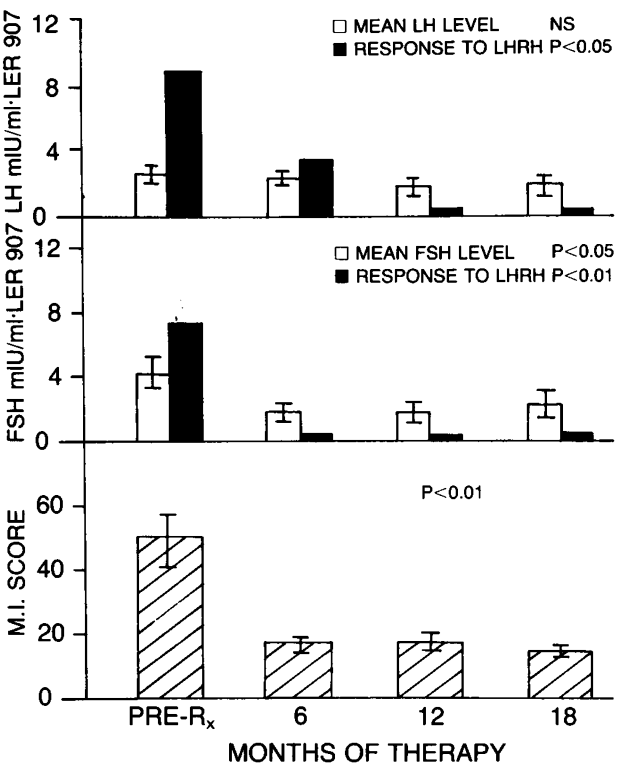

Figure 1. Effect of $LHRH_a$ Therapy on Maturation-Index (M.I.) Scores and Levels of Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH) in Nine Girls with Precocity.

Values are expressed as means ±S.E.M. Statistical comparisons were made between values before therapy (PRE-Rx) and values at each point during therapy.

Basal levels of FSH and LH are represented as open bars, and the peak responses of these gonadotropins to LHRH as solid bars. Levels are expressed in terms of the reference preparation LER 907 (1 mg = 60 IU of LH and 20 IU of FSH of the Second International Reference Preparation of human menopausal gonadotropin) obtained from the National Pituitary Agency.[24]

Before treatment, all patients had serum gonadotropin levels that were elevated into the pubertal ranges, with detectable nocturnal gonadotropin pulsations and a brisk rise in gonadotropin levels in response to LHRH administration. Initiation of treatment with $LHRH_a$ did not change mean base-line levels of LH (2.53±0.43 vs. 1.92±0.24 mIU per milliliter; F = 1.11, df = 3.32, P not significant) (Fig. 1). However, spontaneous LH and FSH pulsations were abolished throughout the treatment period in all patients. The LH response to LHRH fell significantly, from 8.86±2.11 mIU per milliliter before treatment to 0.32±0.14 mIU per milliliter after 18 months of therapy (F = 4.27, df = 3.32, P<0.05). Mean FSH levels fell from 4.08±0.88 mIU per milliliter before treatment to 2.39±0.56 mIU per milliliter at 18 months of therapy (F = 3.28, df = 3.32, P<0.05). FSH responses to LHRH were also abolished during $LHRH_a$ treatment (pretreatment vs. post-treatment values,

7.32±2.27 vs. 0.43±0.31 mIU per milliliter; F = 9.03, df = 3.32, P<0.005).

Before $LHRH_a$ therapy, five of seven previously untreated children had serum estradiol levels above 20 pg per milliliter (normal range during early follicular phase, 23 to 56 pg per milliliter in our laboratory), and all nine had estrogenization of their vaginal smears. In the two patients who had had previous therapy, estrogen levels and maturation indexes were still suppressed at the start of $LHRH_a$ therapy, even though gonadotropin pulsations and pubertal LHRH responses were present in both patients. Estradiol levels fell from a pretreatment mean of 28.4±3.7 pg per milliliter to less than 20 pg per milliliter (the normal prepubertal level) in all subjects after three months of therapy (F = 3.65, df = 3.30, P<0.05). Vaginal-maturation scores declined from a mean value of 50.7±6.6 to 14.6±0.56 at 18 months of therapy (F = 9.06, df = 3.30, P<0.005). The normal prepubertal maturation-index score[27] is less than 36. After three months of therapy, four of nine patients had complete suppression of maturation-index scores (scores below 9), which was maintained throughout the 18 months of treatment with a dose of 4 μg of $LHRH_a$ per kilogram. Four other subjects required 8 μg per kilogram per day for complete suppression. A single patient (Patient 9), who received 8 μg per kilogram, had a continued estrogen effect as demonstrated by vaginal-maturation scores. This effect notwithstanding, breast development regressed, estradiol levels were suppressed to less than 20 pg per milliliter, and both gonadotropin pulsations and responses to LHRH testing were abolished throughout the treatment period.

Growth Velocity and Skeletal Maturation

Mean growth velocity in the six previously untreated patients for whom data on pretreatment growth were available was 9.35±0.64 cm per year during the 19 months before $LHRH_a$ therapy (the normal pubertal peak height velocity for girls averages 9.0±0.16 cm per year).[20] During the 18 months of therapy, the mean growth rate in these six girls fell significantly, to 4.58±0.60 cm per year (F = 12.07, df = 1.10, P<0.005) (Fig. 2).

For each of these six patients, predicted growth velocity was calculated from the Tanner growth-velocity tables[20] both for chronologic age and developmental (skeletal) age, by using the prepubertal-height centile for four patients and mean parental-height centile for the two patients who had entered puberty in their first year of life. The mean expected growth velocity as related to the patients' chronologic ages was 5.75±0.28 cm per year, and the mean expected growth velocity according to their developmental ages was 5.38±0.38 cm per year.

Growth velocity declined during the first six months of $LHRH_a$ therapy, stabilizing at a mean rate of 3.35±0.32 cm per year after 6 to 18 months of treatment. During the 18 months of therapy the two patients who had been given other treatment during the 19 months before starting $LHRH_a$ had declines in growth rates similar to those in the previously untreated patients (pretreatment vs. post-treatment values, 9.66±0.25 vs. 5.20±0.33 cm per year).

Bone ages advanced 9.4±3.3 months during the 18 months of $LHRH_a$ therapy. The slowing of bone maturation relative to chronologic age is shown in Figure 3. It is evident that before $LHRH_a$ therapy, bone age had progressed more rapidly than chronologic age in all patients. In contrast, during $LHRH_a$ therapy, bone ages advanced less than chronologic ages in eight patients — i.e., patients began to "catch up" to their bone ages. The two patients with the greatest advance in bone age during $LHRH_a$ therapy (17 months in Patient 3, and 22 months in Patient 8) were the only patients with dehydroepiandrosterone sulfate levels higher than 100 μg per deciliter before therapy. Gonadotropin pulsations, estradiol levels, and maturation-index scores were well controlled in both these patients at a dose of 4 μg of $LHRH_a$ per kilogram.

Predicted Height

In the seven previously untreated patients, predicted adult height increased from a mean of 146.6±4.4 cm before therapy to a mean of 149.9±6.3 cm after 18 months of treatment with $LHRH_a$ — an average increase of 3.3 cm (P<0.01). The two patients who had received medroxyprogesterone or cyproterone before $LHRH_a$ had gains of 6.2 cm and 3.9 cm, respectively.

Adverse Reactions

No families elected to discontinue $LHRH_a$ therapy during the treatment period, and no adverse psycho-

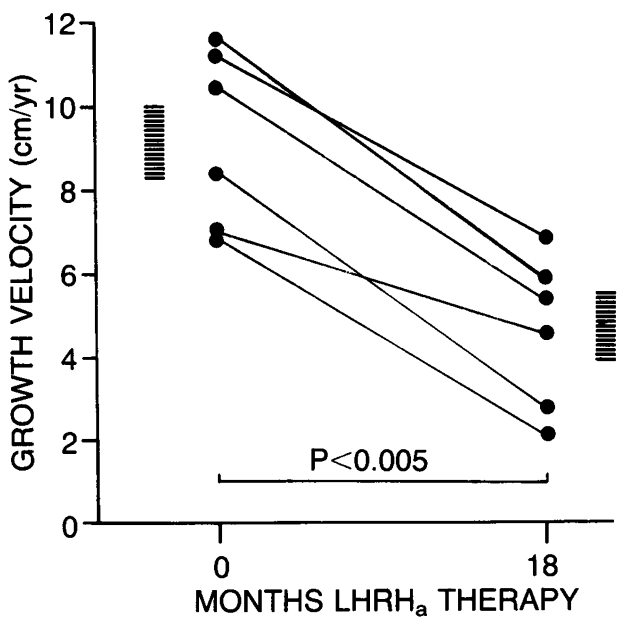

Figure 2. Effect of $LHRH_a$ Therapy on Growth Velocities of Six Previously Untreated Girls.

Data on growth before and during the 18 months of therapy were available for these six subjects. The hatched bars indicate means ±S.E.M. before and during therapy.

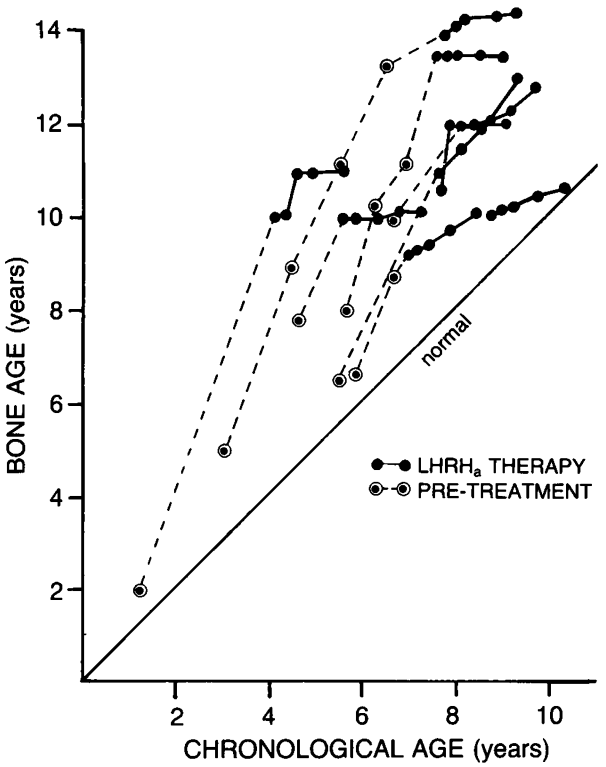

Figure 3. Bone Age Plotted against Chronologic Age in Nine Girls. Data on bone age before (PRE-TREATMENT) and during ($LHRH_a$ THERAPY) hormone administration were available for seven subjects. The line marked "normal" represents the synchronous progression characteristic of normal development for bone age and chronologic age.

social effects were noted by the investigators, parents, or patient advocates during the study. Formal behavioral and psychological testing was not undertaken in this study. Parents, however, regularly commented that the excessive activity and emotional lability that had characterized their child's behavior before treatment decreased during $LHRH_a$ therapy, thereby reducing behavioral problems at home and at school.

No evidence of secondary drug failure occurred with $LHRH_a$, and no antibodies to LHRH or $LHRH_a$ were demonstrable in any subject during $LHRH_a$ administration. No major allergic reactions were encountered; occasional transient redness at the injection site was reported by some patients. There were no significant changes in the results of the routine tests used to monitor hematologic, hepatic, renal, and gonadal function. One patient had a slight elevation of serum aspartate aminotransferase (40 IU per liter; normal, 5 to 30) and alanine aminotransferase (28 U per liter; normal, 1 to 21) before therapy, which persisted during treatment. There was no clinical evidence of liver dysfunction, and no change in other liver-function test results.

Reversibility of Drug Effect

In one patient (Patient 9), $LHRH_a$ therapy was terminated after 18 months when treatment goals were met (a normal age for puberty and a predicted height of more than 152 cm). This patient was restudied 6 and 12 months after therapy; these studies showed that secondary sexual development had progressed, gonadotropin pulsations and LHRH responses had returned to the pubertal pattern, estradiol levels had risen to 55 pg per milliliter, and bone age had advanced 22 months in one year. The other eight patients are still receiving therapy. Subsequently, in two other patients not involved in this study, the normal clinical and biochemical features of puberty developed after $LHRH_a$ therapy for precocity.

Discussion

The present study demonstrates that the previously recognized[17,18] $LHRH_a$-induced pituitary desensitization and suppression of pubertal gonadotropins and sex-steroid secretion, achieved in short-term treatment of children with precocity, can be maintained during 18 months of continuous treatment. In addition, during the prolonged quiescence of pituitary gonadotropin secretion induced by $LHRH_a$ administration, skeletal maturation slowed dramatically. This combination of continuing growth, although at a decreased velocity, with slowed bone maturation during the 18 months of treatment resulted in a mean gain of 3.3 cm in the projected final height of these children. These results are especially noteworthy since in untreated precocious puberty, bone age usually advances more rapidly than height age, resulting in a decrease in estimates of predicted adult height with time.[28]

It is of interest that the growth velocity of our patients declined during the 18-month treatment period to a mean velocity of 3.3 cm per year during the last 12 months of therapy. This rate is below the expected prepubertal rate of growth (5.75 cm per year).[20] This slowing of growth was greatest in the two patients whose bone ages were 13.5 and 14 years at the start of therapy. Progressive slowing of growth rates would be expected in these patients, whose skeletal maturation at the beginning of therapy had already passed the point of peak height velocity. However, the growth rates of all patients were lower than the rates expected for their prepubertal height centiles or their advanced maturational age (bone age) during the 6- to 18-month treatment period. The slower rate of growth observed from the 6th to the 18th month of therapy may have been due to a pause in growth as patients returned to genetically determined growth trajectories in the absence of sex steroids. Another possible explanation may be that some sex steroids are required to maintain a growth rate appropriate for chronologic age in the advanced state of skeletal maturation induced by prior precocity. Longer-term data on growth in a larger group of patients will be required to distinguish between these possibilities.

Although no serious adverse effects of $LHRH_a$ therapy were seen in this study, concerns about the use of a relatively new medication in a pediatric population have been raised in response to previous reports.[29]

The return of puberty after therapy in the patients with precocity in whom $LHRH_a$ was discontinued after 18 months is consistent with long-term data in adults[30-32] and animals[33] and short-term data in children[17,18] showing complete reversibility of gonadal suppression after analogue therapy. Long-term follow-up of the patients in this study is planned.

In this paper we have reported the results of $LHRH_a$ treatment of girls with precocity; our findings in male subjects with premature activation of the pituitary–gonadal axis treated with $LHRH_a$ have been similar.

In these nine girls, the selective, reversible suppression of gonadotropin and sex-steroid secretion induced by $LHRH_a$ therapy was sufficient to cause a regression of secondary sexual characteristics, cessation of menstruation, return to age-appropriate behavior, decrease of growth velocity, and slowing of skeletal maturation. Moreover, it appears that these last two effects lead to an increase in adult height, bringing these patients closer to their genetic height potential.

The ability of $LHRH_a$ to inhibit gonadarche selectively and to slow bone maturation makes it a unique therapeutic probe into the pubertal process, offering a novel approach to the study of the interrelations between gonadarche and other facets of puberty such as skeletal growth, bone maturation, and adrenarche.

We are indebted to Dr. Robert Neer and the staff of the Clinical Research Center at the Massachusetts General Hospital for their assistance; to Dr. Jurgen Sandow for his determination of LHRH and $LHRH_a$ antibodies; to Patricia Meara for calculation of growth velocity by computer program; to Judy Donnelly, Carolyn Albers, and Steven Trigilio for special technical assistance; to Nancy Delaney-Perry and Lyn Russo for helping prepare the manuscript; and to the referring physicians, Drs. Wayne Moore, Olga Tatpati, and Jay Orsen, for their invaluable help with the study.

References

1. Kaplan SA, Ling SM, Irani NG. Idiopathic isosexual precocity: therapy with medroxyprogesterone. Am J Dis Child 1968; 116:591-8.
2. Collipp PJ, Kaplan SA, Boyle DC, Plachte F, Kogut MD. Constitutional isosexual precocious puberty: effects of medroxyprogesterone acetate therapy. Am J Dis Child 1964; 108:399-405.
3. Werder EA, Mürset G, Zachmann M, Brook CGD, Prader A. Treatment of precocious puberty with cyproterone acetate. Pediatr Res 1974; 8:248-56.
4. Kauli R, Pertzelan A, Prager-Lewin R, Grünebaum M, Laron Z. Cyproterone acetate in treatment of precocious puberty. Arch Dis Child 1976; 51:202-8.
5. Smith CS, Harris F. Preliminary experience with danazol in children with precocious puberty. J Int Med Res 1977; 5: Suppl 3:109-13.
6. *Idem*. The role of danazol in the management of precocious puberty. Postgrad Med J 1979; 55: Suppl 5:81-6.
7. Lee PA, Thompson RG, Migeon CJ, Blizzard RM. The effect of danazol in sexual precocity. Johns Hopkins Med J 1975; 137:265-8.
8. Richman RA, Underwood LE, French FS, Van Wyk JJ. Adverse effects of large doses of medroxyprogesterone in idiopathic isosexual precocity. J Pediatr 1971; 79:963-71.
9. Lemli L, Aron M, Smith DW. The action of depo-provera in 3 girls with idiopathic isosexual precocity: decrease in estrogen effect without urinary gonadotropin reduction. J Pediatr 1964; 65:888-94.
10. Sadeghi-Nejad A, Kaplan SL, Grumbach MM. The effect of medroxyprogesterone acetate on adrenocortical function in children with precocious puberty. J Pediatr 1971; 78:616-24.
11. Cloutier MD, Hayles AB. Precocious puberty. Adv Pediatr 1970; 17:125-38.
12. Mathews JH, Abrams CAL, Morishima A. Pituitary-adrenal function in ten patients receiving medroxyprogesterone acetate for true precocious puberty. J Clin Endocrinol Metab 1970; 30:653-8.
13. Camacho AM, Williams DL, Montalvo JM. Alterations of testicular histology and chromosomes in patients with constitutional sexual precocity treated with medroxyprogesterone acetate. J Clin Endocrinol Metab 1972; 34:279-86.
14. Jeffcoate WJ, Edwards CRW, Rees LH, Besser GM. Cyproterone acetate. Lancet 1976; 2:1140.
15. Heinze F, Teller WM, Fehm HL, Joos A. The effect of cyproterone acetate on adrenal cortical function in children with precocious puberty. Eur J Pediatr 1978; 128:81-8.
16. von Mühlendahl KE, Korth-Schütz S, Müller-Hess R, Helge H, Weber B. Cyproterone acetate and adrenocortical function. Lancet 1977; 1:1160-1.
17. Crowley WF Jr, Comite F, Vale W, Rivier J, Loriaux DL, Cutler GB Jr. Therapeutic use of pituitary densensitization with a long-acting LHRH agonist: a potential new treatment for idiopathic precocious puberty. J Clin Endocrinol Metab 1981; 52:370-2.
18. Comite F, Cutler GB Jr, Rivier J, Vale WW, Loriaux DL, Crowley WF Jr. Short-term treatment of idiopathic precocious puberty with long-acting analogue of luteinizing hormone-releasing hormone: a preliminary report. N Engl J Med 1981; 305:1546-50.
19. Tanner JM. Growth at adolescence. 2d ed. Springfield, Ill.: Charles C Thomas, 1962.
20. Tanner JM, Whitehouse RH, Takaishi M. Standards from birth to maturity for height, weight, height velocity and weight velocity: British children 1965. Part II. Arch Dis Child 1966; 41:613-35.
21. Meisels A. That maturation value. Acta Cytol (Baltimore) 1967; 11:249.
22. Greulich WW, Pyle SI. Radiographic atlas of skeletal development of the hand and wrist. 2d ed. Stanford, Calif.: Stanford University Press, 1950:1959.
23. Bayley N, Pinneau SR. Tables for predicting adult height from skeletal age: revised for use with the Greulich–Pyle hand standards. J Pediatr 1952; 40:423-41.
24. Crowley WF Jr, Beitins IZ, Vale W, et al. The biologic activity of a potent analogue of gonadotropin-releasing hormone in normal and hypogonadotropic men. N Engl J Med 1980; 302:1052-7.
25. Buster JE, Abraham GE. Radioimmunoassay of plasma dehydroepiandrosterone sulfate. Anal Lett 1972; 5:543-51.
26. Santen RJ, Bardin CW. Episodic luteinizing hormone secretion in man: pulse analysis, clinical interpretation, physiologic mechanisms. J Clin Invest 1973; 52:2617-28.
27. Meisels A. Computed cytohormonal findings in 3,307 healthy women. Acta Cytol (Baltimore) 1965; 9:328-33.
28. Bierich JR. Sexual precocity. Clin Endocrinol Metab 1975; 4:107-42.
29. MacGillivray MH. Treatment of idiopathic precocious puberty. N Engl J Med 1982; 306:1109-10.
30. Happ J, Scholz P, Weber T, et al. Gonadotropin secretion in eugonadotropic human males and postmenopausal females under long-term application of a potent analog of gonadotropin-releasing hormone. Fertil Steril 1978; 30:674-8.
31. Bergquist C, Nillius SJ, Wide L. Intranasal gonadotropin-releasing hormone agonist as a contraceptive agent. Lancet 1979; 2:215-7.
32. Linde R, Doelle GC, Alexander N, et al. Reversible inhibition of testicular steroidogenesis and spermatogenesis by a potent gonadotropin-releasing hormone agonist in normal men: an approach toward the development of a male contraceptive. N Engl J Med 1981; 305:663-7.
33. Johnson ES, Gendrich RL, White WF. Delay of puberty and inhibition of reproductive processes in the rat by a gonadotropin-releasing hormone agonist analog. Fertil Steril 1976; 27:853-60.