RESEARCH PLAN: Gonadotropin Releasing Hormone (GnRH) Agonist Test in Disorders of Puberty

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A. Hypotheses and Specific Aims

To test the overall hypothesis that the hormonal responses to injection of a challenge dose of GnRH agonist (GnRHag) will distinguish among disorders of puberty as well as a sleep test. Specifically, we will test the hypotheses that the response to injection of the GnRH agonist leuprolide acetate will:

1. Distinguish among the causes of precocious pubety:
   a. **Patients with incomplete precocity due to idiopathic premature thelarche will have gonadotropin and sex hormone responses to GnRHag lower than those of patients with idiopathic complete precocity but higher than those of normal prepubertal children.**
   b. **Patients with gonadotropin-independent precocious pseudopuberty due to virilizing or feminizing disorders (such as gonadal tumors) will have lower gonadotropin and sex hormone responses to GnRHag than will patients with idiopathic true sexual precocity.**
2. Distinguish among the causes of delayed puberty:
   a. **Patients with gonadotropin deficiency (GnD) will have lower hormonal responses to GnRHag than 14-18 year olds with constitutional delay of puberty (CDP).**

B. Background and Significance

1. **GnRH and GnRHag Testing in Relationship to Pubertal Development.** The hypothalamic-pituitary-gonadal axis is in place before birth and capable of functioning at any age (1). However, the central nervous system of young children suppresses its function. This neural restraint normally begins to wane at about the end of the first decade of life, which allows puberty to commence. The first hormonal change of puberty is sleep-associated GnRH secretion, which is most sensitively indexed by blood levels of the gonadotropin luteinizing hormone (LH).

As a consequence of increasing GnRH secretion as puberty progresses, the LH response to exogenously administered GnRH increases in parallel with pubertal stage (2). A standard GnRH (Factrel®) test dose stimulates only the readily releasable pool of pituitary luteinizing hormone (LH), the blood level of which transiently peaks at 30-60 min (3). In contrast, a single dose of GnRH agonist (GnRHag) additionally creates a “reserve pool” of LH and follicle-stimulating hormone (FSH) by stimulating their synthesis; LH and FSH release peaks at 4 hr and persists for 24 hr. This is sufficient to stimulate gonadal sex hormone secretion within 24 hr in both men (4) and women (5).

LH, FSH, and gonadal sex steroid responsiveness to GnRHag challenge increase significantly with advancing pubertal stage, in both normal and abnormal puberty (6-8). These observations suggested that an acute GnRHag challenge test for pituitary-gonadal function would have diagnostic potential for disorders of puberty. We carried out the initial studies of GnRHag as a diagnostic agent with the injectable GnRHag nafarelin, which never came to market. The GnRHag leuprolide acetate (originally marketed as Lupron Injection®) 10 mcg/kg has been shown to yield a degree of pituitary-gonadal stimulation comparable to nafarelin 1 mcg/kg in adults (3) and children (9). Collaborative preliminary studies (unpublished) show that other pituitary gonadotrope products (free alpha subunit, FAS) (unpublished) and gonadal hormones (inhibin-B) (10) are also released into the circulation.

Obstacles to making a timely diagnosis of disorders of puberty exist. First, manufacturing cut-backs for low-profit drugs lead to frequent unavailability of Factrel for testing purposes. Second, advances in gonadotropin assay methodology have led to diagnostic criteria for disorders of puberty being in a state of flux. Gonadotropins are glycoproteins that exhibit considerable molecular heterogeneity. Within the past decade monoclonal antibody-based “third generation”, immunometric assays of high sensitivity and high specificity have become available for diagnostic purposes to replace the polyclonal radioimmunoassays (RIAs) previously available (11).

We propose to evaluate GnRHag as a diagnostic agent to develop diagnostic criteria that will distinguish among the causes of the most common disorders of puberty, premature (precocious) puberty and delayed puberty; sleep-associated LH characteristics will be the gold standard test for comparison.

2. **Premature (Precocious) Puberty**

Complete ("true") precocious puberty, due to premature activation of the hypothalamic-pituitary-gonadal axis, occurs when neural restraint is lifted or circumvented. It must be distinguished from incomplete or gonadotropin-independent premature puberty. This is ordinarily accomplished by Factrel testing. Lupron depot contains sufficient free leuprolide to confirm the diagnosis once treatment is initiated (12). Thus, GnRHag testing would be expected to yield distinctions as good or better than Factrel testing.

a. Incomplete vs Complete Precocious Puberty. Complete (" true") precocious puberty can be due to organic brain disorders, but is idiopathic in about ninety-five percent of girls. It can occur on an autosomal dominant basis, with apparent incomplete penetrance, particularly in boys (13); the underlying genetic defects have not been identified. The most common types of premature puberty are incomplete forms of puberty, premature thelarche and premature adrenarche, which have been considered to be extreme variants of normal. Considerable evidence suggests that isolated premature thelarche (breast development) may be due to slightly excessive activity of the pituitary-ovarian axis (14). Our pilot studies with the GnRHag nafarelin is compatible with this concept: our patient with premature thelarche had lower LH, FSH, and estradiol responses than did subjects with true precocious puberty (6). Recent reports indeed suggest that responses to the GnRHag leuprolide are lower in premature thelarche and premature adrenarche than in progressive sexual precocity (9, 15). It is possible that abnormal production of neuroendocrine modulators of the pituitary gonadotrope response to GnRH, such as inhibin B (16), may play a role in what has been considered a normal variant. Thus, GnRHag testing has the potential to broaden our understanding of the regulation of the function of this system.

b. Gonadotropin-independent precocity. Conversely, when there is autonomous sex hormone excess, as in tumor, McCune-Albright syndrome or primary Leydig cell hyperplasia, one would expect suppression of gonadotropin secretion (17). Our case studies suggest that the gonadotropin response to GnRHag is suppressed in children with premature sex hormone excess due to such disorders (18). Thus, the GnRHag test may prove to be of diagnostic utility in the work-up of children with precocious puberty.

3. **Delayed Puberty**

Distinguishing CDP from GnD in Teenagers. Delayed puberty is usually due to an extreme variation of normal, termed constitutional delay in growth and pubertal development (CDP). It can occasionally be due to chronic disease. CDP children are "late bloomers" who spontaneously enter puberty in their late teens and go on to grow and mature normally. CDP appears to be due to a prolongation of the normal GnRH- and gonadotropin-deficient prepubertal state, and it is difficult to reliably distinguish CDP from isolated GnD (19-21).

GnD is heterogeneous in etiology. It can be congenital, hereditary, or acquired. Genetic causes of isolated GnD account for less than 10% of cases (22-26). Acquired GnD can be organic, as in the case of brain or pituitary tumor, or it can be functional, as in anorexia nervosa or hypothalamic amenorrhea (a transient form of the latter is "boarding school amenorrhea"). GnD requires long-term sex hormone replacement therapy and, eventually, fertility treatment.

GnD is also heterogeneous in clinical severity, and the degree of GnD as reflected by MRI (27), and LH pulse analysis or responsiveness to a GnRH test (23, 28-35). LH pulse analysis is impractical and those with LH pulses, who represent 15-30% of cases, have normal GnRH tests. Functional hypothalamic GnRH deficiency can cause a wide spectrum in women, ranging from profound sexual infantilism in anorexia nervosa to "hypothalamic amenorrhea," which is characterized by subnormal LH pulsatility and lack of midcycle LH surges to occur in an estrogenized female (36). Nevertheless, the diagnosis of isolated GnD in adults generally does not represent the diagnostic dilemma in adults that it does in the teenage years. In otherwise healthy men, GnD is diagnosed by the presence of small testes and a subnormal testosterone level, without a compensatory elevation of gonadotropin levels (33). In women, GnD is diagnosed by the presence of amenorrhea and low plasma estradiol levels or estrogenization of the genital tract, without a compensatory elevation of gonadotropin levels.

Teenagers 14-18 years of age represent a special diagnostic problem because isolated GnD so closely resembles CDP, which is more common in boys (37-40). If puberty is delayed beyond 18 years of age in boys (17 yr in girls), GnD exists, with rare exceptions (41). prepubertal child with all the characteristic features of CDP still carries only a presumptive diagnosis and currently requires surveillance until the eventual normal progression into puberty definitively rules-out GnD. Delaying diagnostic procedures beyond 14-15 years of age is unwarranted, however, for psychosocial reasons and because of possible adult osteoporosis (42-44).

The tests available to distinguish isolated GnD from CDP have limitations (45, 46). Plasma sex steroid

levels are not useful diagnostically in early puberty because of diurnal (47, 48) and cyclic (females) (38) variations in LH and FSH secretion. The first hormonal event of puberty is an increase in sleep-associated, episodic LH secretion (49, 50). A sleep test, measuring LH levels at 20 min intervals overnight, distinguished 31 of 32 subjects (10 GnD and 22 CDP) by in increase of LH (ΔLH $\geq$ 0.35 IU/L) (51). However, the sleep test is impractical as a routine diagnostic tool.

Though GnRH (Factrel®) testing is FDA-approved for assessing the functional capacity of the gonadotropes, the responses to this test are consistently flat only when the pituitary gland is extensively damaged (52). GnRHag assessment of the gonadotrope "reserve" pools seems to enhance the ability to distinguish GnD from CDP. We found that the LH (4hr) response to GnRHag testing distinguished 9 of the 10 GnD from the 11 CDP patients in boys 13.25-17.6 yr old; this discrimination equalled that of the sleep test (51). While these results were initially confirmed (53), another group found overlap in the responses of GnD and boys (54) and another group found an indirect test to be better (55). Indeed, when we switched from RIA to monoclonal antibody-based LH and FSH immunometric assays, the distinction between GnD and CDP was blurred.

FAS cross-reacted 50% in our LH RIA, and so was a candidate discriminant. Pilot studies showed that the FAS level 4 hr post-GnRHag discriminated 3 GnD from 8 CDP boys. This is consistent with the recent report of significantly higher FAS responses to GnRH in CDP than GnD (56) and contrasts with the finding that GnD men treated with prolonged pulsatile GnRH secretion develop higher FAS levels than normal men (57). These data suggest that the regulation of FAS is abnormal in GnD. Therefore, we propose that the FAS response to GnRHag will distinguish CDP from GnD better than the response to GnRH itself. Other possible hormonal discriminants between GnD and CDP exist, such as inhibin B (58, 59).

C. Preliminary Results

Included in **Background**.

D. Research Design and Methods

1. Selection and definition of study groups

a. Control subjects

1) Prepubertal healthy volunteers, consisting of 20 boys (9-13 years old) and 20 girls (8-12 years old). Boys will have prepubertal size testes (less than 2.5 cm in long diameter) and 0800 hr plasma testosterone levels of less than 30 ng/dl. Girls will lack breast development and have 0800 hr plasma estradiol < 9 pg/ml.

2) Early pubertal healthy volunteers, consisting of 20 boys and 20 girls with chronologic and bone ages of 9-15 years of age in genital or breast stage 3 or 4 (60), will be solicited; the girls will be premenarcheal.

b. Patients.

Re Specific Aim 1: Premature Puberty

Premature puberty is defined as breast development of onset from 6 months to 8 years of age (girls) or pubic hair development or testicular enlargement of onset from 6 months to 9 years of age (boys) (1, 61). The critical samples (4 hr nocturnal, and 0, 1, 4, 20 hr post-GnRHag) can be obtained from children as small as 10 kg.

1) Premature thelarche -- The 20 girls to be studied will have premature breast development as an isolated phenomenon, bone age within 2 S.D. of average for age, and plasma estradiol levels below 9 pg/ml.

2) Complete (gonadotropin-dependent) precocious puberty -- the 20 children of each sex to be studied will have pubertal sex steroid levels (estradiol over 9 pg/ml in girls and testosterone over 20 ng/dl in boys) with bone age more than 2 SD advanced . The diagnosis will be confirmed by this study to include pubertal sleep-related LH increases.

3) Gonadotropin-independent precocity -- 20 children of either sex, younger than 8 (girls) or 9 (boys) years of age. They will have such disorders as McCune-Albright syndrome, primary Leydig cell hyperplasia, tumors, and congenital adrenal hyperplasia. The diagnosis of these disorders will be established independently by the pattern of sex hormone secretion, absence of sleep-related LH rises, and ultrasound or other radiologic imaging procedures as indicated clinically.

Re Specific Aim 2: Delayed Puberty

Delayed puberty criteria will be retardation of both pubertal milestones (62) and bone age (63) by two or more years at 14 through 17 years of age.[~~?~~*sic*]

1) Criteria for CDP: Otherwise healthy boys (20 prepubertal and 20 early pubertal) and girls (10 prepubertal and 10 early pubertal) (subgroups defined as above) will be studied. Chronic systemic,

metabolic, and endocrine disease will be excluded by history, physical examination, complete blood count, erythrocyte sedimentation rate, comprehensive metabolic panel, thyroxine, and somatomedin-C determinations. The diagnosis will be supported independently initially by sleep test criteria and confirmed by spontaneous progress of puberty upon follow-up.

2) Criteria for GnD: Males (20 prepubertal and 20 partial/pubertal) and females (20 prepubertal and 20 partial/pubertal) will be studied. Since GnD is a rare ("orphan") disorder, adult patients will be included in this study population for comparison. The diagnosis of GnD will be provisionally assigned to teenagers if delayed puberty is associated with (1) anterior panhypopituitarism, (2) a hypothalamic-pituitary mass upon magnetic resonance imaging (MRI), (3) cranial irradiation therapy, (4) anosmia ± MRI evidence of Kallmann's syndrome (27), (5) or congenital micropenis without evidence of primary hypogonadism. Confirmation of the diagnosis of GnD will be by lack of onset of puberty by 18 years of age in males (17 years in females) or lack of spontaneous progression of puberty, upon re-evaluation within 1 year after completion of one or more courses of replacement sex steroid therapy (64).

2. Exclusion criteria: Sex hormone usage within 2 months.
3. Conduct of study:

a. **Admission procedures.** History and physical examination, including height, weight, and pubertal staging.

b. **Sleep test**. Protocol attached. Blood sampling will commence at approximately 1900 hr and continue for 12 hr up to 0700 hr. Approximately 2.5 cc heparinized blood will be collected for LH, FSH, and sex steroids over sequential 20-min intervals from an indwelling intravenous line by constant withdrawal pump.

c. **GnRH agonist test**.

1) Blood sampling will commence at approximately 0700 hr with baseline blood samples obtained at 20 min intervals x 4 (-60 to 0 time).

2) Leuprolide acetate injection: 10 mcg/kg subcutaneous at 0 time.

3) Blood sampling will continue after the leuprolide dose: 0.5, 1, 2, 3, 4, 8, 12, 16, 20, and 24 hr. LH and FSH will be measured in all samples; testosterone (boys) and estradiol (boys and girls) at 0, 16, 20, and 24 hr. Extra blood will be obtained at 4 and 24 hr for special studies (e.g., FAS, inhibin-B)

d. **Miscellaneous procedures.**

1) Bone age radiograph will be taken if not performed within 3 months.
2) Blood will be obtained for DNA (15-30 cc).
3) Discharge on prophylactic ferrous sulfate (300 mg daily for 1 month).

4. Data collection:

Hormone assays will be performed in the University of Chicago Hospital Endocrine Laboratory. Estradiol will be assayed by a modification of a Pantex kit method, dehydroepiandrosterone (DHEA)-sulfate by a Diagnostic Systems kit (3), total testosterone by a nonchromatographic method using a Diagnostics Products kit, and free testosterone from competitive protein binding analysis with a sensitivity of 3 pg/ml, precision of 13% (3, 65, 66). LH and FSH ultrasensitive (0.15 IU/L) immunometric Delfia® assays will be performed (11).

Serum will be stored for assay of inhibin-B, activin, and FAS (16, 57).

Molecular genetic studies. Blood (5-10 cc x 3, volume considerations permitting) will be collected in EDTA to extract DNA, prepare a lymphoblastoid cell line, and freeze in the CRC Core Laboratory for potential studies of the molecular genetic basis of disorders of puberty. DNA will be extracted by standard methods; in the past this has been by salt extraction and ethanol precipitation, and recently Quiagen kits were introduced. One of these tubes will be used for the creation of an EBV-transformed lymphoblastoid cell line (67, 68), which will serve as a permanent back-up source of DNA should we run short of frozen extract, since there does not appear to be a single, common genetic basis for these rare disorders.

5. Data analysis:

a. Variables.

1) Sleep test. The gold standard test for the diagnosis of GnD will be demonstration of a subnormal sleep-associated increase in LH level. The onset of true puberty will be defined as a significant sleep-related rise in LH (ΔLH, mean sleep minus mean pre-sleep) $\geq$ 0.35 IU/L; normal ΔLH for pubertal children will be $\geq$0.8 IU/L IU/L (51). These cut-offs are provisional, based on RIA. Normal LH pulse frequency (69) is > 2 pulses/6 hr (33, 70).

2) GnRHag test. Plasma concentrations of LH, FSH, estradiol and/or testosterone in samples collected before and after GnRHag. Baseline, "early" (30-60 min), 4 hr, and peak values will be analyzed as

levels and responses (∆). The primary variable for the delayed puberty study will the FAS response at 4 hr.

b. Comparison of groups.

1) Either the 5th or 95th percentiles of the separate prepubertal and pubertal groups of healthy volunteers will define the lower or upper limit of normal range for the test, depending upon the study group in question. This fixes the specificity of the test at 95%. A similar range will be constructed for girls with central precocious puberty and CDP boys.

2) Re premature puberty. Variables will be compared among the controls and the subgroups of patients with premature puberty to determine the statistical significance of differences (by analysis of variance and unpaired t-tests, as appropriate, with correction for multiple comparisons where indicated). We expect that variables for patients with complete precocious puberty will not be significantly different than those of sex-matched pubertal controls. If so, the data for patients with idiopathic precocious puberty will be pooled with that of the healthy conrols of appropriate sex.

3) Re delayed puberty. The responses of normal prepubertal boys will be compared to those of prepubertal CDP and GnD boys, and likewise early pubertal normal, CDP, and GnD boys will be compared. The mean, S.D., and shape of the distribution curve will be determined for each variable above for each test (sleep, GnRHag). Responses will be compared to determine whether differences among the prepubertal groups (normal, CDP, GnD) are statistically significant ($p < .05$) by analysis of variance, with post-hoc Sheffe's test to correct for multiple comparisons, and likewise for the comparisons among the early pubertal groups. Girls' responses will be similarly analyzed. However, because so few girls present with CDP, if the normal and CDP prepubertal groups are not significantly different, their data will be pooled into a prepubertal control group, and likewise with the respective early pubertal groups. Then two-sample t-tests will be used for comparison of prepubertal and early pubertal GnD girls with pooled prepubertal and early pubertal control groups, respectively.

c. Specificity and sensitivity of GnRHag test for diagnosis of central precocious puberty (CPP).

The principal control group will be the healthy prepubertal controls of the same sex. CPP will be provisionally diagnosed based on the sleep test. The 95th percentile will be calculated for each GnRHag test variable at each time point separately in prepubertal and CPP patients. This fixes the specificity of the test at 95%. The sensitivity of the GnRHag test will be determined by the fraction of CPP patients lying above the 5th percentile of the prepubertal control group of like sex.

d. Specificity and sensitivity of GnRHag test for diagnosis of GnD.

1) For boys the principal control group will be the stage-matched CDP patients. CDP will be provisionally diagnosed based on the sleep test. The 5th percentile will be calculated for each variable at each time point separately in prepubertal and early pubertal CDP boys. The sensitivity of the GnRHag test will be determined by the fraction of GnD patients lying below the 5th percentile of the stage-matched control group. For girls the principal control group will be the normal volunteers, or the pool of normals and CDP, since so few girls present with CDP.

2) Upon conclusion of the study, after the final categorization of patients as GnD or CDP by the criterion of progression of puberty, the specificity and sensitivity of the GnRHag test will be compared with those of the sleep test. We expect that the sleep test and GnRHag test will have equivalent sensitivity in the diagnosis.

6. Interpretation of data:

Diagnostic Accuracy

a. Recruitment of some groups may not be optimal. For example, central precocious puberty is less common in boys than girls, while conversely CDP is seen more often in boys than girls. In addition, incomplete (gonadotropin-independent) precocious puberty and partial gonadotropin deficiency are especially rare. In such situations as these, the small numbers may not permit assignment of sensitivity of the GnRHag test for a particular condition, but we anticipate that the numbers will permit statistical comparison of group means for each test, albeit of low power. The following Table shows estimates of the allowance for error (AE) at different sensitivities of the test for different numbers of subjects.

Table. 95% Allowance for Error

	Range of Sensitivity	
Number	80%	90%
5	± 35%	± 27%

10	± 25%	± 19%
20	± 18%	± 13%

b. The sensitivities of the GnRHag and sleep tests will be compared using McNemar's test for paired binomial data (71). Due to the small sample size in some groups, the power of this comparison is low and differences will not likely reach statistical significance. Although it is anticipated that the diagnostic accuracy of the GnRHag test will prove to be no greater than that of the sleep test, it is clearly less expensive and more practical because it does not require overnight hospitalization, the intensive effort involved in sampling every 20 minutes, nor the cost of assaying so many samples.

Re Specific Aim 1: Precocious Puberty

a. We expect that children with idiopathic premature thelarche will have gonadotropin and sex hormone responses to GnRHag lower than those of patients with idiopathic true sexual precocity, but higher than those of healthy prepubertal children. We expect to see little overlap between the variables for complete and incomplete precocious puberty for either sex.

b. We expect that patients with gonadotropin-independent precocity will have lower gonadotropin and sex hormone responses to GnRHag than will healthy pubertal controls and patients with central precocious puberty and similar to those of healthy prepubertal children. We expect to see little overlap with the pubertal groups.

Re Specific Aim 2: Delayed Puberty

We expect that the hormonal response to a single leuprolide dose of 10 mcg/kg SC will a) be significantly lower in GnD than in CDP, and b) when the specificity of this GnRHag test is set at 95%, the sensitivity will be 90% or more for the diagnosis of GnD. Primarily, we expect that an optimal window of discrimination will be established for FAS responses at 4 hr, at which time we will observe 9 of 10 prepubertal GnD patients to have a ΔFAS below the 5th percentile cut-off for prepubertal CDP boys.

7. Alternative Considerations.

a. The importance of the recruitment of healthy control children is to provide normative data; this may eventually be pooled with the CDP data, if the statistical comparison of stage-matched groups shows the responses to be similar, and thus improve the statistical power of the study. Ultimately, positive results would point the direction for a larger scale, multi-center study.

b. Sleep onset time will be determined from nursing notes rather than from formal EEG monitoringboth to facilitate scheduling and because of cost saving. We do not expect this to affect the accuracy of the determination of the hormonal changes during sleep because acute deviation from the usual sleep pattern does not eliminate the increased nocturnal LH secretion of pubertal children (50).

E. Human Subjects

1. Characteristics of the study population. Each study population consists of 20 subjects at most. Patients will be those presenting to the Pediatric Endocrinology Clinics of the University of Chicago Medical Center. The disorders under study are uncommon, and the literature cited in **Background** does not suggest any unusual demographic distribution. We will attempt to recruit all subjects meeting eligibility criteria by speaking with them directly in the clinic. African-American girls appear to be at particular risk for premature puberty (62, 72). Otherwise, the demographics of the study population will reflect the demographics of the US population as determined in the 1990 US Census, i.e., 0.8% Alaskan/American Indian, 2.9% Asian, 12.1% African-American, 9.0% Hispanic, 80.3% Caucasian, (some minorities double-counted) (73). Our accrual to the immediate predecessor versions of this protocol has been 1% Asian, 24% Afro-American, 7% Hispanic, and 67% White, with 60% boys and 40% girls (n=88).
2. Source of research materials. Blood specimens will be obtained prospectively in living human subjects after obtaining informed consent.
3. Consent procedure. The principal investigator, co-investigator, or R.N. associate will obtain written consent after informing subjects fully about the project, procedures associated with it, and all experimental procedures. The subject will have the opportunity to ask questions concerning any and all aspects of the project and any procedures involved. Possible risks, discomfort and their consequences will be explained and the subjects will be told that they may withdraw their consent at any time and that such withdrawal will not restrict access to health care services at the University of Chicago Hospitals. No guarantee or assurance as to the results to be obtained will be given.

Informed consent forms are attached for parents or guardians of the children.

4. Potential risks. These studies are of two types: first, multiple blood sampling alone, and second, blood sampling after administration of a test substance. Blood is withdrawn constantly over a period from 4 hr (small children) to 36 hr (teenagers). The intravenous line may be removed during part of this time if that is the child's preference or if there are access limitations; removal of the line is more common in younger children. The amount of blood removed is about a half-pint, less than 10% of blood volume over a 36-hr period. The test substance to be given is a GnRHag; this is a synthetic decapeptide which is identical to natural GnRH except for a change in a central amino acid that slows its degradation. It is identical to a depot preparation marketed for the long-term treatment of precocious puberty; the preparations differ only in that the depot preparation is in a vehicle that slows its absorption. Allergic reactions to the depot are rare (1 case report) and unheard of in response to the short-acting form used for these studies. We have performed 246 leuprolide diagnostic tests in children (6-17 years old) and 211 in adults (≥ 18 years old) with no adverse events. There are no known side effects from a single GnRHag injection other than those related to elevating estrogen to midcycle levels in sexually mature women.

This study involves overnight hospitalization, at considerable inconvenience, involving even the parents who must accompany their volunteer children to the hospital for admission and discharge.

Thus, this research carries minimal increase over minimal risk. The research involves medical risks not unusual for children. A survey in pediatricians' offices showed that at age 7 years, 27% of African-American girls and 6.7% of White girls had breast or pubic hair development and at age 8 years these figures had respectively risen to 48% and 14% (72). Approximately 5% of boys do not show signs of puberty by 14 years of age (74).

5. Procedures for minimizing potential risk. The repetitive blood sampling procedures will be performed after the patient is admitted to the General Clinical Research Center of the University of Chicago Hospitals to the Pediatric Endocrinology (General Pediatric) Service, with a nurse in constant attendance, a resident in pediatrics available in-house, and the Pediatric Endocrinology service on call. Blood volumes will be adjusted so that no more than 5% of blood volume will be removed in 24 hr, 10% in a month. Iron stores will be repleted by prescribing ferrous sulfate.

6. Potential benefits. Healthy volunteers will be compensated $50 for completion of the sleep test, $100 for completion of the GnRHag test, or $150 for completing both the sleep test and the GnRHag test. Volunteers will also be given one parking sticker, allowing free parking, for each day they are in the Hospital during the study. Volunteer children will be reimbursed by check in their own name.

Patients will not be paid for participating in the study. Patients with disorders of puberty benefit from the diagnostic accuracy of the sleep test for the subtle hormonal changes of early puberty, as well as the typically obvious interpretation of the results of GnRHag test. There is often not an available alternative to the GnRHag test because the Factrel® (GnRH itself) supply is erratic; like so many marginally profitable drugs, this drug is often unavailable at the time of need for prompt diagnosis. Factrel is the standard diagnostic agent used by pediatric endocrinologists to attempt to distinguish among disorders of puberty.

This study is potentially beneficial to society by improving diagnostic accuracy of disorders of puberty. There is a pressing need for sex-, age- and pubertal stage-specific normative data. Our published data represents a substantial portion of the medical literature on this subject; however, they were obtained in the era of polyclonal radioimmunoassay, which yielded considerable interassay variation because of differing degrees of non-specificity, particularly at the low gonadotropin levels found until mid-puberty, and different patterns of epitope specificity among antisera (11). With the widespread availability in the last several years of specific monoclonal-based immunometric assays for gonadotropin ß-subunits, these variations have become minimal.

7. Inclusion of women and minorities. Not applicable, since there are no such exclusions.

8. Inclusion of children. Children will be included in the research as outlined above in the research plan. The investigative team consists of trained pediatric physicians and nurses. The Clinical Research Center is approved to administer pediatric care for the age groups defined in this study.

9. Data safety and monitoring plan attached.

F. VERTEBRATE ANIMALS. None.

G. LITERATURE CITED

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H. Need for General Clinical Research Center (CRC) Resources

1. Justification for CRC Utilization.

The GnRHag test and the comparison sleep test are not standard diagnostic procedures. No GnRHag, including leuprolide acetate, is approved for the diagnostic indication being evaluated, so we are investigating its diagnostic efficacy and safety. Normal volunteers and patients will be admitted for determination of the full 24-hour hormonal profile of hormonal responses to leuprolide, which have not been established. Nursing services are required for repetitive blood sampling and patient monitoring so as to minimize risks and record the (unlikely) possibility of adverse events. Nursing will also be responsible for obtaining a urine for pregnancy test on pubertal or post-menarcheal females.

2. Number of Subjects and CRC Days.

1. Subjects: Of the 80 healthy volunteers needed for this protocol, 12 have been studied as of 8/31/04, leaving 68. Of the 80 children required for premature puberty, 11 have been studied, leaving 69. O the 80 required for the study of delayed puberty, 6 have been studied, leaving 74 remaining. Thus, subjects total 211.

2. Days: Type A bed days x 2 per subject x 211 subjects = 422 A bed days; thus, 84 bed days yearly.

3. Other CRC Resources Required.

a. Core Laboratory. Blood samples will be aliquoted for the LH, FSH, and steroid assays which are to be carried out in the Hospital Endocrine Laboratory. The remainder of the serum will be frozen for shipment in batches to collaborators for FAS and inhibin-B assays.

b. Ancillary Funds. Funds will be required for bone age radiographs on the normal volunteers studied as Type A researcsubjects, in addition to endocrine assays performed on all subjects by the Hospital Endocrine Laboratory.

c. Biostatistician. The CRC biostatistician will consult on the statistical analysis of data as needed..

d. Bioinformatics. The Bioinformatics Core will develop programs so that raw data for this protocol will be collected by the hospital's Endocrinology Laboratory and put into a specialized database, from which reports can be generated and data can be exported for statistical analysis. This system will save time and increase accuracy by eliminating the need to re-enter data into several different systems.

4. Resources Provided by PI**.** None.