THE VALUE OF PULSATILE LH-RH ADMINISTRATION AND HCG THERAPY IN THE INVESTIGATION AND TREATMENT OF THE HYPOGONADAL MALE.

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DECLARATION

All patients who underwent pulsatility studies were seen by myself and all blood samples for gonadotrophin profiles were drawn by myself.

Approximately 20% of the patients in the HCG study had attended the Endocrine Clinic at the Royal Infirmary prior to my appointment as endocrine registrar. These patients had been seen by Dr. H N Cohen at their clinic attendances. All these patients were recalled to the clinic and seen by myself and all subsequent patients referred to the clinic were seen at each attendance by myself.

LIST OF PUBLISHED WORK AND PRESENTATIONS

The following list of papers and presentations include information from some of the studies embodied in this thesis.

Published Work.

Gordon, D., Cohen, H.N., Beastall, G.H., Hay, I.D. & Thomson, J.A. (1984)
Contrasting effects of subcutaneous pulsatile GnRH therapy in congenital adrenal hypoplasia and Kallmann's syndrome.
Clinical Endocrinology, 21, 597 - 603.

Gordon, D., Cohen, H.N., Beastall G.H., Perry, B. & Thomson, J.A. (1988)
Hormonal responses in pubertal males to pulsatile gonadotropin releasing hormone (GnRH) administration.
Journal of Endocrinological Investigation, 11, 77 - 83.

Presentations.

Prolonged pulsatile LH-RH administration identifies heterogeneity in hypogonadotrophic hypogonadism.
Caledonian Society for Endocrinology. Bowness, October, 1984.

Failure to achieve maturation of the hypothalamic-pituitary axis of boys with delayed puberty following one week of pulsatile subcutaneous gonadotrophin releasing hormone therapy.
Caledonian Society for Endocrinology. St. Andrews, May, 1985.

Factors of value in predicting response to human chorionic gonadotrophin therapy in boys with delayed puberty.
Scottish Society of Experimental Medicine. Glasgow, February, 1986.

A critical assessment of human chorionic gonadotrophin therapy in boys with retarded pubertal development.
British Endocrine Societies (Joint Meeting). Sheffield, April, 1986.

Enhanced prolactin secrtetion in response to TRH, following pulsatile GnRH treatment.
Association of Clinical Biochemistry, National Meeting. Glasgow, May, 1986.

Varied gonadotrophin responses to short-term pulsatile GnRH administration in patients with delayed puberty and idiopathic hypogonadotrophic hypogonadism.

Caledonian Society for Endocrinology. Dunblane, May, 1986.

Hormonal responses to pulsatile gonadotrophin releasing hormone (GnRH) administration to pubertal patients.
Royal Society of Medicine. London, June, 1986.

The effects of pulsatile LH-RH infusion on nocturnal hormone concentrations in pubertal males.
Caledonian Society for Endocrinology. Creiff, April, 1987.

Night-time hormone profiles during pulsatile LH-RH infusion to pubertal boys.
Scottish Society for Experimental Medicine. Glasgow, May, 1987.

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SUMMARY

The present work investigates the value of pulsatile administration of LH-RH to hypogonadal males. In particular, the value of pulsatile LH-RH infusions in the differentiation of constitutional delay of puberty, short stature and hypogonadotrophic hypogonadism is assessed. Both the early endocrine effects and the effect of prolonged infusions on induction of fertility are studied. In addition, the effects of human chorionic gonadotrophin (HCG) on the pubertal development of boys presenting with delayed sexual development are evaluated. Prior relevant research and work published during the course of the present study are reviewed.

Nocturnal surges in serum gonadotrophin and testosterone concentrations in pubertal boys are confirmed. Following six days of pulsatile LH-RH infusions at pulse doses ranging from 2.5 to 15ug/pulse subcutaneously at 90 min intervals, the naturally occuring diurnal hormone rhythms are pertubed. Exogenous administration of LH-RH stimulates gonadotrophin secretion above pre-infusion concentrations only at times of the day when endogenous secretion is at a minimum. At times of night-time hormone surges the effect of exogenous LH-RH administration is variable; the net effect on a group of pubertal boys was to leave nocturnal LH concentrations unaltered.

Despite the alteration in gonadotrophin fluctuations throughout the day and night caused by LH-RH administration serum testosterone concentration continued to show marked diurnal variation with post-infusion concentrations stimulated above pre-infusion levels throughout the 24 hour periods.

The dosage of LH-RH per pulse over the range 2.5 to 15ug/pulse did not appear to significantly alter the gonadotrophin response to LH-RH, given over 6 day periods.

Mean gonadotrophin concentrations measured by sampling blood every 15 minutes, over 3 hour periods in the afternoon when endogenous secretion is low, showed a linear relationship between pre- and post-infusion concentrations. Patients with constitutional delay of puberty and boys with short stature showed similar increments in gonadotrophins following pulsatile LH-RH administration. However, males with hypogonadotrophic hypogonadism showed quantitatively greater increments when compared with the other two groups.

The gonadotrophin responses to standard bolus injections (100ug I.V.) of LH-RH failed to differentiate between the boys with delayed puberty and short stature and the patients with hypogonadotrophic hypogonadism. Following pulsatile infusions of LH-RH to patients with short stature and delayed puberty, there were no significant differences in basal or peak LH

concentrations, or increments in LH during LH-RH bolus tests. Patients with hypogonadotrophic hypogonadism, on the other hand, had significantly elevated basal and peak LH concentrations to the bolus tests. Increments in LH in the post-infusion bolus tests, however remained unchanged from the basal values.

Peak FSH concentrations tended to fall in the post-infusion bolus LH-RH tests compared to pre-therapy tests in the short stature and delayed puberty groups. Basal and peak FSH concentrations rose in the hypogonadotrophic males in the post-infusion bolus tests. However, overlap between the groups prevented this test from adequately discriminating between the groups.

Increments in serum testosterone following 6 days of pulsatile LH-RH administration were not different between the three patient groups; delayed puberty, short stature and hypogonadotrophic hypogonadism. The stimulated testosterone concentration showed a relationship to the basal testosterone and the patient's bone age. Little or no increment in testosterone occurred with pulsatile LH-RH infusions if the basal testosterone was undetectable or the patient's bone age was less than 12 years.

The present work has confirmed that the prolactin response to TRH (200ug I.V.) was unable to differentiate between patients with pubertal delay or permanent hypogonadotrophic hypogonadism. Following pulsatile LH-RH

infusions there were no significant differences in the basal, peak or incremental prolactin responses to TRH in either group of patients despite increases in both serum LH and testosterone in both groups. There is therefore no obvious relationship between exogenous LH-RH concentrations and the prolactin response to TRH.

The present work confirms the ability of prolonged pulsatile infusion of LH-RH to induce the endocrine changes of puberty. However, difficulties with patient compliance, inadequate testicular function or sensitization to the LH-RH preparation, prevented successful sperm production in most patients with hypothalamic hypogonadism. Standard bolus LH-RH tests and HCG stimulation tests are poor predictors of pituitary and testicular response to this treatment.

Prolonged pulsatile infusion of LH-RH at increasing pulse doses failed to stimulate gonadotrophin secretion in one patient with congenital adrenal hypoplasia. This result would suggest that the hypogonadotrophic hypogonadism in this condition is of pituitary rather than hypothalamic origin.

Human chorionic gonadotrophin was shown to be a potent stimulator of growth and sexual development in pubertal males. Genital development was accelerated particularly in boys at genital stages 1 and 2, when compared with matched controls. It was difficult, however to demonstrate any significant HCG effect on testicular

size when compared with untreated controls.

Patients with height velocities of 7cm/year or less showed significantly accelerated growth during HCG therapy. Height velocities fell in most patients following withdrawal of HCG. Patients growing initially at greater than 7cm/year had more variable responses to HCG therapy. Pre-pubertal patients showed the greatest acceleration in height velocity when compared with untreated patients at the same genital stage.

Only one of 8 treated patients followed to adulthood, achieved a final height greater than predicted prior to instigation of HCG treatment. Only one of 11 patients with initial skeletal ages less than 11.5 years showed an increased, final expected height after therapy. On the other hand, there was no significant difference between the pre-treatment predicted adult heights and either the post-therapy predicted adult height or final height outcome in patients with initial bone ages greater than 11.5 years.

In conclusion therefore, pulsatile LH-RH infusions to patients with pubertal delay can allow successful identification of patients with permanent hypogonadotrophic hypogonadism. However, responses to this therapy are hetergeneous and dependent upon the degree of endogenous LH-RH release. Prolonged infusions for induction of fertility require high patient motivation and are often unsuccessful. HCG therapy for

induction of puberty is most effective for pre-pubertal patients and patients at stage 2 puberty. It can be associated in eventual stunting of growth if given to patients with bone ages less than 11.5 years.

CHAPTER 1

LITERATURE REVIEW

1.1) THE PHYSIOLOGY OF LH-RH SECRETION AND ITS THERAPEUTIC USE

The hypothalamo-hypophyseal portal system of blood vessels was first described in 1930 (Popa and Fielding) but it was not until 17 years later that the physiological significance of this system was appreciated by Green and Harris (1947). They proposed that the multitude of nerve fibres, terminating in the vicinity of capillary loops of the median eminence of the hypothalamus, liberated substances which were transported by these vessels to the anterior pituitary gland and stimulated hormone release.

In 1967 Schally et al demonstrated that the human hypothalamus contained factors which released gonadotrophins from animal pituitary glands and in 1969 Kastin et al demonstrated that extracts of porcine hypothalami released gonadotrophins from the pituitary gland in man. In the same year Root et al (1969) demonstrated that ovine hypothalamic extract released LH in two infants with lethal chromosomal anomalies and

multiple congenital abnormalities and in one child with cerebral dysfunction of unknown aetiology.

In 1971 the decapeptide structure of luteinizing hormone-releasing hormone was characterised by Schally, Nair and Redding (1971b) and later confirmed by synthesis (Schally, Kastin and Arimura, 1972). In these first studies its biological activity was demonstrated both in animals and in man. It also became clear that the one hormone could release both LH and FSH (Schally et al, 1971a) and despite a considerable amount of work since, there is still no conclusive evidence for separate LH and FSH releasing hormones.

The availability of LH-RH has allowed its administration to become an established method for assessment of pituitary gonadotroph secretory reserve in normal and abnormal clinical states (Job et al, 1972; Hashimoto et al, 1972; Roth et al, 1972; Espiner and Donald, 1973; Mortimer et al, 1973a). The value of single LH-RH injections in the assessment of hypogonadal states will be discussed below.

Early attempts to use LH-RH for the treatment of male and female infertility has been reviewed by Mortimer, Besser and McNeilly, (1975). Initially promising results using high dose twice daily intramuscular injections of LH-RH were reported (Mortimer et al, 1974).However, Davies et al (1977) subsequently demonstrated reduced gonadotrophin response to LH-RH after chronic

administration to impotent men. Brook and Dombey (1979) demonstrated an initial response to twice daily LH-RH in 4 patients with hypogonadotrophic hypogonadism who were treated for more than one year. Rabin and McNeil (1981) also showed only a transient rise in serum testosterone in 2 of 4 males with isolated gonadotrophin deficiency treated with high dose (1mg, twice or thrice daily) LH-RH given subcutaneously.

These early attempts at treatment of hypogonadotrophic hypogonadism with LH-RH replacement used unphysiological amounts of hormone given infrequently. Because of the short half-life of exogenous synthetic LH-RH in serum (3.6 to 8 mins [Arimura et al, 1974; Jeffcoate, Greenwood and Holland, 1974; Pimstone et al, 1977, Barron, Millar and Searle, 1982]) potent long acting analogues of LH-RH were developed (Coy et al, 1976; Schally, Kastin and Coy, 1976). Initial studies using such analogues in both animals and humans again demonstrated the ability of these hormones to cause only transient rises in gonadotrophins and testosterone.

The explanation for this initial stimulation followed by inhibition of gonadotrophin release using the protocols described above had to await a better understanding of the basic physiology. Although it was known that serum LH levels exhibited episodic fluctuations (Nankin and Troen, 1971; Yen et al, 1972; Santen and Bardin, 1973; Boyar et al, 1978) the

physiological significance of this was not appreciated until the work of Knobil and his colleagues who carried out a series of pioneering experiments on the Rhesus monkey.

Dierschke et al (1970) first showed that plasma LH concentrations oscillated with a frequency of approximately 1 hour in 13 ovariectomised and 7 intact female Rhesus monkeys. The oscillations showed a rapid rise of serum LH followed by a decay curve, thus suggesting a pulsatile release of the gonadotrophin from the pituitary gland. Plant et al (1978) were able to obliterate endogenous gonadotrophin secretion in the female Rhesus monkey with bilateral radiofrequency lesions in the region of the arcuate nucleus. Using this technique, Belchetz et al (1978) were able to study the effects of LH-RH infusions on the Rhesus monkey without endogenous LH-RH secretion. They showed that only when the infusion was given as an intermittent delivery was gonadotrophin secretion stimulated and maintained. Continuous infusion resulted in return of gonadotrophin concentrations to basal levels whereupon further intermittent infusion caused a second stimulation. Wildt et al (1981) were later able to show in the same animal model that alteration of the infusion pulse frequency and amplitude critically influenced the gonadotroph response to LH-RH. When LH-RH was infused at a frequency of 1 pulse per hour (thus simulating the natural periodicity

in the female rhesus monkey) physiological serum concentrations of LH and FSH were achieved. When the frequency of administration was increased to 2, 3 or 5 pulses per hour, serum LH and FSH declined. A decrease in the frequency to 3 hourly caused a variable reduction in serum LH but a rise in FSH. A decrease in pulse amplitude resulted in a decline in both LH and FSH, while an increase in amplitude caused a drop in FSH only.

Recently similar studies have been reported in human males with isolated gonadotrophin deficiency. Gross, Matsumoto and Bremner, (1987) have shown that as the frequency of LH-RH administration was decreased from every 1 to 2 to 3 hours serum FSH levels progressively increased while serum LH concentrations did not differ significantly. Spratt et al (1987a), on the other hand, demonstrated that as the frequency of LH-RH stimulation increased, mean serum LH concentrations rose. Assuming that each dose of LH-RH causes an equal increase in gonadotrophin secretion, Spratt argued that doubling the frequency of LH-RH administration should cause a doubling of gonadotrophin concentrations. Therefore, an increase in serum gonadotrophins of less than 100% after a doubling of LH-RH frequency should reflect a diminished gonadotroph responsiveness to LH-RH. Thus, while mean LH rose with pulse frequency, this LH rise was less than expected for the increased LH-RH dosage. The amplitude of each LH pulse also fell as frequency increased.

Spratt and colleagues, found no change in mean FSH concentrations, although FSH corrected for LH-RH dosage fell to a greater degree than corrected LH. These two studies differed in several respects. The former study looked at patients 4 days after alterations in infusion frequences, while the latter group studied acute changes immediately following increases in the frequency of pulsatile LH-RH administration.

It has become clear therefore, that stimulation of the pituitary gonadotrophs requires administration of LH-RH in a pulsatile manner mimicking the normal physiology; continuous exposure to LH-RH or its analogues results in down-regulation of the pituitary gonadotrophs. The mechanisms of gonadotroph down-regulation are not the subject of this text and have been adequately reviewed elsewhere. (Clayton, 1987)

1. 2) NORMAL AND DELAYED SEXUAL DEVELOPMENT

1.2.1) General Concepts

The onset of puberty is a consequence of a complex sequence of maturational changes that are incompletely understood (Grumbach et al 1974; Grumbach 1975; Odell and Swerdloff, 1976). It is recognised that the hypothalamic-pituitary-gonadal system operates in fetal life and infancy (Kaplan, Grumbach and Aubert, 1976) but is suppressed to a low level of activity in childhood.

This inhibitory stage is characterised by low gonadotrophin secretion, increased sensitivity to the negative feedback effects of sex steroids, low pituitary responsiveness to LH-RH and only a slight increase in gonadotrophin secretion in functional and surgical castrates (Kelch, Kaplan and Grumbach, 1973; Kelch et al, 1975; Conte et al, 1980).

The timing of pubertal onset and its course are strongly influenced by genetic factors and are modified by a variety of environmental factors such as nutrition, social deprivation and chronic disease. This has been adequately reviewed elsewhere (Grumbach et al, 1974; Grumbach 1975).

Frisch and Revelle (1970) have suggested that, in healthy girls, there is a 'critical weight' which must be reached before menarche can be achieved. However Johnston and colleagues, (1975) in a carefully argued critique of the work of Frisch and Revelle have exposed the errors in their hypothesis which is based upon population means. The large degree of variability in girls' weights at menarche argues against the critical weight theory. Nevertheless, the possibility remains that some component of body composition can provide a metabolic signal to the CNS for the timing of puberty.

1.2.2) The Endocrine Changes of Puberty

Pre-pubertal children appear to release gonadotrophins in a pulsatile manner although the amplitude of the pulses is small and difficult to detect. Reiter, Root and Ducket, (1976) showed low amplitude LH pulses in 4 of 16 pre-pubertal children and Penny, Olambiwonno and Frasier, (1977) noted episodic LH secretion during the day (0800 - 1300hrs) in 3 sexually immature children between 9 and 11 years of age. More recently, Jakacki et al (1982) using a sensitive LH assay, documented pulsatile secretion of LH in 8 of 15 pre-pubertal children with bone ages less than 10 years.

In 1972 Boyar and colleagues discovered a striking nocturnal rise in gonadotrophins which occurs in boys and girls beginning in late pre-puberty. As adolescents reach the later stages of puberty, daytime LH secretory episodes increase in amplitude, but are still less than during sleep. Finally the adult pattern with LH pulses of similar amplitude throughout the 24 hours, is achieved. The pre-pubertal, nocturnal surge in gonadotrophins probably represents an amplification of the pre-existing pattern of gonadotrophin secretion; several authors have reported significant elevation of urinary gonadotrophin concentrations at night-time in pre-pubertal patients (Kulin and Reiter, 1976; Beck and Wuttle, 1980; Jakacki et al, 1982).

In 1932, Hohlweg and Junkman , describing experiments in the rat, advanced the concept of a change at puberty in sensitivity to circulating sex steroids of a 'CNS Sexualzentrum' that regulates gonadotrophin secretion. There is now a substantial body of data in experimental animals and the human to support the hypothesis that the hypothalamic gonadotrophin-regulating mechanism of the pre-pubertal individual is more sensitive to the negative feedback effects of circulating androgens and oestrogens than that of the adult (Donovan and van der Werff ten Bosch, 1965; Critchlow and Bar-Sela, 1967; Ramirez, 1973; Davidson, 1974). This concept of the 'resetting of the gonadostat' is generally accepted as a critical occurrence in the initiation of puberty. The escape of the CNS regulatory centre from feedback inhibition by the sex steroids allows the hypothalamus to secrete increasingly larger amplitude pulses of LH-RH thus stimulating gonadotrophin release and subsequently maturation of the hypothalamic-gonadal axis progresses. The mechanisms initiating this phenomenon and influencing the timing of pubertal onset are unknown.

1.2.3) Delayed puberty

Most boys presenting with delayed puberty have simple (constitutional) delay, and will undergo the normal process of sexual maturation after a variable period. A

much smaller number who present with delayed puberty will have permanent hypogonadotrophic hypogonadism. One form of this condition is Kallmann's syndrome, in which hypogonadism is associated with anosmia and various congenital abnormalities (Kallmann, Schoenfield and Barrera, 1944). Although the association between hypogonadism and anosmia was known according to De Morsier (1954), since the mid nineteenth century, Kallmann et al, were the first to review the literature of these cases and a genetically determined hypothalamic defect was postulated. In 1971, Naftolin, Harris and Bobrow, demonstrated low gonadotrophin levels in these patients.

1.3) THE USE OF ACUTE ADMINISTRATION OF LH-RH IN ATTEMPTS TO DIFFERENTIATE SIMPLE DELAYED PUBERTY FROM HYPOGONADOTROPHIC HYPOGONADISM

1.3.1) The standard bolus LH-RH test

A difficult problem in clinical practice is to distinguish prospectively between hypogonadotrophic hypogonadism and constitutional delay in puberty. Initially it was hoped that gonadotrophin levels following LH-RH bolus administration might differentiate the two conditions. However, several workers (Hashimoto et al, 1972; Roth et al, 1972; Espiner and Donald, 1973;

Mortimer et al, 1973a) have shown that patients with hypogonadotrophic hypogonadism can have normal, minimal or sub-optimal gonadotrophin responses to single bolus injections of LH-RH. Subsequently, Bell et al (1973) concluded that there was too much heterogeneity of response in the patients with hypogonadotrophic hypogonadism to allow such differentiation to be made.

In 1978 de Lang, Snoep and Doorenbos suggested that four hour infusions of 200 ug of LH-RH could identify patients with hypogonadotrophic hypogonadism since LH values following infusion were lower compared to subjects with constitutional delayed puberty. However, the authors did not quote individual values and the large standards deviations of responses suggest that the test would not be sensitive or specific enough to be of any clinical value. Furthermore, the non-physiological nature of this mode of administration is now recognised. The following year, Razdan et al (1979) demonstrated that 4 hour LH-RH infusions produced greater LH reactions than a conventional bolus test in boys with delayed puberty but not hypogonadal males. However the hypogonadal group included patients with known pituitary lesions and this result is therefore not unexpected. More recently Kelch, Hopwood and Marshall, (1980) found standard gonadotrophin releasing hormone tests of no value in the differentiation of obese children with constitutional delayed puberty and hypogonadotrophic hypogonadism in a

well designed five year study examining gonadotrophin releasing hormone responses throughout this period of time.

1.3.2) The effect of supplementary LH-RH on the standard LH-RH bolus test

Hashimoto et al, (1975) investigated the effect of daily intramuscular injections of LH-RH (400ug for 5 days) on standard bolus LH-RH tests in 13 patients with pituitary and suprasellar lesions. A fall in response in the second test occurred in 3 patients with pituitary tumours and in one patient with a suprasellar lesion who had a normal initial LH-RH test. The only patients to show an augmented response in the second test were the two remaining patients with suprasellar lesions. It may well be that this regimen resulted in down-regulation of the gonadotrophs, particularly in patients showing evidence of some persisting gonadotroph function.

Dickerman, Prager-Lewin and Laron, (1976) investigated the effect on a standard LH-RH test after 5 daily intramuscular injections of LH-RH (100ug) in patients with hypogonadotrophic hypogonadism or pituitary deficiencies. However, the authors divided the patients into 3 groups depending on the degree of augmentation of the LH response and it is therefore impossible to know whether there were any differences between the two

clinical groups.

Wentz and Andersen (1980), investigated the effect of giving 3 I.V. boluses of LH-RH at 2 hourly intervals to females with primary and secondary amenorrhoea of hypothalamic origin and found no augmentation of LH release. Similar results were found for patients with pituitary defects. Snyder and colleagues (1979) were able to normalise the LH response to a bolus LH-RH test in 5 patients with hypothalamic hypogonadism following daily infusions of 500ug LH-RH for 7 days. In view of the non-physiological nature of this therapy and the inability of continuous infusions to stimulate gonadotrophin release in the animal model, these results are surprising.

The major fault with all the above studies is the non-physiological mode of LH-RH administration which is likely to have caused variable degrees of pituitary gonadotroph stimulation or down-regulation depending on the exact protocol used. It is not surprising therefore that the studies give variable results.

1.3.3) The effects of more prolonged, repeated administration of LH-RH

Reitano, Caminos-Torres and Snyder, (1975) found increased LH and FSH release in response to 50ug intravenous injections of LH-RH given 4 hourly for 7 days

to 6 patients with hypogonadotrophic hypogonadism. This study demonstrated that pituitary gonadotrophs must be primed by prior exposure to LH-RH before they are capable of responding to LH-RH in a normal adult fashion. The study however used pharmacological doses of LH-RH given at a non-physiological frequency.

Jacobson and colleagues (1979) administered LH-RH at night-time to 2 males with Kallmann's syndrome using a portable infusion pump in an attempt to simulate normal physiology. LH-RH was infused over one minute every 64 minutes for 10 successive nights. Both patients showed significantly elevated serum FSH concentrations during LH-RH infusions on the last night compared with the first night. Gonadotrophin responses to an intravenous infusion of LH-RH (0.55ug/min for 3 hours) were also increased at the end of the pulsatile therapy, although one patient showed a predominant FSH rise and the other, a biphasic LH response. The high FSH levels achieved by the pulsatile infusion may reflect the one hour pulse frequency used or a primary testicular failure with lack of feedback inhibition of FSH. No details of testicular size are however given.

Valk et al, (1980) studied 4 males and 2 females with isolated gonadotrophin deficiency given LH-RH (0.025ug/kg) intravenously every 2 hours for 5 days. LH concentrations and incremental responses to LH-RH boluses (2.5ug/kg) rose throughout the study in all patients. The

males showed increasing and the females decreasing incremental FSH responses to LH-RH and the authors concluded that this reflected the differences normally seen in maturing boys and girls. However, alternative explanations for this difference in FSH response can be made.

Several recent studies have used pulsatile infusions of LH-RH in an attempt to distinguish between patients with constitutional delay of puberty and permanent hypogonadotrophic hypogonadism (Sippell, Hermanussen and Partsch, 1984; Barkan et al 1985; Partsch, Hermanussen and Sippell, 1985; Wagner et al, 1986;) Sippell et al (1984) gave LH-RH over 36 hours intravenously at a dose of 5ug every 90 mins to 18 hypogonadal patients. Their patients were divided into 4 groups; Kallmann's syndrome (4 patients), idiopathic hypopituitarism (6 patients), isolated hypogonadotrophic hypogonadism (3 patients), and constitutional delayed puberty (5 patients). They demonstrated a significant rise in serum FSH in the patients with Kallmann's syndrome and idiopathic hypopituitarism but not in the patients with isolated hypogonadotrophic hypogonadism and only in 3 of 5 patients with delayed puberty. The difference between the patients with Kallmann's syndrome and hypogonadotrophic hypogonadism is difficult to explain, since the latter group had higher serum testosterone and might therefore have been more adequately primed. The small number of

patients in each group is also disconcerting since hypogonadotrophic hypogonadism is a heterogeneous condition and differences between the groups might reflect different degrees of hypogonadism within each group. A further study by the same workers (Partsch et al, 1985) is open to similar criticism. Using the same protocol, they reported a predominant LH response to short-term pulsatile LH-RH accompanied by a rise in serum testosterone in a group of 9 patients with constitutional delayed puberty whereas an FSH response without LH or testosterone occurred in 8 patients with hypogonadotrophic hypogonadism. This latter pattern in boys is typical of the early normal pubertal response while the former is more like that seen in the later stages of normal puberty. The former group contained 4 patients with testicular volumes of 9mls or greater and it is likely that the differences between the groups is a result of the relative maturity of the boys with delayed puberty who were studied.

Variation in response to pulsatile LH-RH administration in patients with different degrees of hypogonadism was further confirmed by Barkan et al (1985) who divided their patients into partial or complete hypogonadotrophic hypogonadism depending on the presence or absence of secretory LH pulses during 24 hr hormone profiles. They showed a predominent LH response only in the patients with partial hypogonadotrophic hypogonadism.

This study did not include any patients with constitutional delay of puberty for comparison.

Wagner et al (1985) concluded that they were unable to distinguish between temporary and permanent hypothalamic hypogonadism by means of the FSH/LH ratio following pulsatile LH-RH infusions. Their groups were also defined by the presence or absence of nocturnal LH secretory pulses. Their results however show a much greater FSH increment in the patients with absent nocturnal pulses, a feature which might reflect a less advanced state of maturation of the reproductive system in these patients. Since their groups were defined in terms of the initial 24 hour LH secretory profiles and since they compared post-infusion concentrations during two 4 hour periods with these pre-infusion results their data is strongly biased: It is clearly easier to achieve significant rises in serum LH and FSH in the group which had no night-time hormone rises. Since the follow up period of the study was only 90 days the classification of the groups into temporary and permanent hypogonadism may be inaccurate.

1.4) PROLONGED PULSATILE LH-RH INFUSIONS FOR THE INDUCTION OF FERTILITY

As early as 1979, small, portable infusion pumps were used for delivering LH-RH in a pulsatile manner (Jacobson

et al, 1979). Using such technology, chronic pulsatile LH-RH infusions have been used to induce all the changes of puberty in men with hypothalamic hypogonadism, including spermatogenesis (Hoffman and Crowley, 1982; Skarin et al, 1982; Morris et al, 1984; Santoro, Filicori and Crowly, 1986;)

Hoffman and Crowley (1982) reported the response to prolonged pulsatile LH-RH therapy in 6 males with hypogonadotrophic hypogonadism. All patients showed rises in serum gonadotrophins by 2 weeks but in only 4 patients did testosterone achieve adult concentrations and only 3 patients demonstrated maturation of spermatogenesis after 4 to 43 weeks of treatment. Post treatment sperm counts were as low as 2.3 million/ml and only one patient successfully fathered a child. Failure to normalise serum testosterone may have been due to inadequate doses of LH-RH, since a subsequent report by the same group (Santoro et al, 1986) demonstrated normal testosterone concentrations in all 13 patients studied, if the LH-RH pulses were increased in some patients to a maximum of 200ng/kg.

Morris et al (1984) reported normalisation of testosterone in 3 of 10 patients with hypogonadotrophic hypogonadism. All 3 patients achieved fertility although sperm counts were as low as 1.5million/ml and paternity was not tested. These 3 patients had all previously been unsuccessfully treated with the more conventional therapy

of HCG and HMG.

Five patients in the series of Morris showed a declining hormone response to LH-RH during a year's treatment despite increasing the pulse dosage from 2.5 to 20ug. This desensitisation may indicate that pulse dosage or frequency should be specifically tailored to the patient. Stanhope and colleagues (1985a) have described a similar arrest in clinical induction of puberty in a 16.9yr old female with hypogonadotrohpic hypogonadism, following 3 months of pulsatile LH-RH therapy at a dose of 15ug/pulse. They attempt to explain the decreased pituitary responsiveness of this patient as resulting from down-regulation of LH-RH receptors or depletion of gonadotrophin stores or both. They have no evidence however for such mechanisms and after such a prolonged period of treatment, development of antibodies to LH-RH would also be an alternative mechanism.

Morris et al (1984) described a local skin reaction in one patient in their series. Subsequent skin testing confirmed sensitisation to the LH-RH solution. Santoro et al (1986) demonstrated antibodies to LH-RH in 1 of 13 males who failed to respond to pulsatile therapy. Similarly we have been able to show skin sensitivity in one of six patients who recieved prolonged LH-RH therapy. The only indication of sensitisation in this patient during treatment was a progressive decline in gonadotrophin response. If an allergic reaction to the

LH-RH solution was responsible for the decline in LH response in some or all of the 5 patients described by Morris et al and the single patient described by Stanhope et al (1987), then the incidence of this complication may be as high as 8 in 68 (11%) when all the above series are taken into consideration.

Stanhope et al, (1984, 1987) have attempted to simulate normal pubertal development by induction of puberty using 2-4g LH-RH pulses every 90 minutes at night time only, until mid-puberty. When testicular volumes reached 8mls treatment was altered to pulsatile administration throughout 24hrs. Despite this physiological approach, puberty was accelerated 2-3fold compared with the normal rate of pubertal changes (Marshall and Tanner, 1970). The relative merits of this regimen, therefore remain to be established.

The end point in the study of Stanhope et al (1987) was induction of puberty and not fertility. However the results of this study are relevent to the dicussion on hormonal responses to prolonged pulsatile LH-RH administration. Seventeen patients with either delayed puberty or hypogonadotrophic hypogonadism were studied. Puberty was successfully induced in 11. Of the remaining 6 patients, 3 (18%) were non-compliant, 1 patient with Kallmann's syndrome showed 'absent testicular response' and 2 patients had probable pituitary defects. Poor compliance and presumably (although not clearly stated)

absent testosterone production limited the value of this treatment.

The relative merits of LH-RH therapy over more conventional treatment remain to be established. The response in the 3 patients of Morris following failure of HCG and HMG is encouraging. Donald et al (1983) reported a case resistant to HCG but responsive to pulsatile LH-RH. A 30 year old patient with hypogonadotrophic hypogonadism failed to demonstrate a testosterone response to 10 months of HCG therapy (1500 units twice weekly) and remained azoospermic. LH-RH given for 6 months as 6, daily I.V. boluses (5ug per injection) resulted in increased testicular volume and the development of spermatazoa. After changing to hourly 3-min infusions of 2.7ug given subcutaneously, the patient's serum testosterone became normal and sperm count reached 11 million/ml. The reason for failure of testicular response to HCG in this patient is not clear.

Finkel, Phillips and Snyder, (1985) in a carefully executed study of conventional therapy (HCG alone or with HMG) showed that in patients who developed hypogonadism after puberty, fertility was achieved with HCG alone. Five of 7 patients with pre-pubertal hypogonadism responded to combined therapy while only 1 of 7 patients responded to HCG and HMG if there was a history of pre-pubertal hypogonadism and cryptorchism. It is clearly important, therefore, when assessing response to LH-RH

therapy that such factors are taken into consideration. No history of testicular descent was given on any of the patients in the series of Morris et al (1984), Santoro et al(1986) or Stanhope et al (1987).

1.5) FAMILIAL CYTOMEGALIC ADRENOCORTICAL HYPOPLASIA

This condition was first recognised by Sikl in 1948 and later the histological features were described (Kerenyi, 1961). These consist of adrenal cortices with absent zonal differentiation and the presence therein of large vacuolated cells resembling those seen in the normal fetal adrenal gland. A family with 2 affected brothers was first described in 1959 by Mitchell and Rhaney and in 1968, the X-linked inheritance of the condition was demonstrated (Dacou and Di George, 1968).

In 1975, Prader, Zachmann and Illig described a boy with congenital adrenal hypoplasia who failed to show signs of puberty by age 16 years and at age 18 years was shown to have hypogonadotrophic hypogonadism. During 1977, 3 more cases of this association were reported (Black, Brook and Cox, 1977; Golden, Lippe and Kaplan, 1977; Kelly, Joplin and Pearson, 1977)

Kruse, Sippell and Schnakenburg (1984) recently reviewed the response to bolus LH-RH tests in 14 previously reported cases and included results for 2 patients of their own. In 15 of the 16 cases there were

absent or markedly impaired increments of both LH and FSH. The single case with normal peak LH and FSH increments was reported by Petersen et al (1982) and followed only until the age of 14 years at which time he remained prepubertal and had a bone age delay of 2.5 years. It is therefore not known whether this patient spontaneously entered puberty or not. Black et al (1977) and Richards et al (1978) each reported minimally improved gonadotrophin responses to a bolus LH-RH test following intermittent LH-RH stimulation for up to a week. Richards et al were led to conclude from these findings that the hypogonadism in their patient was due to LH-RH deficiency. Similarly, Kelch et al (1984) have suggested on the basis of similar findings that the hypogonadism of this condition is of hypothalamic origin. However, Hay, Smail and Forsyth, (1981) reported the responses to LH-RH tests in 3 patients in whom the tests were repeated several months apart, and variations in the magnitude of responses were similar to those reported by Black et al and Richards et al suggesting that the improvements in gonadotrophin increments which they observed may have occured by chance.

Kruse et al (1984) reported the gonadotrophin response in two patients with presumed congenital adrenal hypoplasia following low dose pulsatile LH-RH infusions for 26 hours. They reported a rise in serum LH and FSH in 1 patient and serum FSH alone in the second patient.

However their data is unconvincing. The changes in gonadotrophin concentrations were small and pulsatile infusions were commenced shortly after standard LH-RH tests had been undertaken. The gonadotrophin concentrations were elevated at the start of the infusion periods compared with the results prior to the LH-RH bolus tests and when this is taken into consideration it is unlikely that there was any change in LH concentrations during the infusions and FSH levels only rose minimally. There was also no significant improvement in response to a second bolus LH-RH test following the period of pulsatile infusion indicating a lack of ability to prime the gonadotroph in these patients.

More recently, Kikuchi et al (1987) have reported failure to induce puberty in another case of congenital adrenal hypoplasia, following 13 weeks of pulsatile LH-RH therapy. They demonstrated a minimal increase in serum FSH alone while serum LH remained unchanged and testosterone remained undetectable. There was a small improvement of the FSH response to a standard LH-RH bolus by day 21 of the infusion, although the response remained subnormal and there was no further improvement.

The data of Kikuchi et al would therefore favour a pituitary basis for the hypogonadism and this is of interest in view of the report of Marsden and Zakhour (1978) showing similar cytomegalic changes in the pituitary of a patient with cytomegalic adrenal

hypoplasia.

1.6) HUMAN CHORIONIC GONADOTROPHIN THERAPY FOR INDUCTION OF PUBERTY

In 1904, Halban observed that the genital organs of newborn infants of both sexes showed evidence of hypertrophy. He speculated that this temporary development of the genitalia was due to the action of a then unknown secretion of the placenta. In 1932, Engle demonstrated that an extract of urine from pregnant women was able to increase the size of the testes in immature rats and monkeys. The urinary extract was therefore similar in effect to anterior pituitary gland extract which was also shown to cause growth of testicular interstitial cells and tubules. The following year, Goldman and Stern (1933), demonstrated the effectiveness of anterior pituitary-like gonadotrophic hormone on the undescended testes of two adolescent boys.

Later, Sexton (1934) showed the ability of this urinary extract to stimulate testicular development in patients with cryptorchism and hypogonadal states. In a series of papers between 1935 and 1942, Dorff confirmed the ability of extracts from pregnant urine to stimulate testicular growth along with the development of secondary sexual characteristics in groups of boys with hypogonadism (1935a, 1935b, 1936, 1937, 1938, 1940, 1942)

The studies of Dorff and others were carried out in heterogeneous groups of children at a time when it was not yet possible to confirm endocrine pathology by measurement of serum hormone concentrations. It is therefore likely that many of the children with hypogonadism were suffering from a number of different hormone problems such as hypothyroidism and hypopituitarism as well as primary hypogonadism. This is of particular importance when the data on growth (Dorff 1940, 1942) are discussed, since the presence of other endocrine pathology is likely to have influenced the results of HCG treatment.

In 1940, Dorff described growth data in 9 boys aged between 3.25 and 8 years who were treated with a variety of HCG preparations for periods ranging from 5 to 25 months. Four patients who were obese also received dessicated thyroid. In most cases there was an increase in height velocity accompanied by more rapid advancement of skeletal age than chronological age. However, uncertainty about the underlying diagnoses, the possible effect of thyroid hormone on growth, and the inability at the time to accurately assess skeletal age makes the findings difficult to interpret.

A further paper by Dorff in 1942 describes growth in 4 boys aged 3.25 to 21 years who received HCG for varying periods for cryptorchidism (2 boys), hypogonadism and short stature. Again acceleration in growth was

demonstrated during treatment. However, in 2 cases accelerated growth continued for many years after treatment stopped and it is therefore difficult to believe that this continued rate of height gain was due to the HCG. Skeletal age data are given on only one patient and it is difficult to assess the accuracy of such information.

Reiss et al (1965), described the growth promoting action of HCG in 24 mentally and physically retarded boys aged 7.5 to 13.5 years. Since this is the only other work describing growth during HCG treatment, it demands some attention.

HCG was given in doses between 400 and 2500 IU daily for 2 to 7 months. Growth rates were considerably increased during the treatment periods and in the majority of cases height velocities exceeded that seen during a normal pubertal growth spurt. Growth rates immediately after treatment was stopped were recorded in most patients although data on periods of longer than one month were available on only 9 patients. All patients showed reduced growth following therapy although 8 of 9 patients were growing faster after treatment than before therapy was begun.

Follow up data, 3 to 5 years after treatment was available on 17 patients. Twelve of the patients showed a height percentile which was higher than the pre-treatment range. No information, however was available on skeletal

Authors	No of patients	Age (yrs)	Diagnosis	Therapy Regimen	Length of Follow-up	Comments
Sobel et al 1956	13	5.1-10	S.S.	5-40 mg/day for 6 months	Up to 24 months	Δ BA > ΔHA
Bayley et al 1957	46	10-18	C.D.	5-20 mg/day for 6 months+	Up to 19 yrs	During first year, mean
	5		C.D.	30 mg/day for 6 months+	20 patients achieved	Δ BA = 1.4 yr
	25		Controls	None	F.A.H.	Mean FAH > mean PAH in 20 patients
Foss 1965	30	Mean age 12.1 yr	S.S.	5-30 mg/day for 2-14 months	Up to 18 years	No effect on FAH in low dosage
	37	Mean age 14.3 yr	C.D.	or 75 mg/day intermittently for 30 months	FAH known for 50 controls and 64	FAH PAH with implants+ oral MT or IM inj
	21	12.8-25	Hypogonadism	Implants + oral MT or IM inj	treated patients	
	120	9-17	Controls	None		
Kaplan et al 1973	9	13.3-16.3	C.D.	10-20 mg for 3-6 months	Up to 15 yrs	PAH overestimated FAH of controls
	12	11.7-16.3	C.D. controls	None		Treated patients closer to P.A.H.
	4	13.4-16.6	D.P.	20 mg for 2-6 months		
	9	11.5-15.3	D.P. controls	None		
Sharma et al 1974	109	10-18	C.D.	5-10 mg/day for 6-24 months	No post-therapy follow-up	10-14 yr Mean PAH +1.1 14-18 yr Mean PAH +0.8

TABLE 1

Growth studies with methyltestosterone

S.S. = short stature
C.D. = constitutional delayed puberty
B.A. = bone age
H.A. = height age
F.A.H. = final adult height
P.A.H. = predicted adult height
S.D.S. = standard deviation score

Authors	No of patients	Age (yrs)	Diagnosis	Therapy Regimen	Length of follow-up	Comments
Mellman et al 1961	10	4.7-13.7	C.D.	2-5 mg/day for 3-14 months	No post therapy follow-up	$\Delta BA > \Delta HA$ 4 patients $\Delta BA \leq \Delta HA$ 6 patients
Reilly and Gordan 1961	1 11	14.1 9.9-15.9	C.D. S.S.	5 mg/day for 12 months 5-15 mg/day for 6-41 months	No post therapy follow-up	Δ mean PAH + 2.5 inches Δ mean PAH + 1.4 inches
Laron 1961	13	1.8-10.8	C.D.	0.1-0.15 mg/kg/day for 3 months 0.25 mg/kg/day for 3 months	2-4.5 months	B.A. not accelerated during treatment
Laron 1963	43	3.4-14.8	C.D.	0.05-0.25 mg/kg/day for 6-7 months	2-17 months	$\Delta BA \leq \Delta HA$
Kaplan et al 1973	4	13.8-15.9	C.D.	5-10 mg/day for 4-6 months	Up to 15 years	FAH obtained by communication mean FAH 1.3 in > mean PAH treated patients mean FAH 1.1 in < mean PAH controls
Lenko et al 1982	61 29	9.2-19.3	C.D. Controls	0.05-0.24 mg/kg/day for 5-42 months	6-45 months	No reduction in PAH when BA > 10.5 yrs
Stanhope et al 1985	17	12.8-17.5	C.D.	2.5-5 mg/day for 0.23-0.58 yrs	2-16 months	Mean height for BA SDS remained unchanged

TABLE 2

Growth studies to fluoxymesterone

Authors	No of Patients	Age (yrs)	Diagnosis	Therapy Regimen	Length of follow-up	Comments
Danowski et al 1967	11	3.8-17.8	C.D.	10-80 mg/day for 6-44 months	No follow-up following therapy	
Zangeneh and Steiner 1967	10	0.9-8.1	C.D.	0.1 mg/kg/day for 16 months	7-31 months	Δ BA > ΔHA
	8	11-15	C.D.	0.1 mg/kg/day for 16 months		Δ BA = ΔHA
Geller 1968	20	4.5-15.5	S.S.(13CD)	0.15-0.25 mg/kg/day for 1 yr or intermittently for 2 yrs	0-6 months	Δ BA $\leq$ ΔHA (continuous therapy) Δ BA > ΔHA (intermittent therapy)
Bettman et al 1971	27	3.6-17	S.S.(17CD)	0.05-0.2 mg/kg/day for 6 months	6-12 months	Decrease in PAH in half patients when BA < 9 yrs PAH unchanged or increased when BA > 9 yrs
Limbeck et al 1971	25	5.8-15.7	C.D.	0.25 mg/kg/day for 3-21 months	4-7 months data on 7 patients only	Δ BA < ΔHA in 14 Δ BA > ΔHA in 7 Patients with BA < 10 not compromised
Jackson et al 1973	9	8.6-14.8	C.D.	0.25 mg/kg/day intermittently for 2 yrs	No follow up following therapy	Decreased PAH in 5 patients greatest in those with BA 6-7 yrs
Marti-Henneberg et al 1975	9	11.2-13.3	C.D.	0.1 mg/kg/day for 6-33 months	No follow-up following therapy	No HA or BA acceleration compared to controls
	8	11.3-13	C.D.(controls)	None		
Stanhope and Brooks 1985	14	(12.7-16.7)	C.D.	2.5 mg/day for 0.21-0.65 yrs	3-13 months	No change in height for bone age SDS
	10		S.S.			

TABLE 3

Growth studies with oxandrolone

maturity or final height outcome.

Similarly, no data was given regarding the androgenising effects of this therapy or the endocrinology of the patients. It is notable that 5 patients received thyroid replacement therapy in the follow up period and this is likely to have affected growth in patients who were hypothyroid.

In 1941, McCullagh and Rossmiller reported the effect of methyltestosterone on growth and metabolism. However concern arose over reports that large doses of this androgenic compound stimulated osseous maturation disproportionately to linear growth. (Talbot and Sobel, 1947; Deamer, 1948; Sobel et al, 1956). Despite these reports, other drugs which might have improved anabolic:androgenic ratios were sought and many derivatives of testosterone were produced. This has been adequately reviewed elsewhere (Hopwood and Kelch, 1976). While it is outwith the scope of this text to review in detail the literature on anabolic steroids it is important to compare the effects of these agents with the data on HCG therapy. For this reason tables 1 - 3 list information on the larger clinical trials in patients with delayed puberty, of the three most widely used of these compounds, i.e. methyltestosterone, fluoxymesterone and oxandrolone.

It is immediately clear from these tables that many studies contained small numbers of patients, of whom some

had short stature and some delayed pubertal development. There is a wide range of age groups treated in the different studies. In addition, the doses and the treatment duration of the anabolic agents used, varied considerably between studies and often within studies. The lengths of periods following cessation of treatments has often been short and therefore inadequate for assessing effects on future growth. It is not surprising therefore that there is a general lack of consensus regarding the relative effects of these agents on height growth and skeletal maturation.

Furthermore, few papers include adequate control data. This is of particular importance when the age group studied is likely to enter spontaneous puberty during the period of treatment. For example, Stanhope, Bommen and Brook, (1985b) have suggested that a 3 month course of fluoxymesterone was sufficient to accelerate growth which was maintained after treatment was stopped. However, the mean testicular volume of their patients, at the end of the treatment period was 9.9 mls suggesting that perhaps half the patients studied had entered a spontaneous growth spurt during this period. This would also explain the continuing rapid growth after treatment was stopped. Without the inclusion of a control group, it is therefore impossible to demonstrate a therapeutic effect in this study. On the other hand, Lenko, Maenpaa and Perheentupa (1982), in a much larger study, using control patients

was clearly able to demonstrate an acceleration in growth velocity in the treated group.

Some authors compare change in bone age with change in height age after therapy. Height age is the chronological age at which the particular height measured would lie on the 50th percentile. Height age, however disregards the individuality of height potential (half the population never reaches so-called adult height i.e. 177cm for males) and the variability of growth during the pubertal years ; thus conclusions based on height age changes may be highly misleading.

Similarly, prediction of adult height using the tables of Bayley and Pinneau (1952), is beset by problems. These tables are less accurate if bone age retardation is greater than 2 years, as is the case in many patients undergoing anabolic steroid treatment. This might lead to a tendency to either over- or under-estimate final adult heights compared with later predictions during treatment, when bone age delay is likely to be smaller.

Several limited conclusions can therefore be made from the previous work with anabolic steroids. It is likely that high doses of anabolic steroids used for long periods adversely affect final height outcome. Reilly and Gordan (1961) have shown that the effect of fluoxymesterone on epiphyseal maturation is dose related but growth acceleration is not. In a very large,

controlled study, Foss (1965) showed that patients treated with methyltestosterone for periods between 2 and 14 months at doses less than 30mg daily achieved expected final heights. However patients treated continuously with either implants, oral testosterone or frequent injections of testosterone esters showed a significant reduction in final height compared with initial predictions.

The age of the patient and in particular, the skeletal age at the time of therapy may also be important. Zangeneh and Steiner (1967) found disproportionate bone acceleration in children between 0.9 and 8.1 years treated with oxandrolone. This did not occur in older children. Similarly, Jackson et al (1973) found the greatest reduction in predicted adult height in patients with the earliest bone ages who received oxandrolone therapy. However Limbeck et al (1971) using height age determinations did not detect a disproportionate skeletal maturation in patients with bone ages less than 10 years.

1.7) AIMS OF THE PRESENT STUDY

The present study set out to investigate the value of pulsatile LH-RH infusions in the investigation of delayed pubertal states and to assess HCG as a therapeutic agent for the treatment of constitutional delayed puberty. In view of the lack of consensus in the literature regarding

the short-term hormonal responses to pulsatile LH-RH administration in different groups of hypogonadal patients, an attempt has been made to take account of the effects of LH-RH dosage, timing of studies and, most importantly, the heterogeneity of the clinical conditions studied.

An attempt has also been made to assess both the immediate and long-term effects of HCG therapy, on the growth of a typical group of patients presenting to an endocrine clinic with delayed puberty.

During the course of this work other papers have been published which are relevant to the present thesis. These publications are discussed in the relevant chapters of this thesis along with the author's own data.

CHAPTER 2

EXPERIMENTAL SECTION

2.1) SUBJECTS AND METHODS

2.1.1) Acquisition of patients

The Glasgow Royal Infirmary offers an endocrinological service to the West of Scotland and, in particular, the eastern area of Glasgow. Patients in this study were usually referred by their General Practitioner. Occasionally patients were referred by the school Medical Officer or by other hospital specialists. All patients in this study were male and referred for investigation of pubertal delay or shortness of stature. Some adults with hypogonadotrophic hypogonadism were referred by consultant endocrinologists from other hospitals in Glasgow and Falkirk.

2.1.2) Initial presentation and clinical examination

The patients were initially seen at the Endocrine Clinic at the Royal Infirmary, Glasgow and a full clinical history was recorded in the presence of one or both parents. A full medical examination including measurement of standing height (method of Tanner and Whitehouse,

1975) was undertaken. Genital and pubic hair development were assessed on a five point scale devised by Tanner (1962). The scale is described below:

Boys : genital development : (G)

Stage 1 Pre-adolescent. Testes, scrotum and penis are of about the same size and proportion as in early childhood.

Stage 2 Enlargement of scrotum and testes. Skin of scrotum reddens and changes in texture. Little or no enlargement of penis at this stage.

Stage 3 Enlargement of penis, which occurs at first mainly in length. Further growth of testes and scrotum.

Stage 4 Increased size of penis with growth in breadth and development of glans. Testes and scrotum larger; scrotal skin darker.

Stage 5 Genitalia adult in size and shape.

Pubic hair : (P)

Stage 1 Pre adolescent. The vellus over the pubes is not futher developed than that over the abdominal wall, i.e. no pubic hair.

Stage 2 Sparse growth of long, slightly pigmented

downy hair, straight or slightly curled, chiefly at the base of the penis.

Stage 3 Considerably darker and coarser and more curled. The hair spreads over the junction of the pubes.

Stage 4 Hair now adult in type, but area covered is still considerably smaller than in the adult. No spread to the medial surface of thighs.

Stage 5 Adult in quality and type with distribution of the horizontal (or clasically 'feminine') pattern. Spread to medial surface of not up linea alba or elsewhere above the base of the inverse triangle (spread up linea alba occurs late and is rated 6).

Testicular volume (T.V.) was measured using a Prader orchidometer (Prader, 1966). All measurements of height, weight and genital development were made by one observer.

2.1.3) Assessment of bone age

In all patients, bone age was assessed routinely by Greulich-Pyle (1959) hand standards. Those patients who were included in the study of HCG therapy had skeletal age estimated in a retrospective manner, by one observer,

using both the Greulich-Pyle atlas and also the TW2 method of Tanner et al (1975). Estimates of final heights were made using the method of Bayley and Pinneau (1952) revised for use with the Greulich-Pyle atlas and also the method of Tanner et al (1975) using the TW2 bone ages.

2.1.4) Interpretation of clinical measurements

The height standard deviation score was determined according to the formula X-x/Sx where X is the patient's standing height, x the mean height at that age and Sx is the standard deviation for height at that age. The last two measurements were obtained from data according to Tanner, Whitehouse and Takaishi, (1966). The patients' heights and weights were plotted on the growth charts of Tanner and Whitehouse (1975). Height age was defined as the age at which a particular height would lie on the 50th centile according to the above charts. The degree of sexual maturation was determined from the percentile charts for testicular volume and genital and pubic hair development according to Tanner and Whitehouse (1976); data also from Marshall and Tanner (1970) and Zachmann et al (1974).

2.1.5) Selection of patients for study

Subjects of chronological age of 14 years or older

were assessed if height was below the third percentile and if genital staging and or testicular volumes were below the third percentile. In addition, subjects of chronological age 12.5 to 14 years who were pre-pubertal (i.e. G1 P1 T.V. < 4 ml) were studied if height was below the third percentile and if there was a bone age deficit compared to chronological age of at least 2 years.

For the main pulsatile LH-RH studies comparing patients with permanent hypogonadotrophic hypogonadism with patients with delayed puberty (chapters 5 - 7) the boys were divided into two groups; those with no signs of puberty beyond Tanner stage 2 by the age of 14 years or greater were defined as 'delayed puberty'. The remainder of the boys whose height was below the third percentile and whose puberty was delayed but whose pubertal staging was still above the 3rd percentile according to Tanner were defined as 'short stature'.

Hypogonadotrophic hypogonadism was diagnosed by absence of pubertal changes during follow-up to chronological age 21 years. In addition, the finding of low basal serum gonadotrophin levels relative to the low serum testosterone was required for diagnosis.

Thirty five patients were included in the study comparing gonadotrophin responses to pulsatile LH-RH infusions (chapter 5). The patients' diagnoses are listed in table 4 below. Further clinical details of these patients are given in the relevant chapter and details on

patients who underwent other studies are also given in the approprate chapters.

Table 4

THE FINAL DIAGNOSIS IN 35 PATIENTS UNDERGOING PULSATILE LH-RH THERAPY

Simple Delayed Puberty	9
Short Stature	14
Idiopathic Hypogonadotrophic Hypogonadism	7
Kallmann's Syndrome	4
Anorchia	1

Adult patients with idiopathic hypogonadotrophic hypogonadism who expressed a desire for fertility were offered therapy initially by pulsatile infusion of LH-RH.

2.2) CLINICAL INVESTIGATIONS.

2.2.1) Initial endocrine assessment

All subjects underwent a combined anterior pituitary function test. In addition serum adrenal and testicular androgens, dihydroepiandrosterone (DHA) dihydroepiandrosterone sulphate (DHAS) and testosterone were estimated. Chromosome analysis was undertaken in every patient.

2.2.2) Hormone estimations

All hormone estimations were performed in the Endocrine Section of the Department of Clinical Biochemistry, Royal Infirmary, Glasgow. All hormones were measured by radioimmunoassay techniques and a summary of these methods is given in Table 5. The assay methods remained constant throughout the study.

2.2.3) Anterior pituitary function test (Mortimer et al, 1973b)

Following an overnight fast in hospital 0.2 units/kg body weight of soluble insulin, 100ug gonadotrophin

Analyte	Antibody	Final Titre (x 1/1000)	Standard	Radioligand	Mass (pg) Counts Added (cpm)	Separation	Incubation (hrs)	Mean % Coefficient of variation Intra-assay	Inter-assay	Sensitivity	Adult Male Ref. range
1. GH	Rabbit-Hunter 6B (Edinburgh)	1/600	MRC 66/217	MRC 69/46 - local	100 pg	Double Antibody	16 + 6 + 16	6%	9%	0.5 mU/L	10 mU/L
2. LH	Rabbit-Butt F87-2 (Birmingham)	1/1500	MRC 68/40	Butt LH-4 - local	100 pg	"	16 + 16 + 16	8%	12%	1.0 U/L	8.6 U/L
3. FSH	Rabbit-Butt M93 (Birmingham)	1/3000	MRC 78/549	Butt CPDS - local	100 pg	"	16 + 16 + 16	8%	12%	0.5 U/L	4.5 U/L
4. PRL	Goat-Corning (Halstead)	1/125	MRC 75/504	Friesen - local	100 pg	"	16 + 6 + 16	8%	12%	50 mU/L	60-360 mU/L
5. T	Sheep - Fahmy -3-O-(CMO)-BSA Cardiff	1/150	Steraloids	^{125}I Histamine local	2 x 10^4 cpm	"	2 + 16	6%	10%	0.5 nmol/L	11-36 nmol/L
6. A	Rabbit-Guildhay 673-1A - 17 - (CTE)-ovalbumin (Guildford)	1/120	Steraloids	3H - Amersham	2 x 10^4cpm	"	2 + 16	8%	12%	0.5 nmol/L	2.0-11 nmol/L
7.DHAS	Rabbit-Rudd M99 (Birmingham)	1/30	Sigma	3H - New England Nuclear	1 x 10^4 cpm	Double Antibody	2 + 16	8%	12%	0.5 umol/L	2.0-9.0 umol/

TABLE 5

DETAILS OF THE HORMONE ANALYSIS

releasing hormone and 200ug thyrotropin releasing hormone were simultaneously administered intravenously through an indwelling cannula inserted 15 minutes prior to the test. Blood was withdrawn for plasma glucose and hormone analysis at the times shown with a cross below.

Time (mins)	Gluc	Cort	hGH	PRL	LH	FSH	TSH	Testo
0	+	+	+	+	+	+	+	+
15	+	+	+					
30	+	+	+		+	+	+	
45	+	+	+					
60	+	+	+		+	+	+	
90	+	+	+					
120	+	+	+					

The test was only considered as satisfactory for growth hormone reserve when the plasma glucose fell to a value of 2.2 mmol/l or less.

2.2.4) Chromosome Analysis

Chromosome analysis was performed on samples of venous blood by Professor Ferguson-Smith of the Institute of Genetics, Royal Hospital for Sick Children, Yorkhill, Glasgow.

2.2.5) Semen Analysis

Semen analysis was carried out by the staff of the Department of Pathology at the Royal Infirmary, Glasgow.

2.2.6) LH-RH Infusion Study Protocols

The basic protocol for patients undergoing pulsatile LH-RH infusion studies is described below. Variations upon this basic protocol were made for some studies and the details of these alterations are described in the Patients and Methods section of the relevant chapter.

During the afternoon of the admission day, blood was sampled via an indwelling venous catheter, at 15 minute intervals over a period of 3 hours for subsequent measurement of plasma LH and FSH. The following morning after an overnight fast, pituitary function was assessed as described above. Serum testosterone was measured at this time and a note made of the exact time of sampling. Thereafter subcutaneous pulsatile LH-RH therapy was commenced using an infusion pump (Graseby Medical, type MS 27) with delivery of gonadotrophin-releasing hormone (LH-RH) pulses every 90 minutes at a dose of 240ng/kg/pulse in most studies. The needle was inserted into the subcutaneous tissue of the abdominal wall and the site changed daily.

Following 6 days treatment with LH-RH further venous blood sampling every 15 minutes for 3 hours was undertaken at the same time of day as the preinfusion study, for estimation of LH and FSH. Blood was drawn for measurement of serum testosterone at exactly the same time of day as the pretreatment sample. All samples from each patient were kept frozen at -20 degrees C and assayed in one batch to eliminate inter-assay error.

2.3) PATIENT FOLLOW-UP

2.3.1) Routine post-admission review

All patients attended the out-patient clinic 6 weeks after hospital discharge and if there had been no clinical or endocrine abnormality detected, patients were offered specific therapy to induce secondary sexual development. After full discussion, patients were able to choose between treatment or regular follow-up in the absence of specific therapy.

Patients who received HCG therapy were re-evaluated at 3 monthly intervals during the first year. Clinical examination included reassessment of sexual development and measurement of testicular volume, standing height and weight.

2.3.2) Retrospective studies and patient recall

Case-notes for all patients who had attended the Endocrine Clinic for investigation of short stature or delayed puberty between the years 1979 - 1987 were reviewed. All patients who fell into the selection categories described in the previous section and who had had careful serial measurements at 3 monthly intervals, of height and sexual maturation by one of two observers (D.G. or H.N.C.) were selected. Attempts were made to recall all selected patients in order to determine present height and bone age.

Fifty males who subsequently underwent 6 month's therapy with Human Chorionic Gonadotrophin (HCG) were reviewed. A further 30 males who declined therapy act as a control group.

2.3.3) Male infertility study.

Adult males with either idiopathic hypogonadotrophic hypogonadism or Kallmann's syndrome who expressed a wish for fertility were offered treatment, initially by pulsatile LH-RH infusion. Those patients receiving sex hormone replacement therapy had all treatment stopped at least 6 weeks prior to the study period. LH-RH was administered subcutaneously in pulses every 90 minutes by

pulsatile syringe driver (Graseby, MS27). Doses of LH-RH varied according to hormonal responses. Serum LH and FSH were measured every 15 minutes for 3 hours before starting LH-RH therapy. Each week thereafter blood was withdrawn every 15 minutes, over a 1 hour period for further measurement of serum LH and FSH. Serum testosterone was measured weekly. Where possible, semen samples were obtained by masturbation from patients before treatment and at monthly intervals thereafter.

Treatment was stopped after varying periods of time due to; a)lack of patient compliance with treatment b)patient unwilling to continue with therapy c)impaired or absent hormonal response d)failure to obtain a significant rise in sperm numbers or inability to become fertile. Some patients were subsequently offered therapy with human chorionic gonadotrophin and human menopausal gonadotrophin (Pergonal).

2.4) STATISTICAL ANALYSIS

Comparison between groups has been performed by standard non-parametric testing. The Wilcoxon paired and unpaired tests and the Kruskal-Wallis test were used as appropriate. Relationships between groups were correlated using the Spearsman rank order test.

CHAPTER 3

24 HOUR HORMONE PROFILES DURING PULSATILE LH-RH INFUSIONS.

3.1) INTRODUCTION

Several recent studies have examined the effects of prolonged, pulsatile LH-RH infusions on pituitary gonadotroph response in pre-pubertal and pubertal males (Valk et al, 1980; Barkan et al, 1985; Delemarre-van de Waal et al, 1985; Partsch et al, 1985; Wagner et al, 1986). However results are contradictory and comparison between studies is confounded by the variety of LH-RH infusion protocols used by the different workers. With the exception of the study of Wagner and colleagues (1986), no account has been taken of the time of day at which the study was undertaken.

It is known from the work of Boyar et al (1972) that boys in early puberty exhibit nocturnal surges in LH and testosterone secretion. The nocturnal LH peaks increase in amplitude as puberty progresses and eventually, in late puberty, LH pulses become easily detectable throughout the day. The effect of 'round-the-clock' pulsatile LH-RH administration on the natural, diurnal variation in LH secretion has never been investigated. When assessing the effects of LH-RH infusions it may be

important to take account of the changing baseline of LH and testosterone secretion throughout the day. For these reasons a pilot study was carried out in 12 pubertal patients to assess the effect of dosage and time of day on the response to pulsatile LH-RH administration.

3.2) PATIENTS AND METHODS

Clinical details of the patients undergoing this study are shown in table 6. All patients were of short stature and the 24hour hormone profiles included hourly growth hormone measurements (growth hormone data is not given). Measurement of serum thyroxine, tri-iodothyronine, TSH and the two 24hour hormone profiles replaced the combined anterior pituitary function tests as the method of assessing the pituitary in these patients.

All patients underwent the basic protocol for LH-RH pulsatile infusion over 6 days, as described in the Experimental Section. Twenty-four hour hormone profiles were carried out immediately prior to, and at the end of the pulsatile infusions in the first 6 patients studied. A shorter study period, between the hours of 21.00 and 06.00 the following morning, was utilised in the remaining 6 patients. During the study times, blood was withdrawn every 20 minutes for estimation of serum LH and FSH. Every hour, on the hour, additional blood was drawn

PROTOCOL

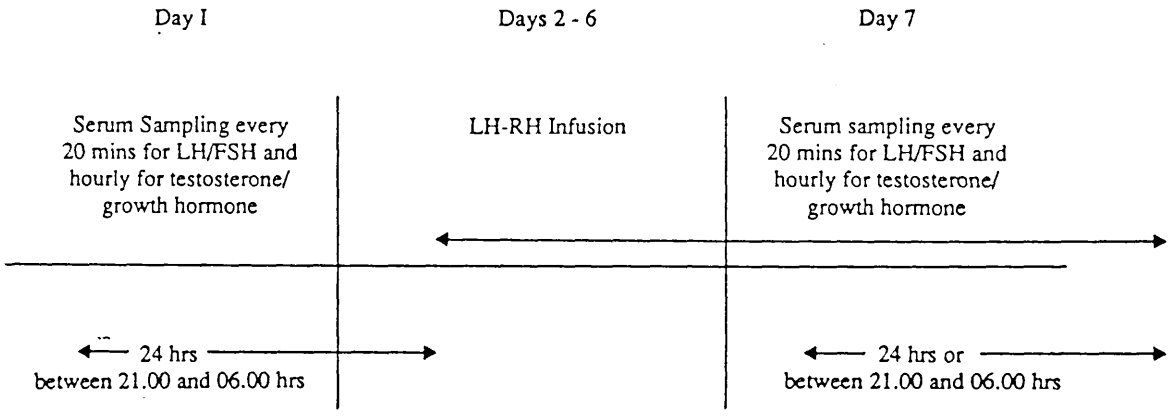


Figure 1
Study protocol.

for measurement of serum testosterone and growth hormone. The study protocol is shown in diagramatic form in figure 1.

LH-RH doses of 2.5, 7.5 and 15 ug/pulse were used in the first 6 patients, whilst patients 7 to 12 all received pulses of 7.5ug LH-RH.

3.3) RESULTS

The results of the pulsatile infusions of LH-RH on serum LH and testosterone concentrations during 24hour periods are shown on figures 2 - 3. All patients showed the characteristic, pubertal night-time surges of LH and testosterone in the pre-treatment profiles. Patient b), who was at genital stage 3 (table 6) also clearly showed LH pulses during the day-time.

Analysis of the LH profiles during the final 24 hours of pulsatile infusion revealed several interesting features: Firstly, despite the continuous nature of the infusion (i.e. pulses were given throughout the 24 hours) LH concentrations in blood continued to show a night-time rise although in most cases this was less marked than prior to therapy. Only patient e) showed stimulation of serum LH concentrations above pre-treatment levels throughout the 24 hour period. The remaining patients showed either broadly unchanged or reduced serum LH during the night.Stimulation of LH secretion occured only

24 Hour Profiles	Genital Staging	Testicular Volumes (mls)	Chronological Age (yrs)	Bone Age (yrs)
Patient a)	G1P1	6/6	15.2	12.6
b)	G3P3	10/10	14.7	13.5
c)	G1P1	3/3	15.1	11.2
d)	G2P2	6/6	14.6	11.6
e)	G2P2	8/10	17.8	14.0
f)	G1P1	5/5	15.8	11.0
Profiles Between 21.00 and 06.00 hours				
Patient g)	G2P2	8/8	17.8	14.0
h)	G1P1	4/3	14.6	11.5
i)	G1P2	5/5	18.1	13.5
j)	G2P2	6/6	14.9	11.5
k)	G1P1	3/3	15.0	11.2
l)	G1P1	6/6	14.9	11.0

TABLE 6

Clinical details of patients undergoing hormone profiles prior to and during LH-RH infusions

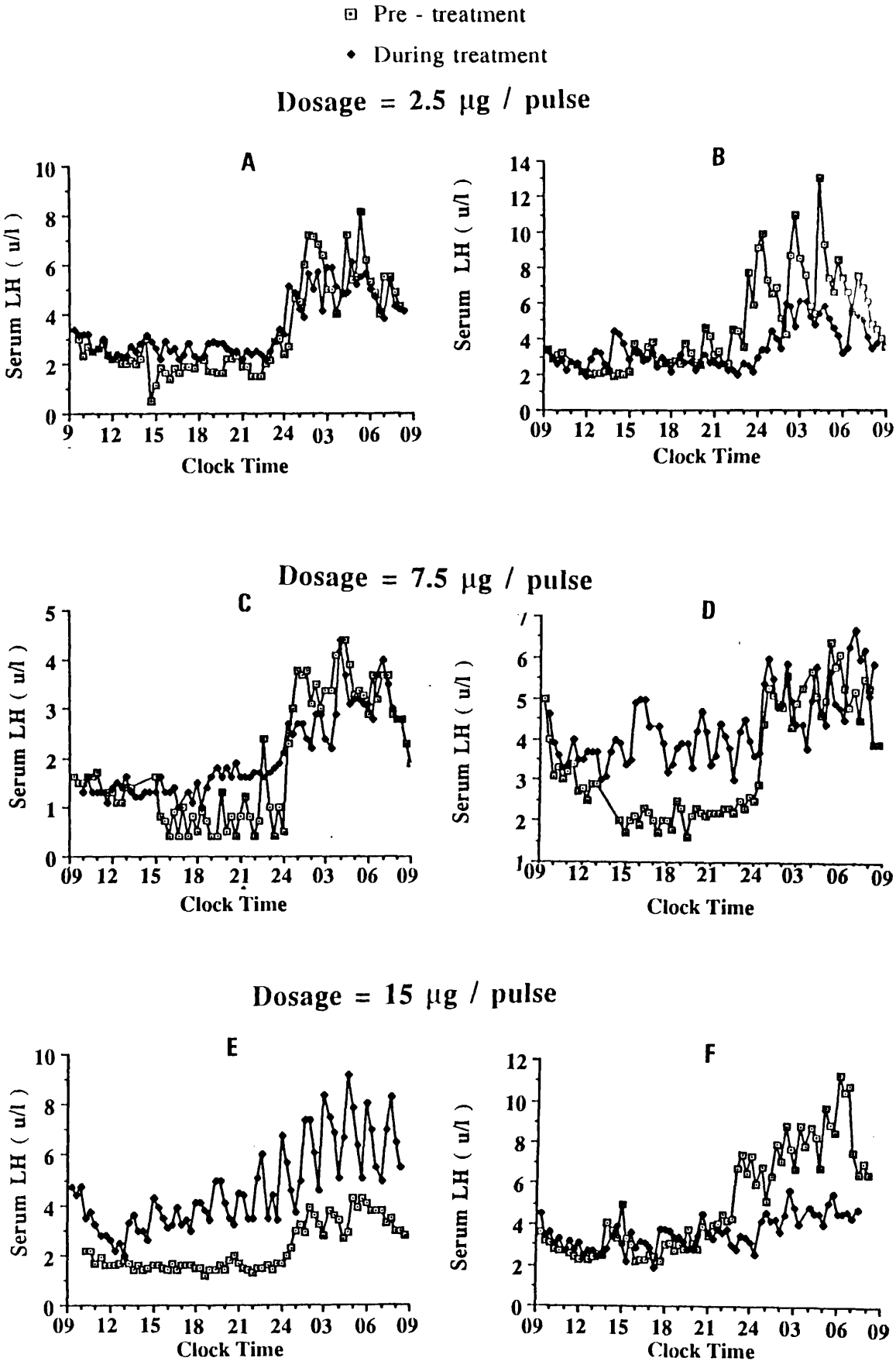


Figure 2

24-hour LH profiles in 6 patients prior to, and during pulsatile LH-RH infusions at doses of 2.5, 7.5 and 15ug/pulse. Pubertal staging data for each patient is given in the text.

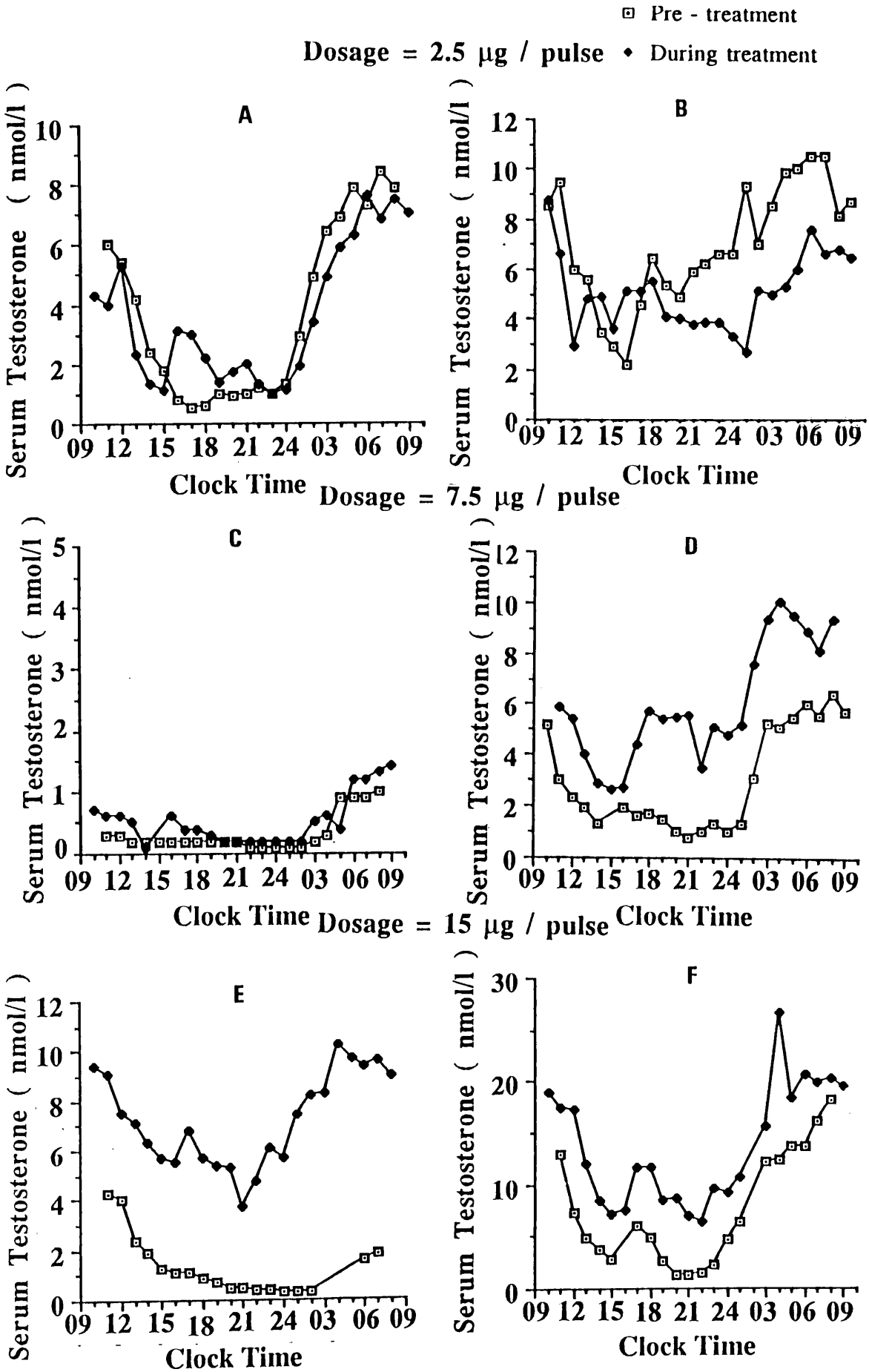


Figure 3

24-hour testosterone profiles in 6 patients prior to, and during pulsatile LH-RH infusions at doses of 2.5, 7.5 and 15ug/pulse.

during the day at times when pre-infusion concentrations were at a nadir. No significant LH stimulation at any time is evident in patient b) who had pulses of LH secretion during the day and night prior to LH-RH infusion.

It is difficult to see any significant effect of pulse dosage on LH concentrations. Whilst the patient showing the greatest stimulation had the highest dose regimen, patient f) who also received 15ug pulses showed marked reduction in LH secretion at night-time.

Despite the 'flattening out' of the LH curves during pulsatile LH-RH infusions, most patients continued to demonstrate marked diurnal variation in serum testosterone during the treatment period. Patient c) who had very low or undetectable serum testosterone concentrations in the pre-infusion profile failed to show any significant stimulation during the study. Patient a) showed elevated testosterone concentrations only between 16.00 and 24.00 hours. Patient b) who was already well established in puberty, had lower serum testosterone at almost all time points, during the second profile.

Similar results were obtained for the 6 patients who underwent the shorter study periods. All 6 patients showed elevated serum LH concentrations compared with pre-infusion results between 21.00 and 24.00 hours. However, after midnight, the stimulation of LH release was more variable (figure 4). Serum testosterone

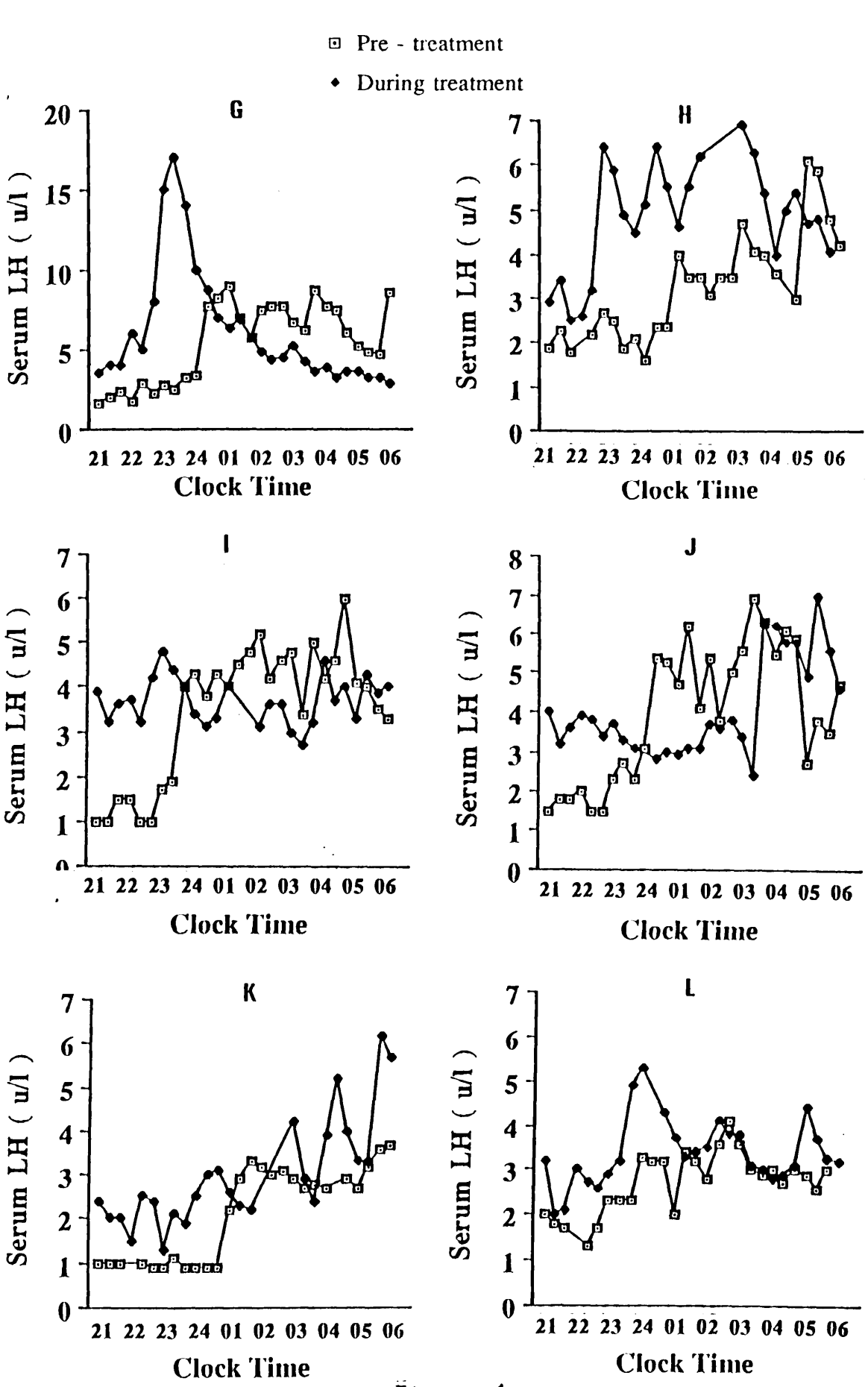
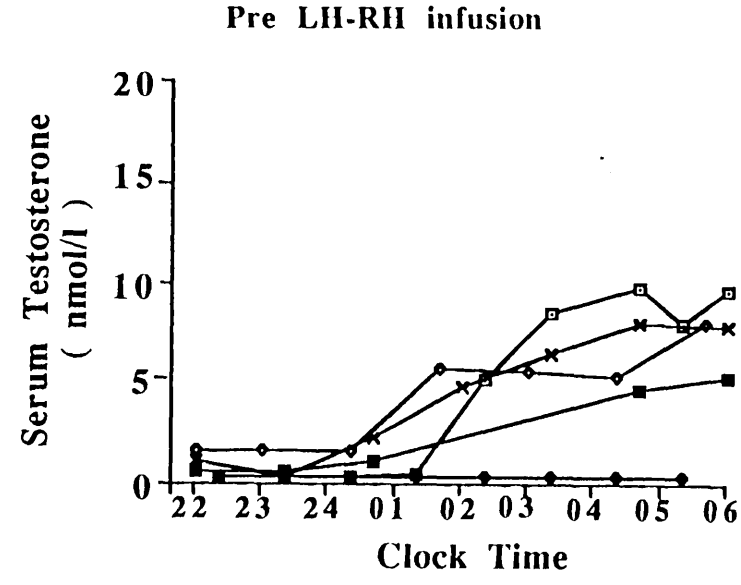


Figure 4

Serum LH profiles between 21.00 and 06.00 hours in 6 patients prior to, and during pulsatile LH-RH infusions at a dose of 7.5ug/pulse. Pubertal staging data for each patient is given in the text.



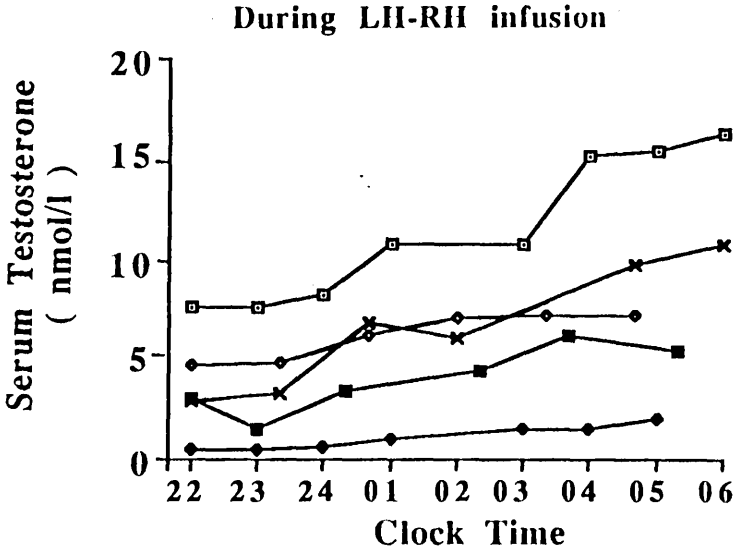


Figure 5
Serum testosterone profiles between 22.00 and 06.00 hours in 5 patients prior to, and during pulsatile LH-RH infusions at a dose of 7.5ug/pulse.

concentrations on the other hand, were elevated in the 5 patients for which results were available, throughout the study periods (figure 5).

When the data from all 12 patients are taken together for the period 21.00 to 06.00 hours on the following day, it can be clearly shown that at all time points between 21.00 and 24.00 hours LH secretion is significantly enhanced by exogenous LH-RH pulses (figure 6). However, between midnight and 6.00a.m. serum LH levels were not significantly different during LH-RH infusions. In contrast, serum testosterone concentrations were significantly elevated by pulsatile LH-RH administration at all times except 3.00 a.m. (figure 7). The testosterone increment was however greater before midnight.

3.4) DISCUSSION

The results of this study suggest that gonadotrophin secretion is enhanced by exogenous pulses of LH-RH, only at times of relative quiescence in endogenous LH-RH release. Wildt and colleagues (1981) have demonstrated in the Rhesus monkey, that the pituitary gonadotroph response to exogenous LH-RH pulses is critically dependent upon the frequency of the pulses. When the pituitary of the monkey was exposed to LH-RH pulses of increased frequency, serum LH and FSH concentrations

fell.

In the present study, patients were exposed to both endogenous and exogenous LH-RH pulses, particularly at night-time when endogenous secretion was maximal. The variable response to the pulsatile infusions at night may depend upon the interaction between endogenous and exogenous pulses. For example, if exogenous pulses happen to coincide with endogenous pulses then one might expect enhanced release of LH. On the other hand, if endogenous and exogenous pulses were asyncronous this would have exposed the pituitary gonadotrophs to an increased rate of pulses resulting in down-regulation.

The serum testosterone concentrations were stimulated by LH-RH infusions throughout the day and night, even in patients who had reduced LH levels at night-time. This result would suggest that testosterone production in early puberty is dependent upon not only the serum concentration of LH but also upon the duration of exposure to luteinizing hormone during the day.

The results of the present study would be consistent with the findings of Wagner et al (1986) who studied 10 boys with delayed puberty. Twenty-four hour gonadotrophin profiles in their patients identified 5 boys with nocturnal elevation of LH and 5 patients with absence of such elevations. All patients underwent pulsatile infusions of LH-RH (25ng/kg/pulse, i.v. every 90 mins for 10 days). On day 5, two 4-hour sampling periods between

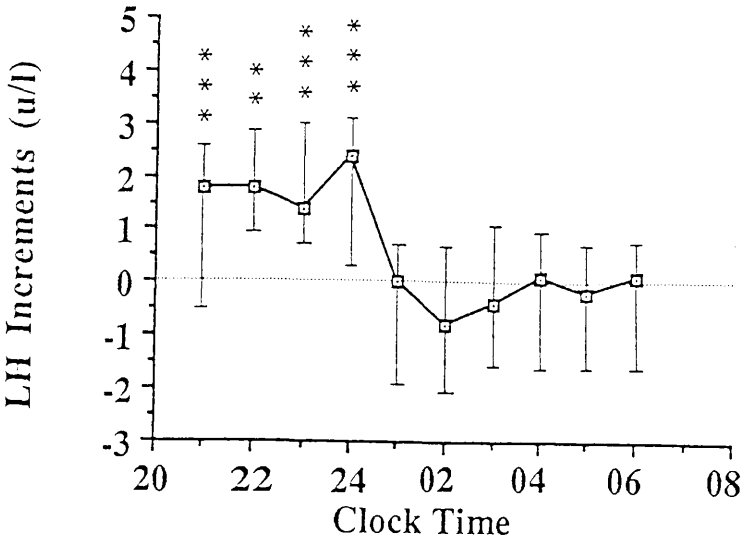


Figure 6

Increments in serum LH concentrations between 21.00 and 06.00 hours for the 12 patients undergoing pulsatile LH-RH infusions. Data are illustrated as medians + interquartile ranges. Only hourly results are shown for clarity. Asterixes refer to comparisons between concentrations before and during infusions.

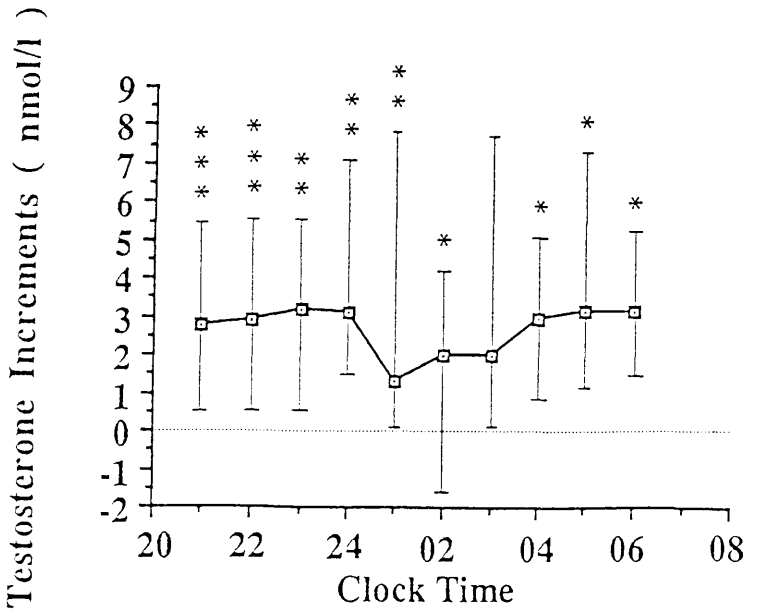


Figure 7

Increments in serum testosterone concentrations between 21.00 and 06.00 hours for the 11 patients undergoing pulsatile LH-RH infusions. Data are illustrated as medians + interquartile ranges. Asterixes refer to comparisons between concentrations before and during infusions.

* = $p < 0.05$
** = $p < 0.02$
*** = $p < 0.01$

the hours 8.00 - 12.00 and 20.00 - 24.00 were carried out. After 10 days of pulsatile therapy a post-treatment 24 hour sampling period was started to include the last 2 to 3 pump pulses.

Only the patients with nocturnal pulses showed further spontaneous pubertal development over the rather short follow up period of only 90 days. The authors were able to demonstrate rises in serum LH only in the group lacking nocturnal LH pulses. All patients however showed rises in serum testosterone during treatment.

The sampling periods on day 5 of the infusion coincided with the times of spontaneous gonadotrophin rises and falls and therefore at times when we also were unable to show consistent LH increments in our patients with delayed puberty. Wagner's group did not sample during treatment, in the afternoon, when natural LH secretion would be at 'baseline' and when stimulation above pre-treatment levels can be demonstrated.

It is also significant to note that Wagner and colleagues were unable to demonstrate the pre-therapy nocturnal LH elevation in the post-treatment profiles, suggesting that there treatment had inhibited this phenomenon even after the infusion had been stopped.

Stanhope et al (1987) have suggested that induction of puberty with pulsatile LH-RH infusions is best carried out when the infusions are given at night-time thus simulating normal pubertal endocrinology. However, this

method may be quite inappropriate for patients with constitutional delayed puberty since nocturnal administration of LH-RH may result in decreased gonadotrophin secretion due to mechanisms described above. It may be more appropriate to administer LH-RH during the afternoon and evening when stimulation of LH secretion can occur and possibly result in increased testosterone throughout 24 hours.

CHAPTER 4

THE EFFECTS OF LH-RH PULSE DOSAGE ON RESPONSE TO PULSATILE LH-RH INFUSIONS

4.1) INTRODUCTION

In view of the variable response to pulsatile LH-RH infusions, during the night and morning, it was decided to undertake further investigations of pulsatile LH-RH administration during 3 hour periods in the afternoons. At this time endogenous LH-RH secretion is at its lowest and unlikely to interfere with the response to endogenous LH-RH administration. A 3 hour study period was choosen to ensure that hormone measurements would include the response to 2 LH-RH pulses.

Previous studies have used a variety of LH-RH pulse doses given by both the intravenous and subcutaneous routes. (Sippell et al, 1984; Barkan et al, 1985;Delemarre-van de Waal et al, 1985; Partsch et al, 1985; Wagner et al, 1986). The effects of the different dosages used, on gonadotrophin response are unknown.

Spratt and colleagues (1986) have investigated the dose response to LH-RH administered at physiological concentrations to patients with hypogonadotrophic hypogonadism. However, their study looked at the gonadotrophin response to a single pulse. The response to

repeated pulses at different doses has never been studied.

4.2) PATIENTS AND METHODS

Twenty two boys underwent pulsatile infusions of LH-RH according to the protocol described in the Experimental Section. Pulse doses of 2.5, 7.5 and 15ug were administered to 7,9 and 6 patients respectively. These doses were chosen in order to cover the likely dosage range which would be used in future studies of young boys and adolescent males. Clinical details of the patients undergoing this study are shown in table 7. There were no significant differences in testicular volumes, chronological or bone ages of the patients in the different dosage groups.

4.3) RESULTS

Figures 8 and 9 show, respectively the serum LH and FSH responses to pulsatile LH-RH infusions in the 3 groups of boys treated with different doses of LH-RH. Results are expressed as mean concentrations of gonadotrophins measured over the 3 hour study periods.

It is clear from figures 8 and 9 that post-infusion gonadotrophin concentrations are dependent upon the pre-treatment levels. The greatest increments in LH and

Pulse dosage group	No of Patients	Genital Stages G1	G2	G3	Testicular Volumes (mls)	Chronological Age (yrs)	Bone Age (yrs)
2.5 ug/pulse	n = 7	3	3	1	5.5 (2-10)	14.7 (13.3-16.9)	12.6 (10.5-13.5)
7.5 ug/pulse	n = 9	4	4	1	5.5 (2-12)	15.0 (14-17.7)	12.0 (10.5-13.5)
15 ug/pulse	n = 6	3	3	0	4.0 (2-9)	14.9 (13.4-17.9)	12.3 (10.0-14.0)

TABLE 7

Clinical details of the patient groups receiving different LH-RH pulse doses

When a patient had testes of differing sizes, a mean of the testicular volumes was used.
Results expressed as median (range).

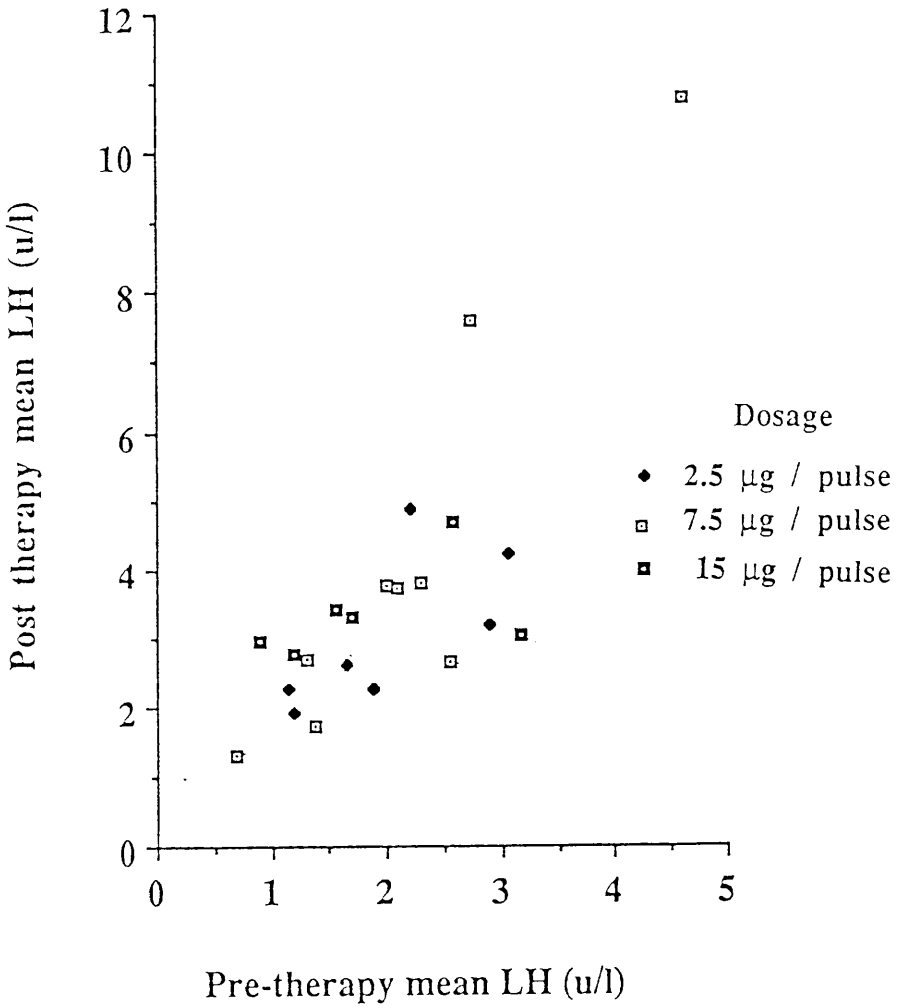


Figure 8

LH dose-response curve for pulsatile infusions of LH-RH over 6 days. Mean serum LH concentrations were calculated from samples taken every 15 mins over 3-hour periods. LH-RH doses of 2.5, 7.5 and 15ug/pulse were used.

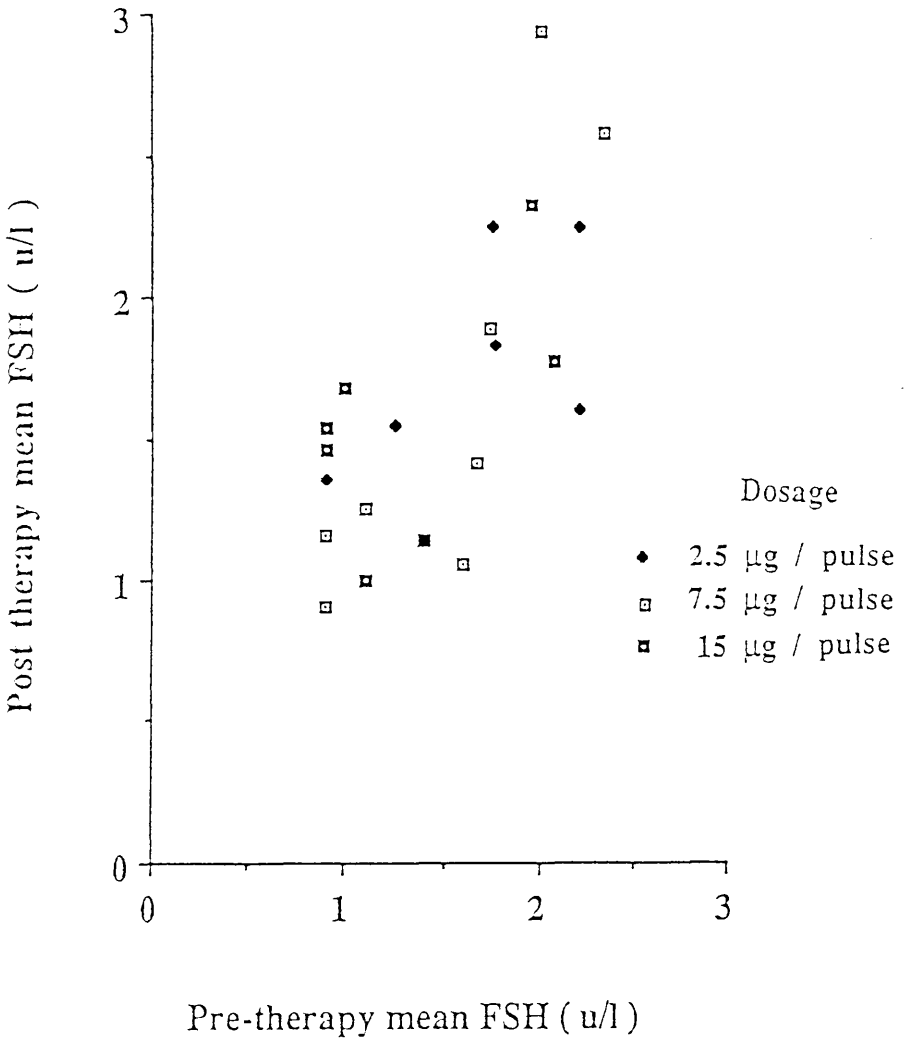


Figure 9

FSH dose-response curve for pulsatile infusions of LH-RH over 6 days. Mean serum FSH concentrations were calculated from samples taken every 15 mins over 3-hour periods. LH-RH doses of 2.5, 7.5 and 15ug/pulse were used.

FSH occured in the patients with the highest pre-infusion concentrations.

There were no significant differences in pre-therapy or post-therapy gonadotrophin concentrations between the dosage groups. Similarly, gonadotrophin increments were not significantly different. However, the small number of patients in each group prevented further statistical analysis of the results.

4.4) DISCUSSION

This study failed to show a relationship between the concentration of gonadotrophins in serum following 6 days of pulsatile LH-RH infusion and the LH-RH pulse dose. However, because of the small numbers of patients in each group, small differences in response to dosage alteration could not be identified.

Spratt et al (1986) have demonstrated a log-linear relationship between LH-RH pulse dose and serum LH response when LH-RH was administered to hypogonadotrophic hypogonadal males in whom pituitary and gonadal function had been normalised by prior LH-RH pulsatile administration. LH-RH was given intravenously at doses between 7.5 and 250ng/kg. Spratt et al measured LH responses to single LH-RH pulses in each patient. They did not investigate the effect of repeated administration of LH-RH at different doses.

Several authors have demonstrated increasing gonadotrophin release in response to repetitive LH-RH administration (Reitano et al, 1975; Valk et al, 1980). Because of this self-priming phenomenon, it is likely that the duration of LH-RH pulsatile infusions rather than pulse dosage is important in influencing gonadotroph response after prolonged administration. The influence of infusion duration on gonadotrophin levels is demonstrated in the chapter dealing with LH-RH treatment of infertility.

CHAPTER 5

SERUM GONADOTROPHIN RESPONSE TO PULSATILE LH-RH ADMINISTRATION IN THE DIFFERENTIAL DIAGNOSIS OF DELAYED PUBERTY AND HYPOGONADOTROPHIC HYPOGONADISM

5.1) INTRODUCTION

The distinction between patients with constitutional delayed puberty and those with idiopathic hypogonadotrophic hypogonadism remains one of the most difficult diagnostic problems in paediatric endocrinology. The gonadotrophin response to a standard bolus injection of LH-RH has proved quite ineffective in differentiating between the two conditions (Bell et al, 1973; Kelch et al, 1980; Mortimer et al, 1973a). More recently, two papers from Sippell's group (Sippell et al, 1984; Partsch et al, 1985) have reported that gonadotrophin responses following pulsatile infusion of LH-RH could differentiate these conditions.

In order to further investigate this, the gonadotroph response to prolonged pulsatile LH-RH infusions was assessed in patients presenting with a variety of abnormalities of growth and development.

PROTOCOL

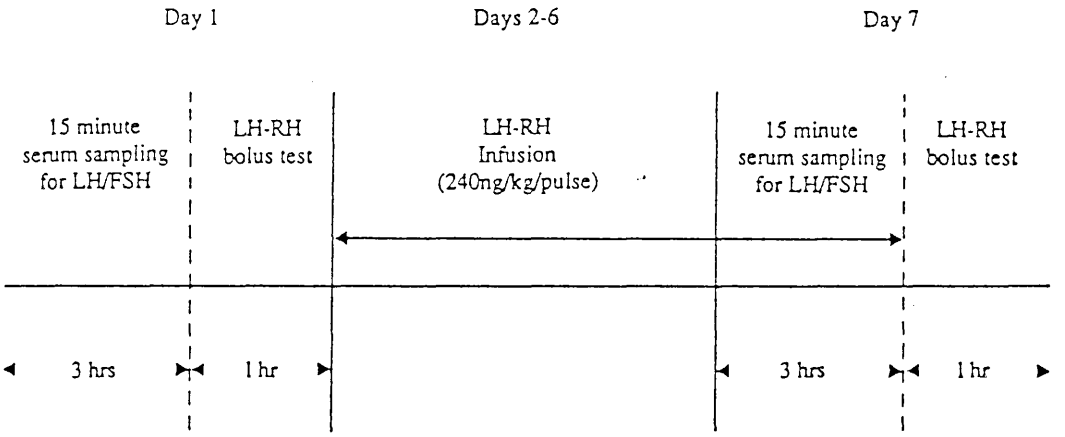


Figure 10
Study protocol.

5.2) PATIENTS AND METHODS

Thirty-five patients referred for investigation of either short stature or delayed puberty were studied. Patients were divided into 3 patient groups according to the criteria outlined in the Experimental Section, namely a) delayed puberty b) short stature or c) hypogonadotrophic hypogonadism. Tables 8 and 9 show the clinical details, including pubertal gradings, testicular volumes and chronological ages of the patients within each group. Also included are the details for one patient subsequently shown to have anorchia.

In view of the results from the preceding study on 24 hour hormone profiles during pulsatile infusions, it was decided to restrict the period of hormone monitoring to 3 hour periods in the afternoons. It was felt impracticable to monitor for 24 hour periods in a large number of patients and furthermore, we have demonstrated that consistent stimulation by pulsatile LH-RH administration occurs only during the afternoon and early evening.

During the afternoon of the admission day, blood was sampled via an indwelling venous cannula, at 15 minute intervals over a period of 3 hours for subsequent measurement of plasma LH and FSH. Thereafter TRH (200ug) was given as an intravenous bolus and blood sampled at 30 minute intervals over 1 hour for estimation of serum prolactin. The following morning after an overnight fast,

Patient	Genital Staging	Testicular Volume (mls)	Chronological Age (yrs)	Bone Age (yrs)
Delayed Puberty				
1	G1P2	2/3	14.8	12.0
2	G2P1	5/5	15.4	12.5
3	G1P1	2/2	14.3	10.0
4	G2P1	6/8	15.6	12.0
5	G1P1	2/2	15.0	11.0
6	G2P1	3/3	14.7	13.5
7	G2P1	3/3	14.1	11.0
8	G2P2	6/6	16.8	13.5
9	G2P2	8/8	17.8	14.0
Short Stature				
10	G2P2	2/2	14.0	13.5
11	G1P1	3/3	12.3	8.0
12	G1P1	2/2	13.0	11.5
13	G1P1	4/4	13.7	12.5
14	G2P2	8/8	14.1	12.5
15	G1P2	3/3	13.3	12.0
16	G3P3	6/6	15.8	13.0
17	G2P2	6/6	14.7	12.5
18	G3P3	4/4	13.9	13.0
19	G2P2	6/6	15.3	13.0
20	G1P2	4/4	15.3	12.0
21	G2P2	6/6	13.9	10.5
22	G3P2	4/3	15.1	13.0
23	G3P2	12/12	17.7	13.0
Anorchia				
24	G1P1	0/0	9.0	-

TABLE 8

Clinical details of males with short stature and delayed puberty undergoing pulsatile LH-RH infusions

Patient	Anosmia	Genital Staging	Testicular Volume (mls)	Chrological age (yrs)	Previous therapy
25	YES	G5P5	12/12	27.8	Prolonged therapy with HCG + sustano
26	NO	G5P5	3/3	26.8	Sustanon
27	NO	G2P1	3/3	20.4	NIL
28	NO	G2P4	3/4	22.5	Sustanon x 3 several years previously
29	NO	G5P5	4/4	25.5	Monthly sustanon for 2.5 years
30	NO	G3P4	10/10	27.9	NIL
31	YES	G2P2	3/3	24.6	NIL
32	NO	G2P1	Inguinal/↑	20.3	NIL
33	NO	G1P2	1/1 Micro-penis hypospadias	17.4	NIL
34	YES	G5P5	0/1	41.1	Sustanon injection for 20+ years
35	HYPOSMIA	G3P3	1/0 R. orchido-pexy at 14 years old	20.5	HCG 3 months Sustanon 3 months

TABLE 9

Clinical details of male patients with hypogonadotrophic hypogonadism undergoing pulsatile LH-RH infusions

pituitary function was assessed as described above. Serum testosterone was estimated at this time and care taken to ensure that subsequent estimates for testosterone were made at the same time of day. Thereafter subcutaneous pulsatile LH-RH therapy was commenced using an infusion pump (Graseby Medical, type MS 27) with delivery of LH-RH pulses every 90 minutes at a dosage of 240ng/kg/pulse.

Following 6 days treatment with LH-RH further venous blood sampling every 15 minutes for 3 hours was undertaken at the same time of day as the pre-infusion study, for estimation of LH and FSH. All samples from each patient were frozen at -20 degrees centigrade and assayed in one batch to eliminate inter-assay error. The pump was then disconnected and intravenous boluses of TRH (200ug) and LH-RH (100ug) were injected 1 hour apart. Blood was sampled at 30 minute intervals for estimation of serum LH, FSH and prolactin. TRH and LH-RH tests were not carried out on all patients. The study protocol is illustrated diagramatically in fig. 10.

5.3) RESULTS

Figures 11-12 show typical examples of the serum concentrations of LH and FSH measured over 3 hour periods immediately before and at the end of 6 days therapy with pulsatile infusion of LH-RH (240ng/kg/pulse) for the three patient groups; a) delayed puberty b) short stature

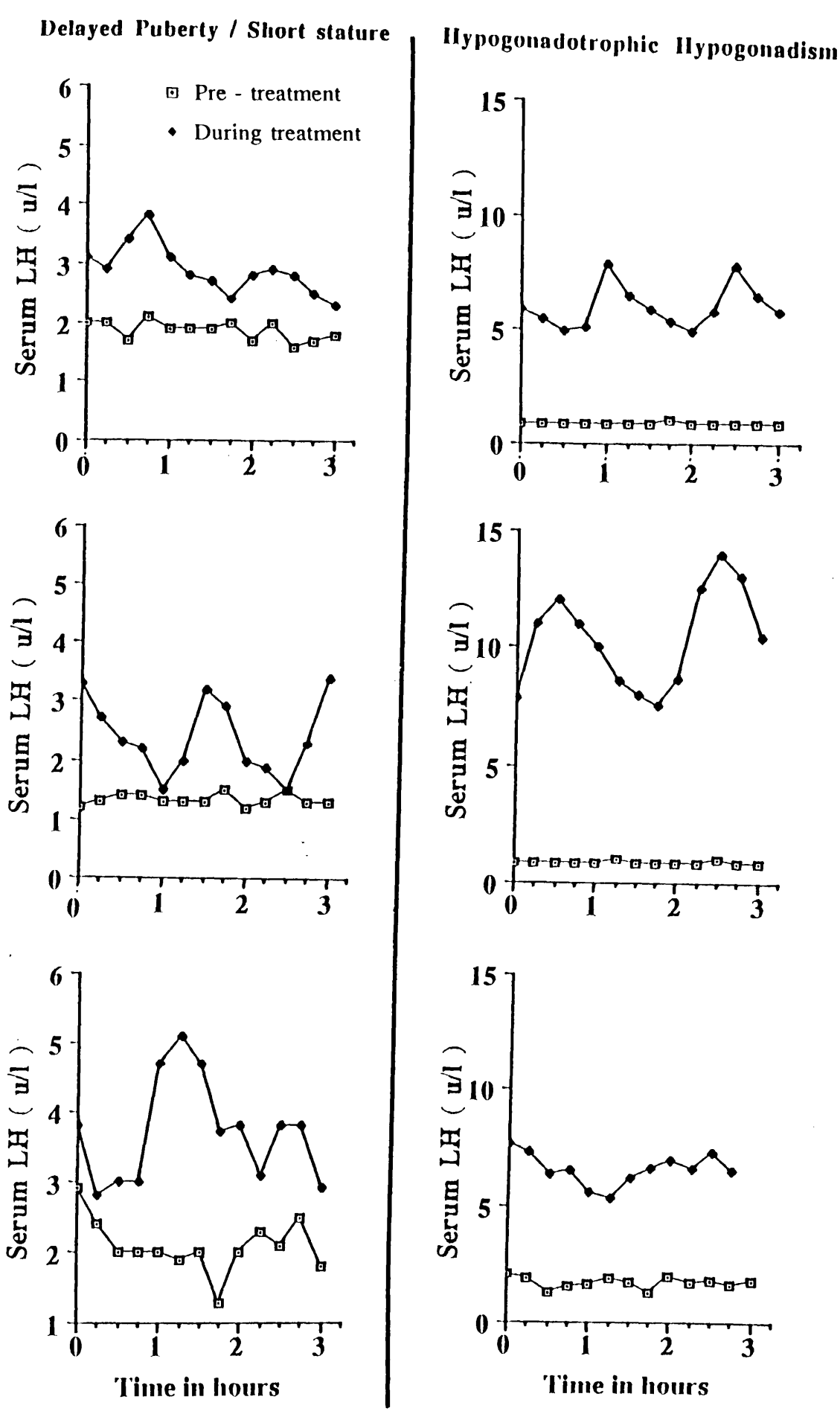


Figure 11

Typical examples of serum LH responses to pulsatile LH-RH infusions in patients with delayed puberty and hypogonadotrophic hypogonadism.

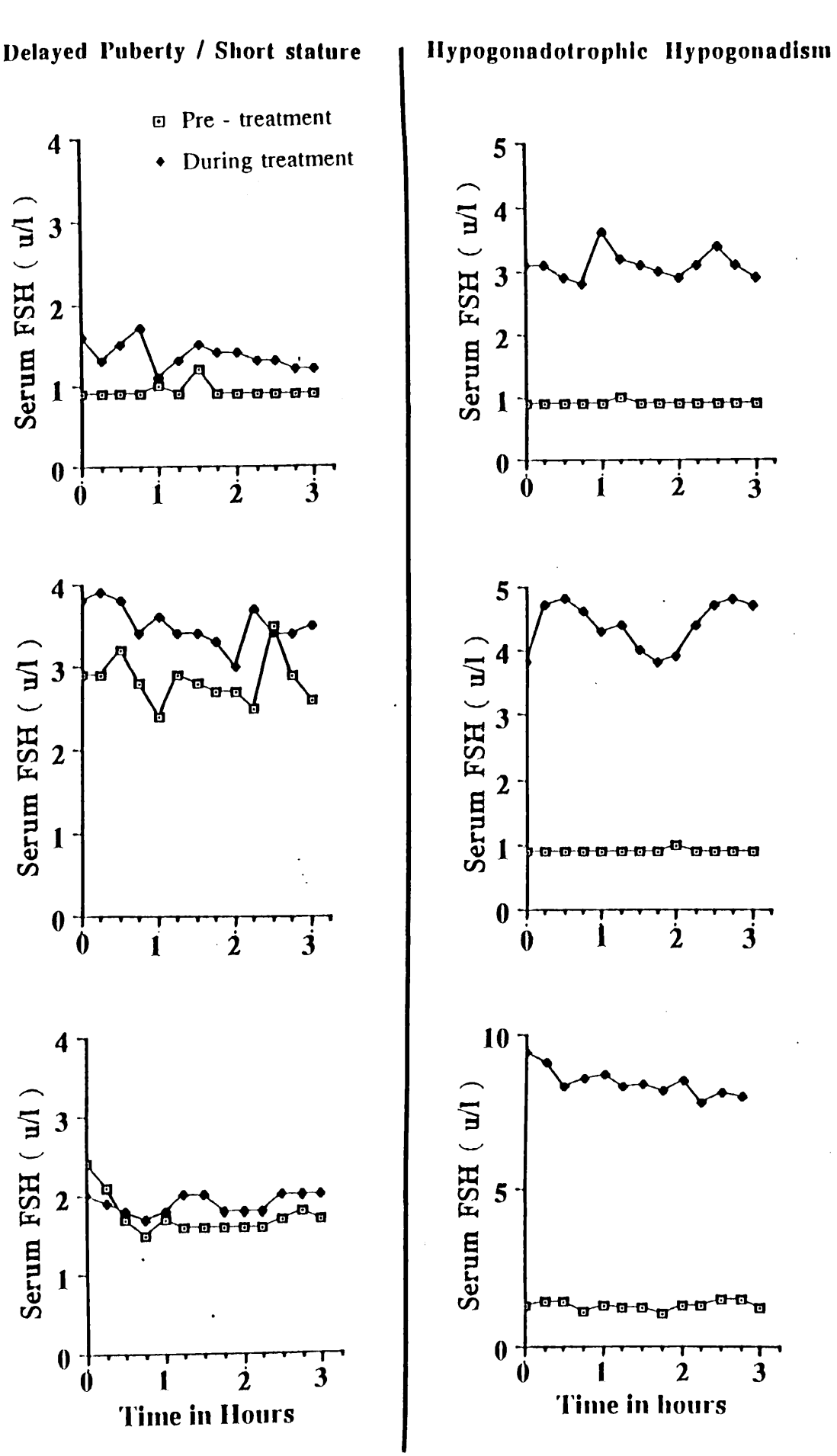


Figure 12

Typical examples of serum FSH responses to pulsatile LH-RH infusions in patients with delayed puberty and hypogonadotrophic hypogonadism.

c) hypogonadotrophic hypogonadism.

The mean LH and FSH concentrations for each patient, measured over the 3 hour periods, before and at the end of the period of pulsatile LH-RH therapy are illustrated in figs. 13 - 14.

There were no significant differences in the mean basal gonadotrophin concentrations between the three groups of patients (table 10). All 9 patients with delayed puberty, 12 of 14 patients with short stature and all 11 patients with hypogonadotrophic hypogonadism showed significant rises in mean LH concentrations at the end of the pulsatile infusion period.

Although there was a significant increase in FSH concentrations after 6 days of pulsatile therapy when the patient groups were taken as a whole, the changes were more variable, with 4 patients showing no change and 6 patients showing a reduced mean FSH concentration after treatment (fig.14). There are significant correlations between the mean pre-treatment LH and FSH concentrations and the mean post-therapy concentrations in the boys with delayed puberty and short stature.

Increments in both mean LH and FSH concentrations were significantly greater for the patients with hypogonadotrophic hypogonadism when compared to the other groups (table 10) Only one patient (patient no. 27, table 9) showed a response characteristic of the younger boys. This patient was, however, grossly obese and poor

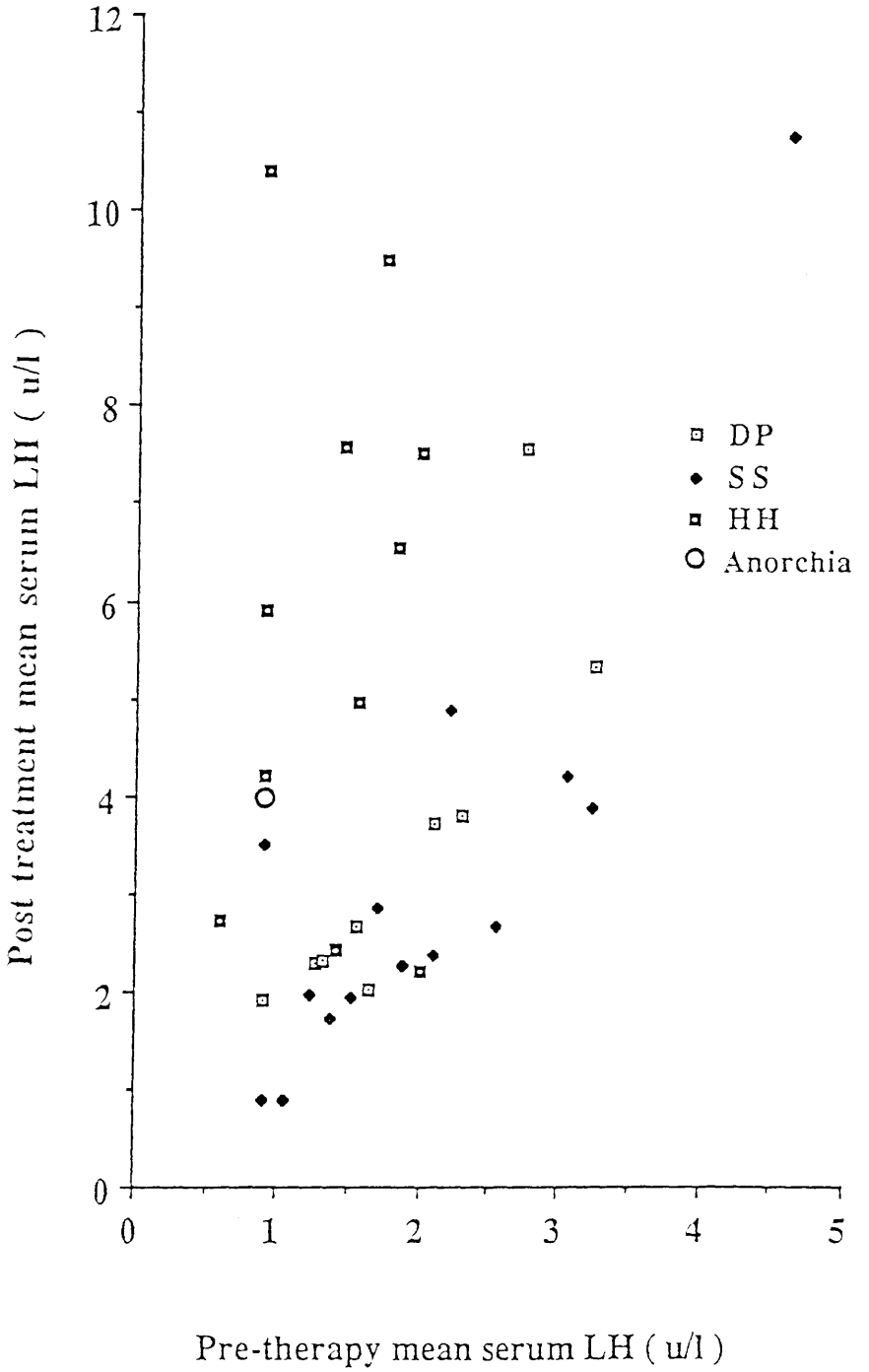


Figure 13
Mean serum LH concentrations prior to, and during pulsatile LH-RH infusions in a) delayed puberty b) short stature and c) hypogonadotrophic hypogonadism. Mean serum LH was calculated from samples taken every 15 mins over 3-hour periods.

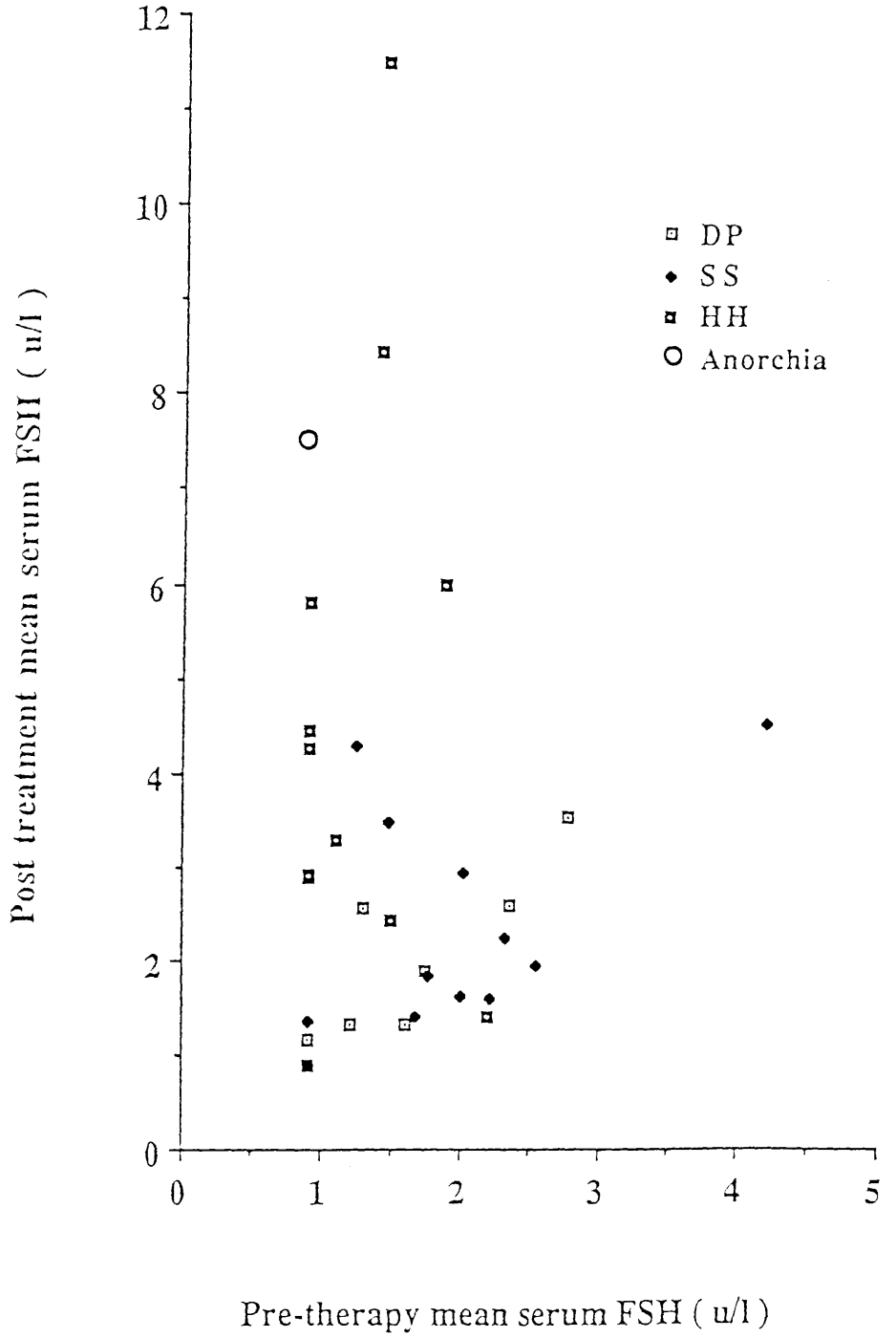


Figure 14
Mean serum FSH concentrations prior to, and during pulsatile LH-RH infusions in a)delayed puberty b) short stature and c) hypogonadotrophic hypogonadism. Mean serum FSH was calculated from samples taken every 15 mins over 3-hour periods.

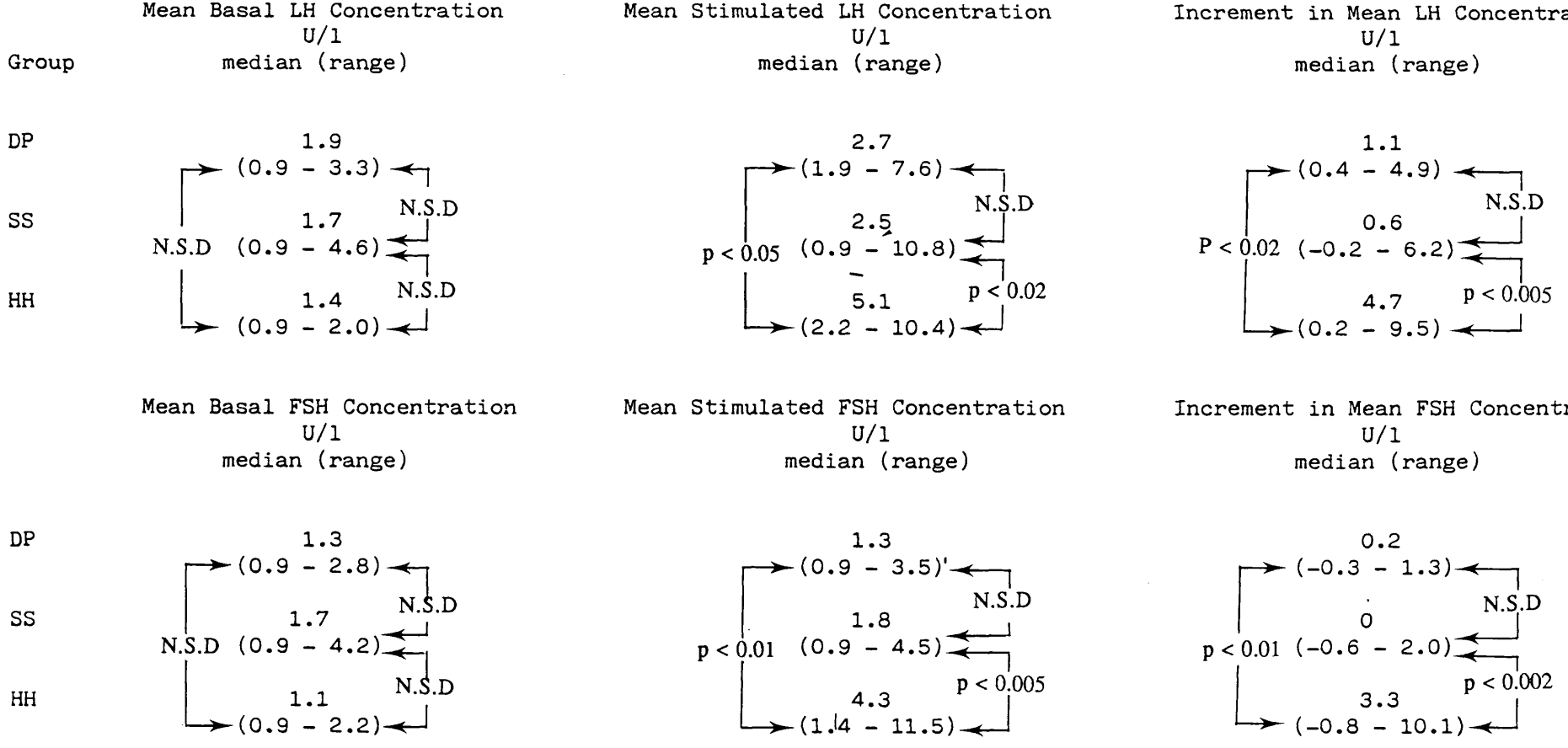


TABLE 10

Changes in mean serum LH and FSH concentrations during pulsatile LH-RH infusions

N.S.D. = not significantly different

DP = constitutional delayed puberty
SS = short stature

HH = hypogonadotrophic hypogonadism

absorption of LH-RH may have occured from the subcutaneous site.

The results for one patient subsequently shown to have anorchia are also shown. In this patient serum gonadotrophin increments were higher than those found in patients with constitutional pubertal delay or short stature. FSH rose to a greater extent than LH and the FSH : LH ratio therefore simulated that found in adult patients with primary testicular failure.

The incremental increase in serum LH during pulsatile LH-RH therapy was also compared with the LH response during the pre-treatment bolus LH-RH test in 8 delayed puberty, 13 short stature and 10 hypogonadotrophic patients. . As can be seen from figure 15, in the delayed puberty and short stature groups of patients there is no significant correlation between the rise in LH during pulsatile therapy and the rise during a bolus test. By contrast, the LH response to pulsatile therapy in the patients with hypogonadotrophic hypogonadism could be predicted by the pre-treatment bolus LH-RH test.

5.4) DISCUSSION

The reasons for the proportionally greater gonadotrophin response to pulsatile therapy in the men with hypogonadotrophic hypogonadism are unknown. It has been shown that pre-treatment with LH-RH augments

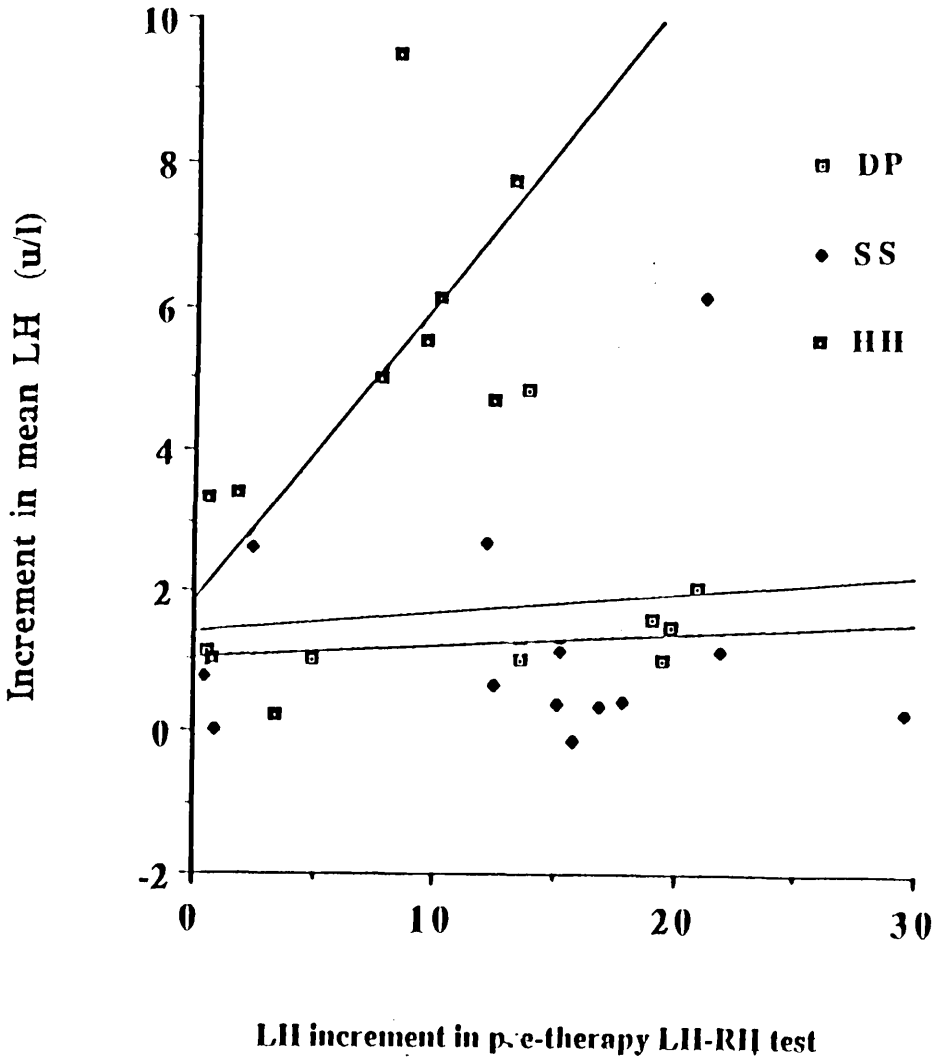


Figure 15
Increments in mean serum LH during pulsatile LH-RH infusions compared with increments after standard bolus LH-RH injections (100ug i.v.) in the 3 patient groups.
Correlation line for HH patients:
$y = 1.8 + 0.4x \quad R = 0.71 \quad p < 0.05$

pituitary responsiveness to the hormone (Reitano et al, 1975; Yoshimoto, Moridera and Imura, 1975; Snyder et al, 1979).Barkan et al (1985) have shown that gonadotrophin responses in 8 men with hypogonadotrophic hypogonadism to pulsatile LH-RH depended upon the degree of endogenous LH-RH deficiency which they characterised by measuring 24 hour secretory pulses. Although many of the men with hypogonadotrophic hypogonadism in this series had undetectable gonadotrophin concentrations throughout the sampling period, 24 hour profiles were not undertaken. This would clearly have enhanced this work, but was not practicable for 1 observer to undertake. Prolonged exposure to intermittent release of LH-RH in our patients with hypogonadotrophic hypogonadism may have resulted in pituitary gonadotroph priming.

Against this hypothesis, however, are the results of the initial bolus LH-RH tests which were undertaken prior to the period of pulsatile infusion. These results showed considerable overlap in response to a standard bolus dose of LH-RH between the three patient groups. Boyar et al (1976) and Santora et al (1986) have demonstrated that the response to a bolus LH-RH test reflects the extent of endogenous LH-RH deficiency and therefore the three groups of hypogonadal patients are likely to have similar degrees of LH-RH deficiency and gonadotroph priming.

A second hypothesis to explain the enhanced gonadotroph response to pulsatile infusion in the

patients with hypogonadotrophic hypogonadism might be reduced feedback inhibition of LH and FSH release by testicular secretions in this group. Previous workers have shown that patients with hypogonadotrophic hypogonadism have impaired testosterone responses during HCG stimulation tests (Bardin et al, 1969; Perheentupa, Dessypris and Aldercreutz, 1972; Santen and Paulsen, 1973; Weinstein and Reitz, 1974; Dunkel, Perheentupa and Sorva, 1985a; Dunkel et al, 1985b). It might therefore be expected that patients with hypogonadotrophic hypogonadism would have impaired testosterone increments in response to pulsatile infusion of LH-RH, and therefore reduced feedback inhibition of LH secretion. In favour of this hypothesis is the clearly enhanced LH and FSH response to pulsatile therapy demonstrated by the patient with anorchia. Testosterone responses to pulsatile therapy are discussed below.

A further hypothesis is based on the results shown in the 24 hour profiles. It appears that exogenous LH-RH causes stimulated LH release at times of hypothalamic dormancy. At periods of greatest hypothalamic activity it is difficult to clearly demonstrate enhanced gonadotrophin secretion with pulsatile therapy. It is possible therefore that there is interaction between endogenous and exogenous pulses resulting in antagonism at pituitary level. Even during times of relative hypothalamic quiesence there may be some LH-RH secretory

pulses in the peripubertal boys. On the other hand the hypogonadotrophic patients may have completely absent or very infrequent pulses (Spratt et al, 1987). Small but undetectable LH-RH pulses able to antagonise the exogenous LH-RH pulses might explain the relatively impaired responses to pulsatile infusion in the peripubertal groups compared with the patients with hypogonadotrophic hypogonadism.

Other factors such as the chronological age of the patient, or previous exposure to HCG or testosterone esters might be important in the response to LH-RH infusion. Although numbers of hypogonadotrophic hypogonadal patients are small these facters do not seem to influence the results. This work therefore is unable to explain the enhanced response to LH-RH pulsatile infusion demonstrated with hypogonadotrophic hypogonadism.

Several studies have compared the gonadotrophin responses to pulsatile LH-RH therapy in patients with delayed puberty and hypogonadotrophic hypogonadism. Valk et al (1980) investigated 4 male and 2 female patients with isolated gonadotrophin deficiency given LH-RH (0.025 ug/kg) i.v. every 2 hours for 5 days. The male patients showed significantly elevated LH and FSH concentrations within 24 hours of treatment, and gonadotrophin concentrations continued to rise over the 5 day periods. FSH concentrations rose to a greater extent than LH

concentrations with FSH achieving concentrations at the upper limit of the adult male range in all patients.

The high initial response in FSH in the 4 patients investigated by Valk may reflect impaired testicular function in the small group studied. Lack of feedback inhibition by inhibin could explain the rise in FSH levels to greater concentrations than those normally found during puberty. A larger study might have shown a more varied FSH response. The magnitude of LH responses is similar to our own results.

Sippell et al (1984) reported the mean FSH rise for 18 hypogonadal patients following 36 hours of pulsatile LH-RH infusion at a dose of 5ug i.v. every 90 minutes. Their patients were divided into 4 clinical groups; Kallmann's syndrome (4 patients), idiopathic hypopituitarism (6 patients), isolated hypogonadotrophic hypogonadism (3 patients), and constitutional delayed puberty (5 patients). They were able to show significant rises in FSH concentrations during the 36 hours of LH-RH infusion, compared with pretreatment nocturnal profiles in all patients with Kallmann's syndrome and idiopathic hypopituitarism. No significant rise was seen in the patients with isolated hypogonadotrophic hypogonadism or in 3 of the 5 patients with constitutional delayed puberty. The difference in responses between the patients with Kallmann's syndrome and idiopathic hypogonadotrophic hypogonadism is difficult to explain since the latter

patients had higher initial testosterone concentrations and might have been expected to be more adequately primed and therefore better able to respond. The variable response in the patients with constitutional delayed puberty may reflect their varied degree of gonadotroph maturation.

A second study by the same group (Partsch et al, 1985) using the same sampling techniques and infusion protocol, reports the results of pulsatile infusion in 8 patients with hypogonadotrophic hypogonadism and 9 with constitutional delayed puberty. This latter group contained 4 patients with testicular volumes of 9 mls. or greater and these patients clearly had the highest initial LH and FSH concentrations and greatest increments in gonadotrophins with therapy. The heterogeneous response in this group is in agreement with our own results. The hypogonadotrophic hypogonadal patients on the other hand did not show the exaggerated response decribed in our present study. The shorter period of treatment and the different dose regime might explain the the differences between the studies.

Barkan et al (1985) studied 8 patients with idiopathic hypogonadotrophic hypogonadism whom they divided into two groups, partial or complete gonadotrophin deficiency, depending upon the presence or absence of secretory LH pulses during 24 hour profiles of gonadotrophin secretion. They were able to show that

pulsatile LH-RH infusion (25ng/kg, i.v., every 2 hours for 4 days) in patients with complete deficiency, resulted in predominant FSH secretion and absent or minimal augmentation of LH secretion. In contrast, predominant LH secetion ocurred in the partial deficiency patients. Both groups of patients achieved supra-physiological concentrations of FSH during LH-RH infusion. Studies in LH-RH deficient animals and man (Wildt et al, 1981; Gross et al, 1987) have demonstrated that administration of LH-RH at rapid pulse frequences stimulates predominant LH secretion and at slower frequences stimulates predominantly FSH secretion. Thus it is possible that the pulse frequency of two hours chosen by Barkan et al was too slow to elicit physiological hormone responses. Recent studies (Matsumato and Bremner, 1984; Veldhuis et al, 1984) using frequent sampling suggest that the physiological pulse interval in man is closer to 90 minutes, which was the frequency used in our study. Barkan's study did not compare results of hypogonadotrophic patients with delayed puberty patients of similar gonadotrophin deficiency.

Wagner et al (1986) investigated two groups of patients with delayed pubertal development whom they defined as either delayed puberty or permanent hypogonadotrophic hypogonadism according to the presence or absence of nocturnal LH pulses.

Nine of the 10 boys studied were under 20 years of age and the follow-up period was only 90 days. The boys with absent nocturnal pulses may be examples of prepubertal boys with simple delayed puberty and might not be expected to show further pubertal development during this short follow-up period. LH secretion was increased in both groups of patients, although this only reached statistical significance for the group with absent nocturnal pulses. Similarly only the latter group showed significant FSH augmentation. The results for individual patients are not given, making further interpretation of the data difficult. If the group of patients showing no night-time pulsatility were indeed patients with permanent hypogonadotrophic hypogonadism the results of Wagner et al suggest that this group of patients, despite having lower endogenous LH-RH secretion, have a greater initial response to pulsatile LH-RH infusion.These results would therefore be in agreement with our own.

Whether classification as either delayed puberty or hypogonadotrophic hypogonadism on the basis of 24 hour LH profiles is justified, is a matter for conjecture. Stanhope et al (1987) demonstrated absent nocturnal LH secretion in a 16 year old female subsequently shown to have constitutional delay of puberty. Spratt et al (1987), in a large series of hypogonadotrophic males, demonstrated nocturnal pulses in 3 of 50 patients

studied. Presence of nocturnal LH pulses may therefore not be a good discriminator between these two conditions.

CHAPTER 6

RESPONSES TO BOLUS INJECTIONS OF LH-RH

6.1) INTRODUCTION

There is general agreement that at puberty LH responsiveness to a bolus dose of LH-RH increases in males at the same time as nocturnal surges of LH appear, as described by Boyar et al (1972). This increase of responsiveness continues throughout puberty. However there is lack of consensus regarding the FSH response to LH-RH. According to some workers responsiveness in puberty remains similar to pre-puberty (Roth et al, 1972; Franchimont et al, 1973) whilst other workers have described pubertal increases of FSH responses (Job et al, 1972; Grumbach et al, 1974; Kastin et al, 1972; Kelch et al, 1975).

The response to bolus LH-RH injections in patients with hypogonadotrophic hypogonadism can be normal, minimal or sub-optimal (Hashimoto et al, 1972; Roth et al, 1972; Espiner and Donald, 1973; Mortimer et al, 1973a). This led Bell et al (1973) to conclude that there was too much heterogeneity in gonadotrophin response to bolus doses of LH-RH to allow identification of this condition .

Several previous studies have shown that prolonged

administration of LH-RH can result in increased secretion of gonadotrophins in patients with hypogonadotrophic hypogonadism (Jacobson et al, 1979; Reitano et al, 1975; Snyder et al, 1979; Yoshimoto et al, 1975). These studies, however used pharmacological doses of LH-RH, administered as either infusions or injections which did not simulate the normal endogenous pulsatile release of LH-RH.

It was therefore of interest to investigate whether augmentation of gonadotrophin response to bolus LH-RH injections occurred following pulsatile LH-RH infusions in pubertal boys and patients with hypogonadotrophic hypogonadism.

6.2) PATIENTS AND METHODS

Standard bolus LH-RH tests were carried out immediately prior to pulsatile infusion of LH-RH in 8 patients with delayed puberty, 13 patients with short stature and 10 patients with hypogonadotrophic hypogonadism. Immediately following the pulsatile infusion further bolus LH-RH tests were carried out in 7 patients with delayed puberty, 12 patients with short stature and 6 patients with hypogonadotrophic hypogonadism. All but one of the patients with post-infusion results had also undergone pre-infusion tests. The protocol for the study is similar to that

PROTOCOL

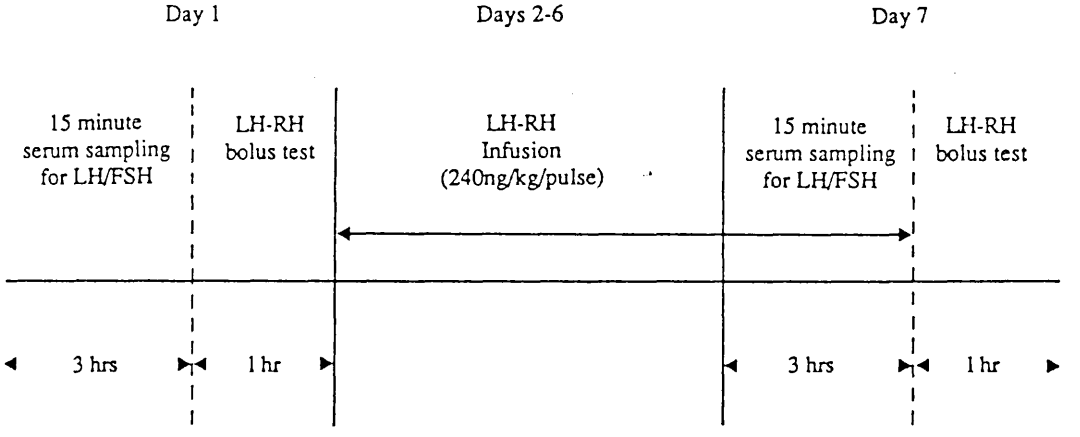


Figure 16
Study protocol.

described in Chapter 5 and is shown in diagrammatical form in figure 16.

6.3) RESULTS

Gonadotrophin responses to bolus tests are compared before and after pulsatile LH-RH infusions both between groups and within groups.

6.3.1) Comparison of LH results between groups

Figures 17 and 18 and table 11 show the LH responses to standard bolus injections of LH-RH immediately prior to and following the periods of pulsatile LH-RH infusion, for the 3 patient groups.

Results of the initial LH-RH bolus tests show considerable overlap of basal, peak and incremental changes in LH between the patient groups (fig 17). There were no significant differences in pre-treatment basal LH concentrations between the groups (table 11). The pre-therapy peak LH and increment in LH were significantly greater in the short stature group when compared with the hypogonadotrophic adults.

Following the treatment period the patients with hypogonadotrophic hypogonadism showed significantly higher basal LH concentrations when compared with the other two groups (table 11). Peak LH concentrations in

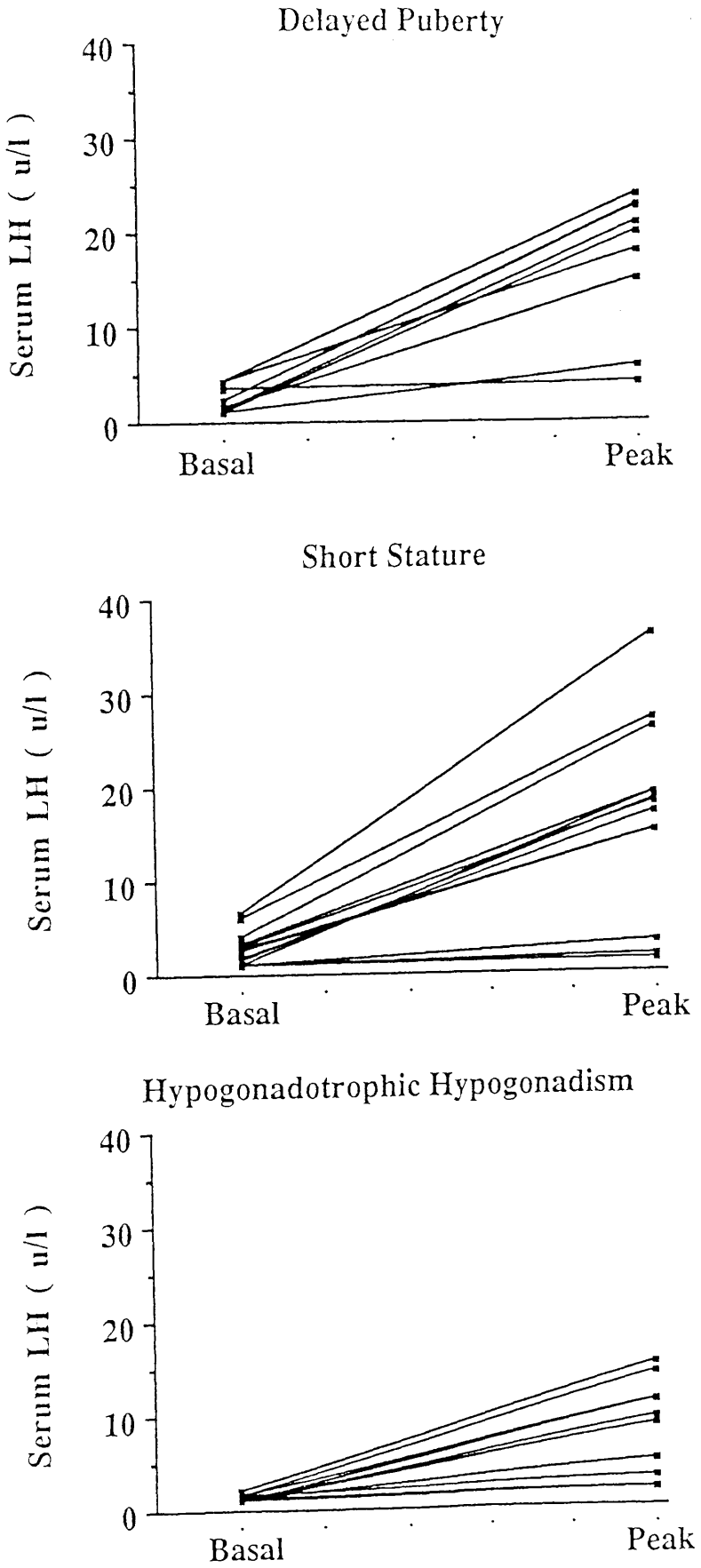


Figure 17
The basal and peak serum LH responses to bolus LH-RH administration (100ug i.v.) prior to pulsatile LH-RH infusions in patients with a) delayed puberty b) short stature and c) hypogonadotrophic hypogonadism.

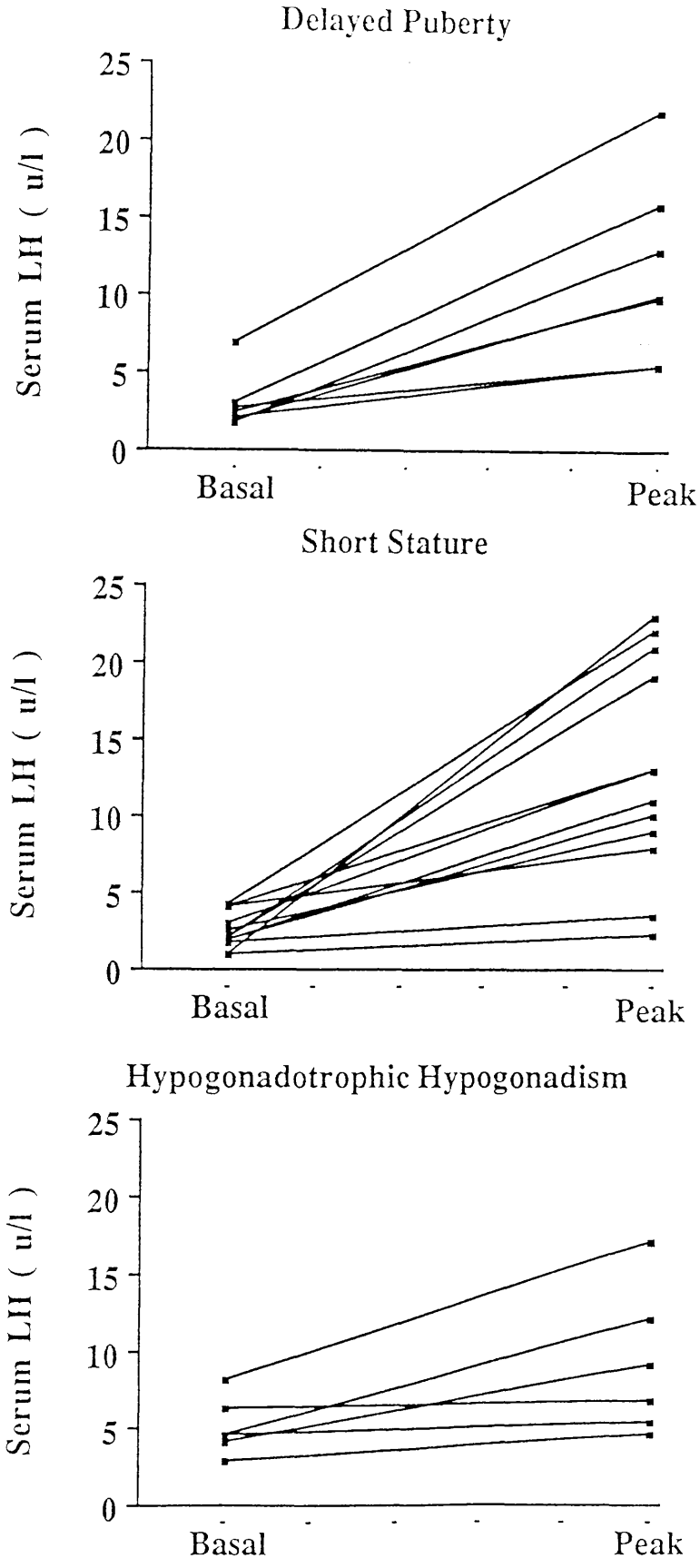
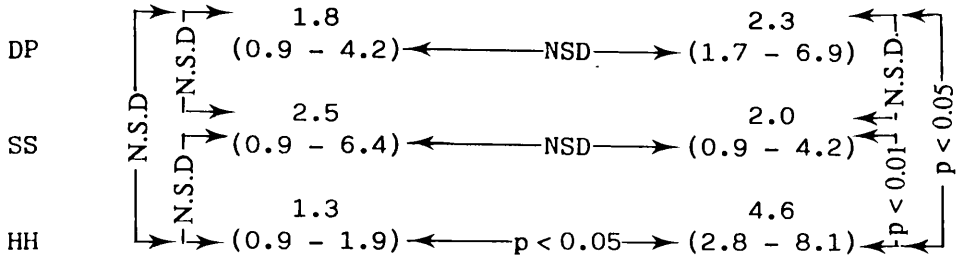
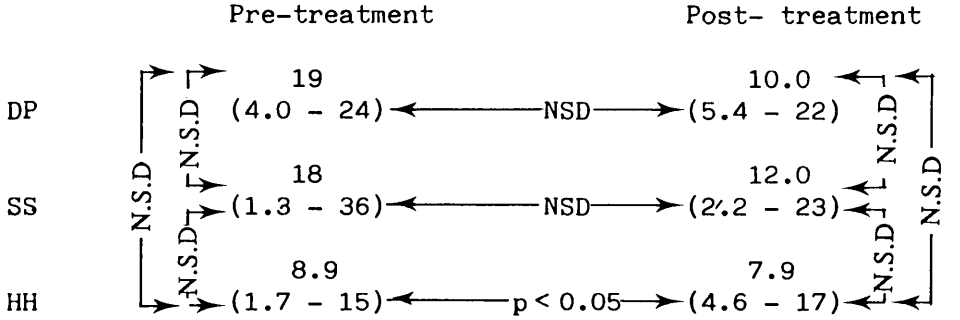


Figure 18
The basal and peak serum LH responses to bolus LH-RH administration (100ug i.v.) following pulsatile LH-RH infusions in patients with a) delayed puberty b) short stature and c) hypogonadotrophic hypogonadism.

BASAL LH (u/l)



PEAK LH (u/l)



INCREMENT IN LH (u/l)

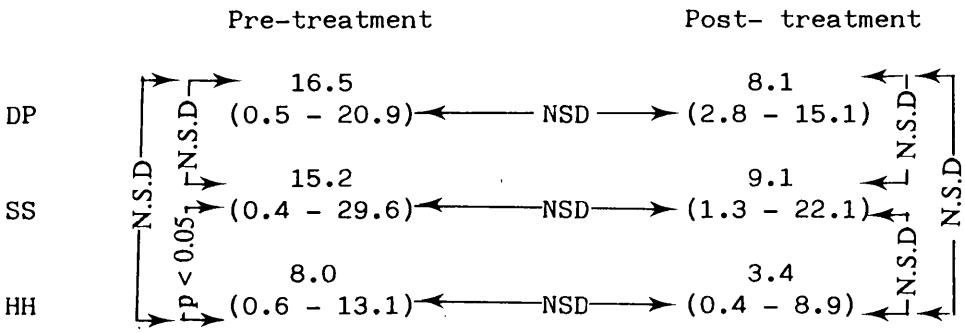


TABLE 11

LH responses to a standard bolus LH-RH test (100 ug IV) prior to, and immediately following 6 days of pulsatile LH-RH infusion.

DP = constitutional delayed puberty
SS = short stature
HH = hypogonadotrophic hypogonadism
NSD= not significantly different

the post-therapy tests were, however not significantly different between the groups (table 11,figure 18).

6.3.2) Comparison of LH results within groups

When the LH responses to bolus tests were compared before and following LH-RH pulsatile administration, the delayed puberty and short stature groups showed no significant differences in basal or peak LH, or increments in LH (table 11).

Patients with hypogonadotrophic hypogonadism, on the other hand, had significantly higher basal and peak LH concentrations in the second bolus tests, although the incremental increases in LH were unchanged. Thus, the higher peaks in the post-infusion tests simply reflect the higher basal concentrations at this time.

6.3.3) Comparison of FSH results between groups

Basal FSH concentrations prior to therapy were lowest in the hypogonadotrophic group and this reached statistical significance when compared with the delayed puberty group. Peak FSH levels in the first bolus tests were significantly reduced in the hypogonadotrophic males when compared with the other two groups. However there was again significant overlap between the groups (fig 19) so that this test could not discriminate well between the

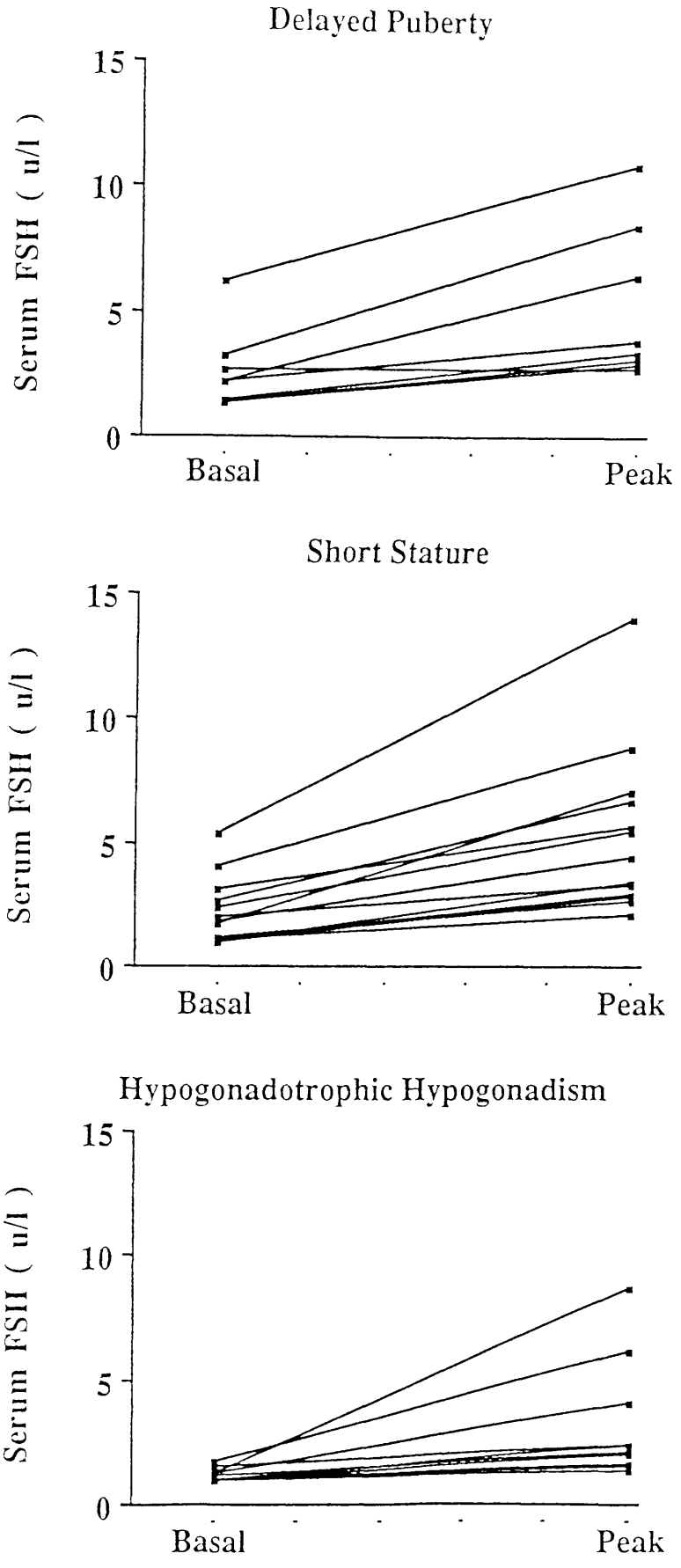


Figure 19

The basal and peak serum FSH responses to bolus LH-RH administration (100ug i.v.) prior to pulsatile LH-RH infusions in patients with a) delayed puberty b) short stature and c) hypogonadotrophic hypogonadism.

groups.

Basal FSH concentrations in the second bolus tests were highest in the hypogonadotrophic patients although this did not reach statistical significance (table 12, fig 20). There were no significant differences in peak FSH or increments in FSH between the groups in the post infusion LH-RH tests (table 12).

6.3.4) Comparison of FSH results within groups

Patients with delayed puberty had significantly reduced basal, peak and incremental changes in FSH in the subsequent LH-RH tests (figs 19 - 20, table 12). The short stature patients showed no changes in basal or peak FSH concentrations. However, the incremental change in FSH was significantly lower in the second bolus test (table 12).

Patients with hypogonadotrophic hypogonadism showed significantly higher basal and peak FSH concentrations, whilst the incremental increases in FSH remained unchanged in the second test. It must be stressed, however, that some of the changes in corresponding FSH concentrations were small and close to the coefficient of variance of the assay.

Figures 21 and 22 compare the incremental changes in LH and FSH before and after the periods of pulsatile infusion. All five patients within the delayed puberty

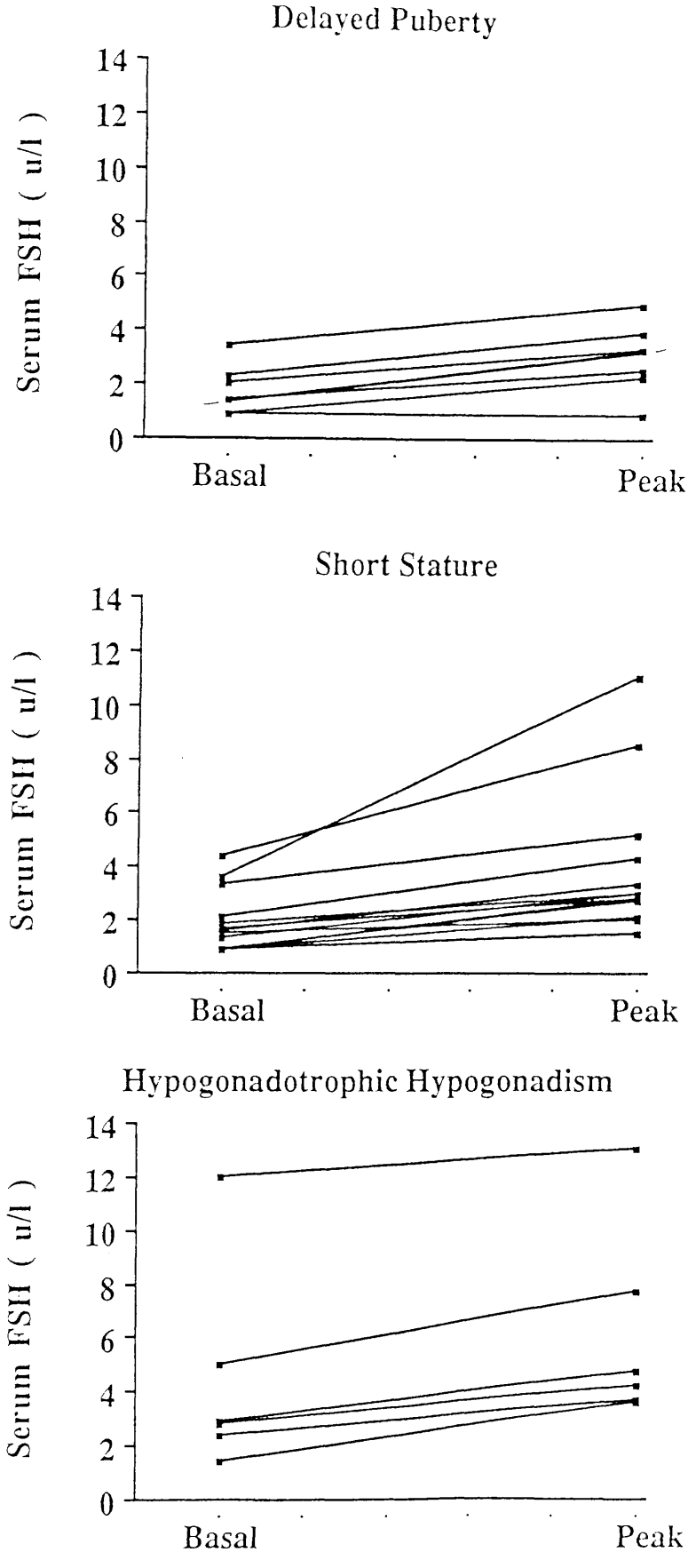
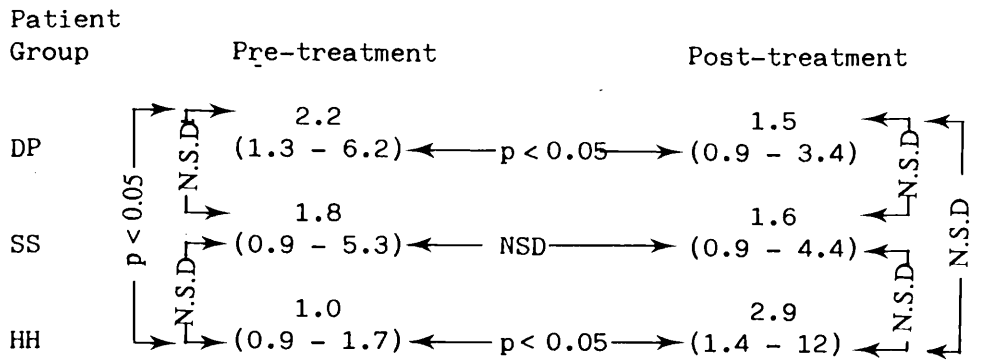


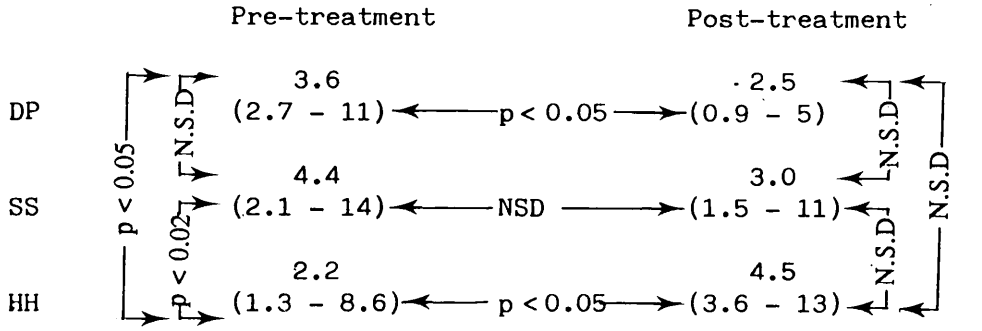
Figure 20

The basal and peak serum FSH responses to bolus LH-RH administration (100ug i.v.) following pulsatile LH-RH infusions in patients with a) delayed puberty b) short stature and c) hypogonadotrophic hypogonadism.

BASAL FSH (u/l)



PEAK FSH (u/l)



INCREMENT IN FSH (u/l)

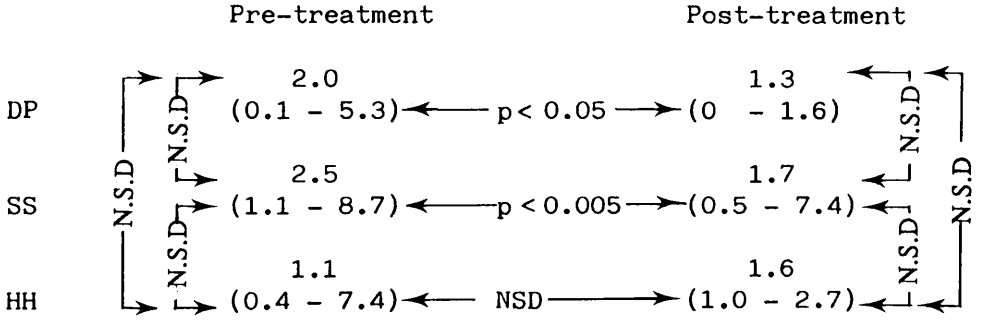


TABLE 12

FSH responses to a standard bolus LH-RH test (100 ug IV) prior to, and immediately following 6 days of pulsatile LH-RH infusion.

DP = constitutional delayed puberty
SS = short stature
HH = hypogonadotrophic hypogonadism
NSD= not significantly different

and short stature groups, who had initial increments in LH < 5mU/L showed increased increments following pulsatile LH-RH. On the other hand, only 2 of 13 patients with initial increments >5 mU/L showed augmented increments in the second bolus test. No such trend was identified with LH increments in the patients with hypogonadotrophic hypogonadism although numbers in this latter group were small.

6.4) DISCUSSION

The results of the pre-infusion LH-RH bolus tests confirm the results of previous workers who have demonstrated that standard LH-RH bolus tests are unable to distinguish between different groups of hypogonadotrophic patients (Job et al, 1976, 1977; Conte et al, 1980).

Valk and colleagues studied LH-RH bolus tests following a period of pulsatile LH-RH infusion in 4 males and 2 females with hypogonadotrophic hypogonadism. LH-RH was given at a dose of 0.025ug/kg/pulse every 2 hours for 5 days. Serum LH concentrations and incremental responses to bolus LH-RH tests rose during the study in both sexes. Males showed increasing and females decreasing incremental FSH responses to LH-RH. Their results therefore simulate the changes previously described during normal pubertal development (Job et al, 1972; Roth

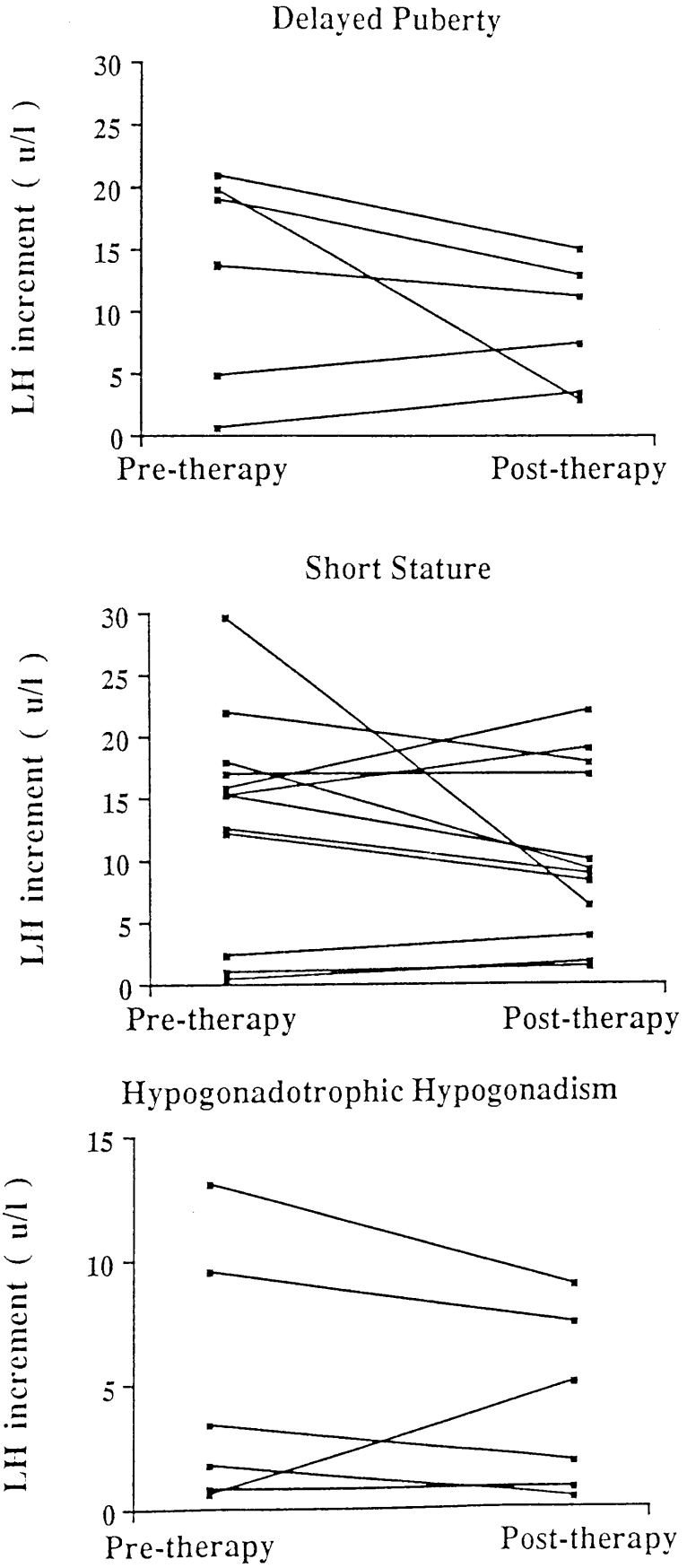


Figure 21

Increments (peak - basal) in serum LH after bolus LH-RH administration to the 3 patient groups prior to, and following pulsatile LH-RH infusions.

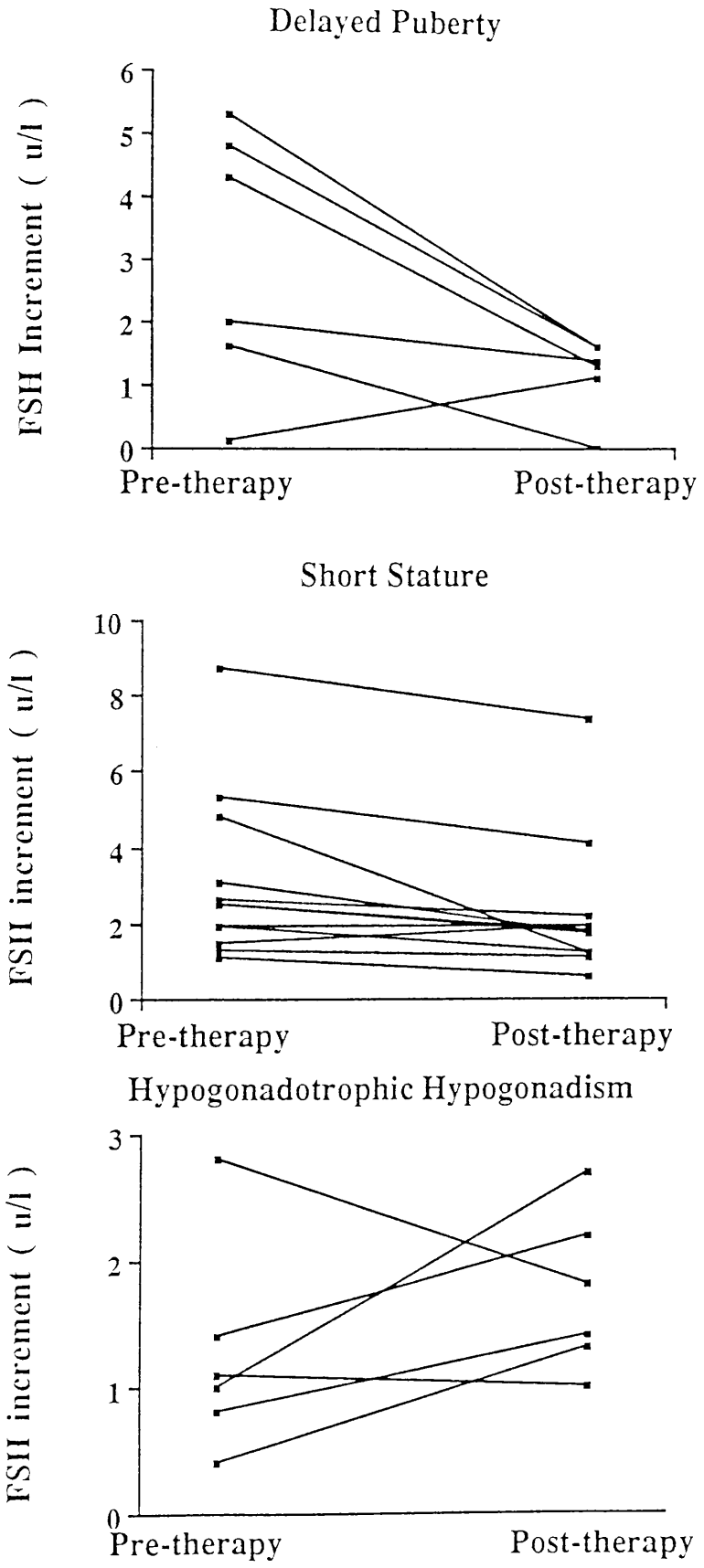


Figure 22
Increments (peak - basal) in serum FSH after bolus LH-RH administration to the 3 patient groups prior to, and following pulsatile LH-RH infusions.

et al, 1972; Franchimont et al, 1974; Garnier et al, 1974) and lead the authors to conclude that repetitive low dose administration of LH-RH induced rapid maturation of pituitary gonadotroph secretion.

Valk's study involved only a small number of patients, all of whom had LH and FSH increments at or below the lower limit of normal for prepubertal patients, during the initial bolus tests. The two female patients studied, however, had higher initial FSH responses and the difference between the males and females may simply reflect the level of initial responses.

Partsch and colleagues (1985) have investigated 8 male patients with hypogonadotrophic hypogonadism and 9 patients with simple delayed puberty using a regimen of LH-RH consisting of 5ug/pulse every 90 minutes for 36 hours. Basal LH concentrations were greater after the period of pulsatile infusion, in both groups, although increments in LH to bolus tests were reduced, particularly in the patients with hypogonadotrophic hypogonadism. Basal and peak FSH concentrations were increased in both groups, although increments in FSH were reduced in both.

Five patients in the group with delayed puberty had basal gonadotrophin and testosterone concentrations within the normal adult range and 4 had testicular volumes of 9mls or greater. Five patients with delayed puberty had initial LH increments > 15mU/L with only one

patient having an increment less than 5mU/L. This study which compared a group of patients with hypogonadotrophic hypogonadism and a group of boys with simple delayed puberty is biased by the presence in the latter group of a majority showing adult or almost adult hormone profiles.

Although it is not possible to make exact comparisons with the present study since Partsch et al have not compared increments in gonadotrophins for individual patients before and after the period of pulsatile infusion, it is clear from the data provided that those patients with simple delayed puberty showing the highest initial increments in LH, had reduced increments in the second bolus test and their results may be in broad agreement with our own.

Delemare-van de Waal, Van den Brande and Schoemaker (1985) investigated the responses to bolus LH-RH tests (three LH-RH injections of 100ug/m^2 i.v. at 2 hourly intervals) before and following pulsatile LH-RH infusion (either 10ug every 90 minutes or 20ug/1.73m^2 every 96 minutes i.v. for 7 days). They studied patients with a large number of hypothalamic-pituitary-gonadal disorders and divided the patients into 4 main groups: Group 1 consisted of 2 girls and 1 boy with primary gonadal failure; group 2 of 1 girl and 2 boys with hypothalamic disfunction; group 3 of 11 girls and 13 boys with various central endocrine disorders including growth hormone

deficiency multiple pituitary hormone deficiencies, delayed puberty and patients with tumours of the pituitary or surrounding area, and group 4 , two patients with pubertal arrest of unknown origin.

Only 4 patients of 32 are classified as simple delayed puberty and two of these patients who were prepubertal at the ages of 17.7 and 18.2 years, may be examples of idiopathic hypogonadotrophic hypogonadism. Although it is difficult to draw comparisons with this study because of the large number of clinical diagnoses within the groups, it appears that the high doses of LH-RH used by the intravenous route in this study resulted in considerable augmentation of gonadotrophin responses to their 'triple LH-RH tests'. Twenty eight of 30 patients achieved increased LH peaks and 27 of 31 patients achieved increased FSH peaks.

The present study suggests that the basal gonadotrophin concentrations during pulsatile LH-RH therapy are better than stimulated levels during bolus LH-RH tests in discriminating between different groups of hypogonadotrophic males.

CHAPTER 7

SERUM TESTOSTERONE RESPONSE TO PULSATILE LH-RH THERAPY

7.1) INTRODUCTION

Previous studies using HCG have suggested that in early puberty there may occur in response to rising serum gonadotrophin levels, a progressive increase in Leydig cell responsiveness to circulating LH (Winter, Taraska and Faiman, 1972; Josefsberg et al, 1976). Winter and colleagues (1972) have shown that following 3 daily injections of HCG (2000 IU), testosterone increments progressively rise with advancing pubertal stages.

Bardin et al (1969) compared the testosterone rise following HCG (4000 IU daily for 4 days) in hypogonadotrophic males, normal adult males and prepubertal boys and incorrectly concluded that patients with hypogonadotrophic hypogonadism had a fixed defect in Leydig cell function. Subsequent studies (Anderson et al, 1970; Boyar et al, 1973) have shown that more prolonged therapy with HCG allows hypogonadotrophic patients to achieve normal serum testosterone concentrations. It would appear therefore, that the extent of prior exposure to gonadotrophins is important in the immediate Leydig cell response to stimulation by HCG. It was of interest therefore to measure the testosterone response to

PROTOCOL

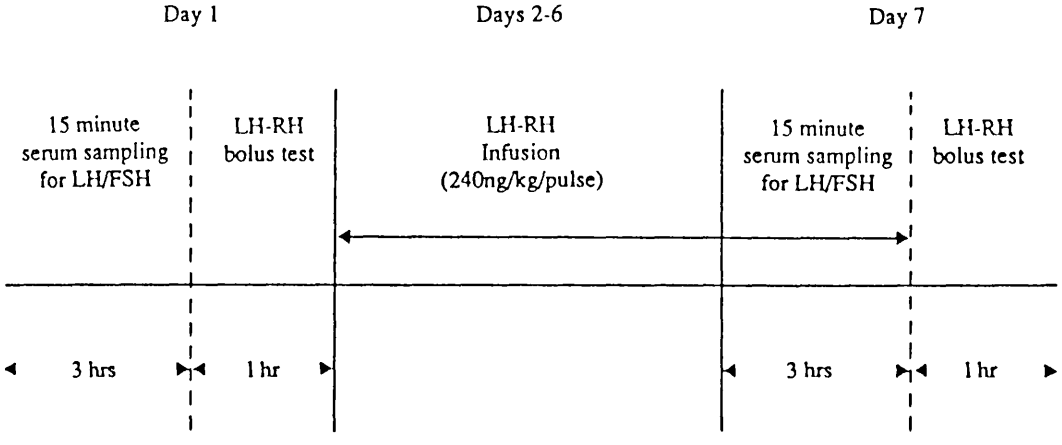


Figure 23
Study protocol

pulsatile LH-RH therapy in order to see whether a similar graded response occured in pubertal boys and to identify any differences between the groups of hypogonadal patients studied.

7.2) PATIENTS AND METHODS

Serum testosterone was measured on 8 of 9 patients with delayed puberty, and all patients with short stature and hypogonadotrophic hypogonadism who underwent the study described in Chapter 5. The protocol for this study is therefore the same as that described in the above chapter. The protocol diagram is reproduced as figure 23 for reference.

In view of the wide diurnal variation in serum testosterone demonstrated in Chapter 3, care was taken to ensure that blood was withdrawn for testosterone estimation at exactly the same time of day, for each patient.

7.3) RESULTS

Figures 24 and 25 show the basal and peak values of testosterone in the delayed puberty and short stature groups following the period of pulsatile LH-RH therapy, as a function of bone age. The results for the patients with hypogonadotrophic hypogonadism are shown in table

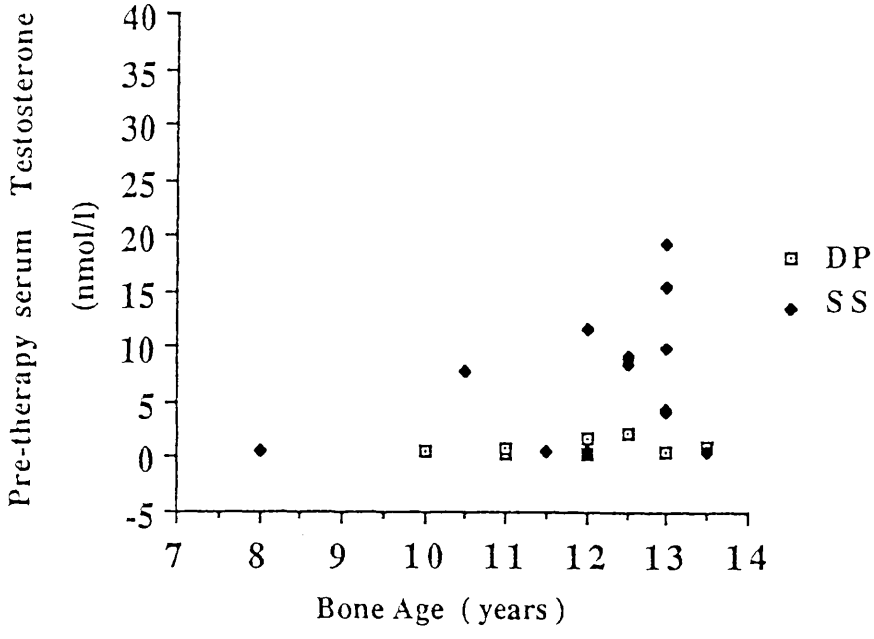


Figure 24

Basal serum testosterone concentrations in patients with delayed puberty and short stature as a function of bone age. Suitably timed serum testosterone samples were not available on all patients in the study.

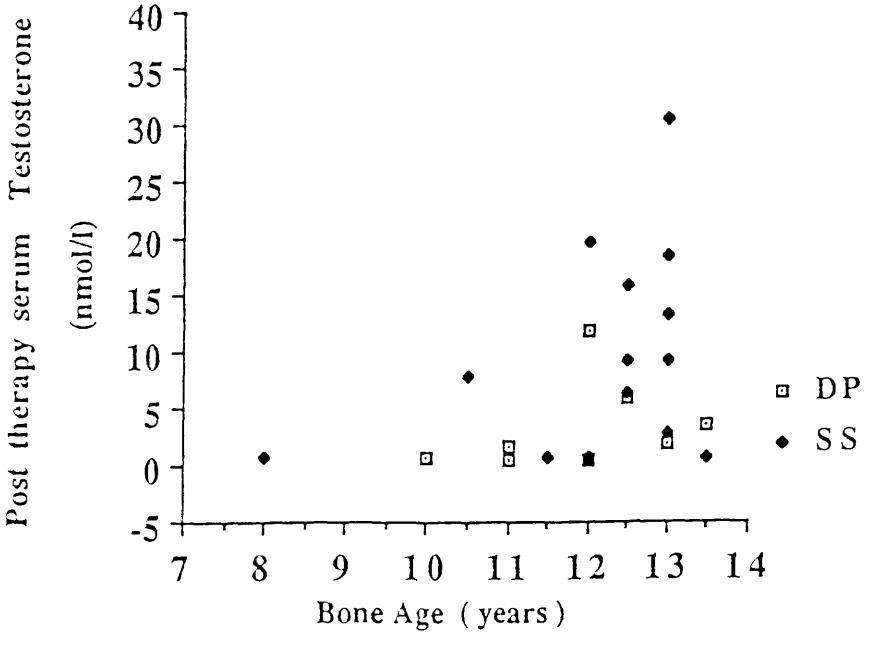


Figure 25

Serum testosterone concentrations following pulsatile LH-RH infusions, as a function of bone age, in patients with delayed puberty and short stature. Suitably timed serum testosterone samples were not available on all patients in the study.

13. It is notable that patients 31 and 32 who both have histories of cryptorchidism, had low basal testosterone concentrations and poor response to LH-RH therapy. However patient 25 who gave no such history failed to demonstrate any testosterone rise during the study period.

Significant moderate correlation was shown between basal values, peak values and increments in testosterone with bone age ($r=0.5$ $p<0.05$, respectively) following pulsatile LH-RH exposure. Basal testosterone appeared to be lower in the delayed puberty group for a given bone age. There did not appear to be any significant differences in incremental testosterone rises following pulsatile LH-RH infusion in the different study groups.

Testosterone values in subjects of bone age >12 years were significantly higher than in subjects with a bone age of 12 years or less ($p<0.05$). The testosterone increments following pulsatile LH-RH infusion also show a significant difference between patients with bone ages of 12 years or greater, and those with bone ages <12 years ($p<0.02$). Only one patient in the present study with a bone age less than 12 years showed a testosterone rise following LH-RH infusion.

7.4) DISCUSSION

The changes seen in the present study, in basal

Patient No	Genital Staging	Initial Testosterone (nmol/l)	Final Testosterone (nmol/l)
25	G5P5 T.V. 12/12	9.2	13.2
26	G5P5 T.V. 3/3	2.1	3.3
27	G2P1 T.V. 3/3	< 0.5	< 0.5
28	G2P4 T.V. 3/4	1.4	6.4
29	G5P5 T.V .4/4	1.5	4.3
30	G3P4 T.V. 10/10	3.9	13.0
31	G2P2 T.V. 3/3	4.8	2.2
32	G1P1 T.V. 1/1	2.4	7.0
33	G2P1 T.V. Ing/↟	0.9	< 0.7
34	G5P5 T.V. 0/1	1.6	2.4
35	G3P3 T.V. 1/0	0.9	1.4

TABLE 13

Serum testosterone response to 6 days pulsatile LH-RH infusion in men with hypogonadotrophic hypogonadism

T.V. = testicular volume. Ing = inguinal ↟ = undescended

testosterone levels with bone age are very similar to those of previous workers, who have shown that testosterone concentrations are fairly constant up to about 12 years of age, following which a sharp rise occurs and continues though the pubertal years. (Winter and Faiman, 1972; Sizonenko and Paunier, 1975; Pakarinen, Hammond and Vihko, 1979).

Valk et al, 1980) in an early study of pulsatile LH-RH administration (using 0.025ug/kg i.v. every 2 hours for 5 days) investigated 4 males with isolated gonadotrophin deficiency. They demonstrated a significant rise in serum testosterone by day 4, when the results for the 4 patients were grouped together. Individual results are not given. Three of the patients studied had bone ages which were advanced by previous therapy with either testosterone or HCG and it is not possible therefore to compare their data directly with the present study.

Sippell et al (1984) compared the testosterone response to i.v. LH-RH, 5ug every 90 minutes for 36 hours, in 13 males with hypogonadotrophic hypogonadism and 5 with delayed puberty. They showed a significant rise in serum testosterone in the patients with delayed puberty only. However it is clear that the delayed puberty group had achieved greater testicular maturation since testicular volumes were greater and basal serum testosterones were significantly higher ($p < 0.005$) in this group.The improved testicular response to pulsatile

LH-RH in the delayed puberty patients may therefore reflect the more advanced testicular development in this group.

A second paper from the same group (Partsch et al, 1985) compared the testosterone response to pulsatile LH-RH administration in 8 men with hypogonadotrophic hypogonadism and 9 males with delayed puberty. Again a significant rise in serum testosterone was found only in the patients with delayed puberty. Only one patient in the delayed puberty group failed to show a significant testosterone rise and this patient had the lowest testicular volume. The hypogonadotrophic hypogonadal patients had a lower mean basal testosterone (mean ± S.E.; 30 ±11 ng/dl) compared with the delayed puberty subjects (mean ± S.E.; 104 ± 40 ng/dl) although the difference did not reach statistical significance. There was also significant overlap of testosterone values after pulsatile LH-RH which included particularly those delayed puberty patients with lower bone ages and lower testicular volumes. This study would also confirm that testicular response to a short course of LH-RH is dependent upon the extent of testicular priming by endogenous gonadotrophins. The lack of testicular response in the hypothalamic hypogonadal patients cannot fully be explained by the immaturity of testicular development in this group and the reason for the difference with the present study is unknown.

Barkan et al (1985) studied testosterone levels after LH-RH therapy in 4 males with 'partial' and 4 with 'complete' hypogonadotrophic hypogonadism as defined by the presence or absence of nocturnal LH pulses in the pretreatment period. Three of the 4 men with complete gonadotrophin deficiency had undetectable basal serum testosterone and no patient in this group showed a rise in testosterone at 4 days. Results for 3 of the 4 patients with partial gonadotrophin deficiency are given and all showed a rise in serum testosterone, with the greatest rise occurring in the patient with the highest initial concentration.

On the other hand, Wagner et al (1986) compared serum testosterone after 10 days exposure to pulsatile LH-RH in 5 patients with spontaneous night-time LH pulses and 5 patients without such pulses and failed to show any significant differences between the two groups. Despite the difference in nocturnal gonadotrophin secretion between the groups, there were no obvious differences in bone age, testicular volumes or basal serum testosterone during HCG stimulation tests between the two groups of patients and this might explain the similar testosterone increments achieved by both groups during LH-RH infusion.

Hawthorne et al (1985a) failed to demonstrate a significant testosterone rise in 5 hypogonadotrophic hypogonadal males undergoing pulsatile LH-RH infusion. Their regimen used a small dose of LH-RH (24ng/kg) given

2 hourly for 72 hours and this may have been insufficient to adequately stimulate the testes. Individual patient results are not given, nor are details of testicular size. Poor testicular development or function in one or more patients might have resulted in insignificant testosterone rises for the group as a whole.

Delemare-van de Waal et al (1985) studied a group of patients with a variety of hypothalamic or pituitary disorders which included 4 patients with delayed puberty and one with Kallmann's syndrome. All patients showed significant testosterone increments but because the number of patients in each group is small, further interpretation of the results is not possible.

CHAPTER 8

THE PROLACTIN RESPONSE TO TRH

8.1) INTRODUCTION

The relationship between various states of hypogonadism and the prolactin response to dynamic tests remains uncertain, due to previous conflicting reports. In the syndrome of isolated gonadotrophin deficiency, both intact and attenuated PRL responses following TRH have been described (Antaki et al, 1974; Winters, Mecklenburg and Sherins, 1978; Yamaji et al, 1977; Spitz, Hirsch and Trestian, 1983; Hawthorne et al, 1985b). Similarly in patients with Klinefelter's syndrome, both normal and exaggerated PRL responses to TRH have been reported (Burman et al, 1975; Spitz et al, 1979). More recently, Gooren et al (1984) reported impaired PRL response to TRH in a group of patients with Kallmann's syndrome when compared with a group of hypergonadotrophic hypogonadal patients with similar serum testosterone concentrations. They postulated a direct relationship between endogenous LH-RH secretion and PRL release.

In order to test this hypothesis and assess the effect of exposure of the pituitary to exogenous LH-RH, the prolactin response to TRH was measured both prior to and following pulsatile LH-RH infusions in patients with

PROTOCOL

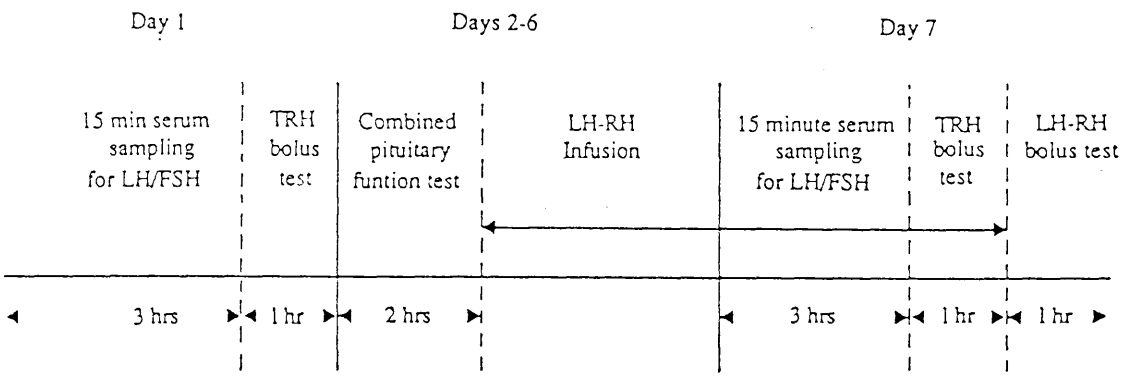


Figure 26
Study protocol

Patient	Genital Staging	Testicular Volume (mls)	Chronological Age (yrs)	Bone Age (yrs)
1	G1P1	2/3	14.8	12.0
2	G2P1	5/5	15.4	12.5
3	G2P1	6/8	15.6	12.0
4	G1P1	2/2	15.0	11.0
5	G2P1	3/3	14.7	13.5
6	G2P1	3/3	14.1	11.0
7	G1P1	3/3	15.0	13.0
8	G2P2	8/8	14.1	12.5
9	G2P2	6/6	14.7	12.5
10	G3P2	4/3	15.1	13.0
11	G3P3	6/6	16.9	13.0

TABLE 14

Clinical details of patients undergoing bolus TRH tests (200 ug IV) prior to, and following 6 days of pulsatile LH-RH infusion

	Pre-LH-RH infusion Serum T (nmol/l)	Post-LH-RH infusion Serum T (nmol/l)	Significance
Peripubertal boys	1.8 (<0.7 - 19.6)	5.9 (<0.7 - 30.6)	$p < 0.02$
Hypogonadotrophic Hypogonadism	2.1 (<0.5 - 9.2)	6.4 (<0.5 - 13.2)	$p < 0.05$

TABLE 15

Serum testosterone concentrations (median and range) for the peripubertal boys and hypogonadotrophic males prior to and following pulsatile LH-RH infusions.

delayed puberty and hypogonadotrophic hypogonadism.

8.2) PATIENTS AND METHODS

The serum prolactin response to an intravenous bolus injection of TRH (200ug) was measured in 11 pre-pubertal and pubertal boys and 7 males with hypogonadotrophic hypogonadism, immediately prior to, and following six days pulsatile LH-RH infusion (240ng/kg/pulse). The study protocol is illustrated in diagramatic form in figure 26.

The clinical details of the peri-pubertal boys are shown in table 14 and the patients with hypogonadotrophic hypogonadism who underwent this study are listed below. (Patient numbers, 25-28, 30, 32, 33, table 9)

8.3) RESULTS

All patients showed a rise in mean serum LH measured by 15 minute sampling over 3 hour periods immediately prior to and at the end of the LH-RH infusions. Both groups of patients showed significant rises in serum testosterone concentrations (table 15).

There was considerable overlap in basal and peak prolactin responses to TRH between both groups of patients both before and following the period of pulsatile LH-RH administration (figs 27 - 28). There were no significant differences in basal or peak serum

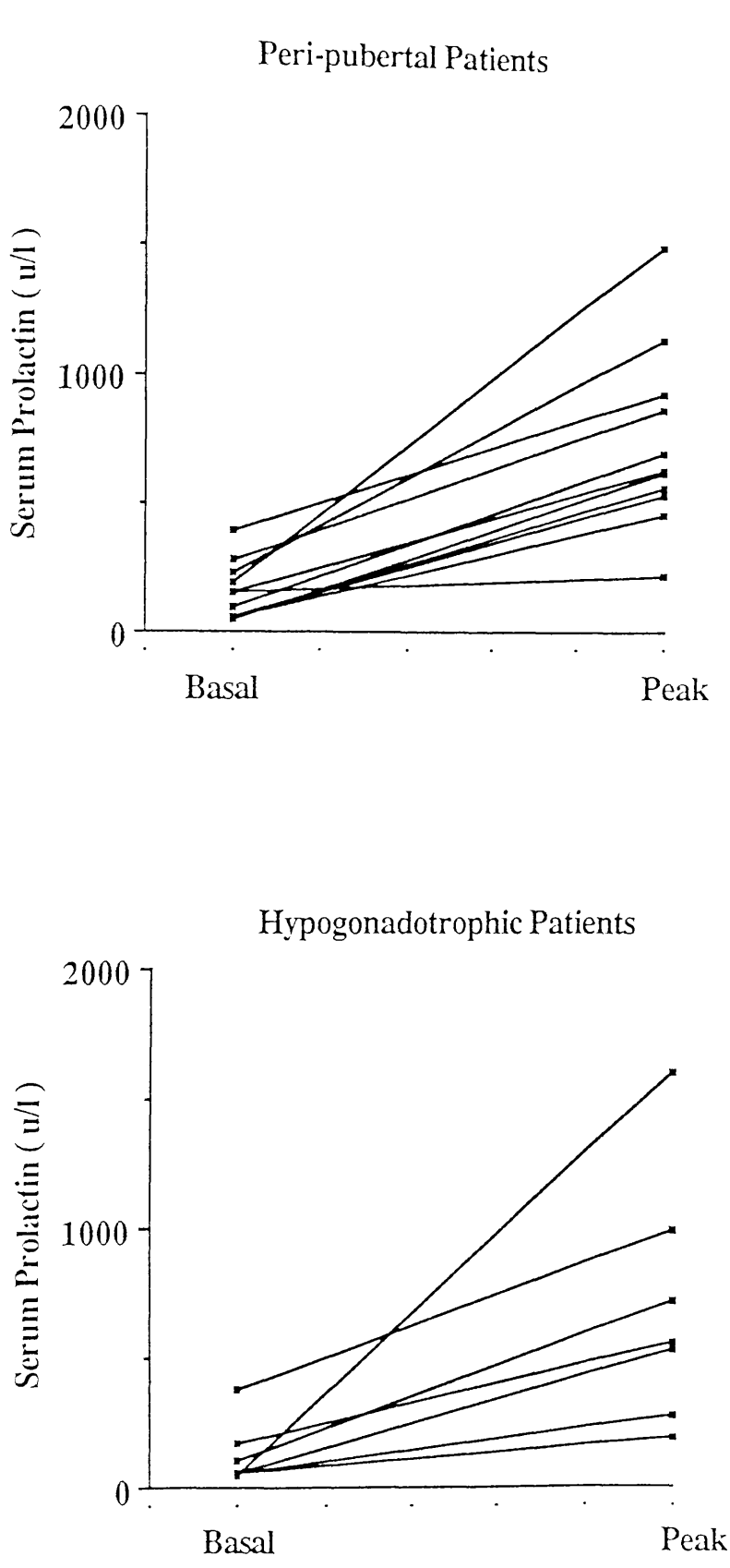


Figure 27
The basal and peak serum prolactin response to TRH (200ug i.v.) in peri-pubertal boys and hypogonadotrophic males, prior to pulsatile LH-RH infusions.

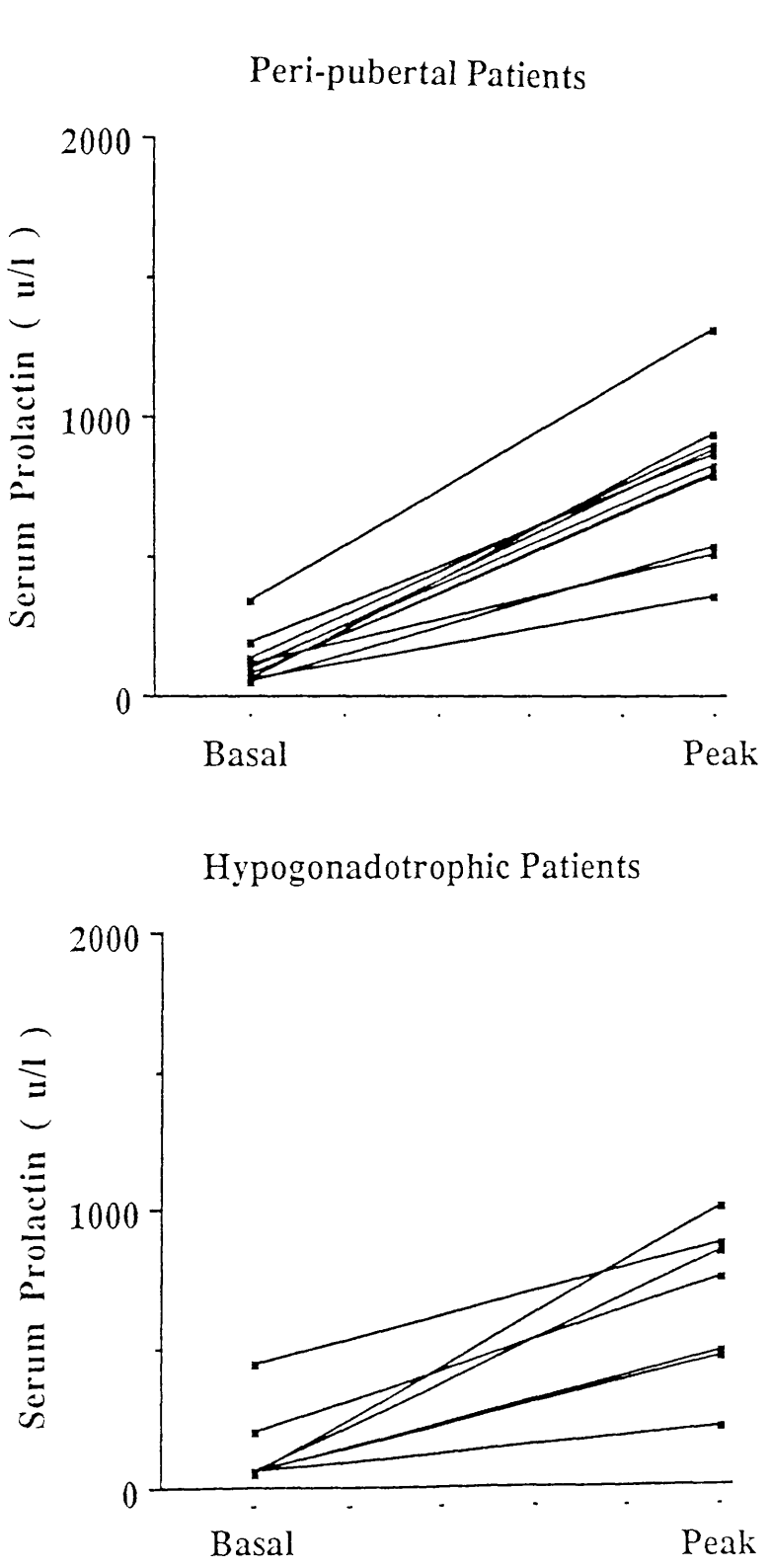


Figure 28
The basal and peak serum prolactin response to TRH (200ug i.v.) in peri-pubertal boys and hypogonadotrophic males, during pulsatile LH-RH infusions.

prolactin or increments in prolactin during the TRH tests following the periods of pulsatile therapy in either group of patients (table 16).

There was no correlation between the changes in prolactin increments and the rises in either serum LH or testosterone demonstrated by both patient groups.

8.4) DISCUSSION

There was considerable overlap between the prolactin responses to TRH in the group of pubertal boys when compared to the males with hypogonadotrophic hypogonadism either before or following exposure to pulsatile LH-RH administration. Spitz et al (1983) showed that patients with constitutional delay of puberty had, after TRH stimulation, a rise above basal in the serum prolactin of greater than 396mU/l, whereas 9 of 10 patients with hypogonadotrophic hypogonadism had an attenuated response. However other workers have reported differing responses in isolated gonadotrophin deficiency. Yamaji et al, (1977) reported an heterogeneous response in untreated males with hypogonadotrophic hypogonadism. In only 4 of 8 such patients was the TRH mediated prolactin release blunted. Hawthorne et al (1985b) found no significant difference in either basal or maximum increment of serum prolactin following TRH between 6 patients with hypogonadotrophic hypogonadism and 8 normal

	Basal Prolactin		Peak Prolactin		Percentage Incremental Change	
	Pre-therapy	Post-therapy	Pre-therapy	Post-therapy	Pre-therapy	Post-therapy
	median (range) (u/l)		median (range) (u/l)		median (range) (u/l)	
Peri-pubertal group	150 (60 - 390)	90 (60 - 340)	700 (220 - 1500)	830 (360 - 1320)	637 (47 - 1140)	730 (288 - 1467)
H.H. group	60 (60 - 380)	60 (60 - 450)	560 (190 - 1600)	750 (210 - 1000)	350 (161 - 3100)	666 (93 - 1900)

TABLE 16

Changes in serum prolactin response to bolus administration of TRH (200 ug) before and after pulsatile LH-RH infusions

$$\text{PERCENTAGE INCREMENTAL CHANGE} = \frac{\text{PEAK} - \text{BASAL}}{\text{BASAL}} \times 100\%$$

adult males. It would appear therefore, that the prolactin response to TRH is variable in hypogonadal states and the small numbers of patients studied in these reports could explain the differences between them.

The relative importance of endogenous LH-RH secretion and the sex steroid concentrations in influencing prolactin remains unknown. Spitz et al (1977, 1979, 1982, 1983) have argued strongly in favour of the steroid milieu having a major influence. Spitz et al (1982) demonstrated that during treatment with HCG, prolactin release could be restored to normal in male patients with hypogonadotrophic hypogonadism. This work, however does not differentiate between an effect due to stimulation of gonadal steroids and a direct effect of HCG which has LH activity. Winters, Johnsonbaugh and Sherins, (1982) have shown that the prolactin response to chlorpromazine was enhanced by HCG or testosterone in 3 of 6 patients with hypogonadotrophic hypogonadism and 10 patients on long-term replacement therapy with HCG or testosterone showed normal prolactin reserve using chlorpromazine or TRH. On the other hand, Yamaji et al (1977) demonstrated reduced prolactin reserve in 4 of 7 patients with hypogonadotrophic hypogonadism on long-term testosterone replacement. In the study of Spitz et al (1983) the impaired prolactin response in idiopathic hypogonadotrophic hypogonadism compared to pubertal boys cannot be explained by altered sex steroid milieu since

both groups had similar serum testosterone concentrations. Elevation of oestradiol is a major factor in establishing prolactin responsiveness in both man and the rat (Jaques and Gala, 1979; Spitz et al, 1982) and since the major source is from aromatization of testosterone, it is unlikely to have been significantly different in the two groups studied by Spitz et al in 1983.

Gooren et al (1984) compared the prolactin response to TRH in patients with Kallmann's syndrome and a group of hypergonadotrophic hypogonadal patients having comparable serum testosterone concentrations. They demonstrated a direct relationship between LH-RH-stimulated gonadotrophin concentrations and the prolactin response to TRH. They also reported that the prolactin response to TRH was greater following a 4 hour infusion of LH-RH in 8 of 9 normal subjects.

This study led the authors to postulate that LH-RH itself may have an effect on prolactin secretion. Herbet and Rennels, (1977) reported a stimulatory effect of low concentrations (0.08 - 8ng/ml) of LH-RH on prolactin release in a clonal strain of pituitary cells, while at higher concentrations an opposite effect was observed. However, Sandow and Robyn (1973) found a decrease of prolactin after i.v. or subcutaneous or intrapituitary injections of LH-RH in male rats. In man, no change of prolactin levels was measured after administration of a

bolus of LH-RH, even after short-term oestrogen/progesterone pretreatment (Debeljuk, Arimura and Schally, 1972; Kastin et al, 1973). Gooren et al (1984) have postulated that the mechanism of LH-RH regulation of prolactin release is indirect, through an inhibitory effect on dopamine secretion into the hypophoseal portal system. In the median eminence an axo-axonic contact between LH-RH and dopamine-neuronal system has been reported. (McNeill and Sladek, 1978) Increased dopaminergic activity in the median eminence is associated with an inhibition of LH secretion (Lofstrom et al, 1976) via a neuronal interaction between LH-RH and dopamine (Judd, Rakoff and Yen 1978; Quigley et al, 1979) and Gooren et al (1984) have suggested LH-RH tone might lead to decreased dopamine secretion, resulting in decreased inhibition of prolactin synthesis and secretion from the lactotroph.

A further mechanism by which LH-RH can influence lactotroph activity has been proposed by Nikolics et al (1985) who identified a peptide sequence at the carboxy-terminal of the LH-RH precursor which can suppress prolactin.

Hawthorne et al (1985b) compared the prolactin increments to TRH stimulation in patients with hypogonadotrophic hypogonadism and normal controls. It is interesting to note that the three patients with hypogonadotrophic hypogonadism who had not received LH-RH

pulsatile therapy within 3 weeks of the study, had the smallest increments.

More recently Spratt et al (1987) have demonstrated that serum prolactin concentrations were higher in hypogonadotrophic hypogonadal patients with detectable LH pulses than in those patients with complete absence of endogenous pulsatility. This work lends further support to the theory that physiological LH-RH secretion influences prolactin release.

If indeed LH-RH stimulates prolactin release either directly or indirectly, an enhanced secretion in response to TRH would have been expected following prolonged pulsatile administration. A significantly greater effect might have been predicted in the patients with complete hypogonadotrophic hypogonadism and thus absolutely deficient in LH-RH prior to treatment. This study has, however failed to demonstrate any differences in prolactin release between the two patient groups either before or following pulsatile LH-RH administration and no changes within either group following therapy. It would appear therefore that exogenous LH-RH exerts no influence on lactotroph activity. The present work, however does not exclude the possibility that endogenous prolactin synthesis and release could influence prolactin secretion by indirect mechanisms.

CHAPTER 9

INFERTILITY TREATMENT WITH PROLONGED PULSATILE LH-RH ADMINISTRATION IN HYPOGONADOTROPHIC HYPOGONADISM

9.1) INTRODUCTION

Jacobson and colleagues (1979) were first to report the use of portable infusion pumps for delivering LH-RH in a pulsatile manner. More recently, chronic pulsatile infusions of LH-RH have been used to induce all the changes of puberty in men with hypogonadotrophic hypogonadism including spermatogenesis (Hoffman and Crowley, 1982; Skarin et al, 1982; Morris et al, 1984; Santoro et al, 1986). In order to obtain experience of this technique for inducing fertility and to attempt to predict patients likely to benefit from this therapy, hypogonadal adult males who expressed a wish to achieve fertility were offered pulsatile LH-RH as a first-line treatment.

9.2) PATIENTS AND METHODS

Six adult males were subsequently treated. All patients had failed to undergo puberty spontaneously by

the age of 18 years and had low circulating concentrations of gonadotrophins and testosterone. Combined anterior pituitary function testing, as described in the methods section, had demonstrated normal basal and stimulated levels of growth hormone, cortisol, TSH and prolactin in all patients with the exception of Patient 1 who had previously been diagnosed as having the cytomegalic variety of congenital adrenal hypoplasia. This patient was on standard cortisol replacement treatment (hydrocortisone 20mg mane and 10mg nocte) at the time of the present study. Skull x-rays and chromosome analysis was normal in all cases. Patients 2, 3, and 6 had life-long anosmia.

The nature of the treatment protocol and details of alternative therapy with the gonadotrophins HCG and HMG (Pergonal) were explained to each patient prior to obtaining informed consent. Testosterone replacement therapy was withdrawn at least 6 weeks prior to commencing pulsatile LH-RH treatment.

In patients 2 to 6, treatment was started at a dose of 5ug LH-RH/pulse, each pulse occuring every 90 minutes. Patient 1 was commenced on a dose of 2.8ug/pulse increasing in a stepwise fashion to a maximum dose of 22ug/pulse. Treatment was continued for 8 to 34 weeks (median 10.5 weeks).

9.3) CASE HISTORIES AND INDIVIDUAL PATIENT RESULTS

The serum testosterone, LH, and FSH responses to prolonged, pulsatile LH-RH infusion for individual patients are illustrated in figures 29 to 34.

9.3.1) Patient 1

This patient has been the subject of several case reports; Uttley (1968), Hay et al (1981) and Cohen et al (1982).Two of his 8 male siblings had died in the early neonatal period and one male sibling had died at 11 days. Post mortem examination of this last child revealed cytomegalic adrenocortical hypoplasia.

In this patient hypoadrenalism was diagnosed at 5.4 years and he was treated initially with cortisone acetate and fluodrocortisone. After an otherwise uneventful childhood he was reassessed at 15.3 years and found to be below the 3rd percentile in height; he had pre-pubertal genital and pubic hair staging with testicular volumes of 4 mls bilaterally. Bone age was 11.5 years. When aged 16 years he was treated for 5 months with HCG and an attempt to induce puberty with adrenal androgen administration was made at the age of 23 years. At 24 years injections of testosterone esters were commenced and subsequently stopped 8 weeks prior to the present study.

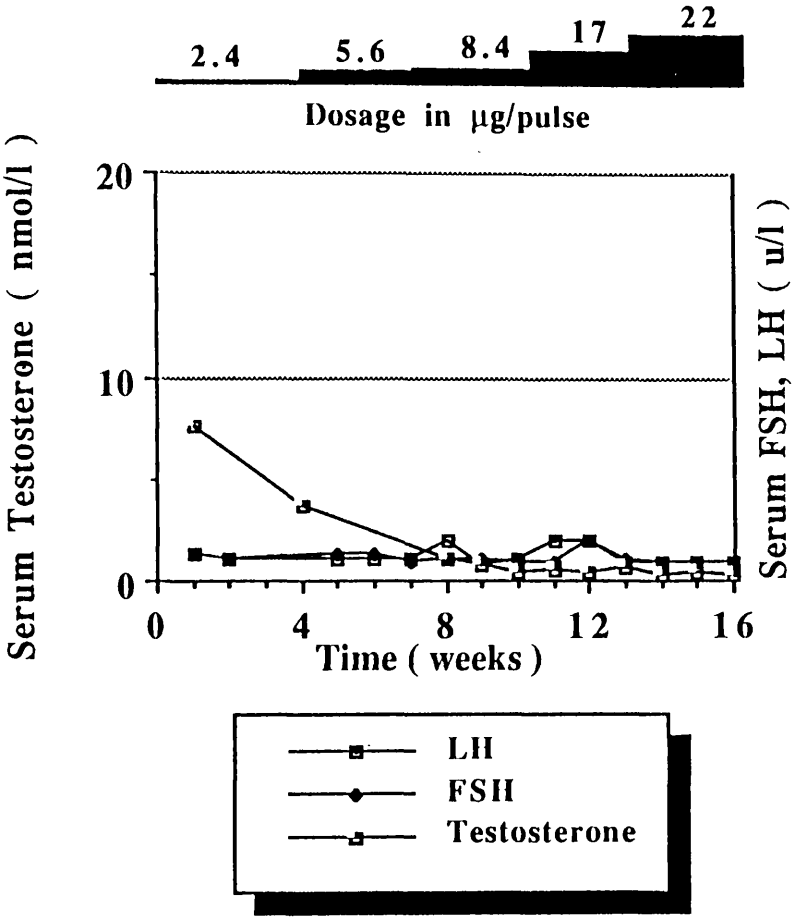


Figure 29

Mean serum LH and FSH, and testosterone responses to prolonged, pulsatile infusion of LH-RH to patient 1. Dosage of LH-RH is also illustrated. Mean serum gonadotrophin concentrations were estimated from samples taken every 15 mins over 3 hours.

Patient 1 was treated for a total of 16 weeks with increasing doses of LH-RH as shown in figure 29. Serum gonadotrophins remained low throughout the treatment period. Basal serum testosterone was measured at 7.6 nmol/l and the concentration of testosterone fell in an exponential fashion during the subsequent weeks reaching the limit of assay detection by week 10. This would reflect the loss of exogenous testosterone following withdrawal of Sustanon (Organon Labs Ltd, Cambridge.) 8 weeks earlier. At no time during the treatment period were sperm detected in ejaculate and there was no change in testicular volume. These results demonstrated a failure in gonadotroph response to pulsatile LH-RH administration over a wide dose range and the treatment was therefore stopped. The patient decided to postpone further attempts at inducing fertility and he was restarted on testosterone replacement therapy.

9.3.2) Patient 2

This patient presented at 19.5 years with delayed pubertal development, and a history of lifelong anosmia. Pubertal development was G2 P2 and testicular volume was 6 mls bilaterally. He defaulted from follow-up but was re-referred at age 25.7 years when he was started on monthly injections of testosterone esters. This therapy was also stopped 8 weeks prior to the present study.

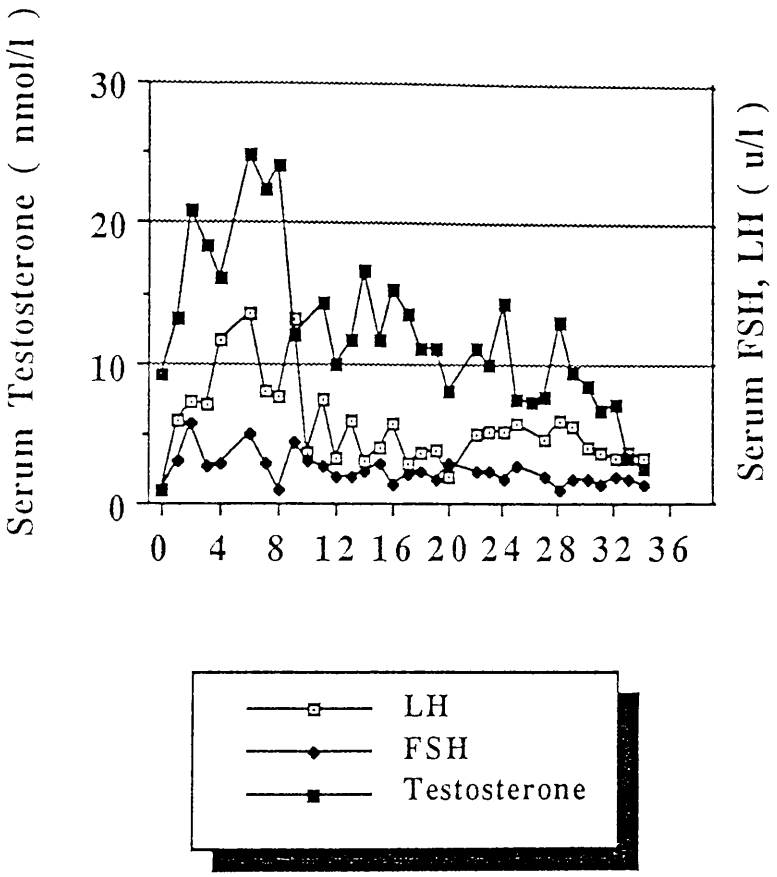


Figure 30

Mean serum LH and FSH, and testosterone responses to prolonged, pulsatile infusion of LH-RH to patient 2. Mean serum gonadotrophin concentrations were estimated from samples taken every 15 mins over 3 hours.

Patient 2 underwent pulsatile LH-RH infusion for a total period of 34 weeks. Basal gonadotrophin concentrations were undetectable throughout the pretreatment sampling period. The basal testosterone of 9.2 nmol/l again reflected the recent withdrawal of sex hormone replacement therapy. Following commencement of LH-RH therapy there was a rise in serum gonadotrophins by week 1 with FSH reaching a peak at week 2 while LH continued to rise achieving supra-physiological levels by week 4 and peaking at week 6. Thereafter the gonadotrophin levels declined into the normal adult range (fig 30).

Serum testosterone also rose gradually until week 6 falling to low normal values by week 9. From week 28 however, there was a gradual decline in LH and testosterone despite increasing the dose of LH-RH administered.

Analysis of seminal fluid showed no sperm present prior to therapy. Sperm became detectable at 4 weeks achieving a peak count of 6 million/ml at 12 weeks. Thereafter sperm count ranged from 3 to 5 million/ml until week 32 when the count fell to 1.5 million/ml. As part of a separate study the patient was given a standard bolus intravenous injection of LH-RH at this time. This resulted in an immediate urticarial reaction at the site of previous subcutaneous injections. It was concluded that the patient had developed sensitivity to the

infusion fluid and treatment was stopped. He was subsequently treated by HCG (1500 units three times weekly) and HMG (75-150 units twice weekly).Sperm density again increased to 9 million/ml a further 8 months later at which time his wife conceived and subsequently gave birth to a healthy male child.

9.3.3) Patient 3

Patient 3 was referred at the age of 23.1 years because of lack of pubertal development. At presentation genital development was G1 P3 (female hair distribution), testicular volumes 3mls bilaterally. Although his sister had previously been diagnosed as a case of Kallmann's syndrome he had refused investigation until this time. Following diagnosis he was started on testosterone replacement therapy which was continued for 10 months until it was stopped 6 weeks before entry into the present study.

This patient continued pulsatile LH-RH therapy for a total of 8 weeks at which time he took up a manual form of employment and found wearing the syringe driver impossible.

Serum gonadotrophins rose rapidly reaching peak concentrations on week 5 of treatment with LH-RH. Despite achieving LH and FSH levels above the normal adult range serum testosterone rose only marginally from a baseline

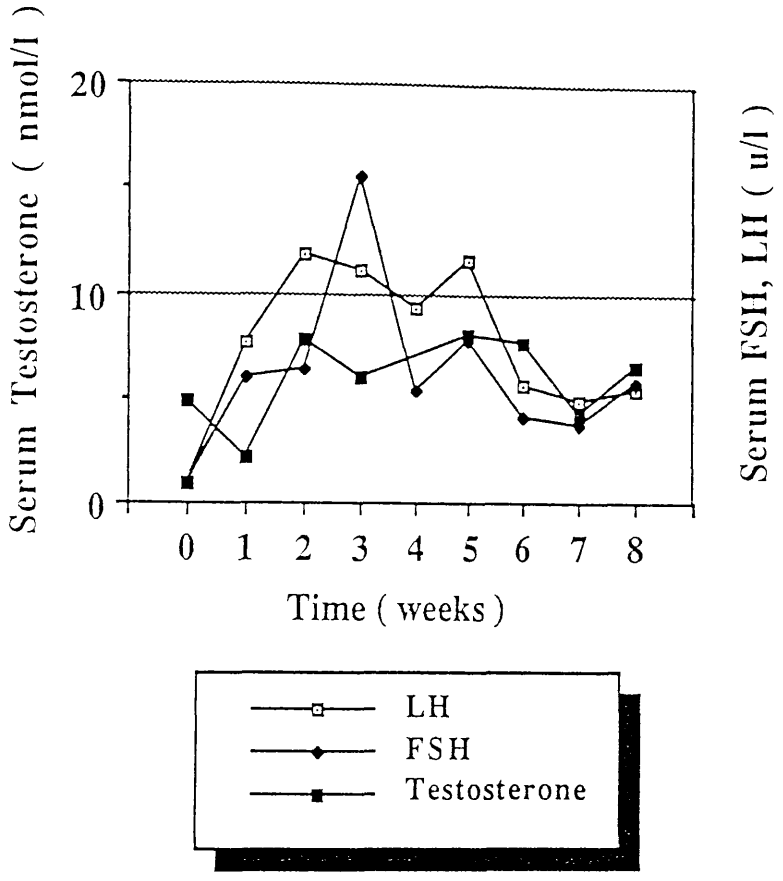


Figure 31
Mean serum LH and FSH, and testosterone responses to prolonged, pulsatile infusion of LH-RH to patient 3. Mean serum gonadotrophin concentrations were estimated from samples taken every 15 mins over 3 hours.

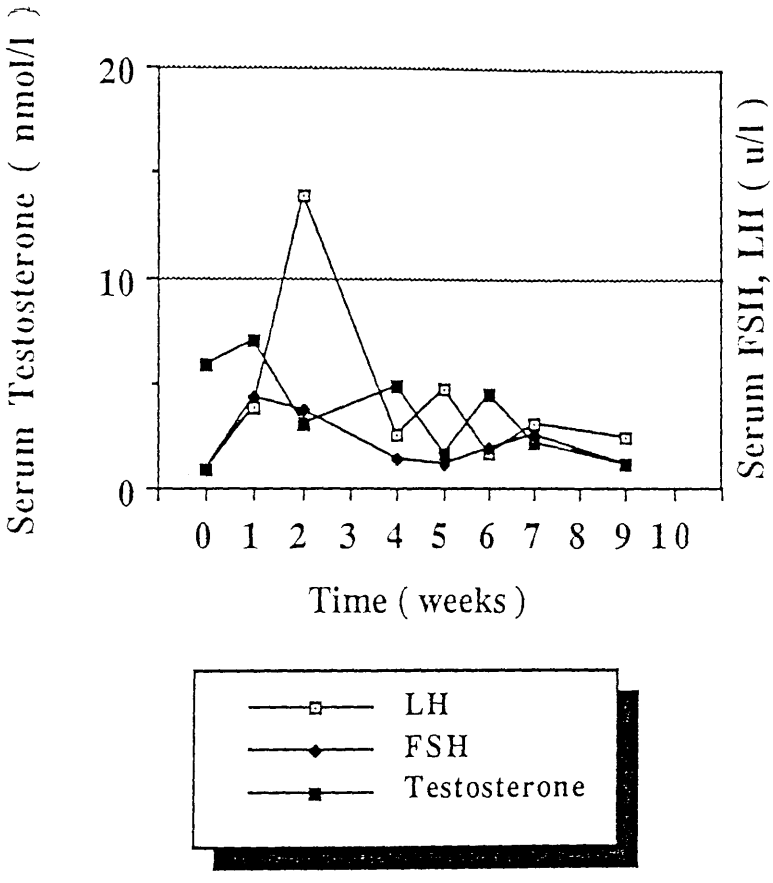


Figure 32
Mean serum LH and FSH, and testosterone responses to prolonged, pulsatile infusion of LH-RH to patient 4. Mean serum gonadotrophin concentrations were estimated from samples taken every 15 mins over 3 hours.

of 4.8 to 7.8 nmol/l. (fig 31). No ejaculate was produced during the time of the infusion. Treatment with HCG and HMG was thereafter started and continued for 16 months. Despite increasing the dose regimen from HCG 5000 units twice weekly plus HMG 75 units three times per week to HCG 5000 units and HMG 150 units thrice weekly a maximum sperm density of only 2 million/ml was achieved and the patient remained infertile. The couple were referred for artificial insemination by donor.

9.3.4) Patient 4

Referral of this patient for investigation of delayed pubertal development was made at the age of 23.1 years. There was no family history of hypogonadism and the patient's sense of smell was intact. Following diagnosis of hypogonadotrophic hypogonadism treatment with Sustanon was started and continued for 12 months before entry into the present study.

Pulsatile LH-RH therapy was administered for 9 weeks when treatment was ceased because it bacame difficult for the patient to continue wearing the pump and undertake the manual tasks required of him while working in the family demolition business.

Basal gonadotrophins were undetectable throughout the initial sampling period and rose to supra-physiological levels on week 2 (fig 32). The testosterone

concentrations fell from 5.9 nmol/l to 1.2 nmol/l during the infusion period (Sustanon had been withdrawn 7 weeks before this investigation).

Ejaculate prior to treatment failed to show the presence of sperm. By week 6 of LH-RH therapy the patient was unable to produce further ejaculate. Following failure of this therapy an attempt at testicular biopsy was made. Unfortunately mainly epididimal tissue was obtained and it was not considered justified to repeat this procedure.

Combined therapy with HCG and HMG was started some 19 months later when the patient again expressed a wish to attain fertility. Despite treatment over a 16 month period, monthly sperm counts showed no significant rise and therapy was abandoned. He and his wife have now applied for adoption.

9.3.5) Patient 5

Patient 5 was referred at the age of 23.4 years because of failure of development of secondary sexual characteristics. There was no significant family history and no anosmia. Pubertal development at presentation was G1 P2 with testicular volumes of 4mls bilaterally. Testosterone replacement therapy in the form of intamuscular injections of Sustanon was given for a total of 23 months and withdrawn 8 weeks prior to LH-RH

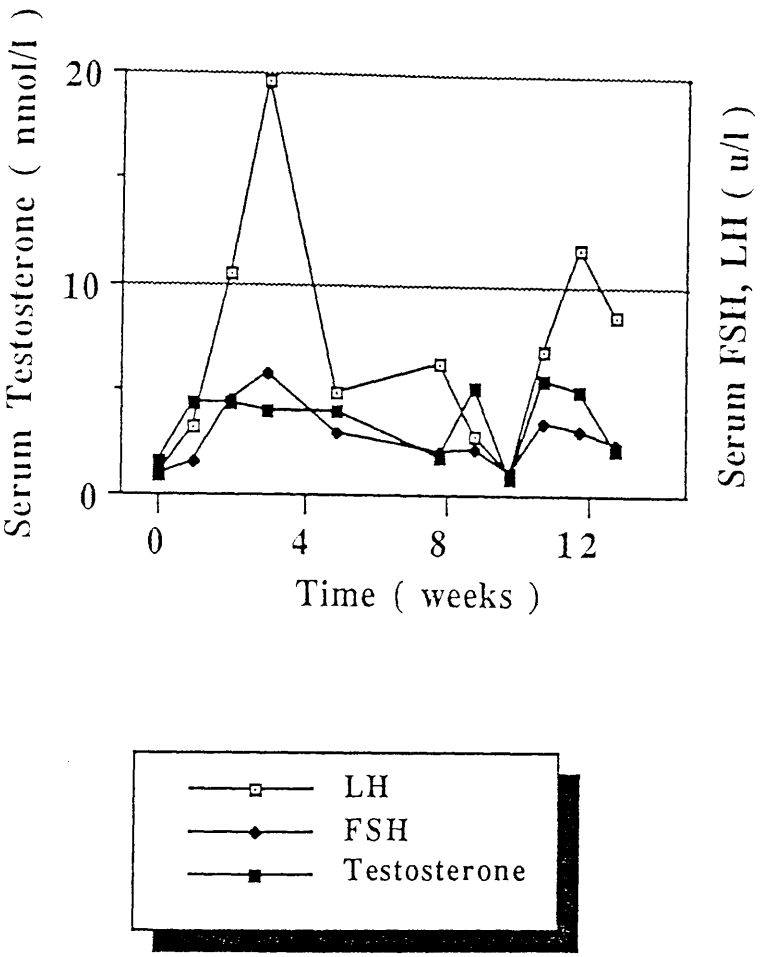


Figure 33
Mean serum LH and FSH, and testosterone responses to prolonged, pulsatile infusion of LH-RH to patient 5. Mean serum gonadotrophin concentrations were estimated from samples taken every 15 mins over 3 hours.

infusion.

The response to pulsatile LH-RH infusion therapy is illustrated in figure 33. Again a peak gonadotrophin response occurred at week 2 after which time the concentrations of LH and FSH fell back to more physiological levels. At week 9 the patient developed an abscess at the infusion site and gonadotrophin concentrations fell, only to rise again, following resiting of the needle, to a second peak after a further 2 weeks.

Serum testosterone rose from a basal level of 1.5 nmol/l to 4.3 nmol/l at the end of the first week. During the entire treatment period the testosterone concentration never achieved normal adult levels and no sperm were identified on semen analysis. Treatment was stopped at the end of 12 weeks because treatment interfered with his work as a welder. He was lost to follow up for 5 months and then restarted on testosterone replacement since he no longer wished to become fertile.

9.3.6) Patient 6

This patient was referred initially in 1970 at the age of 25.3 years when he was found to have failure of pubertal devlopment, an undescended right testis and an atrophic left testis. A diagnosis of hypogonadotrophic hypogonadism was made on the basis of low serum FSH and

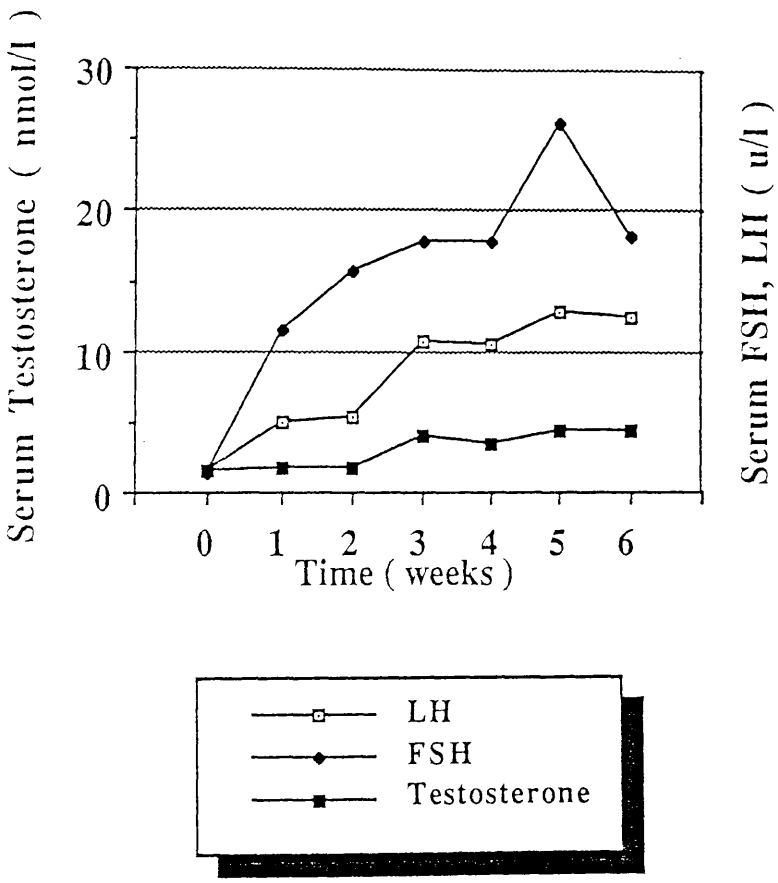


Figure 34
Mean serum LH and FSH, and testosterone responses to prolonged, pulsatile infusion of LH-RH to patient 6. Mean serum gonadotrophin concentrations were estimated from samples taken every 15 mins over 3 hours.

testosterone concentrations. He was found to be azospermic on several semen analyses. He was given a short course of HCG in an attempt to encourage descent of the right testis and was referred for orchidopexy. A right orchidectomy was however performed on an atrophic testis. No histology on this organ was reported. The patient was then commenced on testosterone replacement therapy and lost to follow up until 1986 when a history of lifelong anosmia was obtained. He was reinvestigated prior to starting pulsatile LH-RH treatment which was continued for only 6 weeks.

Serum gonadotrophins rose steadily during the first 5 weeks of treatment before plateauing at significantly elevated concentrations of both LH and FSH. Testosterone rose from 1.6 nmol/l to 4.4 nmol/l during the treatment period and the patient remained azospermic (fig 34). On week 8 the patient developed an abscess at the infusion site requiring admission to his local general hospital. It was then decided to stop treatment because the hormone response suggested poor testicular function. Sustanon injections were restarted and no futher attempts to induce fertility have been made.

9.3.7) The value of stimulation tests in predicting biochemical outcome of pulsatile LH-RH therapy.

All patients underwent standard LH-RH and HCG

stimulation tests prior to pulsatile therapy in an attempt to assess the hypothalamic-pituitary-testicular axis. The results of these investigations are illustrated in table 17. The gonadotrophin responses to LH-RH tests were very variable, a finding which is well recognised in patients with hypogonadotrophic hypogonadism (Bell et al, 1973). The bolus LH-RH tests did not predict the outcome of prolonged pulsatile therapy and this finding is in agreement with the results of other workers. (Morris et al, 1984; Abdulwahid et al, 1985) The bolus LH-RH test correctly predicted the lack of gonadotrophin response in patient 1. However, patient 6 who also had a very flat response to a bolus dose of LH-RH, maintained an exaggerated rise of LH and FSH during treatment. Pulsatile infusion of LH-RH has been shown to increase pituitary gonadotrophin reserve, (Yoshimoto et al, 1975) thus explaining the inability of bolus tests to predict gonadotrophin release after more prolonged exposure.

There is no clearly defined normal response to HCG stimulation tests and many different protocols are used. However, it is usually accepted that a rise of serum testosterone into the normal adult range during the test indicates normal testicular function. Only patient 5 fulfilled this criterion. Despite this, his testosterone rose to a maximum of only 5.5nmol/l during treatment. Santoro et al (1986) have shown that a subthreshold dose of LH-RH (10ng/kg) can result in a rise of gonadotrophins

PATIENT NO	PRIOR TO PULSATILE LH-RH THERAPY							DURING PULSATILE LH-RH THERAPY					
	LH-RH TEST				HCG STIMULATION TEST			MAXIMUM GONADOTROPHIN CONCENTRATIONS		MAXIMUM TESTOSTERONE CONC.	HIGHEST SPERM COUNT	TIME	DURATION OF THERAPY
	BASAL LH (u/l)	PEAK LH (u/l)	BASAL FSH (u/l)	PEAK FSH (u/l)	BASAL T (nmol/l)	PEAK T (nmol/l)	TESTICULAR VOLUME(MLS)	TIME	MEAN LH(u/l)	MEAN FSH(u/l)			
1	1.4	2.2	1.4	1.6	1.0	-	4/4	NO PEAK RESPONSE	2.0	1.3	FALL FROM 7.6 NMOL/L TO UNDETECTABLE	0	AT ALL TIMES	16 WEEKS
2 (25)	1.7	6.1	1.0	2.7	3.7	10.8	12/12	WEEK 6	13.6	4.9	24.0 NMOL/L	6 MILLION /ML	AT 14 WEEKS	34 WEEKS
3 (31)	1.0	11.0	1.2	8.6	0.9	4.8	3/3	WEEK 5	11.6	7.8	7.8 NMOL/L	0	AT ALL TIMES	10 WEEKS
4 (26)	1.0	1.8	1.5	2.3	1.3	2.3	3/3	WEEK 2	13.9	3.7	7.0 NMOL/L AFTER 1 WEEK THEN FALL	0	AT ALL TIMES	9 WEEKS
5 (29)	1.0	9.2	1.0	2.1	1.4	17.8	4/4	WEEK 2	19.6	5.7	5.5 NMOL/L	0	AT ALL TIMES	13 WEEKS
6 (33)	1.4	3.1	1.0	2.0	1.6	3.4	0/1	WEEK 5	12.8	26.2	4.4 NMOL/L	0	AT ALL TIMES	6 WEEKS

TABLE 17

Tests of the pituitary-testicular axis prior to prolonged pulsatile LH-RH therapy and outcome of treatment.

PATIENT NUMBERS IN BRACKETS REFER TO TABLE 9

into the normal adult range without adequate testosterone stimulation. However the dose of LH-RH used in our patients was much greater than 10ng/kg and resulted in supraphysiological serum levels of gonadotrophins. Undertreatment cannot therefore explain the poor testicular resonse to pulsatile therapy in this patient. Patient 2, on the other hand did not quite achieve physiological testosterone concentrations during his HCG test. He did however show a rapid rise of testosterone into the mid-adult range during pulsatile therapy. In this small series of patients, therefore, neither the LH-RH nor the HCG tests were able to adequately predict the biochemical responses to prolonged pulsatile LH-RH exposure.

9.4) DISCUSSION

9.4.1) The outcome of prolonged LH-RH therapy.

Unlike previous workers who have reported ready acceptability of prolonged therapy with LH-RH, (Morris, 1986; Skarin et al, 1982) the patients in this series found the treatment intrusive and in several cases incompatible with a manual occupation. In addition 2 patients developed abscesses at needle sites, in one case requiring hospital admission. One patient developed a hypersensitivity reaction to the gonadotrophin

preparation which was similar to other cases described in small series of patients receiving prolonged treatment and therefore suggesting that this may occur commonly after extended subcutaneous treatment. (Morris et al, 1984; Santoro et al, 1986)

With the exception of patient 1, there was a discernible rise in serum gonadotrophins by the end of the first week of LH-RH treatment. In patients 3, 4, and 5, peak LH levels were achieved by the second week of treatment. The rise of LH in patient 2 was slower with maximal concentrations occurring on week 6. Patient 6 who had one atrophic testis showed a gradual rise in gonadotrophins until week 5 and he showed no subsequent fall thereafter. The decrease in LH after the initial stimulation has been attributed by previous workers to the feedback inhibition of testosterone. (Hoffman and Crowley, 1982) In agreement with this hypothesis would be the results for patient 6 who acheived a testosterone concentration of only 5 nmol/l after 8 weeks of therapy and who demonstrated a continued rise in gonadotrophins which were maintained at supraphysiological levels. It is interesting to note however, that LH stimulation and supression occurred in patient 4 who had falling levels of testosterone during the treatment period and in patient 5 who had only a very modest rise in testosterone during the initial weeks of therapy. This may indicate that there is a short feedback loop at the level of the

pituitary cells; a physiological down regulation in response to pulsatile LH-RH exposure.

The serum testosterone response to LH-RH therapy was also variable with only patients 2 and 3 showing a brisk rise in response to stimulated LH concentrations. This would be in agreement with the results of Morris et al (1984) who were able to demonstrate a testosterone rise in response to increasing gonadotrophin concentrations in only 3 of 10 patients with hypogonadotrophic hypogonadism.

9.4.2) The case of congenital adrenal hypoplasia.

Cytomegalic adrenocortical hypoplasia is a rare X-linked disease associated with hypogonadotrophic hypogonadism (Brook et al, 1973; Sperling, Wolfsen and Fisher, 1973; Kelly et al, 1977; Black et al, 1977; Richards et al, 1978; Hay et al, 1981). A report by Kruse et al (1984) has suggested that a hypothalamic lesion is responsible for the hypogonadal state and that low dose pulsatile LH-RH therapy might be of value in initiating puberty in such patients. The patient in the present study, however, failed to show any increase of serum LH, FSH or testosterone in response to gradually increasing doses of LH-RH given over a 16 week period.

Kruse et al administered LH-RH intravenously at a

dose of 5ug/pulse over a period of 26 hours to 2 patients with congenital adrenal hypoplasia. They inferred from their data that a significant rise of serum LH and FSH occurred in one patient and a significant rise in FSH alone occurred in the second patient. The changes demonstrated however were small. Furthermore, during LH-RH infusion they measured serum gonadotrophin concentrations half an hour after each pulse; a time when peaks would be expected. They therefore compared mainly peak concentrations during infusion with peak and trough concentrations prior to infusion. The data is therefore unconvincing.

A more severe degree of hypogonadism in our patient might, however, explain differences between these studies. Our patient had low basal gonadotrophin concentrations and administration of i.v. LH-RH failed to evoke LH or FSH secretion to within the normal pubertal range. The patients of Kruse et al had pubertal gonadotrophin levels which rose into the normal adult range following 100ug LH-RH given intravenously.

A further difference between the studies was the mode of delivery of LH-RH. Since subcutaneous therapy has been shown to be effective at doses less than 22.4ug/pulse (Valk et al, 1980; Morris et al, 1984; Santoro et al, 1986) we do not believe that this explains the lack of response of our patient.

Prior therapy with testosterone esters in our patient

with congenital adrenal hypoplasia may initially have inhibited the response to low dose pulsatile treatment with LH-RH (Sawin et al, 1978; Lipsett, 1979). However no such inhibitory effect was observed in patients 2 and 4 with hypogonadotrophic hypogonadism.

The lack of LH and FSH response in our patient with congenital adrenal hypoplasia suggests either a diminished pituitary gonadotrophin reserve or decreased ability to secrete FSH and LH in response to LH-RH. Hence it is possible that patients with this condition have a pituitary defect.

CHAPTER 10

EVALUAION OF HUMAN CHORIONIC GONADOTROPHIN (HCG) THERAPY IN BOYS WITH DELAYED PUBERTY

10.1) INTRODUCTION

Following the initial investigations involving pulsatile administration of LH-RH all boys were reassessed at the endocrine clinic 6 weeks later. After full discussion with each boy and his parents a decision was taken either to pursue an expectant policy or to induce puberty with a 6 month course of injections of HCG.

Since Dorff (1940) first described the growth promoting effects of HCG, several authors have described its effect on the development of boys with constitutionally short stature and delayed puberty (Dorff, 1942; Reiss et al, 1965; Van den Bosch et al, 1982). However, little interest has been shown in the factors which might influence response to such treatment, and the use of a control group to help identify and quantitate the acceleration of pubertal development and height velocity has never been reported. Futhermore, the effect of such therapy on the final height outcome of treated patients has never been adequately assessed.

In view of these shortcomings in the literature, it was decided to review retrospectively all patients treated over the previous 4 years with HCG and to study prospectively all patients referred for investigation of pubertal delay.

10.2) PATIENTS AND METHODS

Case-notes for all patients who had attended the Endocrine Clinic for investigation of short stature or delayed puberty between the years 1979 - 1987 were reviewed. All patients who had had careful serial measurements at 3 monthly intervals, of height and sexual maturation by one of two observers (D.G. or H.N.C.) were selected. Attempts were made to recall all selected patients in order to determine present height and bone age.

Fifty males who received HCG therapy either in the previous 4 years or during the study period were reviewed. A further 30 patients who declined treatment acted as a control group.

Bone ages were assessed retrospectively by one person who had no knowledge of the patients' clinical status. The TW2 method of Tanner et al (1975b) was used for bone age estimation and final height estimations were made by the method of Tanner et al (1975).

	Chronological age (yrs)	Height SDS for age	Skeletal age (yrs)	Genital stage G1 G2 G3 unknown
Treated Group	Median 15.2	-3.02	12.0	n=
	Range			
n=50	13.3 - 17.8	-5.35 to +1.5	9.5-14.0	20 22 4 4
Control Group	Median 15.0	-3.42	12.5	n=
n=28	12.4 - 17.5	-5.16 to +0.36	7.0-13.6	9 12 5 2

TABLE 18

Clinical characteristics of the treated and control patient groups

Height SDS = Height standard deviation score. There are no significant differences in age, height SDS, or skeletal maturity between the groups.

10.3) RESULTS

10.3.1) Characteristics of control and treated groups

Table 18 summarises the main characteristics of the two groups of patients. The chronological ages and the height standard deviation scores (SDS) of the two groups were not significantly different ($p>0.05$). There was also no significant difference in skeletal ages between the groups ($p>0.05$). However, skeletal age delay (chronological age - skeletal age) was shorter in the control group reflecting the fact that this group contained more patients who had entered puberty at the start of the study. In order to take account of this difference, results were compared between patients at similar genital development when this was considered appropriate.

10.3.2) Pubertal staging data

Accurate pubertal staging data is available for 45 of 50 patients who received HCG therapy and for 25 of 28 patients in the control group. Figure 35 shows the changes in genital staging during 6 months of HCG therapy in the treated group and during the comparable time interval in the control group. Forty four of the 45

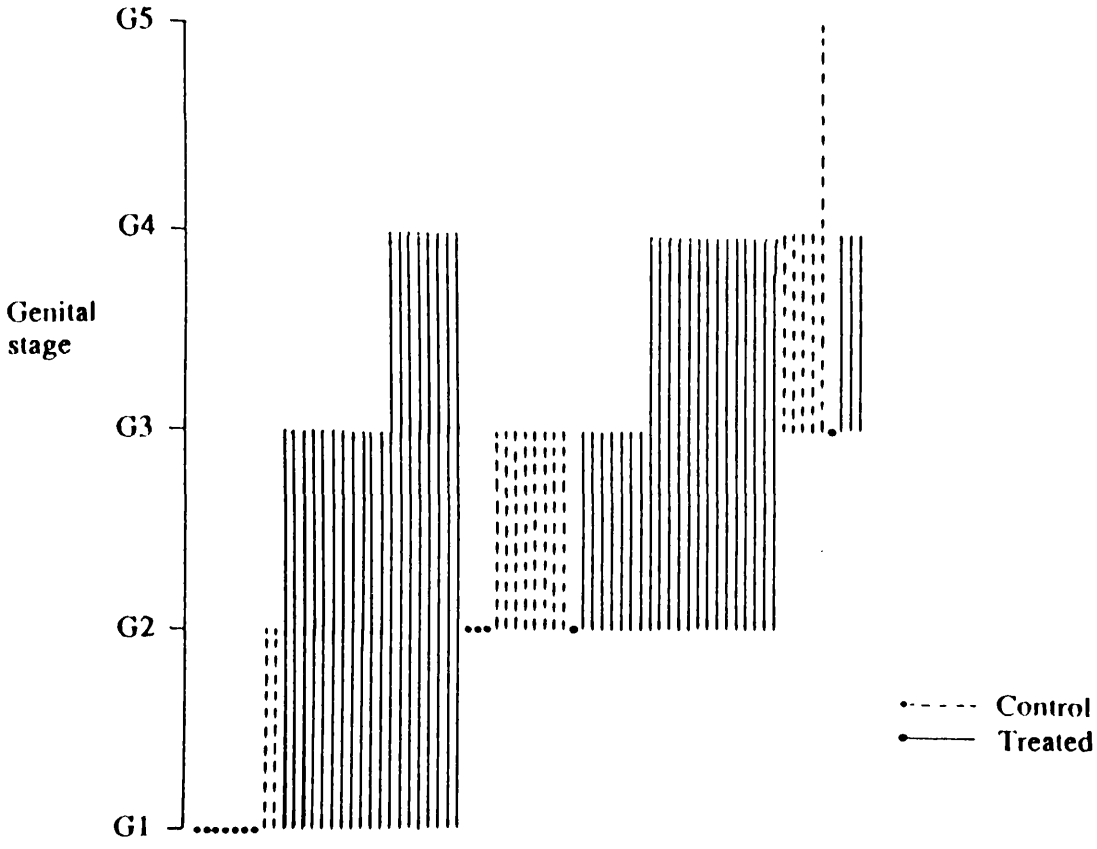


Figure 35
Changes in genital staging of peri-pubertal boys with short stature / delayed puberty during 6 months therapy with HCG and during the comparable time in the control group of untreated boys.

patients in the treated group achieved either genital stage 3 or 4 by the end of the treatment period. The greatest advances in genital developoment therefore occurred in the patients who were pre-pubertal prior to the therapy; most patients in stage G1 advanced 2 or 3 stages. On the other hand, of the 4 HCG treated patients at G3 development, genital staging remained unchanged in 1 and advanced only one stage to G4 in the remainder.

The pattern of pubertal change in the control group during the comparable 6 month period is quite different: Patients either remained unchanged or progressed only 1 genital stage except for one patient who went from G3 to G5 development.

10.3.3) Testicular volumes

Testicular volumes, immediately before and after therapy are known for 43 of the 50 treated patients and in 27 of 28 control patients for the equivalent time period.

Testicular volumes increased in all but 2 patients with HCG therapy from a pre-treatment median volume of 5.0 mls (range 1-12mls) to 9.0mls (range 4-15mls)immediately after HCG. During a comparable period testicular volumes in the control group increased from a median volume of 6.0mls (range 2-10mls) to 9.5mls (range 4-20mls). Two patients in the control group showed no

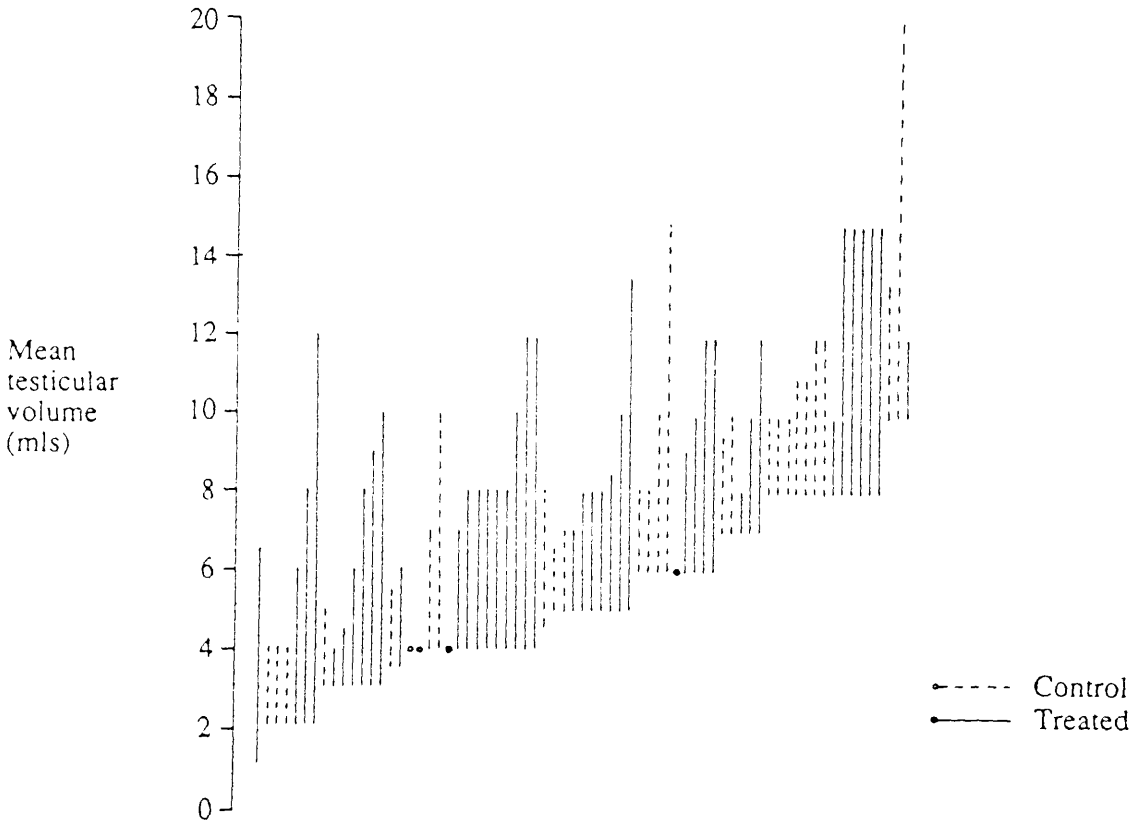


Figure 36
Changes in testicular volume of peri-pubertal boys with short stature / delayed puberty during 6 months therapy with HCG and during the comparable time in the control group of untreated boys.

change in testicular volume during this time.

Figure 36 illustrates the changes in testicular volumes during the study in the two groups. There is considerable variation in testicular growth during the study period and because of the small numbers of patients at each initial testicular volume, it is difficult to demonstrate an effect of HCG on testicular growth with this data.

10.3.4) Height velocity data

Information on height velocities during HCG treatment and in the 3 month periods immediately before and following therapy was available on 40 boys. Similar information on growth velocities over comparable time intervals during the first year of study was obtained for the control group. Relevant growth data were not available for 2 control patients for the second study period and for 3 patients during period 3.

Five patients treated with HCG were growing at rates >7.0cms/yr during the initial 3 month period and may have already been established in a pubertal growth spurt prior to therapy. One of these patients showed decreased growth velocity during the following 6 months, growth in one patient remained unchanged, while 3 patients showed increased growth during HCG therapy. All patients growing initially at < 7.0cm/yr showed marked acceleration in

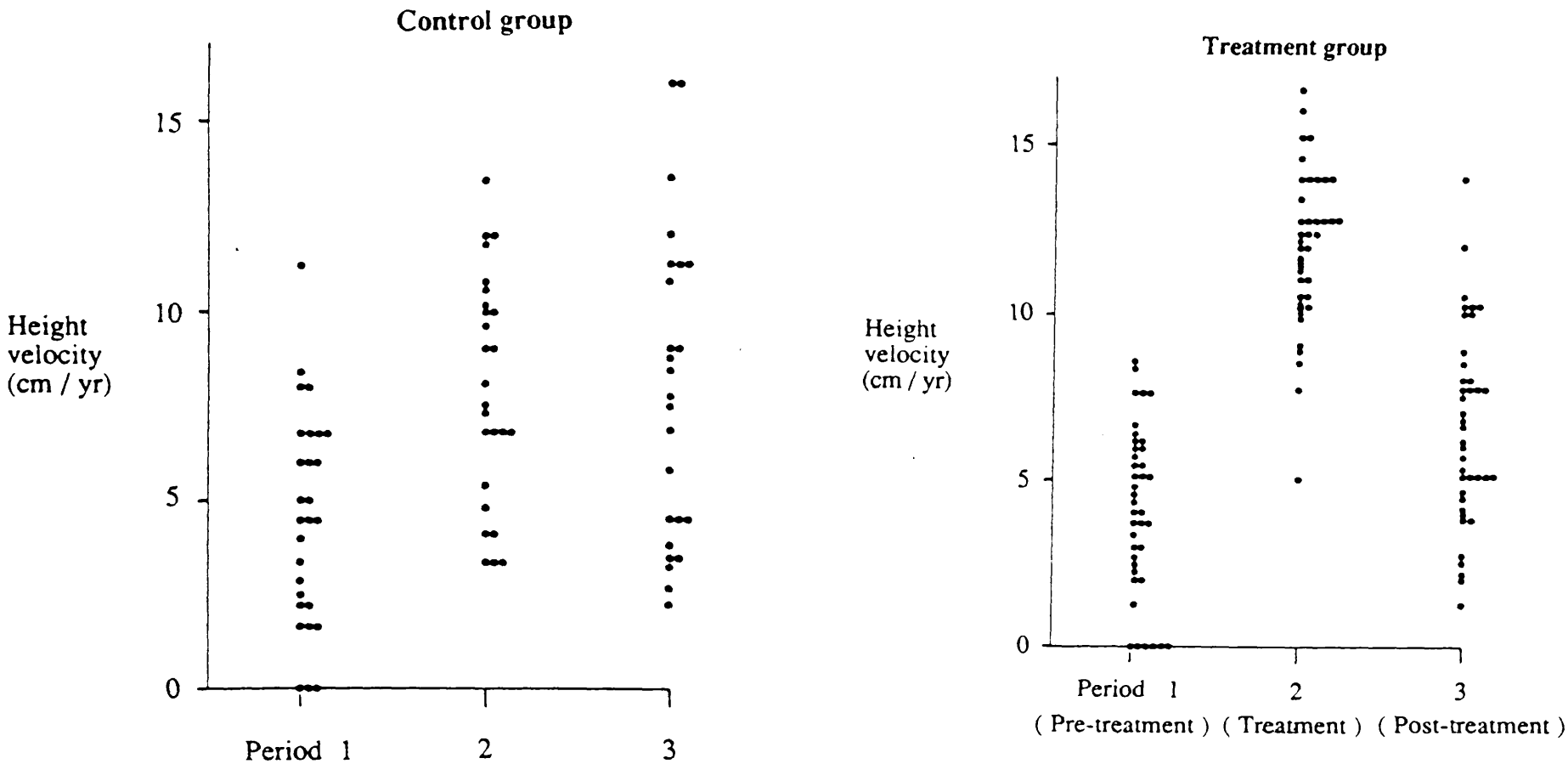


Figure 37
Height velocities in the treated and control groups during the 3 study periods 1) initial 3 months 2) next 6 months 3) final 3 months.

growth (pre-therapy median growth rate 3.9cm/yr [range 0-6.6cm/yr] increasing to a median velocity of 12.7cm/yr [range 8.9-16.8 cm/yr]) (Fig 37), with subsequent deceleration of height velocity in the 3 month period immediately following therapy (median 6.0cm/yr [range 0-12cm/yr]).

Four patients in the untreated group had initial growth velocities > 7.0cm/yr and all showed decreased growth during the subsequent 6 months. Of the remaining 24 patients in this group growing at < 7.0cm/yr, 4 had reduced velocities in the following 6 months, in 2 growth remained essentially unchanged, in 16 height velocity increased and data were not available on 2 (Fig 37).

The annual height velocities during the first year of the study for the two groups of patients on which results are available are shown in table 19. The data is shown separately for each genital stage since, clearly the annual growth velocity in the control group is dependent upon the pubertal development of the patients. The results for the untreated patients illustrate this; the median height velocity for untreated patients who were pre-pubertal at the start of the year was 5.1cm/yr increasing to 7.5cm/yr at G2 development and 8.4 cm/yr for those patients in stage G3 puberty. In the treated group the annual height velocity appears to be independent of pubertal staging with median height velocities for genital stages 1 to 3 of 8.6, 8.3 and

	Annual Height Velocities (cm/yr)		
Genital stage	Treated group	Control group	Significance
G1	Median 8.6 Range 6 - 12.1	Median 5.1 Range 3.6 - 6.4	$p < 0.001$
G2	Median 8.3 Range 4.5 - 12	Median 7.5 Range 4.5 - 10.8	$p > 0.05$
G3	Median 8.3 Range 5.7 - 13	Median 8.4 Range 5.8 - 12	$p > 0.05$

TABLE 19

Annual height velocities in the treated and control groups during the year of study

Each group is sub-divided according to genital stages of the patients at the startof the year. P values relate to comparisons between control and treated groups.

8.3cm/yr respectively. Therefore pre-pubertal patients showed the greatest and most significant response to HCG therapy in terms of increased growth rates.

The annual height velocities for the HCG treated group are considerably lower than the height velocities measured during the treatment periods, reflecting the rapid acceleration and deceleration in growth which occured during and after treatment.

10.3.5) Effect of HCG treatment on skeletal age and final height outcome

Bone age estimations are available on 40 patients prior to and following HCG therapy. In 13 patients the estimations were made at the beginning and end of the year during which treatment was given. In the remainder, the second bone age estimation was made 0.9-7.8years after the initial x-ray and 8 of these patients have achieved bone ages of 18yrs or greater. Table 20 compares the actual final heights achieved by these 8 patients with the predicted heights from the initial bone ages. Only one of these 8 patients who reached adult height during the follow up period, achieved a height greater than that predicted prior to therapy. Figure 38 shows the change in predicted adult height including the information for the patients in whom the final height is known, and compares this data against initial skeletal

Patient	Initial Bone age (yrs)	Height at first bone age (cm)	Estimated final height (cm)	Actual final height (cm)
1	11.0	147	172	166
2	11.5	147	170	170
3	12.5	149	171	166.5
4	11.0	145	171	169
5	13.5	150	165	164.5
6	12.5	147	171	168
7	13.0	165	183	188
8	11.0	141	171	159

TABLE 20

Comparison of actual and predicted final heights in 8 patients treated with HCG

age. For patients with bone ages greater than 11.5 years there is no obvious difference between the predicted adult heights (PAH) and either the post-therapy PAH or final height outcome of the group. However, only one of 11 patients with initial skeletal ages less than 11.5 years showed an improved height expectancy after therapy. The mean reduction in PAH in this group was considerable; -5.1cms. Figure 38 also illustrates the genital staging of the patients in relation to their predicted height outcome and clearly genital stage per se cannot predict the influence of HCG therapy on final height.

10.4) DISCUSSION

HCG is clearly established as a potent stimulator of growth and sexual development in pre-pubertal males (Dorff, 1940; Dorff, 1942; Reiss et al, 1965; Van den Bosch, et al, 1982). However the value of such therapy for influencing both height velocity and genital development in a typical population of boys referred to an endocrine clinic, has never been fully assessed. The present study includes a control group of untreated patients who were self-selected. This control group, however contains a greater number of boys in the later stages of puberty and therefore there is a bias to treat patients in the earlier stages of puberty. In comparing patients in the two groups care has been taken to match

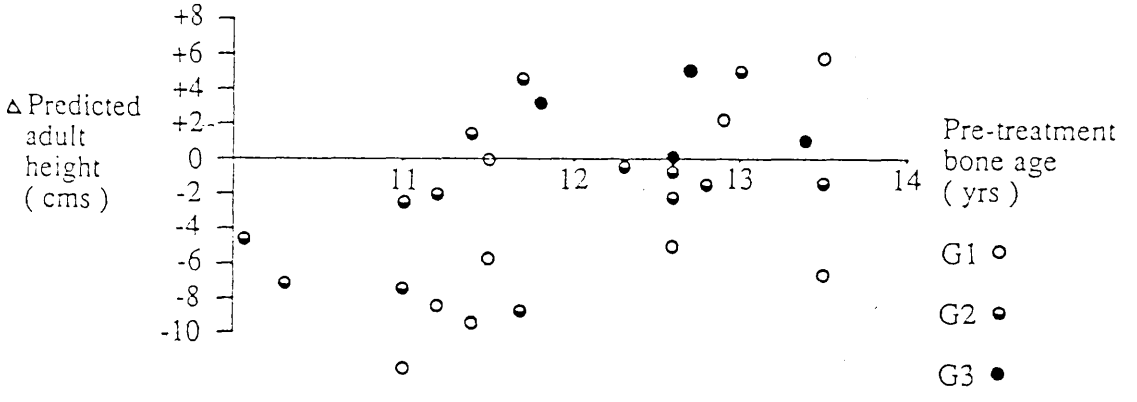


Figure 38
Change in predicted final height following HCG therapy as a function of initial bone age.

patients for genital staging and bone aga or chronological age where relevent and therefore it is hoped that any bias in the study has been eliminated.

We have shown that patients receiving HCG treatment achieve genital stage 3 or 4 by the end of the therapy period with pre-pubertal patients showing the greatest advances. Untreated patients, by contrast, advanced no more than one genital stage during a comparable time period. According to Marshall and Tanner (1970) over a six month period approximately 5% of boys will advance from genital stage 2 (G2) to G3, and approximately 20% will change from G3 to G4. In the present study, even in the control group, a greater than expected number of patients advanced from one genital stage to the next. This would suggest that many of the boys were about to enter the next stage of development at the time of presentation. For the treatment group, even if all the patients who were pre-pubertal at the start of the treatment period were about to enter genital stage 2 imminently, only 5% would be expected to achieve stage 3 puberty in 6 months and none would be expected to advance to G4.

The information on testicular volumes is more difficult to interpret due to the wide range of volumes in the patients studied and the rather poor overlap between the treated and control populations.

The rapid acceleration in growth velocity during HCG

therapy is demonstrated by this work which confirms the early data of Dorff (1940, 1942) and Reiss et al (1965). Most patients grew during the treatment periods at rates which are greater than a normal pubertal growth spurt (Tanner et al, 1966). However when the growth velocities for the whole year of study are computed the difference in height gain between the two groups of patients is much less obvious and the mean growth velocity for the HCG group of 8.7cm/yr is no longer greater than the normal pubertal growth spurt. This is mainly due to the rapid deceleration in growth which occurs on cessation of treatment.

Marshall (1971) has pointed out the difficulties in measuring growth velocities over periods of less than 1 year when errors can arise because of inaccuracies in height measurement and because of variation in growth rates in normal children during the year. These errors, however would not explain the very obvious differences in growth patterns in the two groups of patients.

CONCLUSIONS AND INDICATIONS FOR FURTHER RESEARCH

This work has demonstrated the effects of exogenous pulsatile LH-RH administration upon the endogenous secretion of gonadotrophins throughout 24 hour periods in pubertal boys. Exogenous administration of LH-RH stimulates gonadotrophin secretion above pre-infusion concentrations only at times of the day when endogenous secretion is at a minimum. During the night when natural LH-RH surges occur, the effect of exogenous LH-RH is variable. If endogenous and exogenous LH-RH pulses coincide then further stimulation of the gonadotroph may be expected. However, if endogenous and exogenous pulses are asyncronous then the gonadotroph is exposed to pulses at an increased frequency and this may result in down-regulation of the gonadotroph.

Despite the alteration in gonadotrophin diurnal rhythms caused by pulsatile LH-RH infusions, serum testosterone maintained stimulated levels throughout the 24 hour periods during LH-RH administration. This would suggest that the length of time of exposure to circulating LH as well as the LH concentrations is important for stimulation of testosterone secretion by the Leydig cells.

It would therefore be of interest to investigate the effects of pulsatile LH-RH administration, given over shorter periods during the afternoon and early evening,

upon serum testosterone levels throughout the 24 hour period. It may well be that LH-RH infusions in the afternoon would be sufficient to stimulate testosterone secretion throughout the day and night.

It would also be of interest to investigate the bio-activity of LH pulses during the day and night prior to and during pulsatile LH-RH infusions.

This work failed to show a relationship between LH-RH pulse dosage and gonadotrophin response after 6 days of therapy. The gonadotrophin response to LH-RH infusion may be dependent upon the length of time of LH-RH administration and this requires further investigation.

It has been shown in the present thesis that mean gonadotrophin concentrations following pulsatile LH-RH infusions to hypogonadal males is dependent upon the pre-infusion gonadotrophin concentrations. Patients with permanent hypogonadotrophic hypogonadism showed quantitatively greater gonadotrophin increments when compared with patients with constitutional delay of puberty or short stature. The increased gonadotrophin release in patients with hypothalamic hypogonadism may be due to a lack of interference between endogenous and exogenous LH-RH pulses in these patients. A further possible explanation could be reduced or delayed testicular response to gonadotrophins in the hypogonadotrophic patients. However, no differences in testosterone response to pulsatile LH-RH infusion was

demonstrated between the groups studied. One agonadal patient who was studied, also showed increased gonadotrophin secretion in response to pulsatile LH-RH infusion when compared to delayed puberty or short stature patients. However, in this patient, FSH rose predominantly and the FSH : LH ratio therefore simulated the picture which is often found in adult males with primary testicular failure.

The testosterone response to pulsatile LH-RH infusions was dependent on basal testosterone and the patient's bone age. Most patients with undetectable basal testosterone concentrations or bone ages less than 12 years failed to show significant rises in serum testosterone during the infusion periods. Measurement of serum testosterone concentrations through the night might have shown testosterone responses in some of these patients and further nocturnal profiles in patients in early puberty undergoing pulsatile therapy would be valuable.

The graded testosterone response to LH-RH infusions again demonstrates that the degree of response of the pituitary-gonadal axis is dependent upon prior priming of the axis at all levels.

Standard bolus LH-RH tests (100ug i.v.) failed to differentiate the groups of hypogonadal males both before and following pulsatile LH-RH administration. LH increments to bolus LH-RH tests were unaltered by

pulsatile LH-RH infusions in the 3 patient groups studied. Peak FSH concentrations in bolus LH-RH tests were reduced following pulsatile LH-RH infusions in the delayed puberty and short stature groups. In contrast, peak FSH concentrations in bolus LH-RH tests were increased in hypogonadotrophic hypogonadal patients following pulsatile LH-RH infusions. However the value of this finding is diminished by the overlap in FSH values between the groups. The mean basal gonadotrophin concentrations were better able to discriminate between the groups than stimulated gonadotrophin levels after bolus injections. Whether the diminished FSH responses to bolus LH-RH after pulsatile therapy represents maturation of the hypothalamic-pituitary axis remains conjecture.

The present work has confirmed that the prolactin response to intravenous TRH (200ug) was unable to differentiate between patients with pubertal delay or permanent hypogonadotrophic hypogonadism. The failure of pulsatile LH-RH administration to alter the prolactin response to TRH in either group of patient studied, suggests that there is no significant relationship between exogenous LH-RH and the mechanism by which TRH stimulates prolactin release. This work, however does not exclude the possibility of an association between endogenous LH-RH release and prolactin secretion.

Prolonged pulsatile infusions of LH-RH for the induction of fertility in patients with hypogonadotrophic

hypogonadism produced disappointing results. While the biochemical changes of puberty could be induced in some patients, improvement in sperm count was modest or absent and no patient became fertile during this therapy. The treatment proved intrusive and interfered with the ability of several of the patients to continue their normal work. Impaired testicular function or acquired sensitivity were further problems encountered.

LH-RH and HCG stimulation tests prior to pulsatile treatment were unreliable predictors of response. Further studies using LH-RH infusions to induce fertility are required. Clinical details such as a history of cryptorchidism, patient's age and prior therapy to induce puberty are necessary before the efficacy of this treatment can be fully assessed. A comparison with the conventional treatment (HCG and HMG) is also required.

Prolonged pulsatile LH-RH infusion failed to stimulate gonadotrophin secretion in one patient with congenital adrenal hypoplasia. This result would suggest that the hypogonadism in this rare congenital disorder is of pituitary origin.

Human chorionic gonadotrophin has been shown to accelerate pubertal development in boys at genital stages 1 and 2 when compared with untreated patients of similar sexual maturation. Height velocity is increased during treatment, particularly in patients growing initially at less than 7 cm/yr. Clearly, therefore, boys who are

already well established into puberty show little benefit from treatment. Furthermore children with bone ages of less than 11.5 years at the start of therapy appear to be at risk of diminished final height outcome. There appears therfore, to be a fairly narrow clinical spectrum of patients who show benefit from this treatment without prejudicing their ultimate height. These results with HCG treatment are similar to many of the findings previously shown to occur with androgen therapy for delayed puberty in males.

Perhaps monitoring of serum testosterone concentrations during HCG treatment would help to define an optimum dosage regimen. It would also be of interest to compare response to HCG with the use of less frequent injections of small doses of testosterone itself.

Little is known about the natural maturation of the hypothalamic-pituitary-testicular axis during HCG therapy. With the development of assays for plasma LH-RH and highly specific assays for LH and FSH it may be possible to investigate the effect of exogenous androgenic steroids and HCG upon this axis.

The assessment, management and treatment of delayed sexual development in the male remains a difficult area of paediatric and adolescent endocrinology. Further studies are required in order to establish a simple and rapid method for investigating these patients and for identifying, when considered appropriate, the most

suitable treatment regimen.

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