Current concepts on ultradian rhythms of luteinizing hormone secretion in the human

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A cardinal physiological feature of anterior pituitary hormone secretion is its pulsatile mode of signalling to remote target tissues. The pulsatile release of anterior pituitary hormones is orchestrated by episodic neuronal activation of hypothalamic control centres, which release relevant effector molecules intermittently. The anterior pituitary gland in turn secretes hormones in ultradian bursts, and thereby communicates with and governs the function of peripheral target organs. In the case of the reproductive axis, the release of gonadotrophin-releasing hormone (GnRH) from the hypothalamus in intermittent secretory bursts is a primary neural determinant of pulsatile gonadotrophin [luteinizing hormone (LH) and follicle stimulating hormone (FSH)] secretion. In men, women and pubertal children, the pulsatile mode of GnRH release is critical for sustained physiological function of gonadotroph cells and is an absolute prerequisite for reproductive capability. Furthermore, various clinical pathophysiological states, such as inadequate nutrient intake, stress and uraemia, may dramatically impair the pulsatile release of gonadotrophic hormones. Here, we review some recent studies in reproductive (neuro)endocrinology that illustrate physiological regulation and pathophysiological disruption of pulsatile LH signalling in the human.

Key words: FSH/gonadotrophin/LH/men/pulsatile/women

Pulsatile secretory pattern of gonadotrophin-releasing hormone and luteinizing hormone

Gonadotrophin-releasing hormone (GnRH) is released in a pulsatile manner from hypothalamic neurovascular terminals into the portal circulation (e.g. Levine et al., 1982). The decapetide is transported to its site of action in the anterior lobe of the pituitary gland via the hypothalamic–hypophyseal portal microcirculatory system. The pulsatile release of GnRH is presumably the consequence of a synchronized discharge of scattered GnRH-containing neurons driven by an inferred neural signal-generator or oscillator (Veldhuis, 1990), which is typically referred to as the GnRH pulse generator (Knobil, 1989). Direct monitoring of hypothalamic–pituitary portal blood in the rat, sheep and monkey has demonstrated that GnRH-containing neurons driven by an inferred neural signal-generator or oscillator (Veldhuis, 1990), which is typically referred to as the GnRH pulse generator (Knobil, 1989). Direct monitoring of hypothalamic–pituitary portal blood in the rat, sheep and monkey has demonstrated that secretory bursts of GnRH are followed virtually uniformly by a slightly time-delayed pulse of luteinizing hormone (LH) secretion (Clarke and Cummins, 1982; Levine and Ramirez, 1982). Although there is some evidence that human pituitary tissue produces irregular low-amplitude LH pulses in vitro even without an exogenous GnRH stimulus (Gambacciani et al., 1987), GnRH seems to govern most organized high-amplitude LH pulses. Indeed, en-
dogenous (and small amounts of exogenous) GnRH tends to promote the release of highly bioactive LH as assessed in in-vitro bioassays (Veldhuis et al., 1987c). Occasional disruption of the one-to-one correspondence between GnRH and LH secretory bursts has been inferred recently in the uremic rat, based on hypothalamic–pituitary portal blood sampling (Schaefer et al., 1994). Although such invasive experiments have not been carried out in the human, a clinical thesis in human reproductive neuroendocrinology is that episodes of pulsatile LH secretion from the anterior pituitary gland reflect (indirectly) GnRH pulse generator activity in the hypothalamus, as conditioned by the responsiveness of the gonadotroph cells and both intra-pituitary and extra-pituitary modulators. Of course, many autocrine, paracrine and endocrine factors modulate the biosynthesis, biological activity and molecular isoforms of pituitary LH and follicle stimulating hormone (FSH) (for review, see Dufau and Veldhuis, 1987).

Implications of pulsatile LH secretion

GnRH release from the hypothalamus into the portal circulation is episodic rather than continuous, which in turn causes LH to be released in a series of secretory bursts, resulting in intermittently elevated LH concentrations in the blood [for review, see Veldhuis, 1995, and Human Reproduction (1993), vol. 8 supplement, entitled Central Control of Gonadal Function]. The amplitude of such serum LH concentration peaks in healthy men ranges from 35 to 270% (increase from nadir to peak expressed as a percentage). Although LH pulses do not exhibit strictly regular periodicity, they typically occur with a mean frequency in men and follicular-phase women of approximately one event per hour or one every 90–120 min (Urban et al., 1988a). Hence, this pattern of LH secretion has been termed ultradian, indicating that more than one secretory episode occurs in 24 h, or circhoral (approximately one event per hour) (for review, see Turek and Van Cauter, 1994). This feature of LH neuroregulation is shown in Figure 1.

A major practical implication of this pulsatile mode of LH secretion in clinical practice is that no single blood sample can be used with high reliability to evaluate gonadotrophin pathophysiology, or to diagnose and manage patients with reproductive disorders. For example, if a single blood sample is taken to measure LH, the 95% confidence limits for that sample as an estimate of the mean serum LH concentration span 50–150% of the measured value. If samples are collected at more frequent intervals (e.g. every 20 min for 6 h) and pooled, the mean serum LH concentration estimate is significantly more accurate, with a coefficient of variation of ~10–45% (Santen and Bardin, 1973; Urban et al., 1988a). Hence, either repeated measures of LH on consecutive mornings in the same individual, or repeated blood sampling on the same morning, are recommended to develop a reliable estimate of the mean serum LH concentration in any one patient. In some laboratories, timed urinary LH measurements (e.g. per 3 h or 24 h) are useful. An exception to the need for repeated sampling is the castrate or postmenopausal setting of primary gonadal failure, when marked and sustained gonadotrophin elevations typically occur. However, a blood sample withdrawn during an LH surge may also show 3- to 10-fold elevations in LH and 2- to 5-fold increases in FSH concentration. In addition, because of the extended half-life of urinary-derived FSH, which exceeds 8 h (Urban et al., 1991), a single FSH measurement often provides useful information. The deconvolution-calculated monoexponential half-life reflects the rate of apparent metabolic removal of the endogenous hormone (Veldhuis and Johnson, 1992). Of interest, after i.v. administration, the terminal half-life of exogenous human FSH has been shown to approximate to 24 h in healthy young men and women down-regulated by GnRH agonist (see le Cotonnec et al., 1994). This difference may reflect the different patient populations studied, and/or different durations of in-vivo monitoring, with the latter longer half-lives more closely approximating equilibrium kinetics. On the other hand, Diczfalusy and Harlin (1988), after i.v. injection, also reported an FSH half-life of 7.3 (range 5.5–11) h.

Regulation of pulsatile LH secretion by gonadal steroids

Regulation of the pulsatile LH signal in women in the follicular and luteal phases reflects, as well as directs,
steroid hormone production by the ovary during an ovarian cycle, i.e. circacycle ovarian control. In the late follicular phase, there is a significant (although imperfect) temporal association between LH pulses and oestradiol peaks in the blood (Backstrom et al., 1982; Djahanbakhch et al., 1984; Genazzani et al., 1991). In addition, coordinated release of LH and progesterone or oestradiol has been reported in the mid-luteal phase of the menstrual cycle, with increases and decreases in endogenous sex-steroid concentrations occurring 0–30 min after those of LH release (Backstrom et al., 1982; Filicori et al., 1984; Vel dhuis et al., 1988; Rossmanith et al., 1990). Furthermore, using a new coincidence statistic (Veldhuis et al., 1991), we have found that individual LH secretory bursts are strongly non-randomly associated with individual episodes of progesterone and oestradiol release, even though there is considerably less than 100% concordance between LH and progesterone or oestradiol pulses (Rossmanith et al., 1990; Figure 2). Indeed, substantial autonomous (interpulse or basal) progesterone secretion probably also occurs between consecutive LH secretory events (Hutchinson et al., 1986; Veldhuis et al., 1988, 1991). Although LH and FSH clearly support long-term luteal steroidogenesis and short-term increases in progesterone or oestradiol secretion, various autocrine, paracrine and/or (non-gonadotrophin) endocrine factors also influence progesterone secretion by luteal tissue.

In young men, circulating concentrations of LH and testosterone measured in blood samples collected at 10-min intervals serially over 24 or 36 h show highly significant temporal correlations (Boyar et al., 1973; Veldhuis et al., 1987d). Hence, when serum LH concentrations in the healthy male increase, serum testosterone concentrations rise in parallel after 0–45 min (typically beginning as soon as 20 min; Veldhuis et al., 1987d). In addition, decreases in LH concentrations are strongly temporally connected to later decreases in serum testosterone concentrations (Veldhuis et al., 1987d). Although this correlation is highly significant and non-random, it only explains a portion (~25–60%) of the total 24-h variability in serum testosterone concentrations. Interestingly, this synchrony is lost during ageing in healthy men (see below). However, when catheters are placed into the spermatic vein of young men with varicoceles, a nearly 1:1 correspondence can be seen among testosterone, oestradiol, and α-inhibin pulses, all of which coincide with secretory bursts of LH measured in peripheral blood (Winters and Troen, 1986; Winters, 1990; Figure 3). Strong temporal concordance among secretory pulses of alpha gonadotrophin subunit, testosterone, and both immunoreactive and bioactive LH can also be demonstrated in peripheral blood in healthy young men (Pavlou et al., 1990). These pulsatile events all are GnRH dependent, since their amplitude is markedly attenuated if not abolished following treatment with a GnRH antagonist (Pavlou et al., 1990). LH and FSH release episodes in men are also highly non-randomly associated (Veldhuis et al., 1991), but quite imperfectly, thus strongly suggesting some independent regulation of FSH release.

In summary, both the female and male gonadal axes seem to maintain strong (albeit incomplete) temporal synchrony between the hypothalamus, anterior pituitary gland and gonads, and this synchrony is driven predominantly by the GnRH neuronal ensemble.

**Ontogeny of pulsatile LH secretion**

Episodic LH secretion in response to GnRH release has been shown *in utero* in the sheep and monkey (Huhtaniemi et al., 1979). Throughout fetal life, differences in the time course of LH (and FSH) have been demonstrated in male
versus female animals (Veldhuis, 1991). To our knowledge, there are no studies in the human fetus showing antenatal pulsatile LH release, although human fetal hypothalamic explants in vitro release GnRH in a pulsatile manner (Rasmussen et al., 1986a,b). In the normal human male infant, but not in the female, pulsatile LH secretion is readily demonstrable during the first week of postnatal life (Waldhauser et al., 1981; Veldhuis, 1991) and even on the first day of life (de Zegher et al., 1992; see Figure 4). The apparent half-life of endogenous LH evaluated by the new technique of deconvolution analysis (Veldhuis et al., 1987a; Veldhuis and Johnson, 1992) in the male neonate (de Zegher et al., 1992) approximates the value estimated in healthy children and young and older adults (Urban et al., 1988a, 1989; Veldhuis and Johnson, 1988; Veldhuis et al., 1989, 1993a; Sollenberger et al., 1990b; Evans et al., 1992). The pulse frequency of immunoreactive LH release in male infants is approximately one pulse every 60–90 min, which is a circulatory frequency similar to that in normal men (Urban et al., 1988a; Reyes-Fuentes and Veldhuis, 1993; Veldhuis, 1995). In addition, at 6–12 weeks of age infant boys show notably increased pulsatile LH secretion with pulse amplitudes similar to those observed in healthy adults (Waldhauser et al., 1981). This so-called postnatal surge of LH secretion is accompanied by increased production of testosterone, which documents bioactivity of the released LH and responsiveness of the neonatal Leydig cell to LH. Of note, the female infant does not demonstrate this immediate postnatal surge-like increase in gonadotrophin-driven gonadal steroid secretion (Veldhuis, 1991). However, after 3–6 months of age, infant boys normally show a decrease in this remarkable activity of the hypothalamic–pituitary–gonadal axis. Thereafter, the normal male reproductive axis remains relatively quiescent for at least a decade until the onset of pubertal activation. The importance of the foregoing brief neonatal activation of the male reproductive axis to the further development of the male (human) infant is not known.

**Pubertal transition and puberty**

The activity of the hypothalamic–pituitary component of the human reproductive axis has been thought to be rela-
Ultradian LH rhythms

Figure 5.

Figure 6.

Deconvolution analysis of luteinizing hormone (LH) secretory burst mass across puberty in healthy boys shows 30- to 35-fold amplification of the amount (mass) of LH secreted per burst. In contrast, LH burst frequency rises by ~1.5 fold. Adapted with permission from Wu et al. (1996). IFA = immunofluorometric assay.

tively subdued during the interval beginning 6 months postnatally through prepuberty. However, recent studies using ultrasensitive immunofluorometric assays have shown that circulating gonadotrophin concentrations are detectable in prepubertal children, and increase 30- to 100-fold at the time of pubertal onset (Dunkel et al., 1990; Wu et al., 1991, 1996). Using such assays, a recent study of boys at various stages of puberty demonstrated by deconvolution analysis that pulsatile LH secretion is evident in prepuberty and exhibits no change in calculated half-life, but there is a 30-fold augmentation of secretory burst mass with only a 1.5-fold acceleration of pulse frequency with puberty (Wu et al., 1996; Figure 5). There is evidence that serum concentrations of bioactive LH measured with a Leydig cell bioassay also increase at the onset of puberty (Lucky et al., 1980; Reiter et al., 1987; Huhtaniemi et al., 1996). Additional studies using even more sensitive gonadotrophin assays and more frequently collected blood samples (perhaps every 5–10 min for 8–24 h) will be needed in the future to show clearly whether there is an accompanying increase in low-amplitude pulsatile and/or basal LH secretion. In addition, comparable data are needed in girls.

Extensive experimental studies in rodents and primates have strongly supported the current concept that the GnRH pulse generator prior to puberty is either actively inhibited and/or deficient in relevant activation. According to this hypothesis, the prepubertal reproductive axis is capable of responding in an adult fashion when GnRH is made available. For instance, administration of GnRH or its analogues in a pulsatile manner to prepubertal animals or developmentally prepubertal humans induces complete pubertal maturation and initiates normal functioning of the reproductive axis (Nillius et al., 1975; Marshall and Kelch, 1979; Wildt et al., 1980; Hoffman and Crowley, 1982; Bronson, 1986). GnRH neurons are potentially fully functional prior to puberty since, in the prepubertal monkey, administration of N-methyl-D-aspartate, an excitatory amino acid, stimulates endogenous GnRH release, pulsatile LH secretion and gonadal sex-steroid production (Gay and Plant, 1987, 1988; Plant et al., 1989).

However, little is known about specific activating and inhibiting neurotransmitter systems that trigger pubertal activation (or disinhibition) of the GnRH neuronal population in the human. Studies in experimental animals have suggested that endogenous opioid peptides may play an important role in the regulation of hypothalamic GnRH release at the onset of puberty, but available human studies do not support this hypothesis (Veldhuis et al., 1982; Figure 6). Rather, the inhibitory opiatergic system is only evident in suppressing pulsatile GnRH (and hence LH) release in the latest stage of puberty when gonadal steroid concentrations already approach adult values. The possible roles of dopamine, excitatory amino acids, γ-aminobutyric acid and adrenergic, serotonergic and peptidyl (e.g. neuropeptide Y, galanin) neurotransmitter systems in pubertal awakening in the human are not known (Veldhuis, 1996).

Menstrual cycle

As reviewed elsewhere in detail [see Human Reproduction (1993), vol. 8 supplement, entitled Central Control of Gonadal Function], recent studies utilizing relatively intensive blood sampling (every 10 min) and deconvolution analysis to estimate LH secretion and its half-life from 24-h serum gonadotrophin profiles have
demonstrated that throughout the normal menstrual cycle there is strict stage-of-the-cycle dependent, i.e. circacycle, regulation of the amplitude, duration, mass and frequency of immunoreactive LH secretory bursts (Sollenberger et al., 1990b; Evans et al., 1992; see Figure 7). There is a significant increase in LH secretory pulse frequency during the later follicular phase and preovulatory interval, and a decrease in the number of high-amplitude secretory events in the mid-luteal phase of the normal human menstrual cycle. In addition, the calculated duration of the LH secretory event in the late follicular phase is shorter, suggesting that the amplitude and duration, and hence the mass, of individual LH secretory bursts are under physiological control. During the mid-luteal phase of the menstrual cycle, high-amplitude events of prolonged duration and low frequency are evident. Since the calculated daily LH secretion rate and the apparent half-life of LH are both relatively constant throughout the normal menstrual cycle, this stage-dependent regulation of pulsatile LH secretion is highly specific (Sollenberger et al., 1990b).

Circacycle regulation of LH release presumably involves alterations in both endogenous GnRH release and responsiveness of the gonadotroph population. Thus, i.v. administration of two consecutive and equal doses of GnRH at three different stages of the normal menstrual cycle unmasks a ‘self-priming’ action of successive GnRH stimuli and significant differences in the amplitude, duration and mass of the evoked LH secretory bursts (but not the half-life of LH) in a stage-specific manner (Sollenberger et al., 1990a). Accordingly, pulsatile LH release is highly regulated throughout the normal menstrual cycle, via mechanisms that modulate both the activity of the hypothalamic GnRH pulse generator and the responsiveness of the anterior pituitary gland to GnRH secretory pulses. For instance, pulse frequencies of 90 or 120 min of s.c. GnRH appear to induce more reliably the sequence of follicular development (Letterie et al., 1996). Modulation of responsiveness to GnRH is presumably achieved in large part via oestrogen feedback actions, which are biphasic over time: initially inhibitory (in the early follicular phase) and later (preovulatory interval) facilitative (Veldhuis et al., 1987b). Moreover, amplification by oestrogen of GnRH action (so-called self-priming effect; Veldhuis et al., 1986) is achieved via a specific mechanism of prolonging the GnRH-stimulated LH secretory burst duration and hence increasing the mass of LH secreted per burst (Quyyumi et al., 1993; Figure 8). Furthermore, there are also some interesting recent data concerning the ovarian protein gonadotrophin surge-inhibiting factor or surge-attenuating factor (GnSIF/AF) that suggest that GnSIF/AF eliminates the effect of self-priming by neutralizing the biological activity of so-far unidentified pituitary proteins. The latter are presumably responsible for the increased rate of LH release initiated during surge onset (de Koning, 1995; van Dieten and de Koning, 1995). However, the role of GnSIF/AF in the regulation of the self-priming effect of GnRH needs further studies in the human.

Other studies, as reviewed in Dufau and Veldhuis (1987), indicate that biologically active LH is contained within the anterior pituitary gland and secreted into the blood in a GnRH-dependent manner as a spectrum of biochemical isoforms. In general, more basic isoforms are produced in an oestrogen-rich environment, have greater bioactivity in vitro and have shorter half-lives in vivo. Similarly, recent studies of GnRH-stimulated FSH

Figure 7. Illustrative profiles of serum luteinizing hormone (LH) concentrations and deconvolution-evaluated LH secretory bursts in the early follicular, late follicular, and mid-luteal phases of the normal menstrual cycle. The upper panel of each pair shows the fitted serum LH concentration profiles over 24 h, and the lower panel shows the calculated LH secretory events. Reproduced with permission from Sollenberger et al. (1990b).
isoforms released throughout the menstrual cycle show marked circacycle modulation of FSH bioactivity (Zambrano et al., 1995). In contrast, ovariprival states are accompanied by the release of LH with more acidic composition (more sialic acid additions) and a more prolonged in-vivo half-life, with consequently greater overall bioactivity in vivo and a higher bio:immuno LH ratio in plasma (Dufau et al., 1983; Veldhuis et al., 1984). Similarly, sex steroids also appear to modulate LH and FSH bioactivity in men (Veldhuis and Dufau, 1987; Urban et al., 1992; Veldhuis et al., 1992a).

Ageing and pulsatile secretion of LH

Healthy ageing in men results in changes in the LH pulse signal. The reserve capacity of gonadotroph cells to increase secretion of biologically active LH in older men is decreased. This impaired secretory activity is disclosed by the administration either of a small dose of GnRH late in the day or of an anti-oestrogen, e.g. tamoxifen (Urban et al., 1988b; Reyes-Fuentes and Veldhuis, 1993). A non-steroidal androgen-receptor antagonist can increase the secretion of bioactive LH in young men (Veldhuis et al., 1994) and, interestingly, also in older men. The latter indicates preserved GnRH/LH secretory responsiveness in older individuals when androgen (in contrast to oestrogen) negative feedback is partially blocked (Veldhuis et al., 1994).

Using deconvolution analysis to calculate underlying gonadotrophin secretion and half-life, we have shown that LH secretory burst amplitude and mass decrease progressively with increasing age as well as with obesity. This relative hypogonadotrophism is accompanied by a decrease in serum total and free testosterone concentrations (Veldhuis et al., 1992a). In addition, as serum concentrations of testosterone decline progressively with increasing age, there is a rise in low-amplitude LH secretory burst frequency. This observation has been confirmed recently by overnight sampling every 2.5 min in older (versus young) men, which also unveiled significant and specific blunting of the pulsatile (but not basal) component of testosterone secretion in older men (Mulligan et al., 1995). There is no significant change in the calculated half-life of endogenous LH with ageing, but there are increased amounts of putatively basal LH release as well as possibly an unexpected prolongation of the duration of the computed LH secretion burst (Veldhuis et al., 1992b). Regulation of burst duration also occurs in end-stage renal failure in men (Veldhuis et al., 1993b) and in oestrogen-treated postmenopausal women (Quyyumi et al., 1993). Uraemic men show a decreased mass of LH secreted per burst, which is brought about by a combined decrease in secretory burst amplitude and duration (Veldhuis et al., 1993b). Conversely, in postmenopausal women, oestrogen treatment enhances GnRH action by doubling LH secretory burst duration (Quyyumi et al., 1993). The intrapituitary basis for these inferred changes in LH secretory burst duration is not known.

A recently reported novel finding in healthy older men is increased disorderliness or irregularity of the LH release process, as quantified by an approximate entropy statistic (Pincus et al., 1996). Moreover, this study disclosed that the synchrony between LH and testosterone release is clearly disrupted in older men, even when mean LH and androgen concentrations remain normal. Thus, loss of within-axis coordination may be a hallmark of an ageing male reproductive axis (Pincus et al., 1996).

Nutrition

Diets that are nutritionally inadequate delay and disrupt the pubertal development of the reproductive processes of immature experimental animals and humans, and impair the function of the hypothalamic–pituitary–gonadal axis in adults (for review, see Bergendahl and Veldhuis, 1995). Malnutrition results in decreased serum gonadotrophin
concentrations. Available data suggest that reduced hypothalamic GnRH release is the most important aetiological factor in the fasting-induced suppression of the reproductive axis. Most studies on the effects of malnutrition on gonadotrophin secretion have been performed in women, perhaps because malnutrition in women is promptly accompanied by amenorrhoea, whereas in men hypogonadism develops gradually and becomes clinically evident only following more severe malnutrition.

Reversible hypogonadotrophic hypogonadism in long-term nutrient-restricted women is well known (e.g. Warren, 1983; Beitins et al., 1991). However, there are only a few studies about the effects of short-term fasting on the pulsatile activity of the reproductive axis. In cross-sectional studies, women with exercise- and weight loss-associated amenorrhoea exhibit reduced LH pulse frequencies (Khoury et al., 1987; Loucks et al., 1989; and see below). Other clinical studies have shown that fasting suppresses LH pulsatility in men (see below), but this relationship between dietary intake and LH pulsatility is less clearly defined in women (Beitins et al., 1985; Pirke et al., 1985; Berga et al., 1993). Some earlier studies ignored the phase of the menstrual cycle (Pirke et al., 1985), or used brief and insensitive blood sampling protocols (Beitins et al., 1985). In one negative study, LH pulsatility was assessed repeatedly during the same follicular phase, when LH pulse frequency normally increases (Berga et al., 1985). In another negative study, Loucks et al. (1994), in their recent study of healthy young women in the follicular phase, observed that LH pulse frequency decreased during a 5-day food restriction period especially during waking hours, whereas LH pulse amplitude was increased by 40%, especially during sleep. These findings support the hypothesis that pulsatile secretion of LH depends on energy availability, i.e. calorie availability, in women also.

In men, there are several reports on the suppressive effects of relatively brief periods of fasting on mean and pulsatile LH secretion. Röjdmark (1987b) reported that men of normal weight who fasted for 56 h had a significant decrease in mean circulating values of LH and testosterone. In this experimental context, the responsiveness of the pituitary to exogenous GnRH was increased (Röjdmark, 1987a). We have shown (Veldhuis et al., 1993a) in healthy young men that serum total and free testosterone concentrations decreased by 30–50% and that the 24-h mean concentrations of serum LH fell by 30% during 5 days of total fasting. In this study, deconvolution analysis of blood samples taken at 5-min intervals over 24 h revealed that LH secretory pulse frequency, duration of LH secretory bursts, interburst interval and half-life of LH were unchanged by fasting, but the mass of LH secreted per burst declined significantly (Figure 9). GnRH-stimulated LH release was increased in seven of eight men. In another study, the number of serum LH concentration pulses and the basal LH concentration, but not the serum LH pulse amplitude, decreased during a 2-day fast in healthy men (Cameron et al., 1991). However, this earlier study was limited by a 15-min sampling interval over part of the day only, a relatively insensitive radioimmunoassay for LH measurements (many samples during fasting had undetectable serum LH concentrations), and the absence of calculated LH secretion rates and/or half-life. Thus, nutrient deprivation impairs neuroendocrine activity of the hypothalamic–pituitary–testicular axis, probably by way of suppressing output of the GnRH pulse generator (see below).

Whether the pituitary gland can respond to GnRH in nutrient-deprived humans appears to depend on the type and intensity of the metabolic perturbation. Pituitary responsiveness to GnRH can be decreased in chronically malnourished men with reproductive dysfunction (for review, see Bergendahl and Veldhuis, 1995). In contrast, LH responsiveness to GnRH is typically increased, or not decreased, after a 56-h or 5-day fast in men of normal weight (Röjdmark, 1987a; Veldhuis et al., 1993a). Thus, there is strong indirect support for the notion that fasting-induced hypogonadotrophism in men is due to decreased release of hypothalamic GnRH. Indeed, our recent studies of young men who received pulsatile GnRH
during fasting show complete restoration of pulsatile LH release and mean serum LH and testosterone concentrations (Bergendahl et al., 1996), thus strongly implicating GnRH deficiency in the hypogonadotrophism in men after short-term fasting.

**Stress and exercise**

Stress of various types [e.g. psychological, physical (heat, etc.), metabolic (diabetes mellitus, etc.)], and including physical exercise or endurance training, can be accompanied by delayed puberty or amenorrhea (Feicht et al., 1978; Frisch et al., 1982; McGrady, 1984; MacConnie et al., 1986; Beitins et al., 1991; Rosetta, 1993). A reduction in LH pulse frequency has been reported in some but not all strenuously training men (Rogol et al., 1983; MacConnie et al., 1986), amenorrheic women (Veldhuis et al., 1985), and exercising women who were still eumenorrheic (Cumming et al., 1985). Decreased LH pulsatility during various stresses has been thought to be due to diminished hypothalamic release of GnRH, although the exact pathophysiological mechanism for suppression of the GnRH pulse generator in exercising or stressed individuals is not known. However, a recent 18-month longitudinal study of healthy young women with documented normal pre-training gonadotrophin secretion showed that cyclic ovarian function remained normal despite a total training volume of ~1300 km each (Rogol et al., 1992). In another study of actively training women, whether they trained above or below their individual lactate thresholds did not affect 24-h LH pulsatility, or mean serum LH concentrations (Weltman et al., 1992). These two studies do not support the earlier hypothesis that supervised physical exercise or endurance training alone disturbs the function of the reproductive axis and causes ‘hypothalamic amenorrhea’. The altered pulsatile secretion of LH in the earlier studies may have resulted from multiple concomitant factors, including selection bias, overtraining, perceived stress, altered nutritional intake, or weight loss, rather than the physical training component per se. However, further studies are needed to clarify the exact pathophysiological mechanisms behind multifactorial disruption of neuroendocrine control of the reproductive axis in exhaustively training men, women and pubertal children.

Normal pulsatile GnRH/LH release also is disrupted in a wide variety of pathophysiological settings in women, including other hypothalamic amenorrheic states, polycystic ovaries, prolactinomas, postpartum and insulin-dependent diabetes mellitus. The reader is referred to Crowley et al. (1985), Evans et al. (1992), Griffin et al. (1994) and Diaz et al. (1996) for more detailed reviews.

Moreover, the impact of both androgens (Sir-Petermann et al., 1993) and obesity on gonadotrophin pulsatility requires further studies.

**Summary and conclusions**

Episodic hypothalamic GnRH release drives the pulsatile secretion of LH (and FSH), which is modulated further by intrapituitary and systemic feedback signals [as reviewed earlier in Human Reproduction (1993), vol. 8 supplement, entitled Central Control of Gonadal Function]. Transient activation of the hypothalamic–pituitary–gonadal axis occurs in neonatal boys, followed by a prepubertal hiatus in children of both sexes. Highly sensitive immuno-fluorometric assay of LH and deconvolution analysis have revealed that puberty is heralded by a 30-fold increase in LH secretory burst mass with only a 1.5-fold acceleration in LH pulse frequency, and no evident change in LH half-life. Thereafter, in women of reproductive age, menstrual-cycle-stage-specific regulation of the amplitude, frequency, duration and mass of LH secretory bursts can be demonstrated, which presumptively reflects both a varying GnRH signal and oestrogen-dependent changes in gonadotroph cell sensitivity to GnRH. Before the LH surge, GnRH self-priming, defined by progressively increased LH release in response to fixed doses of GnRH, is evoked by oestrogen-specific prolongation of LH secretory burst duration and mass. Age, starvation, stress, metabolic disorders and systemic illness can bring about hypogonadotrophism via suppression of GnRH pulse generator output. Consequently, the hypothalamus can be viewed as a site of functional integration of diverse internal and external cues that signal either awakening or short-term adaptive suppression of normal reproductive function in children, women and men. Further understanding of how regulatory input to the hypothalamic GnRH pulse generator is controlled in health and disease will likely clarify additional pathophysiologies underlying delayed puberty, male and female infertility, and disease-specific reproductive impairment.

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