Regulation of reproductive hormone secretion in primates by short-term changes in nutrition

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In primates, as in nonprimates, it is well established that periods of chronic or severe undernutrition can lead to a suppression of reproductive hormone secretion. Recent studies in monkeys and humans indicate that reproductive hormone secretion begins to be suppressed with even very brief periods of undernutrition. Specifically, in male monkeys reproductive hormone secretion responds to missing a single meal, eating a single meal, and to a change in the timing of daily meal intake. These findings suggest that nutritional/metabolic signals that are linked to food intake are part of the normal physiological mechanism regulating the daily activity of the reproductive axis, rather than simply signals that influence the activity of the reproductive axis in pathological conditions of severe undernutrition. The nature of the metabolic cue(s) linking reproductive hormone secretion to subtle changes in the metabolic status of the body remains unknown, as does the route by which metabolic information reaches the central hypothalamic neurones governing reproductive hormone secretion. However, recent studies indicate that the metabolic cue transmitting information to the reproductive axis is dependent on calorie intake, but is not dependent on changes in body mass or composition, changes in intake of a specific nutrient, changes in plasma glucose or insulin concentrations, or signals emanating from the taste or smell of food or the physical process of food ingestion.

In primates, as in nonprimates, periods of chronic or severe undernutrition often result in a suppression of reproductive hormone secretion with an accompanying decrease in fertility (Zubiran and Gomez-Mont, 1953; Warren and Vande Wiele, 1973; Smith et al., 1975; Vigersky et al., 1977; Dubey et al., 1986). This sensitivity of the reproductive axis to severe undernutrition would be advantageous, ensuring that fertility is impaired in times when energy resources are limiting for the high energy-requiring tasks of carrying a pregnancy to term and rearing a growing infant. Such a sensitivity to chronic energy availability would be most advantageous for large animals with relatively large energy stores, who could subsist on internal energy stores in times of famine and then reproduce when food is more readily available.

In contrast, much less research has examined the effects of mild changes in food availability on the activity of the reproductive axis. This review will focus on whether mild changes in nutritional intake can regulate reproductive hormone secretion, and will assess our current understanding of the mechanisms by which subtle changes in metabolic status regulate the central neural drive to the reproductive axis.

Specifically, the effects of mild changes in food intake on reproductive hormone secretion in primate species, both humans and nonhumans, will be considered, and hypotheses regarding the utility of a reproductive system that is responsive to subtle metabolic cues are proposed. The physiological regulation of the reproductive axis in Old World macaques, such as rhesus monkeys (Macaca mulatta), is similar to that of humans, with specific similarities in the neuroanatomy of GnRH neurones, the responsiveness of the hypothalamic–pituitary axis to gonadal steroid hormones, and hormonal fluctuations across the menstrual cycle in females (Hotchkiss and Knobil, 1994; Silverman et al., 1994). Of particular relevance here is that the responsiveness of humans and nonhuman primates to mild changes in nutritional status appears to be similar.

Changes in reproductive hormone secretion in response to short-term changes in nutrient intake

Responses to a single day of fasting

For many years it was widely believed that states of undernutrition would impair reproductive hormone secretion only when a significant amount of body mass had been lost (Frisch, 1984). However, numerous studies have rebutted the notion that body mass, or body fat, plays a role in transmitting information about the nutritional status of the body to the reproductive axis (Bronson and Manning, 1991). Perhaps one of the most compelling lines of evidence against the contention that body mass or body fat are mediators of the nutritional suppression of reproductive function has been the finding that very short-term changes in food intake can rapidly modulate reproductive hormone secretion in monkeys before any changes in body mass or composition occur.

Specifically, short periods of undernutrition, as brief as missing a single meal, lead to a significant slowing of pulsatile LH and testosterone secretion in adult male rhesus monkeys (Cameron and Nosbisch, 1991; Helmreich and Cameron, 1992). In male monkeys kept on a standard feeding regimen of one meal per day, LH pulse frequency on a day of normal feeding
is 7 ± 1.16 pulses per 24 h, whereas LH pulse frequency on a
day of fasting (that is, when monkeys miss their single daily
meal) is 3.33 ± 0.33 pulses per 24 h (see Fig. 1; Cameron and
Nosbisch, 1991). This suppression of LH release is already ap-
parent in the first 4 h after a meal is missed (that is, between
12:00 h and 16:00 h), and LH pulses in the first 4 h after a no-
mal meal occur at a rate of 2.0 ± 0.36 pulses per 4 h, whereas
LH pulses in the same 4 h period on a day of fasting occur at
a rate of 0.66 ± 0.42 pulses per 4 h (P ≤ 0.05; Helmreich and
Cameron, 1992). The suppression of pulsatile LH secretion on a
day of fasting is accompanied by a suppression of mean circu-
lating concentrations of LH, FSH and testosterone (Cameron
and Nosbisch, 1991; Helmreich et al., 1991). However, fasting
for 1 day does not lead to suppression of the amplitude of LH
pulses in male monkeys. This finding suggests that fasting
leads to a suppression of gonadotrophin release from the an-
terior pituitary by causing a decrease in the hypothalamic
stimulation of the pituitary, rather than by suppressing pitu-
itary responsiveness to hypothalamic GnRH. This conclusion
is supported by the finding that LH release in response to a very
small dose of exogenous GnRH is not suppressed on a day of
fasting, but rather remains normal compared with the GnRH-
stimulated release of LH occurring on a day of normal feeding
(Cameron and Nosbisch, 1991). It can thus be concluded that,
as in the case of long-term undernutrition, short-term decreases
in nutrient intake lead to a suppression of GnRH neuronal
activity, and ultimately to a decrease in the secretion of all of
the reproductive hormones. Moreover, suppression of repro-
ductive hormone secretion is clearly apparent long before
changes in body mass or composition could occur.

This finding is not unique to nonhuman primates; similar
findings have also been reported in nonprimates, as well as in
humans. In female rats (McClure and Saunders, 1985) and ham-
sters (Morin, 1986), fasting for 1–2 days before the LH surge
can block the midcycle surge. In female rats, Cagampang et al.
(1990, 1991) showed that 2 days of fasting leads to a significant
suppression in the frequency of pulsatile LH secretion, with an
accompanying decrease in LH pulse amplitude. It could be
argued, however, that 2 days of fasting in an animal as small
as a rodent represents a substantial period of undernutrition.

The suppression of LH secretion occurring after 2 days of
fasting in these species is accompanied by a significant loss
of body mass (Cagampang et al., 1990), whereas the slowing of
LH secretion that occurs when monkeys miss a single meal is
accompanied by no change in body mass or composition
(Cameron and Nosbisch, 1991). Thus, it is difficult to assess
whether periods of fasting of several days in duration in ham-
sters and rats should be equated to short- or long-term under-
nutrition in larger animals.

Several groups of investigators have reported that brief
periods of undernutrition in men lead to a suppression of re-
productive hormone secretion. Rojdmark (1987a) reported that
men fasted for 2 days had significant decreases in mean plasma
LH and testosterone concentrations. Rojdmark (1987b) also
found that this suppression of LH secretion occurred with no
impairment in the responsiveness of the pituitary to GnRH. We
reported that 2 days of fasting in men leads to a decrease in the
frequency of pulsatile LH secretion, which is accompanied by a
suppression of mean circulating concentrations of LH, FSH and
testosterone, but with no decrease in LH pulse amplitude
(Cameron et al., 1991). These findings suggest that in men, as
in male monkeys, brief periods of undernutrition lead to a de-
crease in GnRH stimulation to pituitary gonadotrophs. In con-
trast to our findings, however, Veldhuis et al. (1993) reported
that men fasted for 5 days period have a decrease in mean cir-
culating LH concentrations characterized by a suppression of
LH pulse amplitude with no change in LH pulse frequency.
Nevertheless, they conclude that the suppression of LH se-
cretion results from changes in hypothalamic stimulation of
the pituitary, because normal pituitary responsiveness to the
administration of exogenous GnRH is maintained during brief
periods of fasting. Cumulatively, these data indicate that in
men, as in male monkeys, a cue arising in the very early stages
of undernutrition can modulate the central neural drive to the
reproductive axis.

Perhaps surprisingly, a similar sensitivity to brief periods
of fasting has not been found in women. We have studied the re-
response of women in the mid-follicular phase of the menstrual
(menstrual cycle (days 7-9 of the cycle) to a 2 day fast and have found no
change in either mean LH or FSH concentrations, nor a change

Fig. 1. Pulsatile LH secretion in an adult male rhesus monkey on (a) a day of normal feeding (one meal a
day at 11:00 h), (b) a day of fasting and (c) a day of refeeding (a normal daily meal at 11:00 h).
in the frequency or amplitude of pulsatile LH secretion (Berga et al., 1993). Olson et al. (1995) reported that women studied in the late follicular phase do respond to a 2 day fast with a suppression of LH secretion, but this suppression is slight, with LH pulse frequency declining from approximately 5.8 pulses per 8 h to 4.5 pulses per 8 h. Work with female monkeys (Cameron et al., 1993) suggests that the relative insensitivity of women in the follicular phase of the menstrual cycle to brief periods of fasting may be a consequence of the lower circulating steroid hormone concentrations relative to men. Ovariectomized female monkeys have no apparent suppression of LH secretion in response to 2 days of fasting; however, they acquire a responsiveness to brief periods of fasting after gonad-intact concentrations of LH have been restored by supplemental oestradiol implants.

A similar steroid hormone-induced sensitivity to fasting is also apparent in female rats (Cagampang et al., 1991). This same phenomenon is apparent in male monkeys, who show an insensitivity to 2 days of fasting in the castrate state and restoration of sensitivity by implantation of oestradiol-containing capsules that are sufficient to restore gonad-intact concentrations of LH (Cameron et al., 1993). These findings suggest that the presence of steroid hormones may sensitize the central neural components of the reproductive axis to the influence of metabolic cues. A relevant question is thus whether women develop a sensitivity to brief periods of fasting during the luteal phase of the menstrual cycle, when circulating steroid hormone concentrations are high compared with the follicular phase of the cycle. Cagampang et al. (1991) showed, in ovariectomized female rats, that oestradiol plus progesterone increases the sensitivity of the reproductive axis to the effects of fasting more effectively than does oestradiol alone. Further experiments are required but have been difficult to pursue because LH pulse frequency during the luteal phase of the menstrual cycle is already markedly suppressed, even in the well-fed state.

Responses to refeeding after a brief period of fasting

Just as brief periods of fasting can lead to a rapid suppression of reproductive hormone secretion in male monkeys, the re-suppression of food intake after a brief fast can stimulate pulsatile LH secretion rapidly (Cameron and Nosbisch, 1991; Parfitt et al., 1991; Schreinhofer et al., 1993a; Fig. 1). In most monkeys, stimulation of LH secretion is apparent within 2 h of a refeed meal, and often pulses of LH are apparent within 20–40 min of meal consumption. The degree of stimulation of reproductive hormone secretion in response to refeeding is related linearly to the size of the refeed meal (Parfitt et al., 1991). Moreover, stimulation of LH secretion with refeeding occurs even when nutrients are directly infused into the stomach via a gastric cannula; hence the stimulation does not appear to be dependent on the taste, smell or oral consumption of food (Schreinhofer et al., 1993a), but on calorie availability. This contention is supported by experiments demonstrating that infusion of hypertonic saline through gastric cannulae, to create stomach distension without providing calories, does not restore LH and testosterone secretion in fasting male monkeys (Schreinhofer et al., 1993a).

The stimulation of reproductive hormone secretion by refeeding after a period of fasting does not appear to be dependent on the consumption of a specific nutrient. Schreinhofer et al. (1994) found that male monkeys that had been fasted for 1 day before refeeding showed a similar stimulation of LH secretion whether a mixed meal (containing carbohydrate, protein and lipid), a carbohydrate-only meal, or a protein–lipid meal was provided via indwelling gastric cannula. These results argue strongly against the hypothesis that circulating glucose concentrations play a critical role in transmitting metabolic information to the central neural systems regulating the activity of the reproductive axis during periods of fasting and refeeding, because circulating glucose concentrations increased after the two carbohydrate-containing meals but not after the protein–lipid meal. This hypothesis has gained support from experiments in various species, including monkeys, showing that states of hypoglycaemia can lead to a marked decrease in LH secretion (Clarke et al., 1990; Chen et al., 1992). Indeed, insulin-induced hypoglycaemia does lead to a marked suppression of LH secretion; however, the degree of hypoglycaemia is dramatic compared with the degree of hypoglycaemia that occurs with even prolonged periods of undernutrition (Haymond et al., 1982; Dubey et al., 1986). In fact, as will be discussed later in this review, in response to a single day of fasting, male monkeys remain euglycaemic, yet a marked suppression of reproductive hormone secretion is apparent in the early stages of fasting (Helmreich et al., 1991). These two findings, that a metabolic signal capable of suppressing LH secretion is present in the early stages of fasting while euglycaemia is maintained, and that hypoglycaemia that occurs in the later stages of fasting can also suppress LH secretion, imply that multiple metabolic signals may be responsible for the marked suppression of reproductive hormone secretion that occurs with chronic undernutrition.

Such a conclusion is supported by the findings of Schneider and Wade (1989) who showed that, in hamsters, administration of metabolic inhibitors of both glucose and fat utilization can prevent ovulation, but that blocking either one of these pathways alone does not prevent ovulation.

The same signal(s) that stimulates LH secretion during recovery from brief periods of fasting may be responsible for stimulating LH secretion during recovery from periods of chronic undernutrition, as the time course of recovery of reproductive hormone secretion after chronic undernutrition is, in many cases, equally rapid. In castrate adult male monkeys with a marked suppression of circulating LH and FSH resulting from consumption of a low calorie diet for 3–6 weeks, an increase in circulating LH concentrations is evident usually within one to several days after increasing calorie intake (Dubey et al., 1986; Cameron, 1987). Chronically undernourished male rats have a suppression of serum LH and testosterone that is restored to normal, or above normal, concentrations after one day of refeeding (Howland, 1975). In chronically undernourished female rats, in whom puberty has been prevented by low food intake, increasing food intake results in a very rapid increase in pulsatile LH secretion, with some LH pulses evident at 12 h after the initiation of ad libitum food intake, and regular pulses evident within 24 h of increased food intake (Bronson, 1986). Similarly, in chronically undernourished ewes, in whom puberty has been prevented by restricting food intake, increasing food intake results in an increase in pulsatile LH secretion within hours after returning to ad libitum feeding (Foster et al., 1989).

There is one type of undernutrition, however, in which the recovery of reproductive hormone secretion is generally slow,
occurring in parallel with weight gain, and that is the under-
nourished state that occurs in the psychiatric disease, anorexia
nervosa (Beumont et al., 1976; Katz et al., 1978; Pirke et al., 1979; 
Isaacs et al., 1980; McNab and Hawton, 1981; Wheeler et al., 
1983). In both women and men with anorexia nervosa, the time 
course for recovery of normal reproductive hormone secretion 
is usually of the order of several weeks to months. Moreover, a 
significant percentage of patients with anorexia nervosa do not 
develop normal patterns of gonadotrophin secretion even after 
weight restoration (Beumont et al., 1976; Katz et al., 1978; Isaacs 
et al., 1980; McNab and Hawton, 1981; Wheeler et al., 1983; van 
Binsbergen et al., 1990). The reason that normal LH secretory 
patterns are so slow to recover or fail to recover in anorexic 
patients is unknown. However, a number of factors may con-
tribute to the slow recovery, including the very prolonged dur-
ation of undernutrition that anorexic patients have frequently 
experienced, the severity of depletion of energy stores in the 
body, which can be much more marked in anorexic patients
than that generally examined in experimental studies, and the 
continued activation of central stress-related neural systems re-
sulting from the physiological stress of forced weight recovery. 
In addition, it is also possible that some patients with anorexia
may have other underlying reproductive disorders that are not 
diagnosed. Further work is needed to identify the signals re-
sponsible for the restoration of reproductive function in anor-
exic patients and to determine whether they are the same 
signals responsible for the more rapid restoration of reproduc-
tive function reported for most other forms of short and long-
term undernutrition.

The rapid recovery of gonadotrophin and gonadal steroid 
hormone secretion after periods of undernutrition seem likely 
to play an important role in determining how fertility is linked 
to nutritional availability in natural settings. Darwin (1896) ob-
served that fertility in animals is affected by food availability, 
such that fertility is decreased in times when nutritional sup-
plies are scarce and is restored when adequate nutrition again 
becomes available. For survival of a species, it would seem that 
a key regulatory step in the nutritional regulation of reproduc-
tion would be the ability of the reproductive axis to be rapidly 
stimulated when food becomes available, so as to maximize 
fertility as soon as there is an adequate food supply. This re-
quirement provides a reasonable explanation for why the repro-
ductive axis has the capacity to respond to rapid changes in 
food availability. The alternative hypothesis that the reproduc-
tive axis responds quickly to changes in food intake to rapidly 
suppress fertility in times of undernutrition is less likely, as 
there is no evidence that the rapid suppression of gonado-
 trophin secretion that occurs with brief periods of fasting is 
great enough to impair fertility, and it seems unlikely that it 
would be advantageous to suppress fertility during brief 
periods of undernutrition.

The role of daily food intake patterns in determining the daily 
pattern of LH secretion

Further evidence that subtle changes in food intake can serve 
to regulate reproductive hormone secretion within the physio-
logical range comes from work examining how the timing of 
daily meal intake modulates the diurnal pattern of reproductive 
hormone secretion in monkeys. Male rhesus monkeys studied in 
captivity have generally a diurnal rhythm of LH and testoster-
one secretion with an increase in hormone release in the 
evening and at night (Dubey et al., 1981; Plant, 1981; Mattern 
et al., 1993). Unlike the diurnal rhythm in boys, which can be 
shifted by reversing the day–night light cycle (Boyar et al., 1974; 
Kapen et al., 1974), the diurnal rhythm of LH and testosterone 
secretion in male rhesus monkeys remains unchanged when 
monkeys are housed in constant light for 15 days (Dubey et al., 
1981), suggesting that the rhythm may not be controlled by the 
daily light–dark cycle. There are several lines of evidence that 
suggest that the diurnal pattern of LH and testosterone se-
cretion in the male rhesus monkey is regulated by food intake 
(Mattern et al., 1993). First, the timing of the rise in LH and 
testosterone each day occurs just after meal intake and is not 
associated with the timing of lights off. Second, when monkeys 
are fasted for a day, they lose the diurnal rise in LH and testos-
terone secretion. Third, adapting monkeys to a new feeding 
schedule with daily meal intake 6 h later in the day leads to a 
6 h delay in the rise in LH and testosterone secretion (Mattern 
et al., 1993). These results suggest that nutritional/metabolic 
signals that are linked to food intake are part of the normal 
physiological mechanism regulating the daily activity of the 
reproductive axis, rather than simply signals that influence 
the activity of the reproductive axis in pathological situations 
of undernutrition.

Does nutritional intake regulate reproductive hormone 
secretion via a stress signal or a metabolic signal?

It seems plausible that there are at least two types of ‘signal’ 
occurring as a result of decreased food intake involved in sup-
pressing the central drive to the reproductive axis during the 
early stages of fasting. One possibility is that a physiological 
response to the stress of fasting may suppress the activity of 
the reproductive axis. This would suggest that a neural or hor-
monal system that is activated in the early stages of under-
nutrition, which is also activated in response to other types of 
stress (that is, a system not specific to undernutrition), is re-
ponsible for causing the decrease in central neural drive to the 
reproductive axis. An alternative hypothesis is that some meta-
bulic change occurring as the body shifts from a fed to a fasted 
state may be responsible for decreasing the central drive to the 
reproductive axis in the early stages of undernutrition. Several 
experiments have been performed that have helped differenti-
ate between these two possibilities, and the results all suggest 
that a metabolic signal rather than a general stress signal is 
likely to be mediating the suppression of reproductive hor-
mon secretion in at least the early stages of undernutrition.

One strategy to distinguish between these two possible 
types of signals has been to attempt to separate the psychologi-
cal stress of undernutrition from the metabolic changes occur-
ring with undernutrition. When monkeys miss a meal, both 
psychological disturbances and metabolic changes are ap-
parent. Missing a meal is associated with a considerable 
amount of behavioural agitation, marked by significant in-
creases in active behaviour, such as spinning, pacing, banging 
the cage and vocalizing, during the first hour in which the meal 
is missed (Schreiholer et al., 1993a,b). Metabolic changes occur-
ring in monkeys within the first few hours of missing a meal in-
clude a decrease in circulating insulin and thyroid hormone.
concentrations and an increase in growth hormone secretion (Helmreich et al., 1991). To dissociate these two responses to short-term fasting, Schreinhofer et al. (1993a) performed a study in which monkeys were greatly ovied the day before fasting (they were given 100 pellets of monkey chow instead of their standard daily meal of 30 pellets), so that on the following day, they continued to be in a fed state even though their meal was withheld. Monkeys indeed remained in a fed state when their meal was missed after a day of overfeeding (with insulin and tri-iodothyronine concentrations remaining high), but they continued to display behavioural agitation in response to having their meal withheld. In this situation, LH secretion was not suppressed by fasting, indicating that LH secretion was aligned with the metabolic state of the animals rather than their psychological state. A further experiment (Schreinhofer et al., 1993b) took the same approach of separating metabolic from psychological reactions to changes in food intake. Monkeys were fed after a day of fasting with a gastric infusion of nutrients through a chronically indwelling gastric cannula, so that they would receive a meal but would not know that they were being fed, and would thus respond psychologically as if fasted. The monkeys responded behaviourally on a day of refeeding by gastric cannula as if they were being fasted for a second day (that is, with a considerable amount of behavioural agitation). However, they responded metabolically to the gastric feeding and all showed a marked stimulation of LH secretion, comparable with that which occurs with the oral consumption of nutrients on a day of refeeding after a day of fasting. Again, this finding suggests that LH secretion is modulated by the metabolic signals occurring in response to fasting and feeding, rather than by the psychological state associated with changes in food availability. A third piece of data supporting this conclusion is that men who are fasted for two days, but understand that this is part of an experiment and know that they will be fed later and will be paid for participating, do not show any indication of psychological stress on standard psychiatric examinations, but do have metabolic changes associated with fasting and do demonstrate a suppression of reproductive hormone secretion (Cameron et al., 1991).

A second type of experimental strategy has been to determine whether specific neural and hormonal systems, which are frequently activated during various stresses, and are known to be capable of suppressing LH secretion, are causing the suppression of reproductive hormone secretion during fasting. One such neural system studied has been the network of endogenous opioid-containing neurones. A variety of stresses lead to an increase in the synthesis and secretion of endogenous opioid peptides within many neuronal pathways in the central nervous system (Akil et al., 1984). In addition, there is evidence that an increase in the activity of the central endogenous opioid peptide systems plays a role in many forms of stress-induced inhibition of LH secretion, primarily provided by studies examining the ability of naloxone, an opiate receptor antagonist, to reverse stress-induced inhibition of LH secretion. Naloxone has been shown to reverse the slowing of LH secretion induced by foot-shock stress in rats (Petragnani et al., 1986), aggression in marmosets (O'Byrne et al., 1989), and the stress of anaesthetic darting in baboons (Sapolsky and Krey, 1988). However, when similar or greater doses of naloxone are administered to monkeys on a day of fasting, there is no reversal of the suppression of LH secretion by fasting (Helmreich and Cameron, 1992), indicating that endogenous opioid neuronal systems do not play a central role in causing this suppression.

The hypothalamic-pituitary-adrenal axis is another system that is activated in response to a wide variety of stresses. Moreover, at least two secretory products of the adrenal axis, glucocorticoids and corticotrophin releasing hormone (CRH), have been shown to be capable of suppressing the central drive to the reproductive axis when administered pharmacologically (Dubey and Plant, 1985; Olster and Ferin, 1987) or when endogenous release is high (for example, in Cushin's disease; Luton et al., 1977). The adrenal axis is activated only mildly by fasting, in male monkeys experiencing one day of fasting with cortisol concentrations increasing from an average of 16 µg dl⁻¹ to 21 µg dl⁻¹ (Helmreich et al., 1993), and there is no correlation between the magnitude of the fasting-induced rise in cortisol and the degree of fasting-induced suppression of LH secretion. Experiments administering hydrocortisone acetate on a day of normal feeding to mimic the rise in cortisol that occurs with fasting show that this amount of cortisol cannot suppress LH secretion (Helmreich et al., 1993). In addition, administration of dexamethasone, a synthetic glucocorticoid, in doses large enough to suppress the central CRH and vasopressin drive to the adrenal axis completely, does not prevent the fasting-induced suppression of LH secretion (Helmreich et al., 1993). These findings argue strongly against the hypothesis that activation of the adrenal axis causes fasting-induced suppression.

The results of the above experiments provide no support for the hypothesis that a stress signal, independent of metabolic signals provided by fasting, causes the suppression of reproductive hormone secretion that occurs in mild, brief states of undernutrition. Instead, it seems likely, although it remains to be rigorously tested, that in periods of chronic undernutrition, both stress and nutritional signals may act in concert to suppress reproductive secretion. Studies using chronically undernourished models, therefore, may provide little insight into the mechanisms by which mild, physiological changes in metabolic status regulate the activity of the reproductive axis.

Metabolic signals associated with the changes in reproductive hormone secretion occurring with fasting and refeeding

One way to identify the specific metabolic signals providing the critical link between the metabolic status of the body and the amount of central drive to the reproductive axis is to identify metabolic changes linked in time with the changes in reproductive hormone secretion that occur with fasting and refeeding. Results of studies that have used this strategy indicate that monkeys maintain euglycaemia and are not yet ketogenic during the first 4 h after a meal is missed, when a suppression of LH and testosterone secretion first becomes apparent (Helmreich et al., 1991). However, a number of changes are starting to occur in metabolic hormone secretion at this time (Helmreich et al., 1991). Because monkeys have missed a meal, plasma insulin concentrations are low. Plasma tri-iodothyronine concentrations begin to decrease 3 h after a meal is missed and are significantly lower than on a day of normal feeding by 5 h after the meal is missed. The decrease in plasma tri-iodothyronine appears to result from a decrease in its conversion from thyroxine, rather
than as a result of decreased pituitary drive to the thyroid axis, as plasma thyroid stimulating hormone concentrations are not decreased in the first few hours after a meal is missed. Plasma growth hormone (GH) secretion increases during the first 6 h after a meal is missed, with a significant increase in GH pulse frequency but no change in pulse amplitude. Plasma cortisol secretion is also significantly increased, but only by 1–6 µg dl⁻¹, within the first 2 h after missing a meal. These metabolic hormone changes are indicative of the transition of the body from an energy-storing state to an energy-mobilizing state, characterized by low concentrations of insulin and increased concentrations of insulin counter-regulatory hormones, which act to decrease tissue glucose utilization and to mobilize tissue glucose stores. These results indicate that a signal occurring in the early phases of fasting, while the body remains in homeostatic balance with euglycaemia maintained, is capable of suppressing the central neural drive to the reproductive axis (Fig. 2).

With refeeding there is a rapid reversal of the metabolic changes that occur with fasting, and all of these metabolic changes are apparent within the timeframe in which feeding stimulates LH secretion (20 min to 2 h; Helmreich et al., 1991). Feeding after a brief period of fasting leads to a rise in circulating glucose concentrations within minutes, which is rapidly accompanied by a large increase in circulating insulin concentrations. Within a few hours there is a statistically significant increase in circulating tri-iodothyronine and a decrease in circulating GH and cortisol concentrations. These findings strengthen the notion that a cue occurring very early during the transition between an energy-storing state and an energy-mobilizing state can modulate the central neural drive to the reproductive axis. Studies that reverse the metabolic changes associated with fasting and refeeding pharmacologically have been useful in starting to probe the question of which metabolic cues can act in or close to this region to regulate reproductive axis function. As discussed above, pharmacological prevention of the fasting-induced rise in circulating cortisol is not capable of preventing the fasting-induced suppression of LH secretion, ruling out cortisol or its central secretagogues as mediators of the fasting-induced suppression of LH secretion (Helmreich et al., 1993). Similarly, studies by Williams et al. (1995) show that there is no correlation between the magnitude of the feeding-induced increase in insulin secretion and the degree of activation of LH secretion in male monkeys, and that pharmacological suppression of the feeding-induced increase in insulin secretion does not prevent the feeding-induced stimulation of LH secretion. These findings argue strongly against the hypothesis that insulin serves as a critical cue linking reproductive hormone secretion to the metabolic state of the body. The potential roles of tri-iodothyronine and GH as mediators of the metabolic regulation of the reproductive axis remain to be tested; however, it seems unlikely that these hormones, themselves, directly regulate the reproductive axis. It is likely that some, as yet unidentified, signal occurring at the transition from a state of energy storage to a state of energy mobilization will provide the key regulatory signal to the reproductive axis.

Routes by which metabolic cues may influence GnRH neuronal activity

Another useful strategy for trying to dissect the mechanism by which mild changes in metabolic status modulate reproductive hormone secretion may be to test the various routes by which metabolic information could be transmitted to the brain. There are three potential routes by which a metabolic cue(s) could be sensed by the GnRH neuronal system (Fig. 3; Cameron and Schreihofer, 1995). The most direct route would be regulation of GnRH neurones themselves by metabolic signals, such as circulating metabolic substrates or hormones. A likely site for direct regulation would be at the median eminence, where there is convergence of GnRH neurone terminals as they reach toward the hypothalamo–hypophysial portal vasculature. This area is outside the blood–brain barrier, so even large or polar metabolic cues could interact directly with neurones here. There is experimental evidence to support the concept that metabolic cues can act in or close to this region to regulate neuronal activity. For example, insulin receptors and receptors for cholecystokinin (CCK; a gut hormone released by the small intestine with meal intake and also produced within the central nervous system neurones) have been detected in the basal region of the hypothalamus (Havranka et al., 1978; Hill et al., 1990). Electrophysiological and biochemical studies have identified neurones in the hypothalamus that are responsive to metabolic substrates such as glucose (Anand et al., 1964). In addition, neurones have been identified within the medial basal

![Fig. 2. Metabolic changes associated with the suppression of LH secretion in monkeys during the early phases of fasting.](image-url)
hypothalamus, immediately adjacent to the median eminence, that show changes in activity and gene expression in response to changes in food intake (for example neuropeptide Y-containing neurones, Sahu et al., 1988; Schwartz et al., 1992; and CCK-containing neurones, Schick et al., 1987). However, the hypothesis that GnRH neuronal activity can be modulated locally at the median eminence by metabolic cues remains to be tested.

A second route by which metabolic signals could modulate GnRH neuronal activity is through direct influence on the activity of other central neural systems that modulate GnRH neuronal activity. A number of neural systems that are known to be capable of influencing GnRH neuronal activity are known to be responsive to various metabolic signals. For example, central noradrenergic neurones can stimulate GnRH release (Teresawa et al., 1988) and these are known to be responsive to changes in food intake (as demonstrated by an increase in the concentration of the immediate early gene product, FOS, in noradrenergic neurones after food intake; Caston et al., 1994) and to the availability of the precursor amino acid, tyrosine (Fernstrom, 1984). Moreover, data collected by D.A. Schreinhofer and J.L. Cameron (unpublished) indicate that the stimulation of LH secretion in adult male monkeys that occurs with refeeding after a brief period of fasting can be partially prevented by administration of the α-adrenergic antagonist, phenoxybenzamine. In addition, studies in fasted rats have implicated activation of α2-adrenergic receptors in the fasting-induced suppression of LH secretion in this species (Cagampang et al., 1992a). Gruaz et al. (1993) showed that administration of neuropeptide Y antiserum can prevent the induction of LH secretion that occurs when chronically undernourished rats are fed ad libitum. The role of neuropeptide Y in mediating LH secretory responses to fasting and food intake in primates awaits investigation.

A third route by which metabolic signals may influence GnRH neuronal activity is by altering peripheral afferent neural input into the brain. Evidence for the involvement of such a route in mediating metabolic signal regulation of central neural functions comes primarily from studies examining the control of food intake. Such studies show that the ability of a meal to modify food intake (a behaviour that is believed to be regulated, in part, by neural centres in the hypothalamus) can be altered significantly by vagotomy (Rezek and Novin, 1976), suggesting that a metabolic signal occurring with food intake sends information to the brain via the vagus nerves. In rats there is evidence that a vagal pathway may be important for transmitting information regarding metabolic state to the reproductive axis; Cagampang et al. (1992b) have shown that cutting the gastric branch of the vagus can reverse the fasting-induced suppression of LH secretion. The role of the vagus in transmitting information regarding metabolic status to GnRH neurones in primates remains to be examined.

Not only is it possible that metabolic information could reach hypothalamic neurones via any of these three routes, but it is also possible that these routes may be linked to one another. For example, a metabolic signal occurring with food intake may send information via the vagus nerves to noradrenergic neurones in the terminal fields of the vagus nerves (such as within the nucleus tractus solitarius), which may in turn stimulate GnRH neurones in the hypothalamus. In addition, it

![Fig. 3. Potential sites of action of metabolic cues that regulate GnRH neuronal activity.](image-url)
is likely that multiple metabolic signals transmit information simultaneously regarding the metabolic status of the body to GnRH neurones via different neural pathways.

Conclusion

The data reviewed here show that LH secretion can be modified after male monkeys miss a single meal, eat a single meal, or when the timing of meal intake is changed. Similar studies in men, documenting that two days of fasting can suppress LH, FSH and testosterone secretion, show that this phenomenon is not peculiar to monkeys. These findings indicate that mild changes in the metabolic status of the body can serve to regulate the central drive to the reproductive axis, and suggest that nutritional/metabolic signals that are linked to food intake may be part of the normal physiological mechanism regulating the daily activity of the reproductive axis, rather than simply signals that influence the activity of the reproductive axis in pathological situations of severe undernutrition. The ability of food intake to stimulate the activity of the reproductive axis rapidly would be evolutionarily advantageous, so that fertility would be maximized very rapidly when adequate food supplies become available. This capacity to respond to increases in food availability with increased fertility may be the underlying reason why the reproductive axis has the ability to respond to rapid changes in food availability. The alternative hypothesis, that the reproductive axis could respond to a decrease in food availability to decrease fertility rapidly, would be evolutionarily disadvantageous. In fact, there is no evidence that the rapid suppression of reproductive hormone secretion that accompanies short-term fasting causes infertility.

Surprisingly, females in the follicular phase of the menstrual cycle are less sensitive than males to suppression of reproductive function by mild changes in food intake. This decreased sensitivity appears to be related to circulating concentrations of gonadal steroid hormones, with increased oestriadiol concentrations increasing the sensitivity of the reproductive axis to modulation by metabolic state. This finding suggests that females may be more sensitive to suppression of reproductive hormone secretion by mild undernutrition when they are in the luteal phase of the cycle, although this possibility is purely speculative at present. However, perhaps females are protected from environmental fluctuations in food availability during the follicular phase as a consequence of low circulating concentrations of gonadal steroid hormones, which impair the ability of the central components of the reproductive axis to respond to mild changes in food availability.

The nature of the metabolic cue(s) linking GnRH neuronal activity to subtle changes in the metabolic status of the body remains unknown, as does the route by which metabolic information reaches the GnRH neuronal system. However, recent studies clearly indicate that the metabolic cue transmitting information to the GnRH neuronal system is dependent on calorie intake, but is not dependent on changes in intake of a specific nutrient, changes in plasma glucose or insulin concentrations, or signals emanating from the taste or smell of food or the physical process of food ingestion.

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