THE EFFECTS OF NUTRITION ON REPRODUCTIVE ENDOCRINOLOGY

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Summary

There is a wide range of reproductive responses to nutrition, ranging from the advent of puberty to the control of testicular growth and multiple births. Our studies of mature Merino rams have led us to classify the endocrine components of these responses into two types:

- *Gonadotrophin-Releasing Hormone (GnRH)-Dependent* — mediated by the hypothalamic centres controlling reproduction, as evidenced by changes in gonadotrophin secretion;
- *GnRH-Independent* — mediated by unknown endocrine or metabolic systems, and evidenced by changes in gonadal activity that are independent of changes in gonadotrophin secretion.

In mature rams fed to maintenance, supplementation with lupins will increase hypothalamic activity for up to 10 days. However, the testes continue to grow for months after the GnRH-dependent response has ended, so much of the gonadal response is independent of changes gonadotrophin secretion. This observation and others suggest that gonadal activity is also affected by GnRH-independent systems, including growth hormone and insulin-like growth factor-I.

Our studies have also shown gonadotrophin secretion in rams is not affected by large increases in the blood concentrations of glucose and insulin, but is stimulated by infusions of casein or addition of volatile fatty acids to the diet. Thus, both energy and protein substrates are involved in the GnRH-dependent response, but glucose-insulin homeostasis is not. Rather than a simple interaction between a single nutritional cue and the hypothalamic control centres, at least two sites (hypothalamus and testis) are affected by at least two cues (protein and energy) acting through an unknown number of endocrine systems (gonadotrophic and metabolic).

I. INTRODUCTION

(a) Nutritional drive of seasonal cycles

In temperate climates, grazing animals evolved seasonal breeding patterns so their offspring would be born onto abundant spring pastures and therefore have the best chance of survival. The animal times these annual cycles of reproductive activity by using photoperiod, a reliable environmental cue, to detect and predict the seasons. This principle applies to many breeds of domestic sheep, despite millennia of controlled breeding and management of the food supply to overcome some of the constraints of the annual pasture cycle.

There is also a strong seasonal pattern of food supply in with a Mediterranean regions, such as south Western Australia. In contrast with temperate regions, however, the photoperiodic and pasture seasons are out of phase so using photoperiod to ensure birth in spring is not a sensible strategy for survival of the species. Animals that evolved under these conditions tend to be "opportunistic breeders" and use a variety of environmental cues to coordinate their reproductive

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activity (Martin et al. 1992). Thus, Merino sheep in Western Australia respond to social cues (the "male effect", in which rams induce ovulation in acyclic ewes) and the prevailing supply of nutrients (to adjust their fertility and fecundity), so that acute changes in reproductive activity are superimposed on an annual pattern that is controlled by photoperiod (Martin et al. 1992).

This paper will discuss the endocrine mechanisms underlying one of these opportunistic strategies, namely the reproductive response to nutritional cues. There is a wide range of such responses, including the advent of puberty in males and females, the control of testicular growth and multiple ovulations and births in adults. In most of our recent work, we have been studying mature Merino rams so we will concentrate our attention on that animal.

(b) endocrine systems controlling gonadal activity

As background for discussion of the physiological processes through which nutrition might affect the reproductive system of rams, we need to briefly overview the endocrine control of testicular activity (Fig. 1). The primary level of control is based in the neuroendocrine cells of the hypothalamus that secretes gonadotrophin releasing hormone (GnRH). A critical aspect of this system is that the cells fire in unison and so secrete their neurohormone in pulses, and the frequency of these pulses controls gonadal activity. Thus, a high frequency of GnRH pulses will stimulate the pituitary gland and increase the rate of secretion of luteinising hormone (LH) and follicle-stimulating hormone (FSH). These two hormones will increase the activity of the gonads which, in turn, will produce more gametes and also more of their own hormones, inhibin and the sex steroids (eg, testosterone; Fig. 1). Through a negative feedback loop, the gonadal hormones exert homeostatic control on the activity of the hypothalamic-pituitary unit.
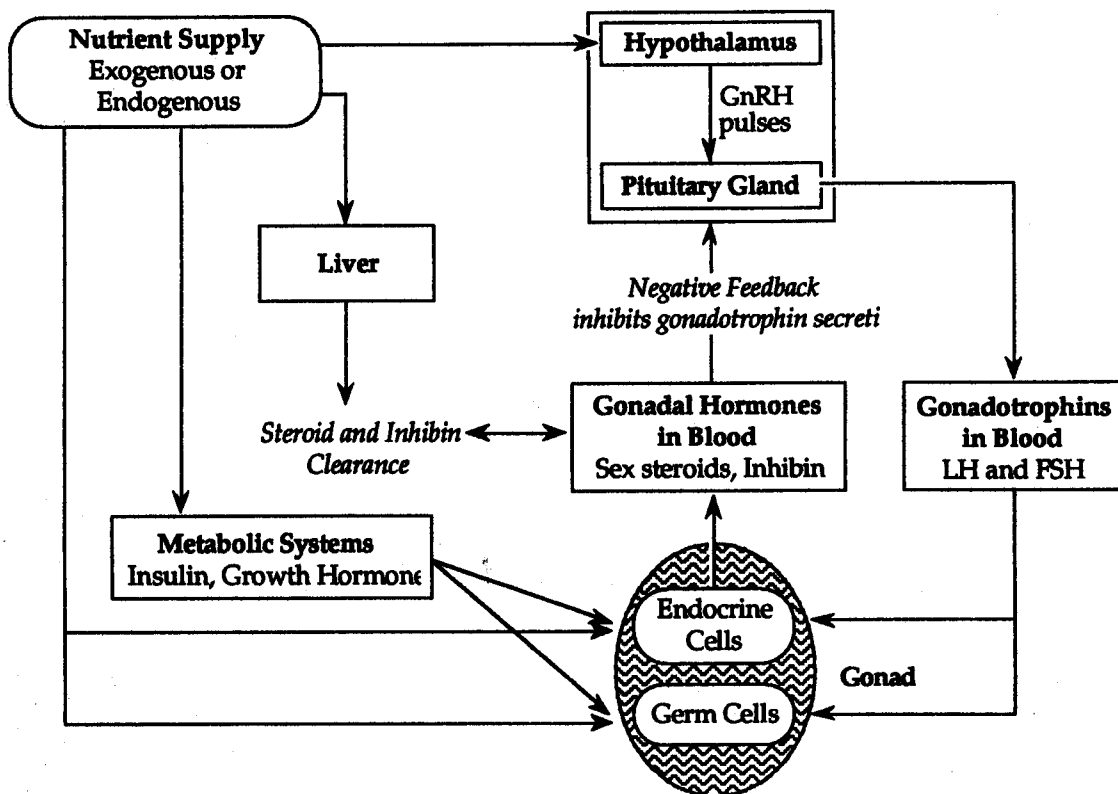

Fig. 1. Hormonal control of testicular activity in the ram, showing negative feedback to the pituitary gland and hypothalamus, and some of the potential sites of action of nutritional stimuli.

The activity of the GnRH cells in the brain is difficult to measure directly, but it is not necessary to do so because each GnRH pulse reflects the coordinated activity of many GnRH cells and the pulse of LH it releases from the pituitary gland is easily detected in the peripheral blood stream. Thus, for many years now, we have been able to use the frequency of the LH pulses as a bioassay of GnRH cellular activity (Thiéry and Martin 1991). Clearly, the pulsatile nature of LH secretion underpins much of our work.

II. POTENTIAL SITES FOR THE ACTION OF NUTRITIONAL STIMULI

Nutritional signals can affect gonadal activity through two types of mechanism:

(a) *GnRH-Dependent Pathways* — such effects would be evidenced by a change in the frequency of GnRH and LH pulses, and in the circulating concentrations of FSH; 'classical' examples of this phenomenon include anorexia nervosa in humans and the delay in puberty in lambs caused by chronic undernutrition (Santen and Bardin 1973; Foster et al. 1986; Fig. 2).

(b) *GnRH-Independent Pathways* — such effects would be evidenced by a change in gonadal activity, without necessarily causing changes in the frequency of GnRH pulses, except as a secondary consequence of a shift in the equilibrium of the feedback system. In mature female sheep, this seems to be the most likely mechanism explaining the effects of nutrition on ovulation rate, the frequency of multiple ovulations (Scaramuzzi and Campbell 1990).
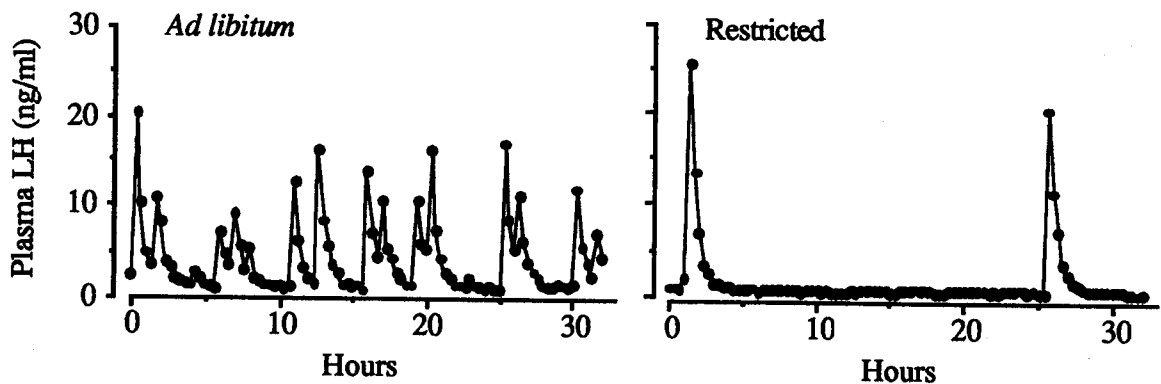

Fig. 2. The plasma concentrations of LH in a peripubertal ram in blood sampled every 20 min for 32 h, before and after dietary restriction. Note the effect of dietary allowance on the frequency of the pulses. Pulse amplitude does not seem to be important in the regulation of gonadal function. From Martin and White (1992).

(a) Evidence for GnRH-dependent pathways

In the mature male sheep, we have shown that changes in nutrition can exert profound effects on testicular size and sperm production (Oldham et al. 1978; Martin et al. 1987). The response of Merino rams fed a supplement of lupin grain was reliable and rapid, so we used it to form the basis of our investigations into the physiological mechanisms involved. Initial attempts to establish whether the hypothalamic pathways played a role produced equivocal results. Ritar et al. (1984) found that rams fed lupins had a higher LH pulse frequency than rams fed to maintenance 5 days after the change in diet, but the effect disappeared over the following 6 weeks. Despite this, the testes in the lupin-fed rams continued to grow. In a similar study, Martin et al. (1987) measured LH pulse frequency 9 weeks after the change in diet, and also found no differences

between maintenance and lupin-supplemented diets. Comparison of these experiments suggested that, if there was any effect on LH pulse frequency, it was short-lived (Martin et al. 1987). A more intensive and comprehensive study resolved the issue — we measured LH pulse frequency over a wider range of times after the dietary changes, we measured FSH as well, and we also tested the effects of a reduction in dietary allowance (Fig. 3).
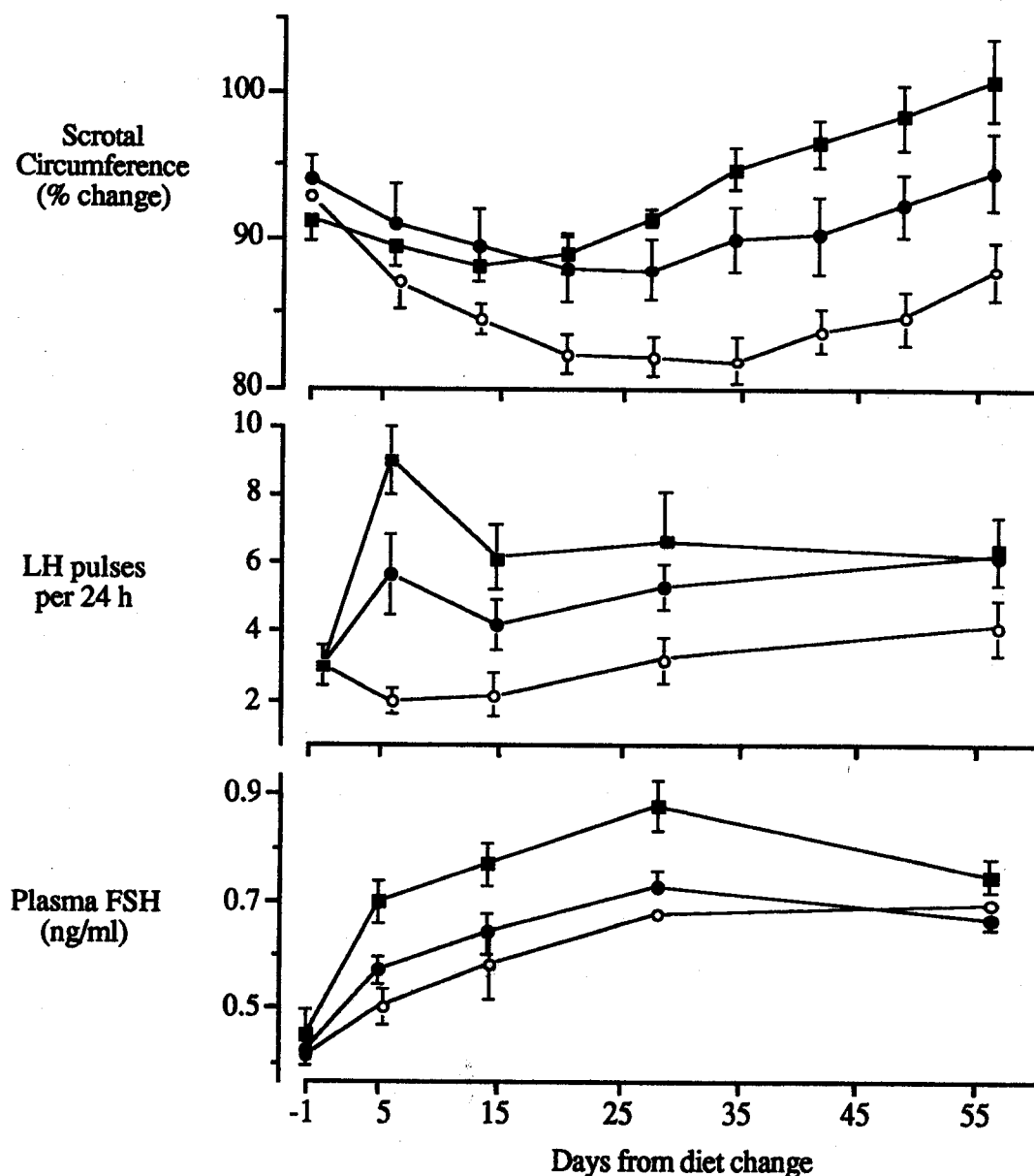

Fig. 3. Effects of nutrition on testicular size (scrotal circumference) and gonadotrophin secretion in mature Merino rams. All values are mean ± sem (n=6). ●—● Maintenance; ■—■ Maintenance + Lupins; o—o Half-Maintenance. From the data of Martin et al. (1989).

When the dietary allocation was reduced below the requirements for maintenance, LH pulse frequency decreased (Fig. 3). This effect was maintained for 6 weeks. Changing the diet from

maintenance to maintenance plus lupins increased LH pulse frequency over the first 3-4 weeks, but thereafter the difference between these dietary treatments disappeared. These observations thus agreed with those by Ritar et al. (1984), and explained those by Martin et al. (1987), who failed to detect an effect of diet on LH pulse frequency because their first measurement was too late.

In recent times, we have completed a series of experiments testing several aspects of the response to lupins and found the increase in LH pulse frequency over the first 3-10 days to be very reliable, in intact rams and in castrated rams undergoing hormone replacement therapy (Martin et al. 1989; Tjondronegoro et al. 1990; Boukhliq et al. 1991). We are now completely satisfied that there is a GnRH-dependent component in the reproductive responses of rams to diet — indeed, the LH response to lupins now forms the basis of the experimental model we are using to determine how dietary factors affect the reproductive control centres in the hypothalamus.

(b) Roles of extra-hypothalamic pathways

The GnRH-dependent response might be reliable, but by no means does it explain all of the effects of diet on testicular growth. When we measured FSH secretion, for example, some surprising findings confronted us (Fig. 3).

The lupin diet increased FSH concentrations, in much the same way that it increased LH pulse frequency (Tjondronegoro et al. 1991). In itself, this observation was not surprising as both gonadotrophins are involved in the control of testicular growth and both are controlled by hypothalamic GnRH pulse frequency (Lincoln 1978, 1979). However, the animals fed less than their requirement for maintenance had similar FSH concentrations to the animals fed to maintenance, despite very different rates of gonadal growth and LH pulse frequencies (Martin et al. 1989; Tjondronegoro et al. 1991). We have not completed our investigations into this phenomenon as yet, but this observation, plus the repeated observation that lupins can only increase LH pulse frequency for a few weeks but stimulate testicular growth for months, are very strong indicators of the importance of GnRH-independent control systems linking nutrition and reproduction. Whether such systems are extra-hypothalamic as well as GnRH-independent, or simply reflect key roles for other hypothalamic controls (such as growth hormone, GH) also remains to be determined.

A role for GnRH-independent systems is also supported by recent advances in our understanding of intratesticular physiology. The effects of gonadotrophins on testicular function are modulated by other hormones that are derived from the systemic circulation, or produced inside the testis itself, where they act as 'local' or 'paracrine' hormones (Sharpe 1984). For example, testicular cells have receptors for several extra-testicular hormones that are affected by nutrition, such as insulin, GH and insulin-like growth factor-I (IGF-I). These promote cell replication and steroid production (Saez et al. 1988) and may mediate the effects of nutrition on testicular function. Metabolic hormones may modulate the actions of gonadotrophins on the testis either through a direct action on common target cells or indirectly, promoting the synthesis of one or several of the hormones produced intra-testicularly. Finally, we cannot rule out the possibility that specific nutrients, such as energy substrates or amino acids, act directly on the testis, improving the efficiency of hormone synthesis and spermatogenesis.

Thus, in the ram, the changes in nutritional status that affect gonadotrophin secretion also alter blood concentrations of glucose and insulin (Fig. 4).
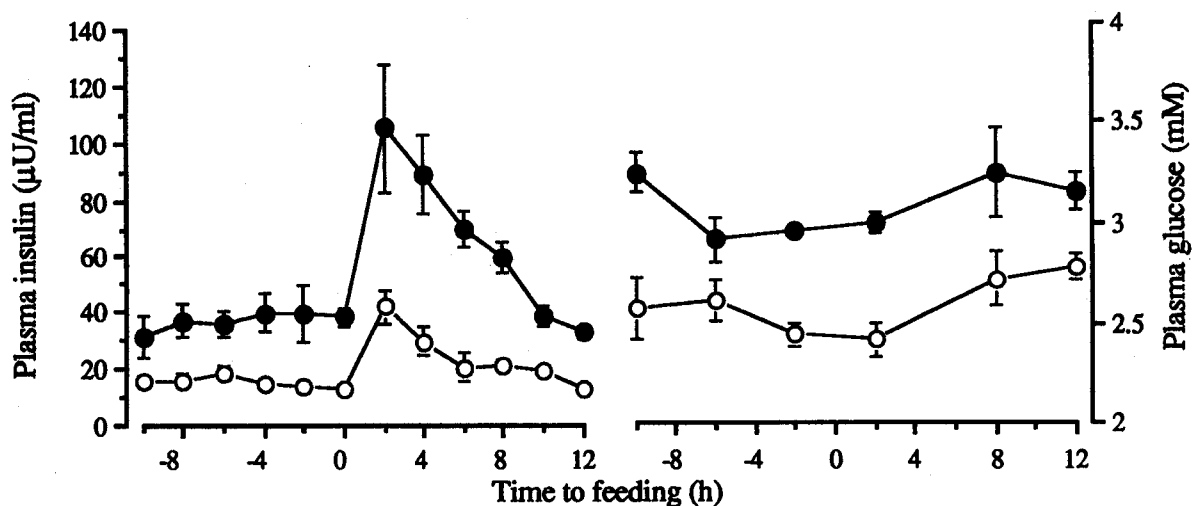
Fig. 4. Blood plasma concentrations of glucose and insulin in rams fed a Maintenance diet (o—o) or the Maintenance diet with 750 g/day of lupins (●—●). All values are mean ± sem (n=6).

We have yet to test the effects of our diets on GH secretion in the mature ram model, but in other studies the blood concentrations seem to be reduced by diets that increase ovulation rate in ewes (Downing and Scaramuzzi 1992), and are inversely related to the energy and protein content of the diet in 10-month-old wethers (Bass et al. 1984). Interestingly, IGF-I secretion by the liver is controlled by GH, yet blood IGF-I concentrations are **directly** related to dietary energy and protein in young wethers (Bass et al. 1984). This might be explained by the effects of nutritional status on the biological activity and metabolic clearance of GH (Gluckman et al. 1987). It might also be explained by an observation in pigs, where high insulin concentrations sensitise hepatic tissue to GH and allow the secretion of IGF-I to be maintained despite a fall in GH concentrations (Kriel et al. 1992). Thus, the lupin-supplemented diet might stimulate testicular growth in rams through increases in the circulating concentrations of insulin and glucose, through changes in the bioactivity or concentration of GH, or through increases in the concentration of IGF-I.

The question we need to answer is whether these events are targeting the gonad.

(c) Hormone Clearance — Passive control?

Endocrinologists tend to regard blood concentrations of hormones as direct reflections of secretory activity, primarily because clearance rates rarely vary and have little impact on the nature of the hormonal signal. However, this is a dangerous assumption in discussions of nutritional responses because of the effects that dietary changes have on the function of the liver, the site of catabolism of many hormones. In sheep, for example, a high plane of nutrition will increase the clearance of progesterone by the liver during the early stages of pregnancy, leading to a decrease in circulating concentrations and an increase in the risk of abortion (Parr et al. 1987). From the feedback system controlling gonadotrophins (Fig. 1) it is evident that similar effects on the clearance of, say, testosterone would alter the secretion of the gonadotrophins and hence testicular activity. This was tested by Parr and Tilbrook (1990), who found no change in testosterone clearance. However, our work with ewes suggests that changes in the clearance or catabolism of oestrogen might explain the effects of lupin supplements on FSH secretion and ovulation rate (Adams and Martin 1990).

Changes in clearance, which could be regarded as a "passive" method of fertility control, do not appear to be a sensible strategy for the survival of the individual or the species. On the other

hand, it is probably rare for animals in the wild that are battling to maintain condition to discover a daily ration of 500 g lupins so they might not have developed physiological mechanisms to cope with such stimuli. When interpreting the responses to large lupin supplements, we need to consider whether we are working with the nutritionist's equivalent of pharmacology.

III. IDENTIFYING THE NUTRIENTS

Identifying the component of the diet that is critical in reproductive responses will help us determine the site of action of nutritional cues and the physiological processes that are involved. The list of potential candidates is very long but, for GnRH-dependent responses at least, we can narrow the field by considering only substances that penetrate the blood-brain barrier, and perhaps by testing substances involved in appetite control. On the simplest level, we need to test whether GnRH activity is affected by changes in energy balance (blood concentrations of glucose or volatile fatty acids) or the hormones that maintain this balance, such as insulin. On a more complex level, we could consider neural interactions between the reproductive control centres and the neuropeptidergic systems controlling appetite, such as cholecystokinin and neuropeptide-Y (Morley 1987).

(a) Protein
If dietary protein is the nutritional cue, the substance involved might be an amino acid that acts as a neurotransmitter (such as aspartate or glutamate), or acts as precursors for the synthesis of neurotransmitters (such as the large neutral amino acids, tryptophan and tyrosine; Growden and Wurtman 1978). Indeed, infusions of such amino acids have triggered LH release and increased ovulation rate in sheep (Foster et al. 1988; Downing et al. 1990; Downing and Scaramuzzi 1992).

One of the problems with many of these hypotheses and those involving synthesis of neurotransmitters from glucose, is the concept that, in non-pathological situations, critical functions of the brain can be determined by the supply of precursors.

(b) Energy
Glucose is the major energy source of all brain tissue and it is the precursor for a number of neurally active amino acids, including gamma aminobutyric acid, glutamate and aspartate. Neurons using such compounds as neurotransmitters are known to affect the activity of the GnRH cells (Thiéry and Martin 1991). Changes in blood glucose concentrations lead to changes in insulin secretion, and there are specific binding sites for insulin on the endothelium of brain microvessels that may aid transcytosis of insulin across the blood-brain barrier. It also binds to receptors in the circumventricular organs, potential pathways by which insulin might affect the activity of the GnRH cells in the hypothalamus (Williamson 1987). Such pathways presumably mediate the stimulatory effects of intracerebroventricular insulin infusion of insulin on LH secretion in the pig (Cox et al. 1989).

We initially tested whether glucose is the energy substrate used by the hypothalamic-pituitary testicular axis as a nutritional cue. Our studies showed that large increases in the blood concentrations of both glucose and insulin, following intravenous or abomasal infusions of glucose, had no detectable effect on gonadotrophin secretion in rams (Fig. 5; Boukhliq et al. 1991; 1992). By contrast, intra-abomasal infusions of casein appear to stimulate gonadotrophin secretion and, when salts of volatile fatty acids were added to the diet of the casein-infused rams, gonadotrophin secretion was increased further (Fig. 5).
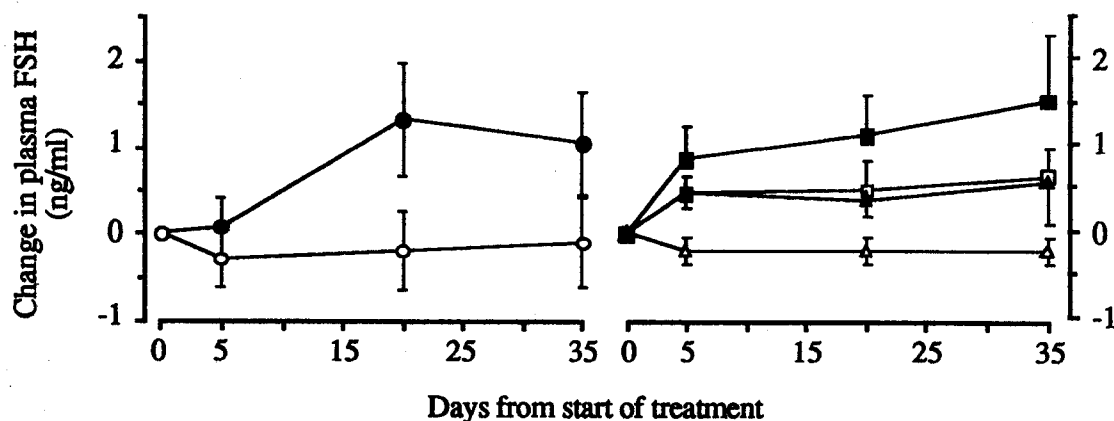
Fig. 5. Plasma FSH concentrations in rams fed a maintenance diet (O—O) or the maintenance diet supplemented with either 750 g/day of lupins (●—●), glucose (△—△), casein (□—□), glucose + casein (▲—▲) or glucose + casein + volatile fatty acid salts (■—■). All values are mean ± sem (n=6). From the work described by Boukhliq et al. (1992).

We have therefore rejected the hypothesis that glucose or insulin, or some measure of the ratio of these two that reflects glucose entry, will be involved, and that the volatile fatty acids play a role (Fig. 5). This is arguably no surprise for ruminants, but rather than simplify the picture, these studies suggest that both energy and protein substrates are involved in the hypothalamo-pituitary responses controlling the reproductive axis.

IV. THE FUTURE

We have really only just begun to penetrate the mechanistic fog surrounding the relationship between nutrition and reproduction, and most of our studies have shown us that this relationship is much more complex than our initial hypotheses suggested. Rather than a simple interaction between a single nutritional cue and the hypothalamic control centres, following the example set by photoperiod, we have at least two sites affected (hypothalamus and testis) by at least two cues (protein and energy) acting through an indeterminate number of endocrine systems (gonadotrophic and metabolic). For our own work, we will try to determine the nutritional signal that affects the brain and induces the GnRH-dependent short-term response, and the metabolic endocrine systems that affect the GnRH-independent testicular response to gonadotrophins. It has become increasingly clear that we reproductive physiologists cannot do this alone.

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