

# Nutritional inputs into the reproductive neuroendocrine control system – a multidimensional perspective

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Evolution has shaped regulatory systems to improve the chance of reproductive success in a somewhat unpredictable environment. One of the more powerful regulators of reproductive function in both sexes is metabolic status, defined as the availability of nutrients and energy to the tissues. Here, we briefly review the basics of the relationship between metabolic status and the activity of the system that controls pulsatile GnRH and LH secretion. We then reflect on these relationships within the framework of a model that comprises four interdependent ‘dimensions’: 1) genetic, 2) structural, 3) communicational, and 4) temporal. Using two major examples, the male sheep and the post-partum dairy cow, we illustrate aspects of each dimension that seemed to have evolved to limit the risks associated with ‘the decision to reproduce’. The results of recent studies have also led us to include in our model the concepts of ‘metabolic memory’ and ‘nutrient sensing’ to help explain some aspects of the temporal dimension. Throughout the review, we propose directions for future research that could shed light on pathways that have evolved to ensure that animals are able to take the least risky ‘decision’.

## Introduction

In this review, we are looking at how nutrition influences reproduction, an old problem that has been researched for decades and reviewed in detail many times (most recent: Butler 2005; Blache *et al.* 2006; Robinson *et al.* 2006). In this instance, we are attempting to analyse the issues from a different perspective – we will consider the multi-dimensional nature of an integrated control system that has developed over an evolutionary timescale to ensure that an animal has the best chance of reproductive success in an unpredictable environment. Of course, for farmed ruminants, the environment is nowadays far more predictable than that with which their ancestors had to cope before domestication. On the other hand, domestication has also led to intense demands, such as selection pressure for increased milk production by modern dairy cows, that greatly increase the energy requirements of the animals – in effect, this is an extreme metabolic stressor that challenges the homeostatic processes developed during evolution.

Before elaborating the notion of a multi-dimensional control system, we will first define several basic concepts in the bioenergetics of reproduction, metabolic status, and their effects on reproduction. Having set the scene, we will consider each of four 'dimensions' of what we see as a 'decision-making system' that allows ruminants to optimise their reproductive success. First, we will briefly look at the genetic dimension, one that sets the limits of responsiveness to nutritional input to animals. Second, we will consider the structural dimension, a growing list of organs and tissues involved in the linkage between metabolic status and reproduction. The third dimension, the communication network of signalling pathways, will be treated in greater detail. The fourth and final dimension is temporal so concerns the role of time on the metabolism-reproduction relationship – and has led us to incorporate 'metabolic memory' and 'nutrient sensing' into our hypothetical control system (Cahill 1980; Obici & Rosetti 2003). To illustrate these concepts, we will mainly use two examples, the male sheep that has been acutely placed on a high plane of nutrition, and the high-output dairy cow that is undergoing a dramatic change in energy balance in the early postpartum period. We will conclude by discussing the four-dimensional model as a conceptual framework upon which we can base further study of the interactions between nutrition and reproduction. Most of the processes involved are autonomic by nature but, to aid clarity of expression, we will occasionally risk the use of anthropomorphic language (eg, 'decision to invest').

## Basic concepts

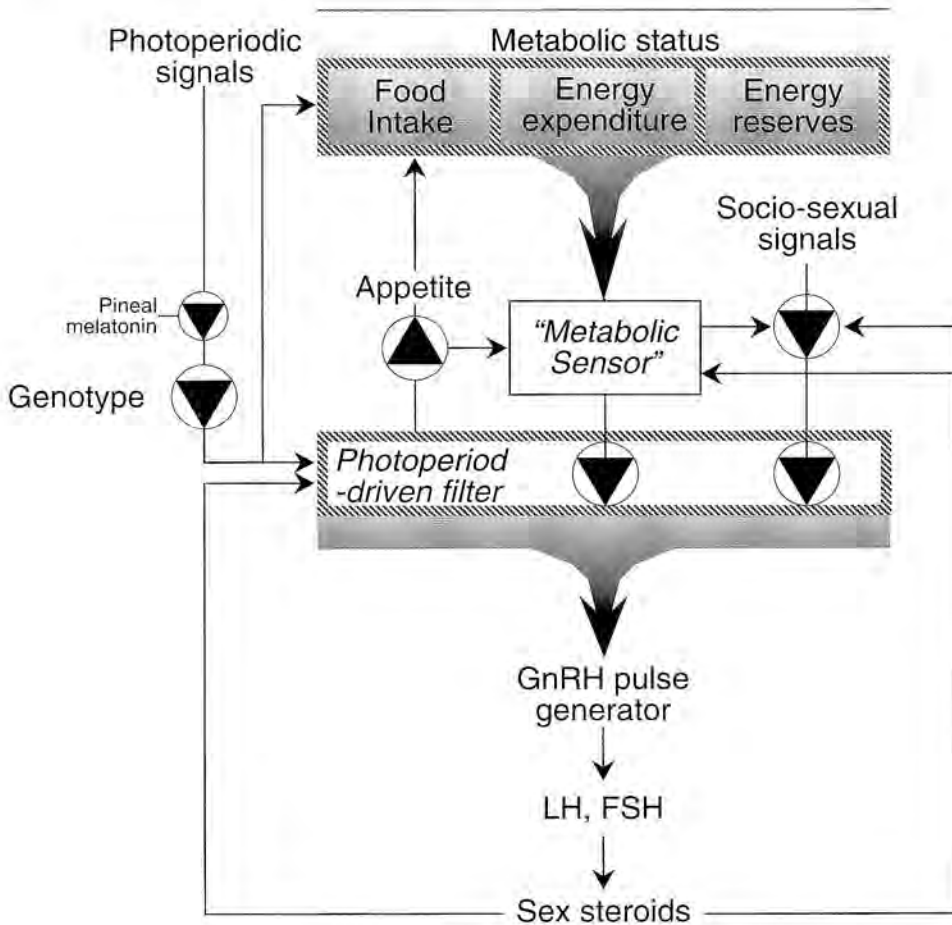
### *Bioenergetics of reproduction*

Energy costs are attached to all components of the reproductive process, from the expression of specific behaviours, such as sexual or maternal behaviour, to the production of morphological elements, such as gametes, fetuses and milk. In mammals, reproduction is energetically more demanding for females than for males because of the requirements for gestational development and the production of milk. The peaks in energy demand for reproduction also differ in timing between the sexes, with the male investing most energy before fertilization, while females invest most energy following fertilization, often with considerable delay (Horton & Rowsemitt 1992). For example, in grazing ewes, gestation costs only 3% of the daily energy expenditure during the first 3 months but this builds rapidly to 20% during the last 2 months (Fierro & Bryant 1990). The energy requirement for dairy cows to ovulate a follicle, form a corpus luteum, and maintain early pregnancy is minuscule compared with the requirement for lactation, which requires up to 50% of the daily energy expenditure (Graham 1964; Fierro & Bryant 1990). For the human female, it has been suggested that the reproductive axis will not be activated until the energy requirements for pregnancy are already in storage (Frisch 1994) but, in a striking contradiction of this logical concept, dairy cows have been successfully selected genetically for their ability to conceive during lactation when their energy balance is negative.

The critical time point in the reproductive cycle seems to be when the animal makes an 'investment decision', accepts the risks inherent in procreation, and 'switches on' its reproductive system. Considering the energetic requirements, the relationship between the metabolic and the reproductive regulatory systems needs to be highly tuned if the probability of success is to be reasonable. As a natural illustration of this matching of potential future resources to potential future demands, the energy regulatory systems of mammals are more strongly tied to reproductive function in females than in males because of the much higher cost for reproduction paid by females.

### The concept of metabolic status

At any given time, for any given animal, the amount of energy available for reproduction depends on the difference between the amount of energy expended, including the demands for maintenance, and pool of disposable energy (Fig. 1). The pool of disposable energy includes the energy derived from feed intake plus the energy stored in body tissues, especially adipose tissue, liver and muscle. The amount of expended energy varies according to the age and the physiological status of the animal and it comprises the energy spent on functions that are responsible for the maintenance of homeostasis, as well as energy spent on extra physiological needs such as growth and reproduction. Most authors refer to 'energy balance' instead of 'metabolic status'. The terms can be seen as interchangeable but 'metabolic status' includes an integrative dimension that 'energy balance' does not.



**Fig. 1:** A schema describing the proposed relationships between photoperiodic, nutritional and social cues and they ways that they interact with genotype and steroid feedback in the control of hypothalamo-pituitary-testicular axis in the male sheep. Nutritional input is via 'metabolic status', a reflection of the difference between energy expenditure and the sum of energy available from food intake and from energy reserves, as measured by a hypothetical 'metabolic sensor'. Redrafted after Blache *et al.* 2003.

*The neuroendocrine system – the fundamental controller of reproduction*

A full reproductive cycle starts with the emergence of sexual activity in future parents and finishes with emergence of sexual activity in the subsequent generation. The various phases of the cycle differ in their duration between the sexes but the secretion of GnRH, and consequently LH and FSH from the pituitary gland, are central to the process in both (Blache *et al.* 2002; Blache *et al.* 2003; 2006). Effectively, the 'decision' of an animal to reproduce or not is implemented by the system, as yet not described, that controls the production of pulses of GnRH – there is a threshold frequency of pulses above which males will produce sperm and females will ovulate. Thus, during postpartum anoestrus in dairy cattle, pulsatile infusion of LH decreases the time delay to first ovulation by stimulating follicular growth and steroidogenesis (Hampton *et al.* 2003). The system that controls GnRH secretion is also the final common pathway via which gonadal activity is usually influenced by external factors (Fig. 1), including socio-sexual cues, photoperiod and energy balance (review: Martin *et al.* 2004). That said, it is important to note that tissues involved in the process of reproduction, including the pituitary gland, gonads and mammary glands, can also respond independently to metabolic inputs, if not to the extent of switching the reproductive process on or off.

*Metabolic status and investment in reproduction*

Because reproduction is very demanding energetically, metabolic status is arguably the most powerful internal regulator of reproductive function and variation in metabolic status can profoundly affect the reproductive cycle at almost any stage. In mature male sheep, an acute increase in the intake of energy and protein induces, first, an increase in the frequency of pulses of GnRH and LH, and then an increase in the tonic secretion of FSH (Martin *et al.* 1994). An acute reduction of feed intake has the opposite effects (Martin & Walkden-Brown 1995). Similarly, in the post-partum cow, the activity of the GnRH neurons is highly positively correlated with energy balance, so pulse frequency increases when energy balance shifts from negative to positive values following an increase in intake or a decrease in expenditure (review: Butler 2000; 2005). Dairy cows have been selected heavily for high milk production and show a high level of nutrient utilisation and adipose tissue mobilization during early lactation, so the impact of milk production on reproduction can be reduced by reducing milking frequency. Reducing milking frequency to once daily increases the rate of spontaneous resumption of oestrous cycles in anoestrous cows (Rhodes *et al.* 1998) independently of any direct effect of diet (Patton *et al.* 2006), although the role played by GnRH pulse frequency in determining the length of postpartum anoestrus interval (PPAI) is yet to be proven directly.

**A 4-dimensional linkage between nutrition and reproduction**

Naturally, animals have evolved to increase their chances of reproducing and regulatory systems have evolved to fulfil that drive. We will now outline a framework within which we can analyse the processes and relationships involved. Following an analogy with theoretical physics, we will consider the regulatory system as having four dimensions: genetic, structural, communicational and temporal.

*1) The genetic dimension*

The effects of metabolic status and dietary manipulation on the reproductive axis differ be-

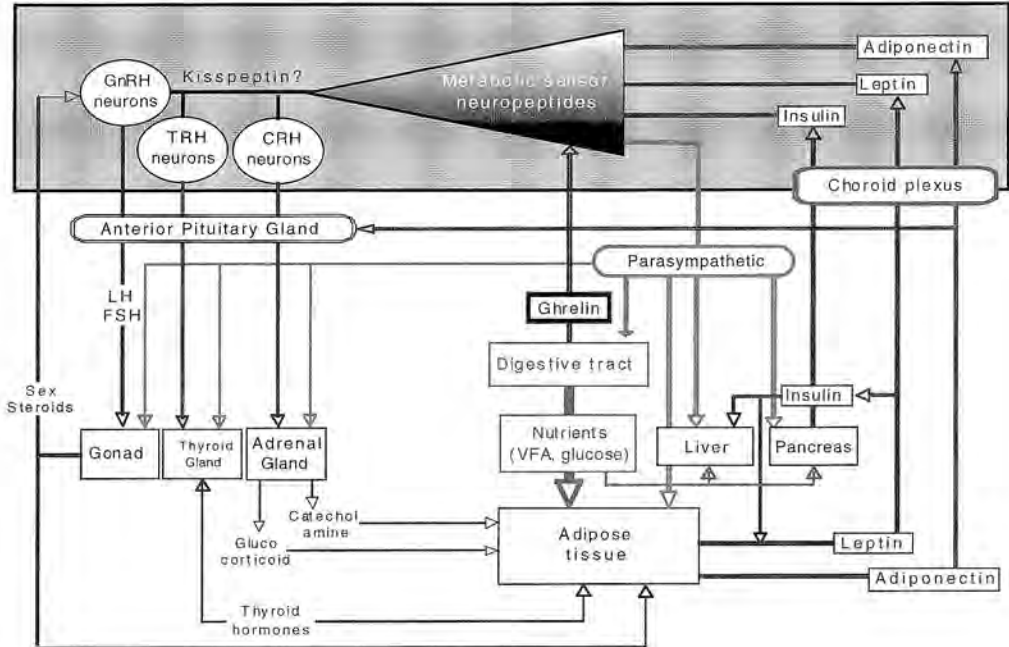
tween genotypes in both sheep and cattle. This genetic dimension accounts for variations in the responses to environmental inputs, whether they be natural (eg, photoperiod) or artificial (eg, genetic selection for high milk production). In our review for the previous symposium in this series, we suggested that photoperiod acts as a 'filter' of the effect of nutrition on the reproductive system and that this filtering effect depends on the genotype of the animal (Blache *et al.* 2003). This is because, in sheep, the effect of nutrition on the reproductive endocrine axis is smaller in breeds that are very responsive to photoperiod than in breeds that are less responsive (Hötzel *et al.* 2003; Martin *et al.* 1999; Martin *et al.* 2002). Similarly, a large body of work in dairy cattle has shown that genotype affects the ability of cows to resume ovulation during the postpartum period. Dairy cows in continuous calving systems that have not ovulated by 44 days postpartum are defined as having a prolonged PPAI (Lamming & Darwash 1998). It seems that the regulatory systems that control metabolism and energy balance can partition energy in different ways in cows of different merit and, in high merit animals, reproductive function has been de-prioritised in favour of milk production. This was clearly demonstrated in a study done in New Zealand that compared the performance of Friesian cows bred from semen collected in 1970s and 1990s – over that 20-year period of selection, the postpartum anovulatory interval increased from 7 to 12 days (McNaughton *et al.* 2003), despite the fact that selection for high milk production was associated with a increase in dry matter intake (Veerkamp *et al.* 2003). The genetic make-up of an animal, whether naturally or artificially acquired, is thus the first regulatory step in the influence of metabolic status on reproductive function.

## 2) The structural dimension

Metabolic status involves inputs from several energy pools that depend on the activity of a variety of organs so it is not surprising that recent decades of research have led to an increasing number and diversity in anatomical sites involved in the relationship between nutrition and reproduction. There has also been a parallel increase our understanding of the precise nature of the role played by each regulatory organ. The brain and the gonads have always been seen as the primary targets for nutritional input into reproductive function but we now accept major roles for the pancreas, liver and adipose tissue (Fig. 2). For adipose tissue, this transformation has been spectacular as it has been elevated from a passive storage site to a vital endocrine organ that produces a number of signals (detailed below). Similarly, the digestive system is also now implicated in the regulatory processes through which nutrition affects reproduction as its endocrine output has been detected and identified. The digestive system also produces very direct metabolic information in the form of energy metabolites (glucose, fatty acids) and amino acids, as detailed in the final section below. In summary, every organ involved in the regulation of the three compartments of metabolic status (intake, reserves, expenditure), is also involved in generating signals that influence reproductive activity (Fig. 2) – some of these have a very specific role (eg, the liver, an organ that processes first most of the circulating nutrients) whereas others have a more general input (eg, the thyroid gland).

## 3) The communicational dimension

The successful implementation of a 'decision to invest' in reproduction depends on the systems that regulate the reproductive axis, via changes in GnRH pulse frequency, responding accurately to changes in metabolic status. These regulatory systems can be classified as endocrine, neural and nutrient-based.



**Fig. 2:** Schematic summary of the potential relationships among the endocrine and neural inputs into the systems that control the reproductive system and mediate the responses to change in metabolic status. For clarity, we have omitted some hormonal systems (GH, prolactin, oxytocin) that are also involved in the control of the metabolic status but do not appear to exert any direct action on GnRH secretion. Within the brain, the neurochemical nature of the hypothetical 'metabolic sensor' that integrates the endocrine and neural inputs is still not known. Kisspeptin is proposed as a possible final link between the neuropeptide systems that respond to insulin and leptin, and perhaps adiponectin, and the GnRH neurons. It should be noted that the pituitary gland might be involved in the response of the reproductive axis to nutrition because it is responsive to many of the same hormonal signals as the hypothalamus.

## Endocrine systems linking metabolic status and GnRH secretion

### Insulin

Insulin is affected by energy balance and seems to be involved in the control of reproduction in male sheep because: 1) a high plane of nutrition leads to high concentrations of insulin in both plasma and CSF (Miller *et al.* 1998; Zhang *et al.* 2004; 2005); 2) with a restricted diet or with diabetes, a low dose of insulin infused into the third ventricle increases LH pulse frequency to values similar to those seen in well-fed animals (Miller *et al.* 1995a; Miller *et al.* 2002; Tanaka *et al.* 2000); 3) insulin receptors are present in the hypothalamus (Blache *et al.* 2002); 4) following an acute increase in dietary allowance, the increase in insulin secretion coincides with the increase in LH pulse frequency (Zhang *et al.* 2004). A central role for insulin in the regulation of GnRH secretion is also supported by studies with neuron-specific, insulin receptor knockout mice (Bruning *et al.* 2000).

In cattle, there are also similar associations: 1) dietary restriction and negative energy balance reduce plasma concentrations of insulin; 2) during post-partum anoestrus, the dominant follicle ovulates in response to restricted suckling only in cattle with high plasma concentrations of insulin (Sinclair *et al.* 2002); 3) dietary manipulation that increases plasma concentrations of propionate and insulin are also associated with a decrease in PPAI without any changes in either milk production or energy balance (Gong *et al.* 2002).

Thus, the accumulated evidence strongly suggests a major role for insulin in the GnRH response to variation in metabolic status, although, in dairy cattle, the evidence for this link is more mixed (review: Butler 2005). For example, during early lactation, infusion of insulin, associated with control of glycaemia, failed to stimulate LH pulse frequency, but did stimulate production of oestradiol by a direct action on the ovary (Butler *et al.* 2004). It appears that insulin does not always stimulate GnRH secretion, especially in presence of strong negative feedback.

### **Growth Hormone (GH)**

The presence of mRNA for GH receptors in the hypothalamus and the pituitary gland (Kirby *et al.* 1996; Lucy *et al.* 1998) suggests that GH could link nutritional inputs to gonadotrophic outputs (Monget & Martin 1997). Energy balance certainly affects plasma concentrations of GH under a wide variety of conditions (Bossis *et al.* 1999) but, in male sheep at least, it is not likely to be involved in the stimulation of GnRH secretion because an increase in nutrition induces a decrease in plasma GH concentrations (Miller *et al.* 1998).

### **Insulin-like growth factor-I (IGF-I)**

In male sheep, circulating concentrations of IGF-I are affected by diet and peripheral administration of physiological doses of IGF-I inhibits LH secretion, but this is probably through an action at pituitary level rather than by a change in GnRH pulse frequency (Adam *et al.* 1998). In addition, concentrations of IGF-I in CSF are not affected by diet (Miller *et al.* 1998) and we have not been able to demonstrate that IGF-I infusion into the third ventricle affects LH pulse frequency (Blache *et al.* 2000). It therefore seems likely that, in male sheep at least, IGF-I is not heavily involved in the effects of changes in nutritional status on the brain's reproductive centres. In the post-partum cow, plasma concentrations of IGF-I are negatively correlated with extended PPAI and positively correlated with body condition and food intake (Beam & Butler 1999). Plasma IGF-I values decrease in food-restricted heifers and, in post-partum dairy cows, IGF-I increases linearly up to the day of first ovulation (Beam & Butler 1997; Diskin *et al.* 2003). Also, IGF-I and its receptor have been found in the hypothalamus of the rat (Bohannon *et al.* 1988; Schechter *et al.* 1994) so IGF-I might play a direct role in the control of GnRH neurons and could be involved in the resumption of cyclicity in lactating cows (Beam & Butler 1997; Diskin *et al.* 2003). However, until this concept is tested directly in cattle, the information from studies with rams leads us to conclude that it is unlikely.

### **Thyroid hormones**

Thyroid hormones play a role in the control of seasonal reproduction in sheep, probably through an input into endogenous cerebral rhythms (Karsch *et al.* 1995). However, in the mature male sheep, the concentrations of thyroid hormones in plasma and CSF are not affected by the acute elevations of nutrition that increase LH pulse frequency, suggesting that they play no role in the rapid stimulation of the GnRH neurons (Miller *et al.* 1998; Zhang *et al.* 2004; 2005). In lactating cows also, there is little or no change in thyroid hormones in relation to selection for

milk production (Veerkamp *et al.* 2003). Overall, there is very little evidence for thyroid hormones being important signals linking energy balance and reproduction. Arguably, this is not surprising because the ubiquitous action of thyroid hormones is not compatible with a very specific action on the reproductive axis.

### **Hormones secreted by adipocytes**

Our view of the role played by adipose tissue in the management of metabolic status has evolved from that of a passive energy reserve to that of a very active endocrine regulator of a multitude of bodily functions, including food intake, metabolism, immunity, thermoregulation and cardiovascular function, as well as reproduction (Ahima 2005; Cinti 2005; Kershaw & Flier 2004). Of the 20 or more endocrine products of adipose tissue, leptin appears to be the most important regulator of reproductive activity in ruminants (review: Chilliard *et al.* 2005), although leptin is also the most studied of the adipocyte hormones and future research might reveal key roles for the others. The expression and release of leptin, and the sensitivity of gonadal and brain tissues to leptin, are all altered by short- and long-term changes in metabolic status. Moreover, numerous experiments in female and male sheep have shown that leptin can affect the neuroendocrine systems that control the activity of the reproductive axis (review: Adam *et al.* 2003; Chilliard *et al.* 2005). A recent study in dairy cattle has illustrated the close link between leptin concentrations and LH pulse frequency during the post-partum period (Kadokawa *et al.* 2006), although a triggering role for leptin in the termination of anoestrus it is not always supported (Chagas *et al.* 2006). Indeed, the consensus of the large amount of leptin literature is that the role of leptin is permissive rather than triggering, and this is logical because adipose tissue is only one of the three compartments of metabolic status.

Another interesting adipose hormone, adiponectin, has yet to receive the same interest as leptin but perhaps should do so because it is exclusively produced by adipose tissue and, in contrast to leptin, it stimulates energy expenditure without any effect on feed intake when it is infused into the cerebral ventricle of the rat (Ahima 2005). In male rat pituitary cells in culture, adiponectin reduces the expression of GnRH receptor and decreases the secretion of LH (Malagon *et al.* 2006). However, an effect of adiponectin on the activity of the GnRH neurons is yet to be demonstrated.

### **Hormones secreted by the gastro-intestinal tract**

A most interesting potential candidate in the regulation of reproduction by metabolic status is ghrelin, a gut hormone that is the endogenous ligand of the GH secretagogue (GHS) receptor. In sheep, ghrelin secretion is inhibited by an increase in feed intake and stimulated by fasting (Sugino *et al.* 2004). In rodents, the distribution of GHS receptors overlaps that of GnRH in the arcuate nucleus (St-Pierre *et al.* 2003) and ghrelin decreases the secretion of LH (Fernandez-Fernandez *et al.* 2004). Interestingly, ghrelin and GHS receptors are expressed in human and rat testis (Tena-Sempere 2005) and ghrelin is found in most reproductive tissues in both male and female sheep (Miller *et al.* 2005). In dairy cattle, Roche *et al.* (2006) measured plasma ghrelin concentration, pre- and post-feeding, during peak lactation (75 days postpartum) in animals of low and high genetic merit for milk production. High-merit cows produced more milk, consumed more pasture, and had higher plasma concentrations of ghrelin and GH, than low-merit cows. Itoh *et al.* (2005) reported a decline in ghrelin concentrations as cows progressed from early to mid- to late lactation, suggesting greater ghrelin production in cows during negative energy balance. Clearly, this should be a high priority area for further study.



### Direct inputs by nutrients

Digestive processes lead to the production of critical nutrients, such as amino acids, fatty acids (volatile or non-volatile) and carbohydrates, that flow into the circulation and can act as signals in their own right. However, in ruminants there is no strong evidence that these molecules are major regulators of the secretion of GnRH pulses. In female sheep, pulsatile LH secretion is not affected by intravenous infusion of precursors of neurotransmitters of large-neutral amino acids (Downing *et al.* 1995; 1996; 1997). In contrast, studies with rams suggest that fatty acids, a major currency in energy transactions in ruminants, affect GnRH secretion (Blache *et al.* 2000). The role of glucose is less clear – in male sheep, intra-abomasal and intravenous infusions of glucose failed to stimulate LH secretion (Boukhliq & Martin 1997; Boukhliq *et al.* 1996; Miller *et al.* 1995b). In dairy cattle, however, twice-daily drenching with propylene glycol, a propionate precursor and thus a source of glucose, can profoundly reduce PPAI in primiparous cows in low body condition (Chagas 2003). Indicators of energy status (body condition score, milk production, glucose, insulin, GH, IGF-I) were not altered by this treatment, but there was an increase in LH pulse frequency and in ovarian activity and, consequently, earlier ovulation postpartum. This suggests that there are nutritional inputs, perhaps some form of 'nutrient sensing or signalling', that are not associated with whole body energy status but can nevertheless stimulate the hypothalamic-pituitary-ovary axis in the early postpartum period (discussed further below). Through such a pathway, nutrients could trigger an endocrine response that would stimulate the reproductive axis. For example, it has been proposed the effects of propylene glycol on resumption of ovulation post-partum are due to the stimulation of an insulin spike (Miyoshi *et al.* 2001) that acts as a signal to increase LH secretion and therefore evoke ovulation.

### Interactions among the elements of the communicational network

Several levels of integration are needed because metabolic status at any given time depends on the status of all three key compartments – intake, storage and expenditure. The necessary interactions are managed by several hormonal systems, perhaps best exemplified by leptin secretion because it is affected by intake and expenditure as well as storage (mass of adipose tissue). In turn, leptin can stimulate the activity of three other endocrine systems involved in controlling the reproductive axis: i) pancreatic insulin in fasted cattle, albeit only at a low dose (20  $\mu\text{g}/\text{kg}$ ; (Zieba *et al.* 2003); ii) pituitary GH in female sheep (Henry *et al.* 2001) but apparently not normal-fed cattle (Zieba *et al.* 2005); and iii) thyroid hormones (Flier *et al.* 2000). In addition, leptin secretion is affected by other inputs, such as the products of digestion and absorption and autonomic neural activity. The autonomic effects are evident in the responses to challenges with beta-adrenergic analogues that decrease leptin sensitivity and stimulate lipolysis (Penicaud *et al.* 2000).

It is important to note that the ultimate level of integration is within the central nervous system (Fig. 2). The brain mechanisms involved in the sensing of metabolic status and in the connection of metabolic status to GnRH neuronal activity are poorly understood. Some sort of 'metabolic sensor' is thought to be localised in the arcuate nucleus and median eminence because receptors for leptin and insulin are found in these areas (Blache *et al.* 2002; Chilliard *et al.* 2005). The search for links between the GnRH cells and the neurons containing insulin and leptin receptors is continuing and there are many candidate intermediaries (see Blache *et al.* 2006). Orexins were initially among the most promising because of their sensitivity to energy balance (Taylor & Samson 2003) but intracerebral injections of orexin A or B inhibit GnRH

activity in mature Merino rams (Blache et al. 2003) so their relevance is questionable. On the other hand, we need to continue investigating the roles of the orexinergic pathways because high intakes of energy and protein dramatically reduce the expression of orexin receptor 2 in the paraventricular nucleus (Blache et al. 2006). We now need to test whether this change in sensitivity to orexins is linked to a change in reproductive activity or a change in food intake.

Recently, a new neuropeptide, kisspeptin, has been shown to stimulate GnRH and FSH secretion in male rodents (Gottsch et al. 2004; Irwig et al. 2004; Navarro et al. 2005) and to stimulate GnRH and LH secretion in female sheep (Messenger et al. 2005). Importantly, the kisspeptin receptor is found in over 75% of GnRH neurons in male rats (Irwig et al. 2004) and kisspeptin can be seen as major player in the processing of inputs into the systems that regulate GnRH secretion (Dungan et al. 2006). Interestingly, the ligand acts through a membrane receptor so kisspeptin pathways could mediate the rapid responses of the GnRH system to acute changes in metabolic status. Research is needed to test whether kisspeptin secretion or expression is regulated by energy balance.

The interactions among peripheral signals (insulin, leptin, ghrelin, adiponectin) and the neuroendocrine systems (kisspeptin) need to be revealed if we are to understand the integratory role of the brain in the process that leads to the 'decision to reproduce'.

#### 4) The temporal dimension

In this section, we consider the dynamic aspects of the responses to nutritional inputs, the effects of time *per se*, as seen in the effects of photoperiod and fetal programming, and, finally, the concepts of 'metabolic memory' and 'nutrient sensing', as we attempt to integrate the four dimensions into a unified theoretical framework.

#### Dynamics of responses to change in nutrition

In the ram, the response of the GnRH neurons to an abrupt change in nutrition is initially rapid and robust, but then fades over the next few weeks (Martin et al. 1994; Zhang et al. 2004). The rapidity of these responses is also consistent with the autonomic nervous system being involved at both brain and adipose levels. This could involve input from the digestive viscera to the brain via the vagus nerve, as is the case for satiety responses following a meal (Woods et al. 2004). Some nutrients that are absorbed very quickly, such as some volatile fatty acids, could also be involved in this rapid response. In fact, plasma concentrations of insulin increase about 3 h before the start of GnRH response to the initial increase in intake (Zhang et al. 2004).

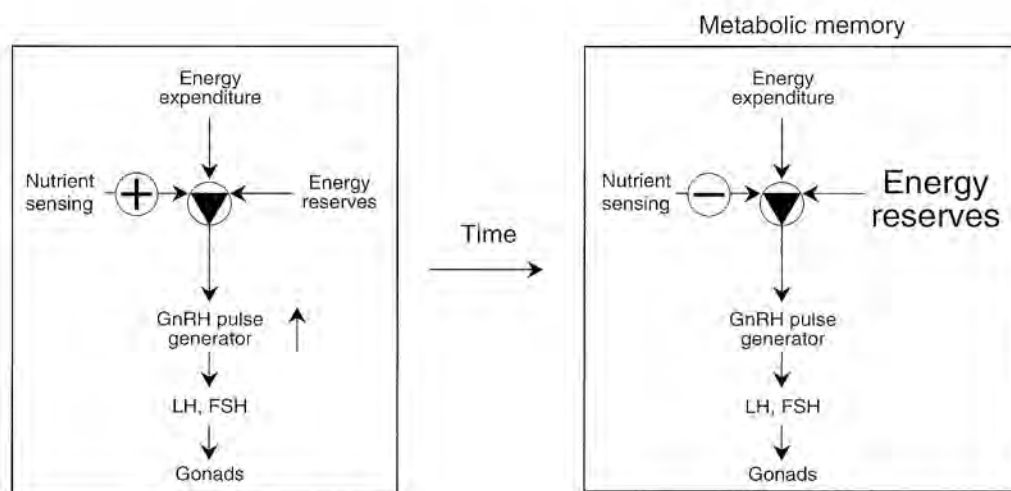
In contrast, the long-term effect of nutrition on the ram testis, measured on a scale of several weeks, seems to be independent of changes in the primary, GnRH-based, control system (Hötzel et al. 1995). The mechanism involved has not been studied further but, again, leptin might be implicated because, in the rat at least, the testis contains leptin receptor and leptin can inhibit testicular steroidogenesis (Tena-Sempere et al. 1999; Tena-Sempere et al. 2001).

On an annual timescale, the role of nutritional inputs, as well as the types of response to those inputs, can vary substantially, especially in genotypes that experience seasonal changes in appetite (Rhind et al. 2002). Some suggestions have been forwarded for control systems that might implement these strategies: i) ghrelin expression in sheep testis responds to changes in photoperiod (Miller et al. 2005); ii) leptin secretion varies among seasons (Alila-Johansson et al. 2004); iii) brain expression of relevant neuropeptides is affected by photoperiod (Adam et al. 2000); iv) adipocytes receive sympathetic and parasympathetic innervations, at least in the Siberian hamster, a seasonal animal (review: Bartness et al. 2002) – the sympathetic input controls several functions (eg, lipolysis, regulation of adipocyte number) including, most inter-

estingly, the secretion of leptin (Bartness *et al.* 2002); v) there are autonomic nervous projections from the adipocytes to the brain (Bartness *et al.* 2005), a connection that could provide metabolic information rapidly and directly to the reproductive centres (Fig. 2).

### Metabolic memory

In both sheep and cattle, previous metabolic status influences the reproductive response of the animals to an increase in energy availability. With respect to adipose stores, mature rams in low body condition, but not rams in high body condition, show a robust and repeatable increase in LH pulse frequency in response to an increase in intake. In low body condition rams, the leptin response is also blunted but the response to insulin is not (Zhang *et al.* 2005). These observations suggest that i) neither insulin nor leptin are necessary for inducing an increase in GnRH pulse frequency in response to an increase in food intake, and ii) leptin secretion does not always respond to an influx of nutrients. In contrast, in mature, grazing dairy cows calving in good body condition, pre-calving dry matter intake has no effect on PPAI or on post-calving plasma concentrations of leptin, IGF-I, or GH (Roche *et al.* 2005). In contrast, in first lactation heifers that have a low body condition score (4 on a scale of 10) 6 weeks prepartum, there are clear responses to an *ad-libitum* intake in the pre-partum period, in both metabolic hormones and LH pulse frequency (Chagas *et al.* 2006). One group of heifers in this study calved with low body condition and also received *ad libitum* feed postpartum, but they did not respond to postpartum feeding and they had low LH pulse frequency and also a longer PPAI. Together, these three studies in cattle and sheep (Chagas *et al.* 2006; Roche *et al.* 2005; Zhang *et al.* 2005) are consistent with the notion of a 'metabolic memory' that modulates the stimulatory effect of nutrient intake according to the level of either energy reserves or energy expenditure (Fig. 3).



**Fig. 3:** A schema describing the potential influence of a 'metabolic memory' (here, an increase in body reserves) on the stimulatory effect of the nutrient influx (via 'nutrient sensing'). On the left panel, the energy reserves are low and the 'nutrient sensor' is able to stimulate the hypothetical 'metabolic sensor' in the brain and thus the GnRH pulse generator. With time, the level of energy reserves may increase, as shown in the right panel, and the input from the nutrient sensor is decreased. Note: nutrients might be still able to stimulate gonadal function by pathways that are GnRH-independent.

Recently, the processes that might underpin 'metabolic memory' have been investigated in sheep and cattle and the data suggest that leptin and insulin are central to the concept (Chilliard *et al.* 2005). For example, leptin stimulates LH secretion from pituitary explants of normal-fed but not fasted cows (Amstalden *et al.* 2003). With respect to energy expenditure, insulin increases leptin secretion in sheep maintained in their thermoneutral zone but not in sheep enduring cold stress (Asakuma *et al.* 2003). In addition, growth factors, such as insulin and growth hormones, also affect leptin secretion in whole animal studies and in isolated adipocytes (Chilliard *et al.* 2005), and could therefore also be part of 'metabolic memory'. Other endocrine factors secreted by adipose tissue or by the digestive system might also be involved: for example, several metabolic effects have been described for adiponectin in rodents, such as increased insulin resistance and fatty acid oxidation, and reduced glucose output by the liver (review: Diez & Iglesias 2003). These interactions between endocrine systems and nutrient supply may effectively act as a sort of peripheral integratory mechanism that complements the brain's integratory mechanisms, with the combination being responsible for 'metabolic memory'.

### 'Nutrient sensing'

Short-chain fatty acids and glucose might also contribute to the 'metabolic memory' because they are known to regulate leptin secretion in cultured ruminant adipocytes (review: Chilliard *et al.* 2005). However, glucose is not always stimulatory in ruminants – for example, intravenous injection of glucose does not stimulate leptin secretion (Kauter *et al.* 2000) but infusion of glucose modifies the post-prandial pattern of leptin secretion in sheep carrying an extra copy of the GH gene (Kadokawa *et al.* 2003). However, in ruminants, volatile fatty acids (acetate, propionate, butyrate) are the main nutrients absorbed from the anterior digestive tract and they are converted to heavier fats for deposition via a well-known pathway of fatty acid synthesis. In rodents, fatty acids are directly involved in the regulation of leptin gene expression and leptin secretion (Shirai *et al.* 2004), and studies using cell culture have led to the development of a model to explain the mechanism by which glucose regulates leptin secretion from adipocytes (Mueller *et al.* 1998; Shirai *et al.* 2004). In this model, glucose, after being metabolised into pyruvate, acts on leptin gene expression and leptin secretion via the malonyl-coenzyme A fatty acid synthesis pathway, a crucial pathway in intracellular signalling in adipocytes (Shirai *et al.* 2004). Should a similar pathway exist in ruminants, it would allow volatile fatty acids produced by the digestive system, such as acetate (Annison *et al.* 2002), to regulate leptin secretion. Interestingly, a recent paper suggested that propionate does not stimulate leptin secretion in lactating cows (Bradford *et al.* 2006), in contrast to the results obtained in sheep (Lee & Hossner 2002). This difference could be caused by differences in metabolic status, and differences in the responsiveness of adipocytes to short chain fatty acids. This issue needs to be resolved by further research.

Finally, nutrients might also be able to act directly on components of the reproductive axis – for example, both acetate and butyrate can modulate the responsiveness of the anterior pituitary gland to leptin, so pituitary tissue could also be a site of interaction between hormones and nutrients in the control of reproductive function. Conversely, pituitary hormones might affect the activity or responsiveness of adipose tissue because cultured adipocytes express receptors for several pituitary hormones, including LH, FSH and GH (Schaffler *et al.* 2005). These receptors need to be investigated but they could be part of a descending pathway which regulates the activity of the adipocytes, not only according to metabolic status, but also according to the 'decision to reproduce'

## Conclusions

The 'decision to reproduce' is controlled by a range of factors and, amongst them, variation in metabolic status is one of the most important, especially for animals that have evolved in an environment where food supply is not predictable. Making a successful 'decision' depends on the integration of a large amount of information coming from a large number of organs. Over the last decade, the number and role of pathways involved in the link between metabolic status and reproductive activity has increased, and each pathway is acting in conjunction with the others. The latest developments suggest that the responses to each pathway vary with time so that, to fully understand the relationship between nutrition and reproduction, the temporal dimension (on scales of hours to years, or even a generation) should be considered because ruminants seem to be able to partition their energy, either from reserves or from nutrient intake, according to their metabolic history.

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