Effects of nutrition on testicular size and the concentrations of gonadotrophins, testosterone and inhibin in plasma of mature male sheep

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The effects of nutrition on the hypothalamo–pituitary–gonadal axis were studied in three groups of six mature Merino rams that were fed for 56 days with a ration that maintained their initial live mass (intermediate diet: 675 g chaff plus 175 g lupins), the same ration with a lupin supplement (high diet: 675 g chaff plus 825 g lupins), or about half of the intermediate ration (low diet: 475 g chaff plus 125 g lupins). Lupin seed provides a highly (95%) digestible source of energy and protein. Plasma concentrations of LH, FSH, testosterone and inhibin were measured in blood samples collected over 24 h on the day before dietary treatments began (day – 1), then on days 0, 1, 5, 14, 28 and 56. Compared with the intermediate diet, the high diet significantly increased live mass within 14 days and testicular size within 28 days, and these differences increased steadily throughout the experiment. Plasma FSH concentrations and LH pulse frequency increased within 5 days, but these effects were maintained for only 14 days. Decreasing the nutritional status reduced live mass and testicular size within 7 days, led to a low LH pulse frequency that persisted throughout the experiment, but did not affect FSH concentrations. Significantly less testosterone was secreted over 24 h in the low dietary group than in the intermediate or high group until day 28. The high group tended to secrete more than the intermediate group, but only at the beginning of the experiment when LH pulse frequencies differed between these groups. The testosterone response to each endogenous LH pulse, or following an injection of ovine LH i.v. (200 ng kg^{–1} live mass), was not related to testicular size or dietary treatment at any stage of the experiment. Similarly, plasma inhibin concentrations were not related to change of diet, despite large differences in testicular size. We concluded that the effects of nutritional status on testicular size in mature rams are at least partly mediated through changes in gonadotrophin secretion. Both increases and decreases in food supply affected LH pulse frequency, suggesting the involvement of hypothalamic mechanisms. However, the lack of an effect of a decrease in nutritional status on the secretion of FSH and inhibin and the inconsistent long-term relationship between LH pulse frequency and testicular size suggest that the effects of diet on testicular growth also involve mechanisms that are independent of changes in gonadotrophin secretion.

Introduction

The testicular size of mature Merino rams increases when the animals are placed on a high plane of nutrition and decreases when they are placed on a low plane of nutrition (Lindsay *et al.*, 1976; Oldham *et al.*, 1978; Masters and Fels, 1984; Thwaites and Hannan, 1989; Murray *et al.*, 1990). These responses are large relative to concomitant changes in body mass, detectable within a few weeks, persist for several months and lead directly

to changes in the rate of production of spermatozoa (Oldham *et al.*, 1978). However, the underlying physiological mechanisms are poorly understood, primarily because few studies have included hormonal measurements. From first principles, we would expect the hypothalamic–pituitary axis, acting primarily through the gonadotrophins, to be involved in testicular responses to food supply in the same way that it is involved in responses to other environmental stimuli, such as photoperiodic and social cues (Lincoln and Short, 1980; Martin *et al.*, 1990). This hypothesis is supported by studies with rats, hamsters and cattle, in which the effects of nutrition on testicular mass have been positively correlated with changes in the circulating concentrations of gonadotrophins and

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testosterone (Howland and Skinner, 1973; Howland, 1975, 1979; Campbell *et al.*, 1977; Eskes, 1983; Gauthier and Coulaud, 1986).

However, for rams the picture is confusing because, in our initial studies, we found that an increase in nutritional status stimulated testicular growth for many weeks but either did not change the frequency of the pulses of LH or increased it only in the first few days after the change of diet (Ritar *et al.*, 1984; Martin *et al.*, 1987a). We therefore need to reassess the role of the hypothalamus, as indicated by LH pulse frequency. Moreover, the circulating concentrations of FSH also control testicular activity and might be affected by food supply, and the effect of nutrition on the secretion of this gonadotrophin has not yet been studied. Finally, the effects of diet on the gametogenic function of the ram testis have been documented in detail (Oldham *et al.*, 1978), as has the potential for dietary management of rams in livestock production (Lindsay *et al.*, 1976), but nutrition-induced changes in endocrine function have not been studied. We therefore measured the secretion of the hormones produced by the Leydig cells (testosterone) and Sertoli cells (inhibin) in the testis.

In the study described here, we tested the hypothesis that in mature Merino rams changes in testicular size caused by increases and decreases in food supply, relative to the requirements for maintenance of live mass, are positively correlated with changes in the secretion of gonadotrophins, testosterone and inhibin. Preliminary reports of this work have been presented elsewhere (Martin *et al.*, 1989, 1992, in press; Tjondronegoro *et al.*, 1990).

Materials and Methods

Animals and treatments

Eighteen intact Poll Merino rams (*Ovis aries*), 18 months old and weighing 49.3 ± 0.7 kg, were brought off lush pasture and placed in single pens in an animal house under natural lighting from October to December (spring and early summer, latitude 32°S). They were acclimatized to a diet calculated to maintain body mass (675 g chaff plus 175 g lupins per ram per day) for 3 weeks, then assigned to three dietary groups of six rams each: 'high diet' (675 g chaff plus 825 g lupins daily); 'intermediate diet' (675 g chaff plus 175 g lupins daily); and 'low diet' (475 g chaff plus 125 g lupins daily). Lupin is a legume (*Lupinus angustifolius*) that produces seed similar to soya beans, which provides a highly digestible (about 95%) source of energy and protein. These diets were fed for 56 days, every morning at about 09:00 h.

Measurements and collection of blood samples

Each week, before feeding, the animals were weighed and testicular size was estimated with the animal in a standing position, by retaining both testes in the base of the scrotum and measuring the combined circumference of scrotal tissue plus the two testes. On days -1, 0, 1, 5, 14, 28 and 56 relative to dietary change (day 0 is the first day of dietary treatment), blood samples were collected at intervals of 20 min for 24 h. Jugular cannulae were inserted the day before sampling. On

day 28, one ram in the group on the low diet could not be cannulated and so was excluded from the remainder of the experiment.

On days 1 and 56, pituitary responsiveness was tested by an i.v. injection of 4 ng GnRH kg^{-1} body mass ('HRF', Ayerst Laboratories Pty Ltd, Parramatta, NSW). The LH response was estimated from blood sampled from the time of injection of GnRH until 2 h later. The first three samples were taken at 10 min intervals and the remainder every 20 min.

On day 55 (1 day before the last 24 h sampling period), testicular responsiveness was tested by an i.v. injection of 200 ng ovine LH kg^{-1} body mass (NIADDK-oLH-25). Testosterone response was estimated from blood sampled from the time of LH injection until 2 h later. The first three samples were taken every 10 min and the rest every 20 min.

Hormone assays

Plasma concentrations of LH were measured in all samples. The assay technique was similar to that reported elsewhere (Martin *et al.*, 1980), except for the use of anti-LH serum R3(1-1) kindly provided by A.S. McNeilly (McNeilly *et al.*, 1976). The preparation CNRS-M3 (biopotency 1.8 iu NIH-LH-S1 mg^{-1}) used for both iodination and reference was kindly supplied by M. Jutisz (Collège de France, Paris). The limit of detection of the standard curve (for all assays) was calculated by subtracting two SDs from the mean counts bound in nine replicates of the zero standard. The samples for LH were assayed as duplicate 100 μl aliquots and the limit of detection was 0.28 $\mu\text{g l}^{-1}$. Intra-assay variation (mean $\pm$ SEM) was estimated in each assay using at least five replicates of three pooled plasma samples containing 0.6 ($21.5 \pm 1.0\%$), 1.43 ($12.3 \pm 0.98\%$) or 7.16 ($7.6 \pm 0.33\%$) $\mu\text{g l}^{-1}$. The interassay coefficients of variation were 36.8%, 24.1% and 8.8%, respectively, and the effect that this would have had on the detection of LH pulses was avoided by assaying all samples from one animal in the same run. The pooled sample with the low concentration was used to improve the reliability of pulse detection around the baseline of each LH profile (see below).

Plasma concentrations of FSH were assayed in hourly samples from the intensive sampling, using a kit kindly supplied by A. F. Parlow of the National Institute of Diabetes, Digestive and Kidney Disease (Baltimore, MD). The kit comprised antiserum oFSH-1, reference preparation oFSH-RP-1 (biopotency 75 iu NIH-FSH-S1 mg^{-1}) and tracer preparation oFSH-I-1. The samples were assayed as duplicate 100 μl aliquots and the limit of detection was 0.27 $\mu\text{g l}^{-1}$. Intra-assay variation was estimated in each assay using at least five replicates of three pooled plasma samples containing 1.8 ($9.5 \pm 1.0\%$), 3.8 ($6.0 \pm 0.43\%$) or 7.51 ($6.4 \pm 0.61\%$) $\mu\text{g l}^{-1}$. The interassay coefficients of variation were 9.6%, 6.9% and 10.1%, respectively.

Concentrations of testosterone were measured in serial samples taken on day -1, 5, 28 and 56. The assay has been described by Martin *et al.* (1987a) and involves extraction of plasma and separation of bound and free fractions with dextran-coated charcoal. The anti-testosterone serum (no. 457) had been raised against testosterone-3-carboxy-methyl-oxime-BSA in sheep and displayed only a few significant

crossreactions: 5 α -dihydrotestosterone (98%); 4-androsten-3 β ,17 β -diol (47%); 4-androsten-3,17-dione (4.7%); 4-androsten-17,19-diol-3-one (3.6%); and androsterone (1.0%). The antiserum and crossreaction data were kindly provided by R. I. Cox (CSIRO Division of Animal Production, Prospect, NSW). The limit of detection was 0.42 ± 0.07 nmol l⁻¹. The non-specific binding was $2.2 \pm 0.13\%$. Included in each assay were six replicates of three pooled plasma samples containing 17.68, 7.70 and 1.98 nmol l⁻¹. They were used to estimate the coefficients of variation within assays (8.7 ± 1.5 , 8.2 ± 1.6 and $12.4 \pm 1.4\%$) and between assays (6.8, 6.3 and 16.4%).

Concentrations of inhibin were measured in samples collected at intervals of 2 h selected from the 24 h serial samples, using the double-antibody radioimmunoassay described in detail by McNeilly *et al.* (1989). This method is briefly described as follows: the antiserum (R150) was raised in a rabbit immunized against a synthetic 1–26 sequence of the α chain of porcine inhibin. At a final dilution of 1:75 000, it specifically bound 24% of labelled antigen. The synthetic peptide was used as reference, and as a tracer after it was labelled with ¹²⁵I by the chloramine-T method. An amount of plasma equivalent to that in the samples was added to each standard. The limit of detection was $0.08 \mu\text{g l}^{-1}$ plasma and nonspecific binding was 2.4%. All samples were measured in a single assay, which included six replicates of three pooled plasma samples containing 2.79, 1.17 and $1.55 \mu\text{g l}^{-1}$. They were used to estimate the intra-assay coefficients of variation (4.8, 6.1 and 4.4%).

Pulse analysis

The serial samples were analysed with a modified version of the 'Pulsar' algorithm developed by Merriam and Wachter (1982) and modified for the Apple Macintosh[®] computer ('Munro', Zaristow Software, West Morham, Haddington, East Lothian), as described by Martin *et al.* (1987b). The G parameters (the number of SDs by which a peak must exceed the baseline in order to be accepted) were 3.98, 2.8, 1.68, 1.34 and 0.93 for G1–G5, these being the requirements for pulses composed of 1–5 samples that exceed the baseline, respectively. The Baxter parameters describing the parabolic relationship between the concentration of hormone in a sample and the SD (assay variation) about that concentration were 0.34862 (b_1 , the y intercept), 0.02548 (b_2 , the x coefficient) and 0.01161 (b_3 , the x^2 coefficient). The pulse frequency, the mean pulse amplitude (the difference between pulse peak and preceding nadir) and the mean concentration of LH were calculated for each profile.

Statistical analysis

All data, excluding those recorded before treatments were imposed (i.e. points leading up to and including day -1), were subjected to repeated measures analysis of variance. Individual means were compared using least significant differences ($P < 0.05$) when the main effects (treatment, time) were significant ($P < 0.05$). The differences between groups in pituitary responsiveness were determined by comparing the amplitude and area of the LH response following GnRH injection. The

Fig. 1. The effect of diet on (a) live mass and (b) scrotal circumference of Merino rams as a percentage of the circumference at week 0. The points on each line are means $\pm$ SEM for the animals fed to maintain their initial body mass [(●) the intermediate diet], to lose body mass [(○) the low diet] or to gain body mass [(■) the high diet].

same technique was used for testicular responsiveness (testosterone production in response to LH). The amount of testosterone produced in each 24 h period was calculated from the sum of concentrations within the area of testosterone pulses (i.e. excluding baseline values in the interpulse periods).

Results

Live mass

Live mass remained constant throughout the treatment period in the group fed the intermediate diet (Fig. 1a). In the other groups, the dietary changes affected live mass within the first 2 weeks ($P < 0.05$), after which the animals fed the high diet gained mass steadily until the end of the experiment. Live mass was reduced by day 7 in the rams fed the low diet ($P < 0.05$ versus intermediate diet) and remained lower than the values for the intermediate group until the end of the experiment. The difference between these two groups was relatively constant for most of the experiment, suggesting they were both being fed to maintenance but at different levels of body condition.

Testicular size

The change from pasture to the animal house diet led to a small decrease in testicular size in all groups during the acclimatization period (Fig. 1b). Thereafter, the scrotal circumferences of all three groups tended to increase as summer began. During the first 4–6 weeks after the imposition of treatments, the effects of diet on scrotal circumference were similar to the effects on live mass. In the group fed the intermediate diet, there was no significant difference between

Fig. 2. The effect of diet on (a) the frequency of LH pulses, (b) LH pulse amplitude, and (c) mean concentrations of FSH in Merino rams. Each point represents the mean $\pm$ SEM for the animals fed to maintain their initial body mass [(●) the intermediate diet], to lose body mass [(○) the low diet] or to gain body mass [(■) the high diet].

the values for day 0 and those for day 56. The testes of the high diet group changed very little in size during the first 3 weeks, then began to grow and, by week 4, were significantly larger than the testes of the intermediate group ($P < 0.05$). They continued to grow steadily throughout the 2-month observation period and always remained significantly larger than those in the intermediate group. In the low diet group, testicular size decreased rapidly after the change of diet and was significantly smaller than that of the intermediate group from 2 weeks until the end of the observation period ($P < 0.05$). During the last month of the experiment, while both of these groups maintained constant live mass, the difference between their testicular sizes remained fairly constant.

Gonadotrophin secretion

LH pulse frequency. There were significant effects of diet ($P = 0.0001$) and time ($P = 0.026$), with increases in nutritional status leading to increased pulse frequency and decreases in nutritional status leading to reduced pulse frequency, relative to the intermediate group. In the intermediate group, pulse frequency changed little throughout the experiment, except at about day 1, when the value was significantly higher than for days -1 and 0 (Fig. 2a). In the group fed the high diet, the addition of the lupin supplement on day 0 was associated with a significant increase in LH pulse frequency above values observed on day -1, before the change of diet (within-diet comparison), but the concurrent changes in the intermediate group meant that the difference between these treatments was

Fig. 3. The effect of diet on the LH response to an injection of GnRH i.v. (4 ng kg^{-1} body mass) on (a) day 1 and (b) day 56 after the change of diet. Each point represents mean $\pm$ SEM for Merino rams fed to maintain their initial body mass [(●) the intermediate diet], to lose body mass [(○) the low diet] or to gain body mass [(■) the high diet].

significant only on day 5. After day 5, the pulse frequency in the high group declined to values that were not significantly different from those in the intermediate group until the end of the experiment. In the low group, dietary changeover significantly reduced LH pulse frequency on the first day ($P = 0.003$, relative to the intermediate group on day 0) and the values in these animals remained significantly lower than for the other two groups until day 28. Over days 14–56, the frequency increased gradually in the low group and it was not significantly different from the other groups on day 56.

LH pulse amplitude and pituitary responsiveness to GnRH.

There were no effects of diet, time or interaction between diet and time on the amplitude of LH pulses (Fig. 2b). The LH responses to exogenous GnRH on days 1 and 56 after dietary treatments (Fig. 3) were similar for the treatment groups. Repeated measures analysis of variance indicated no significant effect of diet on pituitary responsiveness ($P = 0.23$), but significant effects of time ($P = 0.0003$), because the amplitude of the response in all groups was higher on day 56 than on day 1.

Plasma concentrations of FSH. Analysis of variance showed that the gradual increase in FSH concentrations as the experiment progressed was significant ($P = 0.0001$), that the overall effect of diet was not ($P = 0.15$), but that there was an interaction ($P = 0.05$) between diet and time. Between-point comparisons showed that FSH concentrations were significantly greater in the high group than in the intermediate and low groups on days 5 and 14 ($P = 0.04$) only. In the low group, the mean concentrations of FSH tended to be higher than those in the intermediate group throughout the experiment, but the effect was not significant (Fig. 2c).

Secretion of testicular hormones

Inhibin concentrations. There was a significant effect of time ($P = 0.001$) but not of diet, owing to the slow increase in inhibin concentrations as the experiment progressed (Fig. 4a). The tendency for low concentrations of inhibin in the high dietary group reflected chance differences resulting from assignment to groups rather than an effect of diet.

Fig. 4. The effect of diet on (a) the plasma concentrations of inhibin and (b) the total amount of testosterone released in Merino rams during 24 h after change of diet. For the inhibin data, each point represents the mean $\pm$ SEM for the animals fed to maintain their initial body mass [(●) the intermediate diet], to lose body mass [(○) the low diet] or to gain body mass [(■) the high diet]. For the testosterone data, the horizontal shaded area represents the mean $\pm$ SEM ($n = 24$) of the testosterone released on day -1 (before treatment) and the histograms represent means $\pm$ SEM for the animals fed the intermediate diet (▨), the low diet (□) and the high diet (■). *Significantly different ($P < 0.05$) from intermediate and high diets.

Testosterone secretion. Testosterone was measured in serial samples taken on day -1 (before dietary treatment started), day 5 (when differences in LH pulse frequencies between groups were significant), day 28 (when differences in testicular size between groups were significant), and day 56 (when testicular size, but not LH pulse frequency, differed between treatments). The effect of diet on the 24 h production of testosterone at these times, as estimated by the sum of the values in all of the pulses detected, is presented in Fig. 4b. There were significant effects of diet ($P = 0.047$), time ($P = 0.024$) and the interaction between diet and time ($P = 0.034$). There was no significant difference between the intermediate and high dietary groups on any day, although the rams on the high diet had the greatest values on day 5 when the LH pulse frequency for this group was above that for the others. The animals in the low dietary group secreted significantly less testosterone than did the other groups on days 5 and 28 ($P < 0.05$). On day 56, there were no significant differences in testosterone production, just as there were none in the frequency of LH pulses (Fig. 2a).

Testicular responsiveness to LH. Testicular responsiveness, the amount of testosterone released per unit of LH, was estimated by studying the response to endogenous LH pulses and the response to exogenous LH. The endogenous response was calculated by dividing the total area under testosterone pulses by the number of endogenous LH pulses observed in 24 h on days 5, 28 and 56. There were no significant effects of dietary treatment or time (Fig. 5a). The amplitude of the testosterone response to exogenous LH was only studied on day 56, and was not affected by dietary treatment (Fig. 5b, c). As with the endogenous pattern, the highest concentrations were observed in the intermediate dietary group (Fig. 5b) but, in this instance, the area under the curve was significantly larger in these rams than in those on the low and high diets

(Fig. 5c), and the mean concentration after LH injection was also higher ($18.97 \pm 2.25 \text{ nmol l}^{-1}$ compared with 11.72 ± 1.84 and $9.60 \pm 0.31 \text{ nmol l}^{-1}$ for the low and high dietary groups). Between the low and high dietary groups, there were no differences for any of the variables measured.

Discussion

Changes in testicular size that were induced by manipulation of the diet were associated with changes in gonadotrophin secretion, suggesting that the hypothalamic-pituitary axis plays a role in mediating the reproductive responses of mature Merino rams to changes in nutritional status. However, the differences between the effects of increases and decreases in nutritional status on the secretion of LH and FSH, the transitory nature of some of the gonadotrophin responses, and the lack of effects on inhibin and testosterone secretion, suggest that gonadotrophin concentrations in the circulation are not the only factor leading to the changes in the testes.

As well as comparing the responses to both increases and decreases in nutritional status, this study allows comparison of both acute and chronic effects of a change in diet. The acute responses are clearly and consistently associated with changes in gonadotrophin secretion (Martin *et al.*, in press) and so are the simplest to interpret mechanistically. However, with regard to the longer-term effects, it is important to realize that, after a few weeks on the dietary treatments, both the low and the intermediate dietary groups were effectively being fed to maintenance. Thus, differences (or lack of differences) between them probably reflects static differences in body stores and food intake, rather than differences in metabolic state. By contrast, long-term differences between these groups and the high dietary group include an extra level of complexity, because the rams fed the high diet are in an anabolic state,

Fig. 5. The effect of diet on the testosterone response of Merino rams to (a) an endogenous LH pulse on days 5–59 after the change of diet and (b,c) an injection of LH i.v. (200 ng kg^{-1} body mass) on day 59 after the change of diet. The points on each line are means $\pm$ SEM for the animals fed to maintain their initial body mass [the intermediate diet ($\bullet$)], to lose body mass [the low diet ($\circ$)] or to gain body mass [the high diet ($\blacksquare$)]. In (a), the horizontal shaded area represents the mean $\pm$ SEM ($n = 24$) of the testosterone released on day -1 (before treatment). In (a) and (c) the histograms represent mean $\pm$ SEM for the animals fed the intermediate diet ($\hatched$), the low diet ($\square$) and the high diet ($\blacksquare$).

rather than at maintenance. This consideration must be kept in mind when interpreting the gonadotrophin responses to the diets used in this study.

The increase in testicular size in response to the lupin supplement agrees with previous findings (Lindsay *et al.*, 1976; Oldham *et al.*, 1978; Ritar *et al.*, 1984; Martin *et al.*, 1987a; Murray *et al.*, 1990) and is associated with an increase in the frequency of LH pulses observed over the first 5–14 days following lupin supplementation. After the peak on day 5 of lupin supplementation, there was a gradual decline in frequency and control values were re-established by day 28. This finding is in agreement with the observations of both Ritar *et al.* (1984) and Martin *et al.* (1987a), and thus resolves the apparent conflict between these two studies. Clearly, in our earlier study (Martin *et al.*, 1987a), we failed to show a significant increase in LH secretion following lupin supplementation because we did not measure pulse frequency until 9 weeks after the change of diet. By this time, the testes had grown significantly but LH secretion would have reached peak values and returned to control values.

The lupin supplement also increased the concentrations of FSH, a response that has not previously been demonstrated; thus, this gonadotrophin might also have played a role in the induction of testicular growth. Since LH pulses have a one-to-one relationship with GnRH pulses in rams (Caraty and Locatelli, 1988), and GnRH pulse frequency at least partially controls FSH secretion (Lincoln, 1979), it is likely that there is a GnRH-dependent link between nutritional status and reproductive activity in the mature ram. These effects of a high plane of nutrition on gonadotrophin secretion and testicular size are consistent with both the seasonal onset of reproductive activity in mature rams (Lincoln and Short, 1980) and the onset of puberty in immature rams (Olster and Foster, 1986; Martin and White, 1992). However, the disappearance of the LH and FSH responses after only a few weeks in the rams fed the high diet, despite the maintenance of body and testicular growth, is not consistent with these other situations – nor is the lack of effect on testosterone and inhibin production.

Decreasing nutritional status decreased testicular size, in agreement with the findings of Oldham *et al.* (1978) and

Thwaites and Hannan (1989). In the present study, the restriction of food intake led to a rapid reduction in LH pulse frequency, and the maintenance of low body mass was associated with a relatively persistent low frequency of LH pulses. This finding is supported by work on peripubertal Suffolk ewes (Foster and Olster, 1985) and Merino rams (Martin and White, 1992) and, particularly, the data from mature Ile-de-France rams (Lindsay *et al.*, 1984). All of these observations are consistent with the short-term responses to our high diet and reinforce the conclusion that a nutritional cue(s) acts at the hypothalamic level to affect the frequency of GnRH pulses. However, in contrast to the effects of the low diet on LH secretion, as well as the effects of the high diet on FSH concentrations, reducing the plane of nutrition did not decrease FSH secretion – indeed, there was not even a trend in that direction that would encourage further experimentation. This observation also disagrees with those showing that FSH concentrations are reduced by protein deficiency and starvation in male rats (Campbell *et al.*, 1977; Glass *et al.*, 1979, 1982) and by loss of body mass in female sheep (Thomas *et al.*, 1990). This conflict is most likely due to the degree of dietary restriction: as mentioned above, our rams on the low diet were neither starved nor continually lost body mass as the experiment progressed – they were only held at ‘maintenance’ at a lower body mass.

This explanation aside, the difference between the FSH and LH responses to the low diet was not expected, considering the key role played by GnRH pulse frequency in the control of both gonadotrophins, and the lack of any clear effect of diet on the circulating concentrations of inhibin, the testicular hormone that often explains differences between the patterns of secretion of LH and FSH. Possible explanations include the following.

First, there may be differences in the way testicular feedback interacts with nutrition in the control of the two gonadotrophins in intact rams – LH pulse frequency directly reflects the hypothalamic responses to nutritional status, whereas FSH secretion reflects both the response to hypothalamic stimulation and the response to the level of negative feedback by the testes at the pituitary gland. Circulating inhibin concentrations

were not affected by diet but testosterone concentrations over time were, so the tendency for FSH secretion to decrease because of reduced hypothalamic drive on the low diet may have been counteracted by a reduction in the responsiveness of the pituitary gland to the combined action of inhibin and testosterone.

Second, there may be differences between FSH and LH in the nature of their pattern of secretion and their dependence on the GnRH stimulus – LH pulses may be a more sensitive indicator of hypothalamic drive than FSH concentrations. Although the low diet reduced live mass and testicular size in the present study, the difference between the high and the intermediate diets was much greater than the difference between the intermediate and low diets. Thus, to demonstrate the effects of undernutrition on FSH or inhibin secretion in mature rams, we might only have to test planes that sustain loss of body mass, as Thomas *et al.* (1990) did for the ewe, or starvation, as Glass *et al.* (1979) did for male rats.

Changes in nutritional status did not affect pituitary responsiveness to GnRH. This finding is consistent with the lack of effect of diet on the amplitude of LH pulses despite significant differences in their frequency, and suggests that few of the effects of nutritional status on testicular size are mediated by changes at the pituitary level. The same conclusion has also been drawn for ewes (Rhind *et al.*, 1989). In contrast, the effects of photoperiod on LH secretion and testicular size in rams do include an effect on pituitary responsiveness to GnRH (Lincoln, 1976, 1977; Sanford *et al.*, 1984).

The testosterone responses reflected the LH responses to both diets, and this is not surprising, since each pulse of LH induces a pulse of testosterone (Katongole *et al.*, 1974; Sanford *et al.*, 1977). As with the effect on LH secretion, the testosterone response was only short-lived. What was not expected was the lack of an effect on the amount of testosterone produced per LH pulse after the testes had changed in size, an effect that contrasts with the responses to photoperiod in Soay rams (Lincoln, 1976) or puberty in Merino rams (Martin *et al.*, 1994). Similarly, there was no change in the relationship between FSH and inhibin concentrations in the circulation, as was expected from the seasonality studies of Lincoln *et al.* (1990). Hochereau-de Reviers *et al.* (1985) showed that there was no hyperplasia of the Leydig or Sertoli cells during photoperiod-induced growth of the testis, and a similar quantitative histological study is required to test whether the same applies when testicular growth or regression are induced by feeding.

In a review of the role of nutrition in seasonal reproductive cycles in male ruminants (Martin *et al.*, in press), we observed that nutrition and photoperiodic cues will reinforce each other in temperate climates, but act against each other in Mediterranean-type climates. Thus, interactions between nutritional and photoperiodic cues add an extra level of complexity to the control of seasonal reproductive cycles in mature male sheep (Martin *et al.*, 1990). A third level of complexity is added by social cues, because females exert powerful effects on the secretion of GnRH, LH and testosterone. A fourth level of complexity is added by genotype because photoperiodic cues appear to be more dominant in some breeds than in others (Lincoln *et al.*, 1990). Thus, Merino rams in Mediterranean-type environments are photoperiodic with a 'breeding season' in mid-summer (as can be seen in Fig. 1) but do not exhibit strict,

photoperiodically timed patterns of testicular growth. Instead, they are 'opportunistic' breeders with a reproductive strategy that includes short-term responses to nutrition and social factors (Martin *et al.*, 1990, in press).

In conclusion, the effects of changes in nutritional status on testicular size in mature rams are only partly related to changes in the activity of the hypothalamic-pituitary system controlling gonadotrophin secretion. The inconsistencies in the long-term relationship between the patterns of gonadotrophin secretion and testicular size suggest that 'GnRH-independent' mechanisms are also involved in the effects of diet on testicular growth. Moreover, the lack of a relationship between diet, testicular mass and the production of testicular hormones suggests that nutrition affects the somatic and gametogenic compartments of the testis far more readily than it affects the endocrine compartments. Although there is little doubt that the gonadotrophins are essential for testicular growth, development and function, it appears that changes in their secretion are not.

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