Circulating gonadotrophins during a period of restricted energy intake in relation to body condition in heifers

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Summary. Beef heifers, 13 months old, were fed to achieve high (7.6 ± 0.2 units) or low (3.9 ± 0.1 units) body condition by feeding them one of two diets for 20 weeks. During week 17 of the growth phase, all heifers were ovariectomized. From week 20 to week 27 (restriction phase), all heifers were fed a daily diet containing 0.071 MJ metabolizable energy kg^{-1} body weight. At weekly intervals throughout the restriction phase, blood samples were collected at 10-min intervals for 11 h to determine the pattern of secretion of luteinizing hormone (LH), the amount of LH released in response to 750 ng (pituitary responsiveness) and 50 μg LH-releasing hormone (LHRH, releasable stores) and mean concentrations of follicle-stimulating hormone (FSH) in the circulation. Body weight declined during the restriction phase in a similar fashion in heifers with high and low body condition and changes in body weight were unrelated to mean concentrations of LH and FSH and frequency of LH pulses. Amplitude of LH pulses and responsiveness to 750 ng LHRH increased in a linear fashion with weight loss in heifers with low but not in those with high body condition. The amount of LH released in response to 50 μg LHRH decreased with increasing weight loss in heifers with high but not with low body condition, indicating that releasable pools of LH declined with increased weight loss in heifers with high body condition. The results indicated that the pattern of LH in the circulation, responsiveness of the pituitary to LHRH and releasable stores of LH in heifers fed diets of low energy content are modulated by body condition.

Keywords: body condition; energy; luteinizing hormone; follicle-stimulating hormone; heifers

Introduction

Good body condition in cattle reflects high amounts of energy stores in body tissue (Wright & Russel, 1984). Changes in body condition, direction of body weight change and intake of dietary energy influence the duration of anoestrus after parturition (Wiltbank *et al.*, 1962; Dunn & Kaltenbach, 1980) and during the prepubertal period (Kinder *et al.*, 1987) in bovine females. Nutritionally induced anoestrus or a delay in the onset of puberty in heifers that results from feeding them diets with low energy content is mediated by a suppression of luteinizing hormone (LH) in the circulation (Imakawa *et al.*, 1986; Day *et al.*, 1986).

Results from our laboratory support the hypothesis that the direction of change in body weight in heifers of similar body weight influences concentrations of circulating LH (Roberson *et al.*, 1991). The magnitude of suppression of LH in heifers losing body weight is related to body condition at the time that feeding of diets with low energy content is initiated (Roberson *et al.*, 1991).

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The pattern of LH in the circulation changes with altered concentrations of circulating glucose, insulin, nonesterified fatty acids and urea nitrogen when cows (Richards *et al.*, 1989) or heifers (Roberson *et al.*, 1991) are being fed with diets low in energy content. Thus, high stored energy in body tissues available for use in homeorhetic changes in metabolism (Bauman & Currie, 1980) during periods of low energy intake may modulate changes in the concentrations or the pattern of gonadotrophins in the circulation. Our working hypothesis in the present study was that body condition influences mean concentrations and the pattern of gonadotrophins in the circulation, as well as the pituitary response to LH-releasing hormone (LHRH) and releasable LH in heifers fed a diet of low energy content.

Materials and Methods

Animals and diets

Sixteen beef heifers ($\frac{1}{4}$ Angus, $\frac{1}{4}$ Hereford, $\frac{1}{4}$ Red Poll and $\frac{1}{4}$ Pinzgauer), 390 ± 6 days old and weighing 273 ± 3 kg, were fed similar diets before the study. Care was taken to ensure that all heifers were acclimatized to stanchions and human contact to avoid possible stress-related alterations in the pattern of gonadotrophin secretion. Heifers were group fed to achieve high ($n = 8$) or low ($n = 8$) body conditions, evaluated on a scale of 1 (emaciated) to 9 (obese) as reported by Whitman (1975). To attain the target body condition of 7 (high) and 4 (low), heifers were divided into two groups and fed with either an 80% maize and 20% brome grass hay (high) diet or an 80% brome grass hay and 20% maize (low) diet *ad libitum* for 20 weeks (growth phase). Seventeen weeks after initiation of the growth phase (i.e. 17 weeks after the start of the experiment), all heifers were ovariectomized via high lumbar laparotomy under local anaesthesia (lidocaine 2%) to allow evaluation of steroid-independent effects of treatments on secretion of gonadotrophins. From week 20 to week 27 (restriction phase), all heifers were fed daily a diet containing 0.071 MJ metabolizable energy kg^{-1} of body weight. Throughout the restriction phase, daily dry matter intake was 3.9 and 3.5 kg of diet in heifers with high and low body condition, respectively. Heifers were fed individually once a day at 07:00 h during the restriction phase; such feeding has been reported (Roberson *et al.*, 1991) to alter the profiles of gonadotrophins in the circulation of heifers of similar body weight to those in the present study. The diet was composed of brome grass hay (54.5%), maize gluten meal (8.7%), soyabean meal (36.0%) and dicalcium phosphate (0.8%). Heifers with high and low body condition were fed amounts of crude protein each day that met or exceeded National Research Council (1976) recommendations.

Assessment of weight and body condition

Body weights were recorded for individual heifers at weekly intervals before feeding throughout the restriction phase, and weight change was calculated. Body condition was also recorded for individual heifers at weekly intervals during the restriction phase, as the average of three independent measurements.

Blood sampling

At the start of the restriction phase and at weekly intervals thereafter, a catheter was inserted in the jugular vein one day before the start of a regimen of serial blood collection. Blood samples were collected at intervals of 10 min for 11 h beginning 2 h after feeding. LHRH was administered in bolus injections via the jugular catheters at 9 (750 ng) and 10 h (50 μg) after the start of blood collection; the lower dose was used to evaluate pituitary responsiveness to LHRH and the higher dose was used to estimate pituitary stores of LH (Crowder *et al.*, 1982). Blood samples were allowed to clot at room temperature and stored for 24 h at 4°C. Serum was collected after centrifugation at 1520 g for 15 min and stored at -20°C until assayed for LH and follicle-stimulating hormone (FSH). Additional blood samples were collected at hourly intervals within each sampling period into tubes containing heparin (143 U tube $^{-1}$) and sodium fluoride (10 mg ml^{-1} whole blood) to inhibit degradation of glucose by red blood cells. These samples were placed on ice immediately after collection and centrifuged at 1520 g for 15 min within 4 h of collection and plasma stored at -20°C until assayed for urea nitrogen and glucose.

Assays

Concentrations of LH in serum samples were determined by radioimmunoassay (Adams *et al.*, 1975; Wolfe *et al.*, 1989) using a rabbit antiserum against ovine LH (TEA-RAOLH no. 35), highly purified ovine LH (LER-1056-C2) as radiolabelled tracer and NIH-LH-B7 as standard. Intra- and interassay coefficients of variation were 2.0 and 8.9%, respectively. Concentrations of FSH for individual animals were determined in samples pooled within each sampling period (weekly interval) from samples collected at 10-min intervals during the initial 8 h of serial blood collection.

Concentrations of FSH were determined by radioimmunoassay (Acosta *et al.*, 1983; Wolfe *et al.*, 1989) using a rabbit antiserum against ovine FSH (JAD-RAOFSH no. 17-6,7,9) and highly purified ovine FSH (LER-1976-A2) as radiolabelled tracer and standard. Concentrations of FSH were determined in a single assay with an intra-assay coefficient of variation of 4.4%.

Concentrations of plasma urea nitrogen were determined by a colorimetric assay using the methods of Marsh *et al.* (1965). Concentrations of plasma glucose were determined by an assay using glucose oxidase and peroxidase (Gochman & Schmitz, 1972). Coefficients of variation were <2% for both within and between days on which analyses occurred.

Mean concentrations of plasma urea nitrogen and glucose were determined within each blood collection period. The pattern of LH in circulation was characterized by determination of frequency and amplitude of LH pulses. These pulsatile characteristics were determined through the use of algorithms (Pulsar software modified for the IBM-PC by J. F. Gitzen, V. D. Ramirez, Urbana, IL, USA). In addition, maximal response of the pituitary to 750 ng and 50 µg LHRH was determined by Pulsar analysis.

Statistical analysis

Data on changes in body weight and condition and concentrations of urea nitrogen, glucose and gonadotrophins and pattern of gonadotrophin in the circulation were analysed by growth-curve procedures (Allen *et al.*, 1983). The response of individual animals to treatment (hormone variable as compared to weight change) was analysed by polynomial regression. The intercept and slope coefficients from data for individual animals within each treatment group were subsequently subjected to analysis of variance (Proc GLM, SAS, 1985) to determine the effect of treatment.

Results

Body weight and body condition

A quadratic regression of changes in body weight occurred ($P < 0.05$; Fig. 1) during the 20-week growth phase in all heifers. Heifers fed the diet to achieve a high body condition had greater ($P < 0.05$) increases in body weight than those fed to achieve a low body condition. At the start of the restriction phase, average body weight and condition were 395.9 ± 9.5 kg and 7.6 ± 0.2 kg, respectively, in heifers fed the high diet and 343.2 ± 6.5 kg and 3.9 ± 0.1 kg ($P < 0.05$), respectively, in heifers fed the low diet. During the restriction phase, body weight declined in a quadratic fashion ($P < 0.05$; Fig. 1) in all heifers. Body condition declined ($P < 0.05$; Fig. 2) in a cubic fashion during the restriction phase in heifers with high body condition, but changes in body condition were unrelated ($P > 0.05$) to changes in body weight in heifers with low body condition.

Plasma metabolites in the restriction phase

Concentrations of urea nitrogen increased ($P < 0.05$; Fig. 3) in a linear fashion as loss of body weight increased in all heifers but the magnitude of this increase was less ($P < 0.05$) in heifers with high body condition than in those with low body condition. Conversely, concentrations of glucose decreased ($P < 0.05$; Fig. 3) in a linear fashion as weight loss occurred in all heifers.

Gonadotrophins in the restriction phase

Amplitude of LH pulses and pituitary responsiveness to 750 ng LHRH increased ($P < 0.05$; Fig. 4) in a linear fashion with increase in weight loss in heifers with low body condition, but not in those with high body condition. In contrast, amount of LH released in response to 50 µg LHRH declined ($P < 0.05$) in a linear fashion as weight loss increased in heifers with high, but not in those ($P > 0.05$) with low body condition (Fig. 5).

During the 7-week restriction phase, mean concentrations of LH and FSH (3.04 ± 0.35 and 12.27 ± 0.81 ng ml⁻¹ for heifers with high body condition and 3.48 ± 0.45 and 14.13 ± 0.75 ng ml⁻¹ for heifers with low body condition, respectively) and frequency of LH pulses (11.7 ± 0.96 and 10.6 ± 0.86 LH pulses per 8 h in heifers with high and low body condition, respectively) were unrelated ($P > 0.05$) to changes in body weight.

Fig. 1. Regressions of change in body weight over time during the (a) growth and (b) restriction phases in heifers with high (—, $n = 8$) or low (---, $n = 8$) body condition. The distribution of points within each graph depicts actual data used to calculate regression relationships.

Discussion

Body weight stabilized after 5 weeks on the diet of low energy content (restriction phase). This physiological adaptation may result from a reduction in maintenance energy requirements or increased efficiency in utilization of energy (Ledger & Sayers, 1977).

Stored energy in the body, in the form of adipose tissue, acts as a reserve of energy available for use during homeorrhetic changes in metabolism during pregnancy and lactation (Bauman & Currie, 1980). It is likely that these energy reserves are used in a similar fashion during periods when content of energy in the diet is low. The establishment of a significant change in body condition during feed restriction in heifers with an initial high body condition, but not with those of low body condition in the present study, reflects increased mobilization of stored energy (as fat) when the diet limited in energy content was being fed. Heifers with low body condition presumably used other sources for energy during this period and probably used amino acids as shown by the higher concentrations of plasma urea nitrogen in these animals.

An increase in amplitude of LH pulses has been reported in ovariectomized heifers fed diets limited in energy (Imakawa *et al.*, 1987; Roberson *et al.*, 1991). An increased amplitude of LH pulses occurred during the period of feeding the diet containing low energy (restriction phase) in

Fig. 2. Regressions of body condition score (1 emaciated to 9 obese) during the restriction phase in heifers with high (—, $n = 8$) or low (---, $n = 8$) body condition. The distribution of points within each graph depicts actual data used to calculate regression relationships.

Fig. 3. Regressions in heifers with high or low body condition of urea nitrogen in plasma (a and b, respectively) and plasma glucose (c and d, respectively) with daily weight change. The distribution of points within each graph depicts actual data used to calculate regression relationships.

Fig. 4. Regression in heifers with high or low body condition of amplitude of luteinizing hormone (LH) pulses (a and b, respectively) and pituitary responsiveness (amplitude of LH release) to 750 ng LHRH (c and d, respectively) with daily weight change. The distribution of points within each graph depicts actual data used to calculate regression relationships.

Fig. 5. Regressions of pituitary responsiveness (amplitude of luteinizing hormone (LH) release) to 50 µg LHRH in heifers with (a) high and (b) low body condition with daily weight change. The distribution of points within each graph depicts actual data used to calculate regression relationships.

heifers with low body condition in the present study. Changes in amplitude were independent of changes in frequency of LH pulses. One possible explanation is that a direct effect of under-nutrition at the pituitary resulted in the enhanced amplitude of LH pulses. However, an effect on the hypothalamus to increase the amount of LHRH release cannot be discounted.

The results of the present study and those of Roberson *et al.* (1991) provide evidence that, in the absence of gonadal steroids, feeding of a diet with low energy content alters the frequency of LH pulses to only a small degree or not at all. These results, however, are inconsistent with those reported by Imakawa *et al.* (1987) and Foster *et al.* (1989).

The biological significance of the reduction in releasable pools of LH in response to 50 µg LHRH per unit change in body weight in heifers with high body condition in the present study is unknown. Response to administration of a pharmacological dose of LHRH is highly correlated with pituitary stores of LH (Crowder *et al.*, 1982). Whisnant *et al.* (1985) reported a greater release of LH in response to LHRH at 60 days *post partum* in cows fed 80, as compared with 100%, of National Research Council recommendations for energy. It is interesting that responsiveness to 750 ng LHRH increased with weight loss in heifers with low body condition, but that no change in response to this dose of LHRH occurred with weight loss in heifers with high body condition. This indicates that pituitary responsiveness to LHRH in heifers with low body condition was influenced to a greater degree by weight loss in heifers with a low body condition than in heifers with high body condition. However, releasable stores of LH apparently declined with increased weight loss in heifers with high body condition, but were unchanged as a result of weight loss in heifers with low body condition. This is indicated by the amount of LH released in response to 50 µg LHRH in all heifers. A large proportion of the variation in change in pituitary responsiveness to 50 µg LHRH is unaccounted for by changes in body weight. The reasons for the diverse response to the two doses of LHRH in heifers with high and low body condition cannot be explained. These results warrant more intensive studies of the influence of body condition and the feeding of diets containing low energy content on pituitary responsiveness to LHRH and pituitary content of LH in cows.

In well-trained female athletes, amenorrhoea results when energy output exceeds intake of dietary energy (Deuster *et al.*, 1986). Initiation and maintenance of reproductive cycles in women and rats are dependent upon a critical relationship between amount of body fat and lean (Frisch & McArthur, 1974; Frisch, 1985). However, a decline in work output in amenorrhoeic women athletes results in increased secretion of LH and resumption of menstrual cycles without substantial changes in body fat (Warren, 1983). A similar increase in pulsatile release of LH occurs in ewe

lambs in which growth has been restricted and realimentation occurs (Foster *et al.*, 1989). Amount of body fat may, therefore, be an indicator of potential energy status, but resumption of normal reproductive cycles may partially be a function of the net energy status independent of increases in body fat. In the present study, heifers with high body condition had greater availability of stored energy in their bodies during the period of feeding the diet containing low levels of energy. Apparently this enabled heifers with high body condition to maintain a higher net energy status than heifers with low body condition. Amount of body condition therefore influences pattern of LH in circulation, responsiveness of the pituitary to LHRH and releasable stores of LH in heifers fed a diet with low energy content. The feeding of diets with low energy content or increased metabolic demand for energy results in an increase in duration of the prepubertal period in heifers. Heifers with high and low body condition lost equivalent amounts of body weight during the period when a diet was fed that contained low energy content. Restricting the amount of dietary energy consumed by heifers with low body condition resulted in alterations of the pattern of LH in circulation. However, heifers with high body condition were refractory to the restriction in intake of dietary energy with regard to alterations in the pattern of LH in circulation.

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