Precalving Effects on Metabolic Responses and Postpartum Anestrus in Grazing Primiparous Dairy Cows

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ABSTRACT

The effect of increased access to pasture feeding during the last 6 wk of gestation on metabolic responses and postpartum anestrus interval was investigated. Heifers with a body condition score (BCS) of 5.0 (BC5+FF; on a 1-to-10 scale, US = $1.5 + 0.32 \times$ New Zealand) were offered unrestricted pasture, and those with BCS 4.0 were fed either pasture unrestricted (BC4+FF) or restricted (BC4+RES) for the last 6 wk of gestation. After calving, all groups were offered unrestricted pasture. Mean BCS at calving for BC5+FF, BC4+FF, and BC4+RES were 4.7 ± 0.1 , 4.3 ± 0.1 , and 3.5 ± 0.1 , respectively. At 35 d postpartum, LH pulse frequency was lower in BC4+RES than in BC4+FF and BC5+FF, which were similar. At 77 d after calving, 8% of BC4+RES cows had ovulated compared with 75% of BC4+FF and 69% of BC5+FF cows. Metabolic hormonal differences between BC4+FF and BC4+RES were not reflected in the differences between BC4+FF and BC5+FF for LH pulse frequency or ovulation. Unrestricted access to pasture during the final 6 wk of gestation for BC4 heifers reduced the risk of prolonged postpartum anestrus. Systemic factors, tissue sensitivity, and critical developmental set points are probably involved in the integrated control of ovulation by body condition.

Key words: postpartum anestrus, body condition score, milk production, dairy heifer

INTRODUCTION

The postpartum anestrus interval (PPAI) is influenced by cow breed, age, and energy intake (Burke et al., 1995). The majority of studies on PPAI and nutrition have been conducted in intensive high production systems. Dairy production systems of New Zealand are extensive, seasonally based, low production systems using predominantly pasture grazing (Roche et al., 1996).

Young heifers bred to calve as 2 yr olds have a longer PPAI than mature cows (Burke et al., 1995); dietary restriction during the late prepartum period reduces BW and body condition at calving, and extends the PPAI. Holstein-Friesian prepartum BCS and DMI influence LH pulse frequency and therefore, follicular maturation (Roche et al., 1981) and length of PPAI. During the postpartum period, both BCS and dietary energy intake were correlated with LH concentration (Perry et al., 1991). Prepartum dietary energy intake influenced pulsatile LH amplitude and frequency, as well as the time of appearance of large follicles on the ovaries and the interval to first ovulation. The signaling pathways that inform the hypothalamus of energy status and that control GnRH and LH secretion have not been fully elucidated.

Chronic undernutrition and negative energy balance during early lactation, resulting in reduced LH secretion, are associated with changes in the metabolic hormones, reducing plasma concentrations of insulin, IGF-I, and leptin and increasing plasma concentrations of growth hormone (GH; Block et al., 2001). The effect of acute changes in dietary intake on ovarian activity has been correlated with changes in circulating concentrations of metabolic hormones including insulin, IGF-I, GH, and leptin (Armstrong et al., 2003). Undernutrition can cause peripheral resistance to insulin and IGF-I (Thissen et al., 1994), indicating that plasma concentrations and changes in tissue sensitivity could control ovulation directly at the ovary or indirectly through hepatic IGF-I. Lactating cows that are partitioning nutrients away from adipose tissue toward the mammary gland are thought to exhibit insulin resistance by decreasing the sensitivity of adipose and muscle tissue to insulin (Cronjé, 2000). Glucose tolerance tests were used on dairy cows to detect differences in rates of secretion of insulin and use of glucose (Holtenius et al., 2003).

We hypothesized that 1) differing prepartum pasture intake resulted in changes in BCS to affect LH pulsatility postpartum; 2) increased prepartum nutrition of low BCS cows increased plasma concentrations of metabolic hormones (insulin, IGF-I, and leptin) and lowered GH concentration; and 3) low prepartum BCS increased fat mobilization and NEFA along with an increase in

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insulin resistance. The present study intended to determine the effects on PPAI of allowing access to different amounts of pasture before calving to heifers with low BCS.

MATERIALS AND METHODS

This experiment was conducted at Dexcel Dairy no. 5 (Hamilton, New Zealand; 37°46'S 175°18'E). All procedures were approved by the Ruakura Animal Ethics Committee, Hamilton, New Zealand.

Experimental Design and Treatments

Primiparous Holstein-Friesian cows (2 yr of age) that had conceived on a common date following AI to a synchronized estrus were utilized. Pasture allowances were managed during the last 5 mo of gestation such that 6 wk before parturition, 27 heifers had an average BCS of 4.0 (BC4) and 13 had an average BCS of 5.0 (BC5) on a scale of 1 to 10 (1 = emaciated and 10 = obese). In New Zealand, the ideal calving BCS is 5.0 for a mature cow and 5.5 for a 2-yr-old heifer (Macdonald and Roche, 2004). Roche et al. (2004) compared the New Zealand 10-point scale with the US 5-point scale, and presented a regression equation to allow easy conversion between the systems ($US = 1.5 + 0.32 \text{ NZ}$). Allocation to treatments was random and balancing BW and genetic merit for milk production. Animals were weighed and BCS assessed weekly. Six weeks before calving, the heifers with $BCS\ 4.0 \pm 0.1$ were allowed either unrestricted access to pasture until parturition (BC4+FF; $n = 12$) or continued restriction (BC4+RES; $n = 15$; negative control). The animals with $BCS\ 5.0 \pm 0.1$ were offered unrestricted access to pasture (BC5+FF; $n = 13$; positive control). All animals calved within a 10-d period.

Grazing Management

Pasture offered was predominantly perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens*), with <20% weeds and other grasses (*Dactylis glomerata*, *Poa* spp.). Each treatment group grazed separately in 0.25-ha paddocks and a different pasture area was allocated to adjust stocking density (animals/ha per d) thereby achieving a range of DMI. Low postgrazing pasture residuals can be used to restrict DMI in grazing experiments, because dairy stock have difficulty in grazing pasture to ground level (Roche et al., 2005). Offering different grazing area allocations facilitates achieving different cow DMI without confounding factors such as time at pasture or climatic influences.

Before calving, the heifers were allocated fresh pasture each morning. In an attempt to ensure intakes

were different between treatment groups, pregrazing and postgrazing pasture yields were different. Pasture allocations were visually assessed, and assessors were calibrated weekly through cutting a range of pasture yields, representative of pre- and postgrazing yields (O'Donovan, 2000). Precalving group DMI were calculated daily from pregrazing and postgrazing pasture masses (Roche et al., 1996). Pregrazing pasture mass was $3,422 \pm 724$, $4,522 \pm 363$, and $4,424 \pm 336$ kg of DM/ha for BC4+RES, BC4+FF, and BC5+FF, respectively. But postgrazing residual pasture mass increased with desired intakes (724 ± 228 , $1,467 \pm 315$, and $1,534 \pm 345$ kg of DM/ha for BC4+RES, BC4+FF, and BC5+FF, respectively).

After calving, treatment groups were grazed separately. The heifers were allocated fresh pasture following each milking. Pregrazing pasture mass was $3,835 \pm 589$, $3,822 \pm 565$, and $3,799 \pm 602$ kg of DM/ha for BC4+RES, BC4+FF, and BC5+FF, respectively. Postgrazing residual pasture mass was similar ($P > 0.10$) for each treatment group ($2,049 \pm 505$, $2,110 \pm 459$, and $2,101 \pm 475$ kg of DM/ha for BC4+RES, BC4+FF, and BC5+FF, respectively).

Blood Sampling

Coccygeal venipuncture was used to collect blood samples weekly from 6 wk prepartum to 10 wk postpartum to measure concentrations of glucose, NEFA, insulin, IGF-I, GH, and leptin. Blood samples were taken in the morning prepartum (approximately 0730 h) before new pasture was offered, and postpartum before milking and when new pasture was offered.

Profiles of LH release pattern were determined 2 and 5 wk postpartum in blood samples collected at 15-min intervals (commencing at 0700 h) for 16 h, including during milking. Jugular catheters were inserted under local anesthesia to facilitate the frequent collection.

Glucose Tolerance Test

All heifers were subjected to a glucose tolerance test at 2 wk postpartum. This challenge was implemented the day following serial blood sampling for measuring LH secretion and after overnight fasting. Glucose was administered i.v. as a 50% solution (Bomac Laboratories, Auckland, New Zealand) at a dose rate of 300 mg of D-glucose/kg of BW over 1 min; the catheters were flushed with 100 mL of isotonic saline after glucose administration. The glucose dose was chosen to result in a maximum insulin response (Subiyatno et al., 1996). Blood samples were collected at -30, -15, -5, 0, 5, 10, 15, 20, 40, 60, 90, and 120 min relative to the time of glucose administration.

All blood samples were collected into 10-mL Vacutainer tubes containing sodium heparin that were immediately placed in iced water. Blood samples were centrifuged at $3,000 \times g$ for 12 min, within 1 h of collection. Aliquots of plasma were stored at -20°C until assayed for LH, glucose, insulin, IGF-I, GH, and leptin concentrations.

Interval to First Ovulation and Milk Production Measurements

Progesterone concentrations were measured in fresh whole milk samples collected 3 times weekly before the start of each milking. The PPAI was defined as the interval from calving to the first of 2 consecutive sampling days that progesterone concentrations in milk were >1 ng/mL.

Weekly milk yields were measured throughout lactation using inline milk meters (TruTest, Auckland, New Zealand) and subsamples were taken to measure protein, fat, and lactose concentration (MilkoScan FT120, Foss, Hillerød, Denmark).

Hormone and Metabolite Assays

Plasma glucose and NEFA were measured by the hexokinase colorimetric method using a Hitachi 717 analyzer (Roche, Basel, Switzerland) performed at 30°C . The intra- and interassay coefficients of variation (CV) for both assays were 2 and 3%, respectively.

Insulin was measured using a radioimmunoassay (RIA; Hales and Randle, 1963). Insulin antiserum (GP2, 21/7/80) was donated by Peter Wynn (CSIRO Division of Animal Production, NSW, Australia). The intra- and interassay CV were 2 and 3%, respectively. The limit of detection of the assay was $0.89 \mu\text{U/mL}$. Plasma IGF-I was measured by RIA (Gluckman et al., 1983). The intra- and interassay CV were 5.3 and 5.7%, respectively. The limit of detection of the assay was 1 ng/mL. Leptin was measured in duplicate using RIA (Blache et al., 2000). The limit of detection of the assay was 0.1 ng/mL. The intra- and interassay CV were 4.8 and 5.7%, respectively. Plasma GH concentrations were measured using RIA (Downing et al., 1995). The intra- and interassay CV were 6.9 and 8.2%, respectively. The assay detection limit was 0.06 ng/mL.

Plasma concentrations of LH were measured using RIA with rabbit antiserum against ovine LH (AgResearch, Invermay R#2, Mosgiel, New Zealand; McDougall, 1994). The intra- and interassay CV were 8.9 and 17.4%, respectively. The sensitivity of the assay was 0.2 ng/mL.

Concentrations of progesterone in milk were measured using RIA (Coat-A-Count, Diagnostic Products

Corp., Los Angeles, CA; Dieleman and Bevers, 1987). Intra- and interassay CV were 6.1 and 8.6%, for standard concentrations of 4.4, 3.0, and 0.4 ng/mL, respectively.

Statistical Analyses

Differences among treatment groups in BW, BCS, plasma glucose, insulin, IGF-I, GH, leptin, and NEFA were analyzed with a repeated measures analysis using REML to fit a mixed model that included cow and time within cow as random effects and treatment, time, and their interaction as fixed effects. A compound symmetry covariance structure was used to model times within cows, allowing for heterogeneity of the variances at each time point. This analysis was carried out on prepartum and postpartum measurements separately. Milk production for the first 10 wk of lactation was analyzed using this same method. Data at each time point are presented because, in general, there were significant time $\times$ treatment interactions.

The effect of treatment group on the proportion of cows that had ovulated by 77 d after mean calving date was analyzed using generalized linear models with a binomial error distribution and logit link function.

The glucose tolerance test data were analyzed by calculating summary measures of the response curves for each cow and analyzing each individually using ANOVA. Data for each time point individually was analyzed in a univariate ANOVA. The area under the response curve for each cow was calculated using the trapezoidal rule. The clearance rate of glucose was calculated by fitting an exponential curve of the form $a + b \times r^{\text{time}}$ to the glucose data for each cow after time 0, with time, glucose concentration, and the clearance rate being represented by a , b , and r , respectively.

Mean plasma concentration of LH and the frequency and amplitude of LH pulses were determined using a modified version of the algorithm developed by Merriam and Wachter (1982) adapted for an IBM-compatible personal computer (PULSAR, R. Lazarus, Department of Community Medicine, Westmead Hospital, NSW, Australia). The effect of treatment group on LH data was analyzed by calculating the difference for each cow between the LH amplitude, concentration, and frequency at wk 2 and 5 and then analyzing the 2 and 5 wk data and the differences individually using ANOVA. GenStat 8.1 (VSN International Ltd., Hemel Hempstead, UK) was used for all statistical analyses.

RESULTS

BW and BCS

Six weeks before parturition, the BC4 heifers were lighter than BC5 heifers (323 and 407 kg, respectively;

Figure 1. Mean ($\pm$ SEM) for BW (a) and BCS (b) from 10 wk before until 10 wk after calving in heifers with low BCS 6 wk before calving and restricted access to pasture for the final 6 wk prepartum (BC4+RES, Δ ; $n = 15$), low BCS 6 wk before calving and unrestricted access to pasture for the final 6 wk prepartum (BC4+FF, $\circ$; $n = 12$), and moderate BCS 6 wk before calving with unrestricted access to pasture for the final 6 wk prepartum (BC5+FF, $\blacksquare$; $n = 13$).

$P < 0.001$). One week precalving, BW and BCS differed among all treatments with the BC4+RES group having the lowest values ($P < 0.001$; Figure 1). Mean BCS at calving for BC5+FF, BC4+FF, and BC4+RES were 4.7 ± 0.1 , 4.3 ± 0.1 , and 3.5 ± 0.1 , respectively. Associated with the postpartum loss in BW, BC5+FF postpartum had a BCS of 4.2 at 3 wk postpartum, which was similar to BC4+FF (4.1). The BC4+FF retained its BCS of 4, whereas the BCS of BC4+RES dropped to below 3.5 (Figure 1).

Percentage of Ovulating Animals

The percentage of animals that had ovulated by 77 d after the mean calving date was similar for the BC4+FF and BC5+FF treatments (75 and 69%; $P > 0.1$). In contrast, only 1 of 15 cows (8%) in the BC4+RES treatment had ovulated by d 77 ($P < 0.01$; Figure 2).

Figure 2. Percentage of cows cycling during the first 12 wk postpartum in heifers with low BCS 6 wk before calving and restricted access to pasture for the final 6 wk prepartum (BC4+RES, Δ ; $n = 15$), low BCS 6 wk before calving and unrestricted access to pasture for the final 6 wk prepartum (BC4+FF, $\circ$; $n = 12$), and moderate BCS 6 wk before calving with unrestricted access to pasture for the final 6 wk prepartum (BC5+FF, $\blacksquare$; $n = 13$).

Milk Production

There was a significant effect of prepartum nutrition and BCS on milk production (Figure 3). Milk, fat, protein, and lactose yield during the first 10 wk of lactation were less for BC4+RES compared with BC4+FF ($P < 0.05$), which were less than BC5+FF heifers ($P < 0.05$; Figure 3).

Hormonal and Metabolic Measurements

Prepartum Period (Wk -6 to -1). Changes in metabolite concentration are shown in Figure 4. There were no consistent differences associated with BCS and nutrition between BC4+FF and BC4+RES for plasma insulin, glucose, or NEFA. The largest differences associated with nutrition were IGF-I concentrations, with BC4+FF having a marked increase ($P < 0.001$) following increased feed availability. Concentrations of IGF-I in BC5+FF were initially high ($P < 0.001$) reaching around 40 ng/mL, and the IGF-I concentrations of the BC4+FF group were lower ($P < 0.001$) by wk -2.

Concentrations of GH increased in wk -5 in all groups, with BC5+FF and BC4+FF declining after wk -2. In contrast, GH levels in BC4+RES remained constant before increasing ($P < 0.001$) at calving, whereas the BC5 + FF and BC4 + FF, which dipped ($P < 0.001$) before calving, had a similar profile to the BC4+RES postcalving.

Plasma concentrations of leptin were higher for BC5+FF heifers prepartum from wk -6 to -4 ($P < 0.002$), but there were no other treatment effects. Leptin concentrations remained constant for all treatments between wk -6 and +4, after which they began to increase.

Postpartum Period (Wk 0 to 10). Nonesterified fatty acids were not consistently affected by nutrition

Figure 3. Total milk, fat, protein, and lactose yields (kg) during 24 wk of lactation for heifers with low BCS 6 wk before calving and restricted access to pasture for the final 6 wk prepartum (BC4+RES, Δ ; $n = 15$), low BCS 6 wk before calving and access to pasture for the final 6 wk prepartum (BC4+FF, $\circ$; $n = 12$), and moderate BCS 6 wk before calving with unrestricted access to pasture for the final 6 wk prepartum (BC5+FF, $\blacksquare$; $n = 13$).

in the BC4 groups. Group BC5+FF had higher NEFA than the BC4 groups for most of the postpartum period. Insulin was consistently higher in BC4+FF than in BC4+RES, with a peak occurring on wk 1. By 8 wk postpartum, there were no differences between the groups and insulin concentrations had decreased below 8 ng/mL. Glucose was similar in all groups throughout the postpartum period with an increase ($P < 0.05$) at wk 3.

Marked changes in BC4+FF GH occurred at 1 wk postpartum ($P < 0.05$). At wk 4, GH concentration was higher ($P < 0.05$) for the BC4+RES group than in the other groups. After wk 4 there were no differences between any of the groups even though they were producing different quantities of milk and losing BW at different rates.

Leptin concentrations did not show significant changes associated with BCS or prepartum nutrition and were not correlated with the other metabolic hormones, glucose, or NEFA.

LH Measurements

The pulse frequencies of LH secretion (Figure 5a) were similar for all treatments at 2 wk postpartum ($P > 0.10$). Mean pulse frequencies increased for all treatments by 5 wk postpartum ($P < 0.001$). At wk 5, LH pulse frequency for BC4+RES had not increased as much as in the other 2 groups ($P < 0.05$). At 2 wk postpartum, LH pulse amplitude was greater in

BC4+FF than in other treatments, but there were no treatment differences at 5 wk postpartum (Figure 5b). Concentrations of LH across the 16-h sampling periods were similar for all 3 treatments, and did not differ between wk 2 and 5 postpartum (Figure 5c).

Glucose Tolerance Test

The glucose tolerance test at 2 wk postpartum resulted in similar increases in plasma glucose concentrations from 3.41 ± 1.50 to 12.69 ± 0.47 nmol/L by 5 min after the infusion across all treatments (Figure 6a). Clearance of glucose was similar for all treatments (0.8 nmol/L per min); plasma glucose concentrations returned to preinfusion values after 90 min in all treatments.

The peak plasma insulin concentrations associated with the infusion of glucose occurred after 10 min and had returned to preinfusion values at 60 min in all groups (Figure 6b). Plasma insulin following glucose infusion was not statistically different between treatments because of considerable individual cow variability ($P > 0.05$); but there was a tendency for the response to be lower in BC4+RES ($P = 0.07$). Plasma concentrations of IGF-I did not differ with glucose infusion. As with the weekly samples from wk 2 postpartum, concentrations were highest for BC4+FF and lowest for BC4+RES ($P < 0.05$; Figure 6c). Plasma leptin concentrations did not change with glucose infusion, but

Figure 4. Mean plasma concentrations of a) insulin, b) IGF-I, c) leptin, d) growth hormone (GH), e) glucose, and f) NEFA from 6 wk before until 10 wk after calving, in heifers with low BCS 6 wk before calving and restricted access to pasture for the final 6 wk prepartum (BC4+RES, Δ ; $n = 15$), low BCS 6 wk before calving and unrestricted access to pasture for the final 6 wk prepartum (BC4+FF, $\circ$; $n = 12$), and moderate BCS 6 wk before calving with unrestricted access to pasture for the final 6 wk prepartum (BC5+FF, $\blacksquare$; $n = 13$).

tended to be lower ($P = 0.07$) for BC4+RES than for the other 2 treatments (Figure 6d).

DISCUSSION

The outcomes of this study have important practical implications for seasonal pasture-based dairy production systems in which periods of suboptimal nutrition may occur during the 6 wk before calving, generally coinciding with the low growth of winter pasture. Successful pastoral dairy production requires cow nutrient requirements to be aligned with pasture growth and availability.

These results support the hypothesis that increasing pasture intakes during the final 6 wk of gestation for heifers in with low BCS will increase LH secretion patterns in early lactation and reduce the PPAI. Therefore, adjustment of pasture management systems can reduce PPAI and increase the chance of conception within 80 d of calving as heifers. This would have an effect on seasonal calving herds by increasing the possibility of maintaining the 365-d calving interval. Unrestricted

pasture feeding of BC4+FF heifers increased milk production relative to the BC4+RES animals, but did not increase production in relation to heifers calving at BCS 5.0, which were on the same pasture system from 6 wk before and after calving.

Pulsatility of LH, an indicator of future ovulation, increases in response to increased pulses of GnRH, which appear to be under nutritional control, with the nutritional control being at the level of both the GnRH pulse generator as well as LH secretion (Perry et al., 1991). In the present study, LH pulse frequency increased at 5 wk postpartum in both full-fed prepartum groups compared with the BC4+RES group, which was on a low plane of nutrition before parturition. These results may be explained not only by the nutritional level at 6 wk precalving but by the interaction between intake and body condition, both of which are thought to control LH pulsatility (Diskin et al., 2003). However, the BC4+RES group had lower BCS than the other 2 groups. The simplest explanation of these results is that the postpartum differences in BCS during the first 5 wk (achieved by differential prepartum feeding of the

Figure 5. Mean ($\pm$ SEM) for a) LH pulse frequency, b) LH pulse amplitude, and c) mean plasma LH concentrations measured over 16 h at 2 and 5 wk after calving in heifers with low BCS 6 wk before calving and restricted access to pasture for the final 6 wk prepartum (BC4+RES, white bar; $n = 15$), low BCS 6 wk before calving and unrestricted access to pasture for the final 6 wk prepartum (BC4+FF, gray bar; $n = 12$), and moderate BCS 6 wk before calving with unrestricted access to pasture for the final 6 wk prepartum (BC5+FF, black bar; $n = 13$). Superscripts represent differences between treatments ($P < 0.05$).

heifers with BCS of 4) results in increased LH pulse frequency and reduced PPAI, suggesting the possibility of a metabolic memory.

There is considerable evidence that insulin has a major role not only in carbohydrate metabolism, but also in influencing LH release from the anterior pituitary (Monget and Martin, 1997). During early lactation, insulin concentrations tended to be higher in BC4+FF than in BC4+RES until about 42 d postpartum. This is in agreement with observations of Gong et al. (2002) who found that increased insulin postpartum resulted

in a shorter PPAI. Surprisingly, the increased insulin concentrations observed postpartum in the BC4+FF cows did not result in any change in plasma glucose levels postpartum. Butler et al. (2004) reported that increased insulin concentrations stimulated estradiol secretion by the dominant follicle of the first postpartum follicular wave and this is not mediated by changes in LH pulse frequency. This suggests the improved PPAI found in the BC4+FF heifers could be associated with stimuli independent of LH, so the possibility exists that both LH pulse frequency and direct effects on the ovary resulted in early ovulation in the BC4+FF cows.

During the prepartum period there were a number of significant differences between BCS and level of nutrition for GH, IGF-I, NEFA, and glucose. However, at parturition (when all cows were allocated to the same nutritional plane), the differences were reduced and often only seen in the first few weeks postpartum. Postpartum GH is higher in the BC4+RES for the first week although these differences in GH are not reflected in the circulating NEFA. The lack of a NEFA response to GH may account for a lack of insulin resistance, as increased NEFA are known to increase insulin resistance (Boden and Shulman, 2002). Concentrations of IGF-I was higher in BC5 and BC4+FF prepartum, but postpartum IGF-I concentrations were similar, with the BC4+FF then remaining higher than in the other groups. Although low IGF-I concentrations have been associated with extended PPAI (Roberts et al., 1997), the BC4+RES and BC5 groups both had low IGF-I levels but significant differences in PPAI, indicating that the association between IGF-I and PPAI is not found in all situations. However, previous studies have shown that cows with lower concentrations of IGF-I after calving take longer to resume estrous cyclicity (Beam and Butler, 1999). Low plasma concentrations of IGF-I may compromise reproduction because the dominant follicle fails to reach ovulatory size and produce sufficient estradiol to trigger ovulation. A substantial amount of IGF-I in bovine follicular fluid is derived from the peripheral circulation (Echternkamp et al., 1990), and IGF-I has a supporting role in follicular development, influencing and amplifying the effects of FSH and LH on the growth and differentiation of ovarian follicles (Spicer et al., 1993). Leptin has been positively related with plasma insulin and glucose and negatively with GH and NEFA (Block et al., 2001), but in this study, leptin during the first 5 wk postpartum was not different between the groups even though PPAI differences were found. However, leptin did increase after wk 4 in all the groups having higher leptin levels at a time when GH was falling and NEFA concentrations were low. It is possible that leptin concentration does not reflect BCS when the cows have low BCS and that fat

Figure 6. Mean plasma concentrations of a) glucose, b) insulin, c) IGF-I, and d) leptin following infusion of glucose at 0 min in heifers with low BCS 6 wk before calving and restricted access to pasture for the final 6 wk prepartum (BC4+RES, Δ ; n = 15), low BCS 6 wk before calving and unrestricted access to pasture for the final 6 wk prepartum (BC4+FF, $\circ$; n = 12), and moderate BCS 6 wk before calving with unrestricted access to pasture for the final 6 wk prepartum (BC5+FF, $\blacksquare$; n = 13).

deposition was mainly occurring in internal fat depots and not subcutaneous fat depots. Grainger and McGowan (1982) showed that as a cow increases in body condition, the proportion of fat in the bone-free carcass plus gut increased from 10 to 20%, while the proportion of water and protein declined accordingly. Their carcass measurements showed that subcutaneous fat is laid down only when a BCS of 5 is reached. Similarly, in a study of Friesian dairy cows in New Zealand, the relationship between BCS and body composition determined by physical dissection was meager when BCS was low, but as BCS increased, the amount of body fat increased exponentially (Gregory et al., 1998).

This trial demonstrated that combined effects of a low BCS and restricted prepartum energy intake caused changes to the somatotrophic and the gonadotropic axes during the final 6 wk of gestation. These changes are not reversed when the animals are offered unrestricted pasture feeding after calving, suggesting an endocrine memory of the metabolic energy status in heifers. The LH responses associated with differential patterns in metabolic hormone profiles might be mediated through the liver, brain, or ovary, and changes in hormonal sensitivity through receptor regulations need to be examined.

CONCLUSIONS

Prepartum nutrition was manipulated to produce changes in body condition and the differences in BCS at parturition and postpartum directly affected PPAI.

This was associated with differences in LH pulsatility at wk 5 postpartum. The means by which body tissues such as muscle, fat, and liver control PPAI are not clear from the systemic factors measured because differences in hormones between BC4+FF and BC4+RES are not necessarily reflected in the differences between BC4+RES and BC5+FF. There are indications that systemic factors, tissue sensitivity, and critical set points are involved in the integrated control of ovulation by nutrition and body composition.

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REFERENCES

- Armstrong, D. G., J. G. Gong, and R. Webb. 2003. Interactions between nutrition and ovarian activity in cattle: Physiological, cellular and molecular mechanisms. *Reprod. Suppl.* 61:403–414.

- Beam, S. W., and W. R. Butler. 1999. Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. *J. Reprod. Fertil. Suppl.* 54:411–424.
- Blache, D., L. M. Chagas, M. A. Blackberry, P. E. Vercoe, and G. B. Martin. 2000. Metabolic factors affecting the reproductive axis in male sheep. *J. Reprod. Fertil.* 140:1–11.
- Block, S. S., W. R. Butler, R. A. Ehrhardt, A. W. Bell, M. E. Van Amburgh, and Y. R. Boisclair. 2001. Decreased concentration of plasma leptin in periparturient dairy cows is caused by negative energy balance. *J. Endocrinol.* 171:339–348.
- Boden, G., and G. Shulman. 2002. Free fatty acids in obesity and type 2 diabetes: Defining their role in the development of insulin resistance and beta-cell dysfunction. *Eur. J. Clin. Invest.* 32:14–23.
- Burke, C. R., S. McDougall, and K. L. Macmillan. 1995. Effects of breed and calving liveweight on postpartum ovarian activity in pasture-fed dairy heifers. *Proc. N.Z. Soc. Anim. Prod.* 55:76–78.
- Butler, S. T., S. H. Pelton, and W. R. Butler. 2004. Insulin increases 17 beta-estradiol production by the dominant follicle of the first postpartum follicle wave in dairy cows. *Reproduction* 127:537–545.
- Cronjé, P. B. 2000. Nutrient-gene interactions: Future potential and adaptations. Pages 409–422 in *Ruminant Physiology: Digestion, metabolism, growth and reproduction*. P. B. Cronjé, ed. CAB International, Wallingford, UK.
- Dieleman, S. J., and M. M. Bevers. 1987. Effects of monoclonal antibody against PMSG administered shortly after the preovulatory LH surge on time and number of ovulations in PMSG/PG-treated cows. *J. Reprod. Fertil.* 81:533–542.
- Diskin, M., D. Mackey, J. Roche, and J. Sreenan. 2003. Effects of nutrition and metabolic status on circulating hormones and ovarian follicle development in cattle. *Anim. Reprod. Sci.* 78:345–370.
- Downing, J. A., J. Joss, P. Connel, and R. J. Scaramuzzi. 1995. Ovulation rate and the concentration of gonadotrophic and metabolic hormones in ewes fed lupin grain. *J. Reprod. Fertil.* 103:137–145.
- Echternkamp, S. E., L. J. Spicer, K. E. Gregory, S. F. Canning, and J. M. Hammond. 1990. Concentrations of insulin-like growth factor-I in blood and ovarian follicular fluid of cattle selected for twins. *Biol. Reprod.* 43:8–14.
- Gluckman, P. D., J. J. Johnson-Barrett, J. H. Butler, B. W. Edgar, and T. R. Gunn. 1983. Studies of insulin-like growth factor -I and -II by specific radioligand assays in umbilical cord blood. *Clin. Endocrinol.* 19:405–413.
- Gong, J., W. Lee, P. Garnsworthy, and R. Webb. 2002. Effect of dietary-induced increases in circulating insulin concentrations during the early postpartum period on reproductive function in dairy cows. *Reproduction* 123:419–427.
- Grainger, C., and A. A. McGowan. 1982. The significance of pre-calving nutrition of the dairy cow. Pages 135–171 in *Proc. Conf. Dairy Prod. Pasture*. Clark and Matherson Ltd., Hamilton, New Zealand.
- Gregory, N. G., J. K. Robins, D. G. Thomas, and R. W. Purchas. 1998. Relationship between body condition score and body composition in dairy cows. *N.Z. J. Agric. Res.* 41:527–532.
- Hales, C. N., and P. J. Randle. 1963. Immunoassay of insulin with insulin-antibody precipitate. *Biochem. J.* 88:137–146.
- Holtenius, K., S. Agenas, C. Delavaud, and Y. Chilliard. 2003. Effects of feeding intensity during the dry period. 2. Metabolic and hormonal responses. *J. Dairy Sci.* 86:883–891.
- Macdonald, K. A., and J. Roche. 2004. *Condition Scoring Made Easy*. Condition scoring dairy herds. 1st ed. Dexcel Ltd., Hamilton, New Zealand.
- McDougall, S. 1994. Postpartum anoestrus in the pasture grazed New Zealand dairy cow. PhD Thesis, Massey University, New Zealand.
- Merriam, G. R., and K. W. Wachter. 1982. Algorithms for the study of episodic hormone secretion. *Am. J. Physiol.* 243:E310–E318.
- Monget, P., and G. B. Martin. 1997. Involvement of insulin-like growth factors in the interactions between nutrition and reproduction in female mammals. *Hum. Reprod.* 12:33–52.
- O'Donovan, M. 2000. The relationship between the performance of dairy cows and grassland management practise on intensive dairy farms in Ireland. PhD. Diss., National University of Ireland, Galway.
- Perry, R. C., L. R. Corah, R. C. Cochran, W. E. Beal, J. S. Stevenson, J. E. Minton, D. D. Simms, and J. R. Brethour. 1991. Influence of dietary energy on follicular development, serum gonadotropins, and first postpartum ovulation in suckled beef cows. *J. Anim. Sci.* 69:3762–3773.
- Roberts, A. J., R. A. Nugent, J. Klindt, and T. G. Jenkins. 1997. Circulating insulin-like growth factor i, insulin-like growth factor binding proteins, growth hormone, and resumption of estrus in postpartum cows subjected to dietary energy restriction. *J. Anim. Sci.* 75:1909–1917.
- Roche, J., P. Dillon, S. Crosse, and M. Rath. 1996. The effect of closing date of pasture in autumn and turnout date in spring on sward characteristics, dry matter yield, and milk production of spring-calving dairy cows. *Irish J. Agric. Food Res.* 35:127–140.
- Roche, J. F., M. A. Crowe, and M. P. Boland. 1981. Postpartum anoestrus in dairy and beef cattle. *Anim. Reprod. Sci.* 28:371–378.
- Roche, J. R., P. G. Dillon, C. R. Stockdale, L. H. Baumgard, and M. J. VanBaale. 2004. Relationships among international body condition scoring systems. *J. Dairy Sci.* 87:3076–3079.
- Roche, J. R., E. S. Kolver, and J. K. Kay. 2005. Influence of precalving feed allowance on periparturient metabolic and hormonal responses and milk production in grazing dairy cows. *J. Dairy Sci.* 88:677–689.
- Spicer, L. J., E. Alpizar, and S. E. Echternkamp. 1993. Effects of insulin, insulin-like growth factor I, and gonadotropins on bovine granulosa cell proliferation, progesterone production, estradiol production, and (or) insulin-like growth factor I production in vitro. *J. Anim. Sci.* 71:1232–1241.
- Subiyatno, A., D. Mowat, and W. Yang. 1996. Metabolite and hormonal responses to glucose or propionate infusions in periparturient dairy cows supplemented with chromium. *J. Dairy Sci.* 79:1436–1445.
- Thissen, J.-P., J.-M. Ketelslegers, and L. E. Underwood. 1994. Nutritional regulation of the insulin-like growth factors. *Endocrinol. Rev.* 15:80–101.