Plasma insulin, growth hormone, and IGF-1 concentrations of Holstein-Friesian cows of divergent genotype offered varying levels of concentrate in early lactation

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ABSTRACT

This study of insulin and key hormones of the somatotropic axis was made to help explain the milksolids and body condition responses obtained from cows of divergent Holstein-Friesian (HF) genotypes fed differing levels of concentrate. Weekly plasma concentrations of insulin, insulin-like growth factor-1 (IGF-1) and growth hormone (GH) were measured from 57 North American (overseas; OS) and New Zealand (NZ) cows in the first 10 weeks of lactation during the 2002/03 and 2003/04 seasons. Cows of similar breeding worth grazed at a high pasture allowance and were individually fed 0, 3 or 6 kg concentrate DM/cow/day. Compared to NZ HF, OS HF had similar insulin concentrations, lower IGF-1 concentrations, and higher GH concentrations. Feeding 3 or 6 kg concentrate DM/day increased insulin and IGF-1 concentrations to a similar extent, with GH concentrations being reduced at the highest level of supplementation. These results are consistent with the previously reported greater loss of body condition in OS HF, and suggest genotype differences in insulin resistance and rate of recoupling of the somatotropic axis after calving. The reported gain in body condition when concentrates were fed is probably a result of the increased insulin production and a positive effect on liver GH receptor 1A, with the consequent increase in IGF-1 and reduction in GH. Diet only began to affect GH concentrations, and therefore body condition loss, after approximately week 4-6 of lactation. These results have implications for feed management and breeding.

Keywords: Holstein-Friesian; genotype; supplements, hormones.

INTRODUCTION

Dairy cow genotype and level of concentrate feeding have been shown to influence nutrient partitioning between body reserves and milk production in pasture-based farm systems (Kolver *et al*., 2005; Roche *et al*., 2006).

Typically on pasture-based diets, Holstein-Friesian (HF) cows of North American (overseas; OS) ancestry mobilise body reserves for a longer period of time than New Zealand (NZ) HF cows, resulting in a lower nadir body condition in early lactation and a lower BCS throughout lactation. This reflects the inherently greater energy deficit of overseas cows (OS) which results from an inability to consume sufficient pasture (Kolver *et al*., 2005) as well as the selection pressure that has been placed on homeorhetic mechanisms that support milk production at the expense of body condition and reproduction (Lucy, 2004).

A variety of hormones are involved in homeorhesis, but growth hormone (GH) may be the most important as it promotes lipolysis while antagonising lipogenesis and blocking insulin-dependent glucose uptake in peripheral (non-mammary) tissues (Etherton & Bauman, 1998). Recently Lucy *et al*. (2001) proposed that the uncoupling of the somatotropic axis that occurs in postpartum dairy cows is mediated by a decrease in the liver-specific GH receptor 1A (GHR 1A). This in turn decreases the synthesis of insulin-like growth factor-1 (IGF-1) in the liver and reduces blood concentrations of IGF-I. Reduced IGF-1 negative feedback increases post-partum GH concentrations. The homeorhetic effect of GH increases adipose tissue mobilisation and the gluconeogenic rate in liver (Etherton & Bauman, 1998). The somatotropic axis is recoupled when expression of GHR 1A is increased as lactation progresses. The reactivation of GHR 1A by insulin has been proposed by Lucy (2004), and may be influenced by postpartum feeding and energy balance (McGuire *et al*., 1992; Radcliff *et al*., 2006).

This study of insulin and key hormones of the somatotropic axis was made to help explain the milksolids and body condition responses obtained from cows of divergent genotypes (NZ HF and OS HF) and when differing levels of concentrate were supplemented, as previously reported by Kolver *et al*. (2005) and Roche *et al.* (2006).

MATERIALS AND METHODS

Design

Experimental design, cow selection and management, samples and analyses, and production and reproductive performance have previously been described (Kolver *et al.,* 2005; Roche *et al.,* 2006). Briefly, primiparous and multiparous OS and NZ HF grazed pasture and were fed 0, 3, or 6 kg DM/cow/day of a pelleted concentrate supplement (60% maize grain, 31% barley grain, 7% molasses, and 2% broll [wheat bran and pollard] on a DM basis) at the Dexcel Lye Dairy during the 2002/2003 and 2003/2004 seasons. Cows were re-randomised at the beginning of the second season. The six treatments in this 2x2x3 factorial experiment used 54 cows in 2002/2003 and 59 cows in 2003/2004 and were: NZ0 (n=9 2002/2003; n=10 2003/2004); NZ3 (n=9 2002/2003; n=10 2003/2004); NZ6 (n=9 2002/2003; n=10 2003/2004); OS0 (n=8 2002/2003; n=9 2003/2004); OS3 (n=10 2002/2003; n=10 2003/2004); and OS6 (n=9 2002/2003; n=10 2003/2004). Average age distribution within treatments was 19% first-lactation, 14% second-lactation, and 67% mixed age (third- to sixth-lactation) cows.

Each season, treatments were balanced for Breeding Worth ($BW: NZ0 113 ± 35.3; NZ3 115 ± 24.1; NZ6 116 ± 22.7; OS0 97 ± 38.3; OS3 99 ± 33.4; OS6 103 ± 32.3; mean ± SD). Mean $BW of NZ HF was 115 ± 27.4, which was comparable to that of OS HF (100 ± 34.7). Within genotype, treatments were balanced for sire and liveweight. All treatments had a similar mean calving date (NZ0 23 July ± 21.6 days; NZ3 27 July ± 22.6 days; NZ6 21 July ± 21.1 days; OS0 30 July ± 23.4 days; OS3 4 August ± 25.4 days; OS6 29 July ± 24.3 days; mean ± SD), with mean calving date of NZ HF being 24 July ± 21.7 days and OS HF being 31 July ± 24.4 days.

Cow selection

The OS HF genotype had >87.5% OS HF ancestry (North American) and the NZ HF genotype had <12.5% OS HF ancestry based on three-generation pedigrees. Sires used were representative of sires used across the national herd.

Feeding and management

Cows were individually fed 0, 3 or 6 kg DM/cow/day (3.5 or 7 kg fresh matter/cow/day) each day of lactation, with individual residues being measured and sampled each milking. The 6 kg DM/cow/day level of supplementation was the highest rate that could be fed with high quality pasture without incurring protein or fibre deficiencies in the diet.

Cows were grazed as one herd and were offered 50 kg DM/cow/day. Post-grazing residuals were used to determine pasture allocation; 1800 kg DM/ha was targeted during spring and autumn and 2200-2400 kg DM/ha during summer.

Measurements

Blood samples (10 ml) were collected from the coccygeal vessel using heparinised vacutainers immediately after the Thursday AM milking (0730 h) once a week. Collections continued for sufficient time to obtain a blood sample for the first ten weeks of lactation from each cow. Samples were centrifuged at 1120 *g* for 10 mins, and plasma collected and frozen for subsequent analysis.

Plasma concentrations of insulin was measured in duplicate by double-antibody RIA (Hales, 1963) as modified by Basset (1966) and described by Tindal (1978).

The GH assay included six replicates each of three quality control pools. On day 1, plasma samples (100 µl), and standards in 0.05M phosphate buffer + 0.25% BSA (100 µl) were diluted to 400 µl with 0.05M phosphate buffer and 50 µl of antiserum was added and incubated at 4 ^{o}C overnight. On day 2, 50 µl of tracer was added, vortexed and incubated for a further 48 h, after which time donkey anti-rabbit serum (50 µl; 1:7) in 0.05M phosphate buffer was added to all tubes (except control tubes) before centrifugation at 1500 *g* for 25 mins at 4 ^{o}C. The supernatant was decanted off and the activity of the precipitate was determined on a gamma counter.

Plasma concentrations of free IGF-I were measured in duplicate by the chloramine-T RIA method described by Gluckman *et al.* (1983). Interference by binding proteins was minimised by acid-ethanol cryoprecipitation method validated for ruminants by Breier *et al.* (1991).

Statistical analysis

Means for each cow for the first ten weeks of lactation were calculated for each season and analysed using the residual maximum likelihood (REML) procedure of GenStat 8 to fit a mixed model with season, genotype, diet, and interactions as fixed effects, and cow and season within cow as random effects.

The repeated measurements through time (weeks post calving) were modelled using spline models within the linear mixed model framework as described by Verbyla *et al*. (1999). Diet and genotype, linear trend of time and the interaction of the linear trend of time with diet and genotype were included as fixed effects and cow, linear trend of

time within cow, spline and the interaction of diet and genotype with spline were included as random effects. REML was used to fit these models. For the purpose of presentation, the data was analysed at each week individually using REML to fit a mixed model with season, genotype, diet, and interactions as fixed effects, and cow and season within cow as random effects.

Log_{10} transformation was employed because of heterogeneity of variance but back transformed (geometric) means are presented to illustrate treatment effects. Significant effects were declared at $P<0.05$ and trends at $P<0.15$.

RESULTS

No genotype x diet interactions were observed for mean concentrations of plasma insulin, IGF-1, or GH during the first ten weeks of lactation (Table 1).

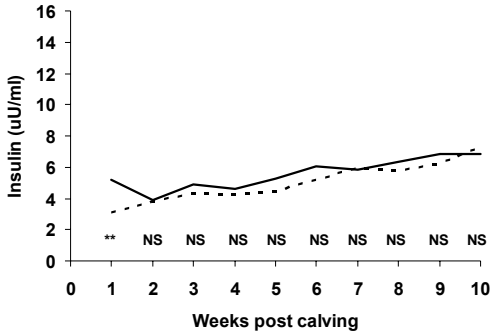
Significant effects of genotype on mean plasma hormone concentrations were observed. Compared to NZ HF, OS HF had a similar mean concentration of insulin, a lower concentration of IGF-1, and a higher concentration of plasma GH during the first ten weeks of lactation (Table 1). Spline modelling of the effects of time (weeks post calving) indicated a genotype x time interaction for insulin ($P<0.05$), IGF-1 ($P<0.05$), and a trend for an interaction for GH ($P=0.14$). Week by week analysis of these time interactions showed that compared to NZ HF, OS HF insulin concentrations were higher on week 1 of lactation but not on subsequent weeks (Figure 1a); IGF-1 concentrations were higher from week 4 onwards (Figure 2a); and GH concentrations were higher throughout early lactation with larger differences occurring after week 3 of lactation (Figure 3a).

Significant effects of diet on mean plasma hormone concentrations were also observed. Compared to the 0 kg concentrate DM/day treatment, the 3 kg concentrate DM/day treatment had higher insulin concentrations, higher IGF-1 concentrations, and similar GH concentrations during the first ten weeks of lactation. Feeding 6 kg concentrate DM/day resulted in a higher insulin concentration compared to the 0 but not 3 kg concentrate DM/day treatment; a higher IGF-1 concentration compared to 0 but not 3 kg concentrate DM/day treatment; and a lower GH concentration compared to either 0 or 3 kg concentrate DM/day treatments (Table 1). Spline modelling of the effects of time (weeks post calving) indicated a diet x time interaction for IGF-1 ($P<0.01$) and GH ($P<0.05$), but not for insulin. Week by week analysis of these time interactions showed that differences between dietary treatments became significant from week 2 onwards for insulin (Figure 1b); week 4 onwards for IGF-1 (Figure 2b); and on week 4, but consistently from week 6 onwards for GH (Figure 3b). Compared to the 0 kg concentrate DM/day treatment, the 3 kg concentrate DM/day treatment had higher insulin concentrations on week 2, 4, 6, 7, and 8 (Figure 1b); higher IGF-1 concentrations on week 4, 5, 6, and 7 (Figure 2b); and similar GH concentrations throughout the first ten weeks of lactation (Figure 3b). Feeding 6 kg concentrate DM/day resulted in higher insulin concentrations in week 2, 3, 4, 5, 6, 7, 8 and 9 compared to the 0 kg concentrate DM/day treatment, and no difference compared to the 3 kg DM concentrate/day treatment (Figure 1b); a higher IGF-1 concentration in week 4, 5, 6, 7, 8, 9 compared to the 0 kg concentrate DM/day treatment, and no difference compared to the 3 kg DM concentrate/day treatment (Figure 2b); and a lower GH concentration in week 7, 8, 9, and 10 compared to the 0 kg concentrate DM/day treatment, and a lower GH concentration in week 4, 6, 7, 9, and 10 compared to the 3 kg concentrate DM/day treatment (Figure 3b).

TABLE 1: Log_{10} and geometric mean plasma concentrations of insulin, growth hormone, and insulin-like growth factor-1 (IGF-1) during the first ten weeks of lactation from New Zealand (NZ) and overseas (OS) Holstein-Friesians (HF) grazing pasture and fed 0, 3, or 6 kg concentrate DM/cow/day of lactation.

	Genotype			**Diet (kg concentrate DM/cow/day)**				**P value**		
	NZ HF	OS HF	SED	0	3	6	SED	Genotype	Diet	GxD
Insulin (μU/ml)										
Log_{10} mean	0.691	0.749	0.039	0.653	0.746	0.760	0.033	NS	<0.01	NS
Geometric mean	4.9	5.6		4.5	5.6	5.8				
IGF-1 (ng/ml)										
Log_{10} mean	0.940	0.838	0.046	0.788	0.929	0.950	0.047	<0.05	<0.01	NS
Geometric mean	8.7	6.9		6.1	8.5	8.9				
Growth hormone (ng/ml)										
Log_{10} mean	0.977	1.096	0.035	1.054	1.074	0.981	0.029	<0.001	<0.05	NS
Geometric mean	9.5	12.5		11.3	11.9	9.6				

FIGURE 1: Geometric mean plasma insulin concentrations.
a) Genotype: New Zealand (dotted line) and overseas (solid line) Holstein-Friesians.



b) Diet: Concentrate level 0 (dotted line), 3 (dashed line), 6 (solid line) kg DM/cow/day.

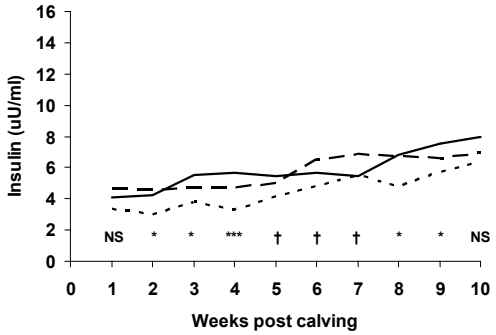
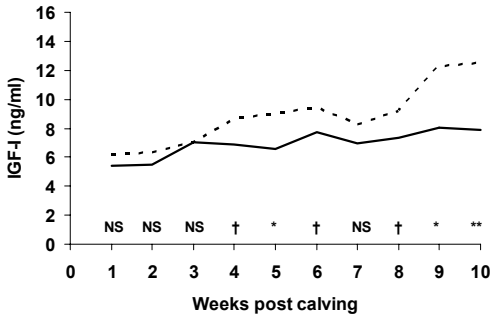


FIGURE 2: Geometric mean plasma insulin-like growth factor-1 (IGF-1) concentrations.
a) Genotype: New Zealand (dotted line) and overseas (solid line) Holstein-Friesians.



b) Diet: Concentrate level 0 (dotted line), 3 (dashed line), 6 (solid line) kg DM/cow/day.

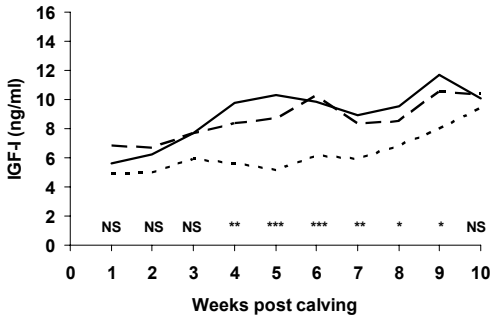
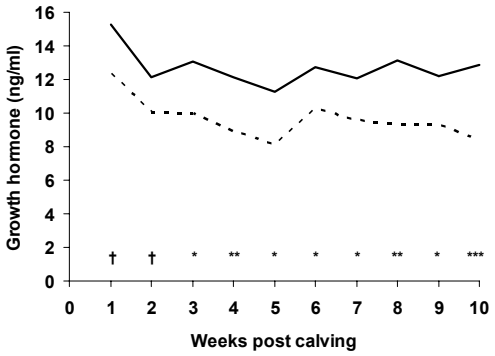
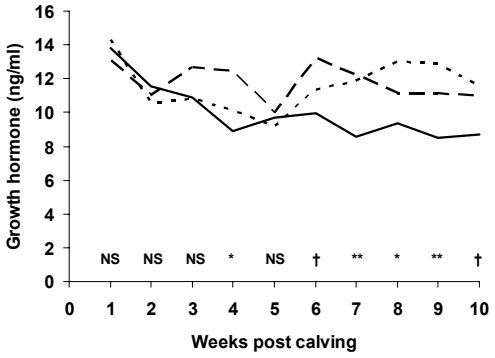


FIGURE 3: Geometric mean plasma growth hormone concentrations.
a) Genotype: New Zealand (dotted line) and overseas (solid line) Holstein-Friesians.



b) Diet: Concentrate level 0 (dotted line), 3 (dashed line), 6 (solid line) kg DM/cow/day.



DISCUSSION

This study provides support for the model of interaction between growth hormone and insulin in postpartum dairy cows proposed by Lucy (2004), and suggests genotype differences exist in tissue resistance to insulin and rate of recoupling of the somatotropic axis in early lactation. During the weeks after calving, insulin and IGF-1 plasma concentrations increased, and GH concentrations decreased. Few studies have measured these hormone profiles during lactation in pasture-based dairy cows. Moyes (2004) reported similar temporal changes in insulin and IGF-1 for dairy cows grazing pasture and supplemented with concentrate.

The genotype differences in hormone concentrations are consistent with the higher production, or greater negative energy deficit, of OS HF compared to NZ HF cows (Kolver *et al*., 2005; Roche *et al*., 2006). Growing evidence suggests that selection of cows for high milk yields at the expense of body condition has resulted in animals with increased tissue resistance to insulin and consequently an uncoupling of the somatotropic axis in early lactation (Cronje 2000; Lucy 2004). The axis can be considered to be re-

coupled when IGF-1 concentrations increase in response to insulin, with a consequent reduction in GH. The OS HF appear to exhibit greater insulin resistance, with a lower IGF-1 concentration occurring despite having similar insulin concentrations as NZ HF. Insulin resistance is known to be created by the antagonising action of high GH concentrations (Dominici & Turyn, 2002), which were apparent in OS HF. The lack of difference in IGF-1 concentrations during the first four weeks, despite OS HF having higher growth hormone concentrations throughout the first ten weeks of lactation, is consistent with an uncoupling of the somatotropic axis. Consistent differences between genotypes in IGF-1 concentration appeared from week 4, possibly indicating the point of axis re-coupling in NZ HF, with re-coupling in OS HF occurring either later in lactation or to a smaller degree.

Feeding level is known to influence the rate at which the somatotropic axis recouples, with higher levels of TMR feeding increasing the rate of GHR 1A proliferation (Radcliff *et al*., 2006).

Results from the present study are consistent with a dietary effect on the recoupling of the somatotropic axis. The provision of concentrate to grazing dairy cows increased insulin from week 2 and IGF-1 from week 4 of lactation. This result may show that GHR 1A expression and IGF-1 synthesis is delayed by up to 2 weeks after insulin concentrations are elevated. This is broadly consistent with Radcliff *et al*. (2006) who reported a delay in the increase of GHR 1A of 7 days after partially restrict-fed cows returned to *ad libitum* feeding. Although both 3 and 6 kg concentrate DM/day elevated insulin and IGF-1 in the present study, only the 6 kg concentrate DM/day treatment resulted in a reduction in GH concentrations relative to the 0 kg concentrate DM/day treatment, with the effect apparent by week 4, but only becoming consistent by week 6. This may imply a dietary effect on the feedback loop from IGF-1 through somatostatin to GH, or by some other unknown mechanism. While this is not consistent with the theory of recoupling of the somatotropic axis, it does reflect the small effect that 3 kg concentrate DM/day had on body condition score throughout early lactation, and the much larger effect achieved by 6 kg concentrate DM/day (Roche *et al*., 2006).

Combined, the genotype and diet effects on insulin and the somatotropic axis suggest management and breeding opportunities for manipulating nutrient partitioning in early lactation. Cows are genetically programmed to mobilise body reserves in early lactation. The point at which the somatotropic axis is recoupled signals the slowing of tissue mobilisation. In production systems where maintenance of body condition is highly valued, selection of animals with low insulin resistance and early recoupling of the somatotropic axis would be desirable. In the present study differences between genotypes appeared after approximately 3 weeks, although the degree of recoupling in OS HF animals appeared minimal during the first ten weeks of lactation. Similarly, nutrition appears to only influence (reduce) GH after about week 4-6. This is because it took the first week of lactation for the supplement to increase insulin, which increased IGF-1 after a 2 week delay, possibly due to a delay in increased GHR 1A. The 0-2 week delay between IGF-1 concentrations being impacted by diet and GH concentrations being reduced may reflect a threshold level of IGF-1 that is required before GH synthesis is down-regulated.

This suggests that management of body condition loss in early lactation, and subsequent carry-over effects on reproduction, can best be managed by genetic selection for early replenishment GHR 1A. For cows with high levels of production on pasture, additional feeding with supplements will not begin to affect body condition loss until after about week 4-6 of lactation. This scenario is consistent with that derived by Roche *et al*. (2006) from this study, based on the profile of body condition loss and milk production.

In conclusion, this study supports the model of interaction between growth hormone and insulin in postpartum dairy cows proposed by Lucy (2004). Results suggest that the lower IGF-1 concentration and consequent higher GH concentration in OSHF is not due to lower plasma insulin. A possible implication is that OS HF have a higher tissue resistance to insulin. The elevated GH results in exacerbated partitioning of nutrients towards milk production at the expense of body reserves. The reported gain in body condition when concentrates are fed is probably a result of the increased insulin production and the positive effect on liver GHR 1A, with the consequent increase in IGF-1 and reduction in GH. These results suggest that recoupling of the somatotropic axis occurred at week 4-6 post-calving, and that while insulin and IGF-1 were elevated with either 3 or 6 kg concentrate DM/day, only the higher feeding rate resulted in the subsequent reduction in GH (and consequent improvement in body condition).

These results provide evidence of physiological responses to genotype and diet which may account for body tissue and production responses observed on-farm. Further studies are required, in particular measurements of liver GHR 1A under different feeding regimens.

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