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Metabolic responses to glucose challenge in New Zealand and overseas Holstein-Friesian dairy cows

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ABSTRACT

North American and Dutch Holstein-Friesian (HF) cattle have undergone genetic selection for increased milk production, however, at the same time, reproductive efficiency has decreased. Overseas HF also have greater propensity to mobilise body reserves during early lactation than New Zealand HF cows. It is possible that reproductive differences are a consequence of metabolic and endocrine differences between the strains. This trial was designed to investigate effects of genotype and diet on endocrine response to a challenge of intravenous glucose in HF cows. Twenty-five HF cows of New Zealand (NZ; n=13) and Overseas (OS; n=12) origin, were fed either pasture (Grass; n=12) or a total mixed ration (TMR; n=13) in a 2x2 factorial design. At two weeks postpartum, they were challenged with 300mg of glucose/kg live weight. Data were analysed for effects of genotype and diet. Following glucose challenge, the NZ TMR cows had higher plasma insulin concentrations than the NZ Grass, resulting in a significant interaction between diet and genotype in the area under the response curve; but the OS groups had similar patterns. Mean plasma leptin concentration was higher in NZ TMR than in NZ Grass, OS TMR or OS Grass. Plasma concentrations of glucose and IGF-I were not affected by genotype or feed regime. These results demonstrated metabolic differences between the strains and also diets. A better understanding of the genetic and nutritional basis of hormonal regulation of nutrient partitioning will be important to develop nutritional strategies to meet the requirements of lactating cows with different genotypes, and to find ways to improve reproductive efficiency.

Keywords: dairy cows; genotype; diet; glucose challenge; postpartum; metabolites.

INTRODUCTION

Continued pressure of selection for milk yield is associated with increased gross feed efficiency. The proportion of North American and Dutch largely by a greater propensity to mobilise body reserves (Veerkamp & Emmans, 1995), and decreased reproductive performance (Butler & Smith, 1989).

These changes have been apparent in the national dairy herd over the las 10 years with the increase use of overseas Holstein-Friesian genetics (OS HF) from North America and the Netherlands (Harris & Kolver, 2001). Kolver *et al*. (2001) reported greater propensity of OS HF to mobilise body reserves during early lactation, in response to an endocrine challenge. The resulting change in energy balance appears to be linked to a lower fertility of the OS HF when compared to the NZ HF (Verkerk *et al.*, 2000). Previous investigations of non-lactation dairy cattle have reported an association between genetic improvement and changes in insulin secretion in response to a glucose challenge (Mackenzie *et al.*, 1988). Lactating cows that are partitioning nutrients away from the adipose tissue and toward mammary gland, are thought to exhibit insulin resistance, by an decrease in the sensitivity of adipose and muscle tissue to insulin (Cronjé, 2000), i.e. more insulin is required to trigger glucose uptake by adipose and muscle tissue.

The present study challenged NZ HF and OS HF with glucose to examine genotype and diet differences in insulin response and the response of other endogenous hormones (IGF-I and leptin) considered to play a role in gluconeogenesis and nutrient partitioning.

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MATERIALS AND METHODS

Experimental design and treatments

This trial was conducted during the third year (2000) of a multi-year trial previously described by Kolver *et al.* (2000). Two HF genotypes of New Zealand and overseas origin (NZ and OS) either grazed pasture at generous allowances (Grass; >45 kg DM allowance/cow/day) or were fed *ad libitum* on a total mixed ration (TMR; 25% maize silage, 21% grass silage, 2% hay, 10% whole cottonseed, 42% concentrate) in a 2x2 factorial design. Twenty-five third-lactation cows were challenged with glucose. The four treatments were NZ Grass (n=6), NZ TMR (n=7), OS Grass (n=6) and OS TMR (n=6).

Live weight was determined weekly and body condition score fortnightly. Two weeks after calving (16 ± 3 d; mean ± SD) all animals were submitted to a glucose challenge. Cows from both the Grass and TMR treatments were withdrawn from their respective feeds the evening before the challenge (approximately 1900 h).

Indwelling jugular catheters were fitted under local anaesthesia the day before the challenge. The challenge started two hours after the morning milking. Glucose solution (50%) was administered via the catheter such that each cow received a dose of 300 mg of D-glucose/kg body weight over a period of 2 min. Blood samples (10 ml) were withdrawn at -30, -15, -5, 0, 5, 10, 15, 20, 30, 40, 60, 90 and 120 min relative to the time of the challenge. After infusion, each catheter was flushed with 100 ml of saline. Blood samples were collected into heparinised vacutainers and immediately placed on ice. Samples were centrifuged at 3000*g* for 10 minutes, within an hour of collection. Plasma was aspirated and samples stored at -20˚C until hormone assay or analysis for glucose concentration. All animal experimentation was performed

following approval by the Ruakura Animal Ethics Committee.

Immunoassays

Plasma glucose concentrations were measured by the hexakinase method using a spectrophotometric auto-analyser: Hitachi 717 (Roche) by Alpha Scientific Ltd (Hamilton, New Zealand). The inter-assay coefficients of variation (CVs) were 3%, and the intra-assay CV's were 2% for the analysis of glucose content.

Leptin in plasma was measured in duplicate by the double-antibody RIA method of Blache *et al.* (2000). The limit of detection of the assay was 0.2 ng/ml; the inter-assay and the intra-assay coefficients of variation were 5.7%in and 4.8%, respectively for pulled samples containing 0.38 and 0.58 ng/ml leptin.

Plasma IGF-I was measured in duplicate by the chloramine-T RIA method (Gluckman *et al.,* 1983). The limit of detection of the assay was 10 ng/ml; the inter-assay and the intra-assay coefficients of variation were 5.7 and 5.3%, respectively for pulled samples containing 7.7 and 27.7 ng/ml IGF-I over 2 assays.

Insulin in plasma was measured in duplicate by double-antibody RIA method of Hales and Randle (1963). Insulin antiserum (GP2, 21/7/80) was kindly donated by Dr. Peter Wynn (CSIRO Division of Animal Production, NSW, Australia). It was raised in guinea pigs using bovine insulin (BI 4499, Ely Lilly Pty Ltd, Australia). The limit of detection of the assay was 0.89 ng/ml for plasma; Six replicates the inter-assay and intra-assay coefficients of variation were 3% and 2%, respectively for pulled samples containing 0.38 and 0.58 ng/ml insulin.

Statistical analyses

Live weight and condition score data, hormone and glucose concentrations at each sample time, and the calculated area under the response curves were analysed by analysis of variance using the statistical package Genstat to examine for effects and interactions of genotype and feeding system.

RESULTS

Mean body condition of cows fed TMR was higher ($P< 0.05$) than cows grazing pasture, both at calving and at the start of the experiment (Table 1). For live weight, there was a significant ($P< 0.05$) effect of genotype, with OS heavier than the NZ cows before calving (Table 1). Two weeks after calving there was a significant ($P< 0.05$) effect of both diet and genotype. Cows fed TMR being heavier than cows fed pasture, and OS were higher than the NZ HF (Table 1). There were no significant interactions.

Plasma glucose concentrations were similar in all the groups throughout the sample collection period (Figure 1a). Infusion of glucose resulted in an immediate elevation in plasma concentrations in all cows, with highest levels measured in the sample taken 5 minutes after infusion (Figure 1a).

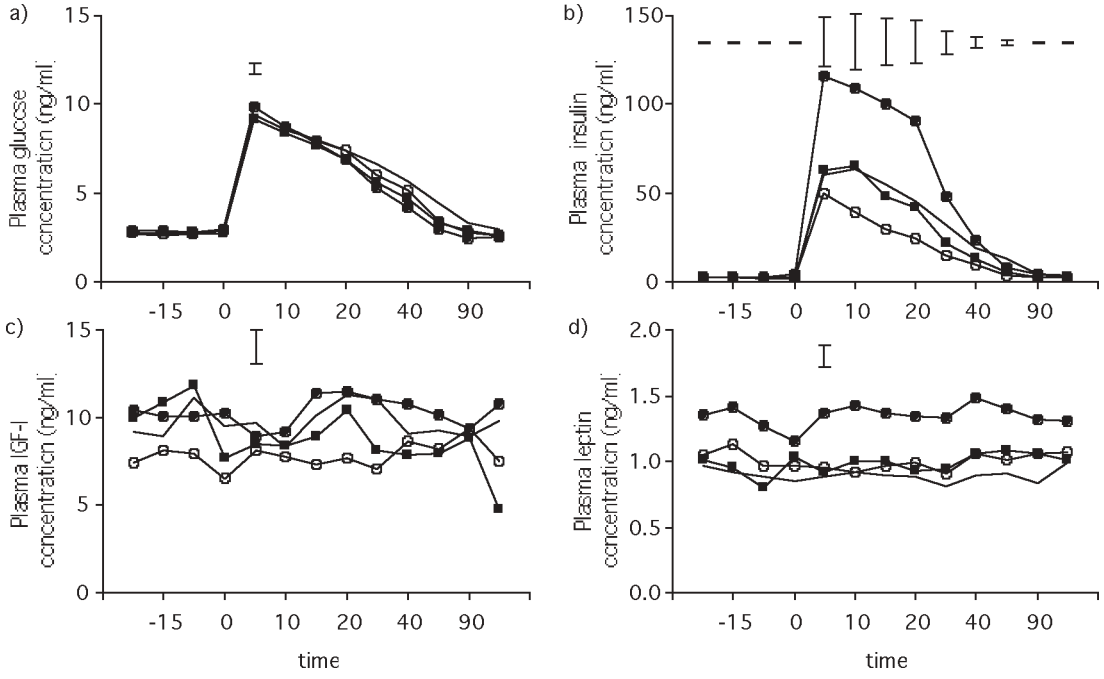
Mean plasma insulin concentrations were similar for all groups before the glucose infusion. The NZ TMR cows

TABLE 1. Mean (± SEM) live weight and body condition score at calving and after 4 weeks in New Zealand (NZ) and Overseas (OS) cows grazing pasture (Grass) or fed total mixed ration.

Group	NZ Grass	NZ TMR	OS Grass	OS TMR	SEM*
Live weight (kg)					
Calving	565	619	686	734	18
Week 2	488	518	564	608	13
Body condition score					
Calving	5.3	6.6	5.3	6.5	0.5
Week 2	4.4	5.9	4.6	5.2	0.4

*Average standard error of means

FIGURE 1. Mean (± SEM) plasma glucose, insulin, IGF-I and leptin concentration during a glucose challenge in New Zealand cows grazing pasture (—○—) or fed total mixed ration (—●—) and Overseas cows grazing pasture (—□—) or fed total mixed ration (—■—).



had higher (P<0.05) plasma insulin concentrations than the NZ Grass following glucose infusion (Fig. 1b). There was a significant (P<0.05) interaction between diet and genotype for the area under the curve (data not shown). Plasma insulin concentrations were similar in both OS HF groups, and both OS HF groups had greater insulin than the NZ Grass. The mean areas under the response curves were 3199, 997,1573 and 2028 ng/ml/min for the NZ TMR, NZ Grass, OS TMR and OS Grass groups, respectively.

Plasma concentrations of IGF-I were similar for all groups, and did not change with the glucose challenge (Fig. 1c).

Glucose infusion did not influence plasma leptin concentrations, however the mean leptin concentration was higher for the NZ TMR than the other groups (P< 0.05, Fig. 1d).

DISCUSSION

New Zealand cows fed total mixed ration (NZ TMR) had a greater insulin response to the glucose challenge than New Zealand cows grazing pasture (NZ Grass), and there was a significant genotype-by-feed-regime interaction. Plasma concentrations of IGF-I did not differ between genotype or feed regime as a consequence of the challenge. Similarly, plasma levels of leptin were not affected by the glucose challenge, but, throughout the sampling period, concentrations in NZ TMR were higher than in the NZ Grass cows, and than in both groups of OS cows.

The NZ TMR cows differed from other groups in their insulin response to glucose challenge. These results agree with earlier reports that insulin release in response to glucose infusion is greater in high-producing cows fed TMR (Xing *et al.,* 1991); however, in the OS TMR cows, which were producing more milk, had a similar response to cows grazing pasture. Thus, there is an effect of both genotype and feed regime on metabolism. The higher insulin concentration required by the NZ TMR cows to clear the same glucose load suggests that NZ TMR cows were insulin resistant. The NZ TMR cows were heavier than the NZ Grass cows at calving, and lost less live weight and body condition than the overseas cows fed either TMR or grass. The plasma concentration of leptin in the NZ TMR group was also higher than that in the other groups. There may be interacting relationships between leptin and insulin sensitivity. Leptin and insulin act centrally as feedback signals, and are sensitive to both body-fat mass and energy balance. In a human study of obese subjects, restriction of energy intake to create a negative energy balance markedly reduced both insulin resistance and leptin levels before there was any substantial weight loss (Assali *et al.,* 2001). This study focused on the role of energy balance on loss in body fat during weight reduction. During early lactation, circulating levels of insulin fall in most species, including cattle; then as lactation progresses and milk yield falls, serum insulin levels recover (Cowie *et al.,* 1980). During early lactation in high-producing dairy cows, total energy expenditure exceeds intake capacity with an increased supply of nutrients required from tissue reserves for milk production (Blake & Custodio, 1984). Selection for milk production is considered to increase the cow's ability to mobilise adipose tissue reserves in early lactation and replace them in late lactation. The decline in insulin levels in early lactation has been attributed to negative energy balance, but these results suggest that, in cattle, there may also be factors that influence the responsiveness of the pancreas to insulinotropic agents.

Plasma concentrations of IGF-I were similar in all groups after calving. This result confirms the observations of Lucy *et al.* (1998) who compared a control line that was analogous to dairy cattle of the 1960s and a selected line that represented modern (1990s) dairy cows. In that study of the consequences of the genetic selection of dairy cattle on reproductive endocrinology, plasma concentrations of IGF-I were similar in both selected and control lines around two weeks after calving. Lucy *et al.* (1998) showed that selection for milk production influenced the dynamics of IGF-I secretion, however the postpartum stage at which this experiment was carried out would likely have preceded those differences that are expressed later in the lactation.

Plasma leptin concentrations did not change during the glucose challenge. Leptin release is regulated by factors other than adiposity, and the homeostatic control mechanism of some metabolites may influence this. For instance, glucose transport and metabolism are implicated in the regulation of leptin expression and secretion in rats (Mueller *et al.,* 1998) and humans (Wellhoener *et al.,* 2000). Kauter *et al.* (2000) investigated the effect of glucose, adrenaline, insulin or glucagon on regulation of plasma leptin in sheep, and found that a single injection of glucose or hormones that affect blood glucose concentrations did not stimulate leptin release. In this experiment, the final sample was taken only 2 hours after glucose infusion so it is possible that, if the time-frame for changes to be induced in plasma leptin concentrations is greater than two hours, then such effects would not have been observed here. Consistent with these results, Gabai *et al.* (2002) observed no effect of infusion of glucose on leptin secretion in early lactating beef cows even though the infusion of glucose resulted in a 2- to 5.5-fold increase in blood glucose which was maintained for 6 hours and elicited a massive pancreatic response. Likewise, the results from the present study indicate that glucose and insulin do not stimulate leptin secretion acutely as previously observed (Kauter *et al.,* 2000; Gabai *et al.,* 2002). Further, these results do not exclude the possibility that the effects of glucose and insulin on leptin release may be modulated by the energy status of the animals. This suggestion is consistent with the fact that the cows fed TMR had higher leptin concentration and also better body condition than all the other cows during the glucose challenge. It also might be due to differences of regulatory mechanism between monogastric and ruminant animals. Leptin levels in cows were observed to decrease during the postpartum period, reflecting negative energy balance status and mobilisation of fat depots, and prolonged postpartum decline in leptin concentrations was associated with decreased reproductive performance (Kadokawa *et al.,* 2001). This

lends further support to the notion that OS cows have greater ability to mobilise body fat reserves for milk production, and that high-producing cows on pasture-only diets are in nutritional deficit during early lactation.

During the early postpartum period, NZ cows fed a total mixed ration had a higher response to an intravenous glucose challenge that either overseas HF grazing pasture or fed a total mixed ration, or NZ HF grazing pasture. Mean plasma concentrations of leptin were also higher in NZ HF fed total mixed ration. Further study is needed to clarify the interactions between the levels of insulin, leptin concentrations and energy balance of dairy cows in pasture-based dairy systems. Understanding the genetic and nutritional basis of regulation of hormones involved in nutrient partitioning is important if management strategies are to be developed to meet the nutritional and reproductive requirements of the modern dairy cows of differing genotype.

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