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Responses of plasma glucose and nonesterified fatty acids to intravenous insulin tolerance tests in dairy cows during a 670-day lactation

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ABSTRACT

The metabolic response of dairy cows undergoing an extended lactation to an insulin tolerance test (ITT) was investigated. Twelve multiparous Holstein-Friesian cows that calved in late winter in a pasture-based system were managed for a 670-d lactation by delaying rebreeding. Four 5-wk experimental periods commenced at approximately 73, 217, 422, and 520 d in milk (DIM). Cows were offered a diet of perennial ryegrass (73 and 422 DIM) or pasture hay and silage (217 and 520 DIM) supplemented with 1 kg dry matter (DM) of grain (control; CON) or 6 kg DM of grain (GRN). Daily energy intake was approximately 160 and 215 MJ of metabolizable energy/cow for CON and GRN, respectively. At all other times, cows were managed as a single herd and grazed pasture supplemented with grain to an estimated daily intake of 180 MJ of metabolizable energy/cow. Cows were fitted with a jugular catheter during the final week of each experimental period. An ITT using 0.12 IU of insulin/kg of body weight (BW) was conducted on each cow at approximately 100, 250, 460, and 560 DIM. Cows in the GRN treatment had greater milk yield, milk solids yield, and BW than cows in the CON treatment. Within treatment, individual cow responses to the ITT were highly variable. Plasma glucose and nonesterified fatty acid (NEFA) concentrations declined at all stages of lactation. The clearance rate of plasma glucose was slower before 300 DIM than after 300 DIM, which indicates greater inhibition of hepatic glucose synthesis and uptake of glucose by insulin-dependent tissues later in the lactation. The clearance rate, area under the curve, and recovery of plasma NEFA were greatest at 100 DIM, indicating greater responsiveness to the antilipolytic effect of insulin in early lactation, but also greater lipolytic responsiveness. The variation in response to the ITT was mostly a result of DIM rather than diet. However, the plasma NEFA response showed interactions between diet and DIM, indicating that energy intake may affect tissue responses to insulin. The responsiveness of peripheral tissues to insulin, primarily adipose tissue, changed throughout a 670-d lactation and contributed to a greater proportion of nutrients being partitioned to body reserves at the expense of milk yield as lactation progressed. Both stage of lactation and dietary intake have a role in the determination of whole-body and peripheral tissue responses to insulin; however, the exact mechanisms in control of this are unclear.
Key words: extended lactation, insulin sensitivity, fatty acid metabolism, nutrient partitioning

INTRODUCTION

There is large variation in the capacity of cows to undergo extended lactations of up to 670 d in the pasture-based dairying systems of Australia (Auldist et al., 2007; Grainger et al., 2009) and New Zealand (Kolver et al., 2007). Some of this may be due to differences in physiological characteristics of cows, leading to variation in the regulation of nutrient partitioning. In previous experiments, cows that were unable to complete a 670-d lactation gained more BW and BCS than those able to continue milking. Those cows that successfully completed an extended lactation also had higher plasma concentrations of growth hormone and nonesterified fatty acids (**NEFA**), but lower concentrations of insulin, glucose, and leptin beyond 300 DIM (Delany et al., 2010; Marett et al., 2011). It has been shown that grazing cows with either restricted or unrestricted feed intakes had similar lactational persistency during extended lactations (Grainger et al., 2009; Delany et al., 2010; Marett et al., 2011).

Insulin is a key regulator of glucose homeostasis in mammals, with blood concentrations shifting with changing physiological priorities of the body (Bauman and Currie, 1980). The actions of insulin are anabolic, promoting the storage of energy substrates, such as glu-

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cose and fatty acids (Blum et al., 1983), and inhibiting hepatic glucose production (Brockman and Laarveld, 1986). Plasma insulin is decreased during times of increased energy requirements for milk production, supporting nutrient partitioning toward the mammary gland for milk production by decreasing the utilization of these nutrients by insulin-dependent tissues (Bauman and Currie, 1980). This occurs because the movement of glucose across the cell membrane for milk production in the mammary gland largely does not require insulin (Collier et al., 1984). Insulin resistance occurs before and after parturition in lactating goats (Debras et al., 1989), sheep (Prior and Christenson, 1978), and cows (Sano et al., 1993; Bell and Bauman, 1997; Vernon and Pond, 1997). Cows with high milk yield have a greater degree of insulin resistance than lower-yielding counterparts, regardless of whether the increased milk yield is due to treatment with recombinant somatotropin (Sechen et al., 1990) or simply genetic merit (Cronje, 2000; Chagas et al., 2009).

Insulin sensitivity, as well as glucose and fatty acid metabolism, have been studied intensively throughout the periparturient period and early lactation in dairy cows. However, there are few data beyond 300 DIM and even fewer in pasture-fed cows. Marett et al. (2015) reported that whole-body sensitivity to insulin following an intravenous glucose tolerance test (**IVGTT**) in dairy cows fed a pasture-based diet and undergoing a 670-d lactation was highly variable. Those authors showed, in response to an IVGTT, a reduction in responsiveness to insulin in terms of glucose clearance but an increase in the antilipolytic response to insulin at 4 stages of an extended lactation. This suggests that the regulation of insulin sensitivity and responsiveness during lactation may vary between insulin-responsive tissues in response to changing mammary gland requirements for milk production. Further, differences may exist in the proportions of the response that are related to either skeletal muscle or adipose tissue at any given stage of lactation or physiological state. Diet may also affect the dairy cow's response to insulin. Although Marett et al. (2015) showed that feeding a diet with a greater proportion of grain decreased whole-body responsiveness to insulin in terms of plasma glucose but not fatty acids response, Schoenberg et al. (2012) reported that insulin sensitivity was not affected by feed intake in nonlactating cows. Thus, it remains unclear whether feed or energy intake affects insulin sensitivity.

To adequately develop nutritional strategies for use during extended lactations, a better understanding of the regulation of nutrient partitioning is required. The insulin tolerance test (**ITT**) investigates the insulin responsiveness of insulin-dependent tissues, primarily adipose and skeletal muscle tissues (Kaneko, 1997). In these experiments, the ITT was conducted at 4 stages of a 670-d lactation to investigate insulin responsiveness in terms of plasma glucose and fatty acids responses. In particular, we aimed to investigate the response of peripheral tissues to exogenous insulin in terms of glucose clearance and the inhibition of lipolysis. These experiments aimed to identify sources of variation in metabolic responses to hyperinsulinemia in cows undergoing an extended lactation and determine whether the responses were affected by cereal grain intake. We hypothesized (1) that the estimated responsiveness to insulin, in terms of the glucose and fatty acids response to an exogenous insulin infusion, would increase with increasing DIM; and (2) that the dietary intake of cereal grain would not affect either the plasma glucose response or the plasma fatty acids response to the ITT.

MATERIALS AND METHODS

Location

This experiment was conducted at the Department of Economic Development, Jobs, Transport and Resources (DEDJTR) Ellinbank Centre in Victoria, Australia (38°14′S, 145°56′E). All procedures were approved by the DEDJTR Animal Ethics Committee.

Cows and Management

The experimental design was described previously by Marett et al. (2015). Briefly, the experiment used 12 multiparous Holstein-Friesian cows of mixed age that calved in late July (winter). They were managed for an extended lactation by delaying breeding until ~450 DIM for a target lactation length of 670 d. They were managed as a single herd for the majority of their lactations and grazed perennial ryegrass (*Lolium perenne*) pasture supplemented with cereal grain fed twice daily in the parlor at milking times. When pasture was limiting during the summer and autumn months, pasture hay (approximately 6 kg of DM/cow per day) and pasture silage (approximately 10 kg of DM/cow per day) were offered to achieve an estimated daily intake of 180 MJ of ME/cow.

There were 4 experimental periods of up to 40 d during these lactations, beginning at each of ~73, 217, 422, and 520 (±9.1) DIM (mean ± SD). Two treatments were used, designated control (**CON**) and grain (**GRN**), each allocated randomly to 6 cows. Treatment groups were balanced for parity, calving date, BW, and yields of milk, protein, and fat in the preceding lactation (Baird, 1994). During the experimental periods, CON cows were fed 1 kg (DM) of grain/d and GRN cows were fed 6 kg (DM) of grain/d, with half of the