Short Communication: Regulation of Milk Fat Yield and Fatty Acid Composition by Insulin

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ABSTRACT

Diet-induced milk fat depression in dairy cows has been known for many years and several theories have been proposed. One that continues to receive support is the glucogenic-insulin theory. Previous studies testing this theory using a hyperinsulinemic–euglycemic clamp have had variable results attributable to variability in the use of body fat reserves as a source of milk fatty acids. Our objective was to test the glucogenic-insulin theory using cows immediately postpartum, a period when the use of body fat for milk fat synthesis is greatest. During wk 2 postpartum, 5 cows were given a 2-d baseline period and then clamped for 4 d. Insulin was increased more than 2-fold during the clamp while the blood glucose concentration was maintained. Milk yield was not altered by administration of the clamp (38.7 vs. 39.0 ± 1.4 kg/d); however, the milk fat percentage and yield were reduced by 27% and plasma nonesterified fatty acids were reduced by 68%. Analysis of the milk fatty acid composition revealed that the decrease in milk fat yield during use of the clamp was almost exclusively due to reductions in preformed fatty acids; this is the exact opposite of what is observed with diet-induced milk fat depression. Therefore, our results do not support the glucogenic-insulin theory of diet-induced milk fat depression. The results further indicated that reductions in milk fat observed previously with hyperinsulinemic–euglycemic clamps or with glucose or propionate infusions were most likely consequences of the ability of insulin to inhibit lipolysis, thereby limiting the mammary availability of preformed fatty acids mobilized from body reserves.

Key words: insulin clamp, milk fat depression, milk fat synthesis

Low milk-fat syndrome, commonly known as milk fat depression (**MFD**), is a condition observed in dairy cows

in which milk fat production can decrease up to 50% without any effect on the yield of milk or other milk components. First described over 150 yr ago (Bauman and Griinari, 2001), the nutritional factors associated with diet-induced MFD and management practices to minimize its occurrence have been reviewed (Davis and Brown, 1970; Palmquist et al., 1993). Many theories have been proposed to explain the basis for diet-induced MFD, but most are inadequate (Davis and Brown, 1970; Bauman and Griinari, 2003). One that has some support is the glucogenic-insulin theory, which proposes that MFD is a consequence of a shortage of lipogenic precursors for milk fat synthesis. The mammary gland is relatively unresponsive to insulin and, according to this theory, diets that elevate circulating insulin result in preferential channeling of lipogenic precursors to body fat (Sutton, 1989).

Directly testing the role of insulin as a cause of diet-induced MFD poses significant challenges because of the central role of insulin in the maintenance of glucose homeostasis. Simply injecting insulin leads to hypoglycemia, which results in a marked decline in milk yield (Schmidt, 1966). Similarly, glucose infusions increase circulating insulin concentrations, but results are often confounded by counter-regulatory changes related to maintenance of glucose homeostasis. An approach that avoids these complications is the hyperinsulinemic–euglycemic clamp, by which an infusion of exogenous insulin elevates circulating insulin while euglycemia is simultaneously maintained by infusing glucose at variable rates to preserve homeostasis. Studies using this technique during established lactation have observed reductions in milk fat production averaging about 5% (Griinari et al., 1997; Mackle et al., 1999), although Molento et al. (2005) observed a 15% decrease. Mashek et al. (2002) used a hyperinsulinemic–euglycemic clamp in dairy cows and reported 12 and 5% reductions in milk fat yield at 4 and 17 wk postpartum, respectively. Most of these investigations have examined effects on milk fatty acids; in contrast to diet-induced MFD, the decrease in milk fat has predominantly been associated with a reduction in longer chain fatty acids, and based on this, it has been suggested that MFD is related to a reduction in fatty acids derived from body fat reserves

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(Bauman and Griinari, 2000; 2003; Molento et al., 2005).

The mobilization of body fat reserves and the use of long-chain fatty acids for milk fat synthesis are greatest in early lactation (Bell, 1995). Thus, our objective was to use a hyperinsulinemic–euglycemic clamp to test the glucogenic–insulin theory of MFD in cows during the interval immediately postpartum. All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Cornell University, and details describing the experimental design, conduct of the hyperinsulinemic–euglycemic clamp, and analytical procedures for glucose, insulin, and NEFA were previously reported (Butler et al., 2004). Briefly, 5 lactating Holstein cows, 8 DIM at the initiation of the study, were given ad libitum access to a TMR formulated to meet or exceed requirements. Cows were milked twice daily and the yields recorded. Blood and milk samples were collected during the 2-d baseline period and throughout the 4-d period of the hyperinsulinemic–euglycemic clamp. During administration of the clamp, insulin was continuously infused via a jugular catheter at an hourly rate of 0.3 μ g/kg of BW. To maintain euglycemia ($\pm 10\%$ of baseline glucose concentrations), glucose was continuously infused via a jugular catheter at variable rates, based on glucose concentrations from plasma samples analyzed at frequent intervals throughout the clamp. The milk fatty acid composition was determined by gas chromatography (Kelsey et al., 2003). Data were analyzed by ANOVA using the GLM procedure of SAS (SAS Institute, Inc., Cary, NC). Means for the 2-d baseline period were compared with means for the last 2 d of the hyperinsulinemic–euglycemic clamp period.

The 4-d hyperinsulinemic–euglycemic clamp was executed successfully, as indicated by plasma concentrations of insulin and glucose. The infusion of exogenous insulin elevated plasma insulin concentrations by more than 2-fold while the glucose infusion maintained euglycemia (Table 1). The rate of exogenous glucose infusion to achieve euglycemia was initially 40 g/h and increased progressively over the first 72 h to a relatively constant rate of 97 g/h during the last 24 h. The role of insulin in maintaining energy homeostasis includes the regulation of adipose rates of lipolysis, and this was evident during the hyperinsulinemic–euglycemic clamp period. Insulin infusion reduced plasma NEFA concentrations by 68% compared with the baseline period (Table 1). Over the same time interval, a separate group of cows not receiving the insulin clamp maintained their plasma NEFA concentrations at 96% of baseline values (Butler et al., 2004).

Milk yield (Table 1) and DMI (Butler et al., 2004) were not affected by the insulin clamp. Similarly, the

Table 1. Plasma variables, milk yield, milk components, and milk fatty acid composition of lactating dairy cows before (baseline) and during administration of a 4-d hyperinsulinemic–euglycemic clamp

Parameter	Baseline ¹	Insulin clamp ²	SEM	P
Plasma				
Insulin, ng/mL	0.22	0.70	0.05	0.001
Glucose, mg/dL	41.6	39.9	1.4	0.39
NEFA, μ M	1,046	334	94	0.001
Milk yield, kg/d	38.7	39.0	1.4	0.87
Milk fat				
%	4.96	3.60	0.19	0.001
g/d	1,938	1,414	120	0.01
Milk fatty acids, %				
4:0	4.13	4.02	0.11	0.50
6:0	1.39	2.01	0.08	0.001
8:0	0.57	1.04	0.06	0.001
10:0	0.94	2.09	0.14	0.001
12:0	0.98	2.18	0.16	0.001
14:0	4.46	7.41	0.32	0.001
16:0 + 16:1	23.63	27.44	0.75	0.005
18:0	18.92	14.08	0.75	0.005
18:1, <i>trans</i>	3.01	2.70	0.08	0.02
18:1, <i>cis</i>	33.73	28.65	0.90	0.001
18:2	3.21	3.18	0.14	0.85
18:3	0.42	0.39	0.02	0.37
Others	4.61	4.81	0.08	0.10

¹Baseline values are least squares means for samples collected during the 2 d immediately prior to initiation of the hyperinsulinemic–euglycemic clamp. Plasma samples (4/d) and milk samples (2/d) were collected on each day of the baseline period. Daily means were computed and used for statistical analysis.

²Values for the insulin clamp are least squares means for samples collected during the final 2 d of the 4-d hyperinsulinemic–euglycemic clamp. Plasma samples were collected hourly during the clamp and milk samples were collected from each milking (2/d). Daily means were computed and used for statistical analysis.

milk content of protein and lactose were unaffected (data not shown). However, milk fat was altered, with the milk fat percentage and milk fat yield reduced by 27% (Table 1). The fatty acid composition of milk was altered during use of the hyperinsulinemic–euglycemic clamp toward a lower proportion of long-chain fatty acids. Concomitantly, the milk fat content of fatty acids with 16 carbons or less increased during the hyperinsulinemic–euglycemic clamp period.

Fatty acids comprising milk fat triglycerides originate from 2 different sources. Fatty acids of 4 to 14 carbons and about one-half of the 16-carbon fatty acids are synthesized de novo by the mammary gland from acetate and BHBA (Bauman and Griinari, 2003). In contrast, long-chain fatty acids (>16 carbons) and the remainder of the palmitate (16:0) are derived by mammary uptake from circulation. We examined changes in the pattern of milk fatty acid yield by expressing fatty acids on a molar basis and grouping them by source of origin. The reduction in milk fat yield was gradual over the 4-d clamp period (Figure 1), and it was almost exclusively due to a decrease in the use of the longer chain fatty acids taken up from circulation (Figure 2).

Figure 1. Effects of the hyperinsulinemic-euglycemic clamp on milk fatty acid yield (mmol/d). Fatty acids are grouped by origin: Fatty acids <16 carbons are derived from de novo synthesis, fatty acids >16 carbons are derived from the uptake of preformed fatty acids, and fatty acids with 16 carbons are derived equally from both sources. Values are means $\pm$ SEM.

The sources of plasma fatty acids are dietary and rumen microbial fatty acids absorbed from the small intestine and fatty acids mobilized from body fat reserves. Those from body fat reserves circulate as NEFA,

Figure 2. Change in milk fatty acid yield (mmol/d) during administration of the hyperinsulinemic-euglycemic clamp as compared with the baseline period. Fatty acids are grouped by origin: Fatty acids <16 carbons are derived from de novo synthesis, fatty acids >16 carbons are derived from the uptake of preformed fatty acids, and fatty acids with 16 carbons are derived equally from both sources.

and plasma NEFA concentrations are highly correlated with net energy balance and rates of adipose tissue lipolysis (Bauman et al., 1988). The contribution of adipose tissue lipolysis to milk fatty acids can range from a low of about 4 to 8% in well-fed cows to over 25 to 40% during early lactation or feed restriction (Palmquist and Mattos, 1978; Bell, 1995). In the present study, reductions in plasma NEFA concentration and changes in the pattern of milk fatty acids indicate that the decrease in milk fat synthesis during use of the hyperinsulinemic-euglycemic clamp was a consequence of reduced availability of plasma fatty acids derived from the mobilization of body fat reserves.

According to Bauman and Griinari (2000), the reduction in milk fatty acids with diet-induced MFD is most pronounced for de novo-synthesized fatty acids, although all chain lengths of fatty acids are decreased. This is very different from our observation with the hyperinsulinemic-euglycemic clamp. Rather, the reduction that occurs with elevated insulin is due to reduced rates of adipose lipolysis, thereby limiting the supply of preformed fatty acid precursors, as suggested previously (Bauman and Griinari, 2000, 2003; Molento et al., 2005). Our study using cows in very early lactation, where fatty acids mobilized from body fat reserves make a substantial contribution to milk fatty acids, represents a particularly powerful demonstration of this point. We observed a 27% reduction in milk fat yield during wk 2 postpartum (Table 1) vs. the 5% reduction generally observed in established lactation (Griinari et al., 1997; Mackle et al., 1999). Although Mashek et al. (2002) did not examine the milk fatty acid composition, their results are similar, with an insulin clamp resulting in a 12% reduction in milk fat yield at 4 wk postpartum but only a 5% reduction at 17 wk postpartum. Further, our results suggest that the wide range in effects of propionate infusion [0 to 14% reduction in milk fat; 13 studies summarized by Davis and Brown (1970)] and glucose infusion [0 to 16% reduction in milk fat, as reviewed by Bauman and Griinari (2001)] are likely related to propionate- and glucose-induced pancreatic release of insulin on lipolytic rates and variability in the proportion of milk fatty acids derived from body fat reserves as a consequence of differences in net energy balance.

Overall, our results do not support the glucogenic-insulin theory of MFD and suggest that insulin plays little or no role in the reduction in milk fat that occurs in the low-fat milk syndrome. In contrast, recent research from others provides additional support for the biohydrogenation theory of MFD, although further research and verification are needed to establish whether this represents a unifying theory that is broadly applicable

to explain the low-fat milk syndrome (Griinari and Bauman, 2006).

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