Role of Insulin in the Regulation of Milk Fat Synthesis in Dairy Cows

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ABSTRACT

Five lactating Holstein cows were fitted with rumen fistulas and subjected to a hyperinsulinemic-euglycemic clamp and abomasal casein infusion to examine the effects on milk fat synthesis and the composition of milk fatty acids. The experiment consisted of two periods of abomasal infusions (water or 0.5 kg/d of casein); each period was divided into three 4-d intervals. The initial interval allowed for acclimation, and baseline measurements were established during the second interval. During the third 4-d interval, a hyperinsulinemic-euglycemic clamp was maintained, and insulin was infused continuously at the rate of 1 μg/kg of body weight per h. Circulating concentrations of insulin were increased more than fourfold, and euglycemia was maintained by infusion of glucose at variable rates. Insulin had no effect on milk fat yield but casein infusion increased milk yield and tended to increase fat yield. A trend toward higher milk yield during the clamp, combined with a slight numerical decrease in milk fat yield, resulted in decreased fat percentage. Calculated net energy balance was positive throughout the study, although feed intake decreased during the insulin clamp, particularly for the water infusion period. Minor changes occurred in the composition of milk fatty acids during the clamp when the balance between de novo and preformed fatty acids shifted slightly toward de novo.

INTRODUCTION

Milk fat is composed mainly of triglycerides. Precursors for milk fatty acids—acetate, BHBA, and preformed fatty acids—are taken up from the circulation by the mammary cell. Several reviews (19, 29, 32) have summarized the factors that affect milk fat percentage and yield. Nutrition plays a major role, which is perhaps most evident for milk fat depression. Endocrine status may alter the partitioning of nutrients to specific tissues by affecting rates of utilization, uptake, or release. For example, the effects of insulin on rates of lipogenesis (stimulatory) and lipolysis (inhibitory) in adipose tissue are well documented (5, 6). The glucogenic-insulin theory of milk fat depression, which occurs for cows consuming a high concentrate, low roughage diet, is based on these effects. This theory is based on the competition of organs and tissues for nutrients and the responsiveness in uptake of lipogenic precursors by adipose tissue, but not the mammary gland, to changes in circulating concentrations of insulin. The glucogenic-insulin theory proposes that increased insulin release, which occurs when high concentrate diets are fed, preferentially channels nutrients to adipose tissue, resulting in a shortage of nutrients at the mammary gland and, thus, milk fat depression (1, 9, 17, 30, 34). Other theories suggest that milk fat depression is caused by a direct inhibition at the mammary gland of one or more steps in the synthesis of milk fat (11, 13). According to these theories, the changes in body fat accretion and adipose tissue metabolism are consequences of a more positive energy balance caused
by the reduced output of milk fat and the higher net energy intake typically associated with high concentrate diets. Recent studies (20) using a hyperinsulinemic-euglycemic clamp technique support this conclusion. Although a fivefold increase in circulating insulin concentrations demonstrated typical and expected physiological effects on circulating metabolites, the yield of milk fat was not affected (20).

The objective of the present study was to examine further the role of insulin in the regulation of milk synthesis by investigating the effects of hyperinsulinemia and associated changes in lipid metabolism on milk fat synthesis and the composition of milk fatty acids. Fatty acid composition of milk fat reflects, to a certain degree, the supply of precursors, and milk fat depression has distinct effects on the composition of milk fatty acids (22). By including abomasal infusion of water or casein, we also were able to test the effect of protein supply on milk protein synthesis stimulated by insulin; results specifically related to protein supply are presented elsewhere (15).

MATERIALS AND METHODS

All procedures involving the cows were approved by the Cornell University Institutional Animal Care and Use Committee. Five Holstein cows in their second (n = 4) or third lactation (n = 1) that had been fitted with rumen fistulas were subjected to a hyperinsulinemic-euglycemic clamp and abomasal infusions. At the initiation of the study, cows averaged 558 ± 23 kg of BW and were 184 ± 19 d postpartum (X ± SEM). Abomasal infusions and insulin clamp treatments were arranged in a crossover design. Each cow was clamped twice for two levels of abomasal infusion: water infusion (6 L/d of water) or casein infusion (0.5 kg/d of sodium caseinate solubilized in 6 L of water). Abomasal infusions were administered via nalgene tubing (0.5 mm i.d.) that passed through the rumen fistula and sulcus omasi into the abomasum (27). Infusate was continuously infused by a peristaltic pump (Harvard Apparatus, South Natick, MA) at the rate of 6 L/24 h.

The experiment consisted of two periods (12 d each) of abomasal infusions; 4 d separated the periods. Each period was divided into three 4-d intervals. The initial 4-d interval allowed for acclimation. Baseline measurements were established during the second 4-d interval, and, finally, a hyperinsulinemic-euglycemic clamp was maintained for the third 4-d interval. Cows were fed for ad libitum intake a total mixed diet (Table 1) formulated to meet nutrient requirements of the NRC (21). Equal portions of feed were offered at 2-h intervals to minimize postprandial effects on nutrient supply; water was available at all times. Cows were milked at 0600 and 1800 h daily. Prior to baseline measurements, two indwelling catheters were inserted in each external jugular vein. Glucose and insulin were infused on one side, and blood samples were taken on the contralateral side. Cows were treated daily with 10 ml of procaine G penicillin (3 × 10⁶ units/ml; Pfizer Inc., New York, NY) as a prophylactic measure.

Blood samples were obtained every 6 h during the 4-d baseline interval, and mean glucose concentration was determined. During the interval of the hyperinsulinemic-euglycemic clamp, target glycemia (±10%) was based on the mean concentrations of blood glucose determined for each cow during the baseline interval. The infusion solution of insulin was prepared individually for each cow by diluting bovine insulin (I-5500, lots 44H0263 and 103H0690; Sigma Chemical Co., St. Louis, MO) with sterile saline and 2.5% plasma from that cow. The final concentration for each cow resulted in an insulin infusion rate of 1 µg/kg of BW per h delivered by syringe pump (model SE 400; Vial Medical, Grenoble, France) at a constant rate of 3.66 ml/h. During the 4-d clamp period, euglycemia was maintained by infusion of glucose (50% glucose monohydrate solution, wt/vol; Butler Co., Rochester, NY) at variable rates via a syringe pump (Harvard Apparatus). Initially, blood was sampled at 5- to 15-min intervals until euglycemia and glucose infusion rate were stabilized. Thereafter, blood sampling to ensure euglycemia was less fre-

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**TABLE 1. Composition of total mixed diet**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Composition (g/kg of DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chopped alfalfa hay</td>
<td>466</td>
</tr>
<tr>
<td>High moisture shelled corn</td>
<td>392</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>89</td>
</tr>
<tr>
<td>Soybean expeller meal</td>
<td>32</td>
</tr>
<tr>
<td>Monodicalcium phosphate</td>
<td>7.4</td>
</tr>
<tr>
<td>Calcium sulfate</td>
<td>4.2</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>4.2</td>
</tr>
<tr>
<td>Magnesium oxide</td>
<td>2.1</td>
</tr>
<tr>
<td>Salt plus a trace mineral</td>
<td>2.55</td>
</tr>
<tr>
<td>and vitamin mix²</td>
<td></td>
</tr>
</tbody>
</table>

1Diet was 90.5% DM and contained 16.6% CP and 1.63 Mcal of NE₃/kg of DM.
2Contains 385 g/kg of NaCl and 615 g/kg of a trace mineral and vitamin mix. Trace mineral and vitamin mix contained (per kilogram of mix) 1.1 g of Mn, 1.4 g of Zn, 0.50 g of Fe, 0.25 g of Cu, 0.027 g of I, 0.024 g of Co, 0.007 g of Se, 750,000 IU of vitamin A, 280,000 IU of vitamin D₃, and 2,560 IU of vitamin E.
The maximal sampling interval was 1 h. Blood glucose concentration was determined for each sample using an automatic glucose analyzer (model 27; Yellow Springs Instrument, Yellow Springs, OH). Glucose concentrations were determined within 5 min after blood sampling and glucose infusion rates were adjusted if necessary.

At each milking during the baseline and clamp intervals, yield was determined, and milk was sampled. One sample was taken for routine milk composition (New York DHI Cooperative, Ithaca, NY), and a duplicate sample was pooled daily and stored at −80°C until analyzed for fatty acid composition. Blood samples (100 U of heparin/ml of blood) were also taken every 2 h; plasma was harvested and stored at −20°C until analyzed for insulin, NEFA, and triacylglycerol. Plasma samples were also pooled from samples from each cow during the baseline interval and on the last day of the insulin clamp. Plasma pools were submitted to the New York State Veterinary Diagnostic Laboratory (College of Veterinary Medicine, Cornell University) for a large animal clinical screen; analyses were conducted in accordance with standards of the New York State Department of Agriculture and Markets.

Insulin was determined by a double-antibody radioimmunoassay as described by McGuire et al. (20). Bovine insulin (lot 615-70N-80; Lilly Research Laboratories, Greenfield, IN) was used for iodination and standards. Plasma concentrations of triacylglycerol were determined by enzymatic colorimetric analysis using a commercial kit (Sigma Chemical Co.), and NEFA were determined by enzymatic colorimetric analysis (Wako Pure Chemical Industries, Osaka, Japan) as modified by Sechen et al. (26).

A composite milk sample was formed for the 4-d baseline interval and for each day during the insulin clamp. These samples were used to determine concentrations of fatty acids in milk fat by gas-liquid chromatography (Hewlett Packard 5890 gas chromatograph fitted with automatic sampler 7673A, HP Vectra QS/16S data processor with HP3365 Chem Station software, and FID detector; Hewlett Packard, Palo Alto, CA). Preparation of milk fat samples and specific analytical conditions have been detailed by Sukhija and Palmquist (28).

Data were analyzed by ANOVA, and treatment effects were tested using an F test. Single degree of freedom comparisons were made to evaluate the effects of abomasal infusion, hyperinsulinemic-euglycemic clamp, and the interaction of infusion and insulin clamp.

RESULTS

The temporal patterns of plasma insulin and blood glucose concentrations and the glucose infusion rate required to maintain euglycemia are presented in Figure 1. Casein infusion had no effect on any of these variables (P > 0.20). Plasma insulin concentration averaged 1.6 ng/ml during the baseline interval and increased more than fourfold to 6.8 ng/ml (P < 0.001) during the hyperinsulinemic-euglycemic clamp (SEM = 0.6 ng/ml). Blood glucose concentration averaged 48.0 mg/dl during the 4-d clamp, and euglycemia was maintained within 10% of baseline blood glucose concentrations (49.4 mg/dl; SEM = 1.0 mg/d) by simultaneous infusion of glucose. The rate of glucose infu-
REGULATION OF MILK FAT BY INSULIN

Figure 2. Temporal pattern of milk yield and milk fat yield and concentration in lactating cows for the baseline interval followed by the 4-d hyperinsulinemic-euglycemic clamp. Throughout the experimental period, cows received abomasal infusions of casein (solid line) or water (dashed line); values are means of five cows.

Insulin had no significant effect on milk fat yield, but casein infusion increased milk yield and tended to increase fat yield (Table 3). A trend \( (P = 0.08) \) was observed toward higher milk yield during the clamp. This positive trend, accompanied with the slight numerical decrease in milk fat yield caused by insulin, resulted in decreased fat percentage \( (P < 0.01) \). The temporal pattern of milk and milk fat illustrated the relative consistency of these variables over the course of the baseline interval and during the insulin clamp (Figure 2).

Calculated NE balance was positive during the baseline interval and during the insulin clamp (Table 3). However, feed intake decreased during the insulin clamp, and the decrease was greater for the period of water infusion \( (32\% \text{ on d 4}) \) than for the period of casein infusion \( (8\% \text{ on d 4}) \). The interaction term between the insulin clamp and the abomasal infusion was significant for feed intake \( (P = 0.06) \) and energy balance \( (P < 0.05) \) (Table 3). Plasma NEFA concentrations decreased and remained low throughout the clamp, but plasma triglyceride concentrations were not affected by treatment (Table 2).

Yields of long-chain fatty acids \( (C_{18:0}, C_{18:1}, C_{18:2}, \text{ and } C_{18:3}) \) and short-chain fatty acids \( (C_{4:0} \text{ and } C_{6:0}) \) decreased during the insulin clamp. Yields of medium-chain fatty acids \( (C_{10:0} \text{ and } C_{12:0}) \) increased (Table 4). When expressed on a percentage basis, fatty acid composition of milk fat was nearly identical for the periods of casein or water infusion (data not shown). Insulin treatment did not result in major changes in fatty acid composition; however, a few consistent shifts in relative proportions of fatty acids were observed (Figure 3). The proportion of longer chain fatty acids in milk decreased during the insulin clamp. The relative decrease in stearic acid \( (C_{18:0}) \) was greater than that observed for the other major \( C_{18} \) fatty acid, oleic acid \( (C_{18:1}) \) (Figure 3). The decrease of \( C_{18} \) fatty acids in milk was compensated by an increase in the proportion of fatty acids synthesized de novo, in particular, the medium-chain fatty acids \( (C_{10:0} \text{ to } C_{14:0}) \) and palmitic acid \( (C_{16:0}) \) (Figure 3). Overall, the ratio of saturated to unsaturated long-chain fatty acids in milk increased during the insulin clamp (Table 4).

DISCUSSION

Milk fat depression caused by high concentrate, low forage diets usually occurs within a few days following dietary changes and is characterized by a substantial reduction in both yield and percentage of milk fat. The glucogenic-insulin theory suggests that insulin has an important role in the etiology of milk fat depression \( (1, 9, 11, 29, 34) \). High concentrate diets result in increased rumen production of propionate and increased hepatic rates of gluconeogenesis, which in turn increase pancreatic release of insulin. The elevated circulating concentrations of insulin, often two- to fivefold increases \( (16, 30) \), enhance uptake of lipogenic precursors and decrease the release of fatty acids from adipose tissue. According to the glucogenic-insulin theory, these overall changes deprive the mammary gland of milk fat precursors.
because of vigorous competition by adipose tissue, resulting in milk fat depression (1, 9, 17, 30, 34).

We evaluated the effects of insulin on the yield and composition of milk fat of lactating dairy cows using a chronic, 4-d hyperinsulinemic-euglycemic clamp. During the insulin clamp, a fourfold increase in circulating insulin concentrations occurred (Figure 1); the maintenance of euglycemia required infusion of exogenous glucose at approximately 2.1 kg/d on the 1st d, increasing to 2.9 kg/d by the 4th d. Cows were in a positive energy balance throughout the clamp, and the amount of exogenous glucose that was infused to maintain euglycemia was quantitatively equal to about 90 to 135% of the normal daily turnover of glucose (6, 7). The infused glucose represented the amount needed to account for glucose uptake by tissues that were sensitive to insulin and to offset insulin inhibition of gluconeogenesis. The increased availability of glucose during the hyperinsulinemic-euglycemic clamp likely would result in the use of more glucose as an oxidative fuel, thus sparing acetate. In the present study, the success of the hyperinsulinemic-euglycemic clamp was demonstrated by the relative stability of euglycemia, milk

### Table 2. Plasma concentrations of metabolites and minerals from cows during the baseline interval and on the last day of a hyperinsulinemic-euglycemic clamp. 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Water</th>
<th>Casein</th>
<th>SEM</th>
<th>INF</th>
<th>INS</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>±INS</td>
<td>+INS</td>
<td></td>
<td>INF</td>
<td>INS</td>
<td></td>
</tr>
<tr>
<td>Metabolite</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NEFA, µeq/L</td>
<td>112</td>
<td>83</td>
<td>113</td>
<td>74</td>
<td>10</td>
<td>***</td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>32.8</td>
<td>34.3</td>
<td>33.1</td>
<td>31.7</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>Mineral</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium, meq/L</td>
<td>3.9</td>
<td>3.7</td>
<td>3.8</td>
<td>3.7</td>
<td>0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Phosphorus, mg/dl</td>
<td>5.2</td>
<td>4.7</td>
<td>5.4</td>
<td>4.9</td>
<td>0.2</td>
<td>NS</td>
</tr>
<tr>
<td>Calcium, mg/dl</td>
<td>9.5</td>
<td>9.2</td>
<td>9.2</td>
<td>9.0</td>
<td>0.2</td>
<td>NS</td>
</tr>
<tr>
<td>Magnesium, meq/L</td>
<td>2.3</td>
<td>2.4</td>
<td>2.2</td>
<td>2.4</td>
<td>0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Sodium, meq/L</td>
<td>139</td>
<td>141</td>
<td>140</td>
<td>139</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td>Chloride, meq/L</td>
<td>100</td>
<td>102</td>
<td>99</td>
<td>100</td>
<td>1</td>
<td>NS</td>
</tr>
</tbody>
</table>

1Abomasal infusions (INF) of casein (0.5 kg/d) or water were administered during the baseline interval (±INS) and during the 4-d hyperinsulinemic-euglycemic clamp (+INS).

2Plasma samples were pooled for the 4-d baseline interval and on the last day of the clamp.

3Determined as total glycerol.

4As-fed basis; mean DM of the ration was 90.1%.

### Table 3. Performance and energy balance during the baseline interval and on the last day of the hyperinsulinemic-euglycemic clamp. 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Water</th>
<th>Casein</th>
<th>SEM</th>
<th>INF</th>
<th>INS</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>±INS</td>
<td>+INS</td>
<td></td>
<td>INF</td>
<td>INS</td>
<td></td>
</tr>
<tr>
<td>Milk yield, kg/d</td>
<td>26.3</td>
<td>27.0</td>
<td>28.6</td>
<td>30.5</td>
<td>0.7</td>
<td>***</td>
</tr>
<tr>
<td>Milk fat, kg/d</td>
<td>0.91</td>
<td>0.85</td>
<td>1.01</td>
<td>0.94</td>
<td>0.04</td>
<td>NS</td>
</tr>
<tr>
<td>%</td>
<td>3.50</td>
<td>3.21</td>
<td>3.58</td>
<td>3.08</td>
<td>0.11</td>
<td>NS</td>
</tr>
<tr>
<td>Feed intake, kg/d</td>
<td>22.2</td>
<td>15.5</td>
<td>21.8</td>
<td>20.4</td>
<td>1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Net energy balance, Mcal/d</td>
<td>5.3</td>
<td>5.1</td>
<td>4.6</td>
<td>10.7</td>
<td>1.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

1Abomasal infusions (INF) of casein (0.5 kg/d) or water were administered during the baseline interval (±INS) and during the 4-d hyperinsulinemic-euglycemic clamp (+INS).

2P > 0.20.

3Calculated as the difference between the estimated net energy content of feeds plus infused glucose and casein and the estimated energy value of milk and net energy requirements for maintenance and growth (21, 33).

4P < 0.10.

5P < 0.05.

6P < 0.01.
yield, and the plasma mineral profile over the 4 d of the clamp. Furthermore, there was no evidence of
down-regulation of tissue response to insulin based on
the stability of the circulating insulin concentrations,
on the glucose infusion required to maintain euglyce-
mia (Figure 1), or on the consistency of the reduc-
tions in circulating concentrations of NEFA (Table 2;
temporal pattern not presented) and urea nitrogen
data not presented). Overall, these data indicate
that the hyperinsulinemic-euglycemic clamp approach
that was used in the present study represented a
rigorous test of the glucogenic-insulin theory of milk
fat depression.

We observed no effect on milk fat yield during the
4-d hyperinsulinemic-euglycemic clamp (Figure 2; Table 3). Milk fat percentage decreased during the
insulin clamp, particularly during casein infusion.
However, this decrease was mainly a dilution effect
related to the increase in milk yield, which was
caused by the combination of casein infusion and the
insulin clamp (Figure 2). According to the

**TABLE 4. Calculated yields of milk fatty acids during the baseline interval and on the last day of the hyperinsulinemic-euglycemic clamp.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Water</th>
<th>Caseinate</th>
<th>SEM</th>
<th>INF</th>
<th>INS</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty acid, g/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>812</td>
<td>760</td>
<td>900</td>
<td>830</td>
<td>39.4</td>
<td>†</td>
</tr>
<tr>
<td>C&lt;sub&gt;4:0&lt;/sub&gt;</td>
<td>44.6</td>
<td>32.4</td>
<td>47.5</td>
<td>34.5</td>
<td>1.62</td>
<td>†</td>
</tr>
<tr>
<td>C&lt;sub&gt;6:0&lt;/sub&gt;</td>
<td>25.5</td>
<td>21.8</td>
<td>28.0</td>
<td>23.5</td>
<td>1.14</td>
<td>†</td>
</tr>
<tr>
<td>C&lt;sub&gt;8:0&lt;/sub&gt;</td>
<td>13.7</td>
<td>13.0</td>
<td>15.5</td>
<td>14.2</td>
<td>0.76</td>
<td>†</td>
</tr>
<tr>
<td>C&lt;sub&gt;10:0&lt;/sub&gt;</td>
<td>30.1</td>
<td>35.0</td>
<td>35.4</td>
<td>38.8</td>
<td>2.27</td>
<td>†</td>
</tr>
<tr>
<td>C&lt;sub&gt;12:0&lt;/sub&gt;</td>
<td>32.0</td>
<td>44.3</td>
<td>38.2</td>
<td>49.4</td>
<td>3.39</td>
<td>†</td>
</tr>
<tr>
<td>C&lt;sub&gt;14:0&lt;/sub&gt;</td>
<td>97.9</td>
<td>102.2</td>
<td>114.3</td>
<td>112.6</td>
<td>5.46</td>
<td>†</td>
</tr>
<tr>
<td>C&lt;sub&gt;14:1&lt;/sub&gt;</td>
<td>11.1</td>
<td>12.6</td>
<td>12.4</td>
<td>14.0</td>
<td>0.58</td>
<td>†</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:0&lt;/sub&gt;</td>
<td>9.6</td>
<td>10.9</td>
<td>10.8</td>
<td>11.7</td>
<td>0.68</td>
<td>†</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:1&lt;/sub&gt;</td>
<td>224.4</td>
<td>259.0</td>
<td>256.3</td>
<td>271.2</td>
<td>18.25</td>
<td>†</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:0&lt;/sub&gt;</td>
<td>11.7</td>
<td>14.9</td>
<td>12.6</td>
<td>16.1</td>
<td>1.19</td>
<td>†</td>
</tr>
<tr>
<td>C&lt;sub&gt;17:0&lt;/sub&gt;</td>
<td>4.5</td>
<td>3.8</td>
<td>4.9</td>
<td>4.0</td>
<td>0.19</td>
<td>†</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:0&lt;/sub&gt;</td>
<td>100.8</td>
<td>56.3</td>
<td>109.8</td>
<td>62.8</td>
<td>4.74</td>
<td>†</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:1&lt;/sub&gt;</td>
<td>15.9</td>
<td>11.0</td>
<td>17.7</td>
<td>12.5</td>
<td>0.56</td>
<td>†</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:2&lt;/sub&gt;</td>
<td>150.0</td>
<td>114.3</td>
<td>155.4</td>
<td>136.8</td>
<td>6.65</td>
<td>†</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:3&lt;/sub&gt;</td>
<td>24.8</td>
<td>17.3</td>
<td>25.6</td>
<td>20.6</td>
<td>0.82</td>
<td>†</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:4&lt;/sub&gt;</td>
<td>5.8</td>
<td>3.7</td>
<td>6.0</td>
<td>4.4</td>
<td>0.19</td>
<td>†</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:1&lt;/sub&gt;:C&lt;sub&gt;18:0&lt;/sub&gt;</td>
<td>4.5</td>
<td>4.1</td>
<td>4.4</td>
<td>4.6</td>
<td>0.25</td>
<td>†</td>
</tr>
<tr>
<td>Total C&lt;sub&gt;4-16&lt;/sub&gt; g/d</td>
<td>501</td>
<td>546</td>
<td>571</td>
<td>586</td>
<td>32.4</td>
<td>†</td>
</tr>
<tr>
<td>Total C&lt;sub&gt;18&lt;/sub&gt; g/d</td>
<td>297</td>
<td>203</td>
<td>314</td>
<td>237</td>
<td>11.3</td>
<td>†</td>
</tr>
</tbody>
</table>

1Abomasal infusions (INF) of sodium caseinate (0.5 kg/d) or water were administered during the baseline interval (–INS) and during the 4-d hyperinsulinemic-euglycemic clamp (+INS).

2Assumes fatty acids represent 89% of total milk fat weight.

3P > 0.20.

4Conjugated linoleic acid (mainly cis-9, trans-11 C<sub>18:2</sub>)

†P < 0.10.

*P < 0.05.

**P < 0.01.

***P < 0.001.
lipoprotein lipase in adipose tissue (12), thus facilitating uptake of fatty acids from circulating triglycerides and incorporation of these fatty acids into body fat reserves. Third, insulin inhibits lipolysis and increased fatty acid reesterification in adipose tissue (5, 6). These effects would reduce fatty acid mobilization, as was indicated in this study by the reduced circulating concentrations of NEFA (Table 3), limiting this source of fatty acids for milk fat synthesis. The same metabolic effects that occur during the hyperinsulinemic-euglycemic clamp also would occur in milk fat depression during which they are assumed to be preferentially diverting nutrients to adipose tissue, resulting in a shortage of precursors for the synthesis of milk fat by the mammary gland. In our study, the reduced availability of acetate, BHBA, and preformed fatty acids was also exacerbated by the modest reduction in feed intake that occurred over the course of the 4-d clamp. Because the supply of metabolites used for fat synthesis by the mammary gland was potentially constrained by decreased adipose tissue mobilization, greater uptake by adipose tissue, and decreased dietary supply, it is remarkable that milk fat yield was relatively constant during the hyperinsulinemic-euglycemic clamp.

Changes in the composition of milk fatty acids were examined to determine the mechanism that allowed the rate of milk fat synthesis to be maintained under hyperinsulinemic-euglycemic conditions. Short-chain fatty acids (C_4:0 through C_8:0) and medium-chain fatty acids (C_10:0 through C_14:0) arise from de novo fatty acid synthesis in the mammary gland, but long-chain fatty acids (total C_18 and greater) arise from the uptake of circulating fatty acids that originate from dietary lipids or mobilization of body reserves (4). Palmitic acid (C_16:0) arises from both sources. Milk fat depression consistently results in a decreased proportion of short- and medium-chain fatty acids relative to long-chain fatty acids (22). The fatty acid composition of milk fat during the hyperinsulinemic-euglycemic clamp (Table 4) was very different from the changes that occurred with milk fat depression. Nevertheless, minor changes were observed during the clamp when the balance between de novo and preformed fatty acids in milk fat shifted slightly toward those synthesized de novo (Table 4). Among the fatty acids arising from de novo synthesis, butyrate and hexanoate were decreased, octanoate remained the same, and the medium-chain fatty acids and C_16:0 represented greater proportions of the total (Figure 3). These changes did not occur immediately but developed gradually over the 4 d of hyperinsulinemia (data not presented). The increase in palmitic acid likely arose from a net increase in de novo synthesis, because mammary uptake of preformed palmitate would be expected to be reduced to the same extent.
extent as other long-chain fatty acids (Figure 3). During the clamp, an increase in the level of saturation in milk fat occurred that was associated with an increased ratio of oleic to stearic acid (Table 4). This shift was consistent with the suggested role of the desaturase enzyme in maintaining fluidity of milk fat (8, 18).

Calculated yields of fatty acids demonstrated that the modest changes in the composition of milk fatty acids during the clamp were mainly due to a decreased yield of long-chain fatty acids (Table 4). However, a relative shift toward longer chain de novo fatty acids was also observed (Figure 3). Overall, changes in the composition of milk fatty acids during the clamp were quite similar to changes that occurred when lactating cows were fed a diet of minimal fat content (10), which suggests that the modest changes in composition during the insulin clamp in the current study might have arisen from limitations in the mammary gland supply of preformed fatty acids. This limited supply of preformed fatty acids would be consistent with predicted changes in the uptake of preformed fatty acids by adipose tissue, the observed decrease in plasma NEFA (Table 2), and reduced feed intake (Table 3). The minor increases in proportions of de novo fatty acids in milk fat demonstrated that the availability of acetate was less affected than that of preformed fatty acids, perhaps because of increased use of glucose as an oxidative fuel. Milk fat depression, which results when high concentrate, low forage diets are fed, also resulted in increased availability of glucose, which could spare acetate oxidation. Also, acetate and BHBA account for almost all of the carbon in de novo synthesis of fatty acids by the mammary gland under normal situations as well as during milk fat depression (4, 23, 25), thereby leaving very little potential for the use of glucose as a carbon source for fatty acids.

The glucogenic-insulin theory is probably the most widely accepted theory of milk fat depression (1, 9, 29, 34), and the mechanism also has been built into current mechanistic models that describe the partitioning of absorbed nutrients in lactation (2). When dealing with negative data, one can never unequivocally disprove a theory. However, our study was a direct and rigorous test of the effects of insulin, and we found no support for the role of insulin in milk fat depression. Other studies (24, 31) have also examined the effect of insulin on milk fat synthesis by infusing insulin alone and have found no support for the role of insulin in milk fat depression. However, those studies were confounded by the hypoglycemia and a subsequent decline in milk yield that resulted from insulin infusion. Similar to the methodology used in the current study, McGuire et al. (20) avoided this complication by using a hyperinsulinemic-euglycemic clamp and also found no impact of elevated insulin on milk fat synthesis.

Support for the glucogenic-insulin theory comes largely from associated changes in blood insulin concentration, increased fat synthesis in adipose tissue, and reduced milk fat synthesis. Although these changes have been generally interpreted to represent cause, they could also be a consequence of milk fat depression. If milk fat depression was the result of a direct inhibition of fat synthesis in the mammary gland, the decrease in milk fat synthesis and the higher net energy intake typically associated with diets that depress milk fat would put the cow in a more positive energy balance. Therefore, circulating insulin and lipid deposition in adipose tissue would increase as a consequence of milk fat depression. Consistent with this theory, others (13, 14) have proposed mechanisms involving direct inhibition at the mammary gland. Results from the present study suggest that these proposed mechanisms offer promise. In another study, Griinari et al. (14) observed elevated concentrations of circulating insulin without milk fat depression when the diet did not provide substrate for the formation of trans-C18:1 fatty acids, which are putative inhibitors of milk fat synthesis.

Several reviews (3, 6, 35) have emphasized that the regulation of nutrient utilization during lactation involves a coordination of metabolic and physiological processes, which is in contrast to the glucogenic-insulin theory of milk fat depression. The glucogenic-insulin theory assumes that tissues compete for nutrient use and that insulin causes nutrients to be diverted to adipose tissue at the expense of the mammary gland. In contrast, our results are consistent with the regulation of nutrient utilization based on coordination among tissues. Insulin secretion responds to an increase in energy supply in excess of requirements and represents a coordinated response to allow for maintenance of essential physiological processes (e.g., synthesis of milk and milk components) while simultaneously allowing for storage of excess energy. Our data demonstrate that a similar coordinated response to insulin occurs during the hyperinsulinemic-euglycemic clamp.

**CONCLUSIONS**

The hyperinsulinemic-euglycemic clamp technique allows a rigorous test of the glucogenic-insulin theory of milk fat depression. The chronic elevation of cir-
Calculating insulin concentration stimulates lipid synthesis in adipose tissue and inhibits lipid mobilization. Despite the challenge to mammary supply of lipogenic precursors, the rate of milk fat synthesis was relatively constant throughout the 4-d insulin clamp, and changes in the composition of milk fatty acids were not characteristic of the changes observed during milk fat depression. Overall, our results demonstrated that the regulation of nutrient use is coordinated so that milk fat synthesis is maintained during periods of hyperinsulinemia, thereby offering no support for the glucogenic-insulin theory of milk fat depression.

ACKNOWLEDGMENTS

The authors dedicate this paper to the late Richard E. Brown who was an early pioneer in the area of milk fat synthesis and milk fat depression. The skilled assistance of Robert Harrell, Scott Butler, Dan Smith, Julie Berry, Alison Smith, Dottie Ceurter, and Donna Kinsey is appreciated.

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