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Changes in Milk Protein and Amino Acid Composition of Dairy Cows in Response to Fatty Liver and Intravenous Glucagon

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Summary
Intravenous glucagon cures fatty liver by improving glucose bioavailability in early lactation. Amino acids, which would be otherwise used for milk protein synthesis, are metabolized to glucose. The objective of this study was to examine whether intravenous glucagon and fatty liver change milk protein and amino acid composition in dairy cows. Multiparous Holstein cows (n=25) were designated as either normal or susceptible to fatty liver and ketosis as based on the ratio of liver triacylglycerol to glycogen being smaller or greater than 2.0 at d 6 postpartum. Cows susceptible to fatty liver were subjected for 3 weeks to a protocol consisting of feed restriction and dietary 1,3-butanediol beginning at d 14 postpartum, which induced fatty liver and ketosis. Normal cows and cows with fatty liver were infused with glucagon for 14 d at 0 or 10 mg/d beginning at d 21 postpartum. Composite milk samples were obtained at d 20, 22, 34, and 36 postpartum and analyzed for milk protein and amino acid composition. Fatty liver decreased milk yield but had little effect on milk protein and amino acid composition except for increasing the proportion of glycosylated κ-casein. Intravenous glucagon decreased total milk protein concentrations and the proportion of α-lactalbumin and increased the proportion of glycosylated κ-casein, total κ-casein, and αs1-casein. Intravenous glucagon had little effect on milk amino acid composition. Our results suggest that milk protein and amino acid composition are under tight concomitant hormonal control and are affected little by changes in amino acid availability and/or insulin to glucagon ratio.

Introduction
Our group showed previously that continuous intravenous infusions of glucagon for 14 days can cure fatty liver and ketosis. Fatty liver is a major metabolic disease that costs U.S. dairy farmers millions of dollars. Glucagon increases glucose availability for early lactation cows by promoting the release and use of gluconeogenic precursors for gluconeogenesis, e.g., deamination of free amino acids in the blood. As a result, less amino acids are available for extraction by the mammary gland and the milk protein content is decreased. The availability of individual amino acids for extraction is not equally limited because leucine and lysine cannot be used as gluconeogenic precursors.

Altering the milk protein composition can improve the physical and manufacturing properties of dairy products; however, little is known about how to change milk protein composition. Insulin and casein infusions increase and atropine infusions decrease total protein content but have little effect on milk protein composition. Infusion or feeding rumen-protected individual amino acids does not consistently alter milk protein composition. Therefore, the question for this study was: How does intravenous glucagon affect the milk protein and amino acid composition?

Materials and Methods
On the basis of their body condition score and their history of ketosis, multiparous Holstein cows (n=25) at the Iowa State University Teaching Herd in Ames were divided into normal or susceptible to fatty liver. To induce fatty liver, cows (n=11) with a history of ketosis or a body condition score of at least 3.5 were fed 5-6 kg of cracked corn daily in addition to their dry cow ration for 30 d prior to expected calving, whereas normal cows received only their dry cow ration. At day 6 postpartum, a liver sample was collected by puncture biopsy and analyzed for total lipids, triacylglycerol, and glycogen. Cows (n=11) with a ratio of liver triacylglycerol to glycogen greater than 2.0 started at d 14 postpartum on a feed restriction (80% of NRC recommendation) and dietary 1,3-butanediol (0.25-1.4 L/d) protocol to induce persistent ketonemia and hypoglycemia for the next three weeks.

1. Normal Glucagon: Starting at d 21 postpartum, cows (n=8) received intravenously 10 mg/d glucagon dissolved in 0.15 M NaCl through catheters at 20.83 mg/L and 20 mL/h continuously for 14 days.
2. Susceptible Glucagon: Starting at d 14 postpartum, cows (n=7) were on the ketosis induction protocol for 3 weeks. Starting at d 21 postpartum, cows received intravenously 10 mg/d glucagon dissolved in 0.15 M NaCl through catheters at 20.83 mg/L and 20 mL/h continuously for 14 days.
3. Normal Saline: Starting at d 21 postpartum, cows (n=6) received intravenously 20 mL/h 0.15 M NaCl continuously for 14 days.
4. Susceptible Saline: Starting at d 14 postpartum, cows (n=4) were on the ketosis induction protocol for 3 weeks. Starting at d 21 postpartum, cows received intravenously 20 mL/h of 0.15 M NaCl continuously for 14 days.

Composite milk samples from cows were collected one day prior and after the beginning and end of the infusion period, respectively (d 20, 22, 34, and 36 postpartum). Milk samples were sent refrigerated at 4°C to Dairy Lab Services (Dubuque, IA) to be analyzed for fat, protein, and lactose by
mid-infrared spectrophotometry (Milk-O-Scan 203, Foss Food Technology Corp., Eden Prairie, MN) and for SCC by using a Fossomatic 90 (Foss Food Technology Corp.). Concentrations of the casein proteins αS1-casein (αS1–CN), β-casein (β-CN), κ-casein (κ-CN), and αS2-casein (αS2-CN) and the whey proteins β-lactoglobulin (β–LG) and α-lactalbumin (α–LA) were determined by reversed-phase high performance liquid chromatography (RP-HPLC). After acid hydrolysis of milk samples, the amino acid composition of milk was determined by RP-HPLC.

Data were analyzed statistically by using mixed models procedures. The fixed effects in the model were time postpartum (d 20, 22, 34, and 35), treatment (normal glucagon, susceptible glucagon, normal saline, susceptible saline), and the interaction of time with treatment. A completely unrestricted variance-covariance matrix was used to account for correlations between samples from the same cow. Effects of intravenous glucagon were determined by using a two-sided t-test comparing the milk protein and amino acid composition during vs. before and after the infusion period. Effects of fatty liver were determined by comparing normal vs. susceptible cows. Figure 1 shows least squares means and their standard errors.

**Results and Discussion**

Intravenous glucagon did not affect milk yield (Figure 1A); however, it decreased protein content in milk by 0.45 ± 0.04% (P < 0.0001; Figure 1B). The decrease in protein content was not altered by fatty liver and ketosis induction. Intravenous glucagon resulted in small but highly significant changes in milk protein composition. The proportion of glycosylated κ-CN and total κ-CN decreased by 1.21 ± 0.14% (P < 0.0001; Figure 1C) and 1.81 ± 0.42% (P = 0.004; Figure 1D) of total milk protein, respectively. Furthermore, intravenous glucagon increased the proportion of αS2-CN by 1.93 ± 0.49% (P = 0.007; Figure 1E) and decreased the proportion of α–LA 0.90 ± 0.34% (P = 0.02; Figure 1F). No significant changes in milk amino acid composition by intravenous glucagon were observed.

The lack of significant changes in milk amino acid composition was somewhat surprising, as intravenous glucagon has been reported to alter the composition of plasma amino acids. Our results would suggest that plasma amino acid availability affect milk protein content but has limited influence on milk protein composition. Similar findings have been reported for atropine infusion, which is believed to act at least partly through decreased insulin concentrations. In our study, insulin concentrations were not decreased by intravenous glucagon, which suggest that the ratio of insulin to glucagon might affect milk protein content.

The changes in milk protein composition were rather small, which suggests that intravenous glucagon does not strongly alter milk protein composition. Similarly to our results, atropine infusion decreased concentrations of α–LA in milk the strongest and decreased concentrations of αS–CN and κ-CN in milk the least. Our results suggest a tight concomitant hormonal control of milk protein synthesis, which allows only minor changes in milk protein composition.

Fatty liver and ketosis decreased milk production (Figure 1A) but had little effect on milk protein content and composition. The only exception is the proportion of glycosylated κ-CN in total milk protein, which was 1.50 ± 0.36% (P = 0.0006; Figure 1C) higher in cows with fatty liver. Our results suggest that the proportion of glycosylated κ-CN is controlled at least partly independently of other milk protein fractions. The post-translational glycosylation of κ-CN is a potential molecular target for the independent control.

**Conclusion**

Intravenous glucagon decreases milk protein content but results in little changes in milk protein and amino acid composition. Therefore, we conclude that milk protein and amino acid composition are under tight concomitant hormonal control and are affected little by changes in amino acid availability and/or insulin to glucagon ratio.

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Figure 1. Changes in A) milk yield, B) total protein content, C) proportion of glycosylated \( \kappa \)-CN, D) proportion of total \( \kappa \)-CN, E) proportion of \( \alpha_S2 \)-CN, and F) proportion of \( \alpha \)-LA in Holstein dairy cows in response to fatty liver and ketosis induction and/or intravenous glucagon at 10 mg/d for 14 days.