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Negative energy balance increases periprandial ghrelin and growth hormone concentrations in lactating dairy cows

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Abstract

The reported effects of feeding on growth hormone (GH) secretion in ruminants have been inconsistent, and are likely influenced by energy status of animals. High-producing dairy cows in early lactation and late lactation were used to assess the effects of energy balance on temporal variation of plasma metabolites and hormones. Cows were fed a single diet once daily, and feed was withdrawn for 90 min prior to feeding. Beginning at the time of feed withdrawal, plasma samples were collected via jugular catheters hourly for 24 h. Concentrations of non-esterified fatty acids and GH were measured for all samples, while insulin, glucose, and acylated (active) ghrelin were quantified for four sample times around feeding. As expected, calculated energy balance was significantly lower in early lactation than late lactation cows (−43.5 MJ retained/day versus 7.2 MJ retained/day). Following the primary meal of the day, a GH surge was observed in early lactation but not in late lactation cows. This difference was not explained by temporal patterns in non-esterified fatty acid, insulin, or glucose concentrations. However, a preprandial ghrelin surge was observed in early lactation only, suggesting that ghrelin was responsible for the prandial GH surge in this group. Results of a stepwise regression statistical analysis showed that both preprandial ghrelin concentration and energy balance were significant predictors of prandial GH increase over baseline. Adaptations to negative energy balance in lactating dairy cattle likely include enhanced ghrelin secretion and greater GH response to ghrelin.

Keywords: Somatotropin; Growth hormone secretagogue; Homeorhesis; Dairy cow; Negative energy balance

1. Introduction

The initiation of lactation is a time of rapid adaptation. The dramatic increase in energy requirements of high-producing dairy cows during this period requires homeorhetic control of metabolism to direct endogenous and dietary nutrients to the mammary gland for lactogenesis [1,2]. Growth hormone (GH) plays a central role in this process, inhibiting lipid storage in adipose tissue and increasing blood flow to the mammary gland, among other effects. Therefore, regulation of GH secretion has been the focus of intensive research for years [3].

Ghrelin is a 28-amino acid, octanoylated peptide which is secreted primarily by cells in the abomasum in ruminants [4,5]. Ghrelin concentrations increase prior to scheduled meals [6] and in response to fasting [7] in ruminants, and feeding suppresses ghrelin secretion [7]. These observations suggest a role for ghrelin in stimulating feed intake, and responses to ghrelin administration have supported this hypothesis [7,8]. In addition to effects on feed intake, ghrelin has been shown to decrease fat oxidation [8], enhance insulin secretion

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[9] and influence a wide variety of processes related to energy balance [10]. Finally, as the endogenous ligand for the growth hormone secretagogue receptor [11], ghrelin has provided a new avenue for investigating regulation of GH secretion.

Infusion experiments have been invaluable for identifying metabolic [12] and endocrine [9,13] effects of ghrelin. However, these studies are not adequate to determine whether ghrelin plays an important role in normal ruminant physiology, nor whether metabolic state influences ghrelin secretion. Recent experiments have suggested that ghrelin contributes to adaptations that occur during long-term fasting [7], but no association between endogenous ghrelin release and GH secretion was found in mature dairy cattle fed to nutrient requirements [14]. Our aim in this work was to assess the influence of energy status on periprandial GH and ghrelin concentrations in lactating dairy cattle managed in a typical production environment.

2. Materials and methods

Experimental procedures were approved by the All-University Committee on Animal Use and Care at Michigan State University.

2.1. Design and treatments

Multiparous Holstein cows were selected from the Michigan State University Dairy Cattle Teaching and Research Center and assigned to blocks of early lactation ($n = 6$; 19 ± 6 days in milk; 657 ± 68 kg body weight; mean $\pm$ S.D.) and late lactation ($n = 5$; 229 ± 20 days in milk; 701 ± 80 kg body weight) cows. Stalls were assigned to block and treatment sequence to assure balance within blocks, and cows were randomly assigned to stalls within block. Treatments were arranged in a crossover design; phlorizin (Sigma Chemical Co., St. Louis, MO) was administered via subcutaneous injection at the rate of 4 g/day, with propylene glycol (16 mL/day) as vehicle and control. Treatment periods were 7 days, and injections were given every 6 h during these periods. Animals were adapted to a single diet for a 7-day period prior to the first treatment period, and a 7-day rest period was included between the two treatment periods. Phlorizin is an inhibitor of renal glucose reabsorption which causes urinary loss of glucose and artificially increases glucose demand [15]. Phlorizin treatment was used to manipulate hepatic nutrient metabolism, and responses to phlorizin have been reported [16]; however, differences between stages of lactation reported here are independent of phlorizin treatment.

Table 1
Ingredients and nutrient composition of experimental diet

Diet ingredients	
Corn silage	31.8
High moisture corn grain	29.1
Alfalfa haylage	11.5
Soybean meal	11.8
Modified expeller soybean meal[a]	9.0
Mineral and vitamin mix[b]	6.9
Nutrient composition	
Dry matter, % as-fed	43.4
Organic matter	94.0
Starch	28.2
Neutral detergent fiber	27.1
Crude protein	17.8
Lipid	3.7

Values other than dry matter are expressed as % of dietary DM.
[a] SoyPlus (West Central Soy, Ralston, IA).
[b] Mineral and vitamin mix contained 74.7% dry ground corn, 10.9% limestone, 5.5% salt, 5.3% dicalcium phosphate, 1.8% magnesium oxide, 1.4% trace mineral premix, and 0.4% vitamin ADE premix.

2.2. Data and sample collection

Throughout the experiment, cows were housed in tie stalls and fed an experimental diet (Table 1) as a total mixed ration once daily (11:30 h) at 115% of expected daily intake. Cows were not allowed access to feed from 10:00 to 11:30 h, during which time feed refused and the amount of feed offered were weighed for each cow daily. During the final 4 days of each treatment period, samples of all dietary ingredients (0.5 kg) were collected and frozen for later analysis. Cows were milked twice daily in a milking parlor during rest periods and in the tie stalls during treatment periods; milk yield was recorded and milk samples were collected for analysis at each milking on the final 4 days of each treatment period. Starting on day 4 (10:00 h) of each experimental period, blood was sampled hourly from indwelling jugular catheters for 24 h. Collected blood was immediately emptied into two tubes, one containing potassium EDTA and the other containing potassium oxalate with sodium fluoride as a glycolytic inhibitor (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). Both were centrifuged at $2000 \times g$ for 15 min immediately after sample collection, and plasma was harvested and frozen at $-20\,^{\circ}C$ until analysis.

2.3. Sample analysis

Diet ingredients were dried in a 55 °C forced-air oven for 72 h and analyzed for dry matter concentration. Samples were ground with a Wiley mill (1 mm screen; Arthur H. Thomas, Philadelphia, PA) and analyzed for ash,