J. Dairy Sci. 96:7565–7577
http://dx.doi.org/10.3168/jds.2012-6498

Long-acting insulins alter milk composition and metabolism of lactating dairy cows

L. A. Winkelman and T. R. Overton[1]
Department of Animal Science, Cornell University, Ithaca, NY 14853

ABSTRACT

This study investigated the effect of 2 different types of long-acting insulin on milk production, milk composition, and metabolism in lactating dairy cows. Multiparous cows (n = 30) averaging 88 d in milk were assigned to one of 3 treatments in a completely randomized design. Treatments consisted of control (C), Humulin-N (H; Eli Lilly and Company, Indianapolis, IN), and insulin glargine (L). The H and L treatments were administered twice daily at 12-h intervals via subcutaneous injection for 10 d. Cows were milked twice daily, and milk composition was determined every other day. Mammary biopsies were conducted on d 11, and mammary proteins extracted from the biopsies were analyzed by Western blot for components of insulin and mammalian target of rapamycin signaling pathways. Treatment had no effect on dry matter intake or milk yield. Treatment with both forms of long-acting insulin increased milk protein content and tended to increase milk protein yield over the 10-d treatment period. Analysis of milk N fractions from samples collected on d 10 of treatment suggested that cows administered L tended to have higher yields of milk protein fractions than cows administered H. Milk fat content and yield tended to be increased for cows administered long-acting insulins. Lactose content and yields were decreased by treatment with long-acting insulins. Administration of long-acting insulins, particularly L, tended to shift milk fatty acid composition toward increased short- and medium-chain fatty acids and decreased long-chain fatty acids. Plasma concentrations of glucose and urea N were lower for cows administered long-acting insulins; interactions of treatment and sampling time were indicative of more pronounced effects of L than H on these metabolites. Concentrations of nonesterified fatty acids and insulin were increased in cows administered long-acting insulins. Decreased concentrations of urea N in both plasma and milk suggested more efficient use of N in cows administered long-acting insulins. Western blot analysis of mammary tissue collected by biopsy indicated that the ratios of phosphorylated protein kinase b (Akt) to total Akt and phosphorylated ribosomal protein S6 (rpS6) to total rpS6 were not affected by long-acting insulins. Modestly elevating insulin activity in lactating dairy cows using long-acting insulins altered milk composition and metabolism. Future research should explore mechanisms by which either insulin concentrations or insulin signaling pathways in the mammary gland can be altered to enhance milk fat and protein production.

Key words: milk protein, insulin, dairy cow

INTRODUCTION

Insulin is a potent regulator of energy and protein metabolism in mammals, and it exerts anabolic effects on tissues in both nonruminants and ruminants. Although mammary uptake of glucose is insulin-independent (Laarveld et al., 1981; Zhao and Keating, 2007), insulin is an important hormone in milk and milk component synthesis because of its roles in nutrient partitioning and energy metabolism (Brockman and Laarveld, 1986). Studying the effects of insulin in ruminants and dairy cows is a challenge because hypoglycemia can result when substantial doses of exogenous insulin are administered, resulting in decreased milk yield and DMI (e.g., Kronfeld et al., 1963; Schmidt, 1966).

To overcome the confounding effects of hypoglycemia induced by insulin treatment, hyperinsulinemic-euglycemic clamps have been used during the last 30 yr as an in vivo model to examine the effects of insulin on whole-body metabolism. In several studies (McGuire et al., 1995; Griinari et al., 1997a; Mackle et al., 1999), yields of milk protein were increased when cows were subjected to hyperinsulinemic-euglycemic clamps; the response typically was greater when accompanied by abomasal infusion of casein or AA. Insulin and other lactogenic hormones have been reported to regulate initiation of protein synthesis in multiple cell culture systems and experiments using lactating dairy cows (Hayashi et al., 2009; Toerien et al., 2010; Burgos and Cant, 2010; Burgos et al., 2010). Insulin may exert its effects on milk protein synthesis by activation of the

Received December 18, 2012.
Accepted August 20, 2013.
[1]Corresponding author: tro2@cornell.edu

mammalian target of rapamycin (**mTOR**) signaling cascade (Campbell et al., 1999; Proud, 2006, 2007). The roles of insulin and AA as they interact with mTOR signaling in the lactating dairy cow mammary gland have been examined to some extent (Rius et al., 2010) but are not fully understood.

Interpretation of results from hyperinsulinemic-euglycemic clamps is confounded by the co-infusion of both insulin and large amounts of exogenous glucose, which makes it difficult to determine if the results are due to the effects of insulin, glucose, or both. During hyperinsulinemic-euglycemic clamps, significant amounts of glucose are co-infused into the cow. As an example, in the study by Mackle et al. (1999), the amount of glucose infused per day (3,336 g on d 4 of the clamp) was about 50% greater than the irreversible loss of glucose (2,170 g/d) measured (Bauman et al., 1988) in cows producing similar amounts of milk (~27 kg/d).

Recently, we determined that injection of 2 different forms of long-acting insulin could be used to elevate insulin activity in dairy cows over a 24-h period without concurrent administration of glucose and without significant hypoglycemia (Winkelman and Overton, 2012). Therefore, the objectives of the present study were to evaluate the effects of these forms of long-acting insulin on milk yield and composition by lactating dairy cows over a 10-d treatment period. We hypothesized that milk protein content and yield would be increased by treatment with both types of long-acting insulin and that changes in milk protein and component synthesis would be the result of stimulation of the insulin and mTOR signaling pathways in the mammary gland.

MATERIALS AND METHODS

Animals, Experimental Design, and Treatments

The Cornell University Institutional Animal Care and Use Committee approved all procedures involving animals before the onset of the experiment. Thirty multiparous Holstein cows ranging from 52 to 130 DIM (average 88 ± 25 DIM, mean ± SD) at the onset of the experiment were assigned randomly to 1 of 3 treatments in a completely randomized design. Treatments consisted of control (**C**), 0.2 IU/kg of BW Humulin-N [**H**; 100 IU/mL human insulin (rDNA origin) isophane suspension, provided by Eli Lilly and Company, Indianapolis, IN], and 0.2 IU/kg of BW Lantus [**L**; 100 IU/mL insulin glargine (rDNA origin), Sanofi-Aventis, Bridgewater, NJ]. The H and L treatments were given 2×/d via subcutaneous injection, resulting in a total daily dose of 0.4 IU/kg of BW for both treatments. We reported previously that these forms of long-acting insulin have insulin activity in lactating dairy cattle (Winkelman and Overton, 2012). The administration scheme for the current experiment was modified from our dose-response experiment (same total dosage but divided into 2 doses per day administered at 12-h intervals versus a single daily dose in the previous experiment) in an attempt to spread the insulin activity over a longer duration of the day.

Beginning at least 7 d before the onset of the experiment, cows were fed a common TMR (Table 1) formulated using the Cornell Net Carbohydrate and Protein System, version 6.1 (Tylutki et al., 2008) and designed to provide MP, Met, and Lys in excess of requirements. Cows were fed daily at 0900 h, and orts were removed before feeding. Amounts of feed offered and refused were measured daily, and samples of the TMR were collected once each week and dried at 55°C until static weight was reached for measurement of DM content. Daily DMI were calculated for each cow using the respective amounts of feed consumed and the appropriate weekly DM content of the TMR. Samples of the TMR were sent to Cumberland Valley Analytical Services (Maugansville, MD) for analysis using wet chemistry techniques for CP (AOAC International, 2000), acid detergent insoluble CP [CP determined using AOAC International (2000) after acid detergent extraction (AOAC International, 2000)], neutral detergent insoluble CP [AOAC International (2000) method for CP after neutral detergent extraction (Van Soest et al., 1991)], soluble CP (Krishnamoorthy et al., 1982), ADF (AOAC International, 2000), NDF (AOAC International, 2000), lignin (Goering and Van Soest, 1970), starch (Hall, 2009), sugar (Dubois et al., 1956), ether extract (AOAC International, 2000), and ash (AOAC International, 2000). Chemical composition of the basal TMR, based on the composition of the components, is reported in Table 1. Individual feed ingredients were also dried, sampled weekly, and analyzed in the same manner as the TMR described above. Free choice water was available at all times during the experiment.

Cows were weighed twice before the experiment commenced, and the average BW for each cow was used to calculate the daily dose of long-acting insulin that would be administered to each cow for the 10-d treatment period. Covariate data and samples were collected before first treatment dose for all cows and included blood samples, DMI, and milk yield and composition. Treatments were administered for 10 d at 12-h intervals beginning after milking on d 1. Blood samples were taken twice daily via coccygeal venipuncture into evacuated tubes containing sodium heparin (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) immediately before the 1000 h dose and again at 1600 h. Samples were placed on ice until centrifugation (3,000 × *g* for 20