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Coordination of lipid droplet-associated proteins during the transition period of Holstein dairy cows

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ABSTRACT

Dairy cows often experience negative energy balance with the onset of lactation, and severe or prolonged negative energy balance can contribute to declines in overall fitness. Energy stores, in the form of adipose tissue triacylglycerides, are mobilized during times of energy deficit, and recent research has implicated several proteins associated with the lipid droplet as lipolytic regulators. The objective of this study was to determine if these novel proteins associated with lipolytic regulation are altered with the changing metabolic demands of lactation. Weekly blood samples were collected from 26 Holstein cows from 21 d before expected parturition through 28 d postpartum, and again at 150 d postpartum. Serum nonesterified fatty acids, glycerol, and β-hydroxybutyrate were measured. Energy balance was calculated from daily feed intake and milk yield, weekly body weight, and monthly milk component measurements. Adipose tissue biopsies were taken 21 d before expected parturition (−21 d) and at 5, 21, and 150 d postpartum. Semiquantitative Western blotting was used to measure abundance of hormone-sensitive lipase (HSL), phosphorylated HSL, perilipin, phosphorylated perilipin (PPLIN), adipose triglyceride lipase (ATGL), and comparative gene identity-58 (CGI-58). Abundance of ATGL was less at 5 and 21 d in milk (DIM) compared with −21 and 150 DIM, even though cows were in negative energy balance and experiencing increased rates of lipolysis in early lactation. In contrast, phosphorylated HSL and PPLIN increased with increasing lipolysis immediately after parturition. Additionally, PPLIN was negatively correlated with milk yield at 5, 21, and 150 d postpartum, and negatively correlated with feed intake and energy balance at 21 d postpartum. This result is consistent with the hypothesis that phosphorylation of perilipin is responsive to signals for increased triaclyglyceride mobilization. Finally, a consistent negative correlation between abundance of perilipin and CGI-58 proteins was observed throughout the transition period. These results confirm that novel lipolytic proteins in adipose tissue are regulated at the level of protein abundance and phosphorylation during the periparturient period and into mid lactation.
Key words: perilipin, adipose triglyceride lipase, transition period, energy balance

INTRODUCTION

Milk production per cow has doubled over the last 40 yr, resulting in increased energy requirements throughout lactation (AIPL, 2010). It is widely recognized that energy demands due to the initiation of milk synthesis in early lactation are often not met by energy intake, causing cows to experience negative energy balance. Although a period of negative energy balance is a natural part of the lactation cycle, severe and prolonged negative energy balance can contribute to declines in fitness traits, including fertility (Veerkamp et al., 2000; Lopez et al., 2005), udder health (Banos et al., 2006), locomotive problems (Collard et al., 2000), and disease susceptibility (Hammon et al., 2006; van Knegsel et al., 2007).

During times of negative energy balance, mobilization of energy reserves from adipose tissue is a primary mechanism by which dairy cows compensate for their energy deficit. It is well established that activation of hormone-sensitive lipase (**HSL**) via protein kinase A (**PKA**) is a critical pathway in the regulation of lipolysis. More recently, additional proteins have been associated with lipolytic regulation (Figure 1). Perilipin (**PLIN**) is a phosphoprotein that associates with lipid droplets. In the basal state, PLIN protects the lipid droplet from HSL-mediated lipolysis (Brasaemle et al., 2000) and co-localizes with the protein comparative gene identity-58 (**CGI-58**; Subramanian et al., 2004; Yamaguchi et al., 2006). The phosphorylation of PLIN by PKA stimulates a conformational change in PLIN that provides HSL access to the lipid droplet and facilitates the interaction between HSL and its lipid substrate (Miyoshi et al., 2006). Additionally, phosphorylation of PLIN results in the release of CGI-58 from the lipid droplet. When CGI-58 is not associated with PLIN, it acts as an activator of adipose triglyceride

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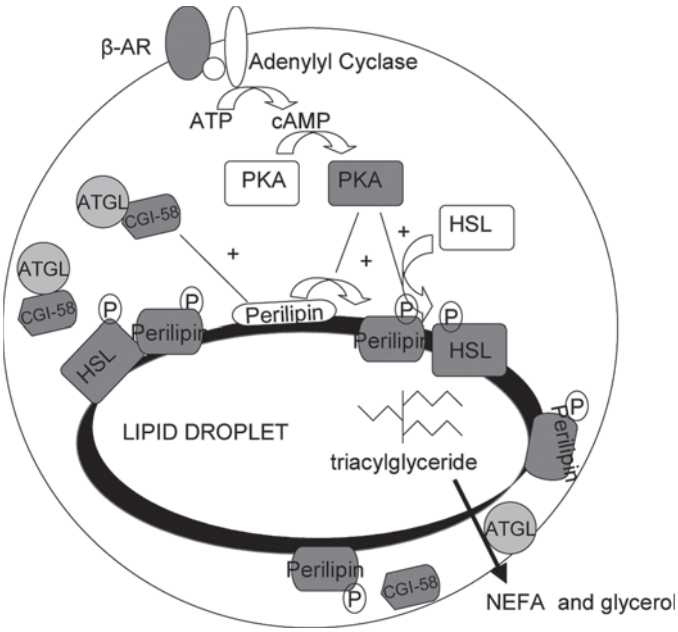

Figure 1. Working model of lipolysis. Beta-adrenergic receptors (β-AR) are activated by the binding of catecholamines. This stimulation results in activation of adenylyl cyclase, which converts ATP to cyclic AMP (cAMP). Increased intracellular concentration of cAMP activates protein kinase A (PKA), which phosphorylates hormone-sensitive lipase (HSL) and perilipin. Phosphorylated HSL translocates to the lipid droplet to hydrolyze triacylglycerides to free fatty acids (FFA) and glycerol. Phosphorylation of perilipin (1) facilitates interactions between phosphorylated HSL and its lipid substrate, and (2) releases comparative gene identity-58 (CGI-58) from the lipid droplet. Then, CGI-58 translocates to the cytosol where it interacts with adipose triglyceride lipase (ATGL). The translocation of ATGL to the lipid droplet allows for the hydrolysis of triacylglycerides to diacylglycerides. Lines with + represent a positive action, a circled 'P' represents a phosphorylation event, and dark gray shapes represent active proteins.

lipase (**ATGL**) in the cytosol. The lipase ATGL can then translocate to the lipid droplet where it hydrolyzes triacylglycerides to diacylglycerides (Granneman et al., 2007; Schweiger et al., 2008; Figure 1). It has been suggested that the hydrolysis of triacylglycerides to diacylglycerides by ATGL is the rate-limiting step in lipolysis (Haemmerle et al., 2006), and that phosphorylation of PLIN is a "master regulator" of lipolysis through its control of both HSL and ATGL, with the latter mediated by CGI-58 (Moore et al., 2005; Miyoshi et al., 2007).

Appropriate regulation of lipolysis is critical for dairy cows to maintain health and productivity in times of negative energy balance. However, the coordinated regulation of HSL, PLIN, ATGL, and CGI-58 has not yet been studied in cattle. Thus, the current research was undertaken to determine if these proteins are altered at the level of protein abundance or phosphorylation with the changing metabolic demands of lactation. First, we quantified changes in protein abundance of HSL, PLIN, ATGL, and CGI-58, as well as phosphorylation of HSL and PLIN, over the transition period. Second, we defined the relationship of these lipolytic proteins with traits influencing energy balance. Finally, we identified correlated changes among the lipolytic regulatory proteins.

MATERIALS AND METHODS

All procedures involving the use of animals were approved by the Iowa State University Institutional Animal Care and Use Committee.

Animals

Individual feed intake was measured daily on 26 multiparous (13, 7, and 6 cows in lactations 2, 3, and 4, respectively) Holstein cows using the Calan Broadbent feeding system (American Calan, Northwood, NH). Feed intake was measured from approximately 30 d before parturition through 150 DIM. Precalving and lactating cows were fed once or twice daily, respectively, and orts were removed and weighed daily. Cows were provided ad libitum access to a consistent TMR formulated to meet or exceed dry or lactating cow nutritional requirements (NRC, 2001). Lactating and nonlactating cows were weighed weekly following their morning milking and in early afternoon, respectively. Body condition scores were determined weekly using a 1 to 5 scale (Elanco, 1996).

Milk Production and Milk Components

Cows were milked twice daily with milk yield recorded at each milking. The software program Bestpred (Cole et al., 2009) was used to predict daily milk fat and protein percentages based on monthly DHIA test-day results. Additional milk samples were taken at the morning milking and analyzed for lactose content by a commercial laboratory (Dairy Lab Services, Dubuque, IA).

Blood and Tissue Sample Collection

Blood was collected weekly via jugular venipuncture beginning at approximately 21 d before parturition through 28 DIM, and again at the completion of the study, at approximately 150 DIM. Serum was stored at −80°C until future analysis. Serum glycerol concentrations were measured using Free Glycerol Reagent (F6428, Sigma Aldrich, St. Louis, MO) according to the manufacturer's protocol. Concentrations of NEFA and BHBA were analyzed from serum using commercially available kits according to the manufacturer's protocol