Phosphorylated Perilipin Abundances Associated with Energy Mobilization in Lactating Dairy Cows

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Summary and Implications

Perilipin, adipose triglyceride lipase (ATGL) and comparative gene identity-58 (CGI-58) are proteins recently associated with energy mobilization from adipose tissue of rodents and humans. Relative protein abundances were measured in 11 early (5-14 DIM) and 9 mid (176-206 DIM) lactation cows. Early lactation cows were mobilizing energy compared to mid lactation cows. Phosphorylation of perilipin was increased in early compared to mid lactation cows, and significantly associated with lipid breakdown. ATGL was significantly increased in mid compared to early lactation, and CGI-58 was similar between stages of lactation. Neither ATGL nor CGI-58 was correlated with lipid breakdown. These results identify perilipin and ATGL as novel proteins involved in the regulation of lipid mobilization throughout lactation.

Introduction

During early lactation, dairy cows often do not consume enough energy to meet their production needs, causing them to experience negative energy balance. Severe or prolonged negative energy balance can result in decreases in fitness which can manifest as decreased reproductive fitness, udder health and increased lameness. To meet energy requirements during negative energy balance, cows mobilize body energy reserves which primarily are stored as triacylglycerides, or lipids, in adipose tissue.

The protein kinase A (PKA) pathway stimulates the mobilization of energy stored in adipose tissue. This pathway is activated by epinephrine and norepinephrine binding to beta-adrenergic receptors (Figure 1), resulting in the activation of PKA by way of activated adenylate cyclase and increases in cyclic adenosine monophosphate. PKA phosphorylates hormone sensitive lipase (HSL) resulting in the translocation of HSL from the cytosol to the lipid droplet, and ultimately the breakdown of triacylglycerides for energy.

Recently, the protein perilipin has been shown to participate in PKA stimulated energy mobilization. Perilipin is a lipid droplet protein that acts as an enhancer and inhibitor of lipid breakdown. When the PKA pathway is not activated, perilipin acts as a barrier and prevents the interaction of HSL with triacylglycerides. When the PKA pathway is activated, PKA will phosphorylate perilipin resulting in a conformational change which allows for phosphorylated HSL to interact with triacylglycerides (Figure 1).

In 2004, an additional lipase was discovered which aided in the breakdown of triacylglycerides. Adipose triglyceride lipase (ATGL) acts as an initial lipase. ATGL is regulated by comparative gene identity-58 (CGI-58). When the PKA pathway is not activated, ATGL resides in the cytosol and at the lipid droplet, whereas CGI-58 resides mostly at the lipid droplet near perilipin. Upon activation of the PKA pathway and phosphorylation of perilipin, CGI-58 translocates from the lipid droplet to interact with ATGL, where the complex translocates to the lipid droplet allowing ATGL to break down triacylglycerides (Figure 1).

These novel proteins of lipid catabolism have been almost exclusively researched in rodent and humans, and to date only mRNA expression of perilipin has been characterized in dairy cattle. With the problems that face the dairy industry associated with negative energy balance, we chose to explore the relationship of perilipin, ATGL, and CGI-58 with energy mobilization during lactation.

Materials and Methods

Twenty Holstein cows were selected based on stage of lactation (early [5-14 DIM] versus mid [176-206 DIM]), current lactation (2-5), body condition score (2.75-4.75), and lack of health concerns. Adipose tissue was removed from the tailhead region for semi-quantitative western blotting for proteins of interest using commercially available antibodies for HSL, perilipin, phosphorylated HSL and perilipin, ATGL and CGI-58. Blood was collected via jugular venipuncture following the biopsy, and non-esterified fatty acids and glycerol were measured using commercially available kits.

Statistical analysis was done to determine differences associated with stage of lactation. Correlations were determined to evaluate relationships between the proteins of interest and indicators of lipid mobilization. Significance was defined as $P<0.05$.

Results and Discussion

Both non-esterified fatty acids and glycerol (products of lipid breakdown) were significantly increased in early compared to mid lactation cows. HSL protein abundance was similar between early and mid lactation, but phosphorylation of HSL was significantly increased in early compared to mid lactation (Figure 2). Significant correlations of HSL protein in early and mid lactation were found with indicators of lipid breakdown (Table 1), and phosphorylation of HSL was correlated with circulating glycerol in early lactation cows. These results suggest that

HSL protein abundance, as well as phosphorylation in early lactation, regulate lipid mobilization.

The abundance of perilipin was not significantly different between stages of lactation. However, phosphorylation of perilipin was significantly increased in early compared to mid lactation and correlated with lipolytic indicators in early and mid lactation cows. These data suggest that phosphorylation of perilipin may be an additional regulator of lipolysis.

Protein abundance of CGI-58 was similar between stages of lactation and lacked correlation with lipolytic indicators, suggesting the role of CGI-58 in lipolysis is not altered by its abundance. ATGL protein abundance was significantly increased in mid compared to early lactation. However, ATGL abundance was not correlated with lipolytic indicators during either stage of lactation. At this time, protein abundance is not a direct measure of ATGL activity. Therefore, it is premature to conclude that the increase in abundance represents increased ATGL lipolytic activity. However, studies from human research suggest ATGL maybe more active during times of neutral or positive energy balance.

In conclusion, this research defines two novel proteins, perilipin and ATGL, as potential regulators of lipid mobilization in dairy cattle. These results expand the current view of mechanisms that regulate this complex process, and may contribute to novel strategies that optimize lipid mobilization in dairy cattle.

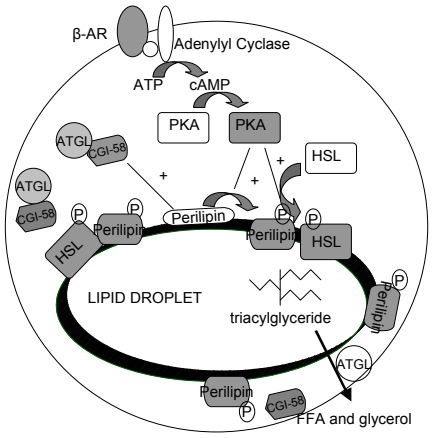

Figure 1. ***PKA pathway in adipose tissue.*** Lines indicated movement/interaction of proteins. Dark filled images indicate activated proteins in the PKA pathway. Encircled P's are proteins activated by phosphorylation. Abbreviations: β-AR, beta adrenergic receptors; ATP, Adenosine triphosphate; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; HSL, hormone sensitive lipase, ATGL, adipose triglyceride lipase; CGI-58, comparative gene identity-58

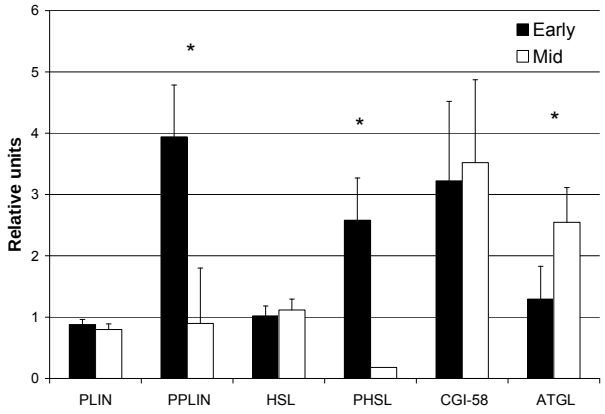

Figure 2. ***Relative Protein Abundances in early (5-4 DIM) and mid (176-206) DIM lactation cows.***
Abbreviations: PLIN, perilipin; PPLIN, phosphorylated perilipin; HSL, hormone sensitive lipase; PHSL, phosphorylated hormone sensitive lipase; CGI-58, comparative gene identity-58; ATGL, adipose triglyceride lipase. Mid lactation had one value for PHSL.
* P<0.05

Table 1. Correlations coefficients (r) of protein and phosphorylation abundance with lipolytic indicators in early (5-14 DIM) and mid (176-206 DIM) lactation cows.

	Early NEFA[a]	Early Glycerol	Mid NEFA	Mid Glycerol
PLIN	-.22	.11	.06	.42
PPLIN	.60*	.73*	.50	.65[#]
HSL	.65*	.65*	.02	.75*
PHSL	.61	.73*	--	--
CGI-58	-.27	-.22	-.16	.01
ATGL	.05	.31	.16	-.47

[a] Abbreviations: NEFA, nonesterified fatty acid; PLIN, perilipin; PPLIN, phosphorylated perilipin; HSL, hormone sensitive lipase; PHSL, phosphorylated hormone sensitive lipase; CGI-58, comparative gene identity-58; ATGL, adipose triglyceride lipase
-- missing data
* P<0.05
[#]P=0.06