Regulation of bovine adipose tissue metabolism during lactation. 7. Metabolism and gene expression as a function of genetic merit and dietary energy intake¹

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ABSTRACT

The regulation of adipose tissue metabolism is critical to the efficient establishment and support of lactation, through both energy supply and several endocrine and cytokine factors. We still lack detailed knowledge of the role of transcription and posttranslational regulation of metabolic flux. We need to quantitatively understand the genetic and environmental (primarily dietary) regulation of adipose tissue to help improve productive efficiency. Therefore, objectives of this project were to help define mechanisms of adipose tissue responses to lactation and energy deficit, including changes in gene expression and their relation to changes in metabolic flux and production. A total of 48 cows were selected for genetic merit based on sire predicted transmitting ability of milk. From 21 d prepartum to 60 d in milk (DIM), cows were fed to energy requirements or to 90% of energy requirements, with content of protein, vitamins, and minerals balanced to be the same for both treatments. Adipose tissue biopsies were taken at 21 and 7 d prepartum and 7, 28, and 56 DIM to determine rates of lipogenesis and lipolysis, and to measure gene expression of proteins controlling lipolysis. The cows on the restricted diet consumed 12% less feed prepartum and 16% less feed postpartum and dietary energy restriction decreased milk production. The slowest rates of lipogenesis occurred at 7 and 28 DIM; higher-merit cows had faster rates of lipogenesis at 7 DIM but slower rates than lower-merit cows at 28 DIM. Energy restriction decreased lipogenesis. Basal and isoproterenol lipolysis increased with higher milk production and was relatively unaffected by dietary energy intake. The expression of genes controlling lipolysis were not

affected by lactation and were slightly increased by dietary restriction, but were not well related to rates of lipolysis. These data confirm and extend previous work that regulation of adipose tissue metabolism in lactation is a function of both diet and genetic merit and is controlled by multiple mechanisms including gene transcription and posttranslational protein modifications.

Key words: adipose tissue, gene expression, lactation

INTRODUCTION

Adipose tissue metabolism in pregnant and lactating dairy cattle is essential in the successful establishment and support of lactational efficiency (McNamara, 2012). We have known for quite some time that specific adaptations in enzymes controlling lipogenesis and lipolysis during lactation help to supply FA to the mammary gland and other organs (Shirley et al., 1973; Yang and Baldwin, 1973). Since this early work, much has been defined of the genetic and environmental effects on adipose metabolism, which, as part of the overall system of the cow, responds to the demand for milk synthesis and attenuates the environmental variation in nutrient availability and intake (McNamara, 2012). The adipose tissue of cows of greater genetic merit for milk production have faster lipolysis, an increased response to β -adrenergic stimulation, increased activity of hormone-sensitive lipase (**LIPE**), and decreased lipogenesis compared with average-genetic merit animals (McNamara, 1989; Smith and McNamara, 1990; Vernon, 2003).

The adaptations of decreased lipogenesis and increased lipolysis to lactation and genetic merit can be attenuated by dietary energy intake (McNamara, 1994; Parmley et al., 1996; Vernon, 2003). During early lactation, a decrease in expression of acetyl-CoA carboxylase (**ACACA**) and lipoprotein lipase (**LPL**), and a diminished response to insulin are common control adaptations for lipogenesis (Sumner-Thomson et al., 2011; McNamara, 2012). Recently, we demonstrated that the mRNA abundance for 5 genes that control lipolysis [3 β -adrenergic receptors: **B1AR**, **B2AR**, and **B3AR**;

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LIPE; and perilipin (**PLIN**)] increased in expression in adipose tissue of dairy cattle during lactation, but not until after peak lactation (Sumner and McNamara, 2007). Gene transcription array work from the present trial has also demonstrated coordinated decreases in genes coding for anabolic pathways in early lactation, but not in genes controlling lipolysis (Sumner-Thomson et al., 2011; Khan et al., 2013). Other recent work has extended our knowledge to show that adipose tissue triacylglycerol lipase is a different enzyme than LIPE, and it appears that adipose tissue triacylglycerol lipase regulates chronic basal lipolysis, whereas hormone-sensitive lipase (**HSL**) is expressed in a mixed pattern of constitutive and responsive elements in which HSL can rapidly activate upon phosphorylation signals from the sympathetic nervous system and other hormones (Koltes and Spurlock, 2011).

The historical and recent data confirm the multifaceted regulation of adipose tissue metabolism and the importance of adipose tissue in the systems biology of the cow. Two main driving forces in metabolic control are the genetic merit of the cow to make milk and the dietary energy available for that production and supporting metabolism. To learn more about specific quantitative regulation of this system and eventually the mechanistic causes of differences in efficiency, the overall hypothesis tested was that animals that vary in genetic merit for milk production and in energy intake will have a different pattern of lipid metabolism in the adipose tissue, including expression of key regulatory genes. The expression of these genes will partially control the balance of lipogenesis and lipolysis, in turn altering the metabolic efficiency of the animals. Thus, the objective of this experiment was to investigate the role of changes in gene expression on the metabolic flux in adipose tissue as affected by the interaction of sire genetic merit for milk production and a mild energy deficit. Such data can be used to construct and update models of metabolic systems control in the cow that are more specific and precise.

MATERIALS AND METHODS

Animals and Treatment Protocol

Forty-eight Holstein cows from the Knott Dairy Herd (Pullman, WA) were selected, blocked by parity (first or second) and by sire genetic merit as predicted transmitting ability for milk (**PTAM**). There were 24 first-lactation and 24 second-lactation animals. Cows were selected for genetic merit (high merit, **HM**; low merit, **LM**) based on their sire PTAM. For the sires of the HM cows, PTAM was 1,913 lb (870 kg) and for the LM cows 832 lb [378 kg; SEM = 45 lb (20.5 kg)]. The 305-d ME

for HM second-lactation animals was 30,582 lb (13,901 kg) and for LM it was 27,997 lb [12,726 kg; SEM = 562 lb (256 kg)]. The rationale for using the sire PTAM was because our original and continuing studies since the 1980s (McNamara and Hillers, 1986a,b) were based on a national study at that time using that criterion and we have used it since. The authors recognize that many other potential genetic traits exist that could be used, but milk production continues to be a major genetic selection criterion. The experiment was approved by the Washington State University Institutional Animal Care and Use Committee (IACUC; project 3478) and was conducted from May 2008 through February 2009.

Dietary treatments were either (1) normal-energy (**NE**) cows fed a TMR to requirements (NRC, 2001) or (2) low-energy (**LE**) cows fed the same TMR (with adjustments to grain mix, see below) at 90% of the intake of the NE group based on intake as a percentage of BW (Table 1). Cows were fed from 21 d prepartum to 60 DIM through Calan gates (American Calan Inc., Northwood, NH); the full daily allotment was fed between 1000 and 1100 h and cows had access to feed at all times. The LE diet was fortified with 10% more protein as well as vitamin and mineral mixes in the grain mix so that these components were consistent across treatments. Dietary ingredients were sampled with each new batch; the TMR andorts were sampled weekly and composited monthly for analysis at Kuo Testing Labs Inc. (Othello, WA) using AOAC International methods for DM, ADF, NDF, CP, fat, Ca, and P (AOAC International, 2000). Some of the data gathered in this experiment have been published previously (McNamara, 2012; Khan et al., 2013); however, this is the first complete publication of the production and metabolism data.

Samples and Measurements

Cows were milked twice per day between 0800 and 1000 h, and between 2000 and 2200 h and yield was recorded daily. Milk composition was determined approximately monthly by DHIA sampling using infrared spectrophotometry at the regional DHIA laboratory in Burlington, Washington (AOAC International, 2000). Body weight and BCS (Waltner et al., 1994) were assessed weekly and these measures were used to calculate body fat (Waltner et al., 1994). Prior to the morning feeding, blood was collected weekly via venipuncture of the coccygeal vessel at 28, 21, 14, 11, 7, and 4 d prepartum and then postpartum at d 1, 3, and 7, and then weekly until wk 8.

Prior to the morning feeding, subcutaneous adipose tissue biopsies were collected at 21 and 7 d prepartum and at 7, 28, and 56 DIM, from the tail head region un-

Table 1. Dietary ingredients and chemical composition¹

Item	Diet ²			
	NE (dry period)	NE (lactation)	LE (dry period)	LE (lactation)
Ingredient, % DM				
Alfalfa hay	—	23.6	—	23.6
Alfalfa haylage	24.0	25.6	24	25.6
Grass hay	60.0	0	60	—
Whole cottonseed	—	7	—	7
Dried distillers grains	—	5.26	—	5.26
Concentrate mix	16	38.6	16	38.6
Concentrate composition, %				
Corn	76	93	86	73
Soybean meal	11	0	0	12.2
Peas	8	0	0	8.9
Limestone	1	1	2	1.1
Salt (trace minerals with selenium)	1	1	2	0.8
Yeast	1	1.4	3	0.67
Ammonium oxide	0	2.5	5	0
Magnesium oxide ³	0.1	0.6	1	0.44
Vitamin D premix ⁴	0.4	0.1	0	0.06
Vitamin A premix ⁴	0.1	0.05	0	0.03
Vitamin E premix ⁴	0	0.1	0	0.01
4-Plex ⁵	0.1	0.2	0	0.11
Chemical analysis, % of DM				
CP	12.4	18.5	12.7	19.0
Crude fiber	2.3	3.8	2.3	3.4
Nitrogen-free extract	50.6	50.0	49.9	51.1
ADF	26.3	19.6	26.3	19.8
NDF	37.1	24.2	37.1	24.1
Lignin	6.9	7.8	7.2	7.6
Ca	0.99	0.87	1.03	1.12
P	0.26	0.37	0.26	0.35
Ash	10.3	9.2	10.6	9.0
NE _L , Mcal/kg	1.25	1.60	1.25	1.60

¹Chemical composition from analysis of individual components (AOAC International, 2000).

²NE = normal energy; LE = low energy.

³Premier Chemicals LLC (West Conshohocken, PA).

⁴Vitamin D premix contained 4 million IU/lb. of vitamin D; vitamin A premix contained 30,000 IU/g of vitamin A-acetate; vitamin E premix contained 227,000 IU/lb of vitamin E.

⁵Zinpro Corp. (Eden Prairie, MN).

der spinal anesthesia (Sumner and McNamara, 2007). Part of the sample was immediately placed in Krebs/HEPES buffer (Sumner and McNamara, 2007) at 37°C for tissue incubations to estimate rates of lipogenesis and lipolysis and part was immediately homogenized in QIAzol reagent (Qiagen 75842; Qiagen Inc., Valencia, CA) and the homogenate frozen until extracted for RNA. For BW, BCS, blood samples, and biopsies, samples were taken up to ± 3 d from the actual DIM. The means of actual days around parturition for the samples was -19 , -5 , 9 , 30 , and 56 d.

Analytical Methods

Blood serum was collected and analyzed for NEFA (NEFA-C kit; Wako Chemicals USA Inc., Richmond, VA) with the modifications published previously (McNamara and Hillers, 1986b) and for glucose [Glucose (HK) Kit; Sigma-Aldrich, St. Louis, MO].

Rates of lipogenesis were measured in vitro using adipose tissue incubated in medium containing Krebs/HEPES buffer containing 2% BSA (FA free); 5 mM glucose; and 0.5, 1, 2, 4, and 8 mmol of acetate at 0.1 μ Ci/mmol of 2-C¹⁴ acetate, at a pH of 7.4 and 37°C for 2 h in triplicate (McNamara and Hillers, 1986a; McNamara and Valdez, 2005). The tissue was sliced to a thickness of approximately 500 μ m and was measured into approximately 80- to 100-mg slices for the triplicate incubations. After incubation, samples were placed in Dole's reagent (McNamara and Hillers, 1986a) to be extracted for total FA synthesis. Rates were reported as millimoles of acetate converted to FA per 2 h per gram of tissue.

Incubations of adipose tissue were used to measure basal and stimulated rates of lipolysis. Adipose tissue was sliced and preincubated in 2 mL of Krebs-HEPES media containing 2% BSA (fatty acid free) for 20 min to remove the effects of handling and slicing (McNa-

Table 2. Reverse-transcriptase PCR primers used for determining gene expression in bovine adipose tissue¹

Gene ²	GenBank accession no.	Sequence (3'-5')
<i>B2AR</i>	NM_194266	CCCCAGGCACCGAAAACT TCCCTTGTGAATCAATGCTATCA
<i>HSL</i>	NM_001080220	GAGTTTGAGCGGATCATTCA TGAGGCCATGTTTGCTAGAG
<i>PLIN</i>	NM_001083699	AGACACTGCCGAGTATGCTG TGGAGGGAGGAGGAACTCTA
<i>BRPS2</i>	NM_001033613	GGAGCATCCCTGAAGGATGA TCCCCGATAGCAACAAACG

¹Primers were designed using Primer Express 2.0 (Applied Biosystems Inc., Foster City, CA) and were ordered from IDT Technologies (Coralville, IA).

²*B2AR* = β -2 adrenergic receptor; *HSL* = hormone-sensitive lipase; *PLIN* = perilipin; *BRPS2* = ribosomal protein S2.

mara and Hillers, 1986b; McNamara and Valdez, 2005; Sumner and McNamara, 2007). The media was then removed and replaced with fresh media and the tissue was incubated another 2 h. Basal media had no added stimulators. The response curve to β -adrenergic receptor binding was conducted using isoproterenol (ISO) at 10^{-8} , 10^{-7} , 10^{-6} , 10^{-5} , and 10^{-4} M concentrations. Adenosine deaminase (6.6 U/mL; #116880; Calbiochem, San Diego, CA) and theophylline (1 mM; no. 200-305-7; Sigma-Aldrich) were included to maximize response to ISO. Rates of lipolysis were expressed as glycerol release in nanomoles per 2 h per gram of tissue.

Gene Expression

Adipose tissue was saved for RNA extraction in duplicate; each duplicate was placed in 5 mL of QIAzol reagent and adipose tissue was immediately homogenized and chilled to -20°C until extraction; it was then extracted using the RNA-easy midi-kit (Qiagen 75842; Qiagen Inc.). The quality of the RNA, once extracted, was determined by resuspending the RNA in RNase-free water and using the NanoDrop 1000 spectrophotometer (Thermo Fischer Scientific, Wilmington, DE) to estimate RNA purity via the ratio of A260/A280 (absorbance at a wavelength of 260 nm/absorbance at a wavelength of 280 nm). First-strand cDNA synthesis was performed once the quality and purity of RNA was assessed and confirmed. A reverse-transcriptase (RT) and a no-RT control were made from each set of cDNA to be run on the ICycler real-time PCR machine (Bio-Rad Laboratories Inc., Hercules, CA). The primers in Table 2 were used to determine gene expression. Real-time RT-PCR analysis was done to determine gene expression of the biopsied bovine adipose tissue. The dye IQ SYBR Green PCR (Bio-Rad Laboratories Inc.) was used to visualize fluorescence of amplified gene products for real-time RT-PCR run on the thermal iCycler from Bio-Rad Laboratories Inc. The system software is designed to generate and plot the data based on the

cycle threshold and normalized fluorescence (ΔRn) over a given number of cycles. Ribosomal protein S2 (*BRPS2*) reference gene was used against the primers to calculate relative gene expression (Table 2).

Experimental Design and Statistical Analysis

The experiment was designed and conducted as a randomized complete block with repeated measures, with sire genetic merit (PTAM) and parity as blocks, dietary energy as treatment, and time around parturition as the repeated measure. Milk and DMI data were analyzed on the daily data; milk composition on the monthly DHIA data; and BW, BCS, blood, and tissue variables as the actual data on the average DIM when samples were taken (DIM as repeated measure). The model for analysis was as follows: $Y_{ijklmn} = \mu + \text{GM}_i + \text{P}_j + \text{D}_k + (\text{GM} \times \text{P})_{ij} + (\text{GM} \times \text{D})_{ik} + (\text{P} \times \text{D})_{jk} + (\text{GM} \times \text{P} \times \text{D})_{ijk} + \text{C}_{(ijk)l} + \gamma_m + \varepsilon_{ijkl}$. In this model, Y_{ijklmn} is the dependent variable, μ is the overall mean, GM_i describes the effect of genetic merit (either high or low), P_j describes the effect of parity (either first lactation or second lactation), and D_k describes the effect of dietary energy (either normal or low). The fixed effect error is $\text{C}_{(ijk)l}$, γ_m describes the repeated measure effect (random) of the DIM or sample date as a continuous variable (DIM) with linear and quadratic effects, and ε_{ijkl} describes the residual error. Data were analyzed using PROC GLM of SAS (SAS Institute, 2005).

RESULTS AND DISCUSSION

Production Variables

In this experiment, the purpose of measuring feed intake, milk production, and body fat was to provide a framework to interpret metabolic adaptations to genetic merit and energy intake, not to retest the well-known effects of genetics and diet on production. When provided with feed ad libitum (Table 3; Figure 1a and

Table 3. Feed intake and milk production of cows varying in genetic merit fed a normal or an energy-restricted diet

Item	High merit, normal diet	High merit, restricted diet	Low merit, normal diet	Low merit, restricted diet	SEM
Feed intake, kg of DM/d					
First lactation					
Prepartum ¹	12.4	12.6	13.0	9.0	0.5
Postpartum ²	18.8	17.7	19.3	14.8	0.5
Second lactation					
Prepartum ¹	15.1	15.5	14.4	13.0	0.8
Postpartum ²	23.9	20.4	25.0	20.2	0.9
Milk production, ³ kg/d					
First lactation					
Overall	26.7	29.7	26.7	23.7	1.2
d 1 to 28	23.0	26.6	24.3	20.8	1.1
d 29 to 56	31.2	33.3	29.5	27.4	1.4
Second lactation					
Overall	35.3	40.9	43.1	35.0	1.8
d 1 to 28	31.0	39.8	38.6	31.9	1.7
d 29 to 56	39.5	43.1	47.1	38.3	2.0

¹Effect of genetic merit: $P < 0.05$, parity: $P < 0.001$, and genetic merit by diet: $P < 0.03$.

²Effect of parity: $P < 0.001$ and diet: $P < 0.001$; trend of genetic merit by diet: $P < 0.09$.

³Effect of parity: $P < 0.0001$, diet: $P < 0.05$, merit: $P < 0.05$, and genetic merit by diet: $P < 0.006$.

1b). As expected, second-parity cows ate more than first-parity cows (Table 3; $P < 0.01$). The cows on the LE diet consumed 12% less feed prepartum and 16% less feed postpartum, regardless of genetic merit or parity (Table 3; Figure 1b).

Cows of HM did not always eat more than LM animals when fed ad libitum, for all time periods. However, between 1 and 10 d postpartum HM cows ate more than LM cows (Figure 1a; $P < 0.05$). When energy was restricted, HM animals consumed more food than LM cows. We do not have a convincing reason for this, but one possibility is that cows of lower genetic merit may not have as high a drive for feed intake so, when challenged, the cows reduced energy utilization and subsequent feed intake. With the number of animals in this trial, however, it is likely that genetic variation among animals was a major reason for variation in intake and we did not make any conclusions about genetic merit for milk production and dietary restriction.

Milk Production

Over both parities, cows of the HM group produced more milk than LM cows and dietary energy restriction did decrease milk production (Table 3; Figure 2a and 2b; main effects of merit and diet: $P < 0.05$). However, the interaction of sire merit and energy intake on milk production was quite interesting. When fed ad libitum, HM cows did not produce more milk than cows of LM. But when feed restricted, primiparous HM cows produced 3 kg/d more than LM cows, and multiparous HM cows produced 8.1 kg/d more than LM cows ($P < 0.03$; effect of genetic merit by diet; Table 3). It may be that

the HM animals can better adapt to an energy deficit to prioritize milk production. The LM animals, however, reacted to a dietary energy restriction by eating even less and producing less milk. Such an adaptation was noted previously (McNamara and Hillers, 1986a,b) and will be discussed further when the data on metabolic flux is presented. Milk fat and protein percentages did not differ between genetic merit groups [HM = 3.98 and 3.2% and LM = 3.94 and 3.12% for fat and protein (SEM = 0.04 and 0.02 for fat and protein)]. Milk fat yield and protein yield of the LE-fed cows were 95 and 93% of the yields of the NE-fed cows, consistent with the difference in milk yield. For the dietary treatment comparison, milk fat percentage and yield were 3.92% and 1.38 kg/d for NE cows and 4.03% and 1.31 kg/d, respectively, for LE cows ($P = 0.32$ for percentage and 0.065 for yield). Milk protein percentage and yield were 3.14% and 1.11 kg/d, respectively, for NE cows and 3.17% and 1.03 kg/d, respectively, for LE cows ($P = 0.04$ and 0.02 for percentage and yield).

BW, BCS, and Body Fat

Cows in their second lactation were heavier and retained BW better than primiparous cows [Table 4; $P < 0.0001$ for BW, BCS, and empty body fat (EBF)]. From 7 d prepartum to 7, 28, or 56 DIM, HM cows lost numerically more weight on the LE diet than the other groups (Table 4; $P < 0.06$). Cows across all genetic merit and diet interaction groups decreased in BW and EBF until 28 DIM, with slight recovery occurring around 56 DIM. Body condition score followed a similar trend or continued to decrease as seen in cows on

Figure 1. Feed intake of cows varying in genetic merit fed a normal or an energy-restricted diet (main effect of dietary energy intake). (a) Diamonds represent normal-energy (NE) animals and open triangles represent low-energy (LE) animals. Normally fed intake line is represented by the equation $y = 4.79 \ln(x) + 1.39$ (with an R^2 of 0.87). Lower energy intake line is represented by the equation $y = 4.25 \ln(x) + 0.82$ (with an R^2 of 0.85). Main effect of diet: $P < 0.0001$. (b) Diamonds represent HM animals and open triangles represent LM animals. Higher genetic merit (HM) line is represented by the equation $y = 4.54 \ln(x) + 0.89$ (with an R^2 of 0.89). Lower genetic merit (LM) line is represented by the equation $y = 4.61 \ln(x) + 0.84$ (with an R^2 of 0.83). Effect of genetic merit: $P < 0.05$.

the LE diet ($P < 0.02$; quadratic effect of DIM). From 28 to 56 DIM, most animals regained some body fat; however, HM animals fed the LE diet did not (Table 4). These differences were consistent with the feed intake and milk production.

In brief summary of the production data, HM first-parity animals ate a similar amount of feed and gave slightly more milk when fed ad libitum, but second-parity HM animals actually produced less milk than second-parity LM animals. When feed restricted, all groups tended to give less milk, but in fact, the LM animals of both parities were much more depressed in milk production than were HM animals. Changes in BW, BCS, and EBF were, in general, consistent with the differences in energy intake and milk production.

Figure 2. Milk yield (kg/d) of cows of varying genetic merit fed a normal or an energy-restricted diet (main effect of energy intake). (a) Diamonds represent normal-energy (NE) animals and open triangles represent low-energy (LE) animals. Normally fed intake line is represented by the equation $y = 6.19 \ln(x) + 12.07$ (with an R^2 of 0.96). Lower energy intake line is represented by the equation $y = 5.40 \ln(x) + 13.32$ (with an R^2 of 0.94). Main and interaction effects of genetic merit and diet: $P < 0.0001$. (b) Diamonds represent HM animals and open triangles represent LM animals. Higher genetic merit (HM) line is represented by the equation $y = 6.30 \ln(x) + 12.30$ (with an R^2 of 0.95). Lower genetic merit (LM) line is represented by the equation: $y = 5.44 \ln(x) + 13.09$ (with an R^2 of 0.94). Main and interaction effects of genetic merit and diet: $P < 0.0001$.

Blood Nutrients

Glucose. No clear effects of merit and energy intake on blood glucose were observed throughout the trial; however, HM cows had decreased serum glucose concentrations compared with LM cows when fed ad libitum, but not when restricted (Table 5; $P < 0.02$ for interaction). Primiparous cows trended to have increased serum glucose compared with second-lactation cows on either diet ($P = 0.10$; Table 5).

NEFA. The NEFA concentration of all groups was highest at 7 DIM and generally trended down to 56 DIM (Table 6). Cows in their second lactation had the highest serum NEFA concentrations in early lactation.

Table 4. Body weight, BCS, and empty body fat (EBF) of cows varying in genetic merit fed a normal or an energy-restricted diet

Treatment	DIM				
	–21	–7	7	28	56
BW, ¹ kg					
High merit, normal diet	666.9	691.2	618.6	600.5	635.4
High merit, restricted diet	694.1	705.8	614.8	585.8	602.8
Low merit, normal diet	679.5	668.6	615.2	586.8	606.2
Low merit, restricted diet	676.8	677.5	631.6	582.0	631.5
SEM	11.2	12.4	14.8	10.9	12.7
BCS ²					
High merit, normal diet	3.4	3.4	3.1	3.0	3.2
High merit, restricted diet	3.4	3.5	3.3	3.3	3.0
Low merit, normal diet	3.4	3.4	3.3	3.2	3.3
Low merit, restricted diet	3.4	3.5	3.3	3.2	3.2
SEM	0.0	0.0	0.1	0.1	0.1
EBF, ^{3,4} kg					
High merit, normal diet	141.4	145.0	120.3	112.0	127.3
High merit, restricted diet	146.0	152.1	124.0	117.9	112.5
Low merit, normal diet	144.0	140.7	125.1	115.7	123.0
Low merit, restricted diet	144.0	144.4	127.6	114.7	126.2
SEM	2.6	3.4	3.8	3.0	3.2

¹Effect of parity: $P < 0.0001$, DIM: $P < 0.0001$, genetic merit by diet: $P < 0.06$, and quadratic effect of DIM: $P = 0.0001$; trend of parity by merit by diet: $P = 0.12$ and genetic merit: $P = 0.12$.

²Effect of DIM: $P < 0.0001$, DIM by parity: $P < 0.02$, and quadratic effect of DIM: $P < 0.02$; trend of parity: $P = 0.08$ and quadratic effect of DIM by diet: $P = 0.09$.

³Calculated according to Waltner et al. (1994).

⁴Effect of parity: $P < 0.0001$, DIM: $P < 0.0001$, DIM by parity: $P < 0.06$, and quadratic effect of DIM: $P < 0.0002$.

No main merit or diet effects were observed on serum NEFA concentrations.

Adipose Lipogenesis. Lipogenesis is highly affected by the energy available in an animal to build fat reserves, so the rates of lipogenesis reflect energy availability. Cows tended to have the fastest rates of lipogenesis at 7 d prepartum and the lowest at 21 d prepartum (Figure 3a; $P < 0.05$ for DIM). At d 21

prepartum, many cows were still adapting to eating from the Calan gates, so it is likely that this data are not representative of cows 3 wk prepartum. It is consistent with previous work that rates 7 d prepartum were faster than rates postpartum (Figure 3a).

Cows on the NE diet had faster rates of lipogenesis than cows on the LE diet, regardless of genetic merit, especially at 7 and 28 DIM (Figure 3b). The result

Table 5. Serum glucose (mg/dL) of cows varying in genetic merit fed a normal or an energy-restricted diet

Item	Treatment interaction				SEM ¹
	High merit, normal diet	High merit, restricted diet	Low merit, normal diet	Low merit, restricted diet	
First lactation ²					
–21 DIM	56.5	51.6	63.1	56.3	2.2
–7 DIM	52.3	60.3	57.3	60.9	1.5
7 DIM	53.7	57.5	56.3	36.7	3.6
28 DIM	58.3	58.3	50.8	57.7	2.8
56 DIM	40.1	58.1	58.7	60.6	3.0
Second lactation					
–21 DIM	58.9	62.2	59.7	51.6	6.3
–7 DIM	51.6	60.5	61.0	53.8	3.2
7 DIM	49.4	58.4	53.7	42.6	2.5
28 DIM	51.5	43.1	48.4	41.8	4.8
56 DIM	54.9	65.8	54.1	60.7	2.0

¹The high-merit (HM) normal energy (NE) group only had $n = 3$ compared with 10 to 12 for the time points.

²Effect of DIM: $P < 0.03$, genetic merit by diet: $P < 0.02$, and quadratic effect of DIM: $P < 0.03$; trend of parity: $P = 0.10$.

Table 6. Serum NEFA (μM) concentration of cows varying in genetic merit fed a normal or an energy-restricted diet

Treatment	DIM				
	−21	−7	7	28	56
First lactation ¹					
High merit, normal diet	273.6	123.3	692.2	125.1	383.5
High merit, restricted diet	643.2	295.2	662.1	345.6	177.6
Low merit, normal diet	534.2	290.7	385.1	166.2	206.4
Low merit, restricted diet	368.7	286.7	287.8	159.6	558.0
SEM	78.1	46.8	128.1	35.4	107.4
Second lactation					
High merit, normal diet	521.0	103.6	298.8	168.2	111.7
High merit, restricted diet	139.1	150.6	254.1	461.6	146.4
Low merit, normal diet	102.3	296.5	895.5	637.6	355.1
Low merit, restricted diet	142.8	228.3	870.1	386.3	161.0
SEM	68.3	47.1	205.1	104.5	63.9

¹Effect of DIM by parity: $P < 0.02$, main effect of parity: $P < 0.02$, and parity by genetic merit: $P < 0.04$; trend of quadratic effect of DIM: $P < 0.07$.

that cows on the NE diet at 7 and 28 DIM had faster rates of lipogenesis than cows on the LE diet at 7 and 28 DIM (Figure 4) was expected because cows on the NE diet had more dietary energy available. Dietary energy intake plays a greater role in determining the rates of lipogenesis, as has been noted several times earlier (McNamara and Hillers, 1986a,b; McNamara, 1994; McNamara and Valdez, 2005).

No consistent effect was observed of genetic merit across all time periods; however, an interesting interaction between merit and time again was observed around 7 and 28 DIM (Figure 3c). At 7 DIM, HM cows had faster rates of lipogenesis than LM cows, whereas at 28 DIM, this was reversed and LM cows had faster rates than HM cows. This finding actually agrees well with the intake and production data, as HM cows ate more than LM cows during the first few days of lactation, but by 28 DIM, their intakes were similar. Also, by 28 DIM, HM cows were producing more milk than LM cows. This shows the high sensitivity of adipose tissue lipogenesis to energy status in early lactation as a function of both intake and energy use.

Adipose Lipolysis. Rates of basal lipolysis increased more than 2 fold from 21 d prepartum to 56 DIM (Figure 4a; $P < 0.05$) and cows at 56 DIM had the fastest rates of lipolysis regardless of dietary treatment or genetic merit. We have shown previously that lipolysis in adipose tissue is closely related to milk production; as lactation progresses and milk production peaks, lipolysis increases (McNamara and Hillers, 1986b; McNamara and Valdez, 2005; Sumner and McNamara, 2007). Regardless of genetic merit, cows on the LE diet had almost no difference in basal lipolysis, but approximately 7% faster stimulated lipolysis rates over all days and 26% faster rates (LE > NE) at 28 DIM, which is at least consistent with the 10% reduction in

energy intake. For the effect of merit, as was the case for lipogenesis, the differences in lipolysis between HM and LM groups was most noticeable at 7 and 28 DIM (Figure 4b). Over both DIM and all concentrations of ISO, cows of LM had rates approximately 81% of those of HM ($P < 0.05$ for merit at these 2 time points; trend for merit by DIM interaction over all time points: $P = 0.12$), indicating a positive relationship of lipolysis with the higher milk production of the HM animals. There is inherently higher variation in this measure than in others, but certainly the means and trends are consistent with lipolysis being positively related to milk production and to lower energy intake.

Overall, the data supports the concept previously developed and since supported (McNamara, 1989; Sumner and McNamara, 2007) that energy intake has a larger effect on lipogenesis than on lipolysis, whereas stimulated basal and stimulated lipolysis is more closely related to milk production. These differences are consistent with the separate mechanisms of control of lipogenesis and lipolysis that have been established previously. Rates of lipogenesis generally respond to decreased energy intake with a coordinated decrease in enzyme activity and gene expression for anabolic enzymes and tend not to be as related to rates of milk production. However, rates of lipolysis are much more closely related to rates of milk production as well as energy intake. Lipolysis is controlled partly by mechanisms of gene transcription, and also by rapidly or chronically induced signals from adrenals and the sympathetic nervous system causing posttranslational activation by protein phosphorylation (Sumner and McNamara, 2007; McNamara, 2012). We also understand now that basal lipolysis is probably catalyzed by a different enzyme, adipose tissue triacylglycerol lipase (Elkins and Spurlock, 2009) than LIPE, which catalyzes stimulated lipolysis. Thus, the

Figure 3. Lipogenesis measured in vitro in adipose tissue of dairy cattle in late pregnancy and early lactation (units are nanomoles of acetate converted to FA per 2 h per gram of tissue). Data lines represent the log fit of the individual acetate conversion data. The SEM for each increasing concentration was 17, 37, 70, 95, 153, and 226 nmol/2 h per gram of tissue. (a) Rates for all cows in experiment (note that the values for 7 and 56 DIM are overlaid). Dotted line = 21 d prepartum; solid line = 7 d prepartum; light dashed line = 7 DIM; dash-dot line = 28 DIM; heavy dashed line = 56 DIM. An effect of time was observed ($P < 0.05$). (b) Comparison of interaction of dietary energy and DIM at 7 and 28 DIM. LE = low energy; HE = high energy. (c) Comparison of merit and DIM interaction at 7 and 28 DIM: comparison of high-merit (HM; HIGH) and low-merit (LM; LOW) cows (genetic merit $\times$ day: $P < 0.01$). The interaction of acetate concentration, DIM, and diet was significant at $P < 0.0001$. The interaction of acetate concentration, DIM, and diet was significant at $P < 0.0001$.

question of the importance of gene transcription versus posttranslational activation is relevant to dairy cattle efficiency. The data from this study reported above provide a framework for interpretation of the gene expression data to be discussed next.

Expression of Genes that Control Lipolysis in Adipose Tissue of Dairy Cattle

Three proteins help to control lipolysis in adipose tissue: B2AR, HSL, and PLIN1. The B2AR responds to catecholamine binding and initiates an intracellular cascade, resulting in phosphorylation and activation of HSL to break down triacylglycerol to FFA and glycerol. Phosphorylation of PLIN1 causes translocation of this protein to the lipid droplet surface, allowing catalytic activity of HSL. The quantitative role of transcription of these genes in control of adipose tissue lipolysis during the transition period in dairy cattle has not been fully defined; thus, the transcription of these 3 genes was analyzed for this experiment.

The expression of the genes of interest were compared with the expression of ribosomal subunit S2, generally referred to as ΔCt , where **Ct** is the cycle threshold in the RT-PCR technique ($\Delta Ct = \text{gene of interest Ct} - \text{S2 Ct}$). This normalization provides a comparison of differential control of expression of a gene compared with one that is usually constitutively expressed. In adipose tissue, especially during the adaptation occurring during transition to lactation, it is difficult to determine with certainty any gene or set of genes that are constitutively expressed (Janovick-Guretzky et al., 2007; Sumner and McNamara, 2007). However, previous work in our laboratory with RT-PCR and gene arrays suggested that the ribosomal S2 unit is relatively constantly expressed. Data discussed below from this trial may indicate this may not always be the case.

B2AR

The expression of B2AR generally decreased in the early postpartum period compared with 7 d prepartum, and then rose again to 56 DIM, with some variation among merit and diet groups (Table 7). Cows fed the LE diet typically had higher expression postpartum, but this was mostly attributable to the HM group (3-way interaction: $P < 0.03$). Overall, animals fed an NE diet generally decreased expression of this gene in early lactation regardless of GM. The HM animals had a trend to lower expression than LM animals when fed an NE diet ($P < 0.07$). The only consistent trend was that dietary restriction clearly resulted in an increase in relative expression of the B2AR (Table 7; diet effect: $P < 0.05$) postpartum, regardless of genetic merit. Yet,

Figure 4. Lipolysis measured in vitro in adipose tissue of dairy cattle in late pregnancy and early lactation. Stimulated lipolysis represents the average obtained with 10^{-8} , 10^{-7} , 10^{-6} , 10^{-5} , and 10^{-4} M isoproterenol. (a) Basal and stimulated lipolysis over all cows. Solid line and diamonds = 21 d prepartum; dotted line and squares = 7 d prepartum; dashed line and triangles = 7 DIM; large dashed line and X = 28 DIM; dash-dot line and star symbol = 56 DIM. Effect of DIM: $P < 0.03$. Stimulated lipolysis effect of day: $P < 0.01$; genetic merit by day interaction: $P < 0.10$. (b) Lipolysis at 7 and 28 DIM; comparison of high-merit (HM) and low-merit (LM) cows, HM cows > LM cows: $P < 0.10$. The SEM at each concentration was 208, 300, 328, 288, 310, and 285 nm glycerol/2 h per gram of tissue.

examination of all the data showed that the change in relative expression was due to a decrease in expression of the S2 gene, whereas the absolute expression of the B2AR remained more constant. This actually can be explained by the decrease in all anabolic reactions in adipose tissue postpartum. The finding of a decrease in expression of a ribosomal gene is a novel finding, and suggests that control of anabolism is the major driver in adipose tissue postpartum. Yet, control of transcription of genes regulating lipolysis relative to anabolic genes is also an effect of dietary energy, whereas actual rates of lipolysis have been shown to be related to rates of milk

production (McNamara and Hillers, 1989; Sumner and McNamara, 2007). This is consistent with posttranslational control of lipolysis being the major mechanism in response to increased milk production.

LIPE

The expression patterns of *LIPE* (which is the gene name for the mRNA that codes for the enzyme HSL) were closely consistent with those of the B2AR, with a reduction in expression postpartum and maintenance of higher expression in LE-fed cows (Table 7). Although differences existed due to diet ($P < 0.04$) and interactions of merit and diet (Table 7; $P < 0.001$), a significant amount of variation was observed among time points. Energy-restricted cows maintained higher expression of *LIPE* compared with NE-fed cows from 7 d prepartum to 28 DIM.

PLIN1

Perilipin is the mRNA for a protein associated with the lipolytic pathway, considered the cofactor of HSL (Sumner and McNamara, 2007; Koltjes and Spurlock, 2011). Phosphorylation of PLIN1 allows for access of active (phosphorylated) HSL to the triacylglycerol surface for hydrolysis. Response was observed of ΔCt of PLIN1 to S2 to DIM ($P < 0.04$) and to the interaction of genetic merit and diet ($P < 0.03$). Although a fair bit of variation existed in the data among DIM and merit and dietary groups, the pattern of response was similar to that for B2AR and *LIPE*, in that expression tended to decrease postpartum, but was maintained at generally higher amounts in LE-fed cows.

Taking the expression of these 3 genes overall indicates a coordinated change in relative expression of the 3 key proteins that regulate lipolysis in cows faced with a dietary energy restriction. The data do not support here a major effect of increased genetic merit on increasing expression of these proteins in early lactation, which we did find in a smaller earlier study (Sumner and McNamara, 2007) and have corroborated with a gene expression array from a subset of cows in this study (Khan et al., 2013).

CONCLUSIONS

Cows from sires of HM for PTAM ate more feed, gave more milk, and had patterns of metabolism and gene expression in their adipose tissue consistent with both of these traits. Cows from sires of LM ate less, and even responded more negatively to an energy restriction than cows from higher genetic merit sires. Measured changes in lipogenesis and lipolysis were consistent with the dif-

Table 7. Expression of β -adrenergic receptor 2 (B2AR), hormone-sensitive lipase (LIPE), and perilipin 1 (PLIN1) in adipose tissue of cows varying in genetic merit fed a normal or an energy-restricted diet

Treatment ¹	B2AR ²					LIPE ³					PLIN1 ⁴				
	Days around parturition					Days around parturition					Days around parturition				
	−7	7	28	56	SEM	−7	7	28	56	SEM	−7	7	28	56	SEM
High merit, normal diet															
Δ Ct	1.5	3.1	4.8	7	0.6	1	3.4	2.7	5	0.7	0.6	1.1	5	2.43	0.7
Δ Ct relative to d −7		1.6	3.3	5.5			2.4	1.7	4			0.5	4.4	1.8	
Expression relative to d −7, %		33	10	2			19	31	6			71	5	29	
High merit, restricted diet															
Δ Ct	3.5	3.9	3.6	3.5	0.4	2.5	2.5	2.1	7.5	0.9	2.7	2.4	2.6	4.4	0.6
Δ Ct relative to d −7		0.4	0.1	0			0	−0.4	5			−0.3	−0.1	1.7	
Expression relative to d −7, %		76	93	100			100	132	3			123	107	31	
Low merit, normal diet															
Δ Ct	3.1	5.2	3.6	3	0.3	2.6	6.5	5.6	2.6	0.7	−0.4	3.3	6	2.5	0.9
Δ Ct relative to d −7		2.1	0.5	−0.1			3.9	3	0			3.7	6.4	2.9	
Expression relative to d −7, %		23	71	107			7	13	100			8	1	13	
Low merit, restricted diet															
Δ Ct	4.7	3.4	4.5	5.8	0.6	0.7	0.8	3.3	3.8	0.6	0.3	0.7	2.1	2.1	0.7
Δ Ct relative to d −7		−1.3	−0.2	1.1			0.1	2.6	3.1			0.4	1.8	1.8	
Expression relative to d −7, %		246	115	47			93	16	12			76	29	29	
SEM of Δ Ct	0.5	0.5	0.7	0.7		0.7	0.8	0.6	1.4		0.7	0.7	0.9	1.1	

¹A decrease in Δ Ct (where Ct = cycle threshold) means an increase in mRNA, whereas an increase indicates a decrease in mRNA. The Δ Ct for β_2 to ribosomal S2 is the difference in Ct between gene of interest Ct and S2 Ct (i.e., gene of interest Ct − S2 Ct).

²For B2AR, effect of diet: $P < 0.05$ and DIM by merit by diet: $P < 0.03$; trend for main effect of genetic merit: $P = 0.07$ and effect of DIM by diet: $P = 0.09$.

³For LIPE, effect of diet: $P < 0.04$ and genetic merit and diet: $P < 0.001$; trend for main effect of DIM: $P = 0.09$.

⁴For PLIN1, effect of DIM: $P < 0.04$ and merit by diet: $P < 0.03$.

ferences in energy intake and milk production, in a dynamic fashion as lactation was initiated and progressed. Lipogenesis was more closely related to intake, whereas lipolysis was more closely related to milk production as well as dietary intake. The changes in genes coding for proteins controlling stimulated lipolysis did not appear to be the major factor in lipolytic control; they did not consistently change in response to lactation; however, expression did tend to be higher when cows were challenged with a mild energy restriction. The present data help to explain the mechanisms of adipose tissue metabolic control in the transition period and can be used to improve our systems biology models to more effectively research the mechanisms of metabolic control in dairy cattle.

REFERENCES

- AOAC International. 2000. Official Methods of Analysis. Vol. 1 and 2. AOAC International, Gaithersburg, MD.
- Elkins, D. A., and D. M. Spurlock. 2009. Phosphorylation of perilipin is associated with indicators of lipolysis in Holstein cows. *Horm. Metab. Res.* 41:736–740.
- Janovick-Guretzky, N. A., H. M. Dann, D. B. Carlson, M. R. Murphy, J. J. Loor, and J. K. Drackley. 2007. Housekeeping gene expression in bovine liver is affected by physiological state, feed intake, and dietary treatment. *J. Dairy Sci.* 90:2246–2252.
- Khan, M. J., A. Hosseini, S. Burrell, S. M. Rocco, J. P. McNamara, and J. J. Loor. 2013. Change in subcutaneous adipose tissue metabolism and gene network expression during the transition period in dairy cows, including differences due to sire genetic merit. *J. Dairy Sci.* 96:2171–2182. <http://dx.doi.org/10.3168/jds.2012-5794>.
- Koltes, D. A., and D. M. Spurlock. 2011. Coordination of lipid droplet-associated proteins during the transition period of Holstein dairy cows. *J. Dairy Sci.* 94:1839–1848.
- McNamara, J. P. 1989. Regulation of bovine adipose tissue metabolism during lactation. 5. Relationships of lipid synthesis and lipolysis with energy intake and utilization. *J. Dairy Sci.* 72:407–418.
- McNamara, J. P. 1994. Lipid metabolism in adipose tissue during lactation: A model of a metabolic control system. *J. Nutr.* 124(Suppl.):1383S–1391S.
- McNamara, J. P. 2012. Integrating nutritional, genetic and reproductive management in early lactation dairy cattle. *J. Anim. Sci.* 90:1846–1854.
- McNamara, J. P., and J. K. Hillers. 1986a. Regulation of bovine adipose tissue metabolism during lactation. 1. Lipid synthesis in response to increased milk production and decreased energy intake. *J. Dairy Sci.* 69:3032–3041.
- McNamara, J. P., and J. K. Hillers. 1986b. Regulation of bovine adipose tissue metabolism during lactation. 2. Lipolysis response to milk production and energy intake. *J. Dairy Sci.* 69:3042–3050.
- McNamara, J. P., and J. K. Hillers. 1989. Regulation of bovine adipose tissue metabolism during lactation 5. Relationships of lipid synthesis and lipolysis with energy intake and utilization. *J. Dairy Sci.* 72:407–418.
- McNamara, J. P., and F. Valdez. 2005. Effects of dietary chromium propionate and calcium propionate on adipose tissue metabolism and milk production of dairy cattle in the transition period. *J. Dairy Sci.* 88:2498–2507.
- NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th revised ed. Natl. Acad. Sci., Washington, DC.
- Parmley, K. L. S., and J. P. McNamara. 1996. Lipid metabolism in adipose tissue of pregnant and lactating pigs fed varying amounts of energy. *J. Nutr.* 126:1644–1656.
- SAS Institute. 2005. SAS 9.2. SAS User's Guide. SAS Institute Inc., Cary, NC.
- Shirley, J. E., R. S. Emory, E. M. Convey, and W. D. Oxender. 1973. Enzymic changes in bovine adipose and mammary tissue, serum and mammary tissue hormonal changes with initiation of lactation. *J. Dairy Sci.* 56:569–574.
- Smith, T. R., and J. P. McNamara. 1990. Regulation of bovine adipose tissue metabolism during lactation. 6. Cellularity and hormone-sensitive lipase activity as affected by genetic merit and energy intake. *J. Dairy Sci.* 73:772–783.
- Sumner, J. M., and J. P. McNamara. 2007. Expression of the lipolytic genes in the adipose tissue of pregnant and lactating Holstein dairy cattle. *J. Dairy Sci.* 90:5237–5246.
- Sumner-Thomson, J. M., J. L. Vierck, and J. P. McNamara. 2011. Differential expression of genes in adipose tissue of first-lactation dairy cattle. *J. Dairy Sci.* 94:361–369.
- Vernon, R. G. 2003. Adipose tissue: An expanding role in the regulation of energy metabolism. Pages 451–464 in *Progress in Research on Energy and Protein Metabolism*. W. B. Souffrant and C. C. Metges, ed. Wageningen Academic Publishers, Wageningen, the Netherlands.
- Waltner, S. S., J. P. McNamara, and J. K. Hillers. 1994. Validation of indirect measures of body fat in lactating cows. *J. Dairy Sci.* 77:2570–2579.
- Yang, Y. T., and R. L. Baldwin. 1973. Lipolysis in isolated cow adipose cells. *J. Dairy Sci.* 56:366–374.