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Gene Expression Profiling of Liver and Mammary Tissues of Lactating Dairy Cows

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ABSTRACT : Gene expression profiling is a useful tool for identifying critical genes and pathways in metabolism. The objective of this study was to determine the major differences in the expression of genes associated with metabolism and metabolic regulation in liver and mammary tissues of lactating cows. We used the Michigan State University bovine metabolism (BMET) microarray; previously, we have designed a bovine metabolism-focused microarray containing known genes of metabolic interest using publicly available genomic internet database resources. This is a high-density array of 70mer oligonucleotides representing 2,349 bovine genes. The expression of 922 genes was different at $p<0.05$, and 398 genes (17%) were differentially expressed by two-fold or more with 222 higher in liver and 176 higher in mammary tissue. Gene ontology categories with a high percentage of genes more highly expressed in liver than mammary tissues included carbohydrate metabolism (glycolysis, glucoenogenesis, propanoate metabolism, butanoate metabolism, electron carrier and donor activity), lipid metabolism (fatty acid oxidation, chylomicron/lipid transport, bile acid metabolism, cholesterol metabolism, steroid metabolism, ketone body formation), and amino acid/nitrogen metabolism (amino acid biosynthetic process, amino acid catabolic process, urea cycle, and glutathione metabolic process). Categories with more genes highly expressed in mammary than liver tissue included amino acid and sugar transporters and MAPK, Wnt, and JAK-STAT signaling pathways. Real-time PCR analysis showed consistent results with those of microarray analysis for all 12 genes tested. In conclusion, microarray analyses clearly identified differential gene expression profiles between hepatic and mammary tissues that are consistent with the differences in metabolism of these two tissues. This study enables understanding of the molecular basis of metabolic adaptation of the liver and mammary gland during lactation in bovine species. (**Key Words :** Bovine, Metabolism, Microarray, Gene Expression Profiling, Liver, Mammary Gland)

INTRODUCTION

Liver and mammary gland are two of the most important tissues for metabolism and partitioning of nutrients in lactating dairy cows. The liver transforms dietary nutrients into the fuels and precursors required by other tissues, and exports them via the blood. The demand of extrahepatic tissues for nutrients varies with physiological and nutritional state of the animal. To meet these changing circumstances, the liver has remarkable metabolic flexibility. Little is understood about the metabolic adaptation in the liver during lactation in cattle. The lactating mammary gland of a high producing dairy cow takes as much as 80% of the metabolites exported from gut and liver tissues and synthesizes milk constituents. The primary substrates needed by the lactating mammary gland are glucose, acetate, long-chain fatty acids, and amino acids for the synthesis of lactose, milk fat, and milk protein. The mammary gland is able to generate and maintain large Na^+, K^+, and Cl^- gradients between milk and blood. A considerable amount of phosphate and calcium transport is required for milk secretion and special transport mechanisms are involved. Relatively few studies have been reported on the transcriptional regulation of nutrient metabolism and nutrient transport systems in the liver and mammary gland of lactating cows.

Microarray technology provides a high-throughput functional genomics approach toward a greater understanding of the complex and reciprocal interactions within the genome at the molecular level (Stover, 2004). A

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recent microarray study demonstrates a strong inhibition of gene expression for cell proliferation and increased gene expression in metabolism at onset of lactation in the bovine mammary gland (Finucane et al., 2008). Recently, Rudolph and coworkers (2007) reported a transcriptome analysis between the liver and mammary gland in mice using the Affymetrix microarray chip (Rudolph et al., 2007); they focused on understanding the transcriptional regulation of lactose and lipid synthesis in mammary tissues. No study on global transcriptome analysis between the liver and mammary gland for nutrient metabolism (carbohydrate, lipid, and protein) and their transport systems has been done in bovine tissues.

To enhance our understanding of metabolic processes in cattle, a set of cDNA sequences encoding most of the bovine metabolome as well as interacting signal transduction pathway genes was generated. From these sequences, we previously developed the bovine metabolism (BMET)-focused microarray containing known genes for metabolism and its regulation using publicly available genomic internet database resources (Etchebarne et al., 2004). BMET microarray analyses may be an effective system for understanding differential transcriptional regulation of metabolic genes for various tissues and in various metabolic states. The purpose of this study is to understand differential transcriptional regulation of genes for metabolism and its regulation between the liver and mammary gland of dairy cows during lactation.

MATERIALS AND METHODS

Tissues samples and RNA isolation

Liver and mammary tissue samples were used from previous work (Binelli et al., 1995). We used tissues stored from control primiparous Holstein cows. Briefly, cows were slaughtered at 181 d of lactation. Cows were housed in the stalls, exposed to 24 h/d of light, and milked three times per day at 0545, 1430, and 2200 h in a parlor at the Michigan State University Dairy Cattle Teaching and Research Center, MI, USA. Cows were fed a TMR for *ad libitum* intake. The TMR was formulated to provide adequate nutrition for a cow (590 kg of BW) yielding 38.5 kg/d of milk containing 3.5% fat, assuming 22.7 kg of DMI/d. Feed was offered twice daily (0330 and 1630).

Cows were slaughtered (stunned with a captive bolt followed immediately by exsanguination). Liver and mammary tissue was collected within 20 min of slaughter and frozen in liquid nitrogen. Samples were then stored at -80°C until RNA extraction.

Total RNA was extracted from frozen liver and mammary tissues from 3 cows. Frozen tissues (200 mg) were homogenized with Trizol reagent (Invitrogen Life Technologies Corp., Carlsbad, CA). RNA was extracted by phenol/chloroform, precipitated by isopropanol, and dried. The RNA pellet was resuspended in nuclease-free water. The quantity of RNA isolated was determined using the NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE), and quality was checked using the using the RNA 6000 Nano LabChip kit and Agilent 2100 BioAnalyzer (Agilent Technologies, Palo Alto, CA).

BMET microarray hybridization

For cDNA synthesis, 15 μg of sample RNA was used as a template in reverse transcription reactions (*SuperScript III* Fluorescent Labeling Kit L101401; Invitrogen Life Technologies Corp., Carlsbad, CA) in which oligo(dT)15-18 plus random hexamer was used as primers. In the Superscript III system, cDNA is prepared with a randomly incorporated amino-modified dUTP. The first-strand cDNA was purified by using a SNAP column. The purified cDNAs for liver and mammary tissues within an animal were differentially labeled using *N*-hydroxysuccinimide-derivatized Cy3 and Cy5 dyes (Amersham Pharmacia, Ltd., Piscataway, NJ), respectively. The labeled cDNA was purified by using a SNAP column and the unincorporated Cye dye was removed. Differentially labeled cDNAs were combined and concentrated by using Microcon 30 spin concentrators (Millipore Corp., Bedford, MA). SlideHyb-1 hybridization buffer (Ambion Inc., Alameda, CA) was added to the concentrated Cy3-Cy5-labeled probe cDNAs for microarray hybridization. The labeled cDNAs were incubated at 70°C for 5 min just prior to 18-h array hybridization.

To examine gene expression, we used the BMET microarray, which is targeted toward studies on metabolic regulation of bovine tissues (Etchebarne et al., 2004). The BMET array is a high-density array of 70mer oligonucleotides spotted onto glass slides. The BMET array has 2,349 bovine genes. The BMET microarray slide was boiled, dried and installed on GeneTAC HybStation according to the manufacture's instruction, and the probe/hybrization solution was injected into hybridization station. The slide was hybridized for 18 h with three step-down procedures (6 h at 42°C, 6 h 35°C, and 6 h at 30°C). After hybridization, the slide was washed, dried, and scanned with Agilent Microarray Scanner G2565B (Agilent Technologies, Inc., Santa Clara, CA, United States). GenePix Pro 6 software (Axon Instruments, Inc., Union City, CA) was used to process microarray images, find spots, integrate robot spotting files with the microarray image, and finally to create reports of raw spot intensities. Total intensity values for each dye channel are converted to comma-separated value data files and exported into Excel spreadsheets and loaded into SAS for data normalization and analysis. After LOESS adjustment of spots within an

Table 1. Primers used in real-time PCR

Gene name	Accession No.	Primer	Sequence (5'-3')	Product size (bp)
Aldolase B, fructose-bisphosphate	BC102278.1	Forward	GGAGTTGCTCCGCTTGCA	69
		Reverse	GCGTTCAGAAAGGCCATCA	
Alcohol dehydrogenase class II	XM_598754.2	Forward	TGGGCCGTACTCTAACTGGAA	75
		Reverse	AGTCAGCAGCCAGTTTTGGAA	
Ornithine carbamoyltransferase	NM_177487.2	Forward	TGGCATCGAGCTGACAGACT	63
		Reverse	TGCCCATCCTCGTCATGA	
Phosphoenolpyruvate carboxykinase 1	NM_174737.2	Forward	TGGCATCGAGCTGACAGACT	71
		Reverse	TGCCCATCCTCGTCATGAT	
Glyceraldehyde-3-phosphate dehydrogenase	BTU85042	Forward	GCATCGTGGAGGGACTTATGA	66
		Reverse	GGGCCATCCACAGTCTTCTG	
ATP citrate lyase	BC108138.1	Forward	TCATTGAGATGTGCCTGATGGT	66
		Reverse	TGGTGTTATGAGCCCCAGAGA	
Malic enzyme 1, NADP(+)-dependent, cytosolic	XM_613987.2	Forward	AACCAACTGCCCTCATTGGA	78
		Reverse	TGAAGGCTGCCATGTCTTTG	
Splicing factor 1	XM_880876.1	Forward	AAACATTCTGAAGCAGGGTATCG	75
		Reverse	GCCAACTCTCGAAGTTGCATCT	
Acetyl-Coenzyme A synthetase 2	XM_582906.2	Forward	GCAGACATTGGCTGGATCACT	74
		Reverse	AAAACACTGGTGGCACCATTG	
Isocitrate dehydrogenase 1 (NADP+), soluble	NM_181012.2	Forward	TTTGGGCCTGTAAGAACTATGATG	72
		Reverse	TGCCGAGAGAGCCATAACCT	
Alpha-lactalbumin	NM_174378.2	Forward	CCCCGTGGCTACCTCGTT	67
		Reverse	GGGCCCAGGGCTCAGA	
Fatty acid binding protein 4, adipocyte	NM_174314.2	Forward	GCGTGGGCTTTGCTACCA	68
		Reverse	CCCCATTCAAACTGATGATCAA	
NADH dehydrogenase (ubiquinone) 1 beta	NM_175809.1	Forward	GCCGCAGCATTCATGATG	74
		Reverse	GACAAATCCCATAGGGACAAGTACA	

array and correction for average intensity of blocks within an array, changes in transcript abundance were tested between two tissues. Direct comparisons between two tissues were made using two arrays for each cow comparison with a reversal of dye assignments for the second array; a total of 6 arrays were performed with the BMET array. The model used for the analysis was:

$$Y = \text{tissue}+\text{cow}+\text{tissue}\times\text{cow}$$

The interaction term tissue x cow was used to test for treatment differences. The p-values were not adjusted for false discovery rate.

Real-time PCR

Real-time reverse transcriptase polymerase chain reaction (RT-PCR) was performed to validate the changes in gene expression detected by microarray analysis. This procedure was performed using the ABI PRISM 7000 Sequence Detection System (Perkin Elmer Corp., Foster City, CA). Total RNA was extracted from each tissue from each of the three cows, quantified and quality checked as described previously. RNA was converted into first-strand cDNA by using 2 μg of total RNA with oligo$(dT)_{18}$ primer. The first-strand cDNA was synthesized with Superscript II RNase H reverse transcriptase (Invitrogen Life Technologies).

SYBR Green PCR Master Mix (Perkin Elmer Corp.) and gene-specific primers were used to perform RT-PCR reactions. Primer Express Software (Perkin Elmer Corp.) was used to design all primers, which were then synthesized by a commercial facility (Invitrogen Life Technologies). Primer sequences are shown in Table 1. The amount of primer used was determined by performing an optimization matrix for each primer using three concentrations of primers: 100:100 nM, 600:600 nM, 1,800:1,800 nM. Dissociation curves were similar for all concentrations and the 600:600 nM matrix was chosen, thus 3 μl of primer was used for all experiments. Each gene of interest and the control gene were measured in duplicate. Within each well of a 96-well reaction plate (MicroAmp Optical, Applied Biosystems), 30 ng of sample cDNA (3 μl), 6.5 μl DEPC water, 3 μl of each primer, and 12.5 μl Sybr Green (Applied Biosystems) were added.

To determine an appropriate reference control gene by which relative mRNA abundances for genes of interest could be measured, a number of potential housekeeping genes were screened based on both expression ratio (liver/mammary tissues) and expression intensity of microarray results. These candidate control genes included NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 1 (ndufb1), NADH dehydrogenase (ubiquinone) 1 alpha

Table 2. List of gene ontologies of differential gene expression profile between liver and mammary tissues of lactating dairy cows

Gene ontology	# genes	>2-fold higher in liver	>2-fold higher in mammary tissue
Carbohydrate metabolism	154	74 (49%)	10 (4%)
Glycolysis	27	5 (19%)	1 (4%)
Gluconeogenesis	15	5 (33%)	1 (7%)
Pentose-phophate shunt	5	0 (0%)	0 (0%)
Lactose biosynthetic process	3	0 (0%)	2 (67%)
Propanoate metabolism	7	7 (100%)	0 (0%)
Butanoate metabolism	7	7 (100%)	0 (0%)
Alcohol dehydrogenase and ethanol metabolic process	5	5 (100%)	0 (0%)
Electron carrier and electron donor activity	85	43 (51%)	4 (5%)
Lipid metabolism	212	76 (36%)	28(13%)
Fatty acid biosynthetic process	22	3 (14%)	2 (9%)
Triacylglyceride Synthesis	24	3 (13%)	2 (8%)
Fatty acid oxidation	44	21 (48%)	3 (7%)
Fatty acid alpha-oxidation	1	1 (100%)	0 (0%)
Fatty acid beta-oxidation	32	10 (31%)	3 (9%)
Fatty acid omega-oxidation	11	10 (91%)	0 (0%)
Chylomicron/ lipid transport	13	4 (31%)	0 (0%)
Long chain fatty acid transport	1	0 (0%)	1 (100%)
Lipid transporter activity	3	2 (67%)	0 (0%)
Fatty acid binding	7	1 (14%)	3 (43%)
Bile acid metabolic and biosynthesis process	32	18 (56%)	0 (0%)
Cholesterol metabolic process	23	8 (35%)	0 (0%)
Cholesterol biosynthetic process	16	6 (38%)	0 (0%)
Cholesterol absorption	7	2 (29%)	0 (0%)
Steroid and glucocorticoid metabolism	7	4 (57%)	0 (0%)
Synthesis and degradation of ketone bodies	5	1 (20%)	0 (0%)
Arachidonic acid metabolism	18	0 (0%)	7 (39%)
Phospholipase D activity	2	1 (100%)	0 (0%)
Amino acid/nitrogen metabolism	110	62 (56%)	7 (6%)
Amino acid biosynthetic process	10	6 (60%)	0 (0%)
Amino acid catabolic process	40	31 (78%)	1 (3%)
L-phenylalanine and tyrosine catabolic process	9	6 (67%)	0 (0%)
Lysine degradation	9	9 (100%)	0 (0%)
Valine, leucine and isoleucine degradation	10	10 (100%)	0 (0%)
Arginine catabolic process	7	2 (29%)	1 (14%)
Urea cycle	21	9 (43%)	1 (5%)
Glutathione metabolic process and glutathione transferase activity	21	10 (48%)	4 (19%)
Antioxidant activity and Oxidative stress	23	10 (43%)	1 (4%)
Cytochrome p450, Heme binding, and Response to xenobiotic stimulus	14	13 (93%)	0 (0%)
Blood coagulation	19	11 (58%)	2 (11%)
Transporter	48	9 (19%)	11 (23%)
Sugar porter activity	12	1 (8%)	0 (0%)
Fatty acid transporter	5	2 (40%)	0 (0%)
Amino acid transport	14	0 (0%)	7 (50%)
Symporter activity	12	1 (8%)	1 (8%)
Sodium:phosphate symporter actvity	3	1 (33%)	1 (33%)
Signal transduction	50	12 (24%)	22 (44%)
MAPK signaling	28	10 (36%)	12 (43%)
Wnt signaling	6	0 (0%)	6 (100%)
JAK-STAT signaling	14	2 (14%)	2 (14%)
mTOR/PDK/Akt signaling	2	0 (0%)	2 (100%)
All genes on array	2,349	222 (9%)	176 (7%)

The right columns show the number and percentage of genes that were expressed at >2-fold in liver or mammary tissue at $p<0.05$. Some genes are included in multiple pathways in the gene ontology analysis.

subcomplex (ndufa3) and splicing factor 1 (sf1). RT-PCR threshold cycle (C_T) values for all three genes were consistent for liver and mammary tissues among animals and we used ndufb1 as a control. We did not use GAPDH as a control, since microarray data showed differential expression between liver and mammary tissues. We also confirmed differential expression of GAPDH between liver and mammary tissues by real-time PCR.

The $2^{-\Delta\Delta CT}$ method of RT-PCR analysis was performed as previously described (Livak and Schmittgen, 2001). This method enabled relative gene expression changes across treatments based on quantitative differences in the PCR amplified target reaching a fixed C_T number at a set treatment versus other treatments. Gene-specific standard errors were estimated using independent analyses of variance (ANOVA). All analyses were performed using the SAS 9.1 for windows.

RESULTS AND DISCUSSION

BMET microarray hybridization

Gene expression profiling was compared between liver and mammary tissues of dairy cows by using BMET microarray. Statistical analysis revealed that the 398 genes (17%) out of 2,349 genes were differentially expressed by greater than 2× difference at $p<0.05$. Of these, 222 genes were greater in liver and 176 genes were higher in mammary tissues (Table 2). The distribution of p-values was highly skewed toward the lower p values, indicating a significant tissue effect (Figure 1).

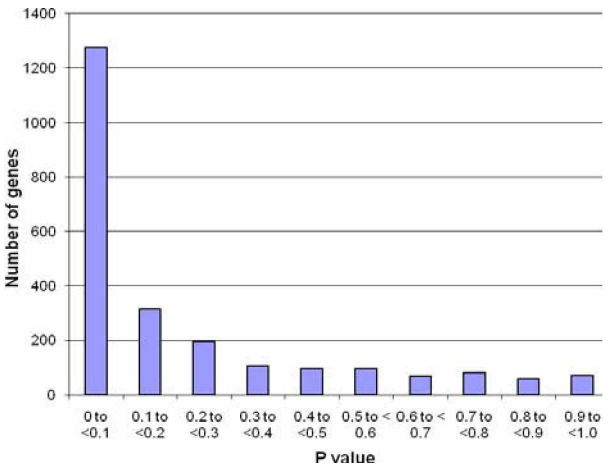

Figure 1. p value distribution plot for all 2,349 genes on the BMET microarray. Individual p value is a significance of difference in gene expression between liver and mammary tissue for each gene.

Validation of the microarray data by real-time PCR

Quantitative real-time PCR of selected genes was performed to confirm microarray results. We selected 12 genes, mostly from the carbohydrate/energy metabolism category: six genes had higher expression in liver, four had higher expression in mammary gland, and two had similar expression between the two tissues. Of these genes, six had over a 10-fold difference between tissues, four had a 2-3 fold difference, and two were not different (Table 3). Real-time PCR analysis showed consistent results with those of microarray analysis for all 12 genes tested (Table 3). However, the real-time PCR analysis was more sensitive than the microarray. The fold difference of expression levels determined by real-time PCR was between 1 and 300,000, while fold difference determined by microarray analysis was between 1 and 58.

Gene expression profiling of liver and mammary tissues of lactating dairy cows

Genes significantly different in mRNA abundance

Table 3. Validation of array-based gene expression profile by real-time PCR

Gene name	Relative expression[1]				Validation (yes/no)
	Microarray[2]	p value	Real-time PCR[3]	p value	
Aldolase B	58	0.01	5,506	<0.001	Y
Alcohol dehydrogenase class II	51	0.003	306,388	<0.001	Y
Ornithine carbamoyltransferase	50	0.006	4,488	0.003	Y
Phosphoenolpyruvate carboxykinase 1	17	0.009	5,493	0.005	Y
Glyceraldehyde-3-phosphate dehydrogenase	3.3	0.003	7	<0.001	Y
ATP citrate lyase	1.8	0.05	6.4	0.001	Y
Malic enzyme 1, NADP(+)-dependent, cytosolic	1.2	0.37	1.9	0.18	Y
Splicing factor 1	1.1	0.4	1.0	0.5	Y
Acetyl-coenzyme A synthetase 2	0.4	0.04	0.26	0.003	Y
Isocitrate dehydrogenase 1 (NADP+), soluble	0.3	0.03	0.22	0.009	Y
Alpha-lactalbumin	0.09	0.007	0.00002	<0.001	Y
Fatty acid binding protein 4, adipocyte	0.04	0.005	0.0003	0.009	Y

[1] Relative expression was calculated as ratio of expression levels in liver/mammary tissues.

[2] Microarray: 6 slides (dye swap) of 3 animals.

[3] Gene expression levels were measured with the real time PCR and normalized to NADH dehydrogenase (ubiquinone) 1 beta (ndufb1) using ddCt method of relative quantification.

between the two tissues were grouped within a number of gene ontologies and pathways using the GenMAPP MAPPFinder 2 program (Dahlquist et al., 2002; Doniger et al., 2003) These gene ontology categories are generalized across species and are not specific for bovine. The BMET analysis was able to differentiate gene expression profiles of major metabolic pathways of liver and mammary tissues (Table 2). Generally, expression profiles of BMET genes were consistent with function of the liver and mammary tissues. As expected, expression levels of genes involved in most of hepatic metabolic functions and their regulation were higher in the liver compared to mammary tissues. Gene ontology categories with a high percentage of genes more highly expressed in liver than mammary tissues included carbohydrate metabolism (glycolysis, glucoenogenesis, propanoate metabolism, butanoate metabolism, electron carrier and donor activity), lipid metabolism (fatty acid oxidation, chylomicron/lipid transport, bile acid metabolism, cholesterol metabolism, steroid metabolism, ketone body formation), amino acid/nitrogen metabolism (amino acid biosynthetic process, amino acid catabolic process, urea cycle, and glutathione metabolic process), cytochrome p450, heme binding, response to xenobiotic stimulus, and blood coagulation. Categories with more genes highly expressed in mammary than liver tissues included nutrient transport systems for milk precursors and milk constituents (amino acid, sugar, sodium and phosphate transporters), lactose synthesis, arachidonic acid metabolism, and genes associated with several signal transduction pathways (MAPK, Wnt, and JAK-STAT).

In addition, BMET microarray was able to identify differential expression profiles of several gene isoforms (Table 4). These include isoforms of the facilitated glucose transporters (GLUT), the glutamate transporters, cationic amino acid transporters, fatty acid transporters, fatty acid binding proteins, aldolases, acyl-Coenzyme A oxidases, long-chain acyl-CoA synthetases, acylglycerol-3-phosphate O-acyltransferases, phosphatidic acid phosphatases, and suppressor of cytokine signaling genes.

Carbohydrate metabolism : Although the Gene Ontology category "glycolysis" had more genes upregulated in liver than mammary tissue, several of these genes encode enzymes that catalyze reversible reactions. Thus, the higher expression of aldolase B, glyceraldehyde-3-P dehydrogenase, 6-phosphofructo-2-kinase, and triosephosphate isomerase are actually consistent with the hepatic focus on gluconeogenesis. Vertebrates have 3 aldolase isozymes, and aldolase B is considered the predominant form in liver. Our results confirm that aldolase B is the main transcript for liver and show aldolase C is the most abundant of the three isoforms in mammary tissue.

As expected, genes encoding the enzymes of gluconeogenesis and propanoate metabolism were more highly expressed in liver than mammary tissues. Phosphoenolpyruvate carboxykinase has a cytosolic (PCK1) and mitochondrial (PCK2) form, which are products of two separate genes for rat, bovine, and several other species (Hod et al., 1986); but only the cytosolic form is hormonally regulated (Weldon et al., 1990). Hartwell et al. (1999) indicated a close relationship between total PCK activity and cytosolic PCK mRNA. Consistent with this, we found that PCK1 expression was liver specific, but PCK2 expression showed only a minor difference between the tissues. Transcript abundance of all enzymes for propanoate metabolism (propionyl Coenzyme A carboxylase alpha, methylmalonyl Coenzyme A mutase, etc) were higher in liver than mammary tissues. In addition, we found greater transcript levels in liver for all enzymes of butanoate metabolism (such as butyryl Coenzyme A synthetase 1, enoyl Coenzyme A hydratase, hydroxyacyl-Coenzyme A dehydrogenase, and aldehyde dehydrogenase 1) as well as all five alcohol dehydrogenase (ADH) isoforms for metabolizing ethanol and retinol.

Lipid metabolism : Of the 212 genes on the BMET array that encode enzymes for lipid metabolism, 36% had>two-fold expression in liver and 13% had>two-fold expression in mammary tissue. Both tissues had very low abundance for ATP citrate lyase. The low transcript abundance of this enzyme in mammary tissue is consistent with its low activity level and minor contribution of glucose to fatty acid synthesis via acetyl-CoA in ruminant mammary gland (Hood et al., 1972). Transcript levels of acetyl-CoA carboxylase alpha (ACACA) and fatty acid synthase (FASN) were high in both tissues but even higher in mammary tissues than liver. Bionaz and Loor (2008) reported that ACACA mRNA was up-regulated during lactation more than was FASN in dairy cows. This is consistent with the role of ACACA in the synthesis of malonyl-CoA, the rate-limiting step in milk fatty acid synthesis. In addition, the expression of acetyl-Coenzyme A synthetase 2 (categorized as a "Fatty acid oxidation" gene) was three-fold higher in mammary than liver tissue, consistent with its function of activating acetate for use in ruminant lipid synthesis and fuel support (Smith and Prior, 1986). Isocitrate dehydrogenase generates NADPH to support fatty acid synthesis and was also more highly expressed in mammary than liver tissues. About half of the fatty acids found in milk triglycerides are derived from blood lipids (Moore and Christie, 1979). We found lipoprotein lipase (LPL) expression was >ten times higher in mammary than liver tissue. Recent report demonstrates that expression of LPL significantly increased at 10 day after parturition compared to about 5 day before parturition of Holstein dairy cows (Finucane et al., 2008). We observed

about 10-fold higher expression of acetyl-coenzyme A acetyl transferase 2 (ACAA2) gene in liver relative to mammary tissues. The ACAA2 gene has been down-regulated in the liver of ketotic dairy cows (Xu et al., 2008).

Table 4. Selected gene lists based on pathway analyses[1]

Gene name	Symbol	Ratio (L/MG)[2]	p value	Abundance of expression level[3]	
				Liver	Mammary
Carbohydrate metabolism					
Glycolysis					
aldolase A, fructose-bisphosphate	ALDOA	0.43	0.043	0.13%	0.30%
aldolase B, fructose-bisphosphate	ALDOB	58.3	0.012	23.7%	0.41%
aldolase C, fructose-bisphosphate	ALDOC	0.46	0.108	0.30%	0.65%
glyceraldehyde-3-phosphate dehydrogenase	GAPDH	3.26	0.003	3.10%	0.95%
glucose phosphate isomerase	GPI	1.93	0.006	0.29%	0.15%
hexokinase 3 (white cell)	HK3	2.96	0.008	2.85%	0.96%
6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 1	PFKFB1	8.10	0.020	0.43%	0.05%
pyruvate kinase, liver and RBC	PKLR	2.22	0.061	0.26%	0.12%
pyruvate kinase, muscle	PKM2	0.61	0.055	1.20%	1.95%
triosephosphate isomerase 1	TPI1	2.89	0.004	0.62%	0.21%
Gluconeogenesis					
fructose-1,6-bisphosphatase 1	FBP1	3.41	0.0001	1.05%	0.31%
fructose-1,6-bisphosphatase 2	FBP2	0.75	0.020	0.04%	0.05%
phosphoenolpyruvate carboxykinase 1 (soluble)	PCK1	17.3	0.009	3.53%	0.20%
phosphoenolpyruvate carboxykinase 2 (mitochondrial)	PCK2	1.71	0.071	0.32%	0.19%
6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 1	PFKFB1	8.10	0.020	0.43%	0.05%
triosephosphate isomerase 1	TPI1	2.89	0.004	0.62%	0.21%
glucose-6-phosphatase, catalytic (glycogen) storage disease type I, von Gierke disease)	G6PC	13.1	0.017	2.34%	0.18%
Pentose phosphate pathway					
glucose phosphate isomerase	GPI	1.93	0.006	0.29%	0.15%
glucose-6-phosphate dehydrogenase	G6PD	0.76	0.162	0.20%	0.26%
6-phosphogluconolactonase	PGLS	0.70	0.042	0.38%	0.55%
phosphogluconate dehydrogenase	PGD	0.66	0.055	0.12%	0.18%
transaldolase 1	TALDO1	0.53	0.131	0.22%	0.42%
Lactose biosynthetic process					
UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 1	B4GALT1	0.30	0.086	1.37%	4.49%
lactalbumin, alpha-	LALBA	0.09	0.007	7.90%	85.7%
UDP-glucose pyrophosphorylase 2	UGP2	0.68	0.04	0.50%	0.74%
Lipid metabolism					
Fatty acid biosynthetic process					
ATP citrate lyase	ACLY	1.75	0.05	0.10%	0.06%
acetyl-Coenzyme A carboxylase alpha	ACACA	0.28	0.031	0.53%	1.91%
fatty acid synthase	FASN	0.58	0.020	2.01%	3.49%
acetyl-Coenzyme A acyltransferase 1 (peroxisomal 3-oxoacyl-Coenzyme A thiolase)	ACAA1	4.54	0.007	0.42%	0.09%
acetyl-Coenzyme A acyltransferase 2 (mitochondrial 3-oxoacyl-Coenzyme A thiolase)	ACAA2	9.53	0.000	1.04%	0.11%
enoyl Coenzyme A hydratase, short chain, 1, mitochondrial	ECHS1	2.84	0.048	4.49%	1.58%
isocitrate dehydrogenase 1 (NADP+), soluble	IDH1	0.30	0.023	1.95%	6.43%
Triacylglyceride synthesis					
acyl-CoA synthetase long-chain family member 1	ACSL1	0.76	0.178	0.11%	0.13%
acyl-CoA synthetase long-chain family member 3	ACSL3	0.90	0.467	0.11%	0.12%
acyl-CoA synthetase long-chain family member 4	ACSL4	1.57	0.072	0.21%	0.13%
acyl-CoA synthetase long-chain family member 5	ACSL5	13.9	0.003	1.34%	0.10%
acyl-CoA synthetase long-chain family member 6	ACSL6	1.05	0.754	0.10%	0.10%

Table 4. Selected gene lists based on pathway analyses[1] (Continued)

Gene name	Symbol	Ratio (L/MG)[2]	p value	Abundance of expression level[3]	
				Liver	Mammary
lipoprotein lipase	LPL	0.09	0.031	2.15%	23.89%
lipase, hormone-sensitive	LIPE	0.43	0.033	0.08%	0.18%
lipase, hepatic	LIPC	6.04	0.023	0.87%	0.14%
glycerol-3-phosphate acyltransferase, mitochondrial	GPAM	0.04	0.005	0.29%	7.12%
1-acylglycerol-3-phosphate O-acyltransferase 1 (lysophosphatidic acid acyltransferase, alpha)	AGPAT1	0.62	0.091	0.84%	1.35%
1-acylglycerol-3-phosphate O-acyltransferase 2 (lysophosphatidic acid acyltransferase, beta)	AGPAT2	5.38	0.023	0.17%	0.03%
1-acylglycerol-3-phosphate O-acyltransferase 3	AGPAT3	1.26	0.059	0.27%	0.22%
1-acylglycerol-3-phosphate O-acyltransferase 4 (lysophosphatidic acid acyltransferase, delta)	AGPAT4	0.96	0.684	0.28%	0.29%
Fatty acid oxidation					
hydroxyacid oxidase (glycolate oxidase) 1	HAO1	6.17	0.018	0.94%	0.15%
acyl-Coenzyme A dehydrogenase, C-2 to C-3 short chain	ACADS	7.83	0.003	0.93%	0.12%
acyl-Coenzyme A dehydrogenase, long chain	ACADL	2.13	0.046	0.49%	0.23%
acyl-Coenzyme A dehydrogenase, very long chain	ACADVL	1.83	0.047	0.38%	0.21%
acyl-Coenzyme A oxidase 1, palmitoyl	ACOX1	6.53	0.003	0.66%	0.10%
acyl-Coenzyme A oxidase 2, branched chain	ACOX2	4.69	0.009	0.70%	0.15%
acyl-Coenzyme A oxidase 3, pristanoyl	ACOX3	1.96	0.088	0.08%	0.04%
enoyl Coenzyme A hydratase, short chain, 1, mitochondrial	ECHS1	2.84	0.048	4.49%	1.58%
hydroxyacyl-Coenzyme A dehydrogenase/ 3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), beta subunit	HADHB	10.8	0.011	0.77%	0.07%
triosephosphate isomerase 1	TPI1	2.89	0.004	0.62%	0.21%
acetyl-Coenzyme A synthetase 2 (ADP forming)	ACAS2	0.37	0.042	0.51%	1.37%
2,4-dienoyl CoA reductase 1, mitochondrial	DECR1	4.55	0.001	0.51%	0.11%
peroxisome proliferative activated receptor, gamma, coactivator 1, alpha	PPARGC1A	0.76	0.058	0.47%	0.62%
peroxisome proliferative activated receptor, alpha	PPARA	2.54	0.064	3.91%	1.54%
Long chain fatty acid transport					
adipose differentiation-related protein	ADRP	0.06	0.002	0.19%	3.17%
Fatty acid binding					
fatty acid binding protein 1, liver	FABP1	27.7	0.005	14.2%	0.51%
fatty acid binding protein 2, intestinal	FABP2	1.14	0.369	0.02%	0.02%
fatty acid binding protein 3, muscle and heart (mammary-derived growth inhibitor)	FABP3	0.01	0.003	0.23%	19.23%
fatty acid binding protein 4, adipocyte	FABP4	0.04	0.005	0.08%	2.26%
fatty acid binding protein 5 (psoriasis-associated)	FABP5	0.46	0.273	1.36%	2.99%
fatty acid binding protein 6, ileal (gastrotropin)	FABP6	1.03	0.580	0.11%	0.11%
fatty acid binding protein 7, brain	FABP7	1.25	0.155	0.02%	0.02%
Arachidonic acid metabolism					
arachidonate 12-lipoxygenase	ALOX12	0.37	0.266	1.05%	2.83%
arachidonate 15-lipoxygenase	ALOX15	0.11	0.011	0.08%	0.72%
prostaglandin D2 synthase 21kDa (brain)	PTGDS	0.35	0.044	0.54%	1.57%
prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	PTGS2	0.46	0.025	0.63%	1.37%
Transporter					
Sugar porter					
solute carrier family 2 (facilitated glucose transporter), member 1	SLC2A1 (GLUT1)	0.69	0.092	1.57%	2.26%
solute carrier family 2 (facilitated glucose transporter), member 2	SLC2A2 (GLUT2)	5.61	0.012	0.67%	0.12%

Table 4. Selected gene lists based on pathway analyses[1] (Continued)

Gene name	Symbol	Ratio (L/MG)[2]	p value	Abundance of expression level[3]	
				Liver	Mammary
solute carrier family 2 (facilitated glucose transporter), member 4	SLC2A4 (GLUT4)	0.67	0.018	0.27%	0.40%
solute carrier family 2, (facilitated glucose transporter) member 8	SLC2A8 (GLUT8)	0.90	0.228	0.39%	0.44%
Fatty acid transporter					
solute carrier family 27 (fatty acid transporter), member 2	SLC27A2 (FATP2)	7.41	0.014	0.64%	0.09%
solute carrier family 27 (fatty acid transporter), member 3	SLC27A3 (FATP3)	0.68	0.001	0.24%	0.36%
solute carrier family 27 (fatty acid transporter), member 4	SLC27A4 (FATP4)	0.99	0.61	1.31%	1.35%
solute carrier family 27 (fatty acid transporter), member 5	SLC27A5 (FATP5)	2.49	0.019	0.71%	0.29%
solute carrier family 27 (fatty acid transporter), member 6	SLC27A6 (FATP6)	0.66	0.214	0.15%	0.22%
Amino acid transport					
solute carrier family 38, member 2	SLC38A2	0.24	0.002	0.19%	0.76%
solute carrier family 38, member 3	SLC38A3	0.21	0.023	0.86%	4.11%
solute carrier family 7 (cationic amino acid transporter, y+ system), member 3	SLC7A3	1.05	0.549	0.16%	0.16%
solute carrier family 7 (cationic amino acid transporter, y+ system), member 5	SLC7A5	0.45	0.009	0.31%	0.68%
solute carrier family 7 (cationic amino acid transporter, y+ system), member 7	SLC7A7	0.18	0.035	1.10%	6.22%
solute carrier family 7 (cationic amino acid transporter, y+ system), member 8	SLC7A8	1.67	0.065	1.28%	0.77%
solute carrier family 7 (cationic amino acid transporter, y+ system), member 9	SLC7A9	1.19	0.478	0.88%	0.74%
solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1	SLC1A1	1.23	0.088	0.12%	0.10%
solute carrier family 1 (glial high affinity glutamate transporter), member 2	SLC1A2	0.32	0.023	0.14%	0.43%
solute carrier family 1 (glial high affinity glutamate transporter), member 3	SLC1A3	1.18	0.053	0.12%	0.11%
solute carrier family 1 (glutamate/neutral amino acid transporter), member 4	SLC1A4	0.41	0.038	0.13%	0.33%
solute carrier family 1 (neutral amino acid transporter), member 5	SLC1A5	0.41	0.040	0.53%	1.30%
Symporter					
solute carrier family 23 (nucleobase transporters), member 1	SLC23A1	9.27	0.018	0.32%	0.04%
solute carrier family 23 (nucleobase transporters), member 2	SLC23A2	0.89	0.400	0.04%	0.04%
solute carrier family 34 (sodium phosphate), member 1	SLC34A1	0.99	0.962	2.09%	2.10%
solute carrier family 34 (sodium phosphate), member 2	SLC34A2	0.01	0.003	0.19%	13.1%
solute carrier family 6 (neurotransmitter transporter, GABA), member 1	SLC6A1	0.61	0.046	0.80%	1.31%
solute carrier family 6 (neurotransmitter transporter, noradrenalin), member 2	SLC6A2	0.78	0.385	0.42%	0.54%
solute carrier family 6 (neurotransmitter transporter, dopamine), member 3	SLC6A3	0.91	0.539	0.33%	0.36%
solute carrier family 6 (neurotransmitter transporter, serotonin), member 4	SLC6A4	1.96	0.047	0.24%	0.12%
solute carrier family 6 (neurotransmitter transporter, taurine), member 6	SLC6A6	1.37	0.092	6.88%	5.04%

Table 4. Selected gene lists based on pathway analyses[1] (Continued)

Gene name	Symbol	Ratio (L/MG)[2]	p value	Abundance of expression level[3]	
				Liver	Mammary
Anion:cation symporter					
solute carrier family 17 (sodium phosphate), member 2	SLC17A2	13.15	0.012	1.15%	0.09%
solute carrier family 20 (phosphate transporter), member 1	SLC20A1	1.79	0.056	34.7%	19.4%
solute carrier family 20 (phosphate transporter), member 2	SLC20A2	0.06	0.010	1.14%	17.8%
solute carrier family 9 (sodium/hydrogen exchanger), isoform 3 regulator 1	SLC9A3R1	0.05	0.019	0.98%	20.3%
solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 6	SLC25A6	0.45	0.007	0.46%	1.01%
Signal transduction					
MAPK signaling					
mitogen-activated protein kinase 6	MAPK6	2.84	0.021	0.25%	0.09%
activating transcription factor 4 (tax-responsive enhancer element B67)	ATF4	0.43	0.020	0.76%	1.75%
tumor necrosis factor (TNF superfamily, member 2)	TNF	0.09	0.005	1.69%	19.7%
transforming growth factor, beta 1 (Camurati-Engelmann disease)	TGFB1	0.47	0.082	0.28%	0.60%
transforming growth factor, beta 2	TGFB2	0.43	0.018	0.06%	0.14%
transforming growth factor, beta 3	TGFB3	0.60	0.019	0.14%	0.23%
transforming growth factor, beta receptor III (betaglycan, 300kDa)	TGFBR3	0.60	0.014	0.02%	0.04%
transforming growth factor, beta receptor II (70/80 kDa)	TGFBR2	0.62	0.010	1.34%	2.15%
mitogen-activated protein kinase 12	MAPK12	0.10	0.000	0.42%	4.01%
mitogen-activated protein kinase-activated protein kinase 2	MAPKAPK2	2.16	0.007	0.58%	0.27%
Ras-related associated with diabetes	RRAD	0.09	0.020	0.06%	0.66%
Wnt signaling					
wingless-type MMTV integration site family, member 5A	WNT5A	0.35	0.047	0.45%	1.26%
frizzled homolog 1 (Drosophila)	FZD1	0.50	0.026	0.05%	0.10%
protein kinase C, beta 1	PRKCB1	0.09	0.020	2.70%	29.0%
protein kinase C, eta	PRKCH	0.31	0.015	0.08%	0.24%
v-jun sarcoma virus 17 oncogene homolog (avian)	JUN	0.04	0.001	0.41%	11.12%
plasminogen activator, urokinase	PLAU	0.31	0.007	0.82%	2.65%
JAK-STAT signaling					
suppressor of cytokine signaling 1	SOCS1	0.58	0.033	0.14%	0.24%
suppressor of cytokine signaling 2	SOCS2	0.70	0.040	0.16%	0.23%
suppressor of cytokine signaling 3	SOCS3	0.17	0.006	2.16%	12.9%
suppressor of cytokine signaling 5	SOCS5	0.58	0.056	0.02%	0.03%
mTOR/PDK/Akt signaling					
tuberous sclerosis 1	TSC1	0.14	0.040	0.06%	0.46%
glycogen synthase kinase 3 alpha	GSK3A	2.11	0.001	1.80%	0.85%

[1] Genes that showed over 2 fold difference at $p<0.05$ were presented.
[2] Ratio indicates expression levels in liver (L)/mammary gland (MG).
[3] Abundance of expression level is an estimate of the percent of maximum intensity for a spot, representing abundance of gene in liver or mammary tissue. It gives a value of ratio (L/MG) in the table if we make a ratio of an estimate of the percent of maximum intensity of liver and mammary tissue.

Fatty acids must be activated by acyl-CoA synthethetase (ACSL) prior to their use in triacylglycerol synthesis The BMET microarray contains several ACSL isoforms. The ACSL5 isoform was the most abundant in liver. Among the other four isoforms, only ACSL1 showed even a trend for greater expression in mammary tissue. Recent studies report that ACSL1 is the predominant acyl-CoA synthetase of lactating bovine mammary tissues (Rudolph et al., 2007; Bionaz and Loor, 2008b). The fact that its expression was not greater was surprising.

Glycerol-3-P acyltransferase and 1-acylglycerol-3-P acyltransferase are both required for milk triglyceride synthesis (Moore and Christie, 1979). Two mammalian forms of glycerol-3-P acyltransferase have been identified on the basis of localization to either the endoplasmic reticulum or mitochondria. We observed a 25-fold higher expression of the mitochondrial form in mammary tissue relative to liver. Previously, higher enzymatic activities of

this enzyme were observed in lactating mammary tissue of sheep compared to liver tissue (Vernon et al., 1987); our study suggests that this is transcriptionally regulated. Yamashita et al. (2007) showed the existence of nine mammalian isoforms of 1-acylglycerol-3-phosphate O-acyltransferase (AGPAT), which catalyzes the transfer of fatty acid from fatty acyl-CoA to lysophosphatidic acid, forming phosphatidic acid. The BMET array contains four genes of these isoforms, and AGPAT1 was the most abundant transcript in both tissues. We found a trend for more AGPAT1 transcripts in mammary tissues and a five-fold expression of AGPAT2 in liver. The BMET array does not contain AGPAT6; recently Bionaz and Loor (2008) showed a 10-fold increase in GPAM and 15-fold increase in AGPAT6 mRNAs at 60 d postpartum compared to the late prepartum/non-lactating period. They suggest that APTAT6, and to a lesser extent AGPAT1, are the most important AGPAT isoforms in bovine mammary gland (Bionaz and Loor, 2008a, 2008b).

As expected, hepatic lipase was more highly expressed in liver. We also found that expression of hormone-sensitive lipase was more highly expressed in mammary tissue, which is consistent with reports that hormone sensitive lipase likely plays a role in mammary epithelial cells and is up-regulated during lactation relative to gestation in rodents (Martin-Hidalgo, 2005). Half of the genes categorized as part of fatty acid oxidation were more highly expressed in liver than mammary tissue. Transcript levels of peroxisome proliferative activated receptor alpha, which is a major regulator of lipolysis, were also higher in the liver. These results are consistent with the demonstration of decreased levels of enzymes for β-oxidation in the lactating mammary gland (Rudolph et al., 2007). Consistent with reports that adipose differentiation-related protein localizes to neutral lipid storage droplets and is a component of the milk lipid globule membrane, its transcript was highly abundant in mammary tissue, and it was ten-fold greater than in liver (Heid et al., 1996).

Nine fatty acid-binding proteins (FABPs 1-9) have been identified (Furuhashi and Hotamisligil, 2008), and seven of these are found on the BMET array. The different members of the FABP family exhibit unique patterns of tissue expression and are expressed most abundantly in tissues involved in active lipid metabolism. Expression of FABP1 was much greater in liver, while FABP3 and FABP4 transcripts were much more abundant in mammary tissues. FABP1 is abundant in liver cytoplasm, but is also expressed in splanchnic tissues and lung (Chmurzynska, 2006). FABP3 has been isolated from a wide range of tissues, including muscle, brain, mammary gland, ovary and brown adipose tissue (Chmurzynska, 2006). FABP4 was first detected in mature adipocytes and adipose tissue (Hunt et al., 1986).

Of the 18 genes categorized as part of arachidonic acid metabolism on the BMET, seven were expressed at a >two-fold level in mammary tissue than in liver. These include enzymes for prostaglandin synthesis, such as prostaglandin D2 synthase and prostaglandin-endoperoxide synthase 2 (cyclooxigenase 2, COX2) and enzymes for leukotriene synthesis, such as arachidonate 12-lipoxygenase and arachidonate 15-lipoxygenase. Cyclooxygenase is the key enzyme in prostaglandin biosynthesis and acts both as a dioxygenase and as a peroxidase. Two COX isozymes exist: one constitutive (COX1) and one inducible (COX2). In our study, expression of COX2, but not COX1, was higher in mammary tissue. This is consistent with the postulated roles of COX2 in immune function, inflammation, and carcinogenesis (Pfaffl et al., 2003; Subbaramaiah et al., 2008).

Amino acid/nitrogen metabolism : Of the 110 genes on the BMET array that are included in the amino acid/nitrogen metabolism gene ontology category, 56% were expressed at >two-fold in liver compared to mammary tissue. Transcripts encoding enzymes involved in both amino acid biosynthetic processes (synthesis of arginine, methionine, glutamine, histidine, and sulfur amino acids) and catabolic processes (catabolism of L-phenylalanine, tyrosine, lysine, valine, leucine, isoleucine, and arginine) were generally more abundant in liver than mammary tissues. In addition, about half of the genes involved in the urea cycle were more highly expressed (>2X) in liver relative to mammary tissue.

Glutathione S-transferase functions in the detoxification of electrophilic compounds, including carcinogens and products of oxidative stress. At present, eight distinct classes of the soluble cytoplasmic mammalian glutathione S-transferases have been identified: alpha, kappa, mu, omega, pi, sigma, theta and zeta. The alpha class genes are the most abundantly expressed glutathione S-transferases in liver. Consistent with this, we found an 18-fold higher expression of glutathione S-transferase A1 gene in the liver; the kappa and theta class genes also were over three-more highly expressed in liver compared to mammary tissue.

Transporters : Of the 48 genes on the BMET array that encode transport proteins, 40% were differentially expressed at our threshold value of 2X and $p<0.05$. Of the glucose transporters, GLUT1 was the most abundant transcript in both tissues, and both GLUT1 and GLUT4 were more highly expressed in mammary tissue than liver but not at the two-fold threshold. In the lactating bovine mammary gland, GLUT1 is the predominant glucose transporter, but GLUT3, 4, 5, 8, and 12 are also expressed (Zhao and Keating, 2007). The major sites of GLUT2 expression in human are liver, kidney, and small intestine (Fukumoto et al., 1988). Consistent with this, we found a six-fold expression of GLUT2 in liver compared to

lactating mammary gland in dairy cows. Fatty acid transport proteins (FATP) are transmembrane proteins that enhance the uptake of long-chain and very long chain fatty acids into cells. In humans, FATPs comprise a family of six highly homologous proteins, named FATP 1-6 (Stahl, 2004). FATP2 is found mostly in liver and kidney cortex, FATP5 is found only in liver, and FATP3 shows a broader expression pattern with notably high mRNA and protein levels in the lung (Hirsch et al., 1998). Our study provides, for the first time, the tissue specificity of FATP expression in cattle. The most abundant FATP transcript in both tissues was FATP4, but expression levels of FATP2 and FATP5 genes were higher in liver than mammary tissues, consistent with the data from human studies. Expression FATP3 gene was slightly higher in mammary than liver tissue, and there were no differences in expression of FATP4 and FATP6 between the two tissues.

Of the 14 amino acid tranporters on BMET, 7 had >two-fold expression in mammary tissue compared to liver. There are seven amino acid transporters in solute carrier family 1, five high-affinity glutamate transporters and two neutral amino acid transporters (Kanai and Hediger, 2004). The BMET array had five of these, and three were more highly expressed in mammary tissue. The solute carrier 7 family is divided into two subgroups, the cationic amino acid transporters and the glycoprotein-associated amino acid transporters, also called catalytic chains of the hetero(di)meric amino acid transporters (Verrey et al., 2004). The BMET array contained four of these, and two were more highly expressed in mammary tissue. Expression levels of two amino acid transporters of family 38 were also greater in mammary tissues than liver. Thus, our results clearly demonstrate that several amino acid transporter genes are actively transcribed in lactating mammary gland, but little has been done to understand their role in milk synthesis.

We observed significant differences in expression of sodium potassium transporters. Of note, solute carrier family 34 member 2 was expressed 100-fold greater in mammary tissues than in liver. This transporter, also known as NaPi-IIb, was expressed apically in lactating mouse mammary gland but not in virgin mammary gland and is a potential marker of secretory function (Miyoshi et al., 2001) and mammary gland differentiation (Evarts et al., 2004). Recently, NaPi-IIb was found in goat mammary gland (Muscher et al., 2008). We suggest that NaPi-IIb may also function as a differentiation marker of mammary gland in lactating dairy cows. Solute carrier family 20 member 2 was also highly expressed in mammary tissues (17 fold higher than in liver). Shillingford et al. (1996) have suggested that this phosphate transporter provides basolateral uptake of Pi from the blood for subsequent secretion into the lumen in Pi secreting glands, such as the lactating mammary gland. Solute carrier family 9 (sodium/hydrogen exchanger), isoform 3 regulator 1 also was much more highly expressed in mammary tissues. The role and expression patterns of most of sodium potassium transport systems have not been reported. Our analyses provide data for the first time that genes for specific sodium and potassium transporter systems are actively transcribed for milk synthesis and secretion in bovine lactating mammary gland.

Signal transduction : Of the 50 signal transduction genes examined, 34 were differentially expressed >two-fold in these tissues. The BMET contains oligos for 28 mitogen-activated protein kinases (MAPK) and nearly all of these were differentially expressed. Transcript levels of MAPK6 were higher in liver, while those of MAPK12 were higher in mammary tissues. One of the genes whose expression is under tight control by JNK is the c-jun gene. MAPKs of the JNK family rapidly phosphorylate c-jun proteins already present in the cell in response to extracellular stimuli (Pulverer et al., 1991). BMET analysis showed 25-fold higher mRNA levels of jun in mammary tissues. MAPKs, which include the extracellular signal-regulated protein kinases (ERK1 and ERK2), c-jun N-terminal kinases (JNK1, JNK2, JNK3), and p38s and ERK5, are a family of serine/threonine kinases that play an essential role in signal transduction by modulating gene transcription in the nucleus in response to changes in the cellular environment.

We found greater transcript levels in mammary tissue, compared to liver, for genes in the TGF-β pathway including TGF-β isoforms 1, 2, and 3 and transforming growth factor-beta receptors II and III. Binding of transforming growth factor beta (TGF-β) to the TGF-β receptor complex activates both Smad and MAPK pathways.

There are three major Wnt signaling pathways: a canonical Wnt/β-catenin pathway, Wnt/Ca^{2+} pathway and Wnt/PCP (Planar Cell Polarity) pathway (Turashvili et al., 2006). The Wnt/Ca^{2+} pathway involves Frizzled and Dishevelled proteins and leads to the release of intracellular calcium and thereby affects the activity of calcium-modulated kinases, including calcium/calmodulin-dependent protein kinase II and protein kinase C. Our analysis shows higher mRNA levels of genes for the Wnt/Ca^{2+} pathway including wingless-type MMTV integration site family, member 5A (Wnt5A), frizzled homolog 1 (FZD1), and protein kinase C genes in mammary tissues compared to liver. This suggests that the Wnt/Ca^{2+} pathway has an active functional role in mammary gland. Wnt signals are strongly implicated in initial development of the mammary rudiments, and in the ductal branching and alveolar morphogenesis that occurs during pregnancy (Brennan and Brown, 2004).

The suppressor of cytokine signaling (SOCS) proteins function in a negative feedback loop regulating cytokine JAK-STAT signal transduction. We found differential

expression of several SOCS isoform genes between the two tissues: mRNA levels of SOCS 3 gene were five-fold higher in lactating mammary tissues compared to liver, while those of SOCS 1, 2, and 5 genes were 40 to 70% greater in mammary tissue. SOCS can modulate prolactin signaling in mammary tissues. During lactation, high levels of circulating prolactin may modulate up-regulation of SOCS 3 gene expression. Mammary transcription of mRNA for SOCS 2 and 3 proteins was low during the dry period but increased in lactation in dairy cows (Wall et al., 2005). SOCS 2 mRNA increased after parturition in the liver of dairy cows (Winkelman et al., 2008). In liver, growth hormone is shown to induce a transient expression of SOCS 3 (Adams et al., 1998).

CONCLUSION

Previously, there was no report on expression data for some genes because bovine cDNA sequences were not available. For the first time, we were able to detect expression profiles of several genes by using bioinformatics. This was possible because we designed bovine oligonucleotide sequences based on comparison of bovine EST sequences and human cDNA database for the genes that bovine cDNA sequences were not available. These include ACLY, GLUT2, SLC1 family genes, and most genes for sodium potassium transport systems.

In conclusion, BMET microarray analyses were able to clearly identify differential gene expression profiles between liver and mammary tissues of high-producing lactating dairy cows. Many of these differences were consistent with the differences in metabolism of the two tissues. Thus, as expected, many of the metabolic functions of the two tissues can be explained by differences in gene transcription. The use of microarrays to help understand complex gene expression patterns in various tissue types in cattle is becoming increasingly helpful in understanding phenotype and genotype interactions. Further studies on the regulation of transporter proteins and signaling molecules are warranted and may help to improve the production and quality of milk in the future.

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