Inflammation- and lipid metabolism-related gene network expression in visceral and subcutaneous adipose depots of Holstein cows¹

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ABSTRACT

This experiment was conducted to determine the effects of energy overfeeding on gene expression in mesenteric (MAT), omental (OAT), and subcutaneous (SAT) adipose tissue (AT) from nonpregnant and nonlactating Holstein cows. Eighteen cows were randomly assigned to either a controlled energy [LE, net energy for lactation (NE_L) = 1.35 Mcal/kg of dry matter (DM)] or moderate energy-overfed group (HE, NE_L = 1.62 Mcal/kg of DM) for 8 wk. Cows were then euthanized and subsamples of MAT, OAT, and SAT were harvested for transcript profiling via quantitative PCR of 34 genes involved in lipogenesis, triacylglycerol (TAG) synthesis, lactate signaling, hepatokine signaling, lipolysis, transcription regulation, and inflammation. The interaction of dietary energy and adipose depot was not significant for any gene analyzed except *LPL*, which indicated a consistent response to diet. Expression of *ACACA* and *FASN* was greater in SAT than MAT, whereas expression of *SCD* and *ADFP* were greatest in SAT, intermediate in OAT, and lowest in MAT. However, the 2 visceral depots had greater expression of *THrsp*, *ACLY*, *LPL*, *FABP4*, *GPAM*, and *LPIN1* compared with SAT. The transcription factor *SREBF1* was more highly expressed in MAT and SAT than in OAT. The expression of *PNPLA2* was greater in visceral AT sites than in SAT, but other lipolysis-related genes were not differentially expressed among AT depots. Visceral AT depots had greater expression of *LEP*, *ADIPOQ*, and *SAA3* compared with SAT. Moreover, MAT had greater expression than SAT of proinflammatory cytokines (*IL1B* and *IL6*), IL6 receptor (*IL6R*), and chemokines (*CCL2* and *CCL5*). However, *TNF* expression was greatest in SAT, lowest in OAT, and intermediate in MAT. Overall, results indicated that visceral AT might be more active in uptake of preformed long-chain fatty acids than SAT, whereas de novo fatty acid synthesis

could make a greater contribution to the intracellular pool of fatty acids in SAT than in visceral AT. The visceral AT compared with SAT seem to have a greater capacity for expression (and potentially for secretion) of proinflammatory cytokines; thus, excessive accumulation of visceral lipid due to a long-term overfeeding energy may be detrimental to liver function and overall health of dairy cows, particularly during the transition period.

Key words: visceral adipose tissue, gene expression, lipogenesis, inflammation

INTRODUCTION

Adipose tissue (**AT**) carries out important functions, including (1) lipogenesis to store surplus energy in the form of triacylglycerol (**TAG**); (2) lipolysis to release energy during periods of undernutrition in the form of NEFA and glycerol; and (3) secretion of a broad spectrum of cytokines, chemokines, and acute phase proteins, which links metabolism with the innate immune system. Adipose tissues from different sites of the body differ in metabolic activity, such as sensitivity to lipolytic stimuli (Giorgino et al., 2005), as well as secretory characteristics. For example, in humans, visceral AT (**VAT**) produces more proinflammatory cytokines than subcutaneous AT (**SAT**; Hamdy et al., 2006; Fontana et al., 2007). These differences result from anatomical location, relative proportions of various cell types (preadipocytes and mature adipocytes, resident macrophages), and the composition of extracellular matrix (Hassan et al., 2012). Obesity in nonruminants, particularly over-accumulation of VAT, is associated with chronic low-grade inflammation that is causally linked to many metabolic diseases (e.g., insulin resistance, diabetes, and cardiovascular disease) in rodent models and humans (Hotamisligil, 2006).

The use of BCS has been widely adopted as a practical way to monitor the degree of body fat storage and loss at different stages of lactation (Roche et al., 2009). Despite the reasonable accuracy for assessment of SAT stores, BCS may be a less effective predictor of internal and intramuscular fat storage (Wright and Russell, 1984). Reynolds et al. (2003) observed that

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VAT associated with the portal-drained viscera had a high degree of lipolytic activity during early lactation. A previous study from our laboratory demonstrated that proinflammatory cytokine mRNA expression in VAT and SAT responded differently to an *in vitro* LPS challenge (Mukesh et al., 2010), which may be due partly to the difference in immune cell infiltration in various AT sites that has been shown in obese mice (Weisberg et al., 2003) and human (Lim et al., 2013). Together, the limited data available indicate that VAT of dairy cattle may have a significant effect on whole-body metabolic responses, and particularly liver, due to the direct portal drainage.

Characterization of potential differences between VAT and SAT of dairy cows is important to improve understanding of lipid metabolism in each depot, which in the long-term might help decrease the incidence of lipid-related metabolic disorders. We used nonpregnant, nonlactating cows to replicate the effects of overfeeding in typical production systems without the confounding hormonal changes that occur around parturition. The present paper describes differences in expression of genes associated with adipogenesis, lipogenesis, lipolysis, and inflammation in 2 VAT (mesenteric and omental AT) and SAT. Results pertaining to the main effect of dietary energy intake on gene expression are presented in a companion paper (Ji et al., 2014).

MATERIALS AND METHODS

Experimental Design, Animals, Diets, and Sampling

All procedures were conducted under protocols approved by the University of Illinois Institutional Animal Care and Use Committee (Protocol No. 06194) and have been described in detail elsewhere (Drackley et al., 2014; Ji et al., 2014). Briefly, 18 nonpregnant, nonlactating Holstein cows (BW = 656 ± 29 kg; BCS = 3.04 ± 0.25 ; dried off at 60 to 130 d postpartum) were randomly assigned for 8 wk to a moderate energy diet (**HE**; $NE_L = 1.62$ Mcal/kg of DM) or a controlled energy diet (**LE**; $NE_L = 1.35$ Mcal/kg of DM) provided as TMR for *ad libitum* intake. Cows were blocked by initial BCS and previous experimental treatment (proprietary). The HE diet contained 73.8% forage from alfalfa silage, alfalfa hay, and corn silage, whereas the LE diet contained 81.7% forage, including 40.5% wheat straw (DM basis, see detailed ingredient composition and nutrient profile of diets in Drackley et al., 2014). At the end the 8-wk experiment, cows were euthanized at the College of Veterinary Medicine, University of Illinois, diagnostic facilities (Urbana). All visceral organs, omental AT (**OAT**), mesenteric AT (**MAT**), and perirenal AT were dissected and weighed. Subsamples

of SAT from the left side of tail-head region, OAT, and MAT were harvested immediately following euthanasia and snap-frozen in liquid N_2 until RNA extraction.

RNA Extraction and Quality Assessment, Primer Design and Evaluation, Internal Control Gene Selection, and Quantitative PCR

Complete details of these procedures are reported elsewhere (Ji et al., 2014). Briefly, tissue was weighed (~ 2.0 g) and immediately placed into ice-cold TRIzol reagent (~ 15 mL; Invitrogen, Carlsbad, CA) with 1 μ L of linear acrylamide (Ambion Inc., Austin, TX). Total RNA was cleaned using RNeasy mini kit columns and genomic DNA was removed using the RNase-Free DNase Set (Qiagen, Valencia, CA). A portion of the RNA was diluted to 100 ng/ μ L with DNase- and RNase-free water before reverse transcription for synthesis of cDNA (Ji et al., 2014).

Primer Design and Evaluation. Genes tested in the current study are listed in Table 1. Primers were designed and evaluated as previously described (Bionaz and Looor, 2008). The details of primer design, sequences, verification, and the description of genes measured are reported in a companion paper (Ji et al., 2014). Briefly, primers were designed using Primer Express 3.0 (Applied Biosystems, Carlsbad, CA) with minimum amplicon size of 80 bp (amplicons of 100 to 120 bp were used, if possible) and limited 3' G + C percentage. Primer sets were intentionally designed to span exon-exon junctions.

Quantitative PCR. The quantitative (q)PCR analyses were performed in a MicroAmp Optical 384-Well Reaction Plate (Applied Biosystems). Within each well, 4 μ L of diluted cDNA combined with 6 μ L of mixture composed of 5 μ L of 1 $\times$ SYBR Green master mix (Applied Biosystems), 0.4 μ L each of 10 μ M forward and reverse primers, and 0.2 μ L of DNase- and RNase-free water were added. Three replicates and a 6-point standard curve plus the nontemplate control were run for each sample to test the relative expression level (User Bulletin #2, Applied Biosystems). The qPCR was conducted using an ABI Prism 7900 HT SDS instrument (Applied Biosystems) following conditions of 2 min at 50°C, 10 min at 95°C, 40 cycles of 15 s at 95°C (denaturation), and 1 min at 60°C (annealing + extension). The presence of a single PCR product was verified by the dissociation protocol using incremental temperatures to 95°C for 15 s, and then 65°C for 15 s. The development of the standard curve was reported in the companion manuscript (Ji et al., 2014).

Selection and Evaluation of Internal Control Genes. Complete details of this procedure are reported in a companion paper (Ji et al., 2014). Briefly,

Table 1. The gene symbol for full gene names recorded in National Center for Biotechnology Information (NCBI; <http://www.ncbi.nlm.nih.gov/gene>)

Gene symbol	Gene name in NCBI
<i>ABHD5</i>	Abhydrolase domain containing 5
<i>ACACA</i>	Acetyl-Co A carboxylase α
<i>ACLY</i>	ATP citrate lyase
<i>ACSL1</i>	Acyl-CoA synthetase long-chain family member 1
<i>ADRB2</i>	Adrenoceptor β 2, suface
<i>ADFP/PLIN2</i>	Perilipin 2
<i>ADIPOQ</i>	Adiponectin
<i>CD36</i>	Thrombospondin receptor
<i>CCL2</i>	Chemokine (C-C motif) ligand 2
<i>CCL5</i>	Chemokine (C-C motif) ligand 5
<i>ELOVL6</i>	Elongation of long chain fatty acids family member 6
<i>FASN</i>	Fatty acid synthase
<i>FABP4</i>	Adipocyte fatty acid binding protein 4
<i>GPAM</i>	Mitochondrial glycerol-3-phosphate acyltransferase
<i>HCAR1/GPR81</i>	Hydroxycarboxylic acid receptor 1
<i>IL1B</i>	Interleukin-1 β
<i>IL6</i>	Interleukin-6
<i>IL6R</i>	Interleukin-6 receptor
<i>LEP</i>	Leptin
<i>LIPE</i>	Hormone sensitive lipase
<i>LPIN1</i>	Lipin 1
<i>LPL</i>	Lipoprotein lipase
<i>MGLL</i>	Monoglyceride lipase
<i>MLXIPL/ChREBP</i>	MLX interacting protein-like
<i>PDE3B</i>	Phosphodiesterase 3B, cGMP-inhibited
<i>PLIN1</i>	Perilipin 1
<i>PNPLA2</i>	Patatin-like phospholipase domain containing 2
<i>PPARG</i>	Peroxisome proliferator-activated receptor gamma
<i>SAA3</i>	Acute-phase serum amyloid A3
<i>SCD</i>	Stearoyl-CoA desaturase
<i>SREBF1</i>	Sterol regulatory element binding transcription factor 1
<i>THrsp</i>	Thyroid hormone responsive SPOT 14
<i>TLR4</i>	Toll-like receptor 4
<i>TNF</i>	Tumor necrosis factor α

candidate internal control genes were selected following the procedures described previously by Mukesh et al. (2010). The 3 internal control genes chosen from 10 candidate genes were kelch-like ECH-associated protein 1 (*KEAP1*), tripartite motif-containing 41 (*TRIM41*), and mitochondrial ribosomal protein 63 (*MRP63*).

Data Processing and Statistical Analysis

The gene expression data reported by Ji et al. (2014) and those in this paper were analyzed as a 2 $\times$ 3 factorial arrangement in a completely randomized design. The normalized qPCR data (relative mRNA expression) were subjected to tests of normality and homogeneity of variance using the UNIVARIATE and GLM procedures, respectively. The data were tested with ANOVA using the MIXED procedure of SAS (version 9.1; SAS Institute Inc., Cary, NC) using a model that included the fixed effect of diet, AT depot, and their interaction. Cow within treatment was random term. The data were expressed as least squares means with standard errors of the mean (Figures 1, 2, and 3). Significance was declared when $P < 0.05$.

RESULTS AND DISCUSSION

Dietary energy intake exerted a consistent effect on gene expression in both VAT and SAT depots, which is described in a companion manuscript (Ji et al., 2014). Because interactions of adipose site and diet were not significant for any genes except *LPL*, differentially expressed genes among the 3 AT sites are discussed here. Differences reflected unique characteristics of each depot examined.

Lipid Metabolism

Lipogenesis. With exception of *ELOVL6* ($P = 0.17$) and *CD36* ($P = 0.85$), all genes associated with processes leading to TAG synthesis were differentially expressed ($P < 0.05$) among the 3 AT sites, with the largest differences always detected between MAT and SAT (Figure 1). Compared with the VAT, SAT exhibited greater expression of *ACACA* ($P = 0.001$), *FASN* ($P = 0.0001$), and *SCD* ($P = 0.0001$), which encode the major enzymes responsible for de novo FA synthesis and desaturation. The enzyme product of *ACLY*, ATP

Figure 1. Main effect of adipose site on mRNA expression of genes involved in lipid metabolism (panel A) and transcription regulation (panel B). Within genes, different letters indicate differential expression among adipose sites ($P < 0.05$). The x-axis indicates the official gene symbol from the National Center for Biotechnology Information (NCBI; <http://www.ncbi.nlm.nih.gov/gene>; see Table 1 for gene names); the y-axis indicates relative mRNA expression value from natural logarithmic transformed data. MAT = mesenteric adipose tissue; OAT = omental adipose tissue; and SAT = subcutaneous adipose tissue.

citrate lyase, converts glucose-derived citrate to cytosolic acetyl-CoA, which can then be used as substrate for de novo FA synthesis, despite the low preference for glucose as a lipogenic substrate in ruminant AT (Ingle et al., 1972). It is noteworthy that the expression of *ACLY* was greater in VAT than SAT ($P < 0.05$), which was opposite to the expression pattern of *ACACA* and *FASN*. In contrast to FA synthesis, genes involved in long-chain FA (LCFA) uptake (*LPL*), intracellular activation and transport (*ACSL1* and *FABP4*), and TAG

synthesis (*GPAM* and *LPIN1*) had greater expression in VAT, particularly in MAT ($P < 0.05$; Figure 1).

From a gene transcription standpoint, our results indicate that AT from different anatomic locations of dairy cattle varies in the relative enrichment of the pathways leading to TAG synthesis. Such depot-specific expression patterns also have been observed in beef cattle. For instance, Baldwin et al. (2007) observed that the general pattern of in vitro incorporation rate of acetate into FA per unit of cell volume from AT depots of beef steers was SAT > OAT > MAT. Eguinoa et al. (2003) reported that activities of glycerol-3-phosphate dehydrogenase and fatty acid synthase were greater in SAT than OAT or perirenal AT when adjusted for cell size. Because transcriptional regulation is the primary determinant of protein abundance of these enzymes in nonruminants (Foufelle and Ferre, 2002), the greater expression of *ACACA* and *FASN* in our study suggested that SAT has a potentially greater capacity for de novo FA synthesis than VAT.

The coordinated upregulation of *ACLY* with *ACACA* and *FASN* regardless of AT site in the energy-overfed (HE) group compared with the LE group (Ji et al., 2014) suggested the existence of co-regulation in response to greater dietary energy intake. However, the physiological reason for the difference in expression pattern between SAT and VAT of *ACLY* compared with the other FA biosynthetic genes (*ACACA* and *FASN*) could not be discerned from our data.

Lipoprotein lipase is responsible for releasing LCFA from lipoprotein-associated TAG in blood, which then must be activated once inside cells by LCFA-CoA ligases before metabolism. Hocquette et al. (1998) reported that both *LPL* mRNA expression and enzymatic activity in male calves were greater in internal AT depots (perirenal AT and OAT) than in SAT. Among all the LCFA ligase isoforms, *ACSL1* was identified as the most highly expressed in mammary gland and SAT of dairy cows (Bionaz and Looor, 2008). Both *GPAM* and *LPIN1* encode enzymes that participate in TAG synthesis (Takeuchi and Reue, 2009). The greater expression of these genes (*LPL*, *ACSL1*, *GPAM*, and *LPIN1*) in VAT, and especially in MAT, seems to highlight a greater potential for utilizing preformed LCFA for TAG synthesis in comparison with SAT.

Consistent with apparent differences in de novo FA synthesis, the expression of *SCD* was greatest in SAT, lowest in MAT, and intermediate in OAT (Figure 1). This difference indicates that SAT may have a greater capacity for desaturating 16:0 and 18:0 to MUFA. Therefore, SAT appears to have not only a greater lipogenic capacity but also a greater ability to synthesize oleic acid (the primary FA found in SAT of dairy cows; Douglas et al., 2007) for esterification into

TAG. Martin et al. (1999) observed that the peak of *SCD* mRNA expression preceded a significant increase in lipogenesis and lipid filling in SAT of yearling steers. They concluded that the level of *SCD* expression was indicative of the extent of terminal differentiation in bovine tail-head SAT.

The greater expression ($P = 0.0002$) of *FABP4* in VAT compared with SAT deserves particular attention because this protein acts as a chaperone for intracellular LCFA, thus, facilitating their uptake but also their intracellular fate (e.g., esterification versus oxidation). Fatty acid binding protein 4 (**FABP4**) is a cytosolic lipid-binding protein highly expressed in adipocytes (Shen et al., 2001). The response for this gene in VAT was consistent with greater expression of *LPL*, *ACSL1*, *GPAM*, and *LPIN1*, all of which would favor the channeling of LCFA toward TAG synthesis.

Lipolysis. A greater ($P = 0.0003$) expression of *PNPLA2* was detected in both VAT sites compared with SAT (Figure 2). The expression of the lipolysis-activator protein *ABHD5* exhibited a pattern similar ($P = 0.04$) to that of *PNPLA2* specifically in MAT compared with SAT. However, the expression of *PLIN2* (also known as *ADFP*) was greatest ($P = 0.0001$) in SAT, intermediate in OAT, and lowest in MAT. Expression of *ADRB2* ($P = 0.23$), *PLIN1* ($P = 0.63$), *LIPE* ($P = 0.25$), and *MGLL* ($P = 0.61$) did not differ among AT sites.

During lipolysis, FABP4 helps shuttle intracellular LCFA toward the cellular membrane through its interaction with hormone-sensitive lipase (Furuhashi and Hotamisligil, 2008). Hence, FABP4 content appears to be proportional to the rates of FA metabolism (both mobilization and uptake or flux), which also is consistent with greater expression of *PNPLA2* in VAT than in SAT. Targeted disruption of *FABP4* attenuates both basal and stimulated lipolysis (Scheja et al., 1999). Interestingly, FABP4 is abundantly expressed in activated macrophages that reside within AT stores and are functionally associated with many immune-mediated responses during metabolic disease states (e.g., insulin resistance; Makowski and Hotamisligil, 2004). Whether the greater expression of *FABP4* in VAT in the current study was immune cell- or adipocyte-specific warrants further research.

Previous studies from our laboratory and others have demonstrated that hepatic TAG, hepatic total lipid content, and serum NEFA concentration during the dry period were not different in cows overfed energy compared with controls (Dann et al., 2006; Janovick et al., 2011). However, those studies demonstrated that cows overfed prepartum were predisposed to a more dramatic increase of hepatic lipid accumulation, circulating NEFA, and incidence of ketosis at the initiation

of lactation. In cows overfed energy during the close-up dry period, greater expression of *PNPLA2* (formerly *ATGL*), *ABDH5*, and *PLIN2* in SAT at 10 d prepartum persisted at 7 d postpartum, thus serving as a mechanism responsible at least in part for the increased NEFA mobilization postpartum (Ji et al., 2014). However, how VAT would respond to energy overfeeding before parturition in terms of lipolysis cannot be discerned from the present study.

Two classic studies by Reynolds et al. (2003, 2004) focusing on visceral organ metabolism of dairy cows during the transition period revealed that the net release of NEFA from portal-drained viscera increased nearly 3 times at 11 DIM compared with that at -7 DIM, and the total mass of VAT (MAT and OAT) was significantly reduced during the first 22 DIM compared with that in late gestation. In the present study, the greater expression of *PNPLA2* in VAT compared with SAT underscored the potential for greater lipolytic activity per unit of VAT. Therefore, even though no diet $\times$ AT depot interaction was observed in our study (Ji et al., 2014), the 92% increase in MAT mass and the 76% increase in OAT mass in cows fed HE (Drackley et al., 2014) might have predisposed them to a much greater hepatic NEFA flux had they undergone a period of negative energy balance. It is possible, then, that if similar changes in AT mass are achieved in late-pregnant transition cows because of energy overfeed-

Figure 2. Main effect of adipose site on mRNA expression of lipolysis-related genes. Within genes, different letters indicate differential expression among adipose sites ($P < 0.05$). The x-axis indicates the official gene symbol from the National Center for Biotechnology Information (NCBI; <http://www.ncbi.nlm.nih.gov/gene>; see Table 1 for gene names); the y-axis indicates relative mRNA expression value from natural logarithmic transformed data. MAT = mesenteric adipose tissue; OAT = omental adipose tissue; and SAT = subcutaneous adipose tissue.

ing, they could be predisposed to developing metabolic disorders postpartum.

Transcriptional Regulation of Lipogenesis

Expression of *SREBF1* was greater ($P = 0.0001$) in SAT and MAT than in OAT, whereas *PPARG* ($P = 0.17$) and *MLXIPL* ($P = 0.16$) were consistently expressed among AT depots (Figure 1). In rodent liver, SREBF1 is a well-established control point of lipogenic gene expression (e.g., *FASN*, *SCD*) in direct response to an increase in insulin and the ensuing signaling cascade leading to its maturation and activation (Horton et al., 2002). More recent work revealed that MLX interacting protein-like (MLXIPL), which is activated by the glycolytic intermediate ribulose-5-phosphate, could control approximately 50% of hepatic lipogenesis by regulating glycolytic and lipogenic gene expression (Iizuka and Horikawa, 2008) through binding to the carbohydrate response element in the promoter region of its target genes (Yamashita et al., 2001), including *THRSP* (Shih et al., 1995), *ACACA*, and *FASN* (Towle et al., 1997). Clearly, both of these transcription factors could overlap in terms of their gene targets in bovine AT, but it is likely that SREBF1 would be more biologically relevant because glucose is not a primary lipogenic substrate in bovine AT (Ingle et al., 1972).

It is well-established in rodents that peroxisome proliferator-activated receptor gamma ($PPAR\gamma$) and CCAAT/enhancer-binding protein alpha (C/EBP α) coordinate expression of adipogenic genes [e.g., *FABP4* and insulin-induced glucose transporter (*SLC2A4*, formerly known as *GLUT4*); Rosen and Spiegelman, 2000], and also SREBF1 and the adipokine adiponectin (Walkey and Spiegelman, 2008). Although the role of $PPAR\gamma$ in the control of adipogenesis is crucial, the fact that the heterodimer $PPAR\gamma$:RXR can bind to thousands of response elements particularly abundant in the vicinity of genes involved in lipid and glucose metabolism in rodent adipocytes (Lefterova et al., 2008) suggests that it may also play a role in promoting lipogenesis; for example, partly through SREBF1 and directly through effects on specific target genes (e.g., *LPL*, *ACSL1*, *FABP4*, *LPIN1*). From a practical standpoint, we have speculated that $PPAR\gamma$ activation via specific dietary LCFA (e.g., SFA and marine PUFA; Schmitt et al., 2011) could aid in manipulating AT deposition or insulin sensitivity during the transition from pregnancy into lactation.

The expression of *THRSP* differed ($P = 0.0003$) among AT depots, with greater values in VAT than SAT (Figure 3), which may underscore its importance in the ability of VAT (especially MAT) depots to ac-

crete lipid. This depot-specific expression pattern might be induced by differing numbers of thyroid hormone receptors (TR) and the different ratios of TR isoforms among fat depots, which are required for transcriptional activation of *THRSP* (Cunningham et al., 1998). In humans, expression of TR α (an isomer of TR) and *THRSP* differed between SAT and OAT, and *THRSP* expression was significantly correlated with TR α mRNA level and with the TR α 1:TR α 2 ratio (Ortega et al., 2009). However, in contrast to our study, Ortega et al. (2009) found greater expression of these genes in SAT than in OAT, which may be associated with inherent species differences.

Adipokines and Proinflammatory Cytokines

In the current study, the expression of *ADIPOQ* and *SAA3* was greater ($P = 0.00001$) in both VAT depots than in SAT, with no statistical difference between MAT and OAT (Figure 3). Leptin was differentially expressed ($P = 0.0001$) among the 3 depots, with the highest mRNA expression in OAT and the lowest in SAT. Mesenteric AT exhibited the highest expression of *IL1B* ($P = 0.0001$) and *IL6R* ($P = 0.0001$) among depots, and expression did not differ between OAT and SAT. The expression of chemokines (*CCL2* and *CCL5*) was greater ($P = 0.001$) in MAT than in SAT. Although

Figure 3. Main effect of adipose site on mRNA expression of adipokines. Within genes, different letters indicate differential expression among adipose sites ($P < 0.05$). The x-axis indicates the official gene symbol from the National Center for Biotechnology Information (NCBI; <http://www.ncbi.nlm.nih.gov/gene>; see Table 1 for gene names); the y-axis indicates relative mRNA expression value from natural logarithmic transformed data. MAT = mesenteric adipose tissue; OAT = omental adipose tissue; and SAT = subcutaneous adipose tissue.

expression of *IL6* did not differ ($P = 0.26$) among depots, *TNF α* expression was greater ($P = 0.005$) in SAT than in OAT.

In human and rodent models, visceral obesity is associated with chronic systemic low-grade inflammation (Fontana et al., 2007). Evidence also indicates that visceral obesity results in an increase in macrophage infiltration in VAT, which is causally linked with inflammation, liver disease, and insulin resistance (Hotamisligil, 2006). Our results seem to suggest that VAT, and especially MAT, of dairy cows might be more prone to produce most of the adipokines, proinflammatory cytokines, and chemokines. However, we could not determine if this was due to greater immune cell infiltration or to imprinted characteristics of VAT. Because the mass of MAT increased more than that of OAT as BCS increased (Drackley et al., 2014), this potential proinflammatory role of MAT in dairy cows could play a role in metabolic pathophysiology associated with overfeeding and excessive BCS.

Recently, Akter et al. (2012) utilized immunohistochemistry to determine potential differences in macrophage recruitment capacity between VAT and SAT of early-lactation Holstein cows. Only 7 out of 25 cows had positive staining for the macrophage marker CD68 in at least one AT depot, with numerically greater counts in VAT than in SAT. The authors ascribed these results to a greater metabolic rate and chemokine production of VAT. However, the authors acknowledged that the degree of fatness (BCS 3.0) might not have been enough to stimulate significant infiltration (Akter et al., 2012).

It is well established in nonruminants that adipocytes express several receptors for cytokines, including tumor necrosis factor- α and IL-1 β (Fain et al., 2004). Therefore, an initially greater proinflammatory state due to more immune cells, greater cytokine secretory activity of VAT adipocytes, or simply greater VAT mass (Drackley et al., 2014) could exaggerate the inflammatory response of those depots through autocrine mechanisms. Upon hepatic delivery through the portal vein, these cytokines (e.g., IL-1 β and TNF α) can then stimulate an acute phase response and alter hepatic function, thus contributing to a state of systemic inflammation, which would provide a feedback mechanism on AT depots. Further research is needed to confirm the immuno-endocrine activity of VAT and SAT as well as their link with peripheral tissues in periparturient dairy cows.

CONCLUSIONS

The greater mRNA expression of genes for lipolytic enzymes, proinflammatory cytokines, chemokines, and acute phase proteins in VAT along with the greater mass of VAT (49.0 and 25.7 ± 2.76 kg for the sum

of MAT and OAT in HE and LE cows, respectively; Drackley et al., 2014) suggests that overfeeding energy may predispose dairy cows to inflammation and potentially impaired liver function. Despite the limitations of deducing physiological changes by focusing on mRNA profiles, a logical implication of our results is that the effect of VAT metabolism on liver function and health may be much more prominent in cows overfed energy during the transition from late pregnancy into lactation. These findings are particularly important because of the poor association between BCS and VAT mass in the present work. Therefore, research to establish how nutritional management affects VAT metabolism and its immuno-endocrine functions during the periparturient period is warranted.

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