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Inflammation- and lipid metabolism-related gene network expression in visceral and subcutaneous adipose depots of Holstein cows[1]

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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 μL 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/μL 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 Loor, 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 to120 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 μL of diluted cDNA combined with 6 μL of mixture composed of 5 μL of 1× SYBR Green master mix (Applied Biosystems), 0.4 μL each of 10 μ*M* forward and reverse primers, and 0.2 μL 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,