1-2016

Body condition score and plane of nutrition prepartum affect adipose tissue transcriptome regulators of metabolism and inflammation in grazing dairy cows during the transition period

M. Vailati-Riboni
University of Illinois at Urbana-Champaign

M. Kanwal
University of Illinois at Urbana-Champaign

O. Bulgari
University of Illinois at Urbana-Champaign

S. Meier
DairyNZ Limited

N. V. Priest
DairyNZ Limited

See next page for additional authors

Recommended Citation

Vailati-Riboni, M., Kanwal, M., Bulgari, O., Meier, S., Priest, N. V., Burke, C. R., ... & Crookenden, M. (2016). Body condition score and plane of nutrition prepartum affect adipose tissue transcriptome regulators of metabolism and inflammation in grazing dairy cows during the transition period. Journal of dairy science, 99(1), 758-770. DOI: https://doi.org/10.3168/jds.2015-10046

J. Dairy Sci. 99:758–770
http://dx.doi.org/10.3168/jds.2015-10046

Body condition score and plane of nutrition prepartum affect adipose tissue transcriptome regulators of metabolism and inflammation in grazing dairy cows during the transition period

M. Vailati-Riboni,* M. Kanwal,* O. Bulgari,*† S. Meier,‡ N. V. Priest,‡ C. R. Burke,‡ J. K. Kay,‡ S. McDougall,§ M. D. Mitchell,# C. G. Walker,‡ M. Crookenden,‡ A. Heiser,‖ J. R. Roche,‡ and J. J. Loor*[1]

*Department of Animal Sciences, University of Illinois, Urbana 61801
†Department of Molecular and Translational Medicine, University of Brescia, Brescia 25123, Italy
‡DairyNZ Limited, Private Bag 3221, Hamilton 3240, New Zealand
§Cognosco, Anexa Animal Health, PO Box 21, Morrinsville 3300, New Zealand
#University of Queensland, Centre for Clinical Research, Royal Brisbane & Women's Hospital Campus, Herston, Queensland 4029, Australia
‖AgResearch, Hopkirk Research Institute, Grasslands Research Centre, Palmerston North 4442, New Zealand

ABSTRACT

Recent studies demonstrating a higher incidence of metabolic disorders after calving have challenged the management practice of increasing dietary energy density during the last ~3 wk prepartum. Despite our knowledge at the whole-animal level, the tissue-level mechanisms that are altered in response to feeding management prepartum remain unclear. Our hypothesis was that prepartum body condition score (BCS), in combination with feeding management, plays a central role in the peripartum changes associated with energy balance and inflammatory state. Twenty-eight mid-lactation grazing dairy cows of mixed age and breed were randomly allocated to 1 of 4 treatment groups in a 2 × 2 factorial arrangement: 2 prepartum BCS categories (4.0 and 5.0, based on a 10-point scale; BCS4, BCS5) obtained via differential feeding management during late-lactation, and 2 levels of energy intake during the 3 wk preceding calving (75 and 125% of estimated requirements). Subcutaneous adipose tissue was harvested via biopsy at −1, 1, and 4 wk relative to parturition. Quantitative polymerase chain reaction was used to measure mRNA and microRNA (miRNA) expression of targets related to fatty acid metabolism (lipogenesis, lipolysis), adipokine synthesis, and inflammation. Both prepartum BCS and feeding management had a significant effect on mRNA and miRNA expression throughout the peripartum period. Overfed BCS5 cows had the greatest prepartum expression of fatty acid synthase (*FASN*) and an overall greater expression of leptin (*LEP*); BCS5 was also associated with greater overall adiponectin (*ADIPOQ*) and peroxisome proliferator-activated receptor gamma (*PPARG*), whereas overfeeding upregulated expression of proadipogenic miRNA. Higher postpartum expression of chemokine ligand 5 (*CCL5*) and the cytokines interleukin 6 (*IL6*) and tumor necrosis factor (*TNF*) was detected in overfed BCS5 cows. Feed-restricted BCS4 cows had the highest overall interleukin 1 (*IL1B*) expression. Prepartum feed restriction resulted in greater chemokine ligand 2 (*CCL2*) expression. Overall, changes in mRNA expression were consistent with the expression pattern of inflammation-related miRNA. These data shed light on molecular mechanisms underlying the effect of prepartum BCS and feeding management on metabolic and inflammatory status of adipose tissue during the peripartum period. Data support the use of a controlled feed restriction prepartum in optimally conditioned cows, as well as the use of a higher level of dietary energy in under-conditioned cows.

Key words: transition cow, nutrition, inflammation, immune response

INTRODUCTION

Cows in early lactation experience a period of negative energy balance (**NEB**) due to the sudden increase in requirements of the mammary gland for milk production. Nutritional management of the late-pregnant nonlactating cow has been examined as a way to improve DMI, energy balance, immunometabolism, and health during the transition period. Traditional management provides far-off dry cows with a high-fiber, low-energy density ration, whereas in the last month of gestation (close-up dry period) the ration increases in energy density with a lower fiber content. However, studies from different research groups have demonstrated that prepartum overfeeding of energy often results in prepartum hyperglycemia and hyperinsulinemia and

Received July 1, 2015.
Accepted September 8, 2015.
[1]Corresponding author: jloor@illinois.edu

marked postpartum adipose tissue mobilization (i.e., greater blood fatty acid concentration) (Janovick et al., 2011; Ji et al., 2014a; Khan et al., 2014). In addition, higher-energy close-up diets also have been associated with negative effects in postpartum health indices, underscoring possible detrimental effects of this management approach (Dann et al., 2006; Graugnard et al., 2013; Shahzad et al., 2014).

Although several studies aimed at understanding metabolic and molecular changes associated with dietary energy intake before calving have been performed, the contributing factors remain poorly understood. One animal factor that could potentially interact with prepartum level of feeding is cow BCS. The BCS provides a qualitative assessment of body fat and, due to its association with production parameters and the chances for a successful lactation (Roche et al., 2005; Pires et al., 2013; Randall et al., 2015), it is recognized as an important variable in transition dairy cattle management (Roche et al., 2009). For example, not only fat cows are at a greater risk of metabolic disorders postpartum (Randall et al., 2015), but thin cows can also be susceptible to failure to transition (Pires et al., 2013).

Despite the fact BCS plays an important role in the metabolic response of the animal to lactation and its level is regulated through nutrition, cows with different level of adiposity are generally managed similarly during the prepartum period. Both BCS and feeding management can have a great effect on cow fat depots. Adipose tissue is an active component of the regulation of animal reserves through production of adipokines (McGown et al., 2014; Musi and Guardado-Mendoza, 2014). One of its features is the control of inflammation in a localized manner through recruitment and regulation of the innate immune system, hence, making it an active immunological organ (Grant and Dixit, 2015).

Besides the well-established role of changes in mRNA expression in controlling cellular pathways, recent studies have underscored that microRNA (**miRNA**) also are important for fat cell formation (adipogenesis) and regulation of their metabolic and endocrine functions (Arner and Kulyte, 2015). For example, beef cattle adipose miRNA profiling was recently correlated with fat depot location and function, underscoring the importance of miRNA (e.g., miR-378 and miR-143) in regulating adipocyte metabolism (Jin et al., 2010, 2009), as well as in response to diet (Romao et al., 2012).

The recognition that cows experience a degree of inflammation around parturition (Bertoni et al., 2008; Trevisi et al., 2012) has led to the hypothesis of a homeorhetic role of inflammation as a physiological adaptation to lactation (Farney et al., 2013; Vailati Riboni et al., 2015). Furthermore, in the context of inflammation, the miRNA signaling through complex networks involving transcription factors has been demonstrated (Arner and Kulyte, 2015). A good example of this is the miRNA regulatory circuits composed by miR-92a, miR-126, and miR-193b that control levels of the chemokine CCL2 in human adipose tissue (Arner et al., 2012). However, regarding inflammation, miRNA not only regulate gene expression, but their expression patterns have been associated with levels of inflammatory molecules such as cytokines and the degree of immune cell infiltration (Klöting et al., 2009).

In the present study, gene and miRNA expression profiling were used to better understand the interaction between precalving BCS and plane of nutrition in the metabolism of adipose tissue. Specifically, we were interested in how these factors influence the adipose response to the physiological changes induced by the high metabolic demands of early lactation.

MATERIALS AND METHODS

Animal Management

Complete details of the experimental design have been published elsewhere (Roche et al., 2015). Briefly, a group of 150 mid-lactation grazing dairy cows of mixed age and breed were enrolled in the experiment on January 21, 2013. Animals were allocated randomly to 1 of 6 treatment groups (25 cows per group) in a 2 × 3 factorial arrangement: 2 precalving BCS categories [4.0 and 5.0 (**BCS4** and **BCS5**, respectively), based on a 10-point scale, where 1 is emaciated and 10 obese; Roche et al., 2004] and 3 levels of energy intake during the 3 wk preceding calving (75, 100, and 125% of estimated requirements; Roche et al., 2005). The different levels of energy intake were obtained by daily manipulation of pasture allowance, adjusting area allocation (m^2/cow) in each group (Roche et al., 2015). Cows were randomly assigned to the 6 groups, balanced for age, breed (Holstein and Holstein × Jersey), BCS at the time of enrollment, and expected calving date. For the current study only 4 groups with a subset of 28 animals (7 cows per group) were considered. These were cows with prepartum BCS4 fed to meet 75 (**B4F75**) or 125% (**B4F125**) of requirements, and cows with prepartum BCS5 fed to meet 75 (**B5F75**) or 125% (**B5F125**) of requirements. These subsets were still balance for breed, age, and calving date. Average age and day in gestation at enrollment was 6.20 ± 2.18 yr and 259.32 ± 1.86 d.

RNA Extraction and Quantitative PCR

Adipose Biopsy. Tissue was harvested during wk −1, 1, and 4 relative to parturition as described previ-

ously (Grala et al., 2013). Briefly, subcutaneous adipose tissue was collected posterior to the shoulder blade and approximately 10 cm down the withers. The site was clipped and cleansed with iodine before administering a local anesthetic (2% lignocaine). A 3-cm incision was made through the skin and 30 to 80 mg of adipose tissue removed by blunt dissection. Biopsies were immediately placed in screw-capped, microcentrifuge tubes, snap-frozen in liquid nitrogen, and stored at −80°C until RNA extraction.

Total RNA Extraction, Target Genes, and Quantitative PCR. Complete details of these procedures are included in the supplemental material. Briefly, RNA samples were extracted from 0.2 g of frozen adipose tissue and used for cDNA synthesis using established protocols in our laboratory (Khan et al., 2014). The Quanta qScript microRNA cDNA Synthesis Kit (Quanta BioSciences, Inc., Gaithersburg, MD) was used for miRNA following the manufacturer's protocols. The quantitative PCR performed was based on SYBR Green (Quanta Bioscience Inc.) using a 7-point standard curve. Genes selected for transcript profiling are associated with fatty acid metabolism: fatty acid synthase (*FASN*) and peroxisome proliferator-activated receptor gamma (*PPARG*); adipokines: adiponectin (*ADIPOQ*) and leptin (*LEP*); and inflammation: chemokine (C-C motif) ligand 2 (*CCL2*), chemokine (C-C motif) ligand 5 (*CCL5*), haptoglobin (*HP*), interleukin-1β (*IL1B*), inteleukin-6 (*IL6*), inteleukin-6 receptor (*IL6R*), retinoid X receptor α (*RXRA*), serum amyloid A3 (*SAA3*), toll-like receptor 4 (*TLR4*), and tumor necrosis factor α (*TNF*). The miRNA selected for expression profiling (Table 1) are associated with immune cell infiltration (miR-26b, miR-126, miR-132, miR-155, and miR-193), inflammation and lipolysis (miR-99a, miR-145, and miR-221), and positive regulation of adipogenesis (miR-103, miR-143, miR-378).

Blood Collection and Analysis

Blood was sampled by coccygeal venipuncture in correspondence to biopsy, using evacuated blood tubes containing a lithium heparin anticoagulant. Samples were placed immediately on ice and centrifuged within 30 min at 1,500 × *g* for 12 min at 4°C. Following centrifugation, aspirated plasma was stored at −20°C until assayed.

Blood fatty acids and BHB analysis was performed by Gribbles Veterinary Pathology Ltd. (Auckland, New Zealand). Blood metabolites were assayed using colorimetric techniques at 37°C with a Hitachi Modular P800 analyzer (Roche Diagnostics, Indianapolis, IN). Plasma fatty acid concentration (mmol/L) was measured using Wako Chemicals (Osaka, Japan) kit NEFA HR2 measuring oxidative condensation of 3-methyl-*N*-ethyl-*N*-β hydroxyethyl aniline with 4-aminoantipyrine, whereas plasma BHB (mmol/L) concentrations were assessed using Roche reagent kits measuring the reduction of NAD to NADH during oxidation of D-3-hydroxybutyrate to acetoacetate.

Statistical Analysis

Both expression data sets (mRNA and miRNA) and blood data were analyzed in the same fashion. After normalization with the geometric mean of the internal control genes, the quantitative PCR data were $\log_2$ transformed before statistical analysis to obtain a normal distribution, whereas fatty acids and BHB data were used as is. Statistical analysis was performed with

Table 1. Details and functions of the microRNA (miRNA) targets analyzed in the current study

miRNA	Function or expression pattern
Infiltration of immune cells	
miR-26b	Expression is associated with the number of macrophages infiltrating the fat depot. Affected by levels of circulating tumor necrosis factor (TNF), leptin, and resistin.
miR-126	Directly inhibits chemokine ligand 2 (CCL2) expression
miR-132	Expression levels are associated with the number of macrophages infiltrating fat depots. Activates nuclear factor-κB signaling and the transcription of IL-8 and CCL2. Lower expression is associated with increased secretion of IL-6.
miR-155	Expression levels are associated with the number of macrophages infiltrating fat depots
miR-193	Indirectly inhibits CCL2 expression through a network of transcription factors
Inflammation and lipolysis	
miR-99a	Negative correlation with secretion of IL-6 and level of fatty acids
miR-145	Affects secretion of TNFα, regulating lipolysis
miR-221	Lower expression is associated with high levels of TNFα
Proadipogenic	
miR-103	Regulates expression of PPARG, PANK1, CAV1, FASN, ADIPOQ, and FABP4
miR-143	Regulates expression of ERK5, SLC2A4, TFAP2A, LIPE, PPARG, CEBPA, and FABP4
miR-378	Targets PPARG expression through the MAPK1 pathway

SAS (v 9.3; SAS Institute Inc., Cary, NC). Normalized data, $\log_2$-transformed expression data, and blood metabolites data were subjected to ANOVA and analyzed using repeated measures ANOVA with PROC MIXED. The statistical model included time (**T**; −1, 1 and 4 wk postpartum), BCS (**B**; 4 and 5), feeding (**F**, 75% and 125%), and their interactions (B × T, F × T and B × F × T) as fixed effects. Cow, nested within treatment, was the random effect. The Kenward-Roger statement was used for computing the denominator degrees of freedom, whereas spatial power was used as the covariance structure. Data were considered significant at a $P \leq 0.05$ using the PDIFF statement in SAS. For ease of interpretation, the expression data reported in Tables 3 through 6 are the $\log_2$ back-transformed least squares means that resulted from the statistical analysis.

RESULTS

Fatty Acids and BHB

Both fatty acids and BHB were affected by BCS ($P < 0.05$), with a greater overall concentration in BCS5 animals compared with BCS4, independently from prepartum feeding regimen (Table 2). In contrast, feeding only affected BHB concentration, with a greater overall response in animals fed 75% compared with 125%. Despite the main effects, the interaction was not significant (B × F, $P > 0.05$) for either biomarker. As expected, time ($P < 0.05$) was significant for both biomarkers.

Gene Expression

Adipokines and Fatty Acid Metabolism. The gene *ADIPOQ* was increased by BCS (B, $P < 0.0001$) due to a greater expression ($P < 0.05$) in BCS5. Likewise, *LEP* was affected by BCS, precalving plane of nutrition, and their interaction (B, F, B × F, $P < 0.003$; Table 3). Its expression was greater in B5F75 and B5F125 cows, whereas B4F75 cows had the lowest expression and B4F125 cows an intermediate expression. Among the adipokines, time was significant only for *LEP* (T, $P < 0.0001$) due to a decrease in expression postpartum (Table 4). However, the interaction with BCS ($P < 0.004$) revealed that from 1 to 4 wk BCS4 cows had decreased expression whereas BCS5 cows had similar *LEP* expression ($P < 0.05$).

Among the adipogenic or lipogenic genes, *PPARG* expression was greater ($P < 0.05$) in BCS5 (Table 3) compared with BCS4 cows. Further, *PPARG* expression was higher (T, $P < 0.05$) around parturition (−1 and 1 wk) compared with wk 4 of lactation (Table 4). There was a triple interaction (B × F × T, $P = 0.02$)

Table 2. Effect of prepartum BCS and feeding management on plasma concentrations of fatty acids and BHB in dairy cows during the transition period

	B			F			B × F					*P*-value[1]					
							4		5								
Item, mmol/L	4	5	SE	75%	125%	SE	75%	125%	75%	125%	SE	B	F	B × F	T	B × T	F × T	B × F × T
Fatty acid	0.50[x]	0.72[y]	0.04	0.59	0.62	0.04	0.48[x]	0.51[x]	0.70[y]	0.73[y]	0.05	0.0004	0.62	0.99	<0.0001	0.05	0.26	0.99
BHB	0.46[x]	0.60[y]	0.03	0.57[x]	0.49[y]	0.03	0.51[x–z]	0.42[x]	0.64[y]	0.56[x–z]	0.04	0.004	0.05	0.83	<0.0001	0.10	0.01	0.57

[x–z]Different letters represent significant differences among groups ($P < 0.05$).

[1]T = time (week relative to parturition); B = BCS (1–10 scale); F = level of feeding prepartum relative to requirement (%); B × F × T = interaction of BCS, level of feeding prepartum relative to requirements (%), and time around parturition.

for *FASN* expression due to differences among feeding management groups at −1 and 4 wk around parturition coupled with the general decrease in expression postpartum for all cows (Table 4). Precalving, F125 cows had a greater ($P < 0.05$) expression (~3 fold) compared with cows fed 75% of requirements regardless of BCS. However, when fed 75% of requirements, the BCS5 cows had a greater expression compared with BCS4. In contrast, at 4 wk of lactation *FASN* expression was greater ($P < 0.05$) for B4F75 cows compared with B4F125 and B5F75. Compared with all other groups, B5F125 cows had an intermediate level of expression (Table 4).

RNA Expression of Inflammation and Immune-Related Proteins and Receptors. Among cytokine-encoding genes, *IL1B* was affected only by the interaction between BCS and feeding management (B × F, $P = 0.05$) due to the greater ($P < 0.05$) expression in B4F75 compared with B4F125 cows (Table 3). Cows with BCS5 had an intermediate expression level that did not differ ($P > 0.05$) due to feeding. A 3-way interaction was detected (B × F × T, $P < 0.005$) for *IL6* and *TNF* (Table 4). Both genes increased ($P < 0.05$) in expression postpartum across all groups except for B5F75, in which expression of *TNF* was constant over time ($P > 0.05$). Expression of *IL6* decreased immediately postpartum compared with −1 wk and then peaked at 4 wk of lactation (Table 4). Independent of feeding management, prepartum expression of *IL6* was greater ($P < 0.05$) in BCS5 compared with BCS4 cows, whereas B5F75 cows had the greatest ($P < 0.05$) expression of *TNF*. At 1 wk postpartum, both B4F75 and B5F125 groups had a higher ($P < 0.05$) expression of both *IL6* and *TNF*, but at 4 wk only *TNF* remained higher whereas *IL6* expression was greater only in B5F75 cows (Table 4).

Both acute-phase protein-related genes *HP* and *SAA3* were affected by time (T, $P < 0.004$), as their expression increased after calving (Table 4). A 3-way interaction (B × F × T, $P = 0.006$) and an effect of feeding level (F, $P = 0.02$) were detected for *HP*. In all groups, *HP* expression was higher at 1 and 4 wk postpartum compared with prepartum. In BCS4 animals, disregarding feeding regimen, the postpartum expression remained constant. However, in the BCS5F75 group the expression increased at 4 wk compared with 1 wk, whereas in BCS5F125 it peaked at 1 wk followed by a decrease at 4 wk to an intermediate level compared with the other 2 time points (Table 4).

Both *IL6R* and *TLR4*, cytokine receptor-encoding genes, were affected by time (T, $P < 0.0001$) due to greater expression postpartum. Both BCS and feeding management (B, F, $P < 0.004$) also affected *IL6R* and *TLR4*, as BCS5 or cows fed 75% of requirements had an overall greater ($P < 0.05$) expression. However, the interaction with time (B × T or F × T) underscored how this difference was only significant ($P < 0.05$) prepartum (Table 4). A 3-way interaction was detected for *TLR4*, revealing how the response in B5F75 cows due

Table 3. Effect of prepartum BCS and feeding management on subcutaneous adipose tissue expression ($\log_2$ back-transformed LSM) in dairy cows during the transition period

					B × F							
	B		F		4		5			*P*-value[2]		
Gene	4	5	75%	125%	75%	125%	75%	125%	SEM[1]	B	F	B × F
Inflammation												
CCL2	2.16	2.82	3.39^{x}	1.79^{y}	2.97	1.57	3.88	2.04	0.44	0.13	0.0005	0.99
CCL5	1.34^{x}	2.88^{y}	1.89	2.04	1.39	1.29	2.56	3.24	0.33	<0.0001	0.58	0.28
HP	0.17	0.25	0.28^{x}	0.16^{y}	0.26	0.11	0.30	0.22	0.17	0.13	0.02	0.31
IL1B	0.99	0.93	1.05	0.88	1.21^{x}	0.8^{y}	0.91^{xy}	0.96^{xy}	0.10	0.66	0.13	0.05
IL6	0.48^{x}	0.79^{y}	0.70	0.54	0.57	0.40	0.86	0.72	0.10	0.006	0.15	0.63
IL6R	0.82^{x}	1.08^{y}	1.08^{x}	0.82^{y}	0.90	0.75	1.30	0.90	0.11	0.005	0.004	0.32
RXRA	0.74^{x}	1.19^{y}	0.86^{x}	1.02^{y}	0.62^{x}	0.87^{y}	1.19^{z}	1.19^{z}	0.07	<0.0001	0.03	0.03
SAA3	0.53	0.39	0.42	0.49	0.52	0.55	0.34	0.43	0.12	0.27	0.61	0.77
TLR4	1.02^{x}	1.25^{y}	1.22^{x}	1.04^{y}	1.09	0.95	1.38	1.14	0.08	0.0005	0.007	0.65
TNF	0.78	0.93	0.87	0.83	0.90^{x}	0.68^{y}	0.84^{xy}	1.03^{x}	0.10	0.07	0.66	0.01
Adipokines and fatty acid metabolism												
ADIPOQ	0.72^{x}	1.28^{y}	0.87	1.05	0.59	0.88	1.29	1.27	0.11	<0.0001	0.09	0.07
FASN	0.15	0.17	0.13^{x}	0.21^{y}	0.12	0.19	0.13	0.23	0.17	0.33	0.0001	0.55
LEP	0.12^{x}	0.65^{y}	0.17^{x}	0.44^{y}	0.05^{x}	0.26^{y}	0.57^{z}	0.74^{z}	0.33	<0.0001	<0.0001	0.003
PPARG	0.86^{x}	1.08^{y}	0.94	0.99	0.81	0.93	1.10	1.07	0.09	0.008	0.52	0.33

[x–z]Different letters represent significant differences among groups ($P < 0.05$).

[1]SEM = greatest standard error of the mean.

[2]B = BCS (1–10 scale); F = level of feeding prepartum relative to requirements (%); B × F = interaction of BCS and level of feeding prepartum relative to requirements (%).

Table 4. Effect of the interaction among prepartum BCS, prepartum feeding management, and time around parturition on subcutaneous adipose tissue gene expression (log_2 back-transformed LSM) in dairy cows during the transition period

							B × F × T								
			B × T		F × T		4		5			*P*-value[2]			
Gene	Week	T[1]	4	5	75%	125%	75%	125%	75%	125%	SEM[1]	T	B × T	F × T	B × F × T
Inflammation															
CCL2	−1	2.11	1.63	2.72	2.41	1.84	1.57	1.70	3.72	1.99	1.57	0.28	0.17	0.23	0.19
	1	2.48	2.65	2.32	3.58	1.72	4.46	1.57	2.87	1.88					
	4	2.87	2.32	3.54	4.52	1.82	3.74	1.44	5.46	2.29					
CCL5	−1	1.09^{a}	0.69	1.75	1.18	1.01	$0.52^{a,x}$	$0.91^{a,x–z}$	2.71^{y}	$1.12^{a,z}$	1.53	<0.0001	0.25	0.33	<0.0001
	1	2.23^{b}	1.71	2.91	2.04	2.43	$2.11^{b,x}$	$1.38^{b,x}$	1.98^{x}	$4.26^{b,y}$					
	4	3.11^{c}	2.05	4.71	2.78	3.47	$2.48^{b,xy}$	$1.70^{b,x}$	3.12^{y}	$7.10^{b,z}$					
HP	−1	0.04^{a}	0.04	0.05	0.06	0.03	$0.06^{a,xy}$	$0.02^{a,x}$	$0.07^{a,y}$	$0.04^{a,xy}$	0.46	<0.0001	0.83	0.2	0.0006
	1	0.46^{b}	0.35	0.61	0.49	0.43	$0.68^{b,x–z}$	$0.18^{b,y}$	$0.36^{b,xy}$	$1.05^{b,z}$					
	4	0.46^{b}	0.40	0.54	0.73	0.29	$0.46^{b,x}$	$0.34^{b,x}$	$1.17^{c,y}$	$0.25^{c,x}$					
IL1B	−1	0.93	1.05	0.82	1.03	0.84	1.36	0.81	0.78	0.87	0.29	0.7	0.29	0.08	0.81
	1	0.93	0.85	1.03	1.18	0.73	1.15	0.62	1.22	0.87					
	4	1.02	1.08	0.96	0.96	1.09	1.15	1.01	0.80	1.16					
IL6	−1	0.53	0.34	0.82	0.62	0.46	$0.4^{a,xy}$	$0.29^{a,x}$	$0.96^{a,y}$	$0.71^{a,xy}$	0.41	0.44	0.16	0.29	0.002
	1	0.66	0.52	0.83	0.64	0.68	$0.76^{b,x–z}$	$0.36^{b,y}$	$0.53^{b,xy}$	$1.31^{b,z}$					
	4	0.66	0.62	0.71	0.86	0.50	$0.61^{b,xy}$	$0.63^{b,y}$	$1.23^{c,x}$	$0.41^{c,yz}$					
IL6R	−1	0.46^{a}	0.39	0.55	$0.64^{a,x}$	$0.33^{a,y}$	0.49	0.31	0.85	0.36	0.28	<0.0001	0.52	0.011	0.73
	1	1.38^{b}	1.30	1.47	1.52^{b}	1.26^{b}	1.41	1.21	1.64	1.32					
	4	1.31^{b}	1.11	1.54	1.30^{b}	1.31^{b}	1.08	1.14	1.58	1.51					
RXRA	−1	1.02	0.66^{x}	$1.59^{a,y}$	0.79^{x}	$1.32^{a,y}$	$0.40^{a,x}$	$1.07^{a,y}$	$1.55^{a,z}$	$1.63^{a,z}$	0.20	0.08	0.0002	0.001	0.01
	1	0.94	0.81^{x}	$1.08^{b,y}$	0.92	0.96^{b}	$0.79^{b,x}$	$0.83^{ab,x}$	$1.06^{b,x}$	$1.11^{b,x}$					
	4	0.86	0.75^{x}	$0.98^{b,y}$	0.88	0.83^{b}	$0.75^{b,xy}$	$0.75^{a,y}$	$1.04^{b,x}$	$0.93^{b,xy}$					
SAA3	−1	0.31^{a}	0.32	0.29	0.37	0.25	0.35	0.30	0.40	0.21	0.37	0.004	0.7	0.06	0.4
	1	0.60^{b}	0.71	0.50	0.45	0.79	0.58	0.89	0.36	0.71					
	4	0.51^{b}	0.65	0.40	0.45	0.58	0.69	0.62	0.29	0.55					
TLR4	−1	0.72^{a}	$0.55^{a,x}$	$0.93^{a,y}$	$0.87^{a,x}$	$0.59^{a,y}$	$0.59^{a,x}$	$0.52^{a,x}$	1.29^{y}	$0.67^{a,x}$	0.19	<0.0001	0.003	0.04	0.03
	1	1.27^{b}	1.24^{b}	1.30^{b}	1.32^{b}	1.22^{b}	1.38^{b}	1.12^{b}	1.26	1.33^{b}					
	4	1.59^{c}	1.54^{c}	1.63^{c}	1.60^{b}	1.57^{b}	1.60^{b}	1.48^{b}	1.60	1.67^{b}					
TNF	−1	0.58^{a}	0.51	0.65	0.68	0.49	$0.55^{a,xy}$	$0.48^{a,y}$	0.86^{x}	$0.49^{a,y}$	0.27	<0.0001	0.87	0.11	0.005
	1	1.10^{b}	1.04	1.16	1.04	1.16	$1.24^{b,xy}$	$0.88^{b,y}$	0.88^{y}	$1.53^{b,x}$					
	4	0.97^{b}	0.88	1.07	0.92	1.02	$1.07^{b,xy}$	$0.73^{ab,y}$	0.79^{y}	$1.44^{b,x}$					
Adipokines and fatty acid metabolism															
ADIPOQ	−1	1.13	0.95	1.35	0.99	1.29	0.69	1.32	1.43	1.26	0.26	0.07	0.21	0.49	0.24
	1	0.86	0.63	1.16	0.74	0.99	0.48	0.83	1.14	1.17					
	4	0.91	0.61	1.34	0.90	0.92	0.61	0.62	1.31	1.37					
FASN	−1	1.47^{a}	1.22	1.78	$0.68^{a,x}$	$3.22^{a,y}$	$0.47^{a,x}$	$3.15^{a,y}$	$0.97^{a,z}$	$3.29^{a,y}$	0.78	<0.0001	0.38	<0.0001	0.02
	1	0.05^{b}	0.04	0.05	0.04^{b}	0.05^{b}	0.04^{b}	0.05^{b}	0.05^{b}	0.05^{b}					
	4	0.06^{c}	0.07	0.06	0.07^{b}	0.06^{b}	$0.09^{c,x}$	$0.05^{b,y}$	$0.05^{b,y}$	$0.08^{b,xy}$					
LEP	−1	1.83^{a}	$0.96^{a,x}$	$3.49^{a,y}$	1.03	3.28	0.28	3.26	3.70	3.29	1.32	<0.0001	0.004	0.62	0.07
	1	0.20^{b}	$0.11^{b,x}$	$0.36^{b,y}$	0.14	0.29	0.07	0.17	0.28	0.47					
	4	0.06^{c}	$0.02^{c,x}$	$0.21^{b,y}$	0.04	0.09	0.01	0.03	0.17	0.27					
PPARG	−1	1.15^{a}	1.03	1.28	1.07	1.23	0.88	1.21	1.30	1.26	0.19	0.0004	0.92	0.72	0.7361
	1	1.03^{a}	0.93	1.13	1.00	1.05	0.89	0.97	1.13	1.12					
	4	0.77^{b}	0.67	0.88	0.78	0.76	0.67	0.67	0.90	0.86					

[a–c]Different letters represent significant differences among time points, within group ($P < 0.05$).
[x–z]Different letters represent significant differences among groups within the same week relative to parturition ($P < 0.05$).
[1]SEM = greatest standard error of the mean.
[2]T = time (week relative to parturition); B = BCS (1–10 scale); F = level of feeding prepartum relative to requirement (%); B × F × T = interaction of BCS, level of feeding prepartum relative to requirements (%), and time around parturition.

to the higher ($P < 0.05$) expression at −1 wk drove the difference prepartum between management groups. The expression of *TLR4* in B5F75 cows was stable over time and without changes ($P > 0.05$) among time points.

The expression of *CCL2* was only affected by feeding management (F, $P = 0.0005$) due to higher expression in F75 cows. Expression of *CCL5* had a significant BCS, time and 3-way interaction (B, T, B × F × T, $P < 0.0001$; Tables 3 and 4). Its expression was overall higher ($P < 0.05$) postpartum and greater ($P < 0.05$) in BCS5 cows independent of feeding, but the greatest ($P < 0.05$) expression was detected postpartum in B5F125 cows.

All factors except time affected *RXRA* ($P < 0.03$), for time only a tendency was detected ($P = 0.08$; Tables 3 and 4). Its expression was overall greater (B, F, $P < 0.05$) in BCS5 or cows fed 125% of requirements, and this was observed primarily prepartum.

MicroRNA Expression

Proadipogenic miRNA. Plane of nutrition affected both miR-378 and miR-143 (F, $P < 0.004$) due to greater expression ($P < 0.05$) in cows fed 125% of requirements (Table 5). The expression of miR-143 also had a significant 3-way interaction (B × F × T, $P = 0.03$) due to greater expression over time in B5F125 cows. The expression of miR-103 was affected by BCS alone (B, $P = 0.007$) or by the interaction with feeding (B × F, $P = 0.004$). Its expression was overall greater in BCS5 cows, and even greater when these animals were fed 125% of requirements during the dry period (B5F125). An effect of time (T, $P < 0.01$) was detected for the expression of miR-378 and miR-103 due to a decrease ($P < 0.05$) postpartum (Table 6).

The expression of miR-103 was greater overall in BCS5 cows (B, $P = 0.007$), and even greater when these animals were fed 125% of requirements during the dry period (B5F125; B × F, $P = 0.004$). The expression of miR-103 was greatest in B5F125 cows (B × F, $P = 0.004$).

Inflammation and Lipolysis. Expression of miR-99a (T, B × T, $P < 0.02$) and miR-145 (F × T, $P = 0.04$) decreased ($P < 0.05$) postpartum for BCS4 or F75 animals (Table 6). Feeding management affected expression (F, $P = 0.02$) of miR-145 and miR-99a in opposite directions [i.e., F75 increased ($P < 0.05$) miR-145 and decreased ($P < 0.05$) miR-99a relative to F125 cows (Table 5)]. For miR-145, the difference was mainly due to responses in the prepartum period (F × T, $P = 0.04$). For the expression of miR-221, BCS and feeding interacted differently across the groups (B × F, $P = 0.01$), as its expression was greater ($P < 0.05$) in B4F75 and B5F125 cows.

Adipose Infiltration of Immune Cells. MicroRNA-193 was only affected by time (T, $P < 0.0001$), as its expression decreased ($P < 0.05$) gradually from −1 to 4 wk relative to parturition (Table 6). The 3-way interaction was significant (B × F × T, $P < 0.04$) for miR-155 and miR-26b. For miR-155, this was due to an increase in expression ($P < 0.05$) postpartum and overall greater ($P < 0.05$) expression in BCS4

Table 5. Effect of prepartum BCS and feeding management on subcutaneous adipose tissue microRNA expression ($\log_2$ back-transformed LSM) in dairy cows during the transition period

	B		F		B × F							
					4		5			*P*-value[2]		
Gene	4	5	75%	125%	75%	125%	75%	125%	SEM[1]	B	F	B × F
Infiltration of immune cells												
miR-26b	1.35	1.52	1.65^{x}	1.24^{y}	1.64	1.11	1.65	1.39	0.17	0.27	0.007	0.30
miR-126	0.72	0.88	0.67^{x}	0.94^{y}	0.66	0.79	0.68	1.13	0.08	0.08	0.004	0.14
miR-132	1.23	1.46	1.55^{x}	1.15^{y}	1.48	1.03	1.63	1.30	0.12	0.07	0.002	0.45
miR-155	1.48^{x}	1.19^{y}	1.74^{x}	1.01^{y}	2.03	1.07	1.50	0.95	0.15	0.04	<0.0001	0.37
miR-193	1.05	1.05	1.08	1.03	1.02	1.09	1.15	0.97	0.11	0.99	0.50	0.16
Inflammation and lipolysis												
miR-99a	0.96	0.93	0.85^{x}	1.05^{y}	0.88	1.04	0.82	1.05	0.09	0.70	0.02	0.64
miR-145	1.23	1.40	1.50^{x}	1.15^{y}	1.43	1.06	1.56	1.25	0.12	0.23	0.02	0.70
miR-221	1.24	1.33	1.29	1.28	1.37^{xy}	1.11^{x}	1.20^{x}	1.47^{y}	0.09	0.33	0.93	0.01
Proadipogenic												
miR-103	0.97^{x}	1.16^{y}	1.04	1.08	1.05^{x}	0.90^{x}	1.04^{x}	1.30^{y}	0.07	0.007	0.61	0.004
miR-143	0.94	1.08	0.87^{x}	1.16^{y}	0.86	1.02	0.88	1.32	0.08	0.14	0.004	0.21
miR-378	1.06	0.94	0.80^{x}	1.25^{y}	0.87	1.30	0.73	1.21	0.16	0.19	<0.0001	0.58

$^{x-z}$Different letters represent significant differences among groups ($P < 0.05$).

[1]SEM = greatest standard error of the mean.

[2]B = BCS (1–10 scale); F = level of feeding prepartum relative to requirements (%); B × F = interaction of BCS and level of feeding prepartum relative to requirements (%).

Table 6. Effect of the interaction among prepartum BCS, prepartum feeding management, and time around parturition on subcutaneous adipose tissue microRNA expression (log_2 back-transformed LSM) in dairy cows during the transition period

							B × F × T								
			B × T		F × T		4		5			*P* value			
Gene	Week	T[1]	4	5	75%	125%	75%	125%	75%	125%	SEM[2]	T	B × T	F × T	B × F × T
Infiltration of immune cells															
miR-26b	−1	0.91[a]	1.10[x]	0.76[a,y]	1.01	0.82	1.37[x]	0.88[xy]	0.74[a,y]	0.77[a,y]	0.58	<0.0001	0.005	0.07	0.04
	1	1.69[b]	1.49	1.92[b]	2.31	1.24	1.78[x]	1.25[x]	2.99[b,y]	1.24[a,x]					
	4	1.89[b]	1.49[x]	2.39[b,y]	1.93	1.85	1.81[xy]	1.22[y]	2.05[b,x]	2.80[b,x]					
miR-126	−1	0.81[ab]	0.89[a]	0.73[a]	0.58[x]	1.14[a,y]	0.72	1.11	0.46	1.16	0.25	0.007	0.01	0.01	0.28
	1	0.93[a]	0.73[ab]	1.19[b]	0.80[x]	1.09[a,x]	0.62	0.87	1.03	1.37					
	4	0.66[b]	0.57[b]	0.77[a]	0.65	0.67b	0.65	0.50	0.65	0.91					
miR-132	−1	1.48	1.17[x]	1.86[a,y]	1.82	1.20	1.64	0.84	2.02	1.72	0.32	0.21	0.04	0.12	0.10
	1	1.31	1.36	1.28[b]	1.62	1.07	1.77	1.04	1.48	1.10					
	4	1.23	1.17	1.30[b]	1.27	1.20	1.11	1.24	1.45	1.16					
miR-155	−1	0.99[a]	1.01	0.97	1.47	0.67	1.46[a,x]	0.70[a,y]	1.49[ab,x]	0.63[a,y]	0.44	<0.0001	0.24	0.14	0.02
	1	1.30[b]	1.60	1.05	1.65	1.02	2.57[b,x]	1.00[a,y]	1.06[a,y]	1.04[b,y]					
	4	1.82[c]	2.00	1.67	2.19	1.52	2.25[ab,x]	1.77[b,xy]	2.13[b,x]	1.30[b,y]					
miR-193	−1	1.63[a]	1.61	1.65	1.68	1.59	1.65	1.57	1.70	1.60	0.29	<0.0001	0.10	0.66	0.07
	1	1.06[b]	1.18	0.95	1.13	0.99	1.27	1.09	1.01	0.89					
	4	0.68[c]	0.62	0.74	0.67	0.69	0.51	0.75	0.88	0.63					
Inflammation and lipolysis															
miR-99a	−1	1.11[a]	1.36[a,x]	0.91[y]	0.87	1.41	1.09	1.69	0.70	1.17	0.25	0.01	0.01	0.10	0.21
	1	0.93[ab]	0.87[b]	0.99	0.88	0.99	0.77	0.99	1.00	0.99					
	4	0.81[b]	0.75[b]	0.88	0.79	0.83	0.82	0.68	0.77	1.01					
miR-145	−1	1.41	1.26	1.58	1.86[a,x]	1.07[y]	1.80	0.88	1.93	1.30	0.32	0.40	0.62	0.04	0.16
	1	1.21	1.21	1.22	1.35[b]	1.10	1.45	1.01	1.26	1.19					
	4	1.31	1.22	1.42	1.33[b]	1.30	1.13	1.32	1.58	1.27					
miR-221	−1	1.30	1.13	1.49	1.45	1.16[a]	1.49	0.86	1.41	1.57	0.25	0.75	0.14	0.05	0.09
	1	1.23	1.22	1.25	1.24	1.23[ab]	1.42	1.05	1.08	1.45					
	4	1.31	1.36	1.27	1.18	1.46[b]	1.22	1.52	1.15	1.40					
Proadipogenic															
miR-103	−1	1.21[a]	1.08	1.35	1.24	1.17	1.34	0.87	1.16	1.57	0.20	0.01	0.45	0.42	0.15
	1	1.05[ab]	0.93	1.19	1.04	1.06	0.99	0.87	1.09	1.29					
	4	0.95[b]	0.91	0.98	0.88	1.02	0.88	0.95	0.88	1.08					
miR-143	−1	1.05	1.05	1.05	0.81	1.36	0.94[xy]	1.18[ab,x–z]	0.70[a,y]	1.58[z]	0.23	0.44	0.45	0.15	0.03
	1	1.03	0.90	1.18	0.93	1.15	0.74[x]	1.11[a,xy]	1.18[b,y]	1.18[y]					
	4	0.94	0.87	1.01	0.87	1.00	0.92[xy]	0.81[b,x]	0.83[ab,x]	1.24[y]					
miR-378	−1	1.86[a]	2.09	1.65	1.30	2.66	1.62	2.69	1.03	2.63	0.43	<0.0001	0.50	0.09	0.46
	1	1.01[b]	1.09	0.94	0.82	1.25	0.89	1.34	0.76	1.16					
	4	0.53[c]	0.52	0.54	0.47	0.59	0.45	0.61	0.49	0.58					

[a–c]Different letters represent significant differences among time points, within group ($P < 0.05$).

[x–z]Different letters represent significant difference among groups within the same week relative to parturition ($P < 0.05$).

[1]T = time (week relative to parturition); B = BCS (1–10 scale); F = level of feeding prepartum relative to requirement (%); B × F × T = interaction of BCS, level of feeding prepartum relative to requirements (%), and time around parturition.

[2]SEM = greatest standard error of the mean.

compared with BCS5 cows, and in cows fed 75% of requirements compared with 125% of requirements. Expression of miR-132 and miR-126 was affected by plane of nutrition (F, $P < 0.004$; Table 5); the former was greater ($P < 0.05$) in cows fed 75% of requirements whereas the latter was greater in cows fed 125% of requirements (especially prepartum). Both miRNA also were affected by the interaction of BCS with time (B × T, $P < 0.04$). Expression of miR-132 was greater in BCS5 cows, and postpartum its expression decreased to similar levels than BCS4 cows. Expression of miR-126 changed mainly due to time, with a decrease ($P < 0.05$) in expression immediately postpartum followed by an increase ($P < 0.05$) at 4 wk, at which point it reached prepartum levels only in BCS5 cows. This miRNA was also affected by the interaction of plane of nutrition and time (F × T, $P = 0.01$), mainly due to a higher ($P < 0.05$) expression prepartum in F125 cows and to a decrease ($P < 0.05$) in expression at 4 wk. In contrast, cows fed 75% of requirements maintained a constant level of expression ($P > 0.05$) throughout the experimental period.

DISCUSSION

Results from the present study enhance the understanding of molecular mechanisms underlying some of the physiological responses associated with both the independent and combined effects of prepartum BCS and feeding management. The relationship between the immune and metabolic response of the adipose tissue was evident both at the mRNA and miRNA expression level, underscoring the existence of a self-regulatory mechanism within the adipose depot. Together, the data generated could help develop more mechanistic approaches for utilizing dry period feeding and BCS management to manipulate the physiological response of subcutaneous adipose tissue during the transition period.

Adipocyte Metabolism and Adipokines

Around parturition, body reserves are mobilized extensively (i.e., there is little anabolism and substantial catabolism); the expression pattern of *FASN* over time exemplifies this adaptation. This enzyme is mainly regulated through expression rather than post-transcriptional mechanisms (Bionaz and Loor, 2008); thus, mRNA expression is a suitable indicator of its activity within adipocytes. The marked effect of prepartum overfeeding on *FASN* and the proadipogenic miRNA studied (miR-378 and miR-143) appears important for driving adipogenesis in adipose tissue. Furthermore, the greater prepartal *FASN* expression when cows of BCS5 compared with BCS4 were underfed may indicate their adipose depots were primed to deposit fat. This idea is further supported by the effect of BCS on expression of *PPARG* and *RXRA*, 2 nuclear receptors involved in promoting adipocyte differentiation and hypertrophy (Metzger et al., 2005; Bionaz et al., 2013), and also the expression of miR-103, an upregulator of adipogenesis (Romao et al., 2011; Trajkovski et al., 2011).

As exemplified by the responses in the BCS5 compared with BCS4 cows, the evident association between BCS and fat deposition might be due in part to greater flexibility of tissue preadipocytes to differentiate rather than enlargement of existing adipocytes (Kawada et al., 2001). The greater expression of *ADIPOQ* in BCS5 cows lends support to this hypothesis. In nonruminants, adiponectin improves insulin sensitivity and exerts some regulation over fatty acid metabolism (Brochu-Gaudreau et al., 2010). Its expression also is markedly increased during ruminant adipocyte differentiation (Roh et al., 2006). Thus, changes in *ADIPOQ* provide an indication of the degree of preadipocyte differentiation (Soliman et al., 2007).

The BCS, together with feeding management prepartum, also influenced the expression of leptin, another adipokine. Leptin, produced mainly in white adipose tissue, is a protein that, in rodents, is involved in the regulation of energy intake, storage, and expenditure (Chilliard et al., 2005). In cows and heifers, leptin is positively associated with both BCS and nutrient status (Reist et al., 2003; León et al., 2004), which explains its greater expression in both BCS5 and overfed cows. However, cow adiposity had a greater role because BCS alone increased prepartum *LEP* expression by 4-fold. Given that leptin could elicit a lipolytic effect (Harris, 2014), its greater expression postpartum along with higher fatty acids (Table 2) in cows with BCS5 might be beneficial in terms of helping them cope with the typical NEB of early lactation.

Inflammation and the Adipocyte

The mild-to-high systemic inflammation around parturition may impair production (Bertoni et al., 2008). However, recent data suggest that, when controlled, the inflammatory phenomena play a homeorhetic role in the adaptations of the modern dairy cow to transitioning into lactation (Farney et al., 2013; Vailati Riboni et al., 2015). As indicated by the higher *HP* and *SAA3* expression postpartum, cows in the current experiment most likely experienced a degree of peripartum inflammation. Both haptoglobin and serum amyloid A are well known proinflammatory biomarkers (Eckersall and Bell, 2010) mainly synthesized by the liver. However, it is well established in nonruminants that adipocytes

also produce these proteins and are recognized as adipokines (Trayhurn, 2005). The greater *HP* expression early postpartum in both B4F75 and B5F125 groups, compared with their opposite feeding in the same BCS group, is indicative of a higher degree of localized inflammation. Thus, avoiding high dietary energy prepartum (Selim et al., 2014; Shahzad et al., 2014) at an optimal BCS during the dry period (BCS5) while overfeeding under-conditioned cows during the close-up dry period could help alleviate excessive adipose tissue inflammation after calving. As a result of the lower production of proinflammatory mediators, the lipolytic sensitivity of adipose may be reduced. In that context, the peak in *HP* expression detected in optimally conditioned feed-restricted cows (B5F75) at 4 wk of lactation is noteworthy. Further research is needed to understand the biological mechanisms driving this response.

Apart from the systemic environment, nonruminant studies revealed that localized inflammation plays an important role in coordinating the physiology of the adipocyte. For instance, infiltration of fat depots by immune cells in obese individuals is one of the triggers of localized inflammation (Surmi and Hasty, 2008). This occurs via the expression of chemokines (chemoattractant cytokines), such as CCL2/MCP-1 (C-C motif chemokine ligand 2/macrophage chemoattractant protein-1) or CCL5/RANTES (C-C motif chemokine ligand 5/regulated on activation, normal T cell expressed and secreted; Neels and Olefsky, 2006). Although overfeeding generally increases chemokine expression, both in human and animal models (Lionetti et al., 2009; Ji et al., 2014b), feed-restriction in lactating goats also upregulated *CCL2* in adipose tissue (Faulconnier et al., 2011).

Underfed animals are generally in NEB and rely heavily on body reserves. Because inflammation induces lipolysis and insulin-resistance in nonruminant adipocytes (Tilg and Moschen, 2008), the upregulation of *CCL2* could serve as a homeostatic mechanism to reduce utilization of nutrients by adipose depots, hence, directing the greatest amount of glucose toward maintenance or milk synthesis. This idea is supported by the upregulation of miR-155, miR-26b, and miR-132 [all of which are markers of macrophage infiltration (Klöting et al., 2009)] in feed-restricted cows independently of BCS. The upregulation of these miRNA postpartum in all cows supports the idea of inflammation as a response to the physiological adaptations to lactation.

In contrast to other markers of immune cell infiltration, an additive effect of dry period conditioning on miR-155 was noted, as in early lactation the feed-restricted BCS4 cows had the greatest expression among all groups. Thus, feed-restricting thin cows prepartum could lead to an excess of localized inflammation that might impair the normal adaptation of the tissue to lactation. Based on the treatment effects detected, the expression of miR-126 (upregulated in overfed animals) and miR-193 (no change in relation to feeding management) did not seem to be associated with *CCL2*. In humans, both of these miRNA are involved in the transcriptional regulation of *CCL2* (Arner et al., 2012). This leads us to speculate that the same mechanism might not be present in bovine adipocytes.

The downregulation of miR-99a along with upregulation of miR-145, especially prepartum in feed-restricted cows, offers support to the hypothesis of induced local inflammation via the recruitment of immune cells, likely augmenting tissue lipolytic sensitivity. Because in humans miR-99a abundance is negatively correlated with concentration of fatty acids within the adipocyte (Klöting et al., 2009) and miR-45 regulates adipocyte lipolysis through different mechanisms (Lorente-Cebrián et al., 2014), their expression patterns imply a higher degree of mobilization in feed-restricted cows, which is partly supported by the higher fatty acids prepartum for the entire group of cows reported in Roche et al. (2015).

In contrast to feeding level being a primary driver of *CCL2* expression, prepartum cow adiposity (BCS) appeared to be the main effector of *CCL5* expression. Compared with CCL2, CCL5 is a chemokine with a broader spectrum that is not only able to recruit monocytes and promote their survival (Keophiphath et al., 2010), but also mediates the trafficking and homing of T cells, basophils, eosinophils, natural killer cells, dendritic cells, and mast cells (Appay and Rowland-Jones, 2001). As in the obesity model (Huber et al., 2008), the effect of BCS5 on *CCL5* expression could be attributed in part to the higher adiposity, because *CCL2* also was numerically higher at BCS5. Its expression was further increased postpartum in cows that underwent overfeeding at BCS5 over the precalving period.

The nuclear receptor *RXRA* can play a key role in the regulation of innate immunity by modulating expression of other chemokines (e.g., CCL6, CCL9; Nuñez et al., 2010). The fact that its expression followed the same trend as *CCL5*, with increased expression in BCS5 cows and overfed cows, especially prepartum, could have contributed to excessive localized inflammation, greater insulin resistance, and greater lipolytic sensitivity. Such effects could be even greater in cows with BCS higher than 5, as overconditioned cows seem to mobilize more body reserves; hence, they are more prone to metabolic disorders, such as ketosis (Roche et al., 2009; Pires et al., 2013).

Adipose tissue is an active producer of cytokines, primarily by nonfat cells, and their release is enhanced in response to local inflammation (Fain, 2006). In turn,

the cytokines released can affect adipose metabolism (Coppack, 2001). Because tumor necrosis factor α is one of the main cytokines produced by infiltrated immune cells, especially macrophages (Weisberg et al., 2003; Xu et al., 2003), and it increases lipolysis (Cawthorn and Sethi, 2008), the greater expression of *TNF* in cows with higher BCS that were overfed indicates they might be most susceptible to having pathological increases in blood fatty acids. Interleukin-6, another cytokine known in nonruminants for its lipolytic effects (Yang et al., 2008), also was upregulated early postpartum in the same animals. Regardless of time around parturition, the upregulation of *IL6R* in BCS5 cows indicates that they were more susceptible to IL-6. Thus, as proven in overconditioned cows at parturition, an increase in the local sensitivity to IL-6 would lead to excess lipolysis in early lactation and threaten the liver (e.g., increasing triacylglycerol accumulation and impairing its functions; Roche et al., 2009; Akbar et al., 2015). In contrast to overfeeding, the postpartum prolipolytic effect of feed restriction seems to be increased at a lower degree of adiposity. For example, expression of *IL1B*, encoding a cytokine involved in insulin resistance and lipolysis in adipocytes (Lagathu et al., 2006), was markedly increased in feed-restricted BCS4 cows. This is another example of a prolonged effect of prepartum feeding management into early lactation.

Despite what is known thus far regarding the effect of BCS and plane of nutrition in the dry period, the combination of greater BCS (BCS5) with feed-restriction prepartum seems to affect precalving expression of *CCL5*, *TLR4*, and *IL6*. Of particular interest, from an inflammation standpoint, is the LPS receptor TLR4, because in nonruminants it links innate immunity with fatty acid-induced insulin resistance (Shi et al., 2006). The prepartal response in *CCL5* and *IL6* would be expected to trigger an inflammatory response close to calving, and the upregulation of *TLR4* would induce insulin resistance, both of which favor lipid mobilization mechanisms in cows with optimal BCS. As such, the adaptations in these gene networks serve to prime the adipocytes for the demands of early lactation. It is noteworthy that the transcriptional response of these genes was lost postpartum. Thus, the link between these genes in relationship with BCS and dietary management prepartum needs further research.

CONCLUSIONS

Overfeeding optimally conditioned cows during the last 3 wk before parturition primed adipose tissue for accretion of lipid and a robust localized inflammatory response, which upon parturition increases the probability for metabolic disorders; that is, localized inflammation renders the adipocyte more susceptible to lipolytic signals that could result in greater flow of fatty acids into liver. Similarly, prepartum nutrient restriction of thinner cows enhances the localized proinflammatory response of adipocytes, hence eliciting a similar negative outcome. Overall, the combined data indicate that a regimen of nutrient restriction prepartum in optimally conditioned cows avoids detrimental effects at the adipose tissue level, hence physiologically priming the cow to the demands of lactation and avoiding a metabolically lazy phenotype. Instead, thinner animals seem to benefit from a higher plane of nutrition, with beneficial effects in terms of controlling localized inflammation.

ACKNOWLEDGMENTS

This research was supported by New Zealand dairy farmers through DairyNZ Inc. (RD1403), and the Ministry of Business, Innovation and Employment (Wellington, New Zealand; DRCX1201). The authors thank Jason Philips, farm manager, Scott Farm, DairyNZ, and his capable team, as well as Stu Morgan, Kate Watkins, Hamish Hodgson, and Chris Roach for technical support.

REFERENCES

Akbar, H., T. M. Grala, M. V. Riboni, F. C. Cardoso, G. Verkerk, J. McGowan, K. Macdonald, J. Webster, K. Schutz, S. Meier, L. Matthews, J. R. Roche, and J. J. Loor. 2015. Body condition score at calving affects systemic and hepatic transcriptome indicators of inflammation and nutrient metabolism in grazing dairy cows. J. Dairy Sci. 98:1019–1032.

Appay, V., and S. L. Rowland-Jones. 2001. Rantes: A versatile and controversial chemokine. Trends Immunol. 22:83–87.

Arner, E., N. Mejhert, A. Kulyte, P. J. Balwierz, M. Pachkov, M. Cormont, S. Lorente-Cebrian, A. Ehrlund, J. Laurencikiene, P. Heden, K. Dahlman-Wright, J. F. Tanti, Y. Hayashizaki, M. Ryden, I. Dahlman, E. van Nimwegen, C. O. Daub, and P. Arner. 2012. Adipose tissue microRNAs as regulators of CCL2 production in human obesity. Diabetes 61:1986–1993.

Arner, P., and A. Kulyte. 2015. MicroRNA regulatory networks in human adipose tissue and obesity. Nat. Rev. Endocrinol. 11:276–288.

Bertoni, G., E. Trevisi, X. Han, and M. Bionaz. 2008. Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows. J. Dairy Sci. 91:3300–3310.

Bionaz, M., G. J. Hausman, J. J. Loor, and S. Mandard. 2013. Physiological and nutritional roles of PPAR across species. PPAR Res. 2013:807156.

Bionaz, M., and J. J. Loor. 2008. Gene networks driving bovine milk fat synthesis during the lactation cycle. BMC Genomics 9:366.

Brochu-Gaudreau, K., C. Rehfeldt, R. Blouin, V. Bordignon, B. D. Murphy, and M. F. Palin. 2010. Adiponectin action from head to toe. Endocrine 37:11–32.

Cawthorn, W. P., and J. K. Sethi. 2008. TNF-alpha and adipocyte biology. FEBS Lett. 582:117–131.

Chilliard, Y., C. Delavaud, and M. Bonnet. 2005. Leptin expression in ruminants: nutritional and physiological regulations in relation with energy metabolism. Domest. Anim. Endocrinol. 29:3–22.

Coppack, S. W. 2001. Pro-inflammatory cytokines and adipose tissue. Proc. Nutr. Soc. 60:349–356.

Dann, H. M., N. B. Litherland, J. P. Underwood, M. Bionaz, A. D'Angelo, J. W. McFadden, and J. K. Drackley. 2006. Diets during far-off and close-up dry periods affect periparturient metabolism and lactation in multiparous cows. J. Dairy Sci. 89:3563–3577.

Eckersall, P. D., and R. Bell. 2010. Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. Vet. J. 185:23–27.

Fain, J. N. 2006. Release of interleukins and other inflammatory cytokines by human adipose tissue is enhanced in obesity and primarily due to the nonfat cells. Vitam. Horm. 74:443–477.

Farney, J. K., L. K. Mamedova, J. F. Coetzee, B. KuKanich, L. M. Sordillo, S. K. Stoakes, J. E. Minton, L. C. Hollis, and B. J. Bradford. 2013. Anti-inflammatory salicylate treatment alters the metabolic adaptations to lactation in dairy cattle. Am. J. Physiol. Regul. Integr. Comp. Physiol. 305:R110–R117.

Faulconnier, Y., Y. Chilliard, M. B. Torbati, and C. Leroux. 2011. The transcriptomic profiles of adipose tissues are modified by feed deprivation in lactating goats. Comp. Biochem. Physiol. Part D Genomics Proteomics 6:139–149.

Grala, T. M., J. R. Roche, C. V. Phyn, A. G. Rius, R. H. Boyle, R. G. Snell, and J. K. Kay. 2013. Expression of key lipid metabolism genes in adipose tissue is not altered by once-daily milking during a feed restriction of grazing dairy cows. J. Dairy Sci. 96:7753–7764.

Grant, R. W., and V. D. Dixit. 2015. Adipose tissue as an immunological organ. Obesity (Silver Spring) 23:512–518.

Graugnard, D. E., K. M. Moyes, E. Trevisi, M. J. Khan, D. Keisler, J. K. Drackley, G. Bertoni, and J. J. Loor. 2013. Liver lipid content and inflammometabolic indices in peripartal dairy cows are altered in response to prepartal energy intake and postpartal intramammary inflammatory challenge. J. Dairy Sci. 96:918–935.

Harris, R. B. 2014. Direct and indirect effects of leptin on adipocyte metabolism. Biochim. Biophys. Acta 1842:414–423.

Huber, J., F. W. Kiefer, M. Zeyda, B. Ludvik, G. R. Silberhumer, G. Prager, G. J. Zlabinger, and T. M. Stulnig. 2008. CC chemokine and CC chemokine receptor profiles in visceral and subcutaneous adipose tissue are altered in human obesity. J. Clin. Endocrinol. Metab. 93:3215–3221.

Janovick, N. A., Y. R. Boisclair, and J. K. Drackley. 2011. Prepartum dietary energy intake affects metabolism and health during the periparturient period in primiparous and multiparous Holstein cows. J. Dairy Sci. 94:1385–1400.

Ji, P., J. K. Drackley, M. J. Khan, and J. J. Loor. 2014a. Overfeeding energy upregulates peroxisome proliferator-activated receptor (PPAR)gamma-controlled adipogenic and lipolytic gene networks but does not affect proinflammatory markers in visceral and subcutaneous adipose depots of Holstein cows. J. Dairy Sci. 97:3431–3440.

Ji, P., J. K. Drackley, M. J. Khan, and J. J. Loor. 2014b. Overfeeding energy upregulates peroxisome proliferator-activated receptor (PPAR)gamma-controlled adipogenic and lipolytic gene networks but does not affect proinflammatory markers in visceral and subcutaneous adipose depots of Holstein cows. J. Dairy Sci. 97:3431–3440.

Jin, W., M. V. Dodson, S. S. Moore, J. A. Basarab, and L. L. Guan. 2010. Characterization of microRNA expression in bovine adipose tissues: a potential regulatory mechanism of subcutaneous adipose tissue development. BMC Mol. Biol. 11:29.

Jin, W., J. R. Grant, P. Stothard, S. S. Moore, and L. L. Guan. 2009. Characterization of bovine miRNAs by sequencing and bioinformatics analysis. BMC Mol. Biol. 10:90.

Kawada, T., N. Takahashi, and T. Fushiki. 2001. Biochemical and physiological characteristics of fat cell. J. Nutr. Sci. Vitaminol. (Tokyo) 47:1–12.

Keophiphath, M., C. Rouault, A. Divoux, K. Clement, and D. Lacasa. 2010. CCL5 promotes macrophage recruitment and survival in human adipose tissue. Arterioscler. Thromb. Vasc. Biol. 30:39–45.

Khan, M. J., C. B. Jacometo, D. E. Graugnard, M. N. Correa, E. Schmitt, F. Cardoso, and J. J. Loor. 2014. Overfeeding dairy cattle during late-pregnancy alters hepatic PPARalpha-regulated pathways including hepatokines: Impact on metabolism and peripheral insulin sensitivity. Gene Regul. Syst. Bio. 8:97–111.

Klöting, N., S. Berthold, P. Kovacs, M. R. Schon, M. Fasshauer, K. Ruschke, M. Stumvoll, and M. Bluher. 2009. MicroRNA expression in human omental and subcutaneous adipose tissue. PLoS ONE 4:e4699.

Lagathu, C., L. Yvan-Charvet, J. P. Bastard, M. Maachi, A. Quignard-Boulange, J. Capeau, and M. Caron. 2006. Long-term treatment with interleukin-1beta induces insulin resistance in murine and human adipocytes. Diabetologia 49:2162–2173.

León, H. V., J. Hernandez-Ceron, D. H. Keislert, and C. G. Gutierrez. 2004. Plasma concentrations of leptin, insulin-like growth factor-I, and insulin in relation to changes in body condition score in heifers. J. Anim. Sci. 82:445–451.

Lionetti, L., M. P. Mollica, A. Lombardi, G. Cavaliere, G. Gifuni, and A. Barletta. 2009. From chronic overnutrition to insulin resistance: The role of fat-storing capacity and inflammation. Nutr. Metab. Cardiovasc. Dis. 19:146–152.

Lorente-Cebrián, S., N. Mejhert, A. Kulyte, J. Laurencikiene, G. Astrom, P. Heden, M. Ryden, and P. Arner. 2014. MicroRNAs regulate human adipocyte lipolysis: Effects of miR-145 are linked to TNF-alpha. PLoS ONE 9:e86800.

McGown, C., A. Birerdinc, and Z. M. Younossi. 2014. Adipose tissue as an endocrine organ. Clin. Liver Dis. 18:41.

Metzger, D., T. Imai, M. Jiang, R. Takukawa, B. Desvergne, W. Wahli, and P. Chambon. 2005. Functional role of RXRs and PPARgamma in mature adipocytes. Prostaglandins Leukot. Essent. Fatty Acids 73:51–58.

Musi, N., and R. Guardado-Mendoza. 2014. Adipose tissue as an endocrine organ. Cell. Endocr. Health Dis. 2014:229–237.

Neels, J. G., and J. M. Olefsky. 2006. Inflamed fat: What starts the fire? J. Clin. Invest. 116:33–35.

Nuñez, V., D. Alameda, D. Rico, R. Mota, P. Gonzalo, M. Cedenilla, T. Fischer, L. Bosca, C. K. Glass, A. G. Arroyo, and M. Ricote. 2010. Retinoid X receptor alpha controls innate inflammatory responses through the up-regulation of chemokine expression. Proc. Natl. Acad. Sci. USA 107:10626–10631.

Pires, J. A. A., C. Delavaud, Y. Faulconnier, D. Pomies, and Y. Chilliard. 2013. Effects of body condition score at calving on indicators of fat and protein mobilization of periparturient Holstein-Friesian cows. J. Dairy Sci. 96:6423–6439.

Randall, L. V., M. J. Green, M. G. Chagunda, C. Mason, S. C. Archer, L. E. Green, and J. N. Huxley. 2015. Low body condition predisposes cattle to lameness: An 8-year study of one dairy herd. J. Dairy Sci. 98:3766–3777.

Reist, M., D. Erdin, D. von Euw, K. Tschuemperlin, H. Leuenberger, C. Delavaud, Y. Chilliard, H. M. Hammon, N. Kuenzi, and J. W. Blum. 2003. Concentrate feeding strategy in lactating dairy cows: metabolic and endocrine changes with emphasis on leptin. J. Dairy Sci. 86:1690–1706.

Roche, J. R., P. G. Dillon, C. R. Stockdale, L. H. Baumgard, and M. J. VanBaale. 2004. Relationships among international body condition scoring systems. J. Dairy Sci. 87:3076–3079.

Roche, J. R., N. C. Friggens, J. K. Kay, M. W. Fisher, K. J. Stafford, and D. P. Berry. 2009. Invited review: Body condition score and its association with dairy cow productivity, health, and welfare. J. Dairy Sci. 92:5769–5801.

Roche, J. R., E. S. Kolver, and J. K. Kay. 2005. Influence of precalving feed allowance on periparturient metabolic and hormonal responses and milk production in grazing dairy cows. J. Dairy Sci. 88:677–689.

Roche, J. R., S. Meier, A. Heiser, M. D. Mitchell, C. G. Walker, M. A. Crookenden, M. Vailati Riboni, J. J. Loor, and J. K. Kay. 2015. Effects of pre-calving body condition score and prepartum feeding level on production, reproduction, and health parameters in pasture-based transition dairy cows. J. Dairy Sci. In press.

Roh, S.-G., D. Hishikawa, Y.-H. Hong, and S. Sasaki. 2006. Control of adipogenesis in ruminants. Anim. Sci. J. 77:472–477.

Romao, J. M., W. Jin, M. V. Dodson, G. J. Hausman, S. S. Moore, and L. L. Guan. 2011. MicroRNA regulation in mammalian adipogenesis. Exp. Biol. Med. (Maywood) 236:997–1004.

Romao, J. M., W. Jin, M. He, T. McAllister, and L. L. Guan. 2012. Altered microRNA expression in bovine subcutaneous and vis-

ceral adipose tissues from cattle under different diet. PLoS ONE 7:e40605.
Selim, S., S. Salin, J. Taponen, A. Vanhatalo, T. Kokkonen, and K. T. Elo. 2014. Prepartal dietary energy alters transcriptional adaptations of the liver and subcutaneous adipose tissue of dairy cows during the transition period. Physiol. Genomics 46:328–337.
Shahzad, K., M. Bionaz, E. Trevisi, G. Bertoni, S. L. Rodriguez-Zas, and J. J. Loor. 2014. Integrative analyses of hepatic differentially expressed genes and blood biomarkers during the peripartal period between dairy cows overfed or restricted-fed energy prepartum. PLoS ONE 9:e99757.
Shi, H., M. V. Kokoeva, K. Inouye, I. Tzameli, H. Yin, and J. S. Flier. 2006. TLR4 links innate immunity and fatty acid-induced insulin resistance. J. Clin. Invest. 116:3015–3025.
Soliman, M., K. Kimura, M. Ahmed, D. Yamaji, Y. Matsushita, Y. Okamatsu-Ogura, K. Makondo, and M. Saito. 2007. Inverse regulation of leptin mRNA expression by short- and long-chain fatty acids in cultured bovine adipocytes. Domest. Anim. Endocrinol. 33:400–409.
Surmi, B. K., and A. H. Hasty. 2008. Macrophage infiltration into adipose tissue: Initiation, propagation and remodeling. Future Lipidol. 3:545–556.
Tilg, H., and A. R. Moschen. 2008. Insulin resistance, inflammation, and non-alcoholic fatty liver disease. Trends Endocrinol. Metab. 19:371–379.
Trajkovski, M., J. Hausser, J. Soutschek, B. Bhat, A. Akin, M. Zavolan, M. H. Heim, and M. Stoffel. 2011. MicroRNAs 103 and 107 regulate insulin sensitivity. Nature 474:649–653.
Trayhurn, P. 2005. Endocrine and signalling role of adipose tissue: new perspectives on fat. Acta Physiol. Scand. 184:285–293.
Trevisi, E., M. Amadori, S. Cogrossi, E. Razzuoli, and G. Bertoni. 2012. Metabolic stress and inflammatory response in high-yielding, periparturient dairy cows. Res. Vet. Sci. 93:695–704.
Vailati Riboni, M., S. Meier, N. V. Priest, C. R. Burke, J. K. Kay, S. McDougall, M. D. Mitchell, C. G. Walker, M. Crookenden, A. Heiser, J. R. Roche, and J. J. Loor. 2015. Adipose and liver gene expression profiles in response to treatment with a nonsteroidal antiinflammatory drug after calving in grazing dairy cows. J. Dairy Sci. 98:3079–3085.
Weisberg, S. P., D. McCann, M. Desai, M. Rosenbaum, R. L. Leibel, and A. W. Ferrante Jr. 2003. Obesity is associated with macrophage accumulation in adipose tissue. J. Clin. Invest. 112:1796–1808.
Xu, H., G. T. Barnes, Q. Yang, G. Tan, D. S. Yang, C. J. Chou, J. Sole, A. Nichols, J. S. Ross, L. A. Tartaglia, and H. Chen. 2003. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J. Clin. Invest. 112:1821–1830.
Yang, Y., D. Ju, M. Zhang, and G. Yang. 2008. Interleukin-6 stimulates lipolysis in porcine adipocytes. Endocrine 33:261–269.