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Overfeeding a moderate energy diet prepartum does not impair bovine subcutaneous adipose tissue insulin signal transduction and induces marked changes in peripartal gene network expression[1]

P. Ji,*[2] J. S. Osorio,* J. K. Drackley,*† and J. J. Loor*†[3]
*Department of Animal Sciences, and
†Division of Nutritional Sciences, University of Illinois, Urbana 61801

ABSTRACT

Mechanisms regulating subcutaneous adipose tissue (SAT) insulin sensitivity and gene network expression during the peripartal period were evaluated in cows fed to meet or exceed prepartal energy requirements. Holstein cows were dried off at −50 d relative to expected parturition and fed a controlled-energy diet [CON; net energy for lactation = 1.24 Mcal/kg of dry matter (DM); 36% of DM as wheat straw] until −21 d. Cows were then randomly assigned (n = 7/diet) to either the same CON diet or a moderate-energy close-up diet (OVE; net energy for lactation = 1.47 Mcal/kg of DM) until parturition. Biopsies of SAT were harvested at −10, 7, and 21 d for mRNA expression of 48 genes associated with insulin signaling, adipogenesis, and lipolysis. In vitro basal and insulin-stimulated insulin receptor substrate 1 tyrosine phosphorylation (IRS1-PY) was assessed at −10 and 7 d. The OVE led to more positive energy balance and greater serum insulin concentration prepartum. Compared with CON, OVE led to a more drastic increase in serum NEFA and also greater overall serum BHBA postcalving, both of which were associated with greater hepatic total lipid and triacylglycerol concentration. Close-up OVE did not improve any aspect of performance. In prepartal SAT, insulin-stimulated IRS1-PY was greater in OVE than in CON. However, IRS1-PY, serum insulin, and *GLUT4* expression decreased postpartum regardless of prepartal treatment, suggesting a more severe state of insulin resistance. The expression of all genes encoding adipogenic regulators (*PPARG* and *ZFP423*), most lipogenic enzymes/inducers (*FASN*, *SCD*, *DGAT2*, and *INSIG1*), and basal-lipolysis regulators (*ATGL* and *ABDH5*) was greater at −10 d in OVE than in CON. Whereas adipogenic and basal lipolysis regulator expression remained greater in cows fed OVE by 7 d postpartum, expression of all lipogenic enzymes decreased regardless of diet. Despite those responses, the approximately 3-fold increase in expression of *IRS1* and *ZFP423* between 7 and 21 d suggested that insulin responsiveness and adipogenic capacity of SAT were partially restored. Expression of the preadipocyte marker *DLK1*, adiponutrin (*PNPLA3*), and fibroblast growth factor 21 (*FGF21*) was undetectable. Results suggested that close-up energy overfeeding did not exacerbate insulin resistance in SAT. Signs of restored insulin responsiveness (upregulation of *IRS1*, *INSIG2*, *SREBF1*, and *ZFP423*) were apparent as early as 3 wk postpartum. Thus, identifying specific nutrients capable of activating PPARγ after calving in AT might help accelerate its replenishment. A regulatory network encompassing the genes and physiological measurements obtained is proposed.
Key words: adipose tissue, insulin signaling, lipolysis, dietary energy

INTRODUCTION

Insulin resistance (**IR**) has been defined as either decreased sensitivity (the insulin concentration to induce half-maximal response) or responsiveness (the maximal response) of insulin-sensitive tissues [primarily adipose tissue (**AT**) and skeletal muscle] to insulin (Kahn, 1978). The peripartal period in ruminants has long been thought to represent a physiological state of peripheral IR, and considered an important homeorhetic adaptation to the onset of lactation (Bauman and Currie, 1980). Besides the hypoinsulinemia during early lactation, the apparent IR in AT is linked with a sharp increase in circulating NEFA, particularly after parturition (Bell, 1995). Overmobilization of AT stores is one of the major causes of metabolic disorders postpartum including ketosis and fatty liver (Drackley et al., 2001).

The underlying molecular mechanisms associated with peripartal IR remain largely unknown. Vernon and Taylor (1988) observed compromised insulin sensitivity

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[2]Current address: Miner Research Institute, 1034 Miner Farm Road PO Box 100, Chazy, NY 12921.
[3]Corresponding author: jloor@illinois.edu

in AT of lactating compared with dry sheep, which could not be attributed to changes in receptor number or insulin-receptor binding affinity; they suggested the possibility of postreceptor defects, which have not been evaluated in ruminants. Tyrosine phosphorylation (**PY**) of insulin receptor substrates (**IRS**) is the first step initiating intracellular insulin signal transduction. Accumulating evidence from rodent and human studies has revealed that a defect in tyrosine PY of insulin receptor substrate 1 (**IRS1-PY**) is causal of peripheral IR either during late pregnancy (Sevillano et al., 2007) or in cases of obesity-induced type II diabetes (Esposito et al., 2001; Sesti et al., 2001). Hence, the IRS1-PY state in AT could be used as a key parameter to assess IR in AT of peripartal dairy cows.

Dry cow diets have been examined as a way to improve feed intake, energy balance, metabolic profiles, and health during the transition period. The steam-up dietary approach during the last few weeks of gestation (i.e., feeding a higher-energy diet primarily by increasing cereal grains) has been used in the field for several years. However, studies from different research groups demonstrated that prepartal overfeeding of energy with greater NFC has often resulted in prepartal hyperglycemia and hyperinsulinemia and marked adipose tissue mobilization (i.e., greater blood NEFA concentration) at the initiation of lactation (Rukkwamsuk et al., 1999; Holtenius et al., 2003; Janovick et al., 2011). As a consequence, energy-overfed cows often have greater hepatic lipid accumulation and are more susceptible to metabolic disorders postpartum (Dann et al., 2006; Janovick et al., 2011). Together, these observed signs provide some evidence that overfeeding energy during the dry period can affect prepartal IR and probably alters lipid metabolism of AT, of which the carryover effects can persist into early lactation.

The hypotheses that we sought to test in the present research were that prepartal energy overfeeding exacerbates IR in subcutaneous AT (**SAT**) by impairing 1) tyrosine phosphorylation of IRS-1 and altering 2) expression of genes encoding the major adipogenic transcription regulators and enzymes involved in lipogenesis and lipolysis. Specific objectives were to examine the basal and insulin-stimulated phosphorylation state of IRS-1 and corresponding changes in the expression of 48 genes.

MATERIALS AND METHODS

Experimental Design, Diet, and Animals

All procedures were conducted under protocols approved by the University of Illinois Institutional Animal Care and Use Committee. Fourteen multiparous Holstein cows were used in this study. All cows were dried off at d −50 relative to expected parturition and fed a controlled-energy diet (NE_L = 1.24 Mcal/kg of DM; Table 1) containing wheat straw at 36% of DM for ad libitum intake during the far-off dry period (i.e., d −50 to d −21). During the close-up period (i.e., d −21 to parturition), cows were randomly assigned either to a moderate-energy diet (**OVE**; NE_L = 1.47 Mcal/kg of DM) or continuously fed the same controlled-energy diet (**CON**) for ad libitum intake. The same lactation diet (NE_L = 1.65 Mcal/kg of DM) was provided for all animals postpartum until 30 DIM.

Management, Sampling, and Analyses

Complete details are presented in the Supplemental Materials (available online at http://www.journalofdairyscience.org/). Briefly, cows were housed in a freestall barn with Calan gates (American Calan Inc., Northwood, NH) during the dry period (dry-off at −50 d relative to expected parturition). At 3 d before expected parturition, cows were moved to individual maternity pens in the same barn until parturition. After parturition, cows were housed in a tie-stall barn and milked 3 times daily. Prepartal and postpartal diets were fed as a TMR (Table 1). Energy balance was calculated pre- and postpartum individually for each cow based on the equations from NRC (2001; see details in the Supplemental Materials, available online at http://www.journalofdairyscience.org/).

Blood was sampled from the coccygeal vein or artery every Monday and Thursday before the morning feeding from −21 to 30 d. Samples were collected into evacuated serum tubes containing clot activator (BD Vacutainer; BD and Co., Franklin Lakes, NJ). Serum was obtained by centrifugation at 1,300 × *g* for 15 min and frozen at −20°C until later analysis. Serum insulin concentration was analyzed with a commercial bovine insulin ELISA kit (catalog #10–1201–01; Mercodia AB, Uppsala, Sweden). Concentrations of BHBA and NEFA were analyzed using commercial kits at the Veterinary Diagnostics Laboratory, College of Veterinary Medicine, University of Illinois (Urbana).

SAT and Liver Biopsy and Tissue Handling

Complete details are presented in the Supplemental Materials (available online at http://www.journalofdairyscience.org/). Briefly, SAT biopsies were collected before the morning feeding from alternate sides of the tail-head region at −10, 7, and 21 d. Adipose tissue (2 to 4 g) was collected by blunt dissection. The incision

was then closed with surgical staples (Multi-Shot Disposable Skin Stapler; Henry Schein, Melville, NY) and iodine ointment was applied to the wound. A portion of tissue was snap frozen in liquid N_2 until RNA extraction. The remaining tissue was prepared for culture by quickly rinsing in prewarmed (~37°C) sterile 1 × PBS solution and coarsely minced to minimize risk of hypoxia. Liver biopsy was conducted as described previously (Dann et al., 2006). Additional details can be found in the Supplemental Materials (available online at http://www.journalofdairyscience.org/).

SAT Explants and Protein Assay

Complete details are presented in the Supplemental Materials (available online at http://www.journalofdairyscience.org/). Briefly, tissue was carefully processed under a laminar flow hood to remove adjacent nonadipose tissue, chopped into small pieces of ca. 10 mg, and placed into sterile Petri dishes (catalog #5662–7161; Fisher Scientific, Pittsburgh, PA). Samples were then transferred into 12-well culture plates (catalog #CLS3512; Sigma-Aldrich, St. Louis, MO), which contained 1.5 mL of 37°C 1 × Dulbecco's modified Eagle's medium (DMEM) in each well. Tissue was preincubated in a water-jacketed CO_2 incubator (at 37°C with 5% CO_2) for 30 min for adaptation. After 30 min of adaptation, 2 samples were removed and served as duplicate negative controls (i.e., the 0-min sample before insulin challenge). The remaining samples were transferred into a new culture plate, which contained 1.5 mL of 37°C 1 × DMEM in each well with or without addition of 1 μmol/L bovine insulin (catalog #I0516, Sigma-Aldrich; 10 mg/mL of bovine insulin in 25 m*M* HEPES, pH 8.2, sterile-filtered). Culture plates were then incubated in the same conditions as listed above. Duplicate samples with or without insulin were removed from plates at 15, 30, and 60 min of incubation.

After removal, tissue samples were immediately immersed into 1.5 mL of ice-cold 1× cell lysis buffer (catalog #9803; Cell Signaling Technology Inc., Danvers, MA) containing 1 m*M* phenylmethylsulfonyl fluoride (PMSF; protease inhibitor, catalog #P7626; Sigma-Aldrich) and were quickly homogenized. Then, tissue was sonicated in an ice bath 2 times for ca. 10 s each time to break down the nuclear membrane. Samples were then centrifuged at 8,984 × *g* at 4°C for 10 min and the supernatant was carefully transferred into 1.5-mL tubes without disturbing the lipid layer, and stored at −80°C until protein analysis.

Total protein was analyzed with the BCA protein assay kit (Cat. #23227; Thermo Scientific, Fairmont, NJ). Total IRS-1 and IRS-1 pan-tyrosine phosphorylation (IRS1-pY) were analyzed with a PathScan Total IRS-1 Sandwich ELISA kit (catalog #7328; Cell Signaling Technology Inc.) and PathScan Phospho-IRS-1(pan Tyr) Sandwich ELISA kit (catalog #7133; Cell Signaling Technology Inc.) according to the manufacturer's instructions. The phosphorylation level of IRS-1 was calculated by dividing absorbance values of IRS1-pY at 450 nm by absorbance values of total IRS-1 at 450 nm.

Table 1. Ingredient and chemical composition of diets

	Diet[1]		
Component	CON	OVE	Lactation
Ingredient (% of DM)			
Alfalfa silage	12.00	8.20	5.00
Alfalfa hay	—	3.50	4.00
Corn silage	33.00	35.90	33.00
Wheat straw	36.00	15.40	4.00
Cottonseed	—	—	3.50
Wet brewers grains	—	6.00	10.00
Ground shelled corn	4.00	13.00	22.20
Soy hulls	2.00	4.00	4.00
Soybean meal, 48% CP	7.94	3.10	3.30
Expeller soybean meal[2]	—	2.00	6.20
SoyChlor[3]	0.15	3.80	—
Blood meal 85%	1.00	1.00	0.30
Urea	0.45	0.30	0.14
Rumen-inert fat[4]	—	—	1.00
Limestone	1.30	1.30	1.18
Salt (plain)	0.32	0.30	0.27
Sodium bicarbonate	—	—	0.75
Potassium carbonate	—	—	0.10
Calcium sulfate	—	—	0.10
Dicalcium phosphate	0.12	0.18	0.27
Magnesium oxide	0.21	0.08	0.14
Magnesium sulfate	0.91	0.97	—
Mineral-vitamin mix[5]	0.20	0.20	0.20
Vitamin A[6]	0.015	0.015	—
Vitamin D[7]	0.025	0.025	—
Vitamin E[8]	0.38	0.38	—
Biotin	—	0.35	0.35
DM of diet[9] (%)	47.1 ± 2.0	46.6 ± 0.8	45.2 ± 1.5
Chemical analysis			
NE_L (Mcal/kg)	1.24	1.47	1.65
CP (% of DM)	14.6	15.6	16.3
ADF (% of DM)	36.2	30.2	24.1
NDF (% of DM)	52.7	44.7	37.9

[1]The control diet (CON) was fed to all cows during the far-off dry period (−50 to −21 d relative to expected calving); OVE = overfeeding of a moderate-energy diet.

[2]SoyPLUS (West Central Soy, Ralston, IA).

[3]SoyChlor (West Central Soy).

[4]Energy Booster 100 (MSC, Carpentersville, IL).

[5]Contained a minimum of 5% Mg, 10% S, 7.5% K, 2.0% Fe, 3.0% Zn, 3.0% Mn, 5,000 mg of Cu/kg, 250 mg of I/kg, 40 mg of Co/kg, 150 mg of Se/kg, 2,200 kIU of vitamin A/kg, 660 kIU of vitamin D_3/kg, and 7,700 IU of vitamin E/kg.

[6]Contained 30,000 kIU/kg.

[7]Contained 5,009 kIU/kg.

[8]Contained 44,000 IU/kg.

[9]Means ± SD.

RNA Extraction, Primer Design and Evaluation, Internal Control Gene Selection, and Real-Time Quantitative PCR

Complete details are presented in the Supplemental Materials (available online at http://www.journalofdairyscience.org/). Briefly, we evaluated 8 candidate internal control genes (**ICG**) using GeNorm software (Vandesompele et al., 2002). Analysis revealed that genes encoding β actin (***ACTB***), ribosomal protein S9 (***RPS9***) and glyceraldehyde-3-phosphate dehydrogenase (***GAPDH***) were the most stably expressed and were used as ICG. Target genes were normalized with the geometric mean of the 3 ICG. Relative mRNA abundance was calculated for each gene among all of the genes tested in this study to provide additional mechanistic information on the target genes (Bionaz and Loor, 2008).

Statistical Analysis

To avoid problems with fitting covariance structure (Janovick and Drackley, 2010; Janovick et al., 2011), pre- and postpartal data for DMI, NE_L intake, energy balance, serum concentrations of insulin, BHBA, and NEFA were analyzed separately as a completely randomized design using the MIXED procedure of SAS (version 9.1; SAS Institute Inc., Cary, NC). The fixed effects in the model were close-up treatment (**Trt**), day or week, and Trt × time (Trt × d or Trt × wk) for each variable analyzed. The REPEATED statement was used for variables measured over time (DMI, NE_L intake, energy balance, milk yield, milk components, and serum insulin, BHBA, and NEFA). Autoregressive covariance structure was the best fit for these data as determined by the lowest Akaike's information criterion.

Phosphorylation of IRS1-PY from the negative control samples was analyzed as a 2 × 2 factorial arrangement in a completely randomized design using the MIXED procedure of SAS to evaluate the effects of close-up diets (Trt) and day relative to parturition. Cow within treatment and day was used as the random effect. The data for IRS1-PY from samples after insulin challenge were analyzed in a split-plot completely randomized design with the same SAS program to evaluate the main effects of Trt, day, and insulin challenge time (time) and all interactions. The estimate statement was used to analyze the difference of IRS1-PY between the negative control sample (0 min, before insulin challenge) and insulin-challenged samples (15, 30, and 60 min). Cow within treatment and day was designated a random effect. The relative mRNA abundance data of the tested genes were normalized with the geometric mean of 3 ICG. To ensure normal distribution of residuals, the ICG-normalized data were subjected to square root transformation. This final data set was analyzed as a 2 (Trt) × 3 (day) factorial arrangement in a completely randomized design, with day as a repeated measure using a PROC MIXED model. Contrasts were conducted for genes with significant interactions. Gene expression raw *P*-values were adjusted for multiple testing comparisons using false discovery rate (**FDR**) in SAS. Significant difference was declared at $P < 0.05$ and tendency at $P < 0.1$, and for gene expression at an FDR-adjusted $P \leq 0.05$ (raw $P \leq 0.02$).

RESULTS

Performance, Serum Insulin and Metabolites, and Hepatic Lipid Accumulation

Compared with CON, OVE cows had greater DMI ($P = 0.05$), NE_L intake ($P < 0.01$), energy balance ($P < 0.01$), BCS ($P = 0.01$), and serum insulin concentration ($P < 0.01$) during the close-up period and tended ($P = 0.06$) to have greater circulating concentration of BHBA postcalving (Table 2). When comparing changes of serum NEFA from close-up to postpartum within each group, energy overfeeding resulted in greater ($P = 0.04$) NEFA increase postcalving (Table 2; Supplemental Figures 1–4, available online at http://www.journalofdairyscience.org/). That response corresponded with the more drastic decrease in energy balance experienced by OVE cows (Table 2).

No difference in milk performance was observed between the 2 groups except for a tendency ($P = 0.10$) for lower milk protein percentage in OVE cows (Table 2; Supplemental Figure 2, available online at http://www.journalofdairyscience.org/). Although postpartal compared with prepartal hepatic total lipid and triglyceride (**TAG**) concentration was greater in both groups, OVE cows had a more pronounced increase in both indices (Figure 1). Despite the greater liver TAG content, only 2 of the cows in the OVE group were classified as ketotic and 1 of those maintained normal intake relative to the entire group. Per farm protocols, these cows received oral propylene glycol and returned to normal within 5 d of treatment (i.e., before 14 DIM).

IRS-1 Tyrosine Phosphorylation

In the absence of the insulin challenge in vitro, the close-up dietary energy plane did not affect ($P > 0.10$) IRS1 phosphorylation at −10 or 7 d (Figure 2). Supraphysiological bovine insulin challenge significantly

Table 2. Effect of close-up energy overfeeding (OVE, n = 7) or feeding to meet energy requirements (CON, n = 7) on DMI, NE_L intake, blood metabolites, and production of dairy cows (all data are presented as means ± SEM)

	Treatment		*P*-value		
Item	CON	OVE	Treatment (T)	Week (W)	T × W
DMI[1] (kg/d)					
Close-up	12.6 ± 0.70	14.8 ± 0.72	0.05	0.02	0.53
Postpartum, d 1–7	13.0 ± 1.84	15.3 ± 1.85	0.39	<0.01	0.12
Postpartum, d 1–28	16.7 ± 1.84	17.0 ± 1.84	0.92	<0.01	0.02
NE_L intake[1] (MJ/d)					
Close-up	65.4 ± 4.07	91.1 ± 4.07	<0.01	<0.01	0.22
Postpartum	115.4 ± 12.5	117.8 ± 12.5	0.89	<0.01	0.05
Energy balance[2] (% of requirements)					
Close-up	107.9 ± 4.60	139.9 ± 4.11	<0.01	0.03	0.23
Postpartum	60.4 ± 6.59	67.7 ± 6.56	0.67	<0.01	0.13
Change, post vs. close-up	−39.7 ± 5.24	−68.1 ± 4.74	<0.01	—	—
BCS[3] (5-point scale)					
Close-up	3.13 ± 0.05	3.39 ± 0.05	0.01	0.72	0.38
Postpartum	2.72 ± 0.06	2.87 ± 0.06	0.15	<0.01	0.54
Change, post vs. close-up	−0.39 ± 0.06	−0.52 ± 0.06	0.20	—	—
BW (kg)					
Close-up	798.1 ± 7.0	811.5 ± 7.0	0.21	0.36	0.49
Postpartum	684.0 ± 13.5	699.2 ± 13.5	0.46	<0.01	0.05
Change, post vs. close-up	−108.5 ± 10.2	−118.3 ± 10.2	0.48	—	—
Serum insulin (μg/L)					
Close-up	0.37 ± 0.04	0.58 ± 0.03	<0.01	<0.01	0.10
Postpartum	0.14 ± 0.03	0.21 ± 0.03	0.12	0.09	0.65
Serum NEFA (mEq/L)					
Close-up	0.39 ± 0.05	0.27 ± 0.05	0.16	<0.01	0.23
Postpartum	0.79 ± 0.09	1.01 ± 0.09	0.15	0.03	0.24
Change, post- vs. close-up[4]	+0.40 ± 0.09	+0.68 ± 0.09	0.04	—	—
Serum BHBA (mmol/L)					
Close-up	0.47 ± 0.04	0.44 ± 0.04	0.53	0.02	0.42
Postpartum	0.88 ± 0.11	1.20 ± 0.11	0.06	<0.01	0.40
Milk yield[5] (kg/d)	41.7 ± 2.23	41.2 ± 2.23	0.82	<0.01	0.85
3.5% FCM yield[6] (kg/d)	46.4 ± 2.67	47.4 ± 2.45	0.79	<0.01	0.09
ECM yield[7] (kg/d)	47.7 ± 2.43	45.9 ± 2.39	0.61	0.15	0.87
Milk fat					
%	4.47 ± 0.21	4.43 ± 0.19	0.86	0.07	0.94
kg/d	1.78 ± 0.11	1.81 ± 0.10	0.83	0.10	0.27
Milk protein					
%	3.26 ± 0.09	3.04 ± 0.09	0.10	<0.01	0.63
kg/d	1.32 ± 0.07	1.21 ± 0.07	0.32	0.09	0.26
Milk lactose					
%	4.60 ± 0.09	4.71 ± 0.09	0.42	<0.01	<0.01
kg/d	1.89 ± 0.12	1.93 ± 0.12	0.78	<0.01	<0.01

[1]DMI and NE_L intake were analyzed based on daily data from 1 to 28 d.

[2]The energy balance of each animal was calculated weekly for data from −3 to 4 wk relative to parturition.

[3]The BCS of each animal at −4 wk was used as the covariate for both close-up and postpartum data. The postpartum BCS consists of data at 1 to 3 wk. Estimate statement was used to compare the effect of close-up dietary energy planes on serum NEFA change of postpartum vs. close-up period.

[4]Estimate statement was used to compare the effect of close-up dietary energy planes on serum NEFA change of postpartum vs. close-up period. Data set is composed of serum NEFA concentrations at −14, −8, and −3 d for close-up period and 2, 8, 14, and 21 d postpartum of each animal with 7 cows per treatment.

[5]Milk yield was analyzed based on daily data from 1 to 30 d.

[6]Fat-corrected milk = 0.4324 × (milk yield) + 16.2162 × (fat yield). Milk samples from the first week were not used to calculate FCM.

[7]Energy-corrected milk = (0.327 × milk yield) + (12.95 × fat yield) + (7.2 × protein yield).

increased IRS1-PY in tissue from −10 ($P < 0.001$) and 7 d ($P < 0.001$; caption of Figure 2). Lower ($P = 0.05$) IRS1-PY was observed for both groups at 7 compared with −10 d. The same pattern across time points (day, $P = 0.02$) was observed for IRS1-PY in tissues challenged with insulin, although values of IRS1-PY were increased to a greater magnitude in response to insulin (Figure 2).

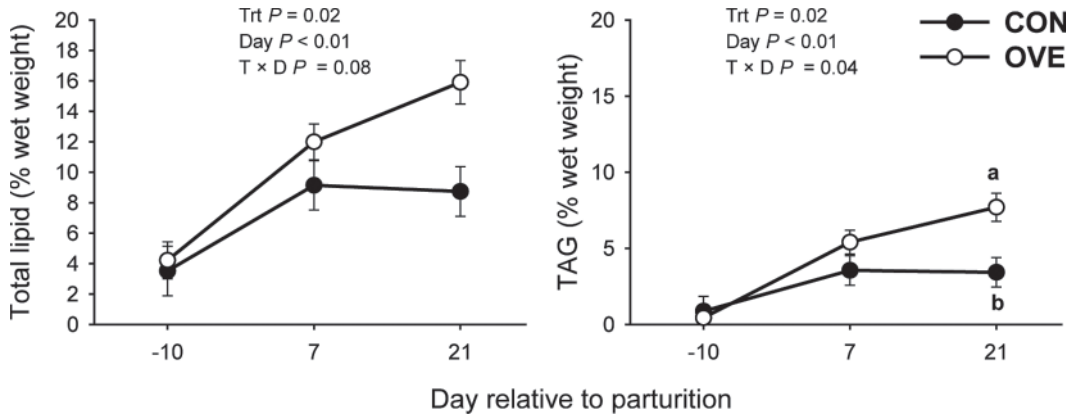

Figure 1. Liver triacylglycerol (TAG) and total lipid content (% of wet tissue) at −10, 7, and 21 d relative to parturition in cows overfed a moderate-energy diet (OVE, n = 7) or fed to meet energy requirements (CON, n = 7) during the close-up period. Values are expressed as mean ± SEM. The *P*-values for main effect of treatment (Trt) and day and interaction of treatment by day (T × D) are shown. A contrast statement was used for mean separation within treatments when the interaction was significant ($P < 0.05$) at the same day relative to parturition. In those instances, significant differences ($P < 0.05$) are denoted with lowercase a and b.

Gene Expression

Expression of the preadipocyte marker delta-like 1 homolog (*DLK1*, or *PREF1*) was only detectable in approximately 30% of samples at −10 and 7 d (data not shown), whereas expression of the triacylglycerol lipase adiponutrin (*ADPN* or *PNPLA3*) and fibroblast growth factor 21 (*FGF21*) was undetectable in most samples. Thus, data for these genes were not analyzed statistically.

Insulin Signaling Pathway. As shown in Figure 3, no treatment or interaction effect (FDR-adjusted $P = 0.10$) was detected for insulin receptor (***INSR***) mRNA expression and other components of postreceptor signaling v-akt murine thymoma viral oncogene homolog 1 (*AKT1* and *AKT2*). Expression of *AKT2* decreased overall after calving (day $P = 0.001$). Overfeeding compared with CON resulted in greater overall ($P \leq 0.05$) 3-phosphoinositide dependent protein kinase-1 (***PDPK1***) and sterol regulatory element-binding protein cleavage-activating protein (***SCAP***) and a tendency ($P = 0.13$) of greater overall *IRS1* expression due to the response at −10 and 7 d for *PDPK1* and *SCAP*, and at 21 d postcalving for *IRS1*. In fact, expression of *IRS1* mRNA exhibited the greatest response among these set of genes because it increased ($P < 0.001$) markedly (>3 fold) due to OVE at 21 compared with 7 d. Despite the greater expression of *SCAP*, a coactivator of sterol regulatory element binding transcription factor 1 (**SREBF1**; Foufelle and Ferré, 2002), the

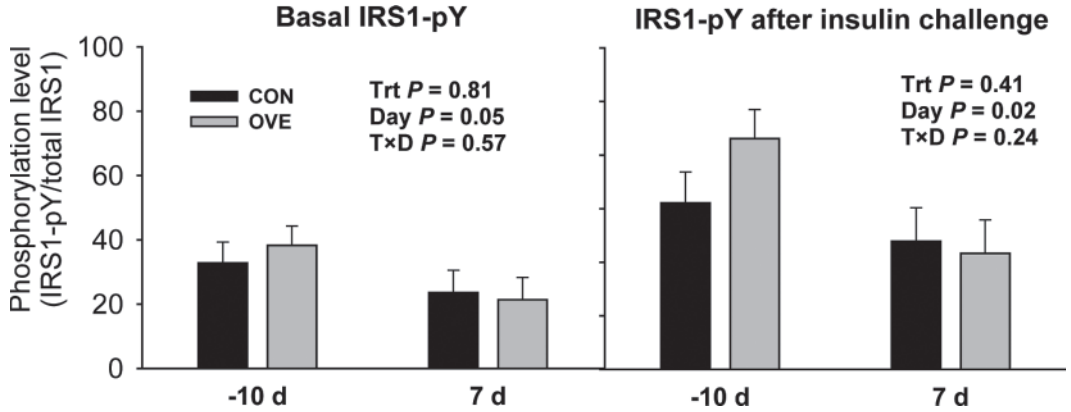

Figure 2. In vitro basal or insulin-stimulated insulin receptor substrate 1 (IRS-1) tyrosine phosphorylation (IRS1-PY) level in adipose tissue (AT) from cows overfed a moderate-energy diet (OVE, n = 7) or fed to meet energy requirements (CON, n = 6) during the close-up period. Values are expressed as mean ± SEM. The difference of IRS1-PY between negative control samples and insulin-challenged samples was −39.2% at −10 d ($P < 0.001$) and −23.1% at 7 d ($P < 0.001$).

expression of the well-established insulin-induced lipogenic transcription regulator *SREBF1* was lower (Trt × day, $P = 0.02$) for OVE at −10 d; cows fed CON experienced a decrease in *SREBF1* expression between −10 and 7 d and even though expression increased by 21 d, it remained lower than at −10 d. The expression of eukaryotic translation initiation factor 4E-binding protein 1 (***EIF4EBP1***) and phosphodiesterase 3B (***PDE3B***) between −10 and 7 d increased (day, $P < 0.001$); however, expression of both genes was greater overall (Trt, $P \leq 0.05$) in response to feeding CON, which was primarily due to levels observed at −10 and 7 d (Figure 3).

Cows fed OVE had greater mRNA expression of the insulin-induced glucose transporter 4 (***GLUT4***) at −10 d ($P < 0.05$), but the expression was sharply downregulated ($P < 0.05$) postpartum in both groups. A Trt × day interaction ($P < 0.05$) was observed for the expression of the facilitated glucose transporter *GLUT1* due to an increase between 7 and 21 d, at which point expression was greater in cows fed CON (Figure 3). It should be noted, however, that the relative mRNA abundance of *GLUT4* was substantially greater than *GLUT1*, underscoring the key role of *GLUT4* in AT glucose uptake (Supplemental Table 1, available online at http://www.journalofdairyscience.org/).

Adipogenesis and Lipogenesis Control. As shown in Figure 4, a significant ($P < 0.05$) main effect of prepartal plane of dietary energy was detected for mRNA expression of the major adipogenic transcription regulator peroxisome proliferator-activated receptor γ (***PPARG***; Rosen and MacDougald, 2006), whereas expression of CCAAT/enhancer-binding protein α (***CEBPA***) tended ($P = 0.08$) to differ. The main effect was primarily due to feeding OVE compared with CON leading to a remarkably greater ($P < 0.05$) expression of *PPARG* at both −10 and 7 d (Figure 4). Similar responses were observed for insulin induced gene 1 (***INSIG1***) and *INSIG2*, 2 key players in preadipocyte differentiation (Rosen and MacDougald, 2006), namely due to greater ($P < 0.02$) expression at −10 d in response to OVE. The PPARγ-regulated adipokine adiponectin (***ADIPOQ***) tended ($P = 0.07$) to be greater overall due to feeding OVE, namely due to responses at −10 and 7 d. Despite the fact that angiopoietin-related protein 4 (***ANGPTL4***) has been recently recognized as a PPARγ target in mice AT (Dutton and Trayhurn, 2008), its expression was lower (Trt × day, $P = 0.01$) at −10 in cows fed OVE compared with those fed CON. Marked upregulation of *ANGPTL4* occurred between −10 and 7 d, regardless of treatment; however, by d 21 expression remained high in cows fed CON but decreased in cows fed OVE leading to lower (Trt × day, $P = 0.01$) expression.

Despite what appeared to be a robust proadipogenic response around parturition, the expression of the recently-discovered PPARγ regulator zinc finger protein 423 (***ZFP423***; Gupta et al., 2010) did not differ due to treatment ($P = 0.98$) or Trt × day ($P = 0.54$) but its expression increased (day $P < 0.001$) 2 fold between −10 through 21 d (Figure 4). Furthermore, the expression of the PPARγ coactivator lipin-1 (***LPIN1***) did not differ prepartum but increased (Trt × day, $P = 0.01$) ca. 2 fold between −10 and 7 d in cows fed OVE compared with CON, followed by a decrease to prepartal levels by 21 d (Figure 4).

FA Uptake, de novo Lipogenesis, and Desaturation. In agreement with the robust proadipogenic response observed prepartum, close-up energy overfeeding resulted in a coordinated upregulation (Trt × day, $P \leq 0.03$) of genes involved in FA uptake and activation [lipoprotein lipase (***LPL***), acyl-CoA synthetase short-chain family member 2 (***ACSS2***), acyl-CoA synthetase long-chain family member 1 (***ACSL1***)], de novo FA synthesis and desaturation [ATP citrate lyase (***ACLY***), acetyl-CoA carboxylase α (***ACACA***), FA synthase (***FASN***), lactate dehydrogenase B (***LDHB***), stearoyl-CoA desaturase (*SCD*)], NADPH production and glyceroneogenesis [glucose-6-phosphate dehydrogenase (*G6PD*), isocitrate dehydrogenase 1 (*IDH1*), and phosphoenolpyruvate carboxykinase 1 (***PCK1***)], and TAG synthesis [glycerol-3-phosphate acyltransferase 1, mitochondrial (***GPAM***), diacylglycerol *O*-acyltransferase 2 (***DGAT2***)] at −10 d (Figure 5). The overall day effect ($P < 0.001$) observed for the expression of these genes clearly was due to the marked decrease in expression in cows fed OVE between −10 and 7 d, after which expression remained unchanged (except for *LDHB*). In cows fed CON, few genes (e.g., *FASN*, *ACSS2*, *GPAM*, and *DGAT2*) had a marked change in expression at −10 vs. 7 and 21 d. Expression of solute carrier family 27 (FA transporter), member 1 (***SLC27A1***), cluster of differentiation 36 (***CD36***), FA-binding protein 4 (***FABP4***), and 1-acylglycerol-3-phosphate *O*-acyltransferase 6 (*AGPAT6*) increased, regardless of treatment (day, $P < 0.02$) by 21 d (Figure 5).

Lipolysis Control. Prepartal energy overfeeding led to greater (Trt × day, $P < 0.05$) mRNA expression at −10 d of the regulatory enzyme controlling basal lipolysis adipose triglyceride lipase (***ATGL***), and despite a decrease in expression by 7 d, cows fed OVE still had greater *ATGL* expression at that point relative to CON (Figure 6). That pattern of expression was accompanied by a greater overall (Trt $P < 0.03$) expression of the coactivator abhydrolase domain containing 5 (***ABHD5***). Similar to *ATGL*, expression of monoglyceride lipase (***MGLL***) was greater (Trt × day, $P < 0.05$) at −10 d and then decreased by 7 d,

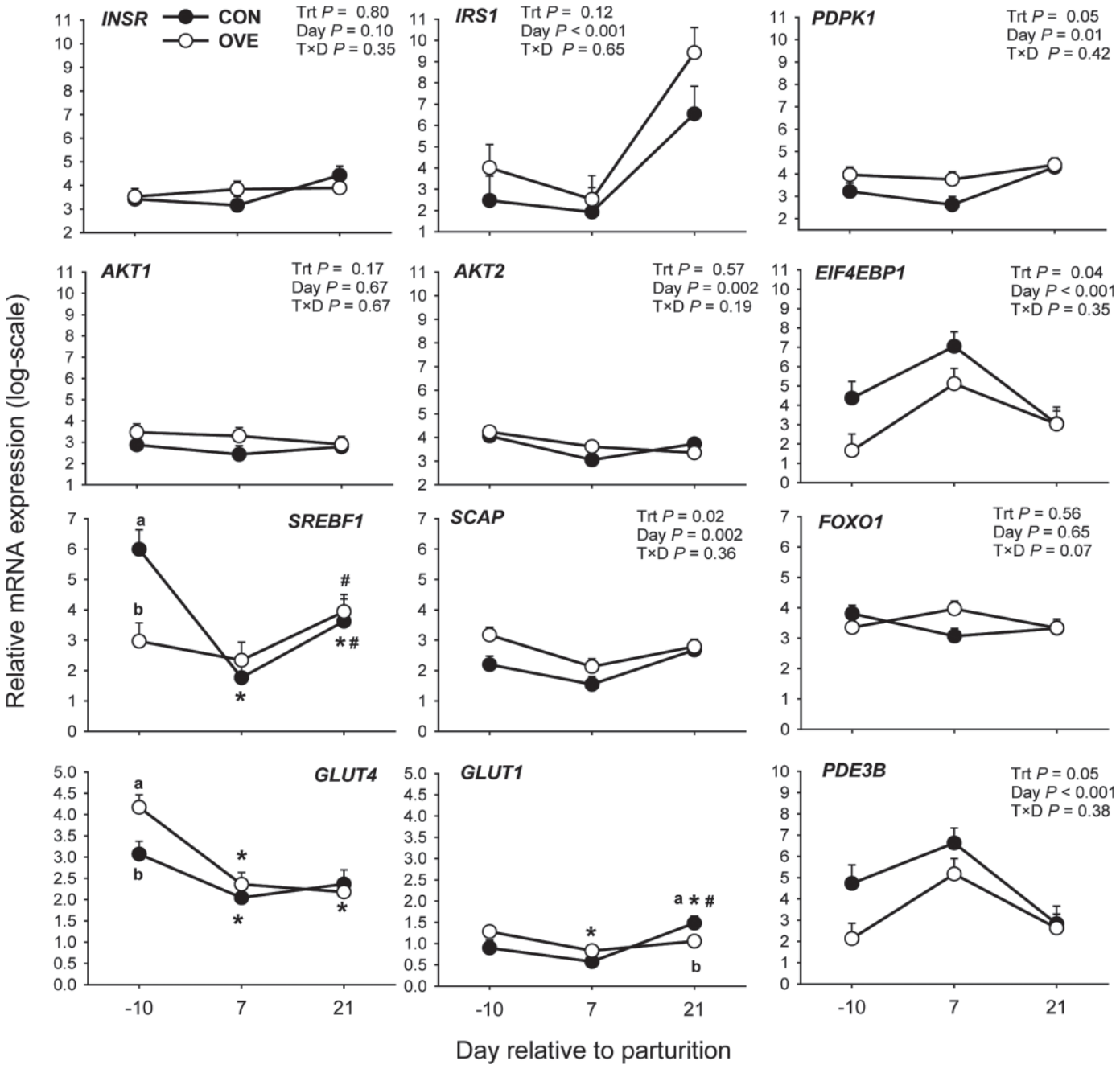

Figure 3. Expression of genes involved in insulin binding [insulin receptor (*INSR*)] and postreceptor signaling [insulin receptor substrate 1 (*IRS1*), 3-phosphoinositide dependent protein kinase-1 (*PDPK1*), v-akt murine thymoma viral oncogene homolog 1 (*AKT1*), *AKT2*, phosphodiesterase 3B (*PDE3B*), eukaryotic translation initiation factor 4E-binding protein 1 (*EIF4EBP1*)], insulin-regulated transcriptional control of lipogenesis [sterol regulatory element-binding protein cleavage-activating protein (*SCAP*) and sterol regulatory element binding transcription factor 1 (*SREBF1*)], and glucose transport [insulin-induced glucose transporter 1 (*GLUT1*) and *GLUT4*] in subcutaneous adipose tissue of cows overfed a moderate-energy diet (OVE, n = 7) or fed to meet energy requirements (CON, n = 7) during the close-up period. Values are expressed as mean ± SEM. The false discovery rate-adjusted *P*-values for main effect of treatment (Trt) and day and interaction of treatment × day (T × D) are shown only for those genes without significant interactions (T × D). For genes with significant interaction ($P < 0.05$), mean separation was evaluated via contrasts at the same day relative to parturition and significant differences ($P < 0.05$) are denoted with lowercase a and b. Mean separation between time points and within treatments were evaluated via contrasts and significant differences ($P < 0.05$) between d −10 and 7 or 21 are denoted with an asterisk (*), and between d 7 and 21 by a pound sign (#).

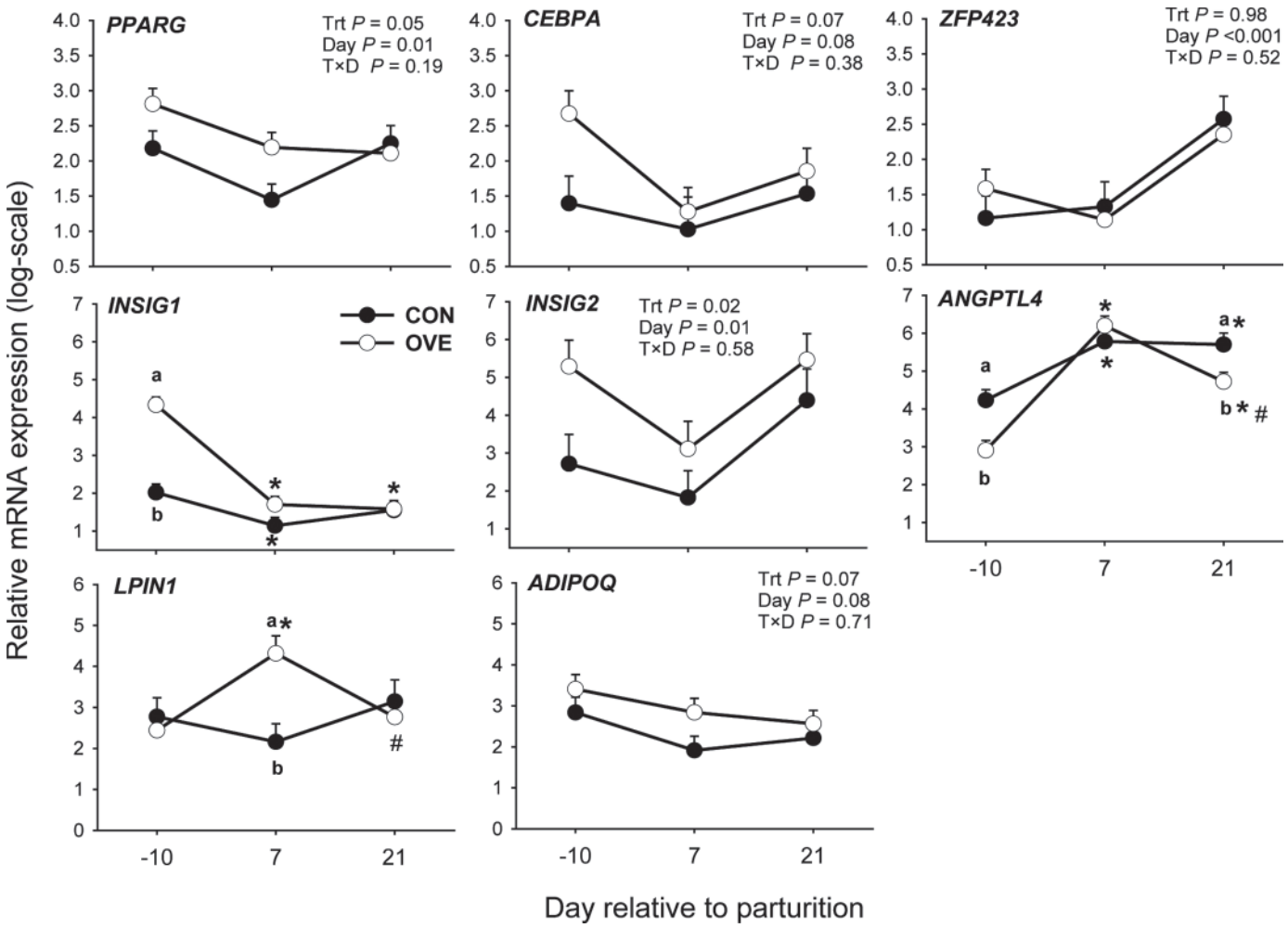

Figure 4. Expression of adipogenic transcription regulators [proliferator-activated receptor γ (*PPARG*), CCAAT/enhancer-binding protein α (*CEBPA*), and zinc finger protein 423 (*ZFP423*)], PPARγ-driven inducers of lipogenesis [insulin induced gene 1 (*INSIG1*) and *INSIG2*], PPARγ- and CEBPα-regulated adipokines [angiopoietin-related protein 4 (*ANGPTL4*) and adiponectin (*ADIPOQ*)], and lipogenic transcription regulator lipin-1 (*LPIN1*) in subcutaneous adipose tissue of cows overfed a moderate-energy diet (OVE, n = 7) or fed to meet energy requirements (CON, n = 7) during the close-up period. Values are expressed as mean ± SEM. The false discovery rate-adjusted *P*-values for main effect of treatment (Trt) and day and interaction of treatment × day (T × D) are shown only for those genes without significant interactions (T × D). For genes with significant interaction ($P < 0.05$), mean separation was evaluated via contrasts at the same day relative to parturition and significant differences ($P < 0.05$) are denoted with lowercase a and b. Mean separation between time points and within treatments were evaluated via contrasts and significant differences ($P < 0.05$) between d −10 and 7 or 21 are denoted with an asterisk (*), and between d 7 and d 21 by a pound sign (#).

after which it remained unchanged in cow fed OVE but increased ca. 3 fold in cows fed CON. The mRNA expression of hormone-sensitive lipase (***HSL***) in SAT was relatively more stable around parturition between treatments, despite a tendency ($P = 0.10$) for a day effect, which was mainly due to the lower expression in CON at 7 d (Figure 6).

Although expression of the lipid droplet-associated protein perilipin 1 (***PLIN1***) was not affected, expression of *PLIN2* (the most-abundant in AT lipid droplets; Duncan et al., 2007) was greater overall ($P = 0.01$) in cows fed OVE compared with CON due to responses at −10 and 7 d. The mRNA expression of growth hormone receptor (***GHR***) was greater ($P = 0.05$) overall in cows fed OVE vs. CON primarily due to the response at −10 and 7 d. In fact, the pattern of expression of *ATGL*, *ABHD5*, and *PLIN2* at −10 and 7 d was strikingly similar to that of *GHR*.

The mRNA expression of β-2 adrenergic receptor (***ADRB2***), encoding the major β-adrenergic receptor in adipocytes, and the glucocorticoid receptor nuclear receptor subfamily 3, group C, member 1 (*NR3C1*) increased ($P \leq 0.001$) at 21 d compared with −10 d regardless of treatment (Figure 6). The *ADRB1* mRNA

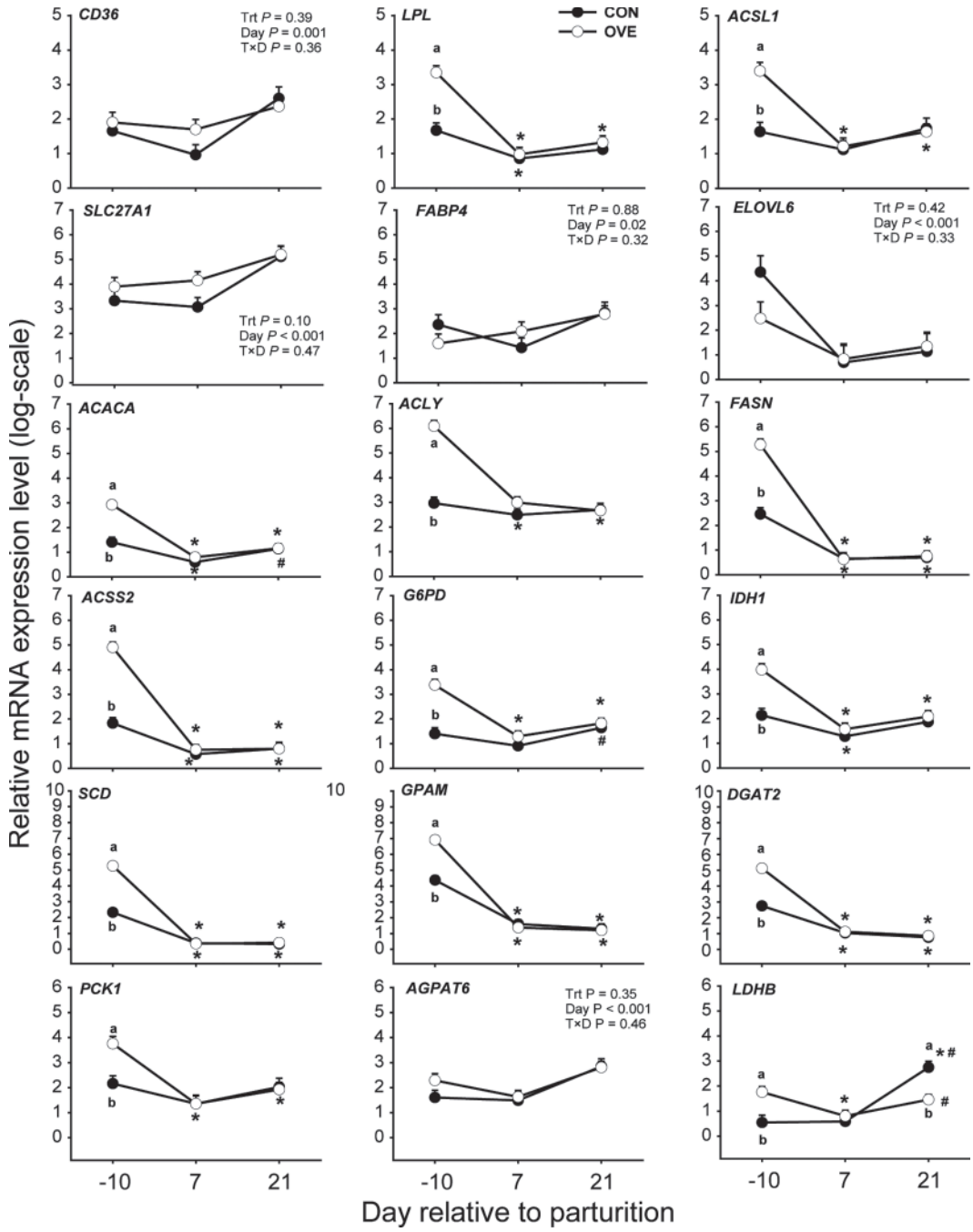

Figure 5. Expression of genes encoding enzymes associated with long-chain FA (LCFA) import and activation [cluster of differentiation 36 (*CD36*); solute carrier family 27 (FA transporter), member 1 (*SLC27A1*); lipoprotein lipase (*LPL*); FA-binding protein 4 (*FABP4*); and acyl-CoA synthetase long-chain family member 1 (*ACSL1*)], de novo FA synthesis [ATP citrate lyase (*ACLY*), acetyl-CoA carboxylase α (*ACACA*), FA synthase (*FASN*), and lactate dehydrogenase B (*LDHB*)], desaturation and elongation [stearoyl-CoA desaturase (*SCD*) and FA elongase 6 (*ELOVL6*)], cytosolic acetate activation [acyl-CoA synthetase short-chain family member 2 (*ACSS2*)], cytosolic NADPH production [glucose-6-phosphate dehydrogenase (*G6PD*) and isocitrate dehydrogenase 1 (*IDH1*)], triglyceride (TAG) synthesis [glycerol-3-phosphate acyltransferase 1, mitochondrial (*GPAM*); 1-acylglycerol-3-phosphate *O*-acyltransferase 6 (*AGPAT6*); and diacylglycerol *O*-acyltransferase 2 (*DGAT2*)], and glyceroneogenesis [phosphoenolpyruvate carboxykinase 1 (*PCK1*)] in subcutaneous adipose tissue of cows overfed a moderate-energy diet (OVE, n = 7) or fed to meet energy requirements (CON, n = 7) during the close-up period. Values are expressed as mean ± SEM. The false discovery rate-adjusted *P*-values for main effect of treatment (Trt) and day and interaction of treatment × day (T × D) are shown only for those genes without significant interactions (T × D). For genes with significant interaction ($P < 0.05$), mean separation was evaluated via contrasts at the same day relative to parturition and significant differences ($P < 0.05$) are denoted with lowercase a and b. Mean separation between time points and within treatments were evaluated via contrasts and significant differences ($P < 0.05$) between d −10 and 7 or 21 are denoted with an asterisk (*), and between d 7 and 21 by a pound sign (#).

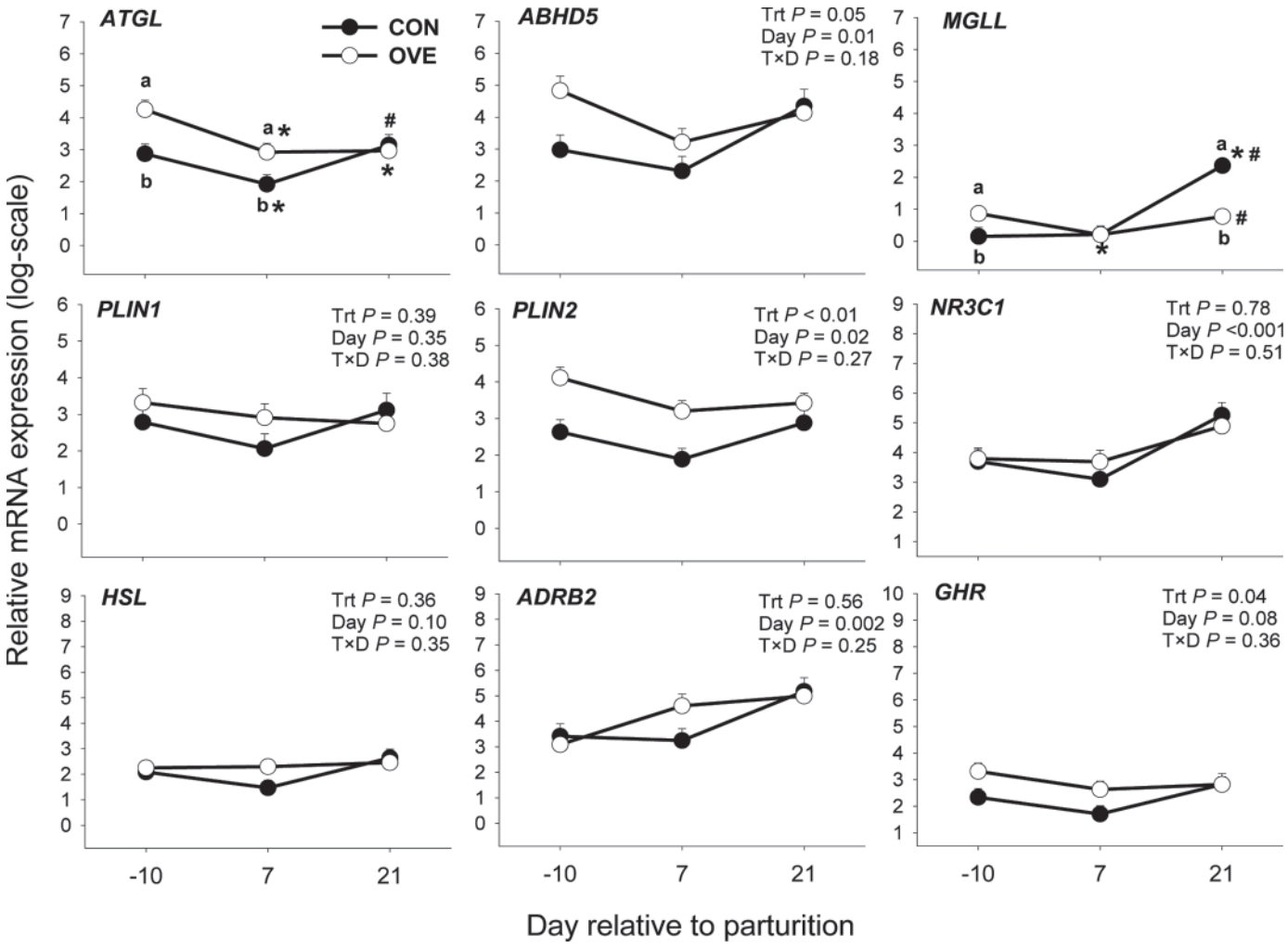

Figure 6. Expression of lipolytic enzymes [adipose triglyceride lipase (*ATGL*), abhydrolase domain containing 5 (*ABHD5*), hormone-sensitive lipase (*HSL*), and monoglyceride lipase (*MGLL*)], lipolytic inducers [growth hormone receptor (*GHR*); nuclear receptor subfamily 3, group C, member 1 (*NR3C1*); and β-2 adrenergic receptor (*ADRB2*)], and lipid droplet-associated proteins [perilipin 1 (*PLIN1*) and *PLIN2*] in subcutaneous adipose tissue of cows overfed a moderate-energy diet (OVE, n = 7) or fed to meet energy requirements (CON, n = 7) during the close-up period. Values are expressed as mean ± SEM. The false discovery rate-adjusted *P*-values for main effect of treatment (Trt) and day and interaction of treatment × day (T × D) are shown only for those genes without significant interactions (T × D). For genes with significant interaction ($P < 0.05$), mean separation was evaluated via contrasts at the same day relative to parturition and significant differences ($P < 0.05$) are denoted with lowercase a and b. Mean separation between time points and within treatments were evaluated via contrasts and significant differences ($P < 0.05$) between d −10 and 7 or 21 are denoted with an asterisk (*), and between d 7 and 21 by a pound sign (#).

abundance was ca. one-hundredth the level of *ADRB2* (Supplemental Table 1, available online at http://www.journalofdairyscience.org/), and *ADRB3* was undetectable in most AT samples (data not shown).

DISCUSSION

Production Responses

Similar to our previous studies (e.g., Richards et al., 2009; Janovick and Drackley, 2010), CON successfully controlled the DMI during the entire dry period and limited NE_L intake close to predicted requirements. Limitation of DMI was due to the bulky nature of wheat straw and the ensuing rumen-fill response. The greater prepartal concentration of insulin in response to energy overfeeding in the present and previous studies (Holtenius et al., 2003; Dann et al., 2006; Janovick et al., 2011) might have been due to 1) greater ground shelled corn consumption, resulting in greater hepatic gluconeogenesis, which subsequently stimulated pancreatic insulin secretion; or 2) the combination of greater gluconeogenesis-stimulated insulin production and exacerbated peripheral IR. Recently, Schoenberg

et al. (2012) used a glucose tolerance test (**GTT**) and hyperinsulinemic-euglycemic clamp (**HEC**) to evaluate the insulin responsiveness of dry cows overfed or restricted fed dietary energy (162 or 90% of requirements) for 14 d . Similar to our study, they observed greater basal plasma insulin in overfed cows as well; however, no differences existed in glucose clearance rate or increased plasma insulin in response to GTT and HEC. As such, those data do not support a compromised IR state in overfed cows.

The same pre- and postpartal patterns in circulating NEFA between OVE and CON cows as in the current study have been observed in previous studies in which overfeeding was implemented for the entire dry period (Janovick et al., 2011; Khan et al., 2011) or only during the close-up period (Vasquez et al., 2011). Rukkwamsuk et al. (1998) reported that SAT biopsied at −1 wk relative to parturition from cows overfed compared with restricted fed energy during the entire dry period had lower basal lipolytic rate in vitro, but tended to have greater noradrenaline-stimulated lipolysis. A second study from the same group demonstrated that SAT from the same overfed cows had greater in vitro rates of basal esterification at −1 wk (Rukkwamsuk et al., 1999), which probably helped recycle mobilized NEFA into TAG (Nye et al., 2008). Both types of adaptations help explain the lower prepartal adipose lipolytic rate in overfed cows (i.e., lower NEFA; Janovick et al., 2011; Vasquez et al., 2011; Khan et al., 2011) and the greater increase in NEFA we observed postpartum compared with prepartum in cows fed OVE.

Despite the fact that postpartal DMI and energy balance did not differ between groups in the current study, OVE cows still experienced a more pronounced increase in serum NEFA, which indicated that a carryover effect of prepartal plane of energy nutrition affects the mechanisms controlling lipolysis, the sensitivity to lipolytic stimulation, or a combination of the 2 aspects. The discussion below expands on the putative molecular mechanisms responsible for these systemic responses.

IRS-1 Tyrosine Phosphorylation and Insulin Signaling

The underlying mechanisms of insulin signaling or IR during the transition period are still unclear. Using the hyperinsulinemic euglycemic clamp Petterson et al. (1993) demonstrated that IR during late pregnancy in sheep was primarily due to impaired peripheral insulin sensitivity, which suggested that the mechanisms involved reduction in INSR binding or dysfunction in early postreceptor signal transduction. Early studies regarding alterations of INSR numbers and binding affinity were mainly conducted with ewes and failed to give conclusive results (Vernon et al., 1981; Vernon and Taylor, 1988; Guesnet et al., 1991). Guesnet et al. (1991) reported a 62% decrease of INSR, accompanied by markedly decreased insulin-stimulated lipogenesis in omental AT of early lactating ewes. Vernon and Taylor (1988) showed that, compared with nonlactating controls, SAT from early lactation ewes had both reduced sensitivity and response to insulin-stimulated glucose uptake and FA synthesis in vitro. However, neither number nor binding affinity of INSR differed between the 2 physiological states. Recently, a similar study showed that the mRNA expression of *INSR* in SAT of peripartal dairy cows remained unchanged from 8 wk pre- to 5 wk postpartum (Sadri et al., 2010). In the current study, with greater degree of variation, *INSR* expression was not affected during the transition period, which a priori highlighted a weak association with control of insulin signaling or IR during the transition period.

Vernon and Taylor (1988) attributed lactational IR to defects in intracellular insulin signaling transduction at the postreceptor level. At the molecular level, IRS proteins carry out the first intracellular step mediating insulin signaling. IRS-1, rather than the other isoforms, is preferentially involved in insulin-induced metabolic actions including glucose uptake (Saltiel and Kahn, 2001). Phosphorylation of IRS-1 on tyrosine residues is required for insulin-stimulated glucose uptake, and a substantial body of research demonstrated a causal relationship between compromised IRS1-PY and AT IR in both late pregnant rodents (Sevillano et al., 2007) and obesity-induced Type II diabetes (Esposito et al., 2001; Sesti et al., 2001). The level of IRS1-PY is relatively sustained even in the presence of phosphatases (Ogihara et al., 1997); thus, we assumed that the phosphorylation of IRS-1 in SAT adapted to culture medium without bovine insulin for 30 min was still representative of the basal physiological state (Figure 2). The lower level of IRS1-PY at 7 compared with −10 d postpartum suggested a decrease in insulin signaling after calving. However, we do not believe that this result alone was indicative of an exacerbated IR state in early lactation because lower IRS1-PY also may have been due to the postpartal hypoinsulinemia observed.

We expected that a supraphysiological challenge with bovine insulin would elicit a maximal response in IRS1-PY, which was clearly shown by the much higher phosphorylation level after insulin challenge (Figure 2), particularly in tissue harvested prepartum. Thus, as suggested previously (Bauman and Currie, 1980), cows seemed to experience a more severe peripheral IR early postpartum regardless of prepartal diet. The lack of

change in *INSR* and *IRS1* mRNA expression between −10 and 7 d suggested that during the short period of time around calving a defect in posttranslational modification of IRS1 likely was a major mechanism exacerbating IR. Such a mechanism is supported by the small change in expression observed for *AKT2* and *PDPK1*, both of which are downstream targets of IRS and mediate intracellular insulin signaling transduction (Foufelle and Ferré, 2002).

The decrease in expression of *GLUT4* by 7 d regardless of prepartal dietary energy is another piece of evidence supporting the diminished responsiveness of SAT to insulin during early lactation. Feed deprivation had been practiced to mimic the postcalving negative energy balance. During an HEC study, Schoenberg et al. (2012) observed a decrease in glucose disposal rate in dry cows deprived of feed for 2 d compared with ad libitum-fed controls. Decreased insulin responsiveness during feed deprivation has been reported in other studies as well (Oikawa and Oetzel, 2006). Whether compromised peripheral insulin responsiveness during acute feed deprivation resulted from decreased *GLUT4* expression warrants further research.

Contrary to our hypothesis, prepartal data on insulin-stimulated IRS1-PY and expression of *GLUT4* mRNA (along with adipogenic and lipogenic genes) suggested that close-up energy overfeeding did not predispose animals to a state with greater IR. If anything, it seemed to have enhanced insulin sensitivity and responsiveness, particularly prepartum, and not to hamper it during the first 21 d postpartum. The data seem to confirm data from Holtenius et al. (2003) who reported a greater glucose clearance rate after a GTT at 3 wk before calving in overfed compared with underfed cows.

Insulin's antilipolytic effect is mediated by PDE3B, which hydrolyzes cAMP into AMP, and consequently reduces the magnitude of hormone-stimulated lipolysis in AT. The increase in *PDE3B*, regardless of diet between −10 and 7 d (Figure 3), might have served as a feedback-regulatory mechanism to prevent excessive lipolysis. We speculate that such a mechanism was activated due to either a blunted antilipolytic response by insulin (e.g., decrease in IRS1-PY postpartum) or increased lipolysis. Thus, it is not surprising that cows fed less energy (CON vs. OVE) had greater expression of *PDE3B* at −10 d to restrict further lipolysis. Despite an increase in both groups, the sustained higher expression of PDE3B in CON cows by 7 d may have contributed to the modest increase in plasma NEFA concentration postpartum, as evidenced in the current and previous studies (Dann et al., 2006; Janovick et al., 2011). Schoenberg et al. (2012) reported that feed-deprived or energy-restricted cows compared with ad libitum-fed or overfed controls were much more sensitive to an inhibition of lipolysis as assessed by a GTT. Those responses might have been at least partly regulated by greater expression of *PDE3B*.

Perhaps the most clear evidence of diminished insulin sensitivity due to change in physiological state was the 3-fold decrease in *SREBF1* and also *GLUT4* expression at 7 d in cows fed CON. Such a response of *SREBF1* was not observed in cows fed OVE, perhaps due to the greater expression of its coactivator *SCAP*. At least in rodent liver, the expression and activity of *SREBF1* is regulated primarily via insulin (Foufelle and Ferré, 2002). Our prepartal data do not seem to support an essentiality for SREBF1 as a regulator of SAT lipogenesis (Sekiya et al., 2007). The mechanistic role of *SREBF1* in coordinating functional adaptations of bovine SAT deserves further study.

Insulin inhibits the expression of *EIF4EBP1*, a response that favors protein translation to proceed (Proud, 2002) and could explain the lower prepartal expression in cows fed OVE (i.e., those with greater blood insulin; Table 2). The sustained upregulation of *EIF4EBP1* soon after calving due to OVE was intriguing but supports the data from other genes examined (e.g., *PPARG* and *CEBPA*), providing evidence that insulin signaling (despite the reduction in IRS1-PY) was not impaired with OVE. Regardless of the diet, the observed increase in both *EIF4EBP1* and *ANGPTL4* expression by 7 d was similar to that observed in human SAT after an 8-wk period of low caloric intake in which fat mass and blood insulin decreased markedly (Franck et al., 2011). Thus, despite prepartal differences in blood insulin due to OVE as compared with CON, the temporal decrease in blood insulin with both treatments agrees with the increase in *EIF4EBP1*. Whether the *EIF4EBP1* response represents another counter-regulatory mechanism to control the overall process of translation in SAT as a way to conserve energy and nutrients during a period of stress (Proud, 2002) remains to be established; however, it could partly explain the almost absent lipogenic enzyme activity that has been reported in SAT during early lactation (McNamara et al., 1995).

Adipogenesis and Lipogenesis

A recent transcriptomics study (Sumner-Thomsen et al., 2011) provided evidence that changes in SAT gene expression during the transition period exert an important level of control over tissue function (i.e., lipogenesis, lipolysis, and remodeling). Despite a lack of direct evidence in bovine adipocytes, Kadegowda et al. (2009) found greater mRNA expression of *ACACA*,

FASN, *DGAT1*, *LPIN1,* and *AGPAT6* in bovine mammary epithelial cells when the culture medium was supplemented with the PPARγ agonist rosiglitazone. Thus, we suggest from our data that expression of lipogenic genes was probably subject to PPARγ transcriptional regulation.

Although no carryover effects of prepartal plane of nutrition were detectable after calving for the lipogenic enzymes, overfeeding energy led to sustained upregulation of *PPARG*, *ADIPOQ*, *INSIG1*, *INSIG2*, and *SCAP* (Figures 3 and 4) through at least 7 d postpartum. Furthermore, the PPARγ and CEBPα coactivator *LPIN1* (most abundant isoform in AT; Csaki and Reue, 2010) almost doubled in expression, probably as a counterregulatory mechanism to help retain an adipogenic potential (Csaki and Reue, 2010) in light of *CEBPA* and *INSIG1* downregulation. The greater *ADIPOQ* expression (likely driven via PPARγ) might have been an additional factor allowing SAT to retain insulin sensitivity as well as help dampen proinflammatory signals (Tishinsky et al., 2011) even after calving.

Together, these data seemed to suggest that adipogenic capacity in SAT (e.g., from existing stem cells or preadipocytes) was maintained despite the substantial release of long-chain FA (**LCFA**) into the circulation (i.e., increased blood NEFA postpartum; Table 2). It is important to note, however, that only differentiated adipocytes would be expected to accrete TAG and release NEFA during lipolysis. The fact that only a fraction of samples at −10 and 7 d expressed *DLK1* (or *PREF1*) suggests that the process of preadipocyte differentiation was actively taking place. The 2.5-fold increase in expression of the PPARγ regulator *ZFP423* (Gupta et al., 2010) between −10 and 7 d compared with 21 d was additional evidence, albeit at the mRNA level, that adipogenic capacity (i.e., proliferation of stem cells to preadipocytes) in SAT of postpartal cows was not entirely lost, as would be expected from classic lipogenic enzyme work (e.g., McNamara et al., 1995). In nonruminant mammals, both *PPARG* and *ZFP423* expression (along with *LPIN1*) is absolutely required for AT differentiation (Rosen and MacDougald, 2006; Csaki and Reue, 2010; Gupta et al., 2010).

The holistic upregulation of most lipogenic genes (Figure 5) at −10 d in response to energy overfeeding underscored the existence of a coregulatory mechanism. The importance of CEBPα in the regulation of adipogenesis in rodents has long been recognized. The presence of CEBPα was required for maintaining PPARγ expression during adipocyte differentiation (Rosen and MacDougald, 2006). Furthermore, blunting *CEBPA* expression led to markedly lower expression of *PPARG*, *FABP4*, *GLUT4,* and *DGAT2* in 3T3-L1 adipocytes (Payne et al., 2010). Our data revealed an identical expression pattern of *CEBPA* (also *INSIG1*) to that of lipogenic genes in response to dietary treatment and the change in physiological state. Thus, just as for PPARγ, we propose that the presence of CEBPα is a key component required to sustain the expression of lipogenic genes in SAT of peripartal dairy cows.

Clearly, the lack of significant change in *CEBPA* (and also *PPARG*) between 7 and 21 d might have curtailed the prolipogenic response because *ZFP423* increased markedly during the same time frame. The presence of CEBPα is required for acquisition of insulin sensitivity in murine adipocytes. Differentiated CEBPα$^{-/-}$ fat cells (induced by ectopic expression of PPARγ) completely lost the ability for insulin-stimulated glucose uptake, which was due to reduced gene expression and tyrosine phosphorylation of *INSR* and *IRS1* (Wu et al., 1999). Whether the reduced adipose IRS1-PY postpartum observed in the current study resulted from the decrease in expression of *CEBPA* warrants further research; however, the response in *CEBPA* (and also *ZFP423*) supports the notion that some degree of insulin responsiveness was restored by 3 wk postpartum. Our data highlighted the interrelationships among transcription regulators and posttranscriptional events in regulating the overall process of adipogenesis and lipogenesis in bovine SAT.

From a mechanistic standpoint, the higher expression of *ACSS2* and *LDHB* indicated that OVE cows had the potential to activate and channel more ruminally-derived acetate and lactate (Hood et al., 1972) from blood toward LCFA during the dry period. In addition to de novo FA synthesis, the process of uptake of preformed FA (*LPL* and *ACSL1*) and esterification (*GPAM*, *DGAT2*, and *PCK1*) likely contributed to TAG accretion in SAT. Although classic in vitro studies showed low activity of ACLY in ruminant AT (Ingle et al., 1972), our data indicated potentially greater glucose use for lipogenesis in OVE cows during pregnancy. The opposite was the case for lactate because expression of *LDHB* increased markedly between 7 and 21 d [i.e., SAT has the potential to adapt to using nutrients (e.g., lactate) that are not preferentially used by the mammary gland for lipogenesis]. More importantly, our data suggested that *ACLY* is adaptable to the amount of intracellular nutrient availability and particularly dietary starch (Graugnard et al., 2010). Together, our data support previous results showing greater basal adipose esterification rates in energy-overfed as compared with restricted-fed cows prepartum (Rukkwamsuk et al., 1999).

The coordinated downregulation of lipogenic genes, especially in the OVE group, at 7 d may be attributed to exacerbated IR in early lactation and reduced availability of lipogenic substrates, which was deduced from

the decrease in expression of *GLUT4*, *PCK1*, *ACSS2*, and *IDH1*. Contrary to previous work with heifers (Sumner-Thomson et al., 2011), it was remarkable that in control cows the degree of downregulation of most lipogenic genes (and also *CEBPA*, *LPIN1*, and *INSIG2*) was quite modest. Despite the marked upregulation of *IRS1* and *SREBF1* expression between 7 and 21 d, the response was insufficient to enhance expression of most lipogenic genes and only those related with FA uptake (*CD36*, *SLC27A1*, and *FABP4*) were upregulated.

Lipolysis: Novel Link with Adipogenesis

The classic lipolytic pathway in response to β-adrenergic stimulation and HSL is well established (McNamara, 1991). Not until recently has accumulating evidence indicated the existence of ATGL, which is highly expressed in white AT and is primarily responsible for both basal and β-adrenergic-stimulated TAG hydrolysis (Duncan et al., 2007). The complete activation of ATGL requires binding to its activator protein ABHD5 after it dissociates from PLIN, followed by phosphorylation induced by β-adrenergic stimulation (Duncan et al., 2007). Due to the specificity or preference of its substrate (TAG), ATGL is now considered the rate-limiting enzyme of lipolysis in mammals (Duncan et al., 2007). The antilipolytic effect of insulin reduces the activity of protein kinase A (PKA) by stimulating PDE3B, which increases catabolism of cytosolic cAMP, thereby preventing phosphorylation of PLIN and effectively reversing the dissociation of PLIN and ABHD5 (Duncan et al., 2007).

Based on the known roles of ATGL and ABDH5, it was interesting that OVE cows had both slightly lower prepartal serum NEFA and higher mRNA expression of *ATGL, ABHD5*, and *MGLL* (the last enzyme in the lipolytic pathway). We speculate that this might be explained by 1) lower hormonal lipolytic signals prepartum; 2) undiminished antilipolytic effect by insulin; and 3) greater capacity of AT from OVE cows to re-esterify the circulating NEFA. Thus, despite the greater mRNA expression of *ATGL* and *ABHD5*, lipolysis prepartum was still tightly controlled in OVE cows. More importantly, however, our data are the first to underscore the existence (as in nonruminants) of a coordinated response among adipogenesis, lipogenesis, and lipolysis, with a strong degree of transcriptional regulation likely via PPARγ and CEBPα. In fully-differentiated 3T3-L1 adipocytes, both *ATGL* mRNA and protein expression were induced by PPARγ agonists in a dose- and time-dependent manner (Kershaw et al., 2007). The same response of *ATGL* was observed in white AT of either lean or obese mice following oral treatment with rosiglitazone (Kershaw et al., 2007).

Although modest, the postpartal decrease in *ATGL* and *ABHD5* mRNA expression in both groups agrees with the lower ATGL protein expression in early lactating as compared with late pregnant dairy cows (Koltes and Spurlock, 2011). Both mRNA and protein expression of *ATGL* in AT were reduced in individuals with obesity-induced IR compared with insulin-sensitive subjects (Jocken et al., 2007). In OVE cows, however, the greater prepartal *ATGL*, *ABHD5*, and *MGLL* was in part due to greater *PPARG* and *CEBPA,* which in human adipocytes regulates expression of these genes (Lo et al., 2011; i.e., substantially-positive energy balance not only promotes lipogenic gene expression but also the expression of *ATGL*). This suggestion also was supported by the parallel postpartal decrease of *ATGL* and *ABHD5* mRNA expression and IRS1-PY [Figure 2; i.e., from a mechanistic standpoint, insulin sensitivity (conferred in part via PPARγ and CEBPα) is probably required to maintain *ATGL* expression in bovine adipocytes]. Similar to adipogenesis/lipogenesis, energy overfeeding (probably via CEBPα and PPARγ) likely allows for SAT remodeling via lipolysis; however, the lower expression of *PCK1* and *LDHB* postpartum hindered the production of glycerol-3-phosphate and allowed for LCFA to exit the cell into the circulation (i.e., increase NEFA in serum). The gradual increase in *ADRB2* expression and the longitudinal NEFA profile after 7 d (Supplemental Figure 4, available online at http://www.journalofdairyscience.org/) seem to support this idea.

The pattern of expression we observed for *GHR*, *ADRB2*, *HSL*, and *NR3C1* suggested that dietary OVE did not alter sensitivity of SAT to lipolytic stimulation postpartum (e.g., via norepinephrine or cortisol). Growth hormone action on AT antagonizes insulin by amplifying the lipolytic response to β-adrenergic signals and inhibiting lipogenesis (Etherton and Bauman, 1998). Rhoads et al. (2004) reported that the abundance of GHR protein in SAT of dairy cows decreased in parallel with the decrease in plasma insulin concentration from late pregnancy (−28 d) to early lactation (10 d). However, administration of exogenous insulin increased adipose GHR protein expression both in late pregnancy and early lactation (Rhoads et al., 2004). Taken together these data seem to support the greater *GHR* expression we observed with OVE. In 3T3 L1 adipocytes, GH exerts an antilipolytic effect by converging on the intracellular insulin signaling pathway through tyrosine phosphorylation of IRS isoforms (Yamauchi et al., 1998). Thus, the greater *GHR* expression in OVE may have served to reduce prepartal lipolysis.

The degree of change in expression we observed postpartum for *ADRB2* was lower than reported previously at 30 compared with −30 d (Sumner and McNamara,

Prepartum	Insulin	IR		Postpartum	Insulin	IR
-10 d vs. 7 d	↑	↓		7 d vs. -10 d	↓	↑
OVE vs. CON	↑	=		OVE vs. CON	=	=

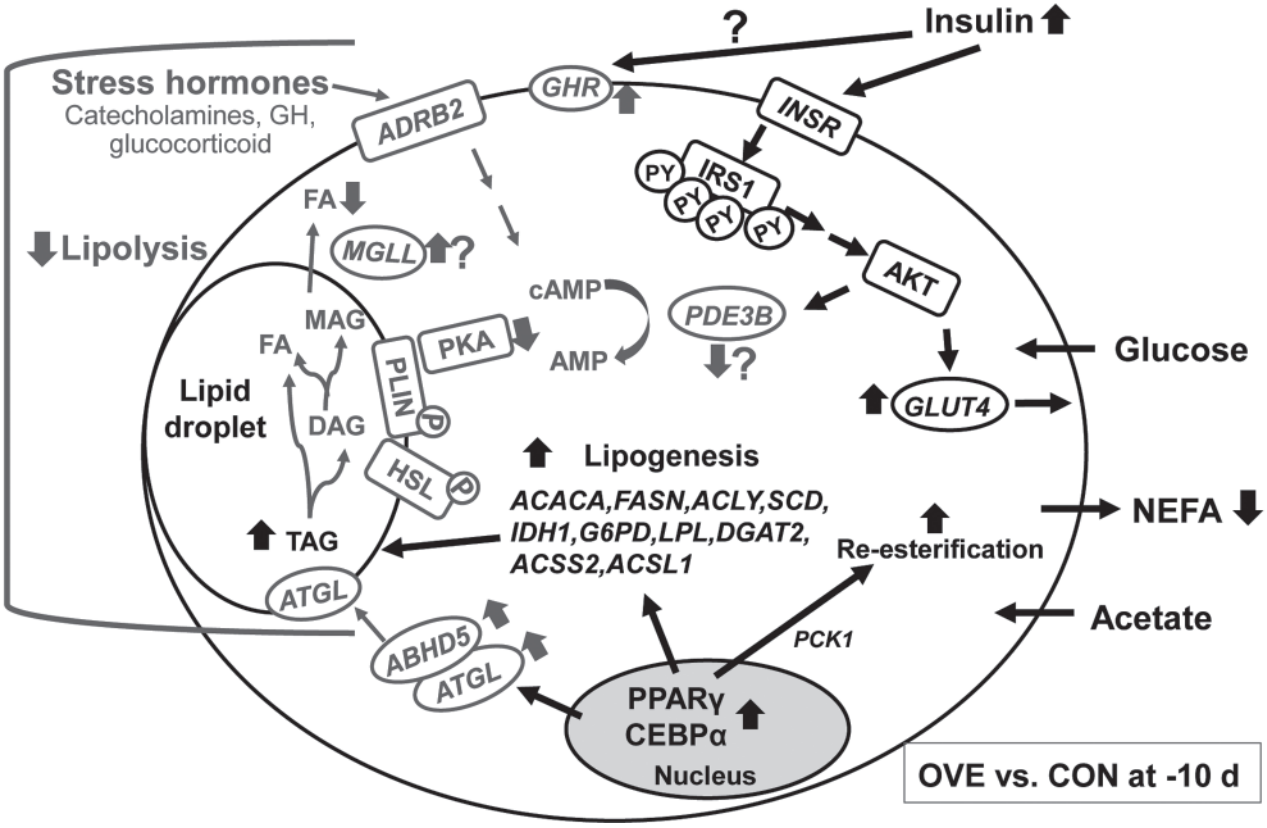

Figure 7. Relative changes in insulin and insulin resistance (IR) in response to plane of dietary energy during the transition period (table insert) and putative regulatory network of pathways involved in metabolism of subcutaneous adipose tissue prepartum. The predominant pathways are depicted in black, whereas the subordinate pathways are in gray. OVE = overfeeding of a moderate-energy diet; CON = controlled-energy diet to meet energy requirements. Legend: equal sign = no difference; arrow shape = increase or decrease; dashed line arrow = transcriptional regulation; solid line arrow = stimulation or activation of a pathway, transport direction of metabolites; spherical shape = gene studied; square shape = protein product of gene. ADRB2 = β-2 adrenergic receptor; GHR = growth hormone (GH) receptor; INSR = insulin receptor; IRS1 = insulin receptor substrate 1; PY = phosphorylation; AKT = v-akt murine thymoma viral oncogene homolog; PDE3B = phosphodiesterase 3B; GLUT4 = glucose transporter 4; MGLL = monoglyceride lipase; PKA = protein kinase A; PLIN = perilipin; HSL = hormone-sensitive lipase; ATGL = adipose triglyceride lipase; MAG = monoacylglycerol; DAG = diacylglycerol; TAG = triglyceride; ABHD5 = abhydrolase domain containing 5; PPARγ = peroxisome proliferator-activated receptor γ; CEBPα = CCAAT/enhancer-binding protein α; ACACA = acetyl-CoA carboxylase α; FASN = FA synthase; ACLY = ATP citrate lyase; SCD = stearoyl-CoA desaturase; IDH1 = isocitrate dehydrogenase 1; G6PD = glucose-6-phosphate dehydrogenase; LPL = lipoprotein lipase; DGAT2 = diacylglycerol *O*-acyltransferase 2; ACSS2 = acyl-CoA synthetase short-chain family member 2; ACSL1 = acyl-CoA synthetase long-chain family member 1.

2007). Furthermore, we found no change in *HSL* or *PLIN1* due to stage of lactation as reported previously (Sumner and McNamara, 2007) and the decrease in *PLIN2* was modest even by 21 d. The sympathetic nervous system in bovine AT might not be as active during lactation as in rats (McNamara and Murray, 2001). The fact that *GHR* was still greater early postpartum coupled with previous data showing greater postpartal blood GH due to prepartal OVE (Khan et al., 2011) seems to argue against the concept of altered sensitivity. However, the postpartal difference in blood NEFA between OVE and CON was minor and nonsignificant (Supplemental Figure 4, available online at http://www.journalofdairyscience.org/).

A potentially important point that is often overlooked when focusing strictly on lipolysis or lipogenesis is that both processes likely are tightly controlled. This was exemplified recently with the discovery that CEBPα

controls transcription of both lipogenic and lipolytic genes in AT (Lo et al., 2011). In the context of post-transcriptional control, it should be noted that, despite unchanged HSL protein expression, Koltes and Spurlock (2011) found increased phosphorylation of HSL in SAT early postcalving compared with late pregnancy, which supported the notion of greater postparturient β-adrenergic-stimulated lipolysis. Clearly, mechanistic regulation occurs at multiple levels and our and previous data allow for a better description of the events controlling blood NEFA in peripartal cows (Holtenius et al., 2003; Dann et al., 2006; Janovick et al., 2011).

CONCLUSIONS

In the current study, close-up energy overfeeding had no benefit on milk production or component yields, yet resulted in greater change between pre- and postpartal NEFA concentration, which underscored the lower efficiency of such a feeding strategy. Such results are consistent with the recent findings from 3 studies in our group, in which the same dietary treatments with larger groups of animals were used (Richards et al., 2009; Khan et al., 2011; Vasquez et al., 2011). Contrary to our hypothesis, overfeeding of the moderate energy diet facilitated rather than compromised the pathway of insulin signaling in SAT particularly during late pregnancy (Figure 7). The result was a robust upregulation of the entire repertoire of adipogenic regulators and lipogenic enzymes (Figure 7), which for the former (e.g., *PPARG*) lasted through at least the first week postpartum. In fact, basal lipolysis was part of the pro-adipogenic/lipogenic response induced by energy overfeeding and might have been under control of both PPARγ and CEBPα even after calving (Figure 7). The fact that the enzymes controlling stimulated lipolysis (*HSL, PLIN1,* and *PLIN2*) are apparently not regulated by PPARγ or CEBPα and that their change in expression after calving was modest suggested that bovine SAT lipolytic control around parturition from a transcriptional standpoint is primarily at the level of basal lipolysis (as in nonruminants). The role of ATGL in controlling lipolysis after calving might also extend to stimulated lipolysis (i.e., once this pathway is activated, greater abundance of ATGL could promote binding to ABHD5, thus enhancing lipolytic activity in SAT). Our data revealed an important link between basal lipolysis and adipogenesis/lipogenesis (Figure 7).

Despite the decrease in serum insulin concentration and insulin sensitivity assessed via IRS1-PY (Figure 7), overall, the data seemed to highlight the fact that negative energy balance after calving does not necessarily correlate with a reduction in insulin-responsive, adipogenic, or lipogenic gene expression in SAT. This idea was further underscored by the modest response observed after calving in cows fed to meet energy requirements prepartum. Therefore, although our data seemed to explain in part the almost complete loss of lipogenic enzyme activity in early lactation (McNamara et al. 1995) it also highlighted that nutrient availability (e.g., acetate) is a major determinant of SAT remodeling/accretion early postpartum. We speculate that postpartal management strategies that could enhance nutrient (e.g., acetate, glucose, and AA) availability to adipose tissue might be effective in accelerating its replenishment.

Our study highlighted a pivotal role of PPARγ and CEBPα as major transcriptional regulators of lipogenic gene expression and basal lipolysis during the peripartal period. Because signs of restored insulin responsiveness (e.g., upregulation of *IRS1*, *INSIG2*, *SREBF1*, *ZFP423*, and *CD36*) were apparent as early as 3 wk postpartum, identifying specific nutrients capable of activating PPARγ and CEBPα specifically in adipose after calving might prove useful in terms of accelerating adipose tissue replenishment.

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