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Prepartal dietary energy alters transcriptional adaptations of the liver and subcutaneous adipose tissue of dairy cows during the transition period

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Selim S, Salin S, Taponen J, Vanhatalo A, Kokkonen T, Elo KT. 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, 2014. First published February 25, 2014; doi:10.1152/physiolgenomics.00115.2013.—Overfeeding during the dry period may predispose cows to increased insulin resistance (IR) with enhanced postpartum lipolysis. We studied gene expression in the liver and subcutaneous adipose tissue (SAT) of 16 Finnish Ayrshire dairy cows fed either a controlled energy diet [Con, 99 MJ/day metabolizable energy (ME)] during the last 6 wk of the dry period or high-energy diet (High, 141 MJ/day ME) for the first 3 wk and then gradually decreasing energy allowance during 3 wk to 99 MJ/day ME before the expected parturition. Tissue biopsies were collected at −10, 1, and 9 days, and blood samples at −10, 1, and 7 days relative to parturition. Overfed cows had greater dry matter, crude protein, and ME intakes and ME balance before parturition. Daily milk yield, live weight, and body condition score were not different between treatments. The High cows tended to have greater plasma insulin and lower glucagon/insulin ratio compared with Con cows. No differences in circulating glucose, glucagon, nonesterified fatty acids and β-hydroxybutyrate concentrations, and hepatic triglyceride contents were observed between treatments. Overfeeding compared with Con resulted in lower *CPT1A* and *PCK1* and a tendency for lower *G6PC* and *PC* expression in the liver. The High group tended to have lower *RETN* expression in SAT than Con. No other effects of overfeeding on the expression of genes related to IR in SAT were observed. In conclusion, overfeeding energy prepartum may have compromised hepatic gluconeogenic capacity and slightly affected IR in SAT based on gene expression.

insulin sensitivity; gluconeogenesis; gene expression; transition dairy cow

THE TRANSITION PERIOD HAS a crucial role in the management of dairy cows. Extensive adipose tissue (AT) mobilization is a key factor in the development of metabolic disorders (16, 52). Hepatic glycogenolysis and gluconeogenesis, and the mobilization of protein reserves from muscle tissue are also augmented during early lactation (4). These adaptations start before parturition and peripheral insulin resistance (IR) may play a role in the initiation of these coordinated changes (20). Earlier studies have shown that prepartal overfeeding resulted in increased AT esterification rate prepartum (43) and lipolytic rate pre- and postpartum (24, 43). Additionally, overfed cows had greater circulating concentrations of insulin, nonesterified fatty acids (NEFA), and β-hydroxybutyrate (BHBA) during early lactation and may have IR around parturition (10, 19).

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In dairy cows, efficient gluconeogenesis is crucial for maintaining a sufficient supply of glucose to the mammary gland. Previous studies by Murondoti et al. (37) and Rukkwamsuk et al. (41) suggest that cows with an elevated liver fat content as a result of overfeeding during the dry period have a diminished capacity for gluconeogenesis during early lactation. However, Hammon et al. (18) report that overfed cows with high liver fat contents had greater expression of hepatic gluconeogenic genes, suggesting higher gluconeogenic capacity in the liver.

As a consequence of overfeeding during the transition period, overconditioned cows may have lower tissue insulin sensitivity than their leaner counterparts. Increased body fatness may not only influence the rate of lipolysis in cows around parturition (19) but also alter the secretion of adipokines, such as adiponectin, tumor necrosis factor alpha, interleukin 6, resistin, leptin, and retinol binding protein 4, which have been indicated as factors affecting AT insulin sensitivity (12, 26, 28, 29, 54).

Voluntary dry matter intake (DMI) depression during the last 2–3 wk of gestation is well known (17). The dip in DMI is more pronounced with high-energy diets than with low-energy diets and may predispose dairy cows to greater lipid mobilization from adipose tissue and lipid-related metabolic disorders around parturition (10, 18, 19). Earlier studies have examined the effects of overfeeding energy during the dry period on lactation performance and metabolic changes before and after parturition (18, 22, 31, 34, 37, 41). However, these studies did not evaluate the effects of overfeeding energy combined with decreasing energy allowance during the last 3 wk of gestation on performance and on hepatic and AT gene expressions in dairy cows during the periparturient period.

The objective of the present study was to assess the effects of overfeeding energy during the first 3 wk of the experimental feeding combined with gradually decreasing energy allowance by parturition on metabolic measurements and on hepatic and subcutaneous adipose tissue (SAT) gene expressions in cows during the periparturient period. The research hypothesis tested was that overfeeding combined with decreasing feed allowance before parturition exacerbates lipid mobilization and reduces SAT insulin sensitivity in dairy cows during the periparturient period as characterized by changes in blood parameters and the mRNA expression of the key candidate genes in SAT. Furthermore, we hypothesized that hepatic gene expression related to glucose metabolism and fatty acid oxidation is impaired as a consequence of overfeeding.

MATERIALS AND METHODS

Animals, diets, and experimental design. The experimental procedures were conducted under the protocols approved by the National Animal Ethics Committee in Finland. Sixteen nonlactating Finnish

Ayrshire dairy cows were selected to participate in the study based on parity (2–4), body weight [693 ± 5.7 kg (mean ± SD)], and body condition score (BCS) using a five-point scaling system (11) [3.7 ± 0.3 (mean ± SD)] in a randomized complete block design. Cows were randomly allocated to two dietary treatment groups of eight cows [44 ± 5 days (mean ± SD) prior to the actual calving date]. They were allocated to the experiment as pairs (one cow per each treatment) based on criteria mentioned above.

The dietary treatments were either a controlled-energy diet [Con; 99 MJ/day metabolizable energy (ME), 100% of energy requirements of pregnant dairy cows (36)] or a high-energy diet (High) during the treatment period. In the High group, average energy intake was 141 MJ/day ME (142% of energy requirements of pregnant dairy cows) during the first 3 wk of experimental feeding and then decreased by 5% on alternate days during the last 3 wk of gestation. Energy allowance of the cows in the High group achieved 100% level of the requirement at the day of the expected parturition. If a cow in the High group calved later than expected, energy allowance was maintained at 100% of the requirement. Both groups were fed grass silage [digestible organic matter 664 g/kg dry matter (DM)] during the early dry period and grass silage supplemented with commercial concentrate (30% of daily ME allowance, Suomen Rehu; Hankkija Agriculture, Hyvinkää, Finland) during the last 3 wk of pregnancy.

Average ME and crude protein (CP) contents of silage during the dry period were 10.6 MJ/kg DM and 155 g/kg DM, respectively. Average ME and CP of concentrate during the last 3 wk of dry period were 12.9 MJ/kg DM and 202 g/kg DM, respectively. Cows were fed individually and had free access to water. After parturition, all cows were offered wilted grass silage ad libitum and an increasing amount of commercial concentrate starting from 5 kg/day at the day of parturition and reaching the amount of 9 kg/day at 9 days postpartum. Average ME and CP of silage after parturition were 11.2 MJ/kg DM and 146 g/kg DM, respectively. After parturition the concentrate ration consisted of cereal concentrate (86%, CP 200 g/kg DM) and protein supplement (14%, CP 303 g/kg DM) (Suomen Rehu, Hankkija Agriculture). Average ME of concentrate after parturition was 12.9 MJ/kg DM. Daily feed intake was measured by roughage intake control system (Insentec, Marknesse, the Netherlands), fitted with separate concentrate troughs. Cows were milked twice daily at 6 AM and at 4.30 PM.

Metabolic measurements. Blood samples were collected at −10.25 ± 5 day (mean ± SD) before the actual parturition day, from the milk vein, and 1 and 7 (± 1) days after parturition from the coccygeal blood vessels into 10 ml vacuum tubes (Vacutainer; BD Medical, Becton Dickinson, Vantaa, Finland) containing EDTA as anticoagulant. Blood samples were cooled on ice. Plasma was separated by centrifugation of blood samples at 2,220 *g* for 10 min and stored at −20°C until analyzed. The plasma concentrations of NEFA and glucose were analyzed as described previously by Salin et al. (46). Intra- and interassay coefficients of variation (CVs) for glucose measurement were 2.85 and 1.95%, respectively. Intra- and interassay CVs for NEFA determination were 2.94 and 1.18%, respectively. The concentration of plasma BHBA was determined with Ranbut kit (Randox Laboratories, Crumlin, UK). Intra- and interassay CVs for BHBA were 2.20 and 1.71%, respectively. Insulin was measured by bovine-specific ELISA (Mercodia, Uppsala, Sweden). Intra-assay CV for insulin determination was 8.22% and interassay CVs were 9.50 and 7.65% for low and medium concentrations, respectively. Plasma glucagon was measured using RIA (GL-32K; Millipore, St. Charles, MO). Intra-assay CVs for glucagon determination were 8.03 and 5.40% for low and medium concentrations, respectively. Interassay CVs for glucagon were 6.54 and 7.26% for low and medium concentrations, respectively. Detection limits for insulin and glucagon assays were 0.62 μIU/ml and 20 pg/ml, respectively. Hepatic triglyceride (TAG) concentration at −10 and 9 days relative to parturition was measured by the methodology described by Nygren et al. (38).

AT and liver collections. AT was collected by biopsy at −10.25 ± 5 days (mean ± SD) before the actual parturition day and 1 and 9 (± 1) days after parturition. After sedation with 0.04 mg/kg of xylazine hydrochloride (20 mg/ml Narcoxyl; Intervet International, Boxmeer, The Netherlands), an epidural anesthesia was induced with 120 mg of lidocaine hydrochloride (20 mg/ml Lidocain; Orion, Espoo, Finland). The biopsy specimen was taken from SAT in the area of ischiorectal fossa, some centimeters caudal from the sacrotuberous ligament. The biopsy region was shaved and washed with soap and disinfectants. A stick incision, ~1 cm long, was made with a scalpel, and AT (~300–500 mg) was harvested with a Weil-Blakesley rongeur. No further suturing or antibiotic therapy was used. Tissue samples were snap-frozen immediately after the sampling in liquid nitrogen and stored thereafter at −80°C until RNA extraction for the gene expression analysis.

The liver biopsy was performed immediately after SAT collection. The biopsy site, the second last intercostal space immediately above the line connecting the tuber coxae and olecranon on the right flank, was shaved and washed with soap and disinfectants. A local anesthesia was induced with 120 mg of lidocaine hydrochloride (20 mg/ml Lidocain, Orion) given subcutaneously and intramuscularly until the peritoneum. A stick incision, ~1 cm long, was made with a scalpel to pierce the skin. The biopsy needle (Surecut, modified Menghini biopsy set) was inserted through the incision into the liver tissue, and the specimen was taken. The procedure was repeated on average four times. No further suturing or any antibiotic therapy after biopsies was used. The liver samples were preserved immediately in Nunc CryoTubes 1.8 ml (Fisher Scientific, Vantaa, Finland) containing protective solution (Allprotect solution; Qiagen, Hilden, Germany), incubated at 4°C overnight and stored at −20°C for subsequent use in RNA analyses.

RNA extraction and cDNA synthesis. Total RNA was extracted from the liver tissue samples using RNeasy Mini Kit and from AT samples using RNeasy Lipid Tissue Kit (Qiagen) according to manufacturer's instructions. Determination of RNA quantity was performed by measuring the absorbance at 260 and 280 nm using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Quality of RNA was assessed using RNA integrity number (RIN) on an Agilent Bioanalyzer 2100 chip electrophoresis system with Agilent RNA 6000 Nano Kit according to manufacturer's instructions (Agilent Technologies, Santa Clara, CA).

Total RNA samples from adipose and liver tissues had 260/280 average absorbance ratios 1.88 and 2.09 and average RIN values 4.91 ± 0.33 and 9.35 ± 0.03 (means ± CV), respectively. The RIN estimates the degradation of total RNA, and thus they do not give absolute estimates of the degradation of mRNA. The correlation between RIN values and gene expression values is ~0.5 (48). First-strand cDNA was synthesized with Anchored-Oligo $(dT)_{18}$ primer using Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics, Mannheim, Germany) in a 20 μl reaction according to manufacturer's instructions. The cDNA was diluted (1:8) with DNase/RNase-free water.

Primer design and quantitative real-time PCR. Primers for 13 genes were designed using the online Primer3 software program (40). The uniqueness of primer sequences was established using BLAST search tool and the National Center for Biotechnology Information's GenBank sequence database. In addition, the expression of six genes was analyzed using primers that were published previously. The DNA sequences for primers, GenBank accession numbers for DNA sequences used, PCR product lengths, analyzed tissue(s), and references for published primers are given in Table 1. The internal control gene used in the current study was eukaryotic translation initiation factor 3 subunit K (*EIF3K*) (7, 23, 47). *EIF3K* has been shown to be one of the most stable internal control genes in bovine liver (7) and AT (7, 47). All oligonucleotides for real-time RT-PCR were commercially synthesized and reverse phase cartridge purified (Oligomer, Helsinki, Finland). The qPCR reactions were performed in a LightCycler 480

Table 1. *DNA sequences (5′-3′) for forward and reverse primers, GenBank accession number, PCR product length, tissue, and source of primers used to analyze gene expression*

Gene ID	Sequence 5′-3′	GenBank Accession No.	Length, bp	Tissue	E	Source
ADIPOQ	For. ATGGCACCCCTGGTGAGAA	XM_005192214	69	SAT	0.99	this paper
	Rev. CACCAGTGTCACCCTTAGGACCA					
ADIPOR1	For. GGCTCTACTACTCCTTCTAC	NM_001034055	144	SAT	0.99	Lemor et al. (28)
	Rev. ACACCCCTGCTCTTGTCTG					
ADIPOR2	For. GGCAACATCTGGACACATC	NM_001040499	200	SAT	0.99	Lemor et al. (28)
	Rev. CTGGAGACCCCTTCTGAG					
CPT1A	For. CCGGTTGCTGATGACGGCTA	XM_002699420	99	liver, SAT	1.00	this paper
	Rev. ATCCGTCTCAGGACAGGAGAACTTG					
EIF3K	For. CCAGGCCCACCAAGAAGAA	NM_001034489	125	liver, SAT	0.99	Kadegowda et al. (23)
	Rev. TTATACCTTCCAGGAGGTCCATGT					
G6PC	For. CTACAGATTTCGTCGGTGCTTGAAT	NM_001076124	140	liver	0.99	this paper
	Rev. AACCGCAATGCCTGACAAGACT					
IRS1	For. AACCGCAGCCTCCTCCACTC	XM_581382	157	liver, SAT	0.99	this paper
	Rev. TGCTGTGATGTCCAGTTGAGCTAC					
IL6	For. GCGCATGGTCGACAAAATCTC	NM_173923	85	SAT	0.99	this paper
	Rev. TGCCAGTGTCTCCTTGCTGCT					
LEP	For. CTGTGCCCATCCGCAAGGT	NM_173928	115	SAT	0.99	this paper
	Rev. CCAGTGACCCTCTGTTTGGAGGA					
LIPE	For. GAGTTTGAGCGGATCATTCA	NM_001080220	102	SAT	0.99	Sumner and McNamara (49)
	Rev. TGAGGCCATGTTTGCTAGAG					
LPL	For. ACACAGCTGAGGACACTTGCC	NM_001075120	101	SAT	1.00	Bionaz and Loor (5)
	Rev. GCCATGGATCACCACAAAGG					
NFKB1	For. CTTCTCAAAGCAGCAGGAGCAGAT	NM_001076409	167	liver	0.97	this paper
	Rev. GGTTTCCCATTTAATATGTCAAATACCTG					
PC	For. CAAAGCAGGTGGGCTACGAGAAC	NM_177946	137	liver	1.00	this paper
	Rev. CAGGTCCACATCTGTGATCTCCTC					
PCK1	For. CCTGTTGGTGTCCCTCTGGTCTAC	NM_174737	117	liver, SAT	0.99	this paper
	Rev. CATGATGACTTTGCCCTTGTACTCC					
PPARG	For. CTTGTGAAGGATGCAAGGGTTTCTT	NM_181024	168	liver, SAT	0.99	this paper
	Rev. CAAACCTGATGGCATTATGAGACA					
RBP4	For. TTAAATAACTGGGACGTGTGTGCAG	NM_001040475	108	liver, SAT	0.99	this paper
	Rev. TTTCTGGAGAAAGGACGCTACGC					
RETN	For. GAGGTCACCACCTCCCTAGTTCCT	NM_183362	102	SAT	0.99	this paper
	Rev. GAAGCCTGAAGGGCAGGTGA					
SCD	For. TCCTGTTGTTGTGCTTCATCC	NM_173959	101	SAT	0.99	Bionaz and Loor (5)
	Rev. GGCATAACGGAATAAGGTGGC					
TNF	For. CCAGAGGGAAGAGCCAGCAGT	NM_173966	124	SAT	0.99	this paper
	Rev. AGAGTTGATGTCGGCTACAACGTG					

ADIPOQ, adiponectin; *ADIPOR1*, adiponectin receptor 1; *ADIPOR2*, adiponectin receptor 2; *CPT1A*, mitochondrial carnitine palmitoyltransferase 1A; *EIF3K*, eukaryotic initiation factor 3 subunit K; *G6PC*, glucose-6-phosphatase catalytic subunit; *IRS1*, insulin receptor substrate 1 transcript variant 1; *IL6*, interleukin 6; *LEP*, leptin; *LIPE*, hormone sensitive lipase; *LPL*, lipoprotein lipase; *NFKB1*, nuclear factor of kappa polypeptide gene enhancer in B-cells 1; *PC*, pyruvate carboxylase; *PCK1*, cytosolic phosphoenolpyruvate carboxykinase 1; *PPARG*, peroxisome proliferator activated receptor-gamma; *RBP4*, retinol binding protein 4; *RETN*, resistin; *SCD*, stearoyl-CoA desaturase; *TNF*, tumor necrosis factor-α. GenBank accession numbers are from the databases of the National Center for Biotechnology Information; all sequences were derived from cattle. For, forward; Rev, reverse; E, approximate amplification efficiency for each gene and tissue calculated from amplification curves using formula $E = (RnA/RnB)^{-(CtA - CtB)} - 1$, where RnA and RnB are arbitrary threshold *points A* and *B* in an individual curve, and CtA and CtB are the threshold cycles at these arbitrary thresholds (32).

optical 384-well plate (Roche Diagnostics) with total volume 10 μl. Using EpMotion automated pipetting system (Eppendorf, Hamburg, Germany), we added 2.5 μl of diluted first-strand cDNA and 7.5 μl of mixture composed of 5 μl 2× SYBR Green master mix (Roche Diagnostics), 0.5 μl (5 pmol/μl) each of forward and reverse primers, and 1.5 μl DNase/RNase-free water into which the final concentration of the primers in the PCR was 0.5 μM.

Quantitative real-time PCR were conducted using LightCycler 480 instrument (Roche Diagnostics), and each sample was run in quadruplicate. The temperature profile of the real-time PCR was as follow: initial denaturation step for 5 min at 95°C, followed by 45 amplification cycles for 20 s at 95°C, 20 s at 60°C, and 20 s at 72°C. A melting curve analysis was run in the end of the PCR program. The mRNA abundance was presented as delta cycle threshold (ΔCt) values (10-ΔCt). The mRNA abundance was calculated relative to the expression of *EIF3K* as an internal control gene; i.e., the calculation of the expression levels was based on the difference between the Ct values of the target genes and the internal control gene.

Approximate amplification efficiencies (E) were calculated from the amplification curves using the method presented by Liu and Saint (32). The following formula was used: $E = (RnA/RnB)^{-(CtA - CtB)} - 1$, where RnA and RnB are arbitrary thresholds in an individual curve, and CtA and CtB are the threshold cycles at these arbitrary thresholds (32). E values varied from 0.97 to 1.0 for studied genes and for the internal control gene, and E values were same for samples from both studied tissues (Table 1). The difference between E values of internal control gene and studied genes varied from 0 to 0.02. The small difference supports our approach to use one internal control gene.

Calculations and statistical analysis. Statistical computations were performed using SAS (release 9.2; SAS Institute, Cary, NC). Prior to statistical analysis, residuals of all data at each time point were checked for normality and outliers using the MIXED and UNIVARIATE procedures of SAS. In this preliminary analysis, statistical model included fixed effects of treatment and time, and a random effect of block. The effect of interval between sampling and actual parturition was included in the initial statistical model as covariate and

Table 2. *Feed intake during the dry period*

Item	Treatment			*P* Value		
	Con	High	SE	Energy Level	Time	Energy Level × Time
Prepartum, week −6 to −4						
Silage DM, kg/day	8.9	12.6	0.26	<0.0001	<0.0001	0.76
ME, MJ/day	97	140	3.5	<0.0001	<0.0001	0.98
Prepartum, week −3 to −1						
Silage DM, kg/day	7.3	8.6	0.18	0.0002	0.0001	0.04
Concentrate DM, kg/day	2.4	2.9	0.09	0.003	0.006	<0.0001

DM, dry matter; ME, metabolizable energy. Cows fed a controlled-energy diet (Con; 99 MJ/day ME, 100%) or ad libitum-energy diet (High; 141 MJ/day ME, 142%) during the early dry period combined with decreasing energy allowance to 99 MJ/day ME during the last 3 wk before the expected parturition.

removed from the model when declared nonsignificant at $P > 0.10$. Blood and production data that were not normally distributed were $\log_2$-transformed to achieve normal residual distribution and presented as $\log_2$-transformed (BHBA, glucagon, and glucagon/insulin ratio). To avoid negative values after $\log_2$-transformation of BHBA and glucagon/insulin ratio, a constant was added to all values before transformation. Liver and SAT gene expression data were presented as $\log_2$-transformed to normalize the data. If data were not normally distributed after $\log_2$-transformation, data points with highest studentized residuals were considered outliers and excluded from analysis. The percentages of observations, which were considered outliers and excluded from analysis, were 2.44 ± 0.08 and 6.07 ± 2.38 (means ± SD) in the liver and SAT gene expression data, respectively.

In the final statistical analysis, repeated-measures ANOVA were conducted using the MIXED procedure of SAS. The statistical model included fixed effects of treatment, time, the interaction between treatment and time, and random effect of block and the interaction between block and time. When the interaction of treatment and time was significant, differences between treatments at each time point were tested for significance using the slice option of the SAS MIXED procedure. For each variable analyzed, cows nested within the treatment were subjected to three covariance structures: compound symmetry (CS), unstructured (UN), and spatial power law SP (POW). The covariance structure that resulted in the smallest Bayesian information criterion was used. Hepatic TAG concentrations at −10 and 9 days relative to parturition were analyzed using the MIXED procedure of SAS, with a model including fixed effects of treatment, day, and the interaction between treatment and day, and a random effect of block. Significance was declared as $P < 0.05$ and trends as $0.15 > P \geq 0.05$.

RESULTS

Performance, blood parameters, and hepatic TAG concentration. Feed intake during the dry period is presented in Table 2. Overfed cows had higher silage and concentrate intakes prepartum than the Con cows. The High cows had greater total DMI compared with the Con cows ($P < 0.05$) (Fig. 1). ME balance (treatment × wk, $P < 0.05$), ME (treatment × wk, $P = 0.05$), and CP intakes (treatment × wk, $P < 0.01$) were greater in High than in Con before parturition, but after parturition there were no differences (Table 3). No significant difference in daily milk yield during the first 14 days postpartum was observed between the two groups (Fig. 2). There were no significant differences in live weights and BCS between High and Con during the dry period and lactation *weeks 1* and *2* (Table 4). No differences in blood parameters were observed between treatments except a tendency for higher plasma insulin concentration ($P = 0.10$) and lower glucagon/insulin ratio (treatment × day, $P = 0.13$) in High cows after parturition (Table 5). Plasma glucose ($P < 0.0001$) and insulin ($P < 0.05$) concentrations were lower after parturition than before parturition in both groups. Plasma NEFA ($P < 0.01$), BHBA ($P < 0.001$), glucagon ($P < 0.0001$), and glucagon/insulin ratio ($P < 0.0001$) increased postpartum (Table 5). Hepatic TAG contents were not different between High and Con cows and increased ($P < 0.001$) postpartum in both groups (Table 5).

Changes in mRNA abundance of genes in the liver. The relative mRNA abundances of hepatic candidate genes are presented in Table 6. There were no significant treatment × time interactions for hepatic genes. The High group had lower *CPT1A* ($P < 0.01$) and *PCK1* ($P < 0.05$) and a tendency for lower *G6PC* ($P = 0.07$) and *PC* ($P = 0.11$) mRNA abundance compared with the Con group. The mRNA expression of *PC* increased ($P < 0.05$) postpartum compared with prepartum across the groups. The prepartal dietary energy level did not affect the mRNA expressions of *IRS1, NFKB1*, or *RBP4* during the sampling period.

Changes in mRNA abundance of genes in AT. Relative gene expression results on SAT candidate genes are presented in Table 7. No significant treatment effect was observed for the mRNA expression of candidate genes in SAT except a tendency ($P = 0.09$) for lower *RETN* and higher *RBP4* ($P = 0.14$) mRNA abundance in the High cows. A treatment × time interaction ($P = 0.11$) was observed for *TNF* characterized by

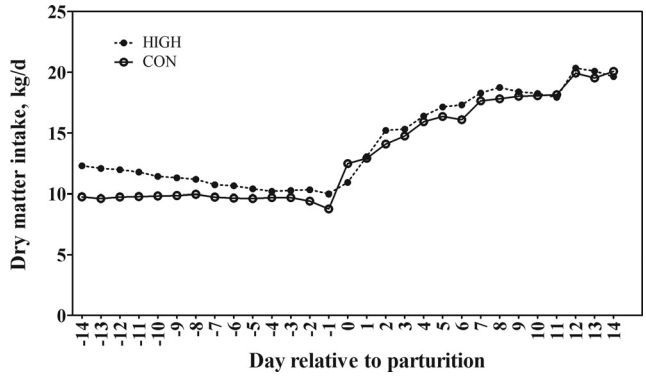

Fig. 1. Daily total dry matter intake [(DMI), kg/day (d)] during the last 2 wk of gestation and first 2 wk of lactation in dairy cows fed a controlled-energy diet [Con; 99 MJ/d metabolizable energy (ME), 100%] or ad libitum-energy diet (High; 141 MJ/d ME, 142%) during the early dry period combined with decreasing energy allowance to 99 MJ/d ME during the last 3 wk before the expected parturition. Values are expressed as least-squares mean; SE for both groups = 0.30. Treatment effect, $P = 0.02$; day, $P < 0.0001$; treatment × day, $P = 0.45$.

Table 3. *Effect of prepartal energy level on ME and CP intakes and ME balance during the transition period*

	Prepartal Energy Level											
	Con				High							
	Weeks Peripartum									*P* Value		
Item	−2	−1	1	2	−2	−1	1	2	SE	Trt	Wk	Trt × Wk
ME intake	109.8	106.9	168.6	207.3	131.1	116.4	170.7	209.7	3.18	0.07	<0.0001	0.05
CP intake	1577	1541	2622	3392	1908	1715	2639	3404	60.20	0.14	<0.0001	0.009
ME balance	4.14	0.63	−81.27	−65.39	24.91	9.40	−80.43	−71.51	4.99	0.39	<0.0001	0.02

ME intake, metabolizable energy intake (MJ/day); CP intake, crude protein intake (g/day). Cows were fed a Con or High diet during the early dry period combined with decreasing feed allowance to 99 MJ/day ME during the last 3 wk before the expected parturition. Trt, treatment; Wk, week.

a higher mRNA abundance in High at 9 days postpartum ($P = 0.07$) compared with Con. In addition, treatment × time interaction ($P < 0.05$) was observed for *SCD* characterized by lower mRNA expression at 1 day postpartum ($P = 0.07$) and higher mRNA expression at 9 days postpartum in the High cows ($P = 0.06$) compared with the Con cows. Lower mRNA abundances of *LEP* ($P < 0.05$), *LPL* ($P < 0.001$), *PCK1* ($P < 0.10$), *SCD* ($P < 0.001$), *PPARG* ($P < 0.10$), and *RBP4* ($P < 0.10$) were observed in SAT postpartum than prepartum in both groups. In contrast, a higher mRNA expression for *RETN* ($P < 0.05$) was detected after parturition than before parturition.

DISCUSSION

The amount of energy intake during the dry period is known to induce changes in blood parameters, and the liver and AT mRNA expression profiles (10, 22, 34). Therefore, the main objectives of this study were to determine the effects of prepartal plane of dietary energy on metabolic measurements and the expression of key genes encoding proteins and nuclear receptors involved in energy metabolism during late gestation through early lactation. The High cows consumed more total DMI, ME, and CP than did the Con cows, which was also reported earlier (17, 41, 42). However, live weights and BCS during the dry period and lactation *weeks 1* and *2* did not significantly differ between the two groups, which is in contrast to these previous studies.

Hepatic gene expression changes. The main finding in the liver gene expression data is that prepartal energy overfeeding decreased the mRNA expression of three important gluconeogenic enzymes (*PC*, *PCK1*, and *G6PC*) during the transition period. The effect of overfeeding of energy on the hepatic gluconeogenic gene expression might have been mediated by a higher plasma insulin concentration postpartum through a feedback mechanism (8, 51). In the current study, the High cows tended to have higher plasma insulin concentrations after parturition compared with the Con cows. Stimulation of hepatic insulin signaling reduces hepatic gluconeogenesis via downregulation of the mRNA expression for *PCK1, G6PC*, and *PC* in dairy cows (8, 18, 34). A recent study by Zachut et al. (55) suggests that cows do not exhibit hepatic insulin resistance during the periparturient period, and thus hepatic insulin signaling is regulated by plasma insulin concentration. Previous studies by Murondoti et al. (37) and Rukkwamsuk et al. (41) reported that the negative effect of dry period overfeeding to hepatic gluconeogenesis is mediated by a high liver fat content. Our results do not support these findings, as there were no differences in the hepatic TAG contents between treatments. A recent study by Hammon et al. (18) observed that high liver lipid does not impair gluconeogenic gene expression.

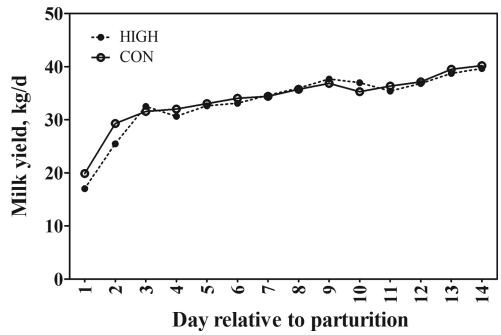

Fig. 2. Daily milk yield (kg/d) during the first 2 wk of lactation in Con or High cows during the early dry period combined with decreasing energy allowance to 99 MJ/d ME during the last 3 wk before the expected parturition. Values are expressed as least-squares mean; SE for the 2 groups = 1.35. Treatment effect, $P = 0.74$; day, $P < 0.0001$; treatment × day, $P = 0.64$.

The increase in mRNA expression for *PC* across the groups after parturition was in line with elevated mRNA level or enzyme activity reported by Greenfield et al. (14), Loor et al. (34), and Murondoti et al. (37), indicating the adaptive adjustments in the liver to meet mammary glucose demands for milk synthesis after parturition (4). As reviewed by Aschenbach et al. (3), the expression of *PC* is regulated by insulin/glucagon ratio and feed restriction. A tendency toward greater glucagon/insulin ratio postpartum in the current study most likely affected the increased *PC* expression and promoted the entry of endogenous substrates (amino acids, lactate) for gluconeogenesis (3, 18) in both groups (more notably in Con). During immediate postpartum period endogenous recycling of carbon through lactate is quantitatively the most important adaptation mechanism to support increased glucose demand (27). The lack of time-related change in *PCK1* supports previous findings (14, 37) showing a lag in the increase of *PCK1* expression during the early days of lactation. Time patterns of gluconeogenic gene expression are most likely coordinated by relative substrate availability. The decreased relative contribution of propionate due to insufficient feed intake favors gene expression of *PC* and *PCK2*, thus facilitating utilization of lactate and alanine (3).

Based on our results and previous studies (31, 34, 37), overfed cows might have a lower hepatic capacity to completely oxidize NEFA (downregulation of *CPT1A*). This might increase esterification of long-chain fatty acids with a resultant increase in the hepatic lipid and TAG accumulations. The

Table 4. *Effect of prepartal energy level on live weight and BCS during the transition period*

Item	Days Peripartum	Prepartal Energy Level		SE	*P* Value		
		Con	High		Trt	Day	Trt × Day
Live weight, kg	−42	694.0	691.9	18.76	0.61	<0.0001	0.51
	−14	721.6	728.8				
	−5	732.9	741.6				
	1	659.2	685.3				
	7	656.4	680.7				
	15	638.5	656.5				
BCS	−42	3.75	3.61	0.11	0.97	<0.0001	0.19
	−14	3.81	3.71				
	−5	3.76	3.70				
	1	3.54	3.52				
	7	3.46	3.45				
	15	3.23	3.29				

Cows were fed Con diet or High diet during the early dry period combined with decreasing feed allowance to 99 MJ/day ME during the last 3 wk before the expected parturition. BCS, body condition score.

decreased *CPT1A* mRNA expression in the High group may be due to an elevated plasma insulin concentration postpartum and lower lipolytic rate prepartum, which in turn would suppress mitochondrial fatty acid β-oxidation. Increased NEFA concentrations very near parturition probably have a strong effect on hepatic TAG accumulation (31). In the current study, plasma NEFA concentration during very early lactation was not affected by prepartal energy intake. Andersen et al. (2) suggested that the decreased hepatic fatty acid oxidation during a hyperinsulinemic-euglycemic clamp is likely via the inhibitory effects of insulin on *CPT1A* and the decrease in NEFA mobilization. Additionally, Litherland et al. (31) and Rukkwamsuk et al. (42) reported that the livers of overfed cows may have been less adapted to oxidize NEFA around parturition as a result of lower basal lipolytic rate prepartum. However, the lack of differences in the liver TAG contents in the present study suggests that hepatic fatty acid oxidation capacity may not be a major factor affecting hepatic TAG concentration.

We did not observe any differences in the mRNA expression of *IRS1* pre- and postpartum between groups. In mice, an acute reduction of *IRS1* expression resulted in an increase in the mRNA abundance of *PCK1* and *G6PC*, indicating the role of *IRS1* mediating insulin signal to regulate gluconeogenesis. In contrast, the lack of treatment- or time-related changes in the present study might indicate that hepatic *IRS1* expression has a small role in hepatic insulin sensitivity dynamics in dairy cows during the periparturient period. In periparturient cow, insulin function in the liver might be regulated by the amount of insulin receptors during the periparturient period (i.e., upregulation of insulin receptors may compensate for the low plasma insulin level during early lactation) (15, 55). The study by Zachut et al. (55) showed that phosphorylation of hepatic insulin receptors is positively related to plasma insulin concentration.

We did not find any time- or treatment-related differences in the mRNA expression of *RBP4* during the course of the study. Recent studies by Abd Eldaim et al. (1) and Rezamand et al. (39) report fluctuation in the plasma *RBP4* concentration during the transition period, with a sharp decline immediately after parturition and rebound toward prepartum levels in early lactation. Our findings are in contrast to the findings of Rezamand et al. (39), who observed that physiological state affected hepatic *RBP4* expression during the periparturient period. Lindberg et al. (30) showed that protein supplementation before parturition induces a more stable plasma *RBP4* concentration near parturition. Therefore, it is possible that different observations of variation of the plasma *RBP4* concentration reflect differences in protein status of cows. In addition to functioning as the main transport system for retinol in circulation, *RBP4* is centrally involved in regulation of glucose

Table 5. *Effect of prepartal dietary energy content on blood parameters and hepatic TAG during the transition period*

Item	Prepartal Energy Level						SE	*P* Value		
	Con			High						
	Days Peripartum									
	−10	1	7/9	−10	1	7/9		Trt	Day	Trt × Day
Glucose, mmol/l	4.4	3.6	2.8	4.1	3.7	3.0	0.08	0.92	<0.0001	0.30
NEFA, mmol/	0.31	0.72	0.57	0.21	0.57	0.54	0.06	0.23	0.003	0.35
Log BHBA, mmol/l	0.80	1.09	1.58	0.65	0.96	1.52	0.08	0.33	0.0001	0.94
Log glucagon, pg/ml	6.37	6.68	6.93	6.51	6.54	6.90	0.12	0.96	<0.0001	0.31
Insulin, μIU/ml	14.0	6.3	5.1	17.5	12.9	9.8	2.09	0.10	0.02	0.51
Log Glucag./Ins. ratio	2.10	2.23	2.48	2.09	2.16	2.31	0.04	0.12	<0.0001	0.13
Log Hepatic TAG	2.48		4.76	2.57		4.99	0.52	0.76	0.0005	0.90

NEFA, nonesterified fatty acids; BHBA, β-hydroxybutyrate; Glucag./Ins. ratio, glucagon/insulin ratio (mol/mol). BHBA, glucagon, glucagon/insulin ratio, and hepatic triglyceride concentrations (TAG) are presented as $\log_2$-transformed to normalize the data. Cows were fed Con or High diet during the early dry period combined with decreasing feed allowance to 99 MJ/day ME during the last 3 wk before the expected calving. Hepatic TAG content was measured at 9 days instead of 7 days after parturition.

Table 6. *Effect of prepartal dietary energy content on the relative mRNA abundance of hepatic genes (log_2) during the transition period*

	Prepartal Energy Level									
	Con			High						
	Days Peripartum							*P* Value		
Gene	−10	1	9	−10	1	9	SE	Trt	Day	Trt × Day
CPT1A	3.79	3.82	3.85	3.61	3.60	3.66	0.04	0.005	0.32	0.85
G6PC	3.99	3.84	3.89	3.73	3.73	3.77	0.06	0.07	0.34	0.46
IRS1	3.61	3.56	3.61	3.50	3.46	3.51	0.06	0.27	0.60	0.99
PC	3.55	3.79	3.68	3.46	3.56	3.55	0.06	0.11	0.01	0.42
PCK1	4.07	4.07	4.14	3.99	3.93	3.94	0.04	0.03	0.58	0.25
NFKB1	3.02	3.09	2.96	2.71	2.82	2.80	0.12	0.18	0.53	0.83
RBP4	4.17	4.21	4.17	4.24	4.12	4.21	0.05	0.88	0.78	0.47

Cows were fed Con or High diet during the early dry period combined with decreasing feed allowance to 99 MJ/day ME during the last 3 wk before the expected parturition.

metabolism. Yang et al. (54) reported that *RBP4* can directly induce *PCK1* expression and reduce insulin action to suppress glucose production in mouse liver. There was a significant positive correlation between *RBP4* and *PCK1* ($r = 0.493$, $P = 0.001$) in the current study, suggesting that *RBP4* might have a role in the regulation of *PCK1* in cows (independent of prepartum energy intake and BW change).

Overfeeding prepartum had no effect on the mRNA expression of *NFKB1* during the transition period. In mice, subacute hepatocellular activation of *NFKB1* induced by a high-fat diet and fatty liver caused hepatic IR and increased proinflammatory cytokine production (9). The absence of a treatment effect for *NFKB1* expression in our study could be related to the lack of differences in energy balance, NEFA concentrations, and liver TAG contents between the dietary groups.

Expression of adipokines in AT. AT plays a crucial role in the regulation of lipid metabolism, and the transition from late gestation to early lactation is characterized by a physiological state of peripheral IR (4). In human obesity a variety of adipokines has been implicated in IR such as *ADIPOQ*, *RBP4*, *LEP*, *RETN*, *IL6*, and *TNF*. However, the mechanisms by which adipokines induce IR are not yet fully understood.

Prepartal energy level did not affect the expression of *ADIPOQ* pre- and postpartum between the two groups. These results are contrary to Ji et al. (22), who observed that overfeeding energy prepartum led to considerable upregulation of *ADIPOQ* until 7 days postpartum, which was driven by increased *PPARG* expression. In addition, several factors may have contributed to the differences in results between the present study and Ji et al. (22), e.g., breed, dietary composition, level and duration of overfeeding, and body fatness before and after treatment. In the study by Ji et al. (22), the diets with different energy contents were fed for the last 3 wk of pregnancy, whereas in the current study high-energy feeding started 6 wk prior to parturition and the difference of energy allowance was gradually decreased during the last 3 wk of gestation.

Our results are similar to Lemor et al. (28) and Sadri et al. (45), who report that treatment- and time-related changes for *ADIPOQ* were not detectable in SAT. We did not observe treatment- or time-related differences in the mRNA abundance

Table 7. *Effect of prepartal dietary energy content on the relative mRNA abundance of subcutaneous adipose tissue genes (log_2) during the transition period*

	Prepartal Energy Level									
	Con			High						
	Days Peripartum							*P* Value		
Gene	−10	1	9	−10	1	9	SE	Trt	Day	Trt × Day
ADIPOQ	4.05	3.96	3.89	3.98	3.96	3.88	0.04	0.59	0.23	0.65
ADIPOR1	3.25	2.95	2.98	3.01	2.95	3.14	0.14	0.88	0.43	0.51
ADIPOR2	3.82	3.65	3.49	3.58	3.42	3.50	0.11	0.20	0.41	0.68
IL6	2.80	2.75	2.53	2.58	2.64	2.70	0.08	0.66	0.72	0.22
IRS1	3.46	3.46	3.29	3.38	3.41	3.37	0.04	0.82	0.27	0.29
LEP	3.69	3.50	3.14	3.66	3.35	3.44	0.09	0.75	0.01	0.13
LIPE	3.32	3.31	3.01	3.28	2.99	3.08	0.15	0.68	0.38	0.49
LPL	3.83	3.56	3.54	3.86	3.49	3.66	0.10	0.84	0.0009	0.39
PCK1	3.30	3.08	2.93	3.44	3.21	2.96	0.11	0.51	0.07	0.95
PPARG	3.67	3.72	3.49	3.69	3.59	3.56	0.04	0.80	0.06	0.45
RBP4	3.98	3.94	3.76	4.01	3.97	3.91	0.03	0.14	0.06	0.65
RETN	2.36	2.44	2.94	1.95	2.49	2.53	0.10	0.09	0.03	0.23
SCD	4.03	3.66	3.38	4.08	3.43	3.73	0.07	0.26	0.0009	0.03
TNF	2.26	1.99	1.88	2.26	1.86	2.41	0.14	0.50	0.24	0.11

Cows were fed Con or High diet during the early dry period combined with decreasing feed allowance to 99 MJ/day ME during the last 3 wk before the expected parturition.

of *ADIPOR1/R2*. Our results are consistent with Giesy et al. (13) and Sadri et al. (45), who found no changes in the expression of *ADIPOR1/R2* from pregnancy to early lactation in SAT. Lemor et al. (28) reports that the mRNA expression of *ADIPOR1/R2* was reduced in SAT postpartum.

In the present study, dietary energy prepartum did not affect the expression of *RBP4* during the sampling period and tended to decrease in both groups postpartum. There is very little information concerning gene expression of *RBP4* and its role in bovine SAT during the transition period. Locher et al. (33) studied *RBP4* expression in different bovine AT and observed that expression in visceral fat was higher than in SAT. A recent study by Ungru et al. (50) in ponies reported a significant reduction in serum *RBP4* concentrations with body weight reduction program, while there were no differences in the mRNA expression of *RBP4* in SAT between insulin-sensitive and insulin-resistant ponies. Furthermore, they observed that serum *RBP4* was closely linked to adiposity probably independently of IR.

In the current study, a trend for lower mRNA expression of *RETN* was observed in High cows, suggesting that overfeeding energy allowance prepartum may not exacerbate IR in SAT. The upregulation of *RETN* postpartum in both groups suggests decreased insulin sensitivity of SAT. Our results are in line with Komatsu et al. (26), who observed that *RETN* mRNA expression in SAT increased in cows during the early phase of lactation.

Our results show that prepartal energy did not affect the expression of *LEP* mRNA. Conversely, Ji (21) observed that overfeeding energy increases the expression of *LEP* mRNA compared with a controlled energy feeding. In the study by Ji (21), overfed cows had higher BCS (even in the beginning of the treatment period) than Con cows, which may explain why *LEP* was higher in overfed cows in their study (and not in the current study, with minor differences in adiposity). The decline of *LEP* expression from pre- to postpartum concurs with earlier studies showing a decrease of plasma *LEP* during late pregnancy through early lactation (6, 19, 24) and paralleled the decrease of plasma insulin during the periparturient period. The ability of insulin to stimulate *LEP* secretion seems to be attenuated during early lactation (28), but other factors may also contribute to low *LEP* expression postpartum.

In the present study, we found no evidence on the effect of prepartal energy overfeeding on the mRNA abundance of *IL6*, suggesting that this adipocytokine does not mediate the potential effects of high-energy feeding on SAT insulin responsiveness. Our results are in line with Ji (21), who reported no difference in *IL6* mRNA between overfed and CON cows. We observed higher mRNA abundance of *TNF* in the High group compared with the Con group at 9 days postpartum, suggesting that overfeeding energy combined with decreasing feed allowance prepartum may exacerbate the inflammation status and might attenuate SAT insulin sensitivity postpartum. Our results are in contrast with Winkelman et al. (53), who found no differences in the plasma *TNF* concentration pre- and postpartum between limited and ad libitum DMI groups.

Expression of genes controlling lipogenesis and lipolysis. At the transcriptional level, we found no difference in mRNA abundance for *IRS1* between groups and no time-related changes during the periparturient period. Lack of changes in *IRS1* mRNA expression between −10 and 7 days relative to parturition in Ji et al. (22) was similar to the current study. In addition, Sadri et al. (44) observed no changes in the mRNA abundance of *IRS1* in a larger time scale, over the dry period and early lactation. The lack of change in *IRS1* expression during the immediate periparturient period supports a notion that a defect in posttranslational modification of *IRS1* after parturition is a major mechanism affecting IR (22). Zachut et al. (55) observed recently that the total amount of insulin receptor protein did not differ pre- and postpartum. Furthermore, they reported differences in insulin signaling in SAT between cows of low and high live weight loss, suggesting that genetic background may affect insulin responsiveness in SAT pre- and postpartum.

Excess dietary energy intake prepartum did not affect the mRNA expression of *LPL* or *PCK1*. A trend for downregulation of *SCD* at 1 day and upregulation at 9 days postpartum in the High cows was observed, suggesting that lipogenesis regarding fatty acid desaturation was temporarily decreased very near parturition in High compared with Con. The lack of response in the expression of *LPL* and *PCK1* was in line with lack of changes in adipokines potentially affecting insulin sensitivity. A coordinated downregulation of genes involved in fatty acid uptake (*LPL*), fatty acid desaturation (*SCD*), and glyceroneogenesis (*PCK1*) postpartum may be attributed to aggravated IR in early lactation (22), and favoring utilization of free fatty acids for TAG synthesis in the mammary gland (52). In agreement with the lack of differences in the expression of lipogenic genes, we observed no significant effect of treatment on the expression of *PPARG* in SAT pre- and postpartum.

Hormone sensitive lipase (HSL) stimulates the lipolysis of diacylglycerides to monoglycerides (35). The present study found no change in the mRNA expression of *LIPE* between treatments over time, which was in agreement with the results of Sadri et al. (45). On the contrary, Sumner and McNamara (49) reported upregulation of *LIPE* postpartum compared with prepartum. It should be noted that posttranscriptional regulation may have an important role in the control of lipolysis, since Koltes and Spurlock (25) observed an increased HSL phosphorylation in SAT early postpartum compared with late pregnancy.

Conclusions

Downregulation of key enzymes linked to gluconeogenesis (*PC*, *PCK1*, and *G6PC*) and fatty acid β-oxidation (*CPT1A*) in the High group indicates that overfeeding energy prepartum impaired liver function compared with the controlled-energy diet, suggesting decreased gluconeogenesis and fatty acid β-oxidation. Based on gene expression, prepartal overfeeding of energy did not exacerbate IR in SAT around parturition. Results of this study indicate that dry-period energy feeding can alter transcriptional adaptations of crucial genes in the liver but much less in SAT during the transition period.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: S. Selim, S. Salin, J.T., A.V., T.K., and K.T.E. performed experiments; S. Selim, S. Salin, J.T., A.V., T.K., and K.T.E. analyzed data; S. Selim, S. Salin, J.T., A.V., T.K., and K.T.E. interpreted results of experiments; S. Selim prepared figures; S. Selim drafted manuscript; S. Selim, S. Salin, J.T., A.V., T.K., and K.T.E. edited and revised manuscript; S. Selim, S. Salin, J.T., A.V., T.K., and K.T.E. approved final version of manuscript; S. Salin, J.T., A.V., T.K., and K.T.E. conception and design of research.

REFERENCES

1. **Abd Eldaim MA, Kamikawa A, Soliman MM, Ahmed MM, Okamatsu-Ogura Y, Terao A, Miyamoto T, Kimura K.** Retinol binding protein 4 in dairy cows: its presence in colostrums and alteration in plasma during fasting, inflammation, and the peripartum period. *J Dairy Res* 77: 27–32, 2010.
2. **Andersen JB, Mashek DG, Larsen T, Nielsen MO, Ingvartsen KL.** Effects of hyperinsulinaemia under euglycaemic condition on liver fat metabolism in dairy cows in early and mid-lactation. *J Vet Med A Physiol Pathol Clin Med* 49: 65–71, 2002.
3. **Aschenbach JR, Kristensen NB, Donkin SS, Hammon HM, Penner GB.** Gluconeogenesis in dairy cows: the secret of making sweet milk from sour dough. *IUBMB Life* 62: 869–877, 2010.
4. **Bell AW, Bauman DE.** Adaptations of glucose metabolism during pregnancy and lactation. *J Mammary Gland Biol Neoplasia* 2: 265–278, 1997.
5. **Bionaz M, Loor JJ.** Gene networks driving bovine milk fat synthesis during the lactation cycle. *BMC Genomics* 9: 366, 2008.
6. **Block SS, Butler WR, Ehrhardt RA, Bell AW, Van Amburgh ME, Boisclair YR.** Decreased concentration of plasma leptin in periparturient dairy cows is caused by negative energy balance. *J Endocrinol* 171: 339–348, 2001.
7. **Bonnet M, Bernard L, Bes S, Leroux C.** Selection of reference genes for quantitative real-time PCR normalisation in adipose tissue, muscle, liver and mammary gland for ruminants. *Animal* 7: 1344–1353, 2013.
8. **Bradford BJ, Allen MS.** Phlorizin administration increases hepatic gluconeogenic enzyme mRNA abundance but not feed intake in late-lactation dairy cows. *J Nutr* 135: 2206–2211, 2005.
9. **Cai D, Yuan M, Frantz DF, Melendez PA, Hansen L, Lee J, Shoelson SE.** Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat Med* 11: 183–190, 2005.
10. **Dann HM, Litherland NB, Underwood JP, Bionaz M, D'Angelo A, McFadden JW, Drackley JK.** Diets during far-off and close-up dry periods affect periparturient metabolism and lactation in multiparous cows. *J Dairy Sci* 89: 3563–3577, 2006.
11. **Edmonson AJ, Lean IJ, Weaver LD, Farver T, Webster G.** A body condition chart of Holstein dairy cows. *J Dairy Sci* 72: 68–78, 1989.
12. **Franckhauser S, Elias I, Rotter Sopasakis V, Ferré T, Nagaev I, Andersson CX, Agudo J, Ruberte J, Bosch F, Smith U.** Overexpression of IL6 leads to hyperinsulinemia, liver inflammation and reduced body weight in mice. *Diabetologia* 51: 1306–1316, 2008.
13. **Giesy SL, Yoon B, Currie WB, Kim JW, Boisclair YR.** Adiponectin deficit during the precarious glucose economy of early lactation in dairy cows. *Endocrinology* 153: 5834–5844, 2012.
14. **Greenfield RB, Cecava MJ, Donkin SS.** Changes in mRNA expression for gluconeogenic enzymes in liver of dairy cattle during the transition to lactation. *J Dairy Sci* 83: 1228–1236, 2000.
15. **Gross J, van Dorland HA, Schwarz FJ, Bruckmaier RM.** Endocrine changes and liver mRNA abundance of somatotropic axis and insulin system constituents during negative energy balance at different stages of lactation in dairy cows. *J Dairy Sci* 94: 3484–3494, 2011.
16. **Grummer RR.** Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. *J Dairy Sci* 73: 2820–2833, 1995.
17. **Grummer RR, Mashek DG, Hayirli A.** Dry matter intake and energy balance in the transition period. *Vet Clin North Am Food Anim Pract* 20: 447–470, 2004.
18. **Hammon HM, Stürmer G, Schneider F, Tuchscherer A, Blum H, Engelhard T, Genzel A, Staufenbiel R, Kanitz W.** Performance and metabolic and endocrine changes with emphasis on glucose metabolism in high-yielding dairy cows with high and low fat content in liver after calving. *J Dairy Sci* 92: 1554–1566, 2009.
19. **Holtenius K, Agenäs S, Delavaud C, Chilliard Y.** Effects of feeding intensity during the dry period. 2. Metabolic and hormonal responses. *J Dairy Sci* 86: 883–891, 2003.
20. **Holtenius P.** Hormonal regulation related to the development of fatty liver and ketosis. *Acta Vet Scand Suppl* 89: 55–60, 1993.
21. **Ji P.** *Transcriptional Adaptation of Adipose Tissue in Dairy Cows in Response to Energy Overfeeding* (PhD Dissertation), Urbana-Champaign: IL: University of Illinois, 2011.
22. **Ji P, Osorio JS, Drackley JK, Loor JJ.** 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. *J Dairy Sci* 95: 4333–4351, 2012.
23. **Kadegowda AK, Bionaz M, Thering B, Piperova LS, Erdman RA, Loor JJ.** Identification of internal control genes for quantitative polymerase chain reaction in mammary tissue of lactating cows receiving lipid supplements. *J Dairy Sci* 92: 2007–2019, 2009.
24. **Kokkonen T, Taponen J, Anttila T, Syrjala-Qvist L, Delavaud C, Chilliard Y, Tuori M, Tesfa AT.** Effect of body fatness and glucogenic supplement on lipid and protein mobilization and plasma leptin in dairy cows. *J Dairy Sci* 88: 1127–1141, 2005.
25. **Koltes DA, Spurlock DM.** Coordination of lipid droplet associated proteins during the transition period of Holstein dairy cows. *J Dairy Sci* 94: 1839–1848, 2011.
26. **Komatsu T, Itoh F, Mikawa S, Hodate K.** Gene expression of resistin in adipose tissue and mammary gland of lactating and non-lactating cows. *J Endocrinol* 178: R1–R5, 2003.
27. **Larsen M, Kristensen NB.** Precursors for liver gluconeogenesis in periparturient dairy cows. *Animal* 7: 1640–1650, 2013.
28. **Lemor A, Hosseini A, Sauerwein H, Mielenz M.** Transition period-related changes in the abundance of the mRNAs of adiponectin and its receptors, of visfatin, and of fatty acid binding receptors in adipose tissue of high-yielding dairy cows. *Domest Anim Endocrinol* 37: 37–44, 2009.
29. **Leury BJ, Baumgard LH, Bloch SS, Segoale N, Ehrhardt RA, Rhoads RP, Bauman DE, Bell AW, Boisclair YR.** Effect of insulin and growth hormone on plasma leptin in periparturient dairy cows. *Am J Physiol Regul Integr Comp Physiol* 285: R1107–R1115, 2003.
30. **Lindberg LA, Sinkkonen H, Pösö AR, Tesfa AT, Schröder J.** Production of monoclonal antibodies and enzyme immunoassay to bovine retinol-binding protein and determination of retinol-binding protein serum levels and retinol concentrations in serum and liver in dairy cows before and after parturition. *Res Vet Sci* 66: 259–263, 1999.
31. **Litherland NB, Dann HM, Drackley JK.** Prepartum nutrient intake alters palmitate metabolism by liver slices from peripartal dairy cows. *J Dairy Sci* 94: 1928–1940, 2011.
32. **Liu W, Saint DA.** A new quantitative method of real time reverse transcription polymerase chain reaction assay based on stimulation of polymerase chain reaction kinetics. *Anal Biochem* 302: 52–59, 2002.
33. **Locher L, Zapfe L, Kern M, Klöting N, Blüher M, Raila J, Fürll M.** Expression of RBP4-mRNA in adipose tissue and RBP4 in serum of healthy dairy cows. In: *Ruminant physiology: Digestion, Metabolism, and Effects Of Nutrition on Reproduction And Welfare*, edited by Chilliard Y, Glasser F, Faulconnier Y, Bocquier F, Veissier I, Doreau M. Wageningen, the Netherlands: Wageningen Academic Publishers, p. 580–581, 2009.
34. **Loor JJ, Dann HM, Guretzky NA, Everts RE, Oliveira R, Green CA, Litherland NB, Rodriguez-Zas SL, Lewin HA, Drackley JK.** Plane of nutrition prepartum alters hepatic gene expression and function in dairy cows as assessed by longitudinal transcript and metabolic profiling. *Physiol Genomics* 27: 29–41, 2006.
35. **McNamara JP.** Adipose tissue during lactation: where do we go from here? *Proc Nutr Soc* 56: 149–167, 1997.
36. **MTT.** *Feed Tables and Nutrient Requirements*. Jokioinen, Finland: MTT Agrifood Research Finland. Available at: http://www.mtt.fi/feedtables, 2011.
37. **Murondoti A, Jorritsma R, Beynen AC, Wensing T, Geelen MJ.** Unrestricted feed intake during the dry period impairs the postpartum oxidation and synthesis of fatty acids in the liver of dairy cows. *J Dairy Sci* 87: 672–679, 2004.
38. **Nygren H, Seppänen-Laakso T, Castillo S, Hyötyläinen T, Orešič M.** Liquid chromatography-mass spectrometry (LC-MS)-based lipidomics for

studies of body fluids and tissues. In: *Metabolic Profiling, Methods in Molecular Biology* 708, edited by Metz TO. Springer Science+Business Media, p. 247–257, 2011.

39. **Rezamand P, Watts JS, Hunt KM, Bradford BJ, Mamedova LK, Morey SD.** Bovine hepatic and adipose retinol-binding protein gene expression and relationship with tumor necrosis factor-α. *J Dairy Sci* 95: 1–8, 2012.
40. **Rozen S, Skaletzky H.** Primer3 on the WWW for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology*, edited by Krawetz S, Misener S. Totowa, NJ: Humana, p. 365–386, 2000.
41. **Rukkwamsuk T, Wensing T, Geelen MJ.** Effect of fatty liver on hepatic gluconeogenesis in periparturient dairy cows. *J Dairy Sci* 82: 500–505, 1999.
42. **Rukkwamsuk T, Wensing T, Geelen MJ.** Effect of overfeeding during the dry period on regulation of adipose tissue metabolism in dairy cows during the periparturient period. *J Dairy Sci* 81: 2904–2911, 1998.
43. **Rukkwamsuk T, Wensing T, Geelen MJ.** Effect of overfeeding during the dry period on the rate of esterification in adipose tissue of dairy cows during the periparturient period. *J Dairy Sci* 82: 1164–1169, 1999.
44. **Sadri H, Bruckmaier RM, Rahmani HR, Ghorbani GR, Morel I, van Dorland HA.** Gene expression of tumour necrosis factor and insulin signalling-related factors in subcutaneous adipose tissue during the dry period and in early lactation in dairy cows. *J Anim Physiol Anim Nutr* 94: 194–202, 2010.
45. **Sadri H, Mielenz M, Morel I, Bruckmaier RM, van Dorland HA.** Plasma leptin and mRNA expression of lipogenesis and lipolysis-related factors in bovine adipose tissue around parturition. *J Anim Physiol Anim Nutr* 95: 790–797, 2011.
46. **Salin S, Taponen J, Elo K, Simpura I, Vanhatalo A, Boston R, Kokkonen T.** Effects of abomasal infusion of tallow or camelina oil on responses to glucose and insulin in dairy cows during late pregnancy. *J Dairy Sci* 95: 3812–3825, 2012.
47. **Saremi B, Sauerwein H, Dänicke S, Mielenz M.** Technical note: identification of reference genes for gene expression studies in different bovine tissues focusing on different fat depots. *J Dairy Sci* 95: 3131–3138, 2012.
48. **Schroeder A, Mueller O, Stocker S, Salowsky R, Leiber M, Gassmann M, Lightfoot S, Menzel W, Granzow M, Ragg T.** The RIN: an RNA integrity number for assigning integrity values to RNA measurements. *BMC Mol Biol* 7: 3, 2006.
49. **Sumner JM, McNamara JP.** Expression of lipolytic genes in the adipose tissue of pregnant and lactating Holstein dairy cattle. *J Dairy Sci* 90: 5237–5246, 2007.
50. **Ungru J, Blüher M, Coenen M, Raila J, Boston R, Vervuert I.** Effects of body weight reduction on blood adipokines and subcutaneous adipose tissue adipokine mRNA expression profiles in obese ponies. *Vet Rec 24:* 171: 528, 2012.
51. **Velez JC, Donkin SS.** Feed restriction induces pyruvate carboxylase but not phosphoenolpyruvate carboxykinase in dairy cows. *J Dairy Sci* 88: 2938–2948, 2005.
52. **Vernon RG.** Lipid metabolism during lactation: a review of adipose tissue-liver interactions and the development of fatty liver. *J Dairy Res* 72: 460–469, 2005.
53. **Winkelman LA, Elsasser TH, Reynolds CK.** Limit-feeding a high-energy diet to meet energy requirements in the dry period alters plasma metabolite concentrations but does not affect intake or milk production in early lactation. *J Dairy Sci* 91: 1067–1079, 2008.
54. **Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, Kotani K, Quadro L, Kahn BB.** Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature* 436: 356–362, 2005.
55. **Zachut M, Honig H, Striem S, Zick Y, Boura-Halfon S, Moallem U.** Periparturient dairy cows do not exhibit hepatic insulin resistance, yet adipose-specific insulin resistance occurs in cows prone to high weight loss. *J Dairy Sci* 96: 5656–5669, 2013.