Longitudinal changes in adipose tissue of dairy cows from late pregnancy to lactation. Part 2: The SIRT-PPARGC1A axis and its relationship with the adiponectin system

M. Weber,* L. Locher,† K. Huber,‡¹ J. Rehage,† R. Tienken,§ U. Meyer,§ S. Dänicke,§ L. Webb,* H. Sauerwein,* and M. Mielenz*^{2,3}

*Institute of Animal Science, Physiology and Hygiene Unit, University of Bonn, 53115 Bonn, Germany

†Clinic for Cattle, University of Veterinary Medicine Hannover, 30173 Hannover, Germany

‡Department of Physiology, University of Veterinary Medicine Hannover, 30173 Hannover, Germany

§Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, 38116 Braunschweig, Germany

ABSTRACT

The transition period in dairy cows is characterized by major changes in glucose and adipose tissue metabolism. The Sirtuin-1 (SIRT1) PPAR γ co-activator 1 α (PPARGC1A) axis might be related to the adiponectin (ADIPOQ) system to orchestrate the regulation of these processes. We aimed to assess the mRNA abundance of the aforementioned components in one visceral and one subcutaneous fat depot, together with the ADIPOQ concentrations in serum of dairy cows from late gestation to early lactation. In addition, the effect of 2 diets differing in energy density was tested. Twenty pluriparous German Holstein cows were all kept on the same silage-based diet until d 42 antepartum. From then on until d 1 antepartum, 10 animals each were assigned to either high-concentrate (60:40 concentrate:roughage) or low-concentrate (30:70) diets. Both groups were further subdivided into a control and a niacin group, the latter receiving 24 g/d nicotinic acid from d –42 until d 24. From d 1 postpartum (p.p.) to d 24 p.p., the concentrate portion was increased from 30 to 50% for all cows. Biopsies of subcutaneous (SCAT) and retroperitoneal adipose tissue (RPAT) were taken at d –42, 1, 21, and 100 relative to parturition. Blood samples were drawn along with the biopsies as well as on d –21, –14, –7, –3, 1, 3, 7, 14, 21, 28, 35, 42, 63, 82, and 100 relative to calving. Quantification of target mRNA was done using quantitative PCR and serum ADIPOQ concentration was measured via ELISA. The feeding regimen did not affect the variables examined. Serum

ADIPOQ concentrations decreased toward parturition, returned to precalving levels within 1 wk after parturition, and remained on a constant level until the end of the experiment. The mRNA abundance of *SIRT1*, *PPARGC1A*, *NAMPT*, and the ADIPOQ receptors 1 (*ADIPOR1*) and 2 (*ADIPOR2*) changed in SCAT and RPAT during the considered time period. Comparing SCAT and RPAT, the mRNA of *SIRT1*, *ADIPOR1*, and *ADIPOR2* were more abundant in RPAT, whereas *PPARGC1A* and *NAMPT* were expressed more highly in SCAT. The protein abundance of SIRT1 tended to increase from d –42 to 21. At d 21 we detected more PPARGC1A protein in the low-concentrate group as compared with the high-concentrate group. The correlations observed point to a link between these factors and might hint to a functional role of the variables in the regulation of glucose metabolism. This study substantiates the existence of the SIRT1-PPARGC1A-axis and indicates a functional relationship between *SIRT1* and *ADIPOR1* in bovine adipose tissue.

Key words: dairy cow, transition period, Sirtuin-1, PPAR γ co-activator 1 α , adiponectin

INTRODUCTION

Dairy cows undergo a plethora of metabolic changes during the transition from late gestation to lactation (Bauman and Currie, 1980), which includes among others the activation of the adrenergic system as most important trigger of lipolysis (McNamara and Hillers, 1986). During late pregnancy, dairy cows experience a period of insulin resistance, which is similar to other mammalian species such as humans, maintained in early lactation (Bell and Bauman, 1997; Lain and Catalano, 2007). The adiponectin system is supposed to be part of the hormonal adaptations regulating insulin sensitivity (Giesy et al., 2012) and some indications are present that this system is possibly connected to the Sirtuin-1 (SIRT1) PPAR γ co-activator 1 α (PPARGC1A) axis

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¹Present address: Institute of Animal Science, University of Hohenheim, 70599 Stuttgart-Hohenheim, Germany.

²Present address: Institute of Nutrition Physiology "Oskar Kellner," Leibniz Institute for Farm Animal Biology (FBN), 18196 Dummerstorf, Germany.

³Corresponding author: mielenz@fhn-dummerstorf.de

(Iwabu et al., 2010). This network is likely involved in the orchestration of the complex metabolic adaptations during the transition period.

Sirtuin-1 belongs to a family of highly conserved NAD⁺-dependent enzymes that deacetylate lysine residues on various proteins (Chaudhary and Pfluger, 2009). Depending on the intracellular NAD⁺/NADH ratio, these enzymes act as sensors to detect the cellular energy availability [i.e., a low energy level characterized by high NAD⁺ and low NADH levels leads to an activation of SIRT1 (Imai et al., 2000; Chaudhary and Pfluger, 2009)]. This activation induces an increased lipolysis via a repression of PPAR γ in differentiated adipocytes. Furthermore, SIRT1 was shown to be a modulator of adipogenesis (Picard et al., 2004). Sirtuin-1 mRNA was shown to be expressed in adipose tissue (AT) of bulls and Holstein male calves and reported to increase with the animals' age (Ghinis-Hozumi et al., 2011; Liu et al., 2014).

One of the target proteins of SIRT1 is PPARGC1A. Activation of PPARGC1A via deacetylation induces gluconeogenic genes in liver, whereas glycolytic genes are downregulated (Rodgers et al., 2005). The PPARGC1A protein was found to be expressed independent of the status of gestation or lactation in liver, kidney, and muscle of beef cows (Wood et al., 2013). A regulatory element of SIRT1 function is nicotinamide phosphoribosyltransferase (NAMPT), the rate-limiting enzyme in the NAD biosynthesis pathway in mammals. Activation of NAMPT leads to an elevation of the cellular NAD⁺ level and subsequently activates SIRT1 (Revollo et al., 2004). Initially characterized as the human pre-B-cell colony-enhancing factor (PBEF; Samal et al., 1994), NAMPT was also identified as an adipokine named visfatin (Hammarstedt et al., 2006). Overexpression of visfatin in rodents activates PPAR γ and ameliorates whole body insulin sensitivity (Sun et al., 2009).

In subcutaneous AT (SCAT) of Holstein dairy cows, visfatin mRNA abundance tended to decrease postpartum (p.p.) (Lemor et al., 2009). A further element involved in the regulation of SIRT1 activity is the adipokine adiponectin (ADIPOQ). Adiponectin is mainly expressed by adipocytes and exerts insulin-sensitizing effects and inhibits lipolysis in AT (Yamauchi et al., 2001; Tschritter et al., 2003). In muscle cells of mice, ADIPOQ was reported to activate SIRT1 by signaling through ADIPOQ receptor 1 (ADIPOR1), which subsequently led to a decreased acetylation of PPARGC1A (Iwabu et al., 2010). Conversely, activation of SIRT1 was shown to decrease the secretion of ADIPOQ (Qiang et al., 2007). In Holstein dairy cows, serum ADIPOQ concentrations decrease toward parturition, reach a nadir at d 1 after calving, and subsequently

increase toward prepartum levels (Singh et al., 2014). Simultaneously, the *ADIPOR1* and *ADIPOR2* mRNA abundances were reported to decrease in subcutaneous AT during the transition period (Lemor et al., 2009).

The existence of the SIRT1-PPARGC1A axis was reported in AT of mice and is assumed to play a role in the regulation of insulin sensitivity (de las Heras et al., 2013). To our knowledge, so far nothing is known about this axis in AT of dairy cows. We assumed that the axis' elements might be involved in the metabolic adaptation processes during the transition period in a tissue-specific manner. Therefore the aim of the present study was to characterize the mRNA abundance of the aforementioned factors as well as PPARGC1A protein expression in SCAT and retroperitoneal AT (RPAT) of Holstein dairy cows during late pregnancy and early lactation and their relationship with the ADIPOQ system. We hypothesized that feeding different portions of concentrate during the prepartum period, resulting in differences in energy balance and a concomitant niacin supplementation, might influence the bovine SIRT1-PPARGC1A axis to support the transition from late pregnancy to lactation.

MATERIALS AND METHODS

Animals, Feeding, and Sampling of AT

Study design, feeding components, AT sample collection, and processing are described in the companion paper by Weber et al. (2016). Table 1 shows the diet fed antepartum and p.p.

Blood Sampling and Analyses

Blood samples were drawn from the jugular vein on d -42, -21, -14, -7, -3, 1, 3, 7, 14, 21, 28, 35, 42, 63, 82, and 100 relative to calving. After clotting, blood was centrifuged at $2,000 \times g$ for 15 min at 15°C and serum was stored at -80°C. Serum adiponectin concentrations were measured in duplicate using an in-house developed bovine-specific ELISA as described in detail earlier (Mielenz et al., 2013). The measuring range as well as the linear range of the assay was 0.07 to 1.0 ng/mL. The intra- and interassay coefficients of variation were 7 and 11%, respectively.

Western Blot Analysis

The SCAT biopsy samples from all 20 cows at d -42 and 21 as well as RPAT samples taken at d 21 were prepared for PPARGC1A Western blot analyses. For SIRT1, RPAT samples from the control cows at d -42,

Table 1. Diet composition antepartum versus postpartum with different portions of concentrate versus roughage in the prepartum period

Nutrient (g/kg of DM)	Concentrate		Roughage	
	Prepartum ¹	Postpartum ²	Corn	Grass
Crude ash	42	47	36	94
CP	230	228	120	136
Ether extract	39	39	29	32
ADF	45	44	222	310
NDF	163	152	461	506

¹Concentrate to roughage ratio: low concentrate group 30:70, high concentrate group 60:40.

²Concentrate portion of 30% for all cows, increased to 50% within 16 d for the low concentrate group and within 24 d for the high concentrate group.

1, and 21 as well as SCAT samples taken at d 21 were analyzed.

Tissue samples were homogenized in homogenization buffer containing 10 mM HEPES pH 7.4 and complete protease inhibitor cocktail (one tablet per 10 mL, Roche, Mannheim, Germany) using the Precellys 24 system (PqLab Biotechnologie GmbH, Erlangen, Germany). Homogenates were centrifuged twice (14,000 × *g*, 4°C for 10 min) to separate the fat layer. Aliquots of the supernatants were stored at −20°C until electrophoresis. The total protein concentration was measured using the Bradford assay (Roti Nanoquant K880, Carl Roth GmbH, Karlsruhe, Germany). For PPARGC1A, the samples were diluted to 0.5 mg/mL with purified water and mixed with loading buffer (final concentrations: 0.1 *M* Tris HCl pH 6.8, 1% SDS, 0.0032% bromophenol blue, 10% glycerol). The samples were loaded in duplicates (17.5 µg of protein per lane) on 8% gels, separated by SDS-PAGE, and transferred onto polyvinylidene difluoride membranes (PVDF, GE Healthcare Europe, Freiburg, Germany) with the use of the Trans-Blot Turbo transfer unit (Bio-Rad Laboratories, Munich, Germany). Membranes were blocked in Tris-buffered saline solution-Tween (**TBS-T**) containing 10% Roti-Block (Carl Roth GmbH) and 0.1% Tween 20 (AppliChem GmbH, Darmstadt, Germany) for 1 h at room temperature. After blocking, a rabbit anti-PPARGC1A antibody (dilution 1:500, Merck Millipore, Merck KGaA, Darmstadt, Germany) was incubated in TBS-T containing 3% fat free milk powder (Carl Roth GmbH) and 0.1% Tween 20 overnight at 4°C. This antibody was already successfully used by Wood et al. (2013) for assessing PPARGC1A protein in bovine liver and muscle tissue. After washing, membranes were incubated with a horseradish peroxidase (**HRP**)-conjugated corresponding secondary antibody (Mouse Anti-Rabbit IgG-HRP, SouthernBiotech, Birmingham, AL, dilution 1:25,000) for 1 h at room temperature in TBS-T containing 10% Roti-Block and 0.1% Tween.

Following another washing step, immunodetection was carried out using an enhanced chemiluminescence detection system (GE Healthcare). Imaging was performed with a VersaDoc MP4000 imaging system (Bio-Rad) and the band intensities were densitometrically analyzed via the Image Lab software (Bio-Rad). After immunodetection, India ink staining was included as a loading and transfer control.

For SIRT1, the samples were diluted to 1 mg/mL and mixed with loading buffer and 0.2 *M* dithiothreitol. The samples were loaded in duplicates (20 µg of protein per lane) on 10% gels and the SDS-PAGE and blotting was performed as described above. Membranes were blocked in TBS solution containing 5% fat-free milk powder (Carl Roth GmbH) and 0.1% Tween 20 (AppliChem GmbH) for 1 h at room temperature and then incubated with primary antibodies against SIRT1 (ab13749, Abcam, Cambridge, UK) in 1:100 dilution overnight at 4°C. After washing, membranes were incubated with a HRP-conjugated corresponding secondary antibody (Goat Anti-Rabbit IgG-HRP, SouthernBiotech, dilution 1:10,000) for 1 h at room temperature. Detection of chemiluminescence and detection of bands carried using the same method as described above. Exemplary membranes showing the India ink staining were included as Supplemental Figures S1 (PPARGC1A) and S2 (SIRT1); <http://dx.doi.org/10.3168/jds.2015-10132>.

Relative Quantification of mRNA

The preparation of the samples including RNA extraction and cDNA synthesis are described in detail in the companion paper by Weber et al. (2016). Relative quantification of the target genes was performed with standard curves based on purified amplicons, except in case of *NAMPT* where serial dilutions of cDNA were used. As controls, a negative template control and a no reverse transcriptase control of the cDNA synthesis were carried along, together with a negative template

control for each PCR run, containing 2 μ L of water instead of diluted template. The reactions were performed in Mx3000P systems (Stratagene, Amsterdam, the Netherlands, and Agilent, Santa Clara, CA) with a total volume of 10 μ L, including 2 μ L of cDNA (diluted 1:4), 1 μ L of primer mix, 2 μ L of water, and 5 μ L of SYBR Green JumpStart Taq Readymix (Sigma-Aldrich, Nümbrecht, Germany). The sequences of the utilized primers and their respective conditions for quantitative PCR are provided in Table 2. Specificity of the PCR products was confirmed for each primer by sequencing.

Reference Gene Stability

Selection, amplification, and evaluation of the final reference genes were realized as described in the companion paper by Weber et al. (2016).

Statistical Analyses

Statistical analyses were performed using SPSS (version 22.0, SPSS Inc., Chicago, IL) on log-transformed mRNA data. No treatment effects were observed. Therefore, data from the 2 feeding groups were merged for further analyses. The repeated comparison for the serum as well as the mRNA data between each time point was done using the mixed model procedure. The examined factors were considered as dependent variables; the level of concentrate and the niacin supplementation were incorporated as fixed effects, sampling day as repeated effect, and their respective interactions were included into the model. As the covariance structure, autoregressive (first-order autoregressive structure with homogeneous variances) followed by Bonferroni correction was used. The Student's *t*-test was applied for assessing differences between SCAT and RPAT at each time point. Pearson correlation coefficient was calculated for assessing correlations. Data are expressed as means $\pm$ standard error of the mean. Statistical significance was set at $P < 0.05$ and P -values < 0.1 were defined as a trend.

RESULTS

Energy Balance and Mobilization of Body Fat

The strategy of feeding different portions of concentrate, supplemented with niacin, did not affect lipomobilization postpartum as indicated by the BCS as well as BHB and fatty acid concentrations. Also the energy balance was not affected by the feeding scheme p.p. (Tienken et al., 2015; Supplemental Tables S1 and S2; <http://dx.doi.org/10.3168/jds.2015-10132>).

Table 2. Sequences of the primers and real-time PCR conditions used for the quantification of the target genes

Gene	Forward primer sequence (5'-3'), reverse primer sequence (5'-3')	Accession no. ¹	bp	Conc. (nM) ²	Mean C _q ³	Annealing ⁴ (s/°C)	Elongation ⁵ (s/°C)	Efficiency
<i>SIRT1</i> ⁶	GGGGTTCTGTTCTTTG ATGGTCTTGGGCTCTTTCTG	NM_001192980.1	139	600	29.3	59/60	60	89.9
<i>PPARGC1A</i> ⁷	GGGGCAAATACACTCTTCCA TGGTCTTGTCTGCTTTGTCG	NM_1777945.3	185	200	32.2	60/60	60	94.5
<i>NAMPT</i> ⁸	TGGCCACCGACTCATACAAG TCGGTCTTCTTTTCACGGCA	NM_001244141.1	97	600	27.1	59/60	60	94.2
<i>ADIPOR1</i> ⁹	GCTGAAGTGAAGGAGAGTC GAGGGAATGGAGTTTATGTC	NM_001034055.1	118	800	26.7	61/35	30	100.3
<i>ADIPOR2</i> ¹⁰	GGCAACATCTGGACACATC CTGGAGACCCCTTCTGAG	NM_001040499.2	200	400	24.5	60/45	30	94.3

¹National Center for Biotechnology Information (NCBI) accession number.

²Concentrations for each primer.

³Mean quantification cycle.

⁴Initial denaturation for 10 min at 95°C; denaturation for 30 s at 95°C.

⁵Elongation at 72°C.

⁶Sirtuin-1.

⁷PPAR γ co-activator 1 α .

⁸Lemor et al., 2009.

⁹Adiponectin receptor 1 (Lemor et al., 2009).

¹⁰Adiponectin receptor 2 (Lemor et al., 2009).

Figure 1. Time course of the adiponectin concentration in serum during late gestation and early lactation of pluriparous dairy cows (means $\pm$ SEM). Different letters (a–d) indicate significant differences between the individual sampling dates ($P < 0.05$).

Adiponectin

The ADIPOQ concentrations in serum were not affected by treatment, but changes with time were observed. The concentrations decreased from d –42 reaching a nadir at d 1 after calving and increased to precalving levels within the first week after parturition. From d 7 onward, the ADIPOQ concentrations remained constant until the end of the experiment (Figure 1).

SIRT1, *PPARGC1A*, *NAMPT*, *ADIPOR1*, and *ADIPOR2* mRNA

No differences were observed between the feeding groups; thus, merged data were analyzed for time-dependent effects.

The expression profiles of all 5 genes, except *ADIPOR2*, differed between SCAT and RPAT. For *SIRT1* mRNA abundance, time-dependent changes were observed in RPAT. *SIRT1* mRNA abundance decreased from d –42 until d 1 and then increased toward d 21. At d 100 *SIRT1* mRNA expression was lower than before parturition in RPAT (Figure 2). For *PPARGC1A* time-dependent changes were restricted to SCAT, with greater mRNA abundance postpartum (Figure 2). The *NAMPT* mRNA expression showed a very weak decrease after parturition on d 1 in SCAT and on d 100 in RPAT (Figure 3). The mRNA abundance of *ADIPOR1* declined from d –42 to d 1 and 21 after parturition

and then increased back to the prepartum level from d 21 to 100 in SCAT. In RPAT, the *ADIPOR1* mRNA abundance at all postpartum sampling dates was less than antepartum (Figure 3). The mRNA expression of *ADIPOR2* was lower after parturition in SCAT and RPAT (Figure 3). Comparing SCAT and RPAT, *SIRT1* showed a higher mRNA abundance in RPAT on d –42 and 21 (Figure 2). For *PPARGC1A* (Figure 2) and *NAMPT* (Figure 3), higher mRNA expression was detected in SCAT. The mRNA abundance of *ADIPOR1* was higher in RPAT on d 21 (Figure 3). The *ADIPOR2* mRNA expression was higher in RPAT postpartum (Figure 3). The correlation coefficients between the variables assessed herein and fatty acids, glucose, and BHB (Tienken et al., 2015) are listed in Table 3. The highest positive correlation was found between *SIRT1* and *ADIPOR1* in both, SCAT ($r = 0.720$) and RPAT ($r = 0.701$). Negative correlations were strongest between fatty acids and visfatin in RPAT ($r = -0.401$); in contrast, this relationship was not significant in SCAT.

PPARGC1A Protein

For *PPARGC1A* protein expression in SCAT, a treatment effect was observed, i.e., the protein expression was higher in the low-concentrate (LC) group as compared with the high-concentrate (HC) group at d 21 after parturition. No differences in *PPARGC1A* protein expression were observed at d 21 between SCAT and RPAT (Figure 4).

Figure 2. mRNA abundance of *SIRT1* and *PPARGC1A* on d -42, 1, 21, and 100 relative to parturition in subcutaneous adipose tissue (SCAT) and retroperitoneal adipose tissue (RPAT) in pluriparous dairy cows (means $\pm$ SEM). Different capital letters (A–C) indicate significant differences between the individual sampling dates in SCAT ($P < 0.05$), whereas different lowercase characters (a–c) designate differences between the sampling dates in RPAT ($P < 0.05$). Asterisks designate differences between SCAT and RPAT at the respective time points (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). For normalization, lipoprotein receptor-related protein-10 (*LRP10*) and emerin (*EMD*) in SCAT, and *LRP10*, *EMD*, RNA polymerase II (*POLR2A*), marvel domain containing 1 (*MARVELD1*), and eukaryotic translation initiation factor 3, subunit K (*EIF3K*) in RPAT were used as reference genes. For comparing SCAT and RPAT, *LRP10*, *EMD*, *POLR2A*, *MARVELD1*, *EIF3K*, and hippocalcin-like 1 (*HPCAL*) were used as reference genes.

SIRT1 Protein

The SIRT1 protein expression showed no changes related to treatment; thus, data were pooled for further analysis. In RPAT, a trend for time-dependent changes of SIRT1 protein expression was detected. No differences were observed when comparing SIRT1 protein expression between SCAT and RPAT at d 21 after calving (Figure 5).

Figure 3. mRNA abundance of *NAMPT*, *ADIPOR1*, and *ADIPOR2* on d -42, 1, 21, and 100 relative to parturition in subcutaneous adipose tissue (SCAT) and retroperitoneal adipose tissue (RPAT) in pluriparous dairy cows (means $\pm$ SEM). Different capital letters (A–C) indicate significant differences between the individual sampling dates in SCAT ($P < 0.05$), whereas different lowercase letters (a,b) designate differences between the sampling dates in RPAT ($P < 0.05$). Asterisks mark a different mRNA abundance between SCAT and RPAT at the respective time points (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). For normalization, lipoprotein receptor-related protein-10 (*LRP10*) and emerin (*EMD*) in SCAT, and *LRP10*, *EMD*, RNA polymerase II (*POLR2A*), marvel domain containing 1 (*MARVELD1*), and eukaryotic translation initiation factor 3, subunit K (*EIF3K*) in RPAT were used as reference genes. For comparing SCAT and RPAT, *LRP10*, *EMD*, *POLR2A*, *MARVELD1*, *EIF3K*, and hippocalcin-like 1 (*HPCAL*) were used as reference genes.

Figure 4. Protein expression of PPARGC1A in subcutaneous adipose tissue (SCAT) on d -42 and 21 and in retroperitoneal adipose tissue (RPAT) on d 21 relative to parturition in pluriparous dairy cows (means ± SEM). An asterisk marks a different protein expression between the individual feeding groups (**P* < 0.05). India ink staining was included as a loading and transfer control. Representative immunoblots are shown below the respective bars.

DISCUSSION

We did not observe any treatment effect for the analyzed factors. This might be associated with the inability to induce differences in the energy balance p.p. by the prepartal feeding scheme we used. The BCS as well as lipolysis indicated by fatty acids and BHB concentrations in blood did not differ between the feeding groups (Tienken et al., 2015).

Figure 5. Protein expression of SIRT1 in retroperitoneal adipose tissue (RPAT) on d -42, 1, and 21 and in subcutaneous adipose tissue (SCAT) on d 21 relative to parturition in pluriparous dairy cows (means ± SEM). A trend was observed for a time effect in SCAT (*P* < 0.1). India ink staining was included as a loading and transfer control. Representative immunoblots are shown below the respective bars.

Table 3. Coefficients of correlation (Pearson) between the serum concentrations of adiponectin (ADIPOQ), glucose, fatty acids, and BHB and the mRNA abundances of *SIRT1*, *PPARGC1A*, *visfatin*, *ADIPOR1*, and *ADIPOR2* in subcutaneous adipose tissue (SCAT) and retroperitoneal adipose tissue (RPAT) of pluriparous dairy cows (*P* < 0.05)

Item	SCAT						RPAT					
	ADIPOQ	ADIPOR1	ADIPOR2	SIRT1	PPARGC1A	NAMPT	ADIPOR1	ADIPOR2	SIRT1	PPARGC1A	NAMPT	
ADIPOQ	NS						NS		NS			
ADIPOR1	NS	0.284					0.511		NS			
ADIPOR2	NS	0.720	NS				0.701	0.357	NS	NS		
SIRT1	NS	NS	-0.288	NS			NS	NS	0.328			
PPARGC1A	NS	-0.388	0.274	-0.661	NS		0.372	0.462	NS	0.246		
NAMPT	NS	0.413	0.366	NS	-0.275	NS	0.422	0.263	NS	NS	0.257	
Glucose	NS	-0.254	NS	NS	NS	NS	-0.286	-0.256	NS	NS	-0.401	
Fatty acids	-0.338			-0.243	NS	NS	NS	NS	NS	NS	NS	
BHB	NS	-0.294	NS				NS		NS			

The serum ADIPOQ concentrations measured herein in pluriparous cows confirm recently published results (Singh et al., 2014) obtained with the same bovine-specific ELISA. The authors likewise reported a negative correlation between fatty acids and adiponectin. Our results are also in accordance with earlier findings (Giesy et al., 2012) and support the authors' hypothesis that the reduced ADIPOQ serum concentrations around parturition contribute to the homeorhetic adaptations during the respective time period and promote insulin resistance.

The decreased *ADIPOR1* and *ADIPOR2* mRNA abundance after parturition in SCAT and RPAT substantiates earlier findings in SCAT (Lemor et al., 2009; Saremi et al., 2014) and supports the authors' assumption of a link to the reduced insulin sensitivity characteristically observed during that time. Adiponectin receptor mRNA expression was shown to be regulated by insulin in AT of mice (Tsuchida et al., 2004), as well as by growth hormone and prolactin in human (Nilsson et al., 2005) and mouse AT (Fasshauer et al., 2004). These hormones are known to change during the transition period (Accorsi et al., 2005). Thus the decreased *ADIPOR1* and *ADIPOR2* mRNA abundance might be the result of a complex regulatory system. High molecular weight ADIPOQ, the main form in dairy cattle (Giesy et al., 2012), was shown to possess a higher affinity to ADIPOR2 than ADIPOR1 (Kadowaki et al., 2006) and is associated with the improvement of insulin sensitivity (Pajvani et al., 2003). The higher *ADIPOR2* mRNA abundance and the higher correlation between the ADIPOQ receptors in RPAT thus point to a tissue-specific regulation of insulin sensitivity. The strongest correlation in this study was observed between *SIRT1* and *ADIPOR1* in SCAT and RPAT. Binding of ADIPOQ to ADIPOR1 was shown to regulate SIRT1 and AMP-activated protein kinase (AMPK) in muscle of mice (Iwabu et al., 2010). Since AMPK was discussed as an anti-lipolytic factor involved in the fine tuning of lipolysis in cows (Locher et al., 2012), this function of AMPK might partly be mediated via ADIPOR1 and SIRT1 in bovine AT, at least during early lactation. The observation that ADIPOQ inhibits lipolysis via an AMPK-dependent pathway (Wedellová et al., 2011) might support this notion. It was further reported, at least in the pancreas, that ADIPOQ inhibits apoptosis via ADIPOR1 and the AMPK SIRT1 pathway (Huang et al., 2014). Because the apoptotic rate does not change during lactation in SCAT of primiparous cows (Häussler et al., 2013), the downregulation of the adiponectin system after parturition and the strong correlation between *ADIPOR1* and *SIRT1* might inhibit adipogenesis to ensure lipolysis. This might be supported by the fact, that SIRT1 attenuates adipogen-

esis (Picard et al., 2004). In this context, the observed correlation between *ADIPOR1* and *NAMPT*, as well as *SIRT1* and *NAMPT*, might display a depot-specific regulatory mechanism. For *SIRT1* and *NAMPT*, a negative correlation was found only in SCAT. During SIRT1-dependent deacetylation processes, NAD is converted to NAM, which inhibits SIRT1 activity (Bitterman et al., 2002). A high amount of NAM would thus lead to the inhibition of SIRT1, while activating the NAD synthetic function of NAMPT. The lacking relationship in RPAT might support our notion of ADIPOR1 and SIRT1 being involved in fat cell turnover, because in RPAT less apoptosis was reported compared with SCAT (Häussler et al., 2013). Song et al. (2013) reported a correlation between *SIRT1* and *ADIPOQ* mRNA abundance in SCAT and RPAT of women, with a higher correlation in SCAT. The authors related this finding to a possible relationship between SIRT1 and ADIPOQ gene transcription as discussed earlier (Qiao and Shao, 2006). The correlation observed herein indicates a similar relationship between SIRT1 and ADIPOR1 in bovine AT.

The mRNA of *ADIPOR1* and *SIRT1* were positively correlated with the anti-lipolytic hydroxycarboxylic acid receptors 1 (*HCAR1*) and 2 (*HCAR2*; Weber et al., 2016) in both SCAT and RPAT, with *ADIPOR1* showing higher correlations in SCAT (Supplemental Table S3; <http://dx.doi.org/10.3168/jds.2015-10132>). The adipokine ADIPOQ was reported to decrease lipolysis in mouse adipocytes (Qiao et al., 2011), and SIRT1 was reported to stimulate lipolysis by integrating β -adrenergic signaling, lipolysis, and oxidative metabolism as an important link (Picard et al., 2004; Khan et al., 2015). The β -adrenergic signaling pathway in controlling lipolysis is well known in dairy cows (Khan et al., 2013) with the β -2 adrenergic receptors as being suggested as the most important subtype (Sumner and McNamara, 2007). During negative energy balance in early lactation, lipogenesis decreases and lipolysis increases (McNamara and Hillers, 1986). Because ADIPOQ showed an increase from d 1 to 21 p.p. and for SIRT1 a trend for a time effect was detected, these variables might be co-regulated in the context of controlling lipolysis in dairy cows. Further, these variables might display a fine-tuning mechanism beside the main regulatory β -adrenergic system.

Higher correlations between *ADIPOR1* and *HCAR1* as well as *HCAR2* in SCAT might hint to a higher lipolytic capacity of the retroperitoneal AT as discussed earlier (Locher et al., 2011).

The catalytic activity of SIRT1 was recently shown to be important for the maintenance of insulin sensitivity in mice (Caron et al., 2014). The observed correlation in both AT depots might indicate a comparable

role in the regulation of insulin sensitivity in cows; the observed trend for a time-dependent change of SIRT1 protein expression in RPAT together with the increasing serum ADIPOQ concentrations might indicate an improvement of insulin sensitivity. In murine hepatocytes, resistin, a regulator of insulin resistance, was reported to decrease SIRT1 mRNA abundance (Yu et al., 2013). This substantiates a relationship between SIRT1 and glucose metabolism, because we found increasing serum resistin concentrations toward parturition (Weber et al., 2016).

During SIRT1-dependent deacetylation processes, NAD is converted to NAM, leading to the inhibition of the enzyme (Bitterman et al., 2002). To maintain SIRT1 activity, NAM has to be reconverted to NAD. The rate-limiting enzyme in this process is NAMPT/visfatin (Revollo et al., 2004). The decreased *NAMPT* mRNA expression in SCAT might lead to a reduced SIRT1 activity, which in turn might contribute to the regulation of insulin sensitivity. The *NAMPT* mRNA abundance in subcutaneous AT detected herein was lower on d 100 p.p. as compared with d 42 antepartum. These results are in accordance with observations from Lemor et al. (2009), who reported a trend toward a decreasing *NAMPT*/visfatin mRNA abundance postpartum. In contrast to this, we could not confirm the observed negative correlations between *NAMPT* and fatty acids and *NAMPT* and BHB. In our study, we likewise detected a negative correlation between *NAMPT* and fatty acids albeit only in RPAT and not in SCAT. The decreasing *NAMPT* mRNA abundance after parturition in RPAT might thus be a tissue-dependent mechanism to decrease insulin-stimulated lipogenesis and to increase lipolysis. This might be supported by results obtained by von Soosten et al. (2011), who reported a higher mobilization of RPAT after parturition as compared with SCAT in primiparous German Holstein cows of rather low BW and milk yield, as well as by Locher et al. (2011), who found a higher HSL expression and phosphorylation in RPAT of pluriparous German Holstein cows, suggesting a higher lipolytic potential for this depot. In addition to its functions as an adipokine improving insulin sensitivity and biosynthetic enzyme, NAMPT/visfatin was also characterized as a pro-inflammatory cytokine (Moschen et al., 2007). Because pro-inflammatory pathways are involved in the homeorhetic adaptations to lactation (Farney et al., 2013), and the higher mRNA in RPAT, NAMPT/visfatin might play a role in this AT depot during this time.

The PPARGC1A protein expression in SCAT was higher in the LC group as compared with the HC group at d 21. Because PPARGC1A is an important mediator of mitochondrial biogenesis (Liang and Ward,

2006), the increased protein expression in the LC group might hint to an increasing number of mitochondria. This might ameliorate the tense metabolic situation, compensating the more negative energy balance in the peripartum period.

CONCLUSIONS

The presented data substantiate the existence of the SIRT1-PPARGC1A axis in bovine AT. Further, a strong relationship between ADIPOR1 and SIRT1 was established at the level of transcription. The results indicate a relationship between the considered factors and point to a regulatory role in glucose metabolism during the transition period.

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