Abundance of adiponectin system and G-protein coupled receptor GPR109A mRNA in adipose tissue and liver of F₂ offspring cows of Charolais × German Holstein crosses that differ in body fat accumulation

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ABSTRACT

In addition to its role in energy storage, adipose tissue (AT) is an important endocrine organ and it secretes adipokines. The adipokine adiponectin improves insulin sensitivity by activation of its receptors AdipoR1 and AdipoR2. Lipolysis in AT is downregulated by the G-protein coupled receptor (GPR109A), which binds the endogenous ligand β -hydroxybutyrate (BHBA). Insulin sensitivity is reduced during the transition from late pregnancy to early lactation in dairy cattle and BHBA is increased postpartum, implying the involvement of the adiponectin system and GPR109A in this process. The aim of the current investigation was to study the effect of the genetic background of cows on the mRNA abundance of the adiponectin system, as well as GPR109A, in an F₂ population of 2 Charolais × German Holstein families. These families were deduced from full- and half-sibs sharing identical but reciprocal paternal and maternal Charolais grandfathers. The animals of the 2 families showed significant differences in fat accretion and milk secretion and were designated fat-type (high fat accretion but low milk production) and lean-type (low fat accretion but high milk production). The mRNA of the adiponectin system and GPR109A were quantified by real-time PCR in different fat depots (subcutaneous from back, mesenteric, kidney) and liver. The mRNA data were correlated with AT masses (intermuscular topside border fat, kidney, mesenteric, omental, total inner fat mass, total subcutaneous fat mass, and total fat mass) and blood parameters (glucose, nonesterified fatty acids, BHBA, urea, insulin, and glucagon). The abundance of adiponectin system mRNA was higher in discrete AT depots of fat-type cows [adiponectin mRNA in mesenteric fat (trend), *AdipoR1* in kidney and mesenteric AT, and *AdipoR2* in subcutaneous fat (trend)] than in lean-type cows. More *GPR109A* mRNA was found in kidney fat of the

lean-type family than in that of the fat-type family. In liver, the abundance of *AdipoR2* and *GPR109A* (trend) mRNA was higher in lean-type than in fat-type cows. Correlation analyses disclosed clear differences between the groups. In total, the results revealed obvious disparities for the mRNA targets between the 2 families with common but reciprocal paternal and maternal genetic backgrounds. Visceral AT mass of both families showed most correlations with the mRNA abundance of the target genes in different AT depots. The effect of adiponectin secretion, especially by visceral AT depots, on liver metabolism should be clarified in further studies.

Key words: adiponectin system, G-protein coupled receptor (GPR109A), cow, genetic background

INTRODUCTION

Adipose tissue (AT), in addition to its function as energy storage, is an important endocrine organ and it secretes adipokines (Ahima, 2006). Adipose tissue is related to different physiological processes such as energy metabolism, insulin sensitivity, and inflammation. One important trigger of these effects is the adipokine adiponectin (**AdipoQ**). Its expression is higher in human visceral (**v.c.**) adipocytes than in subcutaneous (**s.c.**) adipocytes in vitro (Motoshima et al., 2002), as was observed for AdipoQ mRNA in rat v.c. compared with s.c. AT (Atzmon et al., 2002). In pigs, AdipoQ expression is related to genotype; a lean pig breed has higher serum concentrations of AdipoQ than a breed with potential for higher fat accretion (Jacobi et al., 2004). Adiponectin improves insulin sensitivity by enhancing glucose uptake and β -oxidation and decreasing gluconeogenesis in liver or muscle (Berg et al., 2001; Fruebis et al., 2001; Yamauchi et al., 2007). As discussed by Brochu-Gaudreau et al. (2010), AdipoQ might protect against fatty liver disease. For this reason, insights into the AdipoQ system in cattle should be of great importance because negative energy balance during transition from late pregnancy to early lactation of dairy cows is often

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associated with metabolic disorders such as fatty liver disease and ketosis (LeBlanc, 2010).

Overexpression of AdipoQ in mice reduces BW in both sexes as well as NEFA in male transgenic mice. In these transgenic mice, AdipoQ diminishes adipocyte differentiation and leads to a larger number of small adipocytes (Bauche et al., 2007). In isolated porcine adipocytes, in vitro AdipoQ reduces lipogenic activity (Jacobi et al., 2004). In contrast, another transgenic mice model describes the proliferation and increase of tissue mass of defined fat pads (Combs et al., 2004). This study was in line with the observation of other investigators showing that AdipoQ overexpression pushes proliferation and differentiation of 3T3-L1 adipocytes and enhances lipid accumulation (Fu et al., 2005).

Adiponectin signals through the AdipoQ receptors 1 (**AdipoR1**) and 2 (**AdipoR2**), which are the major receptors. The mRNA of the receptor AdipoR1 is ubiquitously expressed and highly abundant in skeletal muscle, whereas that of AdipoR2 is found predominantly in mouse liver (Kadowaki et al., 2006). Both receptors are expressed in AT and downregulated in AdipoQ-overexpressing 3T3-L1 adipocytes (Fu et al., 2005). Downregulation of AdipoR2, but not AdipoR1, was also shown in an AdipoQ-overexpressing transgenic mice model analyzing inguinal and gonadal AT (Bauche et al., 2006). Stimulation of pig adipocytes with insulin alone reduces *AdipoQ* and *AdipoR2* mRNA in vitro (Liu et al., 2008) but increases AdipoQ in 3T3-L1 adipocytes (Scherer et al., 1995).

Adiponectin phosphorylates and thereby activates the 5'-AMP-activated protein kinase (**AMPK**) pathway, which triggers increased fatty acid oxidation, decreases gluconeogenesis, and decreases glucose uptake in muscle or liver (Yamauchi et al., 2002; Guerre-Millo, 2008). For AMPK in AT, the data related to pro- and antilipolytic effects are contradictory (Yin et al., 2003; Daval et al., 2005).

Comparable to AMPK, the G-protein coupled receptor 109A (**GPR109A**; newly named HCA₂) is involved in lipolysis; activation of the receptor results in a decrease in lipolysis. The receptor belongs to the family of hydroxy-carboxylic acid receptors and is activated by niacin as well by BHBA as the endogenous ligand (Offermanns et al., 2011). Reduction of lipolysis by BHBA is thought to be an important negative feedback mechanism during starvation (Gille et al., 2008). In dairy cattle, comparable abundance of the receptor protein was found in liver and in kidney fat but most receptor mRNA was observed in liver (Titgemeyer et al., 2011a). The *GPR109A* mRNA is downregulated during the transition period in s.c. AT of dairy cattle (Lemor et al., 2009).

Less information compared with monogastrics is available about the AdipoQ system in general and its relation to genetic background in the bovine species. Adiponectin mRNA was negatively correlated with back fat thickness in Hereford × Aberdeen Angus as well as in Charolais × Red Angus crossbred steers (Taniguchi et al., 2008). An animal model with differences in fat accretion but shared genetic background could be an important tool in understanding the AdipoQ system in cattle. We established such a model, which comprised a segregating F₂ family structure using the founder breeds Charolais (accretion-type) and German Holstein (secretion-type) by mating Charolais bulls to German Holstein cows to obtain full-sib and half-sib F₂ cows from F₁ intercrosses (Kühn et al., 2002). Differences in milk production, body composition, and glucose metabolism were observed. Cows of the F₂ family that secrete more milk have less fat content in s.c. and v.c. fat depots, a longer glucose half-life, lesser insulin secretion during oral glucose tolerance test, and higher NEFA concentrations compared with cows of the family that secrete less milk and accrete more fat (Hammon et al., 2010). The higher fat deposition in the cow family that accretes more fat might be related to higher insulin concentrations. Insulin diminishes fatty acid oxidation, increases fatty acid esterification in liver (Zammit, 1996), and stimulates lipogenesis in cattle (Etherton and Evock, 1986).

In the present study, we aimed to investigate the effect of a common dairy and beef genetic background on the mRNA expression of the *AdipoQ* system and *GPR109A* in different adipose depots and liver.

MATERIALS AND METHODS

Animals, Sample Collection, and Blood Parameters

The animals were housed at the Leibnitz Institute for Farm Animal Biology (FBN, Dummerstorf, Germany). All procedures were approved by the local authorities of the state Mecklenburg-Vorpommern, Germany. Cows of the F₂ population from the 2 paternal halfsibship families Ab and Ba of Charolais grandsires named A and B crossed with German Holstein cows (parental generation P₀) were used during their second lactation. The uppercase letter represents the paternal origin of the F₁ male and the lowercase letter represents the paternal origin of the F₁ female derived from the P₀ Charolais sires A and B. The animals showed significant differences in fat accretion and milk secretion (Hammon et al., 2010): family Ab, which accretes more fat, was designated fat-type (n = 9), and family Ba, which secretes more energy as milk but accretes less fat, was

designated lean-type ($n = 9$). Cows were part of a large ongoing breeding model described fully by Kühn et al. (2002). The genetic background, husbandry, and feeding of the animals was recently described in detail (Hammon et al., 2010). Analyzed energy and protein contents of TMR were 6.6 MJ of NE_L /kg of DM and 147 g/kg of DM utilizable protein, respectively. The feeding schedule corresponded to the recommendations of the German Society of Nutrition Physiology (GfE, 2001). Blood samples were obtained from the jugular vein at 93 DIM directly before the start of a glucose tolerance test (Hammon et al., 2010). The cows were slaughtered at 100 DIM. After carcass dissection, inner body fat mass (**IF**; sum of omental, abdominal, and retroperitoneal fat around the kidneys), intermuscular topside border fat (**IMtop**), and total cold s.c. fat mass (**SCtot**) were determined. Total fat mass (**FATtot**) was defined as the sum of omental, abdominal, retroperitoneal fat around the kidneys, and s.c. fat. Liver and fat samples were frozen immediately in liquid N_2 and stored at -80°C until used. Details on carcass characteristics and blood metabolites in both families after glucose tolerance tests were published recently (Hammon et al., 2010).

Quantification of Phospho AMPK and AMPK in Liver

Detection of phospho (**p**)AMPK and AMPK was performed according to Kuhla et al. (2009). Briefly, 40 mg of liver tissue (lean-type cows, $n = 9$; fat-type cows, $n = 8$ because of technical difficulties) was homogenized in liquid N_2 and extracted in 400 μL of lysis buffer containing 50 mM Tris-buffered saline, pH 7.6, 1 mM EDTA, 0.1% SDS, and protease inhibitor cocktail. Extracts were quantified by the bicinchoninic acid method (Sigma-Aldrich, Taufkirchen, Germany). After SDS-PAGE, proteins were transferred to nitrocellulose. For detection of AMPK and pAMPK (Thr 172), antibodies at a dilution of 1:500 were used (Cell Signaling, Danvers, MA). First antibodies were detected by a horseradish peroxidase-labeled anti-rabbit antibody (1:1,500, Santa Cruz Biotechnology, Santa Cruz, CA). Hyperfilms were exposed for enhanced chemiluminescence reaction. After scanning, quantification was performed using Image J software (National Institutes of Health, Bethesda, MD).

RNA Extraction and Quantitative Real-Time Reverse Transcription-PCR

Total RNA was extracted from 3 v.c. depots (omental, mesenteric, and kidney), 1 s.c. localization from the back at the level of the 12th rib and the longissimus dorsi muscle (**SCback**), and liver using Trizol (Invitrogen,

Karlsruhe, Germany) according to the manufacturer's instructions. For RNA cleanup, the NucleoSpin RNA II kit (Macherey-Nagel, Düren, Germany) was used according to the manufacturer's instructions, which included DNase I digestion. The concentration of total RNA was checked by using the RiboGreen RNA Assay Kit (Invitrogen) in an Mx3000P-cycler (Stratagene, Amsterdam, the Netherlands). The integrity of the RNA was confirmed by ethidium bromide staining after formaldehyde gel electrophoresis. Reverse transcription was conducted with 200 U of Moloney murine leukemia virus reverse transcriptase, 500 μM of each dNTP, and 20 U of RiboLock ribonuclease inhibitor (all from Fermentas, St. Leon-Rot, Germany), using 250 ng of total RNA according to the manufacturer's instructions, with 250 pmol of random hexamer primers (Sigma) in a 20- μL volume for 10 min at 27°C , 60 min at 42°C , and 1 min at 99°C . Negative controls were performed in the absence of reverse transcriptase and using water instead of total RNA. Quantitative analysis by quantitative real-time reverse transcription PCR (**qPCR**) was carried out in a total volume of 10 μL using SYBR Green JumpStart Taq ReadyMIX (Sigma). In addition to the cDNA controls, a nontemplate PCR control was analyzed. Primers, concentrations, and conditions are shown in Table 1. Primer sequences were published recently (Hosseini et al., 2010, 2012). The quantification of samples was performed against a PCR amplicon standard curve. An inter-run calibrator was run on each plate. After the final PCR cycle, melting curve analysis was performed to verify the PCR products. The abundances of the genes of interest—*AdipoQ*, *AdipoR1*, *AdipoR2*, and *GPR109A*—were normalized as ratios to the geometric mean of the amplified reference genes RNA polymerase II (**Pol II**), β -actin (**ActB**), and *GAPDH*. For qPCR, 2 μL of the reverse transcription reaction mix diluted 1:4 was used. The genes were analyzed under the cycling conditions and annealing temperatures shown in Table 1, after an initial heating step at 95°C for 10 min.

Statistical Analyses

Data were evaluated by independent samples *t*-test or Whitney U-test. Data between the 3 AT depots were analyzed by one-way ANOVA or Kruskal-Wallis one-way ANOVA on ranks followed by Tukey *t*-test to isolate significances. Depending on the data distribution, correlations were calculated by Pearson or Spearman analysis using SigmaStat 3.5 (SPSS Science Software, Erkrath, Germany). Outliers were detected by Dixon test. Therefore, *GPR109A* mRNA abundance in liver of 8 fat-type animals was statistically analyzed. A *P*-value < 0.05 was considered significant and a trend was

Table 1. Primer sequences and quantitative PCR conditions¹

Gene ²	Accession number	Primer sequence (5'-3') ³		Length (bp)	Annealing temperature (°C)/time (s)	Mean cycle threshold
<i>AdipoQ</i>	NM_174742	F	CTGGAGAGAAGGGAGAGAAAAG	204	60/75	24.5
		R	TGGGTACATTGGGAACAGTG			
<i>AdipoR1</i>	NM_001034055	F	GCTGAAGTGAGAGGAAGAGTC	118	60/30	25.7
		R	GAGGGAATGGAGTTTATTGCC			
<i>AdipoR2</i>	NM_001040499	F	GGCAACATCTGGACACATC	200	60/30	25.5
		R	CTGGAGACCCCTTCTGAG			
<i>GPR109A</i>	XR_028237	F	GGACAGCGGGCATCATCTC	140	61/30	28.3
		R	CCAGCGGAAGGCATCACAG			
<i>GAPDH</i>	U85042	F	AATGGAAAGGCCATCACCATC	204	59/75	22.7
		R	GTGGTTCACGCCCATCACA			
<i>Pol II</i>	X63564	F	GAAGGGGGAGAGACAAACTG	86	58/60	24.2
		R	GGGAGGAAGAAGAAAAAGGG			
<i>ActB</i>	AY141970	F	CTCTTCCAGCCTTCCTTCCT	178	60/60	21.1
		R	GGCAGTGATCTCTTTCTGC			

¹Denaturation at 95°C for 60 s and elongation at 72°C for 30 s (or 60 s for *AdipoQ* and *AdipoR2*).

²Target genes: adiponectin (*AdipoQ*), adiponectin receptors 1 and 2 (*AdipoR1* and *AdipoR2*), and G-protein coupled receptor 109A (*GPR109A*); reference genes: *GAPDH*, RNA polymerase II (*Pol II*), and β -actin (*ActB*).

³F = forward; R = reverse.

defined at $0.05 < P < 0.1$. Correlations were defined as follows: $r \leq 0.5$ = weak, $0.7 > r > 0.51$ = moderate, $r > 0.71$ = strong.

RESULTS

Comparing fat masses between families (fat-type vs. lean-type cows), all quantified AT masses were higher in fat-type animals, but IMtop was not. No differences were observed for liver mass or fat and glycogen concentrations in liver (Table 2). At 93 DIM, plasma concentrations of glucose, insulin, and glucagon (as a trend) were lower in lean-type animals (Table 3). Details on carcass characteristics, glucose metabolism, and blood

metabolites in both families after a glucose tolerance test were published recently (Hammon et al., 2010).

Analysis of hepatic AMPK activation, expressed as ratio between pAMPK and AMPK, revealed no differences between the 2 families ($P = 0.81$; data not shown). Differences in expression of the target mRNA within the AT depots in each family revealed comparable patterns between the crossbreeds (Figure 1A). In both families, the abundance of *AdipoQ* mRNA was highest in kidney fat. No differences were observed among different AT depots for *AdipoR2* mRNA. The highest abundance for *AdipoR1* mRNA was found in SCback; this depot also showed the highest values of *GPR109A* mRNA.

Table 2. Adipose tissue and liver masses (kg; means $\pm$ SEM) of fat-type (n = 9) and lean-type (n = 9) cows, and liver fat and glycogen content (% of DM) at 100 DIM used for correlation analyses

Item	Family ¹				P-value
	Fat-type	SEM	Lean-type	SEM	
Kidney (kg)	24.36	1.59	15.36	2.58	0.05
Mesentery (kg)	12.28	1.02	8.44	1.50	0.05
Liver (kg)	10.38	0.45	9.76	0.36	NS
Omentum (kg)	23.30	1.34	16.06	2.51	0.05
IMtop ² (kg)	1.23	0.14	0.90	0.18	NS
Total fat (kg)	101.83	5.99	65.79	9.61	0.05
Total inner fat (kg)	59.94	3.79	39.86	6.24	0.05
Total s.c. fat mass (kg)	41.89	3.08	25.92	3.78	0.05
Liver fat (% of DM)	15.67	0.77	17.26	0.92	NS
Liver glycogen (% of DM)	7.74	1.62	4.98	0.92	NS

¹Cows of the F₂ population from the 2 paternal half-sibship families, fat-type (Ab) and lean-type (Ba), of Charolais grandsires A and B crossed with German Holstein cows, were used during their second lactation. The uppercase letter represents the paternal origin of the F₁ male and the lowercase letter represents paternal origin of the F₁ female derived from the P₀ Charolais sires A and B. For more details on data and data acquisition, please refer to Hammon et al. (2010).

²IMtop = intermuscular fat below the top round.

Table 3. Blood metabolites (means $\pm$ SEM) of fat-type (n = 9) and lean-type (n = 9) cows at 93 DIM used for correlation analyses

Blood metabolite	Family ¹				P-value
	Fat-type	SEM	Lean-type	SEM	
BHBA (mmol/L)	0.44	0.04	0.50	0.06	NS
Glucose (mmol/L)	5.15	0.20	4.18	0.26	0.01
Glucagon ² (ng/L)	108.11	9.19	88.78	11.90	NS
NEFA (mmol/L)	0.67	0.10	0.77	0.10	NS
Insulin (μ g/L)	0.80	0.14	0.33	0.06	0.01
Urea (mmol/L)	4.94	0.32	4.59	0.33	NS

¹Cows of the F₂ population from the 2 paternal halfsibship families, fat-type (Ab) and lean-type (Ba), of Charolais grandsires A and B crossed with German Holstein cows, were used during their second lactation. The uppercase letter represents the paternal origin of the F₁ male and the lowercase letter represents paternal origin of the F₁ female derived from the P₀ Charolais sires A and B. For more details on data and data acquisition, please refer to Hammon et al. (2010).

²A trend of $P = 0.077$ was observed for glucagon.

Between the families, differences in the abundance of the target mRNA were observed in some of the depots (Figure 1A). Expression of *AdipoQ* mRNA tended to be higher in mesenteric AT of fat-type cows compared with lean-type cows. This was also the case for *AdipoR1* mRNA in kidney and mesenteric AT and for *AdipoR2* in s.c. AT from SCback AT, the latter as a trend. In contrast, *GPR109A* mRNA was more abundantly expressed in kidney fat of lean-type cows. Higher values were observed for *AdipoR2* and *GPR109A* mRNA in liver of lean-type versus fat-type cows, although only a trend was observed for *GPR109A* mRNA (Figure 1B).

As illustrated in Figure 2, correlation analysis between the target mRNA and different fat masses showed different patterns between families. Numerically more correlations were found in the lean-type group (Figure 2B). In both families, *AdipoQ* mRNA in mesenteric AT exhibited the most interactions, all of which were positive (Figure 2A and 2B). In both cases, correlations were mostly related to inner fat masses in contrast to s.c. fat and intermuscular fat. Within the fat-type group, *AdipoQ* mRNA in kidney fat was negatively correlated to inner fat mass and moderately correlated with total fat mass. In contrast, *AdipoQ* mRNA abundance in mesenteric fat was positively related to total fat mass, kidney, mesenteric, omental, and IMtop in the fat-type group. For *AdipoR1* and *GPR109A* mRNA, negative correlations with some of the fat masses were observed in this group, which differed depending on the target mRNA. In the lean-type cows, the mRNA abundance of *AdipoQ* was not correlated with IMtop but showed a moderate correlation with SCtot as a trend. Interestingly, *AdipoR2* was not correlated with the different fat masses within the fat-type family, in contrast to the lean-type family. In these animals, *AdipoR2* mRNA in kidney fat was strongly correlated to omental and mesenteric AT. Abundance of *AdipoR2*

mRNA in mesenteric fat was moderately significantly correlated with 6 other compartments. In both families, *AdipoR1* mRNA showed only 3 moderate negative correlations in total. The *GPR109A* mRNA in AT was negatively correlated with the different fat masses in each case. Most correlations between *GPR109A* mRNA abundance and the different fat masses were related to *GPR109A* mRNA in kidney fat. Compared with the fat-type cows, *GPR109A* mRNA in the lean-type group showed numerically more interactions.

Correlation analyses between target mRNA and blood metabolites showed different expression patterns between the cows of the 2 families, which are reported in Figure 3. As observed for fat masses, more correlations were found within the lean-type family (Figure 3B). Remarkably, in lean-type cows, the strongest correlations between the *AdipoQ* system and glucagon were observed; mainly mesenteric AT was involved. Adiponectin mRNA in kidney AT was strongly negatively correlated to glucose concentrations in lean-type animals. The mRNA expression of *AdipoQ*, *AdipoR1* (trend), and *AdipoR2* in mesenteric AT were positively correlated and *AdipoR1* mRNA in SCback AT (trend) was negatively correlated with glucagon. Within the fat-type animals, only 2 positive correlations were observed: between glucagon and *AdipoQ* mRNA in kidney AT and between glucagon and *AdipoR1* mRNA in mesenteric AT as a trend.

In both families, *GPR109A* mRNA in kidney fat was positively correlated with NEFA as a tendency. A positive correlation between this fat depot and insulin was observed in lean-type cows. In fat-type cows, mesenteric *GPR109A* mRNA correlated positively with insulin and negatively with urea on a moderate level (Figure 3A and 3B). In mesenteric fat of the lean-type family, *GPR109A* mRNA showed a strong negative correlation to BHBA. Within the fat-type group, *AdipoQ* mRNA

Figure 1. Messenger RNA abundance of the adiponectin system and G-protein coupled receptor 109A (GPR109A) in fat-type (FAT, $n = 9$) and lean-type (LEAN, $n = 9$) cows at 100 DIM. (A) Comparison among different adipose depots of the individual families (fat-type, $n = 9$; lean-type, $n = 9$) and between adipose depots of the same origin of fat-type and lean-type cows (brackets). (B) Differences in the mRNA abundance of the target genes between families (fat-type, $n = 9$, for *GPR109A*, $n = 8$; lean-type, $n = 9$) in liver. KID = kidney fat; MES = mesenteric adipose tissue (AT); SC = s.c. AT from back. *AdipoQ* = adiponectin, *AdipoR1* and *2* = adiponectin receptor 1 and 2, respectively. Data ($\pm$ SEM) are presented as relative mRNA data expressed as normalized ratio between the target mRNA and geometric mean of the reference genes (*Pol II*, *ActB*, and *GAPDH*). Different letters indicate differences between different adipose depots within each family. Brackets indicate adipose depots differences between the fat-type and lean-type groups; uppercase and lowercase letters indicate differences between adipose depots among the fat-type and lean-type families, respectively; ** $P \leq 0.01$; * $P \leq 0.05$, + $P \leq 0.1$.

Figure 2. Results of the correlation analysis between the mRNA abundance of the adiponectin system and G-protein coupled receptor 109A (GPR109A) in different adipose depots and the adipose tissue (AT) mass (kg) of different localizations in the fat-type (A; n = 9) and lean-type (B; n = 9) cow families at 100 DIM. *AdipoQ* = adiponectin, *AdipoR1* and *AdipoR2* = adiponectin receptors 1 and 2. Data were used as relative data expressed as normalized ratio between the target mRNA and the geometric mean of the reference genes (*Pol II*, *ActB*, and *GAPDH*). KID = AT from kidney; IMtop = intermuscular fat below the top round; MES = mesenteric AT; OM = omental AT; SCback = s.c. AT from back; FATtot = total fat mass; IF = total inner fat mass; SCtot = total s.c. fat mass.

in SCback was strongly related to BHBA, and *AdipoR2* in the same AT depot to NEFA. In the lean-type group, *AdipoQ* mRNA in kidney fat correlated positively with BHBA at a moderate level but correlated strongly and negatively with glucose.

In liver, the amount of *AdipoR2* mRNA was 3-fold higher than that of *AdipoR1* mRNA ($P \leq 0.001$). In liver, *GPR109A* mRNA correlated positively with fat concentration ($P \leq 0.05$; $r = 0.76$) in fat-type animals, whereas *AdipoR1* mRNA tended to correlate negatively with hepatic glycogen content within the lean-type group.

DISCUSSION

In this study, we present data about differential mRNA expression of *AdipoQ*, its receptors, and *GPR109A* mRNA in liver, s.c. fat, and 3 v.c. fat depots of 2 cattle

families that differ in fat accretion but have a common genetic background. Correlation of mRNA abundance data with different fat masses and blood metabolites revealed clear differences between the families. Prior work has shown that abundance of *AdipoQ* mRNA is correlated negatively with subcutaneous AT back fat in cattle (Taniguchi et al., 2008). The *AdipoQ* mRNA correlated less in fatter Hereford × Aberdeen Angus steers than in Charolais × Red Angus steers. However, that study was based on 2 breeds with different genetic backgrounds, and no information was provided about AdipoQ receptors and GPR109A, which are related to insulin sensitivity and energy metabolism. Likewise, v.c. adipose depots were not analyzed but, from studies in humans, it is known that v.c. adipocytes secrete more AdipoQ than do those from s.c. AT (Motoshima et al., 2002) and more *AdipoQ* mRNA is found in v.c. AT compared with s.c. AT in mice (Altomonte et al.,

Figure 3. Results of the correlation analysis between the mRNA abundance of the adiponectin system and G-protein coupled receptor 109A (GPR109A) in different adipose depots at 100 DIM and blood metabolites at 93 DIM in the fat-type (A; $n = 9$) and lean-type (B; $n = 9$) cow families. KID = adipose tissue (AT) from kidney; IMtop = intermuscular fat below the top round; MES = mesenteric AT; OM = omental AT; SCback = s.c. AT from back; FATtot = total fat mass; IF = total inner fat mass; SCtot = total s.c. fat mass; Gluc = glucose; Gluca = glucagon. *AdipoQ* = adiponectin; *AdipoR1* and *AdipoR2* = adiponectin receptors 1 and 2. Data were used as relative data expressed as normalized ratio between the target mRNA and the geometric mean of the reference genes (*Pol II*, *ActB*, and *GAPDH*).

2003). In general, *AdipoQ* expression is positively related to insulin sensitivity and negatively associated with obesity and gluconeogenesis (Combs et al., 2001; Kadowaki et al., 2006).

Differences in Adiponectin System Gene Expression Between Fat-Type and Lean-Type Cows

In the current study, the highest *AdipoQ* mRNA values were found in kidney AT. This observation is similar to that of Locher et al. (2009) but was not confirmed by Ohtani et al. (2011). We observed more *AdipoQ* mRNA as a trend in mesenteric AT of the fat-type cows compared with the lean-type cows but found no differences within the other depots. This result is in contrast to the expected inverse relationship of *AdipoQ* and AT mass in general (Kadowaki et al., 2006) and to the study of Taniguchi et al. (2008) in cattle. The AT sample origin might affect the result, as we have

shown for *AdipoQ* receptor and leptin mRNA in s.c. AT of sheep (Lemor et al., 2010). On the other hand, family-dependent regulation of *AdipoQ* mRNA expression could not be ruled out. A breed-dependent *AdipoQ* mRNA expression mode was described by Daniele et al. (2008) in pigs. In that study, 2 pig lines with differences in fat accretion showed a clear inverse relationship between AT mass and serum *AdipoQ*. Interestingly, in the lean Large White line, more *AdipoQ* protein was found in perirenal AT than in s.c. AT, but the opposite was observed in the fatter Casertana pigs. In the current study, the fat-type line expressed more *AdipoQ* mRNA, at least as a tendency in mesenteric AT, which represented only 12.5% of the total fat mass. Coincidentally, in this group, the abundance of *AdipoR1* mRNA in kidney and mesenteric AT, and the expression of *AdipoR2* mRNA in SCback AT (as a trend) were higher in the fat-type compared with the lean-type family. These observations might be related to positive autocrine and

paracrine effects of AdipoQ on adipogenesis and lipogenesis in the fat-type family, as described in 3T3-L1 cells by Fu et al. (2005), resulting in a higher fat mass observed in the fat-type family, in which cows accrete more adipose tissue. Therefore, our findings might be related to the adaptation to fat accretion in this family, which also had a tendency for higher insulin concentrations than lean-type animals. Higher insulin concentrations despite higher *AdipoQ* receptor mRNA abundance in discrete AT depots in fat-type versus lean-type cows may support our suggestion about metabolic adaptation in the fat-type family. In this regard, it has been shown that insulin decreases *AdipoQ* and *AdipoR2* mRNA in differentiated adipocytes of Chinese Holstein calves in vitro (Sun et al., 2009), whereas it has no effect on *AdipoR1* and *AdipoR2* mRNA expressions in s.c. or v.c. AT explants of Holstein-Friesian cows in vitro (Hosseini et al., 2012).

We observed no differences in the extent of phosphorylation of the downstream effector of AdipoQ, AMPK (Guerre-Millo, 2008), between the families in liver. This might be related to the fact that phosphorylation is more associated with short-term than with long-term regulation because chronic food restriction does not affect AMPK activity in liver (Gonzalez et al., 2004).

Similar to studies in mice (Kadowaki et al., 2006), we observed more *AdipoR2* than *AdipoR1* mRNA in bovine liver. The use of genetic mice models (Yamauchi et al., 2007) has shown that hepatic *AdipoR2* signaling primarily triggers peroxisome proliferator-activated receptor- α (PPAR α), which is involved in improved glucose uptake, reduction of oxidative stress, and inflammation. Higher amounts of *AdipoR2* mRNA were found in liver of the lean-type cows, whereas these animals had lower concentrations of blood glucose and insulin (Hammon et al., 2010). In mice, it was shown that both glucose and insulin are each inversely correlated to *AdipoR1* and *AdipoR2* mRNA expression. Insulin reduces levels of both receptor mRNA in murine AT and hepatocytes (Tsuchida et al., 2004). Based on the published data, we suggest that the regulation of the receptor mRNA expression in liver might be transferable to the current study: improvement of liver metabolism in secretion-type cows by *AdipoR2* is driven by PPAR α -mediated signaling pathways.

Correlation Analysis Between mRNA Abundance of the Adiponectin System and Different Fat Depots in Fat-Type and Lean-Type Cows

Correlation analysis revealed differences between families. The abundance of *AdipoQ* mRNA in mesenteric AT of both families was positively related to mesenteric adipose AT mass but also to the masses

of the other fat compartments, except for SC_{tot} in fat-type cows. Moreover, these correlations were more pronounced in the lean-type cows. Based on our results, we propose that independently of the metabolic differences between both families, mesenteric *AdipoQ* mRNA expression, and potentially AdipoQ protein expression, is an important predictor of total AT mass but only a moderate marker for SC_{tot} and IM_{top} mass in cows. In this context, it is noteworthy that, at least for humans, mesenteric AT has a major role in regulating insulin sensitivity by its gene expression profile (Yang et al., 2008). Less information is available for cattle.

Blood Metabolites in Fat-Type and Lean-Type Cows and Relationship to the Adiponectin System

Glucagon concentrations tended to be lower in the lean-type group compared with the fat-type group. Moreover, the lean-type group showed strong positive correlations between the AdipoQ system, particularly that in mesenteric AT, and glucagon concentrations. Taken together, our results indicate that the AdipoQ system has an important role in determining the AT mass, especially for lean-type cows.

Lower glucagon concentrations but a higher glucagon versus insulin ratio was described by Hammon et al. (2010) for the lean-type family. Glucagon directly stimulates glycerol release in human adipocytes (Perea et al., 1995) and is thus related to lipolysis. Effects of glucagon on lipolysis are less clear in cattle than in monogastric species, and effects on plasma NEFA were seen only by using high glucagon dosages (10 mg/d) in heifers (Hippen et al., 1999; She et al., 1999). Gluconeogenesis, as well as ketone body formation, is positively correlated to NEFA and glucagon concentrations (Herdt, 2000). Indeed, as discussed by Caesar and Drevon (2008), mesenteric and omental AT drain directly to the portal vein of the liver. Thus, adipokines from both depots should have major effects on liver metabolism compared with the other v.c. as well as s.c. AT depots. In cattle, the glucagon:insulin ratio is thought to be central for glucose production (Hammon et al., 2010). Therefore, we suggest that, particularly during phases of low glucagon concentrations but high glucagon:insulin ratios in dairy cows, AdipoQ release from mesenteric and potentially omental AT should be low to reduce its negative effect on gluconeogenesis in bovine liver. This relationship could be a part of a feedback mechanism especially in secreting-type dairy cattle as an adaptation to higher lipid mobilization and glucose production, which is necessary during early lactation. However, further experiments are necessary to examine this hypothesis.

Abundance of *GPR109A* mRNA in Fat-Type and Lean-Type Cows

The hydroxy-carboxylic acid binding receptor GPR109A is expressed in different adipose depots and in liver of cattle (Titgemeyer et al., 2011a). In both families, the highest values of *GPR109A* mRNA were observed in SCback compared with 2 visceral localizations. Thus, we expect that the effect of a BHBA-mediated feedback mechanism on lipolysis might be highest in s.c. AT, independently of the metabolic differences between breeds. We further observed more *GPR109A* mRNA in kidney fat and liver of lean-type than fat-type cows. Therefore, the basal lipolytic activity in kidney fat of lean-type cows, regulated by a BHBA-GPR109A feedback mechanism, might be lower than in the fat-type cows. This mechanism may help to restrict lipolysis from kidney fat during lactation in secreting-type cows. Mobilization of AT is a prerequisite for successful lactation. It was shown recently that only kidney AT mass is significantly mobilized during early lactation, compared with other fat masses in dairy cows (von Soosten et al., 2011). We also observed that the *GPR109A* mRNA in s.c. AT tended to be reduced during the transition period of dairy cows (Lemor et al., 2009), which might support lipid mobilization during early lactation. Stricter regulation of lipolysis in lean-type cows compared with fat-type cows by the GPR109A-mediated feedback mechanism might be a metabolic adaptation to control NEFA concentrations in these animals, which might reduce the metabolic burden in lean-type cows. Knowledge about the regulation and function of the receptor GPR109A during the transition period in kidney fat might be of interest. Nevertheless, stimulation of the receptor with pharmacological amounts of niacin did not totally prevent lipolysis, as shown by Titgemeyer et al. (2011b). The function of GPR109A in liver is not entirely clear, but activation of GPR109A in mouse hepatocytes reduced the amount and activity of cyclic AMP and ATP-binding cassette transporter-1 protein, which is related to a reduction of cholesterol efflux and, consequently, to a lower production rate of high-density lipoprotein cholesterol (Li et al., 2010). Therefore, more *GPR109A* mRNA in lean-type animals might be related to a reduction in cholesterol release. In general, key enzymes of cholesterol synthesis are upregulated after parturition in dairy cattle (Viturro et al., 2009), and cholesterol concentrations are higher in lactating compared with dry cows (Gardner et al., 2003). We observed an association of this receptor with liver lipid metabolism because of its positive correlation to liver fat content in lean-type cows. In steers, expression of this receptor was surprisingly high in liver compared

with other tissues, as discussed by Titgemeyer et al. (2011a). Together with our observations, this may implicate other functions of this receptor in ruminants compared with other mammals and may explain the higher concentrations in secreting-type (lean-type) cows compared with fat-type cows.

Correlation of *GPR109A* mRNA Abundance with Different Fat Depots and Blood Metabolites

All interactions between *GPR109A* mRNA and AT masses were negative. In addition, the abundance of *GPR109A* mRNA in kidney AT was positively correlated to NEFA as a trend in both families, which points to the importance of this receptor in regulation of AT mass and lipolysis in lean-type as well as fat-type cows. As discussed above, the mobilization of kidney AT seems to be mostly affected during early lactation (von Soosten et al., 2011), and upregulation of the receptor might be involved in limiting lipolysis from this tissue in both families. In addition, we observed a strong negative correlation between mesenteric *GPR109A* mRNA expression and BHBA within the lean-type group only. This observation might be related to the fact that, at least in humans, this depot is less sensitive to noradrenergic stimuli compared with s.c. AT (Yang et al., 2008), considering that sympathetic stimulated lipolysis is reduced by GPR109A (Gille et al., 2008). Consequently, downregulation of GPR109A in mesenteric AT of lean-type cows may result in an increased lipolytic rate from this AT depot, despite the presence of BHBA. Therefore, we suggest that lean-type cows are more prone to GPR109A-mediated lipolysis than the fat-type cows, which is AT depot dependent. The importance of this assumption for liver lipid metabolism of cows should be clarified.

CONCLUSIONS

We found clear differences in mRNA abundance of the *AdipoQ* system and *GPR109A* between 2 dairy cow families that differed in body fat accretion and milk secretion but had a common genetic background. Visceral AT in both families was of central importance in interacting with the *AdipoQ* system and *GPR109A* mRNA. The effect of *AdipoQ* secretion, especially by visceral AT depots, on liver metabolism should be clarified. Autocrine as well as paracrine effects in AT might be significant.

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