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 calving¹

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ABSTRACT

Elevated liver fat content occurs in high-yielding dairy cows during the transition from pregnancy to lactation after fat mobilization and may affect hepatic glucose metabolism, but the degree of liver fat storage is highly variable. Therefore, we studied metabolic and endocrine changes and hepatic glucose metabolism in cows that markedly differ in liver fat content. Multiparous cows from the same herd with high (HFL; $n = 10$) and low (LFL; $n = 10$) liver fat contents (mean of d 1, 10, and 21 after calving for each cow, respectively) were studied from 60 d before expected calving to 56 d in milk. Cows were fed ad libitum and all cows received the same diets. Liver samples were taken on d 1, 10, and 21 after calving; mean fat content ($\pm$ SEM) in liver of HFL cows was 174 ± 9.6 mg/g, whereas mean liver fat content in LFL cows was 77 ± 3.3 mg/g. Blood samples were taken 20 and 7 d before expected calving and 0, 7, 14, 28, and 56 d after calving to measure plasma concentrations of nonesterified fatty acids, β -hydroxybutyrate, glucose, insulin, glucagon, insulin-like growth factor-I, and leptin. In liver, glycogen content as well as mRNA levels of phosphoenolpyruvate carboxykinase, pyruvate carboxylase, glucose-6-phosphatase, and glucose transporter were measured by quantitative real-time PCR. Back fat thickness decreased and dry matter intake increased with onset of lactation, and back fat thickness was higher but dry matter intake was lower in HFL than in LFL. Energy-corrected milk yield did not differ between groups, but milk fat content was higher and lactose content was lower in HFL than LFL at the beginning of lactation. Energy balance was more negative in HFL than in LFL. Plasma nonesterified fatty acids

and β -hydroxybutyrate concentrations increased and plasma glucose concentration tended to decrease more in HFL than LFL with onset of lactation. Glucagon to insulin ratios increased more in HFL than LFL with onset of lactation. Hepatic glycogen content was higher in LFL than HFL, whereas mRNA levels of glucose-6-phosphatase and pyruvate carboxylase were higher in HFL than in LFL, and cytosolic phosphoenolpyruvate carboxykinase mRNA level increased similarly after parturition in both groups. In conclusion, an elevated liver fat content was related to greater fat mobilization and reduced feed intake and was associated with effects on hepatic glucose metabolism. As environment and feeding management were the same, individual cow factors were responsible for differences in energy metabolism during the transition period.

Key words: liver fat content, dry matter intake, glucose metabolism, dairy cow

INTRODUCTION

Because of insufficient feed intake to meet energy requirements resulting from high milk production, high-yielding dairy cows often develop severe negative energy balance (**NEB**) during early lactation (Block et al., 2001; Drackley et al., 2001; Grummer et al., 2004). Cows mobilize fat depots to provide NEFA as an energy fuel (Block et al., 2001; Drackley et al., 2001; Kokkonen et al., 2005). Hepatic fat content increases in the postpartum period as a consequence of fat mobilization (Grummer, 1993; Herdt, 2000; Vernon, 2005). Excessive NEFA concentrations and extremely high liver fat content can result in metabolic imbalances that are related to clinical diseases such as ketosis and fatty liver syndrome (Bobe et al., 2004; Ingvarsen, 2006). However, liver fat content shows large variations between cows in practice, and a high liver fat content as well as a high level of body fat may not always lead to disorders related to hepatic metabolism (McNamara, 2000; Jorritsma et al., 2001; Vernon, 2005), but may

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change hepatic energy metabolism (i.e., endogenous glucose production; Rukkwamsuk et al., 1999; Drackley et al., 2001; Ingvarsen, 2006).

Providing adequate amounts of glucose to the mammary gland is essential to enable high milk production in dairy cows (Drackley et al., 2001; Brockman, 2005). As glucose supply in dairy cows depends predominantly on endogenous production in the liver (Nafikov and Beitz, 2007), elevated fat content in liver may interfere with the capacity for hepatic glucose production during early lactation (Rukkwamsuk et al., 1999; Murondoti et al., 2004). However, measurements of impaired hepatic gluconeogenic capacity were mainly taken from cows with induced fatty liver by manipulating feeding regimens. Hepatic glucose production is clearly impaired in cows with fatty liver syndrome (Rukkwamsuk et al., 1999; Bobe et al., 2004) but, in principle, oxidation of NEFA in the liver is needed to provide ATP for gluconeogenesis (Bell, 1995; Drackley et al., 2001; Lam et al., 2003). Therefore, the range between physiological and pathological effects of fatty acids on glucose metabolism in liver is blurred and may vary among cows. In addition, long-chain fatty acids can affect systemic and hepatic glucose metabolism and decrease glucose post-liver supply probably independently from the degree of fat storage in liver (Benson et al., 2002; Hammon et al., 2008).

Hepatic fat content and its effects on liver metabolism seem to be highly variable among cows. This indicates that individual cow factors beyond management and feeding conditions affect hepatic and body fat storage and consequently performance of high-yielding dairy cows during the transition period (McNamara, 2000; Ingvarsen, 2006). Such individual cow factors may be best studied in one herd under identical feeding, housing, and management conditions. We, therefore, have investigated high-yielding dairy cows with low and high liver fat contents after calving. The objective was to study effects on performance and metabolic changes with emphasis on glucose metabolism in dairy cows fed ad libitum and that markedly differed in liver fat content during the transition from pregnancy to lactation. Furthermore, we hypothesized that hepatic gene expression with regard to glucose metabolism may be impaired when high amounts of fat are stored in liver.

MATERIALS AND METHODS

Animals, Husbandry, Feeding, and Measurement of Zootechnical Data

The experimental procedures were supervised by the cattle and swine clinic, Department of Veterinary Medicine, Freie Universität Berlin, Germany, and liver

biopsies were performed for diagnostic purposes. Dairy cows originated from the herd of the State Institute for Agriculture, Forestry and Horticulture of Saxony-Anhalt (Iden, Germany). In total, 48 multiparous (from second to seventh lactation) high-yielding dairy cows ($>11,000$ kg of milk/305 d $\times$ cow) were routinely monitored for liver fat content by liver biopsies on d 1, 10, and 21 after calving. All cows were clinically healthy. Based on mean fat content in liver on d 1, 10, and 21, the 10 cows with the highest fat content in liver after calving (**HFL**) were compared with the 10 cows with the lowest fat content in liver (**LFL**) in the herd. Mean fat content ($\pm$ pooled SEM) in liver of HFL cows was 113, 213, and 197 ± 9.2 mg/g of liver wet weight on d 1, 10, and 21, respectively, whereas mean liver fat content in LFL cows was 70, 82, and 79 ± 5.4 mg/g on d 1, 10, and 21, respectively.

Cows were kept in a free-stall barn, and housing and feeding management was the same for all cows. The dry-off period started 8 wk before expected calving. Feed intake was ad libitum and was measured on an individual basis. Separate rations (TMR) were fed during the dry-off period (wk 8 to 4 before calving), close-up period (wk 3 to 1 before calving), and lactation (wk 1 to 8 after calving). Ingredients and chemical composition of the different diets are shown in Table 1. Cows had free access to water. One week before calving and from wk 2 to 8 of lactation, TMR was placed in troughs on scales, which were connected to a computer, and individual feed intake was calculated for each day. Measurement of individual feed intake around calving was unfortunately not available because the calving area in the barn was not equipped with such troughs. Dry matter content was determined daily for grass and corn silages and weekly for other ingredients. Feed samples for measurement of chemical composition of the diets were collected weekly. Dry matter, CP, crude fiber, starch, and sugar were determined according to the Weender standard procedure (Naumann and Bassler, 1993). The NE_L and utilizable protein in the diets were calculated according to the German Society of Nutrition Physiology (2001).

Body weight was measured on d 60 and 20 before calving, immediately after calving, and on 7, 14, 28, and 56 DIM after morning milking. Cows were milked 3 times daily at 0400, 1200, and 2000 h, and milk yield was recorded daily. Milk samples were taken once weekly for determination of milk protein, milk fat, and lactose by an infrared spectrophotometric method (Milkoscan, Foss Germany, Rellingen, Germany) at the State Institute for Agriculture, Forestry and Horticulture (Iden, Germany). Energy-corrected milk was calculated as follows: ECM (kg) = $(0.038 \times \text{g of crude fat} + 0.024 \times \text{g of CP} + 0.017 \times \text{g of lactose}) \times \text{kg of milk}/3.17$.

Energy balance (**EB**) was calculated thus: $EB = NE_L \text{ intake} - (ECM \times 3.14 + 0.293 \times \text{kg of BW}^{0.75})$ and was expressed in MJ/(d $\times$ cow).

Back-fat thickness (**BFT**) was determined after morning milking by ultrasonic measurement on d 60 and 20 before calving, at calving, and on 7, 14, 28, and 56 DIM in the sacral region according to Schröder and Staufenbiel (2006).

Blood Sampling and Analyses

Coccygeal vein blood samples were taken 20 and 7 d before expected calving, at calving, and on 7, 14, 28, and 56 DIM by venipuncture using tubes containing heparin or no anticoagulant. Blood samples with anticoagulant were immediately put on ice, whereas tubes without anticoagulant were left at room temperature until clotting was complete (within 2 h). Tubes were then centrifuged for 10 min at $4,000 \times g$ and supernatant was stored at -20°C until analyzed.

Serum concentrations of NEFA (kit no. FA 115) and BHBA (kit no. RB 1007) were measured using kits from Randox Laboratories (Crumlin, UK). Analyses were performed using the Hitachi 704 automatic analyzer (Hitachi, Tokyo, Japan). Serum concentrations of glucose were measured photometrically with the Reflotron glucose kit (Roche Diagnostics, Mannheim, Germany) using the Reflovet Plus photometer (Roche Diagnostics).

Insulin was measured in plasma by RIA using a porcine kit (PI-12K, Linco Research, St. Charles, MO; Bellmann et al., 2004). A standard curve was prepared at concentrations from 2 to 200 $\mu\text{U/mL}$. Cross-reactivity with bovine insulin was 90%. Previous findings have shown that this assay exhibited good linearity for concentrations in the range of 25 to 100 $\mu\text{U/mL}$ in bovine plasma samples. The sensitivity of the insulin RIA (the lowest detectable level) was 2 $\mu\text{U/mL}$. Intra- and interassay coefficients of variation (**CV**) were 8.2 and 4.3%, respectively. Glucagon was measured by RIA using a kit from Linco (GL-32K, Linco Research; Hammon and Blum, 1998). Intra- and interassay CV were 9.6 and 5.8%, respectively. The assay is specific for the determination of pancreatic glucagon in serum or plasma in most mammals. Cross-reactivity to oxyntomodulin, which is the primary gut glucagon, is less than 0.1%. Plasma concentrations of leptin were determined in 100- μL aliquots by the multi-species RIA kit (XL-85K, Linco Research). The double-antibody assay utilized an anti-human leptin antibody raised in guinea pigs (primary antibody) and a human standard hormone. According to the manufacturer, the antibody shows wide cross-reactivity to leptin of other species. As indicated in an earlier study (Bellmann et al., 2004)

Table 1. Ingredients and chemical composition of the dry-off, close-up, and lactation diets fed during the study to dairy cows

Item	Diet		
	Far-off ¹	Close-up ¹	Lactation
Ingredient, g/kg of DM			
Corn silage	200	200	240
Grass silage	600	420	300
Straw	120	50	50
Corn cob silage		120	140
Grain (barley, wheat)		60	70
Rapeseed/soy, protected		50	100
Extracted rapeseed meal		80	80
Minerals	10	10	10
Propylene glycol/glycerin		10	10
Chemical analysis			
Utilizable protein, ² g/kg of DM	120	152	175
NE _L , ² MJ/kg of DM	5.7	6.7	7.0
Crude fiber, g/kg of DM	240	183	157
Starch and sugar, g/kg of DM	74	190	215

¹Far-off period was from 8 to 4 wk before calving and close-up period was from 3 to 0 wk before calving.

²German Society of Nutrition Physiology (2001).

several groups of researchers have used the Linco XL-85K kit in cattle and other farm animals. As a result of our own examination, parallelism was demonstrated for a range of volumes between 25 and 100 μL . Intra- and interassay CV were 5 and 8%, respectively. Plasma concentrations of IGF-I were measured by an immunoradiometric assay (DSL-5600 Active IGF-I IRMA; Diagnostic Systems Laboratories, Webster, TX). The first step of the assay procedure is the treatment of the samples with a mixture of ethanol-hydrochloric acid to remove IGF binding proteins. The immunoradiometric assay is a sandwich-type assay with anti-IGF-I-coated tubes and [I-125]-labeled anti-IGF as tracer. Intra- and interassay CV were 4 and 7%, respectively. All immunoassays were adapted for bovine plasma and values measured diluted in parallel with standard curves.

Liver Sampling and Analyses

Liver biopsy samples (about 350 mg) were obtained on d 1, 10, and 21 after calving for analysis of glycogen and fat content as well as mRNA levels of key enzymes in gluconeogenesis and hepatic glucose transport. The biopsy technique was adapted from Gröhn et al. (1982), using the Berliner Model biopsy needle from Eickemeyer (Tuttlingen, Germany). The area around the right 11th and 12th intercostal space on a line from the hip joint to the elbow was clipped, scrubbed with iodine solution, and swabbed with 70% ethanol before being anesthetized with ice (Histofreezer, Braun, Melsungen, Germany). Liver biopsy samples were obtained percutaneously. Samples were frozen in liquid nitrogen. All samples were stored at -80°C until analyzed.

Table 2. Primers used for real-time reverse transcription-PCR to analyze mRNA concentrations of cytosolic (PEPCK-C) and mitochondrial phosphoenolpyruvate carboxykinase (PEPCK-M), pyruvate carboxylase (PC), glucose-6-phosphatase (G6Pase), glucose transporter (GLUT2) in liver

Primer ¹	Primer sequence 5' → 3'	Coding sequence	Length, bp	NCBI ² accession no.
PEPCK-C, for	CTC AGC TGG CAG CAC GGT GTG	1480 to 1500	578	AY145503
PEPCK-C, rev	TGC TCC TGG TGC GTT GTA TGG	2037 to 2057		
PEPCK-M, for	TAT GAG GCC TTC AAC TGG CGC	1490 to 1510	365	XM_583200
PEPCK-M, rev	AGA TCC AAG GCG CCT TCC TTA	1834 to 1854		
PC, for	ACA CCA ACT ACC CCG ACA ATG	2020 to 2040	353	AY185595
PC, rev	CAG CGG GAG GTC AGG GAA G	2354 to 2372		
G6Pase, for	ATG TTG TGG TTG GGA TTC TGG	527 to 547	275	BC114011
G6Pase, rev	CAC CTT CGC TTG GCT TTC TC	782 to 801		
GLUT2, for	ACA GAG GAA TTG CCC ACA AG	193 to 212	242	XM_614140
GLUT2, rev	TTC GAA AAC CCC ATC AAG AG	415 to 434		
GAPDH, for	TAC ATG GTC TAC ATG TTC CAG TAT G	190 to 214	439	NM_001034034
GAPDH, rev	CAG TCT TCT GGG TGG CAG TGA TG	606 to 628		

¹for = forward; rev = reverse.²NCBI = National Center for Biotechnology Information.

Total lipid content in liver was measured gravimetrically as described by Ahmed (2004). A kit (no. 207748, Roche Diagnostics) was used for glycogen measurement in liver. According to the instructions of the company, glycogen was converted to glucose by amyloglucosidase (EC 3.2.1.33) and glucose was measured by the hexokinase method.

For mRNA measurements, total RNA was extracted using TRIzol reagent (Invitrogen, Karlsruhe, Germany) and resuspended in RNase-free water. The integrity and purity of RNA were tested by measurement of optical density (ratios at 260 and 280 nm being greater than 1.9) and by electrophoresis using ethidium bromide staining. Total RNA was reverse transcribed into cDNA using random primers (Random Primers, Invitrogen) as described previously (Hammon et al., 2003).

Real-time reverse transcription-PCR was performed by using a LightCycler (Roche Molecular Biochemicals, Mannheim, Germany) using SYBR Green I as fluorescent dye (Hammon et al., 2003). Primers used for PCR measurement of phosphoenolpyruvate carboxykinase (EC 4.1.1.32; cytosolic: **PEPCK-C**; mitochondrial: **PEPCK-M**), pyruvate carboxylase (**PC**; EC 6.4.1.1), glucose-6-phosphatase (**G6Pase**; EC 3.1.3.9), glucose transporter (**GLUT2**), and GAPDH are shown in Table 2. Primers were designed to flank a region that contains at least 1 intron to ensure that no contaminating genomic DNA was amplified that could lead to false signals.

Melting temperatures (using the LightCycler in a melting curve analysis program after the last amplification cycle) demonstrated specific PCR products. Upon agarose gel electrophoresis, all PCR products moved with one single band and showed the expected sizes (Table 2). Products were verified by sequencing using an ABI Sequencing kit (ABI Big Dye Terminator, Ap-

plied Biosystems, Darmstadt, Germany) and an ABI 310 Genetic Analyzer (Applied Biosystems).

Quantification of mRNA was performed by relative expression using GAPDH as reference gene transcript (Pfaffl, 2001). For each sample, crossing point (**CP**) values, which represent the intersection of a fixed threshold above background fluorescence, and the amplification curve (Rasmussen, 2001) were determined for each enzyme and for GAPDH. Values of CP for enzymes and for GAPDH were corrected for different runs by an internal standard (**ΔCP**). For each sample, ΔCP_{GAPDH} was used for normalization of the amount of every target gene (ΔCP_{target}) of the corresponding sample, which resulted in $\Delta \Delta CP (= \Delta CP_{target} - \Delta CP_{GAPDH})$. Relative expression levels (R) within each enzyme were given by the arithmetic equation $R = 2^{-\Delta \Delta CP}$ (Pfaffl, 2001). The logarithm (log 2) is based on an optimum efficiency (E) of PCR, which is E = 2 when the PCR product is replicated every cycle. The PCR efficiency was calculated based on the slope calculated by LightCycler software 4.05 (Roche Applied Science) using the equation $E = 10[-1/slope]$. Efficiency of PCR was 1.7 to 1.9, and was therefore close to 2. Based on ΔCP evaluation, expression of GAPDH in our study was not affected by fat content in liver or DIM. Inter- and intraassay CV for reverse transcription-PCR of target and reference genes were <1%.

Statistical Analyses

Statistical analyses were done with the SAS System for Windows, release 9.1.3 (SAS Institute, 2004). Body weight, DMI, BFT, milk performance, and plasma concentrations of metabolites and hormones as well as hepatic gene expression and glycogen were assessed by repeated measurements ANOVA using the MIXED pro-

cedure. The ANOVA model used contained the fixed effects liver fat content (groups: LFL, HFL), time during transition (days relative to calving), and the interaction between liver fat content and time during transition and a random cow effect. Because lactation number was different between HFL and LFL cows (4.0 ± 0.4 ranging from second to seventh lactation and 2.7 ± 0.4 ranging from second to sixth lactation for HFL and LFL, respectively), lactation number of the cow was included as a fixed effect in the model. Repeated measures on each animal were taken into account (repeated factor: time during transition). The least squares means (LSM) and their standard errors were computed for each fixed effect in the ANOVA model to display the results. Additionally, all pair-wise differences of these LSM were tested using the Tukey-Kramer procedure. Differences with $P < 0.1$ were considered as trends and with $P < 0.05$ as significant. Significance of NEB was tested by the TTEST procedure.

The CORR procedure was used to calculate and test Pearson correlations between hepatic measurements and plasma concentrations of metabolites and hormones as well as BW, DMI, BFT, and milk performance during first 3 wk after parturition.

RESULTS

Feed Intake, Milk Production, BW, BFT, and EB

Intake of DM increased after parturition in both groups but DMI was lower ($P < 0.05$) before parturition and until 35 DIM in HFL than in LFL (Figure 1). Energy-corrected milk yield increased ($P < 0.01$) after parturition in both groups, but showed no group differences (Figure 2A). Milk fat content was higher ($P < 0.01$) on 14 DIM in HFL than in LFL, decreased ($P < 0.01$) to a greater extent from 14 to 42 DIM in HFL than LFL, and did not differ between groups thereafter (Figure 2B). Milk protein content changed with time ($P < 0.05$), but showed no differences between groups (Figure 2C). Lactose content in milk increased ($P < 0.01$) with onset of lactation and was higher ($P < 0.05$) in LFL than in HFL (Figure 2D). Both groups indicated NEB after parturition lasting up to 28 DIM in LFL and up to 56 DIM in HFL (Figure 3A). However, the degree of NEB on 14 and 28 DIM was much greater ($P < 0.05$) in HFL than in LFL. Body weight and BFT were higher ($P < 0.05$) during the dry period in HFL than in LFL cows (Figure 3B, C). The decrease of BW from d 20 before parturition to 28 DIM was much greater ($P < 0.001$) in HFL than in LFL, resulting in similar BW in both groups on 28 DIM. Back fat thickness was higher ($P < 0.01$) and the decrease of BFT from 0 to 56

Figure 1. Dry matter intake 7 d before to 56 d after calving in cow with low ($\square$) and high ($\blacktriangle$) liver fat content after calving. Data are presented as least squares means $\pm$ SE. Statistically significant ($P < 0.05$) effects of liver fat content, time before and after calving, and fat content $\times$ time interactions are given in the figure.

DIM was much greater ($P < 0.01$) in HFL than in LFL. On 56 DIM BFT was similar in both groups. During the first 3 wk after calving, hepatic fat content was positively related to BFT ($r = 0.34$; $P < 0.01$).

Metabolic and Endocrine Changes

Plasma NEFA and BHBA concentrations increased ($P < 0.05$) around parturition only in HFL, but not significantly in LFL (Figure 4A, B). The LSM of both parameters and values on 0, 7, and 14 DIM for plasma NEFA concentration as well as 7, 14, and 28 DIM for BHBA were higher ($P < 0.05$) in HFL than in LFL, respectively. Plasma glucose concentration decreased ($P < 0.001$) after parturition in both groups, but reached prepartum levels on 56 DIM (Figure 4C). The LSM of glucose concentrations tended to be lower ($P < 0.1$) in HFL than in LFL.

Plasma insulin concentration decreased ($P < 0.01$) and plasma glucagon concentration increased ($P < 0.01$) with onset of lactation, but did not differ between groups in a significant manner (Figure 5A, B). The glucagon/insulin ratio in plasma increased after calving and the LSM were higher ($P < 0.05$) in HFL than in LFL (Figure 5C). Plasma IGF-I and leptin concentrations decreased ($P < 0.01$) around parturition in both groups, but did not differ with regard to hepatic fat status (Figure 6A,B).

Figure 2. Energy-corrected milk (A), milk fat content (B), milk protein content (C), and lactose content (D) on 14 to 56 DIM in cow with low (□) and high (▲) liver fat content after calving. Data are presented as least squares means $\pm$ SE. Statistically significant ($P < 0.05$) effects of liver fat content, time before and after calving, and fat content $\times$ time interactions are given in the figure.

Glycogen Content and Gene Expression of Gluconeogenic Enzymes and GLUT2 in Liver

Glycogen content was higher ($P < 0.05$) on all days in LFL than in HFL (Table 3). Levels of PC mRNA were higher ($P < 0.05$) and levels of G6Pase tended to be higher ($P < 0.1$) in HFL than in LFL. Levels of PEPCK-C increased ($P < 0.05$) with time in both groups, but showed no group differences. Levels of PEPCK-M and GLUT2 mRNA were not affected by time or fat content in liver.

Levels of PC mRNA correlated positively with total fat in liver ($r = 0.48$; $P < 0.05$), with plasma concentrations of NEFA ($r = 0.36$; $P < 0.01$) and BHBA ($r = 0.45$; $P < 0.001$), and with the glucagon to insulin ratio in plasma ($r = 0.3$; $P < 0.05$), but correlated negatively with glycogen content in liver ($r = -0.58$; P

< 0.001) and plasma IGF-I concentration ($r = -0.32$; $P < 0.05$). Levels of G6Pase mRNA correlated negatively with glycogen content in liver ($r = -0.27$; $P < 0.05$). Glycogen content in liver correlated negatively with total fat concentration in liver ($r = -0.56$; $P < 0.001$), with plasma concentrations of NEFA ($r = -0.48$; $P < 0.001$) and BHBA ($r = -0.48$; $P < 0.001$), and with the glucagon to insulin ratio in plasma ($r = -0.37$; $P < 0.01$), but correlated positively with plasma IGF-I concentration ($r = 0.28$; $P < 0.05$). Total liver fat content correlated positively with plasma concentrations of NEFA ($r = 0.4$; $P < 0.01$), BHBA ($r = 0.66$; $P < 0.001$), and glucagon ($r = 0.39$; $P < 0.01$) as well as with the glucagon to insulin ratio in plasma ($r = 0.33$; $P < 0.05$), but correlated negatively with plasma IGF-I concentration ($r = -0.31$; $P < 0.05$).

DISCUSSION

The high-yielding dairy cows investigated in this study were kept under identical environmental conditions and were fed the same diets. No clinical signs of fatty liver syndrome such as sluggish appetite, downer syndrome, or coma were observed in the herd. Based on liver samples routinely taken after calving, we chose the 10 multiparous cows with the highest and lowest fat contents in liver, respectively. The changes of fat in liver after calving were consistent with findings in the literature (Ohgi et al., 2005; Drackley and Andersen, 2006) and result from NEFA that are stored in the liver as triglycerides (Vazquez-Anon et al., 1994; Hayirli and Grummer, 2004; Drackley and Andersen, 2006). Liver fat content of HFL cows corresponded with severe fatty liver and elevated plasma ketone bodies and with reduced DMI, but surprisingly there was no significant reduction of milk production as reviewed recently (Bobe et al., 2004), but numerically a greater ECM yield than in LFL cows.

Interactions of Fat Content in Liver with BFT, Feed Intake, and Fat Metabolism in Early Lactation

Cows with elevated hepatic fat content after calving were older, heavier, and fatter than cows with low hepatic fat content before calving, but differences decreased with onset of lactation because of a greater loss of BW in cows with elevated hepatic fat content. With respect to greater plasma NEFA concentration around calving, fatter cows showed much greater fat mobilization than lean cows, as seen in other studies (Kokkonen et al., 2005; Ohgi et al., 2005). Thereby, the decrement of BW after calving in HFL cows occurred earlier than the loss of subcutaneous fat. Body weight loss after calving is affected by postnatal DMI and gut fill in addition to mobilization of fat depots. Close to calving there may be greater catabolism of visceral fat compared with subcutaneous fat. Although BFT corresponds to total body fat content (Schröder and Staufenbiel, 2006), there are dynamic differences in metabolic activity between visceral fat and subcutaneous fat (Drackley et al., 2001; Drackley and Andersen, 2006; Baldwin et al., 2007).

The decrease of insulin concentration around calving facilitates the mobilization of fat depots, but plasma insulin concentration surprisingly did not differ between HFL and LFL cows. Therefore, plasma insulin concentrations did not reflect the differences in NEB of HFL and LFL cows. Contrary to our findings, plasma insulin concentration corresponded to NEB due to changes in feeding regimen (Reist et al., 2003) or due to prepartum overfeeding and postpartum feed restriction

Figure 3. Energy balance (A) on 14, 28, and 56 DIM in cow with low (white bars) and high (black bars) liver fat content, BW (B), and backfat thickness (C) 60 d before to 56 d after calving in cow with low (□) and high (▲) liver fat content after calving. Data are presented as least squares means $\pm$ SE. Statistically significant ($P < 0.05$) effects of liver fat content, time before and after calving, fat content $\times$ time interactions, and lactation number are given in the figure.

(Kunz et al., 1985). However, in both studies plasma glucose concentration differed markedly with respect to treatment, whereas in our study differences in plasma glucose concentrations were less accentuated. Because differences in fat mobilization of HFL and LFL cows were not mirrored by plasma insulin concentration, an

impaired insulin action in adipose tissues of HFL cows may explain the high fat mobilization in these cows. During early lactation or after induced hepatic lipidosis or hyperlipidemia, reduced insulin action leads to diminished lipogenesis and elevated lipolysis (Bell, 1995; Oikawa and Oetzel, 2006; Pires et al., 2007). Whether the large increase of plasma NEFA concentration in HFL cows around calving was just a consequence of greater body fat storage or an effect of different insulin sensitivity and responsiveness in adipose tissue between fat and lean cows cannot be answered by this study and requires further investigation. As mentioned above, the large increase of plasma NEFA concentration around calving is mainly responsible for the high level of hepatic fat after calving in HFL cows (Drackley et al., 2001; Ohgi et al., 2005; Vernon, 2005). Plasma IGF-I concentrations decrease around calving, but did not differ between groups and did not correspond with differences in DMI and NEB. Because plasma IGF-I concentration is closely related to the insulin status of cows (Butler et al., 2003; Reist et al., 2003), this result confirms the lack of difference in plasma insulin concentration in HFL and LFL cows.

Surprisingly, BFT and BW as well as plasma NEFA and BHBA concentrations did not change after calving in lean cows, although milk production was on a comparably high level in both lean and fat cows. The main reason for high milk production in LFL cows was the much greater DMI compared with HFL cows, already beginning before calving and lasting up to 35 DIM. Although we were not able to measure individual feed intake around calving, we assume that differences in feed intake between HFL and LFL cows were comparable to differences seen 7 d before calving and at 14 DIM and contribute to differences in fat mobilization and liver fat content in HFL and LFL cows. Negative energy balance was much less pronounced in cows with low fat content in liver and diminished 8 wk after calving. It is well established that body condition and fat mobilization affect DMI in high-yielding dairy cows (Garnsworthy and Topps, 1982; Grummer, 1993; McNamara, 2000). Postpartum DMI increases steadily (Ingvarsen and Andersen, 2000; Block et al., 2001; Grummer et al., 2004), but the peak after calving is delayed relative to the peak of milk production, as also seen in our study (Ingvarsen and Andersen, 2000; Block et al., 2001; Hayirli and Grummer, 2004). This delay of feed intake exacerbates NEB and contributes to the consequences of metabolic imbalances (Ingvarsen and Andersen, 2000; Hayirli and Grummer, 2004). In addition, there is good evidence that increasing plasma NEFA and BHBA concentrations may negatively influence DMI (Ingvarsen and Andersen, 2000; Hayirli et al., 2002; Ohgi et al., 2005). As mentioned above, fatty

Figure 4. Plasma concentrations of NEFA (A), BHBA (B), and glucose (C) 20 d before to 56 d after calving in cow with low (□) and high (▲) liver fat content after calving. Data are presented as least squares means $\pm$ SE. Statistically significant ($P < 0.05$) effects of liver fat content, time before and after calving, and fat content $\times$ time interactions are given in the figure.

Figure 5. Plasma concentrations of insulin (A), glucagon (B), and glucagon to insulin ratio (C) 20 d before to 56 d after calving in cow with low (□) and high (▲) liver fat content after calving. Data are presented as least squares means $\pm$ SE. Statistically significant ($P < 0.05$) effects of liver fat content and time before and after calving are given in the figure.

cows mostly lose excess BW and develop a more severe NEB than thin cows (Grummer, 1993; Ingvarsen and Andersen, 2000), although this is not the case if DMI does not decrease (Kunz et al., 1985). Thus, body reserves and degree of fatness seem to be important factors governing feed intake after calving (McNamara, 2000).

Although we have found decreased plasma leptin concentration around calving, supporting previous findings (Block et al., 2001; Reist et al., 2003), plasma leptin concentration was not affected by differences in BFT during the transition to lactation. Furthermore, differences in feed and energy intake in our study cannot be explained by plasma leptin concentration, which is in contrast to other findings (Chilliard et al., 2005). There is still ongoing discussion about metabolic and endocrine signals that may affect DMI regulation during transition and early lactation. Findings in our study may point at metabolites such as NEFA and BHBA, but not leptin, that may affect feed intake in high-yielding dairy cows (Ingvarsen and Andersen, 2000; Allen et al., 2005; Vernon, 2005). Our findings on DMI might, in addition, support the conclusions of Drackley (1999) that the coefficients of variation for ad libitum feed intake at beginning of lactation are in the range of 30 to 40%, whereas DMI after peak lactation typically varies between 6 and 10%. Furthermore, an elevated BCS leads to reduced DMI in dairy cows even before calving (Hayirli et al., 2002), which is in line with the lower DMI in HFL cows. Unfortunately, more data on DMI during the dry period were not available in this study.

Although not significant, slightly lower ECM production in LFL cows supported less severe NEB after calving compared with HFL cows. Elevated fat content in milk of HFL cows was most probably a result of the pronounced plasma NEFA concentration and uptake by the mammary gland increase around calving (Ohgi et al., 2005; Vernon, 2005). Elevated plasma BHBA concentration may have, in addition, stimulated fat synthesis in the mammary gland, but BHBA was probably also secreted with milk (Blum, 2004). The lactose content was reduced in milk of HFL cows at the beginning of lactation, a finding that was also seen in cows that were energetically underfed (Reist et al., 2003). Obviously, components other than lactose had some osmotic effect in milk of HFL cows.

Effects of Fat Content in Liver on Glucose Metabolism in Early Lactation

With the onset of lactation, large amounts of glucose are needed for milk production. Therefore, in addition to a significant increase of endogenous glu-

Figure 6. Plasma concentrations of IGF-I (A) and leptin (B) 20 d before to 56 d after calving in cow with low (□) and high (▲) liver fat content after calving. Data are presented as least squares means $\pm$ SE. Statistically significant ($P < 0.05$) effects of time before and after calving are given in the figure.

cose production, glucose is spared by reducing whole glucose oxidation and more NEFA and BHBA are used as energy-yielding substrates (Bell, 1995; Herdt, 2000; Drackley et al., 2001). Elevated plasma NEFA and BHBA concentrations are usually accompanied by a postpartum decrease of plasma glucose, insulin, and IGF-I concentrations, which reflects the greater glucose demand with the onset of lactation and NEB (Drackley et al., 2001; Reist et al., 2003; Reynolds et al., 2003). Besides lower postpartum plasma glucose concentration, hepatic glycogen content usually decreases during early lactation and is inversely related to hepatic fat content (Drackley and Andersen, 2006).

Lower postpartum plasma glucose concentration as well as reduced hepatic glycogen content in HFL cows was combined with a greater increase of the glucagon to insulin ratio in plasma and greater gene expression of PC and G6Pase in HFL when compared with LFL cows. Because of the narrow time points of liver sampling in our study, mRNA levels of PC and G6Pase did not change with time, but, especially for PC mRNA, the amount seemed to be elevated in our study immediately after calving. Increased mRNA levels or enzyme activities of PC and G6Pase in cows immediately after calving were shown recently (Greenfield et al., 2000; Murondoti et al., 2004; Cedeño et al., 2008), indicating metabolic regulation during transition to lactation in cows. The greater gene expression of PC in HFL cows was possibly the consequence of using relatively more lactate and amino acids as substrates for gluconeogenesis, whereas propionate was likely not sufficiently available because of reduced DMI during calving (Reynolds et al., 2003; Brockman, 2005). Our data support the well-known concept of a shift in gluconeogenic substrates after calving; that is, use of relatively more lactate, glycerol, and amino acids (especially alanine) than propionate for gluconeogenesis (Lomax and Baird, 1983; Drackley et al., 2001; Reynolds et al., 2003). A shift from propionate to alanine when glucose demands increase was also seen in sheep (Overton et al., 1999). The greater glucagon to insulin ratio that particularly affects gene expression of PC in cattle (Donkin, 1999) may also indicate elevated gluconeogenesis from amino acids or lactate, and confirms our finding that the glucagon to insulin ratios were best related to PC mRNA levels.

Levels of PEPCK-C mRNA after calving increased with time, but showed no differences with respect to liver fat content. Similar findings in the literature suggest differences in gluconeogenic enzyme regulation of PC and PEPCK-C in the transition cow (Donkin, 1999; Greenfield et al., 2000). Gene expression of PEPCK may be affected by propionate as well as lactate and amino acid supply and was therefore not different between HFL and LFL cows. Although DMI was much greater in HFL than LFL cows, the rate of gluconeogenesis after calving might not be different between cows, as indicated by similar PEPCK gene expression. The increasing PEPCK-C gene expression with time may represent the incremental glucose demand for milk production (Greenfield et al., 2000). That the greater G6Pase gene expression led to elevated G6Pase activity and a greater glucose output in HFL than LFL cows was unlikely, because plasma glucose concentration was more reduced in HFL than LFL cows during the transition period. Furthermore, mRNA levels of PEPCK-M and GLUT2 in liver were not influenced by time after

Table 3. Glycogen content and mRNA levels of pyruvate carboxylase (PC), mitochondrial (PEPCK-M) and cytosolic phosphoenolpyruvate carboxykinase (PEPCK-C), glucose-6-phosphatase (G6Pase), and glucose transporter (GLUT2) in liver of cow with low (LFL) and high (HFL) fat content in liver

Variable ¹	Day	Group			ANOVA (<i>P</i> -value)		
		LFL	HFL	SE	Liver fat content	Time	Liver fat × time
Glycogen, mg/g of liver wet weight	1	21.2	6.4	0.2	0.001	0.13	0.3
	10	15.2	5.3	0.2			
	21	18.4	9.1	0.2			
mRNA level relative to GAPDH PC	1	2.5	6.0	1.1	0.01	0.8	0.9
	10	1.8	5.8	1.1			
	21	1.9	5.2	1.1			
PEPCK-M	1	4.4	7.0	2.1	0.4	0.6	0.5
	10	2.8	6.5	2.1			
	21	4.8	5.0	2.1			
PEPCK-C	1	8.5	7.2	4.9	0.8	0.01	0.7
	10	12.9	13.4	5.2			
	21	21.2	16.0	5.1			
G6Pase	1	36.0	65.3	10.0	0.09	0.9	0.5
	10	43.2	59.8	10.3			
	21	50.1	56.7	10.6			
GLUT2	1	6.8	6.6	1.8	0.9	0.9	0.9
	10	6.2	6.8	1.9			
	21	6.4	7.1	1.9			

¹Values are least squares means with pooled SE.

calving or by liver fat content and might not, therefore, be regulated by the metabolic status of the cows (Drackley et al., 2001).

Provided that mRNA concentrations of gluconeogenic enzymes reflect their activities (Greenfield et al., 2000), our data do not support recent findings that hepatic gluconeogenic activity is impaired by elevated liver fat content (Rukkwamsuk et al., 1999), although plasma glucose concentration was reduced in HFL cows. However, the reduction of plasma glucose concentration during transition to lactation might have been a consequence of increased availability of fat used as energy fuel, because we have also seen reduced plasma glucose concentration after rumen-protected fat feeding (Hammon et al., 2008). As we have not measured endogenous glucose production in this study, we are not able to conclude whether a greater gene expression of gluconeogenic enzymes in HFL cows implied increased hepatic glucose production. Furthermore, because of greater availability of NEFA and BHBA as energy fuels in peripheral tissues (Bell, 1995; Drackley et al., 2001), HFL cows likely spared glucose, which may have influenced endogenous glucose production as well. On the other hand, hepatic gluconeogenesis requires ATP generated by fatty acids and hepatic fatty acid oxidation increases with onset of lactation (Drackley et al., 2001; Lam et al., 2003; Vernon, 2005). Unfortunately, we could not measure hepatic fatty acid oxidation capacity in these cows. Therefore, interactions between

hepatic glucose and lipid metabolism in the transition cow need further investigations.

In conclusion, elevated fat storage content in liver of HFL cows was a consequence of high body fat and excessive fat mobilization, whereas LFL cows showed smaller changes in metabolic traits during the transition period. In addition, high fat content in the liver did not impair gene expression of enzymes related to gluconeogenesis, as we have hypothesized in this study. Because both lean and fatty cows were able to produce high amounts of milk but differed markedly in the level of DMI, different strategies of nutrient partitioning were used to provide substrates such as glucose for milk production. This is supported by differences in gene expression of gluconeogenic enzymes in HFL and LFL cows, indicating differences in the gluconeogenic pathway.

This study, in addition, pointed out that high fat mobilization, high liver fat content, and severe NEB in high-yielding dairy cows may not always be a consequence of impaired farm management, but might be a consequence of individual cow factors that cannot be easily alleviated by the farmer when using feeding management as described in our study, which is typically implemented in large farms in the United States and Europe. Therefore, individual cow factors should be further investigated to better understand their effects on nutritional partitioning and to better adapt high-yielding dairy cows on common feeding management.

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