Differences in milk production, glucose metabolism, and carcass composition of 2 Charolais × Holstein F₂ families derived from reciprocal paternal and maternal grandsire crosses¹

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ABSTRACT

Two F₂ Charolais × German Holstein families comprising full and half sibs share identical but reciprocal paternal and maternal Charolais grandfathers differ in milk production. We hypothesized that differences in milk production were related to differences in nutritional partitioning revealed by glucose metabolism and carcass composition. In 18 F₂ cows originating from mating Charolais bulls to German Holstein cows and a following intercross of the F₁ individuals (n = 9 each for family Ab and Ba; capital letters indicate the paternal and lowercase letter the maternal grandsire), glucose tolerance tests were performed at 10 d before calving and 30 and 93 d in milk (DIM) during second lactation. Glucose half-time as well as areas under the concentration curve for plasma glucose and insulin were calculated. At 94 DIM cows were infused intravenously with 18.3 μmol of D-[U-¹³C₆]glucose/kg^{0.75} of BW, and blood samples were taken to measure rate of glucose appearance and glucose oxidation as well as plasma concentrations of metabolites and hormones. Cows were slaughtered at 100 DIM and carcass size and composition was evaluated. Liver samples were taken to measure glycogen and fat content, gene expression levels, and enzyme activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, and glucose 6-phosphatase as well as gene expression of glucose transporter 2. Milk yield was higher and milk protein content at 30 DIM was lower in Ba than in Ab cows. Glucose half-life was higher but insulin secretion after glucose challenge was lower in Ba than in Ab cows. Cows of Ab showed higher glucose oxidation, and plasma concentrations at

94 DIM were lower for glucose and insulin, whereas β-hydroxybutyrate was higher in Ba cows. Hepatic gene expression of pyruvate carboxylase, glucose 6-phosphatase, and glucose transporter 2 were higher whereas phosphoenolpyruvate carboxykinase activities were lower in Ba than in Ab cows. Carcass weight as well as fat content of the carcass were higher in Ab than in Ba cows, whereas mammary gland mass was lower in Ab than in Ba cows. Fat classification indicated leaner carcass composition in Ba than in Ab cows. In conclusion, the 2 families showed remarkable differences in milk production that were accompanied by changes in glucose metabolism and body composition, indicating capacity for milk production as main metabolic driving force. Sex chromosomal effects provide an important regulatory mechanism for milk performance and nutrient partitioning that requires further investigation.

Key words: crossbred cow, lactation, glucose metabolism, carcass composition

INTRODUCTION

Differences in milk production indicating divergent nutrient partitioning and its endocrine control are well documented between dairy and beef cows as well as between dairy cows with low or high genetic merit for milk yield (McNamara, 2000; Veerkamp et al., 2003). Number and activity of secretory cells in the mammary gland are markedly greater in dairy than in beef heifers (Keys et al., 1989). Endocrine changes with regard to milk production depend on mammary gland size and function, allowing for prioritized nutrient supply to the mammary gland for maximal milk production (Akers, 2000; Bauman, 2000). Among nutrients and hormones, glucose and insulin are important predictors to explain the variability in milk production (Ingvarsen and Frigens, 2005). Higher plasma insulin concentrations were found in beef when compared with dairy cows (Hart

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Figure 1. Pedigree of the segregating resource family at the Leibniz Institute for Farm Animal Biology (FBN; Dummerstorf, Germany). Charolais bulls A and B were mated to German Holstein cows, followed by intercross of the F₁ individuals to establish full sib and half sib F₂ offspring (Ab, Ba). The capital 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.

et al., 1978; Shingu et al., 2002) and in dairy cows with low genetic merit for milk production versus high genetic merit (Bonczek et al., 1988; Gutierrez et al., 2006).

At the Leibniz Institute for Farm Animal Biology (FBN; Dummerstorf, Germany), an experiment has been initiated using segregating F₂ offspring from the P₀ founder breeds Charolais and German Holstein. Charolais are known for their distinct nutrient accretion and low milk yield. In contrast, Holsteins are known for high milk secretion and low tissue accretion of nutrients (Kühn et al., 2002). Charolais and German Holstein cattle differ in their properties of nutrient transformation and in endocrine regulation of nutrient flow, including differences in insulin-dependent glucose metabolism in Charolais and Holstein cows (Bellmann et al., 2004; Pareek et al., 2007). The segregating family structure of cattle bred in Dummerstorf allows the investigation of nutrient flow regulation in lactating F₂ offspring with a combined dairy and beef genetic background. In this study we have focused on the biology of nutrient partitioning with emphasis on milk production in cattle.

Cows of the 2 families were closely related; namely, they originated from the same 2 grandsire bulls that were mated to Holstein cows to generate an F₁. One male and several females of each F₁ family were bred by crossover design to form the 2 F₂ families. The major difference between these F₂ families is that the same grandsire bulls come either from the paternal or maternal side (Figure 1). These 2 families markedly differed in milk production during their first lactation and these differences corresponded with changes in insulin-dependent glucose metabolism (Hammon et al., 2007). We hypothesize that differences in milk production between these F₂ families with reciprocal grandsire origin, accompanied by differences in systemic and hepatic glucose metabolism and carcass composition, may be caused by sex effects. The present study aimed to investigate the persistence of these effects during second lactation and to expand data on whole-body and hepatic glucose metabolism and carcass composition in cows of the 2 families to confirm the divergent effect of the common but reciprocal specific genetic background in the 2 F₂ families.

MATERIALS AND METHODS

Animals, Husbandry, and Feeding

The experimental procedures were carried out according to the animal care guidelines and were approved by the relevant authorities of the State Mecklenburg-Vorpommern, Germany. The crossbred cows included in this study were generated by mating Charolais bulls (named A and B) to German Holstein cows. An intercross of the F₁ individuals was performed using embryo transfer to German Holstein recipients to establish full sib and half sib F₂ offspring (Figure 1). Cows in this study originated from the 2 paternal halfsibship families [i. e., Ab (n = 9) and Ba (n = 9)]. The capital 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. The breeding model described herein is part of a larger ongoing breeding study that elucidates the genetic and physiological basis of phenotypic variability between animals of different nutrient turnover with respect to accretion and secretion type (Kühn et al., 2002). In this study we used all F₂ cows with the grandsire combination Ab or Ba that were investigated during first lactation (Hammon et al., 2007) and were available during second lactation.

Cows were studied from 10 d before expected calving until 100 DIM of second lactation. During the experimental period cows were fed ad libitum the same TMR ration containing grass (62% of DM) and corn silage

(26% of DM), hay (6% of DM), concentrates (5% of DM; MF 2000, Vollkraft, Güstrow, Germany), and a mineral and vitamin mix (1% of DM; Salvana 9237, Salvana, Sparrieshoop, Germany). When milk yield exceeded 17 kg of milk/d, cows were fed 1 kg of concentrates for every 2 kg of extra milk, respectively. Grass silage contained 151 g of utilizable protein/kg of DM and 6.6 MJ of NE_L/kg of DM, corn silage contained 140 g of utilizable protein/kg of DM and 6.8 MJ of NE_L/kg of DM, and hay contained 113 g of utilizable protein/kg of DM and 4.9 MJ of NE_L/kg of DM. Concentrates mainly contained extracted soy and rapeseed meal, corn, wheat, and sugar-beet pulp (204 g of utilizable protein/kg of DM and 8.0 MJ of NE_L/kg of DM). Analyzed energy and protein content of TMR were 6.6 MJ of NE_L/kg of DM and 147 g/kg of DM utilizable protein, respectively, which corresponds to the recommendations of the German Society of Nutrition Physiology (2001).

Cows were weighed weekly before and during second lactation after morning milking until the end of the study. Milk yield was recorded daily, and milk composition was determined weekly. To avoid possible interference of glucose infusion with milk yield measurement on 30 and 93 DIM because of feed deprivation before glucose infusion, we related average milk yield on d 27 to 29 to results from the glucose tolerance test (GTT) on d 30 and average milk production on d 90 to 92 to GTT on d 93.

Diet and Milk Analyses

Dry matter, crude fat, crude fiber, and crude ashes of freeze-dried dietary components and TMR were determined according to the Weender standard procedure (Naumann and Bassler, 1993). Nitrogen was determined by combustion analysis (Leco CNS-2000, St. Joseph, MI) and CP was then calculated by multiplying the N content by 6.25. The NE_L and utilizable protein in the diet were calculated according to the German Society of Nutrition Physiology (2001).

Milk protein, fat, and lactose concentrations were measured by the infrared spectrophotometric method (MilcoScan, Foss Germany, Rellingen, Germany) according to the MGVO (Milch-Güteverordnung milk quality by-law, Germany) at the local dairy control association (LKV Mecklenburg-Vorpommern, Güstrow, Germany). Energy-corrected milk was calculated according to Hammon et al. (2009).

Infusions, Blood Sampling, and Analyses

An intravenous GTT was performed 2 to 3 h after morning milking at 30 and 93 (±1) DIM and at the same time on d 10 before expected calving. Cows were

equipped with jugular cannulas for blood sampling and, after a period of 12 h without food, glucose (1 g/kg^{0.75} of BW) was infused into the jugular vein (Hammon et al., 2007). Blood samples for determination of plasma glucose and insulin concentrations were collected in tubes containing 1.6 mg of potassium-EDTA/mL of blood (Monovette, Sarstedt, Nümbrecht, Germany) and were put on crushed ice until centrifuged at 1,500 × *g* for 20 min at 4°C. Supernatants were aliquoted and plasma aliquots were stored at -20°C until analyzed.

On 94 DIM after morning milking and after a period of 12 h without food, cows were infused intravenously with 18.3 μmol of D-[U-¹³C₆]glucose (99 atom-%; Chemotrade Ltd., Leipzig, Germany)/kg^{0.75} of BW to measure rate of glucose appearance (Ra_{Gluc}), also indicated as glucose production because of neglecting exogenous glucose uptake in ruminants, and glucose oxidation (GO_x). D-[U-¹³C₆]Glucose was dissolved in 15 mL of 0.9% saline. Blood samples were taken before and 5, 10, 20, 30, 60, 90, 120, 150, and 180 min after D-[U-¹³C₆]glucose injection. Isotopic enrichments of plasma glucose were measured in heparinized plasma using a modified method of Pfaffenberger et al. (1975), as described recently (Hammon et al., 2008). Enrichments are expressed as atom percent excess. The atom percent excess was calculated by difference between the enrichments of each blood sample and the preinfusion baseline abundance.

Determination of Ra_{Gluc} by the isotope dilution of [U-¹³C₆]glucose was as follows: Ra_{Gluc} = D/A(¹³C), where D is the dose of [U-¹³C₆]glucose (mol) and A(¹³C) is the area under the [U-¹³C₆]glucose enrichment-time curve (mol % excess × h). The isotope enrichment of [U-¹³C₆]glucose was calculated from the mass *m* + 6/*m* + 0 isotope ratio at mass/charge ratio (*m/z*) 334/328 (molecular weights of U-¹³C₆]glucose and native glucose, respectively). The value of A(¹³C) was determined as described previously by Junghans et al. (2007). In detail, we applied the sum of 3 exponentials to fit the kinetics of ¹³C enrichments. Nonlinear regression analysis based on the SAAM II software (Barrett et al., 1998) was used to determine the best-fit curve. The GO_x was calculated according to GO_x (mmol/min) = [n(¹³C)_{plateau}/D] × Ra_{Gluc}/c, where n(¹³C)_{plateau} (in mmol) is the plateau value of the cumulated ¹³C amounts in CO₂ released from blood by treatment with lactic acid, D (in mmol) is the ¹³C dose of [U-¹³C₆]glucose administered as single dose, Ra_{Gluc} (in mmol/min) is the glucose appearance rate, and *c* is the recovery factor, which was set at 0.73 (Junghans et al., 2007).

Additional blood samples from the jugular vein were taken before and at 30-min intervals for 3 h after D-[U-¹³C₆]glucose injection and transferred in EDTA tubes to measure plasma concentrations of glucose,

NEFA, BHBA, urea, insulin, and glucagon. The tubes were immediately put on ice and centrifuged (20 min, $1,500 \times g$ at 4°C), and plasma was stored at -20°C until analyzed. Plasma concentrations of glucose and urea were measured using a kit from Labor und Technik Lehmann (Berlin, Germany; #GL 0103 and LT-UR 0010; Hammon et al., 2008). Plasma concentrations of NEFA and BHBA were measured using kits from Randox (Krefeld, Germany; #FA 115 and #RA-RB 1007; Hammon et al., 2008). Analyses were performed at the Landesanstalt für Landwirtschaft (Rostock, Germany) using the automatic analyzer Cobas Mira Plus (Roche, Basle, Switzerland).

Insulin was measured by RIA using a porcine kit (PI-12K, Linco Research, St. Charles, MO; Hammon et al., 2009). Cross-reactivity with bovine insulin was 90%. Intra- and interassay coefficients of variation were 4.3 and 8.2%, respectively. Glucagon was measured by RIA using a kit from Linco (GL-32K, Linco Research; Hammon et al., 2009). Intra- and interassay coefficients of variation were 7.8 and 13.6%, respectively. The assay is specific for the determination of pancreatic glucagon in serum and plasma in most mammals. Cross-reactivity to oxyntomodulin, which is the primary gut glucagon, is less than 0.1%. Plasma IGF-1 concentrations were measured as described by Hammon and Blum (1997).

Carcass Composition, Liver Sampling, and Analyses

Cows were slaughtered at 100 DIM. Carcass was dissected and empty body weight (**EBW**) and hot carcass weight (**HCW**) were measured as described by Pfuhl et al. (2007). Total fat was calculated as the sum of omental, abdominal, perirenal, and subcutaneous fat. Inter- and intramuscular fat were not considered in this calculation. Muscle protein in all cuts (Pfuhl et al., 2007) was determined with near-infrared spectroscopy using an Infratec 1255 Food and Feed Analyzer (Foss Analytical A/S, Hillerød, Denmark). Marbling was scored with photographic standards on a 6-point scale (1 = extremely low marbled, 6 = extremely high marbled; Pfuhl et al., 2007). Furthermore, the fat class was quantified according to the guidelines of the European Union from level 1 to 5 (1 = leanest, 5 = fattest; Pfuhl et al., 2007).

Liver samples were taken for analysis of glycogen and fat content as well as mRNA levels and activities of key enzymes of gluconeogenesis. Samples were frozen in liquid N and stored at -80°C until analyzed. Freeze-dried liver samples were ground in liquid N immediately before analysis. Liver glycogen was determined using a commercial photometric test kit based on amyloglucosidase-catalyzed release of glucose (no.

207748, Boehringer Mannheim, Mannheim, Germany). Determination of liver fat content was done based on carbon and N content, as described by Duske et al. (2009).

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, Karlsruhe, Germany) as described by Hammon et al. (2003, 2009).

Real time reverse transcription PCR was performed by Light Cycler (Roche Molecular Biochemicals, Mannheim, Germany) using SYBR Green I as fluorescence dye (Hammon et al., 2003, 2009). Primers used for PCR quantification 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 were published recently (Hammon et al., 2009). Quantification of mRNA was performed by relative expression using GAPDH as reference gene transcript (Pfaffl, 2001; Hammon et al., 2009). Inter- and intraassay coefficients of variation for reverse transcription PCR of target and reference genes were below 1%.

Hepatic protein content was determined by the method of Bradford (1976) with a Bioquant Protein reagent solution (Merck, Darmstadt, Germany) using BSA as standard. Measurements of total (cytosolic and mitochondrial) PEPCK and PC activities in liver were described recently (Hammon et al., 2003). Liver tissue was homogenized in buffer (wt/vol = 1:3) containing cold 0.1 M sucrose, 50 mM potassium phosphate (pH 7.4), and 0.25 mM EDTA (pH 7.4). Samples were sonicated for 10 s to disrupt mitochondria. The homogenates were kept on ice at all times. Activities of PEPCK were determined in cell homogenates by measuring the carboxylation of phosphoenolpyruvate to oxaloacetate using $\text{NaH}^{14}\text{CO}_3$ (Ballard and Hanson, 1967) with the addition of NADH and malate dehydrogenase to ensure the conversion of the reaction product, oxaloacetate, to malate (Atkin et al., 1979). Activities of PC were assayed in the crude homogenate by $\text{NaH}^{14}\text{CO}_3$ incorporation into oxaloacetate and citrate in the presence of pyruvate (Atkin et al., 1979). Enzyme activity of G6Pase was measured according to Gierow and Jergil (1980). Liver samples were homogenized in 20 mM HEPES buffer containing 100 mM sucrose and 0.25 mM EDTA (wt/vol = 1:10). Phosphate from glucose-6-

phosphate was delivered using ammonium molybdate, and a dilution series of potassium phosphate was used for standard curve (Mahuren et al., 2001).

Statistical Analyses

Data are given as least squares means $\pm$ pooled standard error. For glucose and insulin concentrations during GTT, the area under the curve (AUC_{gluc} and AUC_{Ins} , respectively) was calculated using the GraphPad computer program (GraphPad Software Inc., San Diego, CA). Basal concentrations were subtracted to calculate net area under the curve values. For calculation of glucose half-life in blood during GTT, basal concentrations were subtracted and data were subjected to logarithmic transformation before linear regression from the 7- to 28-min interval (elimination curve) for calculation of kinetic parameters. Half-life (min) was calculated from $\ln 2/k_2$, where k_2 is the slope of the elimination curve.

Statistical analyses were done with SAS (release 9.1.3; SAS Institute, 2004). Data on d -10, 30, and 93 relative to calving for BW, milk production, and GTT as well as differences between first (Hammon et al., 2007) and second lactation with respect to milk performance and metabolic and hormonal measurements during frequent blood sampling on d 94 were assessed by repeated measurements ANOVA using the MIXED procedure of SAS (SAS Institute, 2004). The ANOVA model contained family and time of sampling as fixed effect and a random cow effect. Differences were localized by Tukey *t*-test, with $P < 0.1$ for a trend and $P < 0.05$ for a significant difference.

Data for Ra_{Gluc} and GO_x on d 94 and hepatic and carcass measurements on d 100 were evaluated using the General Linear Model of SAS (SAS Institute, 2004) with family as main effect. Differences were localized by least significant difference *t*-test, with $P < 0.1$ for a trend and $P < 0.05$ for a significant difference. The Wilcoxon rank test was used to analyze differences between families in meat and fat classes as well as marbling score (SAS Institute, 2004). The CORR procedure was used to calculate Pearson correlations between hepatic and carcass measurements and plasma concentrations of metabolites and hormones as well as GTT data and milk performance (SAS Institute, 2004).

RESULTS

BW and Milk Yield

Body weight did not differ during dry period, decreased ($P < 0.05$) from d -10 to d 30 in both groups, and increased ($P < 0.001$) much more in Ab than in

Ba from d 30 to d 93, resulting in greater BW ($P < 0.1$) on d 93 in Ab than in Ba cows (LSM $\pm$ pooled SE of BW: 817 and 825 on d -10, 766 and 731 on d 30, and 841 and 760 $\pm$ 20 on d 93 for Ab and Ba cows, respectively).

Milk yield and ECM decreased ($P < 0.001$) from d 30 to d 93 in both groups; the relative decrease was greater ($P < 0.05$) in Ab than in Ba cows, and both traits were higher ($P < 0.01$) in Ba than in Ab cows on d 30 and 93 (Table 1). Milk protein content increased ($P < 0.05$) during lactation in Ba cows and was lower ($P < 0.01$) on d 30 in Ba than in Ab cows. Overall milk lactose content decreased ($P < 0.05$) during lactation. Milk fat, protein, and lactose yields per day decreased ($P < 0.001$) with increasing DIM; the decrease was relatively greater ($P < 0.01$ for milk fat; $P < 0.05$ for lactose) in Ab than in Ba cows, and milk fat, protein, and lactose yields were higher ($P < 0.01$) in Ba than in Ab cows. Compared with first lactation, ECM increase in second lactation behaved different (family $\times$ time interaction: $P < 0.05$) and was higher ($P < 0.05$) on d 30 in Ba than in Ab cows (10 and 5.2 kg $\pm$ 1.4 kg in Ba and Ab cows, respectively), whereas increase at 93 DIM was the same (mean increase was 3.2 $\pm$ 1.7 kg; $P > 0.2$).

Plasma Glucose and Insulin During GTT

Basal glucose concentrations (i.e., before glucose infusion) increased ($P < 0.05$) from 10 d before parturition to 93 DIM in both families (Table 2). Half-life of plasma glucose during GTT differed between families. Overall AUC_{Ins} was higher ($P < 0.05$) in Ab than in Ba cows. The basal glucose/insulin ratio increased ($P < 0.05$) after calving in Ba cows but did not change significantly in Ab cows. After glucose challenge the glucose/insulin ratio was higher ($P < 0.05$) in Ba than in Ab cows and tended ($P < 0.1$) to increase with time.

Milk yield correlated positively with half-life of plasma glucose ($r = 0.52$, $P < 0.01$) but correlated negatively with basal concentrations of glucose ($r = -0.45$, $P < 0.01$), insulin ($r = -0.57$, $P < 0.01$), and AUC_{Ins} ($r = -0.33$, $P < 0.05$). Glucose half-life correlated negatively with AUC_{Ins} ($r = -0.43$, $P < 0.01$).

Metabolic and Endocrine Measurements During Determination of Glucose Rate of Appearance and Oxidation

Mean plasma concentrations of glucose were lower ($P < 0.05$) but plasma concentrations of BHBA were higher ($P < 0.05$) in Ba than in Ab cows on d 94 during 3-h blood sampling (Table 3). Mean plasma insulin concentrations were lower ($P < 0.05$) and the glucose/insulin and glucagon/insulin ratios were higher ($P <$

Table 1. Milk yield and composition on d 30 and 93 of second lactation in Ab and Ba cows^{1,2}

Trait	DIM ³	Family		SEM	P-value		
		Ab	Ba		Family	DIM	Family × DIM
Milk yield, kg/d	30	9.2	24.1	2.4	0.01	0.001	0.05
	93	4.9	15.3				
ECM, kg/d	30	9.8	25.7	2.6	0.01	0.001	0.05
	93	5.4	16.3				
Milk fat content, %	30	4.9	4.8	0.51	0.4	0.6	0.5
	93	5	4.2				
Milk fat yield, g/d	30	411	1,137	115	0.01	0.001	0.01
	93	234	678				
Milk protein content, %	30	3.6	3.1	0.08	0.01	0.05	0.05
	93	3.6	3.5				
Milk protein yield, g/d	30	320	731	78	0.01	0.001	0.4
	93	182	544				
Milk lactose content, %	30	4.3	4.7	0.27	0.15	0.05	0.7
	93	3.6	4.2				
Milk lactose yield, g/d	30	432	1,140	122	0.01	0.001	0.05
	93	209	718				

¹The capital 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.

²Data are presented as LSM ± SEM.

³To avoid possible interference of glucose infusion with milk production measurements on the day of glucose tolerance test, we used average milk yield on d 27 to 29 as 30 DIM value and average milk production on d 90 to 92 as 93 DIM value.

0.05) in Ba than in Ab cows. No differences were seen for plasma concentrations of NEFA, urea, and IGF-1. The Ra_{Gluc} measured by [U-¹³C₆]glucose enrichment in plasma was not different between families in a significant manner, but GO_x was greater ($P < 0.05$) in Ab than in Ba cows (Table 3).

Milk yield correlated positively with Ra_{Gluc} ($r = 0.78$, $P < 0.01$) and glucagon/insulin ratio ($r = 0.73$, $P < 0.01$) but correlated negatively with plasma concentrations of glucose ($r = -0.43$, $P < 0.1$) and insulin ($r = -0.7$, $P < 0.01$) and GO_x ($r = -0.87$, $P < 0.01$). Additionally, Ra_{Gluc} correlated positively with the glucagon/insulin ratio ($r = 0.59$, $P < 0.01$) but correlated negatively with insulin ($r = -0.67$, $P < 0.01$), and GO_x correlated negatively with Ra_{Gluc} ($r = -0.77$, $P < 0.01$) and the glucagon/insulin ratio ($r = -0.5$, $P < 0.05$) but correlated positively with plasma insulin ($r = 0.57$, $P < 0.05$). There was a positive correlation between plasma concentrations of glucose and insulin ($r = 0.41$, $P < 0.1$).

Glycogen and Fat Content, Gluconeogenic Enzymes, and GLUT2 in Liver

Glycogen and fat content did not differ in a significant manner between families (Table 4). Hepatic mRNA concentrations of PC ($P < 0.01$), G6Pase ($P < 0.01$), and GLUT2 ($P < 0.05$) were higher, whereas mRNA concentrations of PEPCK-C tended to be higher ($P < 0.1$) in Ba than in Ab cows. On the other hand, PEPCK activities in liver were greater ($P < 0.01$) in Ab than in Ba cows.

Milk yield correlated positively with hepatic PC mRNA ($r = 0.54$, $P < 0.05$) and G6Pase mRNA ($r = 0.69$, $P < 0.01$). Plasma glucose concentrations correlated positively with hepatic glycogen content ($r = 0.46$, $P < 0.05$) and hepatic PEPCK activities ($r = 0.49$, $P < 0.05$) but correlated negatively with hepatic G6Pase RNA ($r = -0.66$, $P < 0.01$). Glucose half-life correlated positively with mRNA levels of PC ($r = 0.54$, $P < 0.05$) and G6Pase ($r = 0.74$, $P < 0.01$) but correlated negatively with PEPCK activities ($r = -0.67$, $P < 0.01$). Hepatic glycogen content correlated negatively with hepatic fat content ($r = -0.67$, $P < 0.01$) and with mRNA levels of G6Pase ($r = -0.48$, $P < 0.1$) and GLUT2 ($r = -0.48$, $P < 0.1$). The glucagon/insulin ratio correlated positively with hepatic PC mRNA ($r = 0.69$, $P < 0.01$).

Carcass Composition

Empty body weight and HCW as well as HCW/EBW ratio were higher ($P < 0.01$ and $P < 0.05$) in Ab than in Ba cows (Table 5). Total fat content as well as subcutaneous, omental, abdominal, and perirenal fat and fat class were higher ($P < 0.05$) in Ab than in Ba cows. On the other hand, mammary gland weight was higher ($P < 0.01$) in Ba than in Ab cows. Plasma insulin concentrations correlated positively with EBW ($r = 0.68$, $P < 0.01$) and fat content of the carcass ($r = 0.72$, $P < 0.001$) as well as muscle cross section areas of M. longissimus ($r = 0.46$, $P < 0.05$) but not with muscle protein content of the carcass. In addition, plas-

Table 2. Plasma glucose and insulin concentrations during glucose tolerance test (GTT) on d 10 before parturition and on d 30 and 93 of lactation in Ab and Ba cows^{1,2}

Trait ³	Day relative to parturition	Family		SEM	P-value		
		Ab	Ba		Family	Day	Family × day
Glucose							
Basal concentration, mmol/L	–10	3.43	3.57				
	30	3.95	3.69	0.16	0.3	0.01	0.3
	93	4.3	3.93				
AUC _{Gluc} , mmol/L	–10	139	143				
	30	133	138	5.7	0.9	0.2	0.4
	93	150	141				
Half-life, min	–10	25.7	29.6				
	30	23.1	27.8	2.2	0.01	0.5	0.2
	93	20.1	31.5				
Insulin							
Basal concentration, µg/L	–10	0.68	0.6				
	30	0.29	0.23	0.22	0.3	0.2	0.9
	93	0.87	0.61				
AUC _{Ins} , µg/L	–10	136	80				
	30	129	91	21.2	0.05	0.5	0.7
	93	125	61				
Glucose/insulin							
Basal concentration, mmol/nmol	–10	70.4	58.1				
	30	66.1	111.4	13.2	0.2	0.01	0.05
	93	38.4	63.8				
AUC, mmol/nmol	–10	7.4	12.4	5.5			
	30	7.7	16.6		0.05	0.1	0.3
	93	10.4	31.5				

¹The capital 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.

²Data are presented as LSM ± SEM.

³AUC = area under the curve; half-life = half-life during GTT.

ma insulin correlated negatively with mammary gland mass ($r = -0.59$, $P < 0.01$). Milk production correlated negatively with EBW ($r = -0.82$, $P < 0.001$) and fat content of the carcass ($r = -0.81$, $P < 0.001$) but was not related to the protein content of the carcass.

DISCUSSION

Differences in Milk Production and Carcass Composition Between Families

As expected (Taylor et al., 2006; Wathes et al., 2007), milk yield and ECM increased from first to second lactation in both crossbred families (Hammon et al., 2007) but were still on a low level when compared with cows of dairy breeds. Compared with first lactation, the differences in ECM between the 2 families during second lactation were greater at 30 DIM in Ba cows but remained in the same range at 93 DIM. Milk production decreased in both families from 30 to 93 DIM. Peak lactation in Ab cows was 15.2 ± 2 kg at 8 DIM, which subsequently decreased immediately. In comparison, milk yield in Ba cows was greater than 20 kg for more than 50 DIM, and the decline of milk production was slower in Ba than in Ab cows (data not

shown). The huge difference in milk production was obviously a consequence of much less mammary gland tissue in Ab than in Ba cows, indicated by mammary gland mass at 100 DIM. As already suggested during first lactation, there was a great difference in developing mammary gland tissue between the 2 families that was not a consequence of environmental factors such as feeding, housing, or intrauterine environment because we used embryo transfer to German Holstein recipients to develop F₂ cows. Therefore, genetic factors concerning mammary gland development may be responsible for this discrepancy between Ab and Ba cows, although cows of the 2 families originated from the same grandsire bulls. Beside mammary gland mass, secretion activity of alveolar cells and milk removal may in addition differ between Ab and Ba cows, as seen in beef and dairy heifers (Keys et al., 1989). Variation within breed is unlikely to contribute to the major differences in milk production in the 2 families Ab and Ba because in both families P₀ grandsires A and B are represented from the Charolais side and all P₀ cows were recruited from an average German Holstein dairy herd without any high genetic merit individuals regarding milk production.

Milk in Ba cows contained lower protein at 30 DIM and a trend for lower milk protein at 30 DIM in Ba

Table 3. Plasma concentrations of metabolites and hormones and rate of glucose appearance and glucose oxidation on d 94 of lactation in Ab and Ba cows^{1,2}

Trait ³	Family		SEM	<i>P</i> -value, family
	Ab	Ba		
Plasma concentrations				
Glucose, mmol/L	5.16	4.2	0.25	0.05
NEFA, mmol/L	0.54	0.7	0.08	0.15
BHBA, mmol/L	0.4	0.55	0.04	0.05
Urea, mmol/L	4.53	4.68	0.3	0.8
Insulin, µg/L	0.82	0.36	0.09	0.01
Glucose/insulin, mmol/nmol	43.5	97.6	14.3	0.05
Glucagon, ng/L	106	85	9.5	0.15
Glucagon/insulin ³	0.27	0.54	0.09	0.05
IGF-1, µg/L	117	143	16	0.3
Stable isotope measurements				
Ra _{Gluc} ⁴ µmol/(min × kg ^{0.75})	39.4	45.4	2.95	0.2
GO _x ⁵ % of Ra _{Gluc}	34.9	29.1	2	0.05

¹The capital 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.

²Data are presented as LSM ± SEM.

³The glucagon/insulin ratio was calculated on a molar basis.

⁴Ra_{Gluc} = rate of appearance of glucose.

⁵GO_x = glucose oxidation.

than in Ab cows was already observed during first lactation (Hammon et al., 2007) and when comparing high-yielding and low-yielding dairy cows (Hart et al., 1978). Therefore, changes in milk composition during second lactation within each family were partly mirrored by changes during first lactation (Hammon et al., 2007), indicating that milk performance is reproducible in successive lactation numbers (Taylor et al., 2006).

Carcass data revealed a much higher fat content in Ab than in Ba cows that explained part of the differences in BW in both families at the end of the study.

The greater increase of BW during lactation in Ab cows was obviously attributable to higher body fat accretion. Our data fit to the concept of differences in fat storage or mobilization with respect to differences in genetic merit for milk production (McNamara, 2000). Unfortunately, we were not able to measure DMI of these cows to calculate energy balance. But it is quite obvious that Ab cows stored more energy as fat, whereas Ba cows secreted more energy as milk. Therefore, energy partitioning was clearly different in these 2 families, as known for the founder P₀ breeds Charolais and Ger-

Table 4. Hepatic glycogen and fat content as well as mRNA amounts and enzyme activities of pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PEPCK), and glucose-6 phosphatase (G6Pase) and mRNA concentration of GLUT2 of Ab and Ba cows at 100 DIM^{1,2}

Trait ³	Family		SEM	<i>P</i> -value, family
	Ab	Ba		
Glycogen content, % DM	7.74	4.98	1.3	0.2
Liver fat content, % DM	15.7	17.3	0.9	0.2
mRNA amount relative to GAPDH expression				
PC	0.13	0.32	0.04	0.01
PEPCK-C	1.7	3.52	0.65	0.07
PEPCK-M	0.61	0.59	0.09	0.9
G6Pase	15.1	21.6	1.3	0.01
GLUT2	1.03	1.42	0.1	0.05
Enzyme activities				
PC, nmol/(min × mg of protein)	68.1	73.7	7.4	0.6
PEPCK, nmol/(min × mg of protein)	14.8	11	0.8	0.01
G6Pase, µmol/(min × mg of protein)	1.79	1.8	0.08	0.99

¹The capital 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.

²Data are presented as LSM ± SEM.

³Cytosolic (PEPCK-C) and mitochondrial (PEPCK-M) form of phosphoenolpyruvate carboxykinase.

Table 5. Carcass characteristics in Ab and Ba cows at 100 DIM¹

Trait ²	Family		SEM	P-value, family
	Ab	Ba		
Empty BW, kg	717	635	20.2	0.01
Hot carcass weight, kg	460	399	14.3	0.01
Carcass weight/empty BW, %	64.2	62.8	0.4	0.05
Total fat in carcass, kg	101	65.8	8	0.01
Omental fat, kg	23.3	16.1	2	0.05
Abdominal fat, kg	12.3	8.5	1.3	0.05
Perirenal fat, kg	24.4	15.4	2.1	0.01
Subcutaneous fat, kg	41.9	25.9	3.5	0.01
Muscle protein in carcass, kg	56.9	55.2	2.9	0.7
M. longissimus cross section area, cm ²	99	96.2	4.4	0.7
Meat class, points	3	3.2	0.07	0.15
Fat class, points	3.9	2.8	0.2	0.01
Marbling score, points	3	2.7	0.2	0.2
Liver, kg	10.4	9.8	0.4	0.3
Mammary gland, kg	12.4	18.3	1.3	0.01

¹The capital 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.

²Data are presented as LSM ± SEM or as means ± SEM (meat and fat class, marbling score).

man Holstein (Pareek et al., 2007). On the other hand, muscle protein amount in carcass was not different between families, indicating that protein accretion was less affected by F₂ crossbreeding. In contrast, N balance is greater in Charolais than in German Holstein cows (Pareek et al., 2007). Greater fat accretion in Ab cows was associated with higher insulin levels in this family, and the higher insulin status was probably responsible for increased fat storage in Ab cows. This supports the role of insulin as the main lipogenetic and antilipolytic hormone in ruminants (Vernon, 2005).

Metabolic and Endocrine Changes with Respect to Glucose Metabolism

In Ba cows, glucose half-life during GTT was higher but insulin secretion after glucose challenge was lower when compared with Ab cows. These findings were related to higher milk production in Ba cows. Analogous relationships were observed during first lactation (Hammon et al., 2007) and in dairy cows differing in genetic predisposition regarding milk production (Gutiérrez et al., 2006). Lower insulin plasma concentrations favor glucose flow toward the mammary gland because glucose uptake in the mammary gland does not depend on insulin (Laarveld et al., 1981). Lower insulin plasma concentrations may also explain the increased glucose half-life in Ba cows. Glucose uptake in muscle and fat tissue was faster than in mammary gland tissue. Differences in insulin response after glucose challenge may result from differences in insulin secretion or insulin clearance rate. Insulin secretion is affected by lactational stage (Bossaert et al., 2008) and by the level of

milk production (Hart et al., 1978; Shingu et al., 2002; Hammon et al., 2007). Therefore, we assume that differences in insulin response in our study were probably related to differences in insulin secretion. The reduced insulin secretion after glucose challenge in Ba cows was already seen at 10 d before calving. Reduced insulin secretion in Ba cows was probably related to previous lactation because Ba cows had much more DIM during first lactation than Ab cows (262 ± 30 vs. 128 ± 13 DIM for Ba and Ab cows, respectively). On the other hand, dry period length was not markedly different between groups because of an extended dry period length in both families, which was the consequence of the specific breeding program in this study (dry period length was 291 ± 23 d in Ab cows and 246 ± 15 d in Ba cows). Although differences in insulin status were observed, plasma IGF-1 concentrations did not change in the same way.

Glucose oxidation was less in dairy cows when compared with GO_x in this study (Hammon et al., 2008) and was less in Ba than in Ab cows. Glucose oxidation was inversely related to milk production, indicating that less glucose is used as substrate for energy production and more glucose is available in the mammary gland for lactose synthesis in cows with high capability for milk production. A shift from glucose to fat oxidation and therefore a higher glucose availability for lactose synthesis was also seen in dairy cows after rumen-protected fat feeding, pointing at the glucose sparing effect in cows with increased milk production and increased lactose synthesis (Voigt et al., 2005).

Differences seen for plasma BHBA concentrations were probably not a consequence of different milk pro-

duction level because milk yield was low in both families when compared with dairy cows. Cows, however, were without food for 12 h before blood samples were taken, which may explain relatively high NEFA and BHBA plasma concentrations and, because Ba cows required more energy for milk production, fasting may have led to hepatic ketone body production because of limited hepatic capacity for fatty acid oxidation in Ba cows.

Milk production was highly related to glucose production. These findings point at the importance of glucose supply for milk production because glucose is the primary precursor for mammary lactose synthesis. The amount of available glucose is important to achieve genetically determinate milk production because lactose is the major osmoregulator for mammary uptake of water (Linzell, 1972). A close relationship between whole-body glucose flux and milk volume has been already proposed (Danfaer, 1994). Glucose production was lower in the present study than in German Holstein dairy cows (Hammon et al., 2008), reflecting the lower milk production in F₂ crossbred offspring. Concerning endocrine regulation of glucose production, the glucagon/insulin ratio seems to be most important for stimulation of glucose production, as observed in this study and supported by previous findings (Bines and Hart, 1978; Danfaer, 1994).

Investigations in Liver Tissue

Hepatic fat content was low and did not indicate any metabolic stress caused by high fat mobilization, as seen in high-yielding dairy cows (Duske et al., 2009; Hammon et al., 2009). A negative association between fat and glycogen content in liver as described herein was also seen in dairy cows (Duske et al., 2009; Hammon et al., 2009) and reflects differences in energy substrate mobilization.

Beside PEPCK-M, gene expression of gluconeogenic enzymes and GLUT2 clearly indicated increased expression levels in Ba cows, which in part were associated with the level of milk production. Mitochondrial PEPCK is generally regarded to be constitutively expressed and a reduced effect on PEPCK-M mRNA expression by physiological stage was also observed in dairy cows (Agca et al., 2002). On the other hand, PEPCK enzyme activities were lower in Ba than in Ab cows, indicating that enzyme activities were less related to the respective mRNA levels. Although we could not distinguish PEPCK-M and PEPCK-C activities, PEPCK-M was probably not responsible for increased PEPCK activities in Ab cows because PEPCK-M and PEPCK-C activities are equally distributed in cattle liver (Hamada and Matsumoto, 1984) and PEPCK-M mRNA was not significantly higher in Ab than in Ba

cows. Obviously, there were differences in regulation of gluconeogenic enzymes at the mRNA and protein levels in liver, probably including posttranslational regulation. These results were surprising because gene expression studies in dairy cows showed that PEPCK and PC mRNA expression were closely related to respective enzyme activities (Greenfield et al., 2000). However, because of the different genetic background, hepatic regulation of gluconeogenic enzymes in our F₂ crossbred cows may distinguish from purebred Holstein dairy cows. Measurements of gluconeogenic enzymes in liver of calves also indicated less correlation between mRNA and activity, at least for PEPCK (Hammon et al., 2003).

Cows investigated herein were not exposed to severe metabolic stress as has been shown for dairy cows during early lactation. Hepatic glucose synthesis is regulated by nutritional as well as endocrine factors and may change with altering glucose demands (Danfaer, 1994; Donkin, 1999; Overton et al., 1999), and in dairy cows gluconeogenic enzymes show different time patterns with regard to gene expression during early lactation (Greenfield et al., 2000; Hammon et al., 2009). In our study, transcript levels of PC, or G6Pase, or both, were more related to the glucagon/insulin ratio and level of milk production, whereas PEPCK activities were related to glucose concentration in plasma. The glucagon/insulin ratio particularly affects gene expression of PC in cattle (Donkin, 1999), whereas PEPCK is more regulated by available substrates, mainly propionate, and less by the glucagon/insulin ratio (Danfaer, 1994; Brockman, 2005). Different availability of substrates for gluconeogenesis because of differences in feed intake may also affect changes in hepatic gluconeogenic activities (Brockman, 2005). Because we were not able to measure individual feed intake in this study, we can only speculate that differences in hepatic regulation of gluconeogenesis might also be a result of DMI.

In conclusion, we have found remarkable differences in milk production between the 2 families, and differences in milk production were accompanied by differences in body composition and glucose metabolism. This indicates that the capacity for milk production is the main metabolic driving force, and glucose supply adapts to the level of milk production. Even if we were not able to provide data for DMI, our study indicated that the 2 families markedly differed in nutrient partitioning. Surprising is the fact that these huge differences in milk production and nutrient partitioning were caused by a genetic model that used identical but reciprocal paternal and maternal Charolais grandfathers. Sex chromosomal effects maybe provide an important regulatory mechanism for milk performance and nutrient partitioning. Further investigations should focus on mammary gland

development and sex chromosomal interactions to clarify the importance of sex chromosomal effects on milk performance and related metabolic changes.

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