Intestinal Glucose Absorption but Not Endogenous Glucose Production Differs between Colostrum- and Formula-Fed Neonatal Calves

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Abstract

Glucose supply markedly changes during the transition to extrauterine life. In this study, we investigated diet effects on glucose metabolism in neonatal calves. Calves were fed colostrum (C; n = 7) or milk-based formula (F; n = 7) with similar nutrient content up to d 4 of life. Blood plasma samples were taken daily before feeding and 2 h after feeding on d 4 to measure glucose, lactate, nonesterified fatty acids, protein, urea, insulin, glucagon, and cortisol concentrations. On d 2, additional blood samples were taken to measure glucose first-pass uptake (FPU) and turnover by oral [U-13C]-glucose and i.v. [6,6-2H2]-glucose infusion. On d 3, endogenous glucose production and gluconeogenesis were determined by i.v. [U-13C]-glucose and oral deuterated water administration after overnight feed deprivation. Liver tissue was obtained 2 h after feeding on d 4 and glycogen concentration and activities and mRNA abundance of gluconeogenic enzymes were measured. Plasma glucose and protein concentrations and hepatic glycogen concentration were higher (P < 0.05), whereas plasma urea, glucagon, and cortisol (d 2) concentrations as well as hepatic pyruvate carboxylase mRNA level and activity were lower (P < 0.05) in group C than in group F. Orally administered [U-13C]-glucose in blood was higher (P < 0.05) but FPU tended to be lower (P < 0.1) in group C than in group F. The improved glucose status in group C resulted from enhanced oral glucose absorption. Metabolic and endocrine changes pointed to elevated amino acid degradation in group F, presumably to provide substrates to meet energy requirements and to compensate for impaired oral glucose uptake. J. Nutr. 141: 48–55, 2011.

Introduction

Neonatal calves must adapt to various environmental changes after birth, including nutrition. This includes changes in dietary energy sources and routes, which shifts from a continuous energy supply of glucose via the placenta to a transient fat and carbohydrate supply during suckling (1–3). Because of the discontinuous nutrient supply after birth, endogenous glucose production (eGP) becomes much more important. eGP, especially gluconeogenesis (GNG), is essential in newborns to maintain plasma glucose concentrations, because glycogen storage is limited (3). eGP was recently shown in neonatal calves, but its dependence on type of postnatal feeding is unclear (4). Findings in neonatal piglets indicated stimulating effects of colostrum feeding on gluconeogenic activity in hepatocytes (5).

In calves, first findings pointed at an improved glucose status when colostrum was fed instead of milk replacer or milk-based formulas that possibly resulted from elevated activities of hepatic gluconeogenic enzymes (4,6–9). In addition to high amounts of nutrients and vitamins, colostrum contains bioactive substances such as growth factors and their intake stimulates the development and function of the gastrointestinal tract (GIT) in neonatal calves and pigs (10–12). Development and function of the GIT are important for glucose uptake. With suckling, lactose reaches the intestine and its products, glucose and galactose, are absorbed and delivered by the portal blood to the liver and other tissues (3). The intestinal absorptive capacity for xylose, a pentose sugar that is absorbed by the same transporters as glucose (13), responded to colostrum feeding and was lower in calves fed milk replacer or a milk-based formula with fewer bioactive components (8,14,15). Moreover,
splanchnic tissues may extract a substantial amount of dietary glucose as a fuel for energy supply (16). However, less is known about glucose first-pass uptake (FPU) in newborn calves fed colostrum.

Based on these premises, the goal of this study was to determine the impact of colostrum feeding on oral glucose absorption, FPU, and eGP in neonatal calves. We tested the hypothesis that colostrum compared with formula feeding improves oral glucose absorption and FPU and additionally affects eGP due to stimulation of key enzymes involved in hepatic GNG. The milk-based formula we used in this study had a comparable macronutrient composition to colostrum, especially lactose content, but contained fewer biologically active factors such as insulin-like growth factor-1 or insulin.

Materials and Methods

**Calves and feeding.** The experimental procedures were carried out according to animal care guidelines and were approved by the relevant authorities of the State Mecklenburg-Vorpommern, Germany (LALFF M-V/TS/7221.3–1.1–014/07).

Fourteen calves were randomly assigned to 2 treatment groups [colostrum (C) and formula (F); F: n = 7 each]. Calves in group C received pooled colostrum obtained from milkings 1, 3, and 5 (d 1, 2, and 3 after parturition, respectively) on the first 3 d of life (15). Calves in group F were fed 3 differently composed milk-based formulas on d 1, 2, and 3 that contained amounts of macronutrients comparable to those of the respective colostrum milkings (Supplemental Table 1), as recently described (8,15). Formulas were provided by Bergphor Futtermittelfabrik. To ensure a comparable lactose content in colostrum milkings and related formulas, 77, 39, and 42 g lactose/kg dry matter (DM) was added to colostrum milkings 1, 3, and 5, respectively. On d 4, calves were fed either colostrum of milking 5 or formula of d 3 for groups C and F, respectively. Colostrum and formula amounts fed were targeted to be 8% of body weight (BW) on d 1 and 10% of BW from d 2 on. Calves were bottle-fed twice daily on d 1 and 2 (0800 and 1600 h), once after a tracer test on d 3 (1800 h), and once (0800 h) on d 4. To ensure that all calves received an equal amount of formula or colostrum, calves with reduced appetite were tube fed their residual amount of milk. The calves received their first meal 4.4 ± 0.6 h after birth.

All calves were singletons and spontaneously born to multiparous cows after normal gestation length. Calves were kept in single boxes at the Research Station of the University of Rostock and had free access to water. To protect against infections, all calves were fed chicken egg-derived kg [6 g (d 1), 8 g (d 2), and 10 g (d 3 and 4)], which are barely absorbed by neonatal calves and contain high antibody titer against rotavirus and pathogenic *Escherichia coli* type K 99 (Globigien 88, Erich Westjohn Nutrition). On d 1, 2, and 3, calves were subcutaneously injected with antibiotics (25 mg Enrofloxacin/10 kg BW; Baytril 5%). To avoid iron deficiency, calves received i.m. injections of iron dextran (10 mg/kg BW; Uroserferon 100, Serumwerk Bernburg) before first feed intake. The health, respiratory rate, behavior, nasal discharge, respiratory sounds, fecal consistency, and navel inspection. Navels were disinfected with Betadine (Mundipharma Medical) after birth. BW was determined preprandially on d 1 and 4 immediately before slaughtering.

**Metabolites and hormones.** Basal blood samples from the right jugular vein were taken by Vacutette (Greiner Bio-One International) on d 1 and by catheter (Certofox Mono 340, BRAUN; jugular vein) from d 2 to 4 before morning feeding and 2 h after feed intake on d 4 using S-Monovette (Sarstedt). Tubes containing K3 EDTA (1.8 g/L blood) were used to measure plasma concentrations of glucose, lactate, nonesterified fatty acids (NEFA), protein, urea, insulin, and glucagon. Tubes containing Li-heparin (14–15 kIU/L blood) were used to analyze cortisol. Blood was immediately put on ice and centrifuged at 1500 × g at 4°C for 20 min and the supernatant was stored at −20°C until analyzed.

Plasma metabolites (glucose, urea, NEFA, lactate, protein) were analyzed by the Klinik für Rinder (Stiftung Tierärztliche Hochschule Hannover) using the following kits: glucose (no. 553 – 230) and total protein (Biuret Reaction, no. 553 – 412) from MTL Diagnostics; lactate (no. A11A01721) from Horiba Europe; NEFA (no. 434 – 91795) from Wako Chemicals; and urea (no. LT-UR 0050) from Lehmann. Analyses were performed automatically by spectrophotometer (Pentra 400, Axon Lab). Plasma glucagon (kit from Linco Research) and insulin concentrations were measured by RIA as described (6). The glucagon:insulin ratio was calculated as the glucagon concentration in nmol divided by insulin concentration in nmol. Plasma cortisol concentrations were analyzed in duplicates using a commercially available 125I-RIA kit (DSL) according to the manufacturer’s guidelines. The cross-reactivities of the antibody used in this kit were 33 and 9% to prednisolone and corticosterone, respectively, and to any further competing plasma steroid cross-reactivities was lower than 4%. The assay was validated for use with bovine plasma. The test sensitivity was 7.0 nmol/L and intra- and inter-assay CV were both 6%.

**Glucose FPU.** For determination of glucose FPU, i.e. glucose utilization in the splanchic tissue (intestine and liver) when enterally administered glucose passes the splanchic tissue for the first time, the morning meal on d 2 was divided into 10 small portions (0.5% of BW/portion), which were fed half-hourly over a period of 5 h. Each milk portion contained per kg BW 5.4 μmol/kg [U-13C]-glucose (99 atom% 13C, Chemotrade). Simultaneously, calves received a primed i.v. infusion (Perfuser compact, BRAUN) of [6,6-2H2]-glucose (99 atom% 2H, Chemotrade) [prime, 9.9 μmol/kg; infusion, 15.4 μmol/kg (h) for 5 h] dissolved in isotonic saline and starting 5 min before the first feeding (Supplemental Fig. IA). The tracer was infused through a catheter placed in the left jugular vein. Blood samples were taken from the right jugular catheter in Li-heparinized tubes before tracer application and at 60, 120, 180, 210, 240, 270, and 300 min after the first 13C-labeled meal to measure the 13C and 2H enrichments of plasma glucose and the 13C enrichment of blood CO2 (17,18). Plasma was kept at −80°C until analyzed. Whole blood was taken using Li-heparinized monovettes and stored at −20°C for analysis of 13C blood CO2 enrichment, which was used as a proxy for breath 13CO2. Additional blood samples were collected before and at 60, 120, 180, and 240 min after first feeding to measure plasma concentrations of glucose, insulin, glucagon, and cortisol as described above.

Plasma [6,6-2H2]-glucose enrichments were used to calculate the i.v. glucose rate of appearance (Ra_i) as follows (19):

\[
Ra_i = \frac{I_{IV} \cdot [IE_{IV}/IE_{pl}]}{100} - 1
\]

where I_{IV} is the i.v. [6,6-2H2]-glucose tracer infusion rate [μmol/(kg · h)], IE_{IV} is the isotopic enrichment expressed as mole percent excess (MPE) of the infused tracer, and IE_{pl} is the enrichment (MPE) of [6,6-2H2]-glucose tracer in plasma at tracer steady state (210–300 min after infusion start). The absence of a significant change of plasma enrichment during the above timeframe was confirmed by linear regression (GraphPad Prism Software, version 3.03, GraphPad Software). In addition, enrichments were shown as the area under curve (AUC) from 0 to 300 min after infusion start. The plasma rate of appearance of [U-13C]-glucose from oral tracer administration (Ra_oral) was calculated accordingly using the oral glucose tracer infusion rate [μmol / (kg · h)] and the enrichment (MPE) of [U-13C]-glucose tracer in plasma at isotopic steady state (210–300 min after infusion start).

The splanchic extraction of the oral tracer was calculated as FPU (proportion of administered tracer in percent):

\[
FPU = \frac{(Ra_{oral} - Ra_{i})}{Ra_{oral}} \times 100
\]

as described (20).

The 13C in blood CO2 derived from [U-13C]-glucose oxidation (GOx) was measured as recently described (18).

**eGP.** On d 3, eGP and GNG were determined by a combination of oral intake of deuterated water (2H2O) and i.v. [U-13C]-glucose infusion, as recently (17). After 15 h, feed-deprived calves were tube fed 2 bolus of 2H2O (per bolus: 10 g/kg, 70 atom % 2H, Chemotrade) within 4 h to estimate fractional GNG (Supplemental Fig. 1B). The eGP was deter-
mined using a primed continuous i.v. infusion of [U-13C]-glucose [prime: 4.3 μmol/kg; infusion: 6.4 μmol/(kg · h)] for 4 h dissolved in 0.9% saline and started immediately after the second 2H₂O bolus. Blood samples were collected before and at 180, 240, 300, 360, 390, 420, 450, and 480 min after the first 2H₂O bolus for tracer analysis.

The eGP was calculated as Raₐ for glucose. [U-13C]-glucose infusion as described above. The absolute GNG (GNGabsolute) was calculated as:

\[ \text{GNG}_{\text{absolute}} = \frac{\text{GNG}_{\text{tracer}}}{100} \times \text{eGP}. \]

As indicated by separate tracer studies (Supplemental Fig. 2) (17), there was no increase of m₁, m₂, and m₃ signals derived from primed i.v. [U-13C]-glucose administration alone. The m₁, m₂, and m₃ signals observed are derived from newly synthesized deuterated glucose.

GOx [μmol/(kg · h)] after feeding deprivation was computed according to (21). The CO₂ production was measured by indirect calorimetry (22) in a subset of 5 other newborn calves of the same age and breed and amounted to a mean value of 19.63 mmol/(kg · h) (J. Steinhoff-Wagner, I. Workel, M. Derno, H. M. Hammon, unpublished data), which was comparable to other studies in calves (23).

Plasma concentrations of glucose, NEFA, urea, insulin, glucagon, and cortisol were determined in blood samples taken before and 180, 240, 300, 360, 420, 480, and 540 min after the first 2H₂O bolus as described above.

**Analyses in milk.** Samples were collected during pooling and chemical composition was analyzed by the Qualitätsprüfungs-und Dienstleistungs-gesellschaft Mecklenburg-Vorpommern mbH. DM, crude protein, crude fat, and ash were determined according to the Weender standard procedure (24). Plasma insulin-like growth factor-1 and insulin concentrations were measured by RIA as described (25).

**Analyses in liver.** Calves were slaughtered on d 4 of life at 2 h after feeding and liver samples were snap frozen in liquid nitrogen and stored at −80°C until analysis. Tissue was homogenized under liquid nitrogen. Hepatic glycogen was measured as described (26). The DM concentration was determined by freeze-drying (27). Hepatic protein content was determined by the method of Bradford (28).

Quantitative gene abundance was determined for cytosolic and mitochondrial phosphoenolpyruvate carboxykinase (PEPCK-C and PEPCK-M; EC 4.1.1.32), pyruvate carboxylase (PC; EC 6.4.1.1), and glucose-6-phosphatase (G6Pase; EC 3.1.3.9) by the LightCycler Real-Time PCR System (Roche Applied Science) using SYBR Green I as detection format (7,26). Phosphoglycerate kinase 1 (PGK) was used as a reference gene, because it was not affected by diet. Primer sequences for PGK (forward: CAGTGGAGCCAAGTCACTGTT and reverse: GCCACTGGTGCAAGGAGTA) were designed at the flanking regions of the specific bovine PGK sequence. Metabolite temperatures were checked for specificity of each product. Gel electrophoresis of the PCR product demonstrated only 1 single band of the expected size. Products were verified by sequencing using an ABI Sequencing kit (ABI Big Dye Terminator, Applied Biosystems) and an ABI 310 Genetic Analyzer (Applied Biosystems); Relative quantification of mRNA concentrations was performed as recently described (7,26). Efficiencies of PCR were >1.8 and inter- and intra-assay CV for RT-PCR of gluconeogenic enzymes and PGK genes were <1%.

Measurements of total (cytosolic and mitochondrial) PEPCK and PC activities in liver were performed as described (7). Enzyme activity of G6Pase was measured according to Gierow and Jergil (29). Liver samples were homogenized in 20 mmol/L HEPES buffer containing 100 mmol/L sucrose and 0.25 mmol/L EDTA (wt:vol = 1:10). Glucose-6-phosphate was used as substrate. Produced phosphate concentrations were determined by the reaction with ammoniummolybdate and compared with a phosphate standard curve (30).

**Statistical analyses.** Results are presented as least square means (LSM) ± SEM. Metabolite and hormone data were analyzed by the Mixed Model of SAS with diet, time of blood sampling, and the diet × time interaction as fixed effects and individual calves as random effect. The factor “time” is day in the case of BW, daily preprandial metabolite and hormone plasma concentrations, or hour when comparing the pre- and postprandial metabolite and hormone concentrations on d 4 or during the tracer tests on d 2 and 3. Differences were localized by Tukey t test.

Data concerning cow gestation length and tracer and hepatic measurements were tested with General Linear Model of SAS (SAS, 2004) and diet as main effect. Tracer data of d 2 was analyzed with feeding route (voluntary or by tube) included as covariate in the model.

**Results**

**Gestation length, BW, health status, and feed intake.** Gestation length was 281.1 ± 0.7 d and did not differ between groups. BW on d 1 was 45.9 ± 1.0 kg and BW before slaughtering on d 4 was 46.8 ± 1.2 kg. There was no group effect on BW, but BW increased up to d 4 (P < 0.05). Calves were generally healthy. Tube feeding was partly (C: 3 of 7; F: 4 of 7) necessary to ensure complete tracer ingestion on d 2.

**Preprandial plasma metabolite and hormone concentrations.** Plasma glucose concentrations increased (P < 0.05) from d 1 to 2 but tended to decrease (P < 0.08) from d 2 up to d 4 in both groups and were higher (P < 0.05) in group C than in group F (Table 1). Plasma lactate concentrations decreased in both groups from d 2 to 4. Plasma NEFA concentrations increased (P < 0.01) after birth in both groups (Table 1). Plasma protein concentrations increased (P < 0.01) during the first 24 h in group C and were higher (P < 0.01) from d 2 to 4 in group C than in group F (Table 1). Plasma urea concentrations increased (P < 0.01) during first 3 d in group F and were lower (P < 0.01) from d 2 to 4 in group C than in group F (Table 1). The highest plasma insulin concentrations were determined in both groups at 24 h after birth and insulin concentrations subsequently decreased up to d 4 (P < 0.01) (Table 1). Plasma glucagon concentrations increased (P < 0.01) during the first 24 h much more in group F than in group C and decreased (P < 0.05) in group C from d 2 to 4 and in group F from d 3 to 4, resulting in much higher (P < 0.01) glucagon concentrations in group F than in group C on d 2 and 3 (Table 1). As a consequence, the glucagon:insulin ratio increased (P < 0.01) from d 1 to 3 and decreased (P < 0.05) to d 4 in group F and was higher (P < 0.01) in group F than in group C on d 3. Plasma cortisol concentrations decreased (P < 0.01) from d 1 to 4 in both groups but showed no group differences (Table 1).

**Postprandial changes of plasma metabolites and hormones on d 4.** Plasma glucose concentrations increased (P < 0.01) after feeding in group C and were higher before (P < 0.05) and after (P < 0.01) feeding in group C than in group F (Fig. 1A). Plasma insulin concentrations increased much more (P < 0.05) after feeding in group C than in group F, resulting in higher (P < 0.01) postprandial insulin concentrations in group C than in group F (Fig. 1B). Plasma glucagon concentrations tended to increase (P < 0.09) after feeding only in group F (Fig. 1C), whereas plasma cortisol concentrations decreased (P < 0.1 and P < 0.01) after feeding in both groups (Fig. 1D).

**Metabolic changes and glucose FPU on d 2.** The plasma glucose concentration increased (P < 0.01) with time and was higher (P < 0.05) in group C than in group F (Fig. 2A). Insulin concentrations increased (P < 0.01) with time but did not show diet effects (Fig. 2B). The plasma glucagon concentrations were higher (P < 0.05) in group F than in group C and decreased (P < 0.05) after first feeding in group C. (Fig. 2C). Plasma cortisol...
concentrations decreased \((P < 0.01)\) after feed intake in group C but not in group F, resulting in much higher \((P < 0.01)\) cortisol concentrations in group F than group C (Fig. 2D).

After oral [U-13C]-glucose administration, plasma [U-13C]-glucose enrichment AUC was higher \((P < 0.05)\) and FPU tended to be lower \((P < 0.1)\) in group C than in group F (Table 2). Tube feeding additionally reduced oral [U-13C]-glucose enrichment in plasma and increased \((P < 0.05)\) FPU. The enrichment of 13C in blood CO2 was higher \((P < 0.01)\) in group C than in group F.

Metabolic changes and eGP on d 3. Plasma glucose concentrations decreased \((P < 0.01)\) during feed deprivation in both groups and were higher \((P < 0.01)\) in group C than in group F (Table 3). Plasma NEFA concentrations inconsistently changed with time \((P < 0.01)\) and tended to be higher \((P < 0.07)\) in group C than in group F. Plasma urea concentrations were slightly increased \((P < 0.05)\) during feed deprivation and were much lower \((P < 0.01)\) in group C than in group F. Plasma insulin concentrations decreased \((P < 0.01)\) more in group F than in group C, with the lowest plasma concentrations at the end of feed deprivation in group F. Plasma glucagon concentrations and the glucagon:insulin ratio decreased \((P < 0.01)\) in both groups and were higher \((P < 0.05)\) in group F than in group C. Plasma cortisol concentrations changed with time \((P < 0.01)\) but were not affected by diet.

eGP, GNG\(_{\text{fractional}}\), GNG\(_{\text{absolute}}\), and GOx were not affected by diet [eGP: \(1.4 \pm 0.07\) mmol/(kg · h); GNG\(_{\text{fractional}}\) 58.1 ± 2.4%; GNG\(_{\text{absolute}}\) 807 ± 48 μmol/(kg · h); GOx: 486 ± 19 μmol/(kg · h)].

**TABLE 1** Preprandial plasma concentrations of metabolites and hormones in calves fed either colostrum or formula for 4 d

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1 Values are LSM and pooled SEM, \(n = 7/diet\). *Different from colostrum, \(P < 0.05\). Means in a column with superscripts without a common letter differ, \(P < 0.05\).
intake was similar in Colostrum- and formula-fed calves. Higher plasma glucose concentrations in group C were reflected by a higher postprandial insulin secretion on day 4, in agreement with previous findings on plasma insulin after Colostrum feeding (6–8). In contrast, formula-fed calves partly had higher plasma glucagon concentrations and an elevated glucagon:insulin ratio along with lower plasma glucose concentrations. In addition, plasma cortisol concentrations were markedly higher in formula- than Colostrum-fed calves during interval feeding on day 2. Basically, the same endocrine changes have previously been found (7,8). They may affect protein and glucose metabolism by providing amino acids as substrate for GNG in formula-fed calves and elevated plasma urea concentration after amino acid degradation (31,32).

Absorption and metabolism of glucose in splanchnic tissues. Fractional utilization of ingested glucose in splanchnic tissue as measured by FPU was relatively high in our neonatal calves compared with splanchnic glucose utilization in preterm human neonates (19). In a preliminary study using an identical protocol, we have measured FPU for glucose in two 6-d-old calves, resulting in 32.7 ± 0.1% FPU (J. Steinhoff-Wagner, P. Junghans, S. Görs, C. C. Metges, H. M. Hammon, unpublished data). In piglets, glucose utilization in enterocytes depends on age and was highest in enterocytes of 5-d-old piglets (33). Furthermore, glucose utilization in the portal-drained viscerum of 28-d-old piglets was much lower, because glutamate became the main oxidative substrate in the intestinal mucosa (34). Similar findings have been reported in rats (35). Although glucose utilization in the portal-drained viscerum is different from splanchnic tissue, these studies emphasize age-dependent effects of glucose utilization that also might occur in the splanchnic tissue of neonatal calves.

Studies in ruminal epithelial and duodenal mucosa cells indicated substantial catabolism of glucose to pyruvate and lactate, but carbons from glucose barely reached the tricarboxylic acid cycle, pointing to alternative ways of glucose utilization in bovine gastrointestinal epithelia cells (16). Furthermore, splanchnic glucose utilization in calves might be different from piglets, because glucose could be partly utilized already in the forestomach by ruminal microbiota (36). This possibility exists because microbes colonize the GIT immediately after birth (37). Feeding meals in small portions may have directed milk flow to the rumen instead into the abomasum. Thus, in contrast to suckling, tube feeding can favor ruminal milk drinking. However, glucose FPU was only 10% less in calves voluntarily drinking from the bottle where the esophageal groove reflex should work and avoid ruminal milk drinking (38). Therefore, the reasons for such high glucose FPU in splanchnic tissue of neonatal calves might be different from the reasons for high glucose FPU in splanchnic tissue of preterm human neonates.

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13C enrichment in blood CO₂ was greater in colostrum- than formula-fed calves and assuming that relative amounts of GOx in the enterocytes and liver were the same in groups C and F, we presume that more glucose was available for oxidation in colostrum-fed calves because of higher glucose absorption. The greater glucose absorption in colostrum-fed calves was in line with much the higher hepatic glycogen concentration in group C than in group F calves. The effect of colostrum feeding on hepatic glycogen content was not observed in previous studies, because glycogen measurements in liver were conducted in feed-deprived calves (7). Galactose is preferentially transported to the liver and stored as glycogen (39,40). Because hepatic glycogen content on d 4 was measured in the postprandial state, elevated hepatic glycogen was most probably a result of greater glucose as well as galactose absorption. The postprandial glucose rise on d 4 in plasma of colostrum-fed but not formula-fed calves agrees with this concept. Colostrum contains numerous biologically active factors that stimulate intestinal growth and accelerate mucosal maturation in neonatal calves, leading to a greater intestinal absorptive capacity and activity in the small intestine that may support glucose absorption (4,41–43). Stimulation of mucosal growth by colostrum feeding was also observed in the present study and lactase activities in small intestinal mucosa were greater in group C than in group F calves (J. Steinhoff-Wagner, R. Zitnan, H. M. Hammon, unpublished data).

The lower plasma glucose concentration in group F when fed small portions on d 2 additionally pointed to an inferior glucose status in formula-fed compared with colostrum-fed calves, but glucose turnover was not affected by diet. The higher plasma glucagon and cortisol concentrations in group F than in group C should have favored glucose production in group F (31,32). We recently showed in neonatal calves that glucocorticoid treatment did not affect eGP and GNG, but calves expressed peripheral insulin resistance that resulted in elevated plasma glucose concentrations (7,44). Therefore, higher plasma cortisol concentrations in group F may have acted in the same way and helped to stabilize glucose concentrations in formula-fed calves.

eGP and GNG. Our data indicated high eGP in feed-deprived neonatal calves, which supports previous findings in calves and lambs (40,44). More than one-half of the eGP was produced by GNG, which is higher than previously published (44) but coincides with the concept that GNG in neonates is important (45). Slight differences in relative GNG were probably a result of different methods used to measure GNG; the method used here

### Table 2

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<tr>
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<tr>
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1 Values are LSM and pooled SEM, n = 7/diet. *LSM differ between diets, P < 0.05.

### Table 3

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<tr>
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<td>Diet</td>
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<td>Diet</td>
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<td>Cortisol, nmol/L</td>
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<td>Diet</td>
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1 Values are LSM over 9 h and pooled SEM, n = 7/diet. *LSM differ between diets, P < 0.05.
Enzyme activity
mRNA related to PGK
mg/g DM
Protein, Glycogen, mg/g DM
this reflects differences in eGP during the postprandial state is
be higher in colostrum- than formula-fed calves, but whether
(7). In our study, hepatic G6Pase (on a protein basis) tended to
slightly greater activities in colostrum- than formula-fed calves
measurements in feed-deprived calves of the same age indicated
in the previous study (44) may have resulted from developmental
changes from d 3 to 5. Thus, we have found age-dependent effects of eGP in neonatal calves resulting in higher eGP in 3 d-
than 1-d-old calves using the same method as described herein
(J. Steinhoff-Wagner, C. C. Metges, S. Görs, H. M. Hammon,
unpublished data). Our data clearly show the need for GNG to
maintain glucose homeostasis in neonatal calves, as is generally
known for mammalian neonates (2,3,46).

Neither eGP nor GNG was affected by diet, which was
supported by previous findings (44) but was in contrast to data
from hepatocytes in piglets (5). However, in the pig study, the
authors did not compare different diets but compared different
levels of colostrum and milk feeding (5). Colostrum, with its
high amounts of nonnutritive bioactive components, did not
stimulate eGP and GNG in neonatal calves compared with milk
formulas with a very similar nutrient content. Therefore, we
conclude that nonnutritive factors in colostrum did not affect
eGP and GNG and that the elevated glucose status in colostrum-
fed calves did not result from greater eGP and GNG activities.
As mentioned above, we have found age-dependent effects on
eGP and GNG activities in neonatal calves, resulting in higher
eGP and GNG in 4 d- than 1-d-old calves (J. Steinhoff-Wagner,
C. C. Metges, S. Görs, H. M. Hammon, unpublished data).
Therefore, ontogenetic development and nutrient intake, but not
biologically active components in colostrum, might be most
important for the maturation of neonatal GNG and eGP. In
addition, GOx was not affected by diet and amounted to ~35% of
eGP, similar to GOx values of piglets (34).

Hepatic activities of PEPCK did not differ between diets in
the postprandial state. This confirms our findings that eGP and
GNG were independent from the diet. However, hepatic PEPCK
measurements in feed-deprived calves of the same age indicated
slightly greater activities in colostrum- than formula-fed calves
(7). In our study, hepatic G6Pase (on a protein basis) tended to
be higher in colostrum- than formula-fed calves, but whether
this reflects differences in eGP during the postprandial state is
unclear. In contrast, hepatic PC mRNA abundance and PC
activity on d 4 were higher in formula- than colostrum-fed
calves. Slightly elevated mRNA levels were also observed in
feed-deprived formula-fed calves (7). PC, PEPCK, and G6Pase
are known as rate-limiting enzymes for hepatic GNG, although
substrates can enter the gluconeogenic pathway via oxaloacetate
from tricarboxylic acid cycle independent from PC (45,46).
Lactate is one of the most important substrates for GNG in
neonatal calves (46). Irrespective of diet, plasma lactate
concentrations decreased after birth, as previously reported (6–8).
Elevated PC activity in formula-fed calves may indicate an
increased use of amino acids for GNG (47). These data agree
with the proposed greater amino acid degradation in formula-
than colostrum-fed calves to provide substrates for GNG.
Endocrine changes, especially the elevated glucagon:insulin ratio in formula- than colostrum-fed calves on d 4, would
support such an effect. An increased glucagon:insulin ratio also
results in hepatic PC stimulation (48). However, endocrine
regulation of gluconeogenic enzymes is weak in neonatal calves
(7,44). This is supported in our study by the marked dietary
effects on plasma glucagon that obviously did not stimulate eGP
and GNG, in contrast to adult ruminants (31,32). As stated
earlier, regulation of glucose concentration in neonates is less
controlled by endocrine factors but may be regulated by gluco-
ogenic precursors or by glucose itself (7,44,49).

In conclusion, our data indicate an improved glucose status in
colostrum-fed calves that results primarily from enhanced oral
glucose absorption rather than from colostrum effects on eGP or
GNG. In addition, metabolic and endocrine changes and elevated
amino acid degradation in formula-fed calves point to changes in
energy metabolism due to formula instead of colostrum intake.

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Zbinden (University of Bern, Switzerland) for analytical assist-
cance, as well as Prof. A. Zeyner (University of Rostock,
Germany) for providing the experimental facility. We also thank
J. Aschenbrenner, Bergophor Futtermittelfabrik GmbH (Kulm-
bach, Germany) and Erich Westjohann Nutrition GmbH (Cux-
haven, Germany) for donating the formulas and Globigen 88. H.
M.H. and C.C.M. designed research; J.S-W. performed sampling;
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analysis; S.G., E.K., and R.M.B. performed plasma analysis; S.
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tion; J.S-W. and H.M.H. wrote the manuscript; and H.M.H. had
primary responsibility for the final content. All authors read and
approved the final manuscript.

Literature Cited
during the suckling and weaning period in the newborn. Reprod Nutr
2. Girard J. Gluconeogenesis in late fetal and early neonatal life. Biol
3. Girard J. Metabolic adaptations to changes of nutrition at birth. Biol
5. Lepine AJ, Boyd RD, Whitehead DM. Effect of colostrum intake on
hepatic gluconeogenesis and fatty acid oxidation in the neonatal pig. J
6. Hammon HM, Blum JW. Metabolic and endocrine traits of neonatal calves are influenced by feeding colostrum for different durations or only milk replacer. J Nutr. 1998;128:624–32.


