Expression and activity of key hepatic gluconeogenesis enzymes in response to increasing intravenous infusions of glucose in dairy cows

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ABSTRACT: The present study aimed at investigating whether increasing concentrations of glucose supply have a depressive effect on the mRNA abundance and activity of key gluconeogenic enzymes in dairy cows. Twelve Holstein-Friesian dairy cows in mid-lactation were intravenously infused with saline (SI; n = 6) or a 40% glucose solution (GI; n = 6). For GI cows, the infusion dose increased by 1.25%/d relative to the initial NEr requirement until a maximum dose equating to surplus 30% NEr was reached on d 24. Cows receiving SI received an equivalent volume of 0.9% saline solution. Blood samples were taken every 2 d, and liver biopsies were collected every 8 d. A treatment × quadratic dose interaction (P < 0.01) was observed for the concentration of plasma glucose and serum insulin. The interactions were due to positive quadratic responses of the concentrations of glucose and insulin for GI cows, whereas the concentrations of glucose and insulin did not change over time for SI cows. The concentration of β-hydroxybutyric acid (BHBA) and serum urea nitrogen (BUN) responded in a treatment × quadratic dose manner, such that greater decreases (P < 0.01) in BHBA and BUN concentrations were observed for cows receiving GI than SI as the dosage increased. Serum NEFA concentration tended to follow a similar pattern as serum BHBA and BUN; however, the interaction was not significant (P = 0.07). The mRNA abundance of gluconeogenesis enzymes followed a linear treatment × dose interaction (P < 0.05) for only pyruvate carboxylase (PC), which was paralleled by a trend for a linear treatment × dose interaction (P = 0.13) for PC enzyme activity. The least PC expression and activity were observed at the largest glucose dosage. The activity, but not mRNA abundance, of fructose 1,6-bisphosphatase (FBPase) showed treatment × quadratic dose interactions (P < 0.05) with decreasing activity at increasing glucose dose. Activities and expression of phosphoenolpyruvate carboxykinase and glucose 6-phosphatase were not affected (P > 0.25) by treatment. In conclusion, hepatic gluconeogenic enzymes are only moderately affected by slowly increasing glucose supply, including a translational or posttranslational downregulation of FBPase activity and a decrease in the mRNA abundance of PC with possible consequences for PC enzyme activity.

Key words: dairy cow, enzyme activity, gene expression, glucose infusion, hepatic gluconeogenesis

INTRODUCTION

The majority of ingested carbohydrates are converted to short-chain fatty acids in the forestomach of ruminants (Young, 1977; Seal and Reynolds, 1993). Under many dietary settings, this implies limited availability of glucose for absorption (Baird et al., 1980; Reynolds et al., 1988), thereby generating a need for hepatic gluconeogenesis (Young, 1977). Efficient gluconeogenesis is especially important in dairy cows because it is the major pathway for maintaining adequate glucose
supply for the mammary gland (Reynolds et al., 1988; Huntington et al., 2006). When cows approach peak lactation, insufficient gluconeogenesis might contribute to the occurrence of metabolic disorders, such as ketosis and fatty liver (Mills et al., 1986; Rukkwamsuk et al., 1999; Murondoti et al., 2004). Consequently, knowledge on the regulation of hepatic gluconeogenesis has implications for the performance and health status of dairy cows.

Although surplus supply of glucose has often been shown to downregulate hepatic glucose production (Bartley and Black, 1966; Thompson et al., 1975; Lomax et al., 1977; Rigout et al., 2002), it is a crucial question whether this also involves a downregulation of the involved enzymes. A downregulation of gluconeogenic enzymes could have consequences extending beyond the period of surplus glucose or glucogenic substrate supply. For example, a downregulation of gluconeogenic enzymes in cows overfed prepurum could exacerbate metabolic disturbances during the postparum phase of the transition period (Rukkwamsuk et al., 1999; Murondoti et al., 2004). Therefore, the objective of this study was to investigate the dose effect of surplus glucose on the mRNA abundance and activity of key enzymes of hepatic gluconeogenesis. The hypothesis was that mRNA abundance and activity of hepatic gluconeogenic enzymes would decrease with increasing dosage of glucose and that, at least, the decreases of enzyme activity would persist beyond the actual period of surplus glucose supply.

**MATERIALS AND METHODS**

Experimental procedures were preapproved by the local authorities, Regierungspräsidium Leipzig (reference 24-9168.11, TVV 49/06).

**Animals and Experimental Design**

The use of animals, the general experimental design, and the production traits during the experiment have been described in detail in a previous report (Al-Trad et al., 2009). In brief, experiments were carried out on 12 Holstein-Friesian cows from the dairy herd of the University of Leipzig. Cows were confirmed to be in the 2nd to 4th month of gestation (193 ± 14 DIM) at the start of the experiment and had an average BW and energy-corrected milk yield of 632 ± 33 kg and 30.5 ± 2.3 kg/d, respectively. Cows were housed in individual tie stalls with straw bedding. Twice daily (0600 and 1500 h), cows received a diet based on grass haylage and supplements containing a commercial energy concentrate for lactating dairy cows (Multilac, Leikra GmbH, Leipzig, Germany) and soybean meal. The ingredient and chemical composition of the diet has previously been reported (Al-Trad et al., 2009). The diet was low in starch (11.3% DM) and sugar content (4.4% DM) to minimize the direct entry of glucose from the digestive tract. Water was available for ad libitum intake. Cows were milked at 0630 and 1600 h.

Cows were assigned to a glucose infusion (GI; n = 6) or saline infusion (SI; n = 6) treatment balanced for actual lactation performance and DIM. Infusions were made via a 14-ga, 20-cm jugular catheter (Cavafix Certo Splittocan 338, Melsungen, Germany) that was replaced every 8 d. Glucose infusion treatment consisted of continuous jugular infusions of 40% glucose solution (Serumwerk Bernburg, Bernburg, Germany) over a period of 28 d. The infusion dose was calculated for each animal individually as a percentage of their daily NE\textsubscript{L} requirement at the start of the study (see Calculations and Statistical Analysis). Dosage was 0% of the NE\textsubscript{L} requirement on d 0 and increased linearly by 1.25% each day until a maximum dosage of surplus 30% of the NE\textsubscript{L} requirement was reached at d 24. This was equivalent to 2.65 ± 0.19 kg of glucose per cow per day. After maintaining the infusion dose at 30% of the NE\textsubscript{L} requirement between d 24 and 28, responses to glucose withdrawal were assessed by withholding infusions between d 29 and 32. Infusion canisters were loaded at 1500 h each day, starting in the afternoon of d 0. This ensured that cows had been on the assigned dosage for at least 19 h before the collection of biopsies (see below). The infusion protocol and the individual calculation of infusion volumes were identical for the SI treatment as the GI treatment except that the cows on the SI treatment received a volume-equivalent dose of 0.9% saline (Serumwerk Bernburg, Bernburg, Germany) as a control treatment.

**Sampling and Measurements**

Liver biopsies were obtained between 1000 h and 1200 h on d 0, 8, 16, 24, and 32 with a 2.5-mm wide, 250-mm long biopsy needle (model Berlin, Walter Veterinar-Instrumente, Rietzneuendorf, Germany) under ultrasonography control (Pie Medical Scanner 100 LC; Pie Medical, Maastricht, the Netherlands) as described by Gröhn and Lindberg (1982). After collection, liver samples were washed immediately in ice-cold 0.9% saline solution. Samples for enzyme activity determinations (~200 mg) were snap-frozen in liquid nitrogen and stored at −80°C. Samples (~300 mg) for real-time reverse transcription-PCR (rt-PCR) were transferred into tubes with 3 mL of RNAlater (Qiagen, German-town, MD), placed in a refrigerator for 24 h, and stored at −20°C thereafter.

Blood samples (20 mL for serum plus 20 mL for plasma) were taken from a coccygeal vein every 2 d at 1000 h and processed as described previously. Monovette tubes with heparin (16 IU of lithium heparin/mL of blood; Sarstedt, Nümbrecht, Germany) were used to obtain plasma for glucose analysis. Kaolin-coated Monovette tubes (Sarstedt) were used for serum preparation to determine insulin, serum urea nitrogen (BUN), NEFA, and β-hydroxybutyric acid (BHBA) concentra-
ions. All plasma and serum samples were stored at −20°C.

**Blood Analyses**

An automatic analyzer (Hitachi 912, Boehringer Mannheim, Mannheim, Germany) was used to analyze the concentrations of plasma glucose (glucose/HK kit, Roche Diagnostics GmbH, Mannheim, Germany; Peterson and Young, 1968), serum BUN (urea/BUN kit, Roche; Talke and Schubert, 1965), serum NEFA (NEFA kit, Randox Laboratories Ltd., Crumlin, UK; Matsubara et al., 1983) and serum BHBA (Ranbut/kit, Randox; McMurray et al., 1984). A radiometric immunoassay was used for analysis of serum insulin (INS-IRMA Kit; BioSource Europe SA, Nivelles, Belgium), which has a high specificity for insulin and no significant cross-reactivity to proinsulin (Temple et al., 1990). The kit originally comes with a human calibration standard. However, we routinely prepare calibration standards by diluting bovine insulin in the human insulin-free serum provided with the test to account for the partial differences in the AA sequence between human and bovine insulin.

**Gluconeogenic Enzymes Activity**

The activities of the following key hepatic gluconeogenic enzymes were measured: pyruvate carboxylase (PC, EC 6.4.1.1), phosphoenolpyruvate carboxykinase (PEPCK, EC 4.1.1.32), fructose 1,6-bisphosphatase (FBPase, EC 3.1.3.11), and glucose 6-phosphatase (G6-Pase, EC 3.1.3.9). For PC and PEPCK assays, liver tissues were homogenized with an Ultra-Turrax T25 homogenizer (IKA, Staufen, Germany). Thereafter, mitochondria were disrupted by sonication (Bandelin Sonoplus HD 2070; Bandelin Electronic, Germany) according to the protocol of Agca et al. (2002). Pyruvate carboxylase activity was assayed spectrophotometrically at room temperature using the previously published method of Crabtree et al. (1972). This procedure measures the reduction of 5,5′-dithiobis-2-nitrobenzoic acid by CoA. The latter is released when citrate synthase couples acetyl-CoA to oxaloacetate emerging from the PC reaction. Activity of PEPCK was measured at 37°C based on the 14CO2 incorporation assay of Ballard and Hanson (1967) with the modifications described by Agca et al. (2002). To avoid the release of 14CO2 into the environment, the 14CO2 not incorporated into oxaloacetate was trapped in barium hydroxide (0.2 M) at the end of the procedure.

For FBPase and G6-Pase assays, tissues were homogenized (1:10, wt/vol) in ice-cold homogenization buffer containing 20 mM HEPES, 100 mM sucrose, and 0.25 mM EDTA (pH 7.4). The homogenate was centrifuged for 5 min at 1,000 × g at 4°C to remove cell debris. The supernatant was recentrifuged at 10,000 × g for 10 min at 4°C. The resulting supernatant was used to determine FBPase and G6-Pase activities by the methods of Marcus et al. (1973) and Swanson (1950), respectively. The latter methods spectrophotometrically measure the release of inorganic phosphate from 1,6-bisphosphate or glucose 6-phosphate.

**RNA Isolation and Quantitative rt-PCR**

Ribonucleic acid was extracted from liver tissues stored in RNALater using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. The resulting RNA pellets were dissolved in RNase-free water, and the quantity and quality of the isolated RNA were determined by absorbance at 260 and 280 nm. Total RNA (1 μg) was reverse-transcribed using oligo-(dT)15 primer in a 20-μL reaction according to the manufacturer’s instructions (AMV-RT kit, Roche Diagnostics, Mannheim, Germany). Reverse transcription reactions were carried out at 25°C for 10 min followed by 42°C for 60 min and 95°C for 5 min. The resulting first-strand cDNA was stored at −20°C until use for rt-PCR.

Quantitative rt-PCR was carried out on a Rotor-Gene 6000 (Corbett Research, Australia), using β2-microglobulin (B2M) as a nonregulated reference gene. Primers and dual-labeled fluorescent probes (Table 1) for quantitative rt-PCR were designed using the Web-based quantitative rt-PCR probe design software provided by MWG Biotech AG (Ebersberg, Germany, http://www.eurofinsdna.com/de/home.html) and synthesized by the same company. To ensure the specificity of the primers and probes, a gel was run, in which a single band of expected size was obtained. Primer and probe concentrations were optimized to the concentration that provided the smallest fluorescence threshold cycle (C_T) values and the greatest increase of fluorescence compared with the background. For each sample, the target gene and the control gene were run under duplex reaction conditions in duplicate. The following reagents were used for amplification in 20 μL of final volume: 1 μL of sample cDNA, 2 μL of Mg-free 10X buffer, 0.75 U of Dynazyme II DNA polymerase (Finnzymes, Espoo, Finland), 5.5 mM MgCl2, 0.3 mM dNTP, 900 nM of each primer, and 150 nM of each probe for the genes of interests, and 200 nM of each primer and 50 nM of the probe for the B2M. Amplification conditions for quantification were 95°C for 2 min and 45 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s. After the amplification efficiency of each target and reference gene was validated, the relative gene expression was determined by the 2−ΔΔCT method as described by Livak and Schmittgen (2001). Gene expression was expressed as the normalized ratio of gene expression relative to B2M mRNA using 1 sample from the SI group as an interplate calibrator. The suitability of B2M as a reference gene was checked by demonstrating that B2M C_T values at the beginning of the experiment (i.e., d 0) were not different from those obtained with later samples (d 8, 16, 24, 32) in both groups (P > 0.05; data not shown).
Calculations and Statistical Analysis

Net energy required for maintenance and lactation (MJ/d) was calculated as 4.184 × \{(BW^{0.75} \times 0.08) + milk yield (kg) \times [(0.0929 \times fat \%) + (0.0563 \times protein \%) + (0.0395 \times lactose \%)\} according to the NRC (2001). Based on the energy requirement of the individual cow, infusion dosage (kg of glucose/d) was calculated as follows: designated dose × NE\text{r}/(15.6 MJ/kg).

All data were analyzed using the PROC MIXED procedure (SAS Inst., Inc., Cary, NC) accounting for repeated measures. The model to test for global effects of treatment, dosage, and the dosage × treatment interaction included the fixed effects of treatment (GI vs. SI), dosage (representing dosage of 0, 10, 20, and 30% NE\text{r} requirement), and their interaction. The NE\text{r} calculated before the start of the experiment was used as a covariate. In addition, because cows were gradually exposed to increasing dosage, dosage was included in the model as a repeated measure. The covariate error structure that yielded the smallest Akaike’s and Bayesian information criterion values for each dependent variable was used. Because the first part of the statistical model could not account for the steady increase in dosage over time, we assessed the slope responses of the infusion dosage and their interaction with treatment (i.e., linear vs. quadratic effects) using the same model except that dose was considered to be a continuous variable. The covariate error structure that yielded the smallest Akaike’s and Bayesian information criterion values for each dependent variable was used. Because the first part of the statistical model could not account for the steady increase in dosage over time, we assessed the slope responses of the infusion dosage and their interaction with treatment (i.e., linear vs. quadratic effects) using the same model except that dose was considered to be a continuous variable. The covariate error structure that yielded the smallest Akaike’s and Bayesian information criterion values for each dependent variable was used. 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RESULTS

Production Data

Production data from this study have been reported previously (Al-Trad et al., 2009). Briefly, DMI was not different between the GI and SI groups over the period from d 0 to 24 (period mean ± pooled SEM for GI vs. SI cows: 17.5 vs. 17.7 ± 0.9 kg/d). Correspondingly, surplus glucose linearly improved the energy balance of GI cows between d 0 and 24 (from −14.6 to 35.4 MJ/d), whereas it was unchanged in the SI group during the same period (from −10.4 to −1.8 MJ/d; pooled SEM: 6.3 MJ/d). The improved energy balance was coupled to a net BW gain of 32 kg in GI cows (from 651 to 670 kg) relative to the SI cows (from 614 to 601 kg; pooled SEM: 28 kg), whereas the energy-corrected milk yield was not affected by the treatment (period mean between d 0 and 24 ± pooled SEM: 27.8 and 29.7 ± 1.9 kg/d for GI and SI cows, respectively; Al-Trad et al., 2009).

Plasma and Serum Metabolites and Hormones

Plasma glucose and serum insulin concentrations showed predominantly quadratic dosage effects and treatment × quadratic dose interactions (P < 0.05; Figure 1A and 1B, respectively). Additionally, there was a treatment effect on serum insulin concentration (P = 0.005) and a trend for a treatment effect on plasma glucose concentrations (P = 0.06). These effects were due to increases in plasma glucose and serum insulin concentrations in the GI group occurring mainly when infusion dosages exceeded 20% NE\text{r} requirement, whereas cows receiving the SI treatment had no response to increasing dosage (Figure 1).

Treatment did not affect the concentrations of BUN, BHBA, and NEFA; however, BUN, BHBA, and NEFA decreased in a quadratic manner in response to increasing dosage (P = 0.001; Figure 2A–2C). Additionally, treatment × quadratic dosage interactions were detected for BUN and BHBA (P = 0.001) and tended to be present for NEFA (P = 0.07), indicating that the concentrations of these metabolites decreased specifically with increasing dosage of glucose. In contrast to glucose and insulin concentrations, however, the major part of the decrease in BUN, BHBA, and NEFA concentrations occurred at smaller infusion dosages (<20% NE\text{r} requirement).

Postinfusion values (d 32) were less (P = 0.01) in the GI group for NEFA compared with pretreatment values (d 0). However, glucose, insulin, BHBA, and BUN values were not different between d 0 and 32 in both groups, indicating a quick reversal of the GI effect on these other blood metabolites.

Hepatic mRNA Abundance and Activity of Gluconeogenic Enzymes

Hepatic gluconeogenic enzyme activity and relative mRNA abundance are listed in Table 2. Treatment did not affect the mRNA abundance or enzyme activity measured in this study. However, the relative mRNA abundance of PC showed a linear treatment × dosage interaction (P = 0.048) and a tendency for a quadratic dosage effect (P = 0.06), indicating decreased abundance of PC mRNA with increasing glucose dosage. The decreased abundance of PC mRNA coincided with a numerical decrease in PC activity only at the greatest dosage of glucose (linear treatment × dosage interaction; P = 0.13). The relative mRNA abundance of the mitochondrial isoform of PEPCK (PEPCK-M) decreased already at smaller infusion dosage (10% NE\text{r} requirement) in a quadratic manner (P = 0.01),
<table>
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<tr>
<th>Gene</th>
<th>GenBank accession</th>
<th>Nucleotide range</th>
<th>Primers and probes sequences</th>
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1B2M = β2-microglobulin; PEPCK-C = cytosolic phosphoenolpyruvate carboxykinase; PEPCK-M = mitochondrial phosphoenolpyruvate carboxykinase; PC = pyruvate carboxylase; FBPase = fructose 1,6-bisphosphatase; G6-Pase = glucose 6-phosphatase, catalytic subunit.

2From the database of the National Center for Biotechnology Information (NCBI); all sequences were derived from cattle.

3FAM = 6-carboxy-fluorescein; TAMRA = 6-carboxy-tetramethylrhodamine; JOE = 2,7-dimethoxy-4,5-dichloro-6-carboxyfluorescein; ROX = 6-carboxy-X-rhodamine; BHQ1,2 = black hole quencher-1,2.
## Table 2. Relative mRNA abundance and activity of hepatic gluconeogenic enzymes for cows on saline infusion (SI) or glucose infusion (GI)\(^1\)

<table>
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<tr>
<th>Item</th>
<th>Treatment</th>
<th>Infusion dosage, % of NE(_{\text{d}})</th>
<th>SEM</th>
<th>Treatment</th>
<th>Dosage</th>
<th>Treatment × dosage interaction</th>
<th>Postinfusion</th>
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<tr>
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1\(^{\text{Data are from 6 observations in 6 animals.}}\)

2\(^{\text{Postinfusion samples were collected 4 d after the end of the infusion period at an infusion dosage of 0%.}}\)

3\(^{\text{The values at the infusion dosage of 0% NE\(_{\text{d}}\) listed in column 3 (i.e., preinfusion values) were compared with the postinfusion values by paired Student’s } t \text{-test.}}\)

4\(^{\text{Data are expressed as the normalized ratio of gene abundance relative to } \beta_2 \text{-microglobulin mRNA abundance using 1 sample from SI group as an interplate calibrator (CT = cycle number at the threshold level of log-based fluorescence).}}\)

5\(^{\text{PC = pyruvate carboxylase; PEPCK-C = cytosolic phosphoenolpyruvate carboxykinase; PEPCK-M = mitochondrial phosphoenolpyruvate carboxykinase; FBPase = fructose 1,6-bisphosphatase; G6-Pase = glucose 6-phosphatase, catalytic subunit.}}\)
and the expression of the cytosolic isoform of PEPCK tended to decrease linearly \((P = 0.07)\) with increasing dosage. Corresponding to the decreases in the mRNA abundance for PEPCK, the enzyme assay showed a linear decrease in PEPCK activity with increasing glucose dosage \((P = 0.03)\). However, the decreases in PEPCK mRNA abundance and enzyme activity occurred in the SI and GI groups with no significant treatment \(\times\) dosage interactions, indicating time-dependent or infusion volume-dependent changes with no relationship to glucose treatment. A linear decrease \((P = 0.01)\) in both groups with no treatment \(\times\) dosage interaction was also observed for FBPase mRNA abundance. However, FBPase activity remained stable in the SI group despite the decreasing mRNA abundance but was decreased in the GI group at the largest dosage of 30% NE\textsubscript{l} requirement \((treatment \times quadratic dosage, P = 0.02)\). Treatment did not affect the mRNA abundance of G6-Pase, although a quadratic decrease with increasing dose \((P = 0.02)\) was observed for both treatments. Similarly to PEPCK-M, the steepest decrease in the mRNA abundance of G6-Pase occurred already at smaller infusion dosages of 10% NE\textsubscript{l} requirement. However, the activity of G6-Pase was not affected by treatment, dosage, or their interactions. As such, the changes of mRNA abundance and enzyme activity were uncoordinated for FBPase and G6-Pase, indicating that mRNA abundance had no measurable influence on the activity of these enzymes.

The significantly or numerically decreased activities of FBPase and PC and the decreased transcription of PC in the GI group were restored to preinfusion values by d 32 [i.e., at 4 d after suspending the infusion (Table 2)]. Similarly, PEPCK mRNA abundance and activity was restored to preinfusion values except for a slight remaining reduction in PEPCK activity in the SI group \((P = 0.02)\). However, the activity of G6-Pase was decreased in the GI but not the SI group after stopping infusion \((P = 0.02)\), although G6-Pase mRNA abundance increased to preinfusion values at the same time.

**DISCUSSION**

Hepatic gluconeogenesis is a key component of total glucose entry in cattle (Young, 1977; Huntington et al., 2006). Approximately 60 to 85% of the glucose entry is utilized by the mammary gland in lactating dairy cows (Annison et al., 1974; Bickerstaffe et al., 1974). Consequently, the kinetics and regulation of gluconeogenesis are an area of great interest with regard to milk production. The fact that several previous studies failed to detect a positive influence of surplus glucose supply on milk production (Amaral et al., 1990; Hurtaud et al., 1998; Al-Trad et al., 2009) supports the concept that gluconeogenesis is mostly functioning at a very appropriate amount with regard to lactation requirements (Al-Trad et al., 2009).

As with any biochemical pathway, the regulation of gluconeogenesis may occur at one or more of the following levels: 1) regulation of substrate supply, 2) regulation of activity of catalytic enzymes, and 3) regulation of end product utilization. Of these, glucogenic substrate supply is considered the control point best amenable to feeding management (Veenhuizen et al., 1988; Overton et al., 1999). Increasing the entry rate of propionate and glucogenic AA from the portal-drained viscera has become a successful strategy to support cattle in periods of glucose shortage, especially in the critical period postpartum. Such strategies include measures to increase DMI, to modify fermentation patterns, or to directly add glucogenic precursors to the diet (Overton and Waldron, 2004). On the other hand, upregulation of glucose oxidation or anabolism are primary adaptations of ruminants themselves in times of excessive sup-
ply of glucogenic precursors (Judson and Leng, 1973b; Veenhuizen et al., 1988) or glucose (Amaral et al., 1990; Rigout et al., 2002). However, data are limiting on how the regulation of catalytic activity is utilized to meet imbalances between glucogenic precursor/glucose availability and glucose demand.

The present study was designed to evaluate the effect of increasing glucose supply on 4 key hepatic gluconeogenesis enzymes in mid-lactating dairy cows. The intention was to explore the whole range of nutritionally relevant surplus glucose. When assuming postruminally starch digestion as the major way to provide glucose directly to dairy cows and proceeding from maximum rates of postruminally starch digestion in the order of ~5 kg/d (McCarthy et al., 1989; Taylor and Allen, 2005), a functional maximum uptake capacity (fMUC) of the small intestine can be calculated in the range of ~2.8 kg of glucose/d according to the formula of Cant et al. (1999). In the cows of the present study, this fMUC was modeled by the infusion dosage of 30% NE\textsubscript{i} requirement (i.e., 2.65 kg of glucose/d). It became clear that this infusion dosage may not only be representative for the intestinal limit of glucose absorption. It is also a dose at which cows show dysregulation of glucose homeostasis as evidenced by increased concentrations of blood glucose and insulin. Obviously, the limit of intestinal absorption of glucose and the ability to use the absorbed glucose appear very coordinated in dairy cows.

The present study additionally modeled the whole range of gluconeogenic requirement to meet the glucose demand. Diets were formulated to be low in starch and sugar to provide basal conditions with minimized direct entry of glucose from the digestive tract. Consequently, hepatic gluconeogenesis had to meet the complete demand for glucose entry at the beginning of the experiment. By contrast, the infusion dosage of 2.65 kg of glucose/d at the end of the GI period completely accounted for the glucose demand, which would be 2.27 kg/d at 27.8 kg of energy-corrected milk yield/d according to the formula by Danfær (1994). The latter means that gluconeogenesis was, in theory, completely dispensable at an infusion dosage of 30% NE\textsubscript{i} requirement.

The main finding was that the effect of GI on gluconeogenic enzyme activity was very moderate. Enzymatic activity decreased (or tended to decrease) for only FB-Pase and PC, and only at extremely large surpluses of glucose supply (i.e., infusion of 30% NE\textsubscript{i} requirement). Moreover, both decreases were rapidly reversible within 4 d after the end of infusion. We further elucidated that the glucose-induced decreases in enzyme activity were not linearly related to decreased mRNA abundance because mRNA abundance and enzyme activity changed in a coordinated manner only for PEPCK with a tendency for PC, indicating that the activities of FB-Pase and G6-Pase are predominantly regulated by translational and posttranslational events.

Three of the investigated enzymes, PEPCK, FB-Pase, and G6-Pase, belong to the common gluconeogenic

![Figure 2. Serum urea nitrogen (BUN; panel A), 3-hydroxybutyric acid (BHBA; panel B), and NEFA (panel C) concentrations for blood samples taken every 2 d for cows treated with glucose (■) or saline (●). Open symbols depict pre- and postinfusion values that were not included in statistical trend modeling. Data are expressed as least squares means; pooled SEM, 1.6 mg/dL (A), 0.15 mmol/L (B), 29 μmol/L (C). Probability levels for statistical contrasts were as follows: treatment: 0.22 (A), 0.98 (B), 0.56 (C); dosage linear: 0.001 (A), 0.06 (B), 0.001 (C); dosage quadratic: 0.001 (A), 0.001 (B), 0.001 (C); treatment × dosage linear: 0.12 (A), 0.76 (B), 0.19 (C); treatment × dosage quadratic: 0.001 (A), 0.001 (B), 0.07 (C). Postinfusion values (d 32) were different from preinfusion values (d 0) for only the glucose infusion group in panel C (\(P = 0.01\)).](assets/28618_f2.jpg)
pathway, whereas PC serves to shuttle lactate and glucogenic AA into this pathway (Pilkis and Granner, 1992; Jitrapakdee and Wallace, 1999). Unlike in nonruminant species, lactate and glucogenic AA are not the dominating glucogenic precursors in ruminants where propionate is quantitatively most important (Amaral et al., 1990; Huntington et al., 2006). Propionate enters the common gluconeogenic pathway at PEPCK after initial conversion by propionyl-CoA carboxylase to oxaloacetate (Halarnkar and Blomquist, 1989). Accordingly, PEPCK has been identified as a main rate-limiting enzyme involved in glucose production from propionate in dairy cows (Greenfield et al., 2000). Although the rate of propionate utilization for hepatic gluconeogenesis was not measured in the current study, the nonsignificant treatment × dosage interaction for the expression and activity of PEPCK provides indirect support that infused glucose did not specifically compromise gluconeogenesis from propionate at PEPCK. Previous glucose metabolism studies in ruminants confirm this suggestion by showing that hepatic propionate extraction efficiency and capacity for glucose synthesis from propionate are very resistant to changes in glucose supply (Judson and Leng, 1973a; Baird et al., 1980) and insulin concentrations (Brockman, 1990; Eisemann and Huntington, 1994; Huntington et al., 2006). Therefore, our enzyme measurements in context with previous investigations on glucose metabolism support the view that the liver of ruminants has an increased metabolic priority to utilize propionate for gluconeogenesis (Brockman, 1990; Huntington et al., 2006). This conclusion is not surprising because over 90% of portal propionate is cleared by the liver and most of this amount is used for hepatic glucose synthesis (Armentano, 1992). Efficient propionate clearance and metabolism might not only be important for the glucose balance in lactating dairy cows but may also prevent adverse consequences of propionate accumulation in the blood (e.g., decreased feed intake; Allen et al., 2005).

In contrast to PEPCK, mRNA abundance of PC tended to be specifically suppressed by GI. This confirmed earlier findings that PC mRNA abundance is most consistently changed with varying concentrations of glucose or energy supply in ruminants (Bradford and Allen, 2005; Velez and Donkin, 2005; Loor et al., 2006). Coincidence of a decrease in the mRNA abundance of PC (by 51% at 30% NE\textsubscript{T} requirement) with a numerical decrease in PC activity at the greatest dosage of glucose (by 49% at 30% NE\textsubscript{T} requirement) might suggest some contribution of decreased PC transcription to the decreased enzyme activity. It may be speculated that the observed decrease in PC enzyme activity, although not statistically significant, might have contributed to a decreased deamination of AA because PC is required for hepatic glucose synthesis from glucogenic AA (Greenfield et al., 2000; Velez and Donkin, 2005). A decreased deamination of AA, in turn, was suggested by the decreased serum BUN concentration in the GI group and has the unique metabolic advantage of sparing AA for protein synthesis pathways (e.g., muscle or milk protein synthesis).

An enzyme activity significantly affected by GI was FBPase but only at the greatest dosage of infused glucose (i.e., 30% of the daily NE\textsubscript{T} requirement, equating to 2.65 kg of surplus glucose/d). The linear treatment × dosage interaction was only evident at the enzyme activity level and not at the mRNA level, indicating posttranscriptional regulation of enzyme activity. Fructose 1,6-bisphosphatase is the enzyme that releases fructose 6-phosphate from the gluconeogenic pathway (Pilkis and Granner, 1992), which, after conversion to glucose 6-phosphate, can release glucose by the action of G6-Pase (see below). Fructose 1,6-bisphosphatase thus controls the overall output of gluconeogenesis regardless of the precursors utilized. Consequently, the decrease in FBPase activity might be seen as an effective measure to counteract hyperglycemia occurring at very large infusion dosages. The decrease in FBPase activity could be directly related to the hyperglycemia because excess glucose, together with insulin, stimulates the intracellular accumulation of fructose 2,6-bisphosphate, which is a potent competitive inhibitor of FBPase (Pilkis et al., 1988; Pilkis and Granner, 1992). Additionally, a rapid proteasomal degradation of FBPase has been demonstrated in yeast upon exposure to glucose (Gancedo, 1971; Brown and Chiang, 2009). The rapid translocation of FBPase to the nucleus observed in cultured rat hepatocytes after exposure to glucose or insulin (Yáñez et al., 2004) could indicate that similar proteasomal degradation of FBPase is possible in the mammalian liver during hyperglycemia.

Apart from investigating the dosage effects of glucose, the second intention of the present study was to test the response in gluconeogenic capacity after withdrawing an extremely large glucose load. It became evident that PC mRNA abundance and FBPase activity were fully restored within only 4 d after withdrawing GI. This supports the view that gluconeogenic enzymes in cattle liver adapt rapidly to changes in glucose supply. On the other hand, it indicates that the dysregulation or maladaptation of gluconeogenic enzymes described in postparturient dairy cows are not a primary insufficiency of carbohydrate metabolism but are likely secondary to disturbances of lipid metabolism (Rukkwamsuk et al., 1999; Murondoti et al., 2004). The latter conclusion does not change when considering that withdrawing large-dose GI led to posttranscriptional downregulation of G6-Pase in the present study because the physiological activity of G6-Pase is regulated by the intracellular concentration of its substrate (glucose 6-phosphate; van Schaftingen and Gerin, 2002). The decrease in the activity of G6-Pase after stopping glucose infusion could thus be related to an increased availability of glucose 6-phosphate if glucose 6-phosphate does not only emerge from gluconeogenesis but also from glycogenolysis (Nordlie et al., 1999; van Schaftingen and Gerin, 2002). Because the liver accumulates excess amounts of glycogen during increasing GI (Al-Trad et al., 2009),...
the increased utilization of this glycerone after stopping the infusion could lead to increased availability of glucose 6-phosphate and, subsequently, to a reduction in G6-Pase activity.

In conclusion, our study demonstrated that increases of glucose supply have, in general, no negative effect on the activity of key gluconeogenesis enzymes in mid-lactating dairy cows. Only very large dosages selectively suppress PC mRNA abundance and FBPase activity. Both effects were fully reversed within only 4 d after the end of large-dose GI. The latter indicates that glucose metabolism is rather robust with regard to changes in glucose supply in either direction, at least in the absence of other metabolic disturbances.

LITERATURE CITED


