Increasing intravenous infusions of glucose improve body condition but not lactation performance in midlactation dairy cows

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ABSTRACT

The present study was intended to test whether intravenously applied glucose would elicit dose effects on lactation performance similar to those observed after gastrointestinal glucose application. Six midlactation cows received intravenous glucose infusions (GI), increasing by 1.25% of the calculated net energy for lactation (NE_L) requirement per day, whereas control cows received volume-equivalent saline infusions (SI). Measurements and samples were taken at surplus glucose dose levels of 0, 10, 20, and 30% of the NE_L requirement, respectively. Body weight and backfat thickness increased linearly with increasing glucose dose for cows on GI compared with SI. No differences were observed in daily feed intake, milk energy output, and energy-corrected milk yield between treatments. However, milk protein percentage and yield increased linearly with the dose of glucose infused in the GI group. Although milk lactose was not affected by treatment during the infusion period, milk lactose percentage and yield decreased for GI, but not SI, once infusions ceased. Based on 5 diurnal blood samples, daily mean and maximum concentrations of plasma glucose and serum insulin showed linear increases with increasing GI, whereas their daily minimum concentrations were unaffected. At GI of 30% of the NE_L requirement, marked hyperglycemia and hyperinsulinemia were observed at 1600 h (i.e., 1 h postprandially), coinciding with glucosuria. The revised quantitative insulin-sensitivity check index indicated linear development of insulin resistance for the GI treatment but no such change in SI cows. Glucose infusion decreased daily mean and maximum serum β -hydroxybutyrate and daily minimum nonest-

erified fatty acid concentrations relative to SI, whereas serum urea nitrogen was only numerically decreased by GI. No changes were observed in the serum activities of γ -glutamyl transferase and aspartate transaminase and in the serum concentrations of bilirubin and macrominerals. However, serum phosphorus concentration increased after withdrawal of GI, but not SI. Only in GI cows did glycogen content increase or tend to increase linearly in the liver and skeletal muscle. In conclusion, midlactation dairy cows on an energy-balanced diet directed intravenously infused glucose predominantly to body fat reserves rather than increasing lactation performance. This may suggest that the metabolic fate of glucose is modified by metabolic signals, hormonal signals, or both from the portal-drained viscera when absorbed from the intestine.

Key words: blood metabolite, body condition, glucose infusion, lactation performance

INTRODUCTION

Microbial fermentation in the rumen converts the majority of ingested carbohydrates to short-chain fatty acids (e.g., acetate, propionate, and butyrate; Young, 1977; Reynolds et al., 1994). Consequently, little glucose is available for absorption under many dietary settings, thereby requiring ruminants to compensate with high rates of hepatic gluconeogenesis (Young, 1977). Lactating dairy cows have especially high glucose demands, given the lactose output by the mammary gland (Bickerstaff et al., 1974; Rigout et al., 2002b). A limited availability of glucose in high-yielding cows may not only affect lactation but may also augment metabolic disorders during episodes of negative energy balance (EB; e.g., ketosis; Baird, 1982) and impair fertility (Butler, 2000). Therefore, glucose homeostasis is considered a crucial determinant of metabolic status that affects the performance of dairy cows.

To minimize the adverse consequences of glucose shortage, nutritional strategies to improve glucose homeostasis are an important area of dairy research. Most feeding strategies aim to increase glucose availability

Received March 31, 2009.

Accepted July 30, 2009.

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via postruminally digestible starch. When high-starch diets are fed, a significant amount of starch escapes ruminal fermentation and may be digested in the lower gastrointestinal tract, resulting in the release of glucose monomers for intestinal absorption (Janes et al., 1985; Reynolds, 2006). Intestinal absorption of glucose is energetically more efficient than hepatic gluconeogenesis from propionate (Owens et al., 1986; Reynolds, 2006).

To design a successful nutritional plan, the dose-dependent consequences of additional glucose supply are of considerable interest. Numerous studies have investigated the effects of different levels of intravenous (Fisher and Elliot, 1966; Amaral et al., 1990; Kim et al., 2000) or postruminal glucose infusions (Frobish and Davis, 1977; Hurtaud et al., 1998; Rigout et al., 2002a,b) on lactation performance, metabolic and hormonal profiles, and body glucose metabolism. Lactation performance is clearly one of the primary targets of research, but beneficial effects of glucose supply on milk yield have been the subject of much debate. Some studies have demonstrated that milk yield increases with glucose infusion (Frobish and Davis, 1977; Rigout et al., 2002b), whereas others have shown no change (Amaral et al., 1990; Hurtaud et al., 1998). More consistently, effects on milk composition have been identified, such as decreased milk fat percentage and yield (Fisher and Elliot, 1966; Hurtaud et al., 2000; Rigout et al., 2002a), and increased milk protein yield (Hurtaud et al., 2000; Rulquin et al., 2004). Alternatively, it has been proposed that dairy cows might benefit from increasing the glucose supply by enhancing energy retention in peripheral tissues (e.g., muscle and adipose tissues), without any effect on milk production (Amaral et al., 1990; Reynolds et al., 1994; Reynolds, 2006).

A previous study by Rigout et al. (2002b) using duodenal glucose infusion suggested that the different responses of dairy cows to surplus glucose supply might be linked to dose effects. Moderate amounts of duodenal glucose (493 to 963 g/d) were identified to increase the whole-body appearance rate of glucose and mammary blood flow, leading to increased lactose synthesis and milk output. In contrast, excessive provision of glucose (2,398 g/d) can blunt the increase in milk yield by decreasing the mammary conversion of glucose to glucose-1-phosphate (Rigout et al., 2002b) and by decreasing DMI (Dhiman et al., 1993; Knowlton et al., 1998). Conflicting with the theory above, however, moderate intravenous infusions (100 to 737 g/d) of glucose failed to increase milk yield (Amaral et al., 1990; Kim et al., 2000), indicating that the glucose infusion route may have additional consequences for the response. In this regard, it could be relevant that administration of glucose via the intravenous route lacks first-pass utilization within the portal-drained viscera (Reynolds et al.,

1994) and does not induce the release of gastrointestinal hormones that can affect glucose metabolism and feed intake (e.g., glucagon-like peptide-1; Holst, 2007). Consequently, the aim of the present study was to test the dose effects of glucose on performance specifically at the level of intermediary metabolism by using the intravenous administration route.

MATERIALS AND METHODS

Management of Cows and Experimental Design

All procedures were conducted under protocols pre-approved by the local authorities, Regierungspräsidium Leipzig (reference 24-9168.11, TVV 49/06). The experiment was carried out using 12 Holstein-Friesian cows from the dairy herd of the University of Leipzig. To avoid the influence of reproductive cycling, pregnant (confirmed to be in the second to fourth month of gestation) cows were selected. At the beginning of the experiment, BW and milk yield (means $\pm$ SEM) were 632 ± 33 kg, and 25 ± 3 kg/d, respectively. Health disturbances were excluded in all selected cows by clinical examinations and complete blood counts (CBC). Cows were housed in individual tie stalls with straw bedding. To ensure that the experimental procedures did not affect animal health, clinical examinations (body temperature, rumen motility) were performed daily. Cows were fed a diet based on grass haylage and a concentrate supplement containing Multilac (Leikra GmbH, Leipzig, Germany) and soybean meal (Table 1). The portions of Multilac, soybean meal, and silage were calculated for each individual cow at the beginning of the experiment to meet the NE_L requirement of NRC (2001). However, care was taken to keep starch and sugar low in the diet to minimize the direct entry of glucose from the digestive tract. The concentrate and silage portions were supplied to each cow separately in 2 aliquots daily. The portion sizes calculated initially were held constant over the whole experimental period. Orts (haylage and concentrates separately) were collected before each feeding and weighed. Cows were fed at 0600 and 1500 h and were milked at 0630 and 1600 h. Water was available for ad libitum intake.

Cows were assigned to 1 of 2 treatments balanced for actual lactation performance, with additional care taken for DIM to be approximately equal (means $\pm$ SEM) for GI cows (181 ± 24 d) and SI cows (204 ± 18 d). All cows were aseptically fitted with a 14-gauge, 20-cm jugular catheter (Cavafix Certo Splittocan 338, B. Braun, Melsungen, Germany), which was replaced every 8 d. Cows in the glucose-infusion group (GI; $n = 6$) received a continuous jugular infusion of 40% glucose (40% Glukoselösung, Serumwerk Bernburg, Bernburg,

Table 1. Individual feed components and chemical composition of the diet offered to cows in the glucose-infusion (GI) and saline-infusion (SI) groups¹

Item	GI	SI	SEM
Ingredient, kg DM/d			
Grass haylage	10.91	10.72	0.27
Multilac ²	4.87	5.24	0.39
Soybean meal	2.33	2.56	0.37
NE _L , MJ/d (Mcal/d)	121.8 (29.1)	125.6 (30.0)	6.8 (1.6)
Chemical analysis, % of DM			
CP	20.01	20.41	0.68
Ether extract	3.42	3.23	0.02
Crude ash	9.59	9.49	0.12
ADF	23.47	22.92	0.68
NDF	38.28	37.54	0.97
Sugar	4.30	4.45	0.19
Starch	10.94	11.59	0.63
RNB ³	0.72	0.75	0.06
MP ⁴	13.99	14.08	0.28
Potassium	2.22	2.19	0.02
Calcium	0.76	0.76	0.003
Phosphorus	0.49	0.50	0.01
Sodium	0.10	0.10	0.004
Magnesium	0.24	0.24	0.005
NE _L , MJ/kg of DM (Mcal/kg of DM)	6.69 (1.60)	6.75 (1.61)	0.07 (0.02)

¹There were no significant differences in diet ingredients or chemical constituents between the 2 groups ($n = 6$).

²Energy concentrate containing 22% barley grain, 15% rapeseed expeller, 15% extracted rapeseed meal, 13% palm kernel expeller, 12% wheat bran, 10% wheat middlings bran, 5% beet pulp, 6% molasses, 1.4% calcium carbonate; supplemented with 8,000 IU of vitamin A, 1,500 IU of vitamin D₃, 5 mg of vitamin E, 10 mg of copper, and 0.5 mg of selenium (Leikra GmbH, Leipzig, Germany).

³Ruminal nitrogen balance; (CP – utilizable CP)/6.25.

⁴According to NRC (2001).

Germany) over a period of 28 d. The infusion dose was calculated for each animal individually as a percentage of their daily NE_L requirement at the beginning of the study (see Calculations and Statistical Analysis section). To allow for optimal day-by-day adaptation, an increasing infusion protocol was applied. It began at a dose level of 1.25% of the NE_L requirement on d 1 and gradually increased by 1.25% each day until a maximum dose of 30% of the NE_L requirement was reached on d 24. To assess the metabolic responses to glucose withdrawal, the infusion dose was maintained at 30% of the NE_L requirement until d 28 (i.e., for 4 d) but stopped thereafter (between d 29 and 32). Each day, beginning in the afternoon of d 0, infusion canisters were loaded at 1500 h. This ensured that cows had been on the assigned dose level for at least 19 h before biopsies, which were collected between 1000 and 1200 h. The infusion protocol and the individual calculation of infusion volumes were identical in the second group of cows, except that they received volume-equivalent 0.9% saline infusions (**SI**; $n = 6$; 0.9% Natriumchloridlösung, Serumwerk Bernburg). Because GI and SI cows were maintained on the same feed, the treatment (feed plus infusion) for GI cows was hypercaloric but isonitrogenous compared with that of the SI group. The

cumulative glucose dose infused per cow was (mean $\pm$ SEM) 30.8 ± 1.6 kg/24 d.

Sampling and Measurements

Liver and skeletal muscle biopsies were obtained between 1000 and 1200 h on d 0, 8, 16, 24, and 32, when surplus glucose reached 0, 10, 20, and 30% of the NE_L requirement. The procedure for liver biopsy collection followed the method of Gröhn and Lindberg (1982), using a 2.5-mm-wide, 250-mm-long biopsy needle (Model Berlin, Walter Veterinär-Instrumente, Rietzneuendorf, Germany). The liver was located by ultrasonography (Pie Medical Scanner 100 LC, Pie Medical, Maastricht, the Netherlands) between the 11th and 12th rib on a line just below the tuber coxae on the right side. Approximately 500 mg of liver tissue was collected after administering local anesthesia (procaine, 5 mL of Iso-cain 2%, Selectavet, Weyarn-Holzolling, Germany) to the thoracic wall. Skeletal muscle biopsies were taken alternating from the left to right gluteus medius muscles (approximately 3 g/biopsy) under aseptic conditions after subcutaneous and intramuscular administration of local anesthesia (procaine, 10 mL of Isocain, 2%). An incision approximating 5 cm was made in the center

area between the tuber ischiadicum and tuber coxae, with care taken not to interfere with previous sampling sites or with the measurement site for backfat thickness (BFT), as described below. After collection, liver and skeletal muscle samples were washed immediately in ice-cold saline, snap-frozen in liquid N, and stored at -80°C until analysis for glycogen content. Muscle biopsy sites were closed using a simple continuous suture for muscle and subcutaneous tissue (Surgicryl PGA, USP5, SMI AG, Hünningen, Belgium) and a Ford interlocking suture for skin closure (Dermafil Green Polyester, USP5, SMI AG), whereas the small incisions for liver biopsies did not require surgical closure.

Blood from a coccygeal vein was sampled into two 10-mL evacuated blood tubes containing either anticoagulant (16 IU of lithium heparin/mL of blood) or a clotting activator (both from Sarstedt, Nümbrecht, Germany). Blood samples were taken at 1000, 1600, 2200, 0400, and 1000 h before each biopsy. Tubes with heparin were used for CBC and to obtain plasma for glucose analysis. Tubes without anticoagulant were used for serum preparation to determine all other blood metabolites and hormones (see below). All plasma and serum samples were stored at -20°C until analyses.

Feed samples were collected on d 8 and d 24, and were pooled for feed analysis. Daily feed intake was calculated as the amount of feed offered minus the recordedorts. Milk composition was determined from proportionally composited samples from the afternoon and morning milkings before each biopsy. Midstream urine samples were collected on the mornings of biopsies days. Body weight and BFT were recorded immediately after each biopsy, the latter by ultrasonography (Pie Medical Scanner 100 LC, Pie Medical) according to the method described by Schröder and Staufenbiel (2006).

Laboratory Analyses

Feed ingredients were analyzed by the Agricultural Communication and Service Company Ltd. (LKS-GmbH, Niederwiesa, Germany) according to the standard operating procedures of the Association of German Agricultural Analysis and Research Centres (VdLUGA, Speyer, Germany). Milk fat, protein, lactose, and urea contents were analyzed by infrared spectrophotometry in an accredited laboratory for milk recordings (LKV Sachsen e.V., Lichtenwalde, Germany) using a Milko-Scan FT 6000 instrument (Foss Electric, Hillerød, Denmark). Blood glucose, insulin, NEFA, BHBA, BUN, and cholesterol concentrations were measured in all blood samples, whereas CBC, liver enzymes, bilirubin, and serum minerals were assessed only in blood samples taken at 1000 h on biopsy days. The concentrations of glucose, BUN, cholesterol, and phosphorus, and

the activities of γ -glutamyl transferase and aspartate transaminase were analyzed using commercial kits from Roche Diagnostics GmbH (Mannheim, Germany) on a Hitachi 912 automatic analyzer (Boehringer Mannheim, Mannheim, Germany). The concentrations of NEFA, BHBA, and bilirubin were also measured on a Hitachi 912 analyzer, using commercial kits obtained from Randox Laboratories Ltd. (Crumlin, UK). An RIA was used for analysis of insulin (INS-IRMA, BioSource Europe SA, Nivelles, Belgium). Serum sodium and potassium positive ions were analyzed with a sodium-potassium analyzer KNATM2 (Radiometer, Copenhagen, Denmark). Serum chloride negative ions were determined by a model 925 chloride analyzer (Ciba Corning, Medfield, MA). Samples of frozen liver and skeletal muscle were analyzed for glycogen, using the colorimetric procedure of Lo et al. (1970).

Calculations and Statistical Analysis

Net energy required for maintenance and lactation (MJ/d) was calculated as $4.184 \times (\text{BW}^{0.75} \times 0.08 + \{\text{milk yield (kg)} \times [(0.0929 \times \text{fat \%}) + (0.0563 \times \text{protein \%}) + (0.0395 \times \text{lactose \%})]\})$ according to the NRC (2001). Based on the energy requirement, infusion dose (kg glucose/d) was calculated as follows: designated dose level $\times \text{NE}_L / (15.6 \text{ MJ/kg})$. Energy balance was calculated as $\text{EB} = \text{energy intake by feed and infusion} - \text{energy output for maintenance, lactation, and BW gain}$ according to NRC (2001). The revised quantitative insulin-sensitivity check index (**RQUICKI**) was calculated as $\text{RQUICKI} = 1 / [\log(\text{glucose}) + \log(\text{insulin}) + \log(\text{NEFA})]$ from the plasma concentration of glucose (mg/dL) and the serum concentrations of insulin ($\mu\text{U/mL}$) and NEFA (mmol/L) according to Holtenius and Holtenius (2007). Daily areas under the curves (**AUC**) of blood metabolites were calculated from the set of 5 blood samples taken before each biopsy by using the trapezoidal method. Minimum and maximum values were identified in each of these daily data sets irrespective of the time of sampling. The daily mean values presented in the figures correspond to the 4 blood samples taken closest to each biopsy. When mean values over a period are presented (i.e., daily mean values or infusion period mean values), data were pooled arithmetically per animal before statistical analysis.

All data were analyzed using the MIXED procedure of SAS (version 9.1.3; SAS Institute, 2002) accounting for repeated measures. The model included the fixed effects of treatment (GI vs. SI), dose (representing dose levels of 0, 10, 20, and 30% of the NE_L requirement), and their interaction. The NE_L calculated before the beginning of the experiment was used as a covariate. In addition, because cows were gradually exposed to

increasing dose levels, dose was included in the model as a repeated measure. The covariate error structure that yielded the lowest Akaike's and Bayesian information criterion values for each dependent variable was used. To determine the effect of the infusion dose, the linear and quadratic effects of dose and their interaction with treatment were tested. The model was the same as described above; however, dose was considered a continuous variable. When interpreting the results of statistical analyses, priority was given to linear or quadratic interactions between treatment $\times$ dose because the main effects of dose were partially confounded by changes attributable to the progression of lactation (i.e., time-dependent changes). Comparisons of postinfusion samples (d 32) with preinfusion samples (d 0) were performed using a Student's paired *t*-test. Differences were considered significant when $P < 0.05$.

RESULTS

Animal Health

The results of daily physical examinations and CBC showed that all cows remained in good health status during the whole period of increasing glucose infusions (data not shown). Thereafter, on d 27, one cow in the GI group suffered from acute mastitis, with a sudden decrease in milk yield. Mastitis was treated with intramammary administration of 150 mg/d of cefquinome sulfate (Cobactan LC, Intervet Int., Unterschleissheim, Germany) for 3 successive days. Because all samples relevant for statistical analysis of linear and quadratic effects had been collected in this cow without any problems, a decision was made to extend the infusion period by 3 d for this particular cow, at the infusion level of 30% of NE_L , until she had recovered. The postinfusion values (d 32) were then collected with a delay of 3 d for this cow.

Good health status was confirmed by laboratory analyses, for which mean values and pooled SEM ($n = 6$) over the infusion period from d 0 to d 24 are given in brackets below. Packed cell volume (%) of both groups did not show significant variations (SI, 30.1; GI, 31.7 ± 0.5 ; $P > 0.05$). In addition, no significant changes ($P > 0.05$) were observed in the serum concentrations of the macrominerals (mmol/L), sodium (SI, 140.5; GI, 141.6 ± 0.8), potassium (SI, 3.77; GI, 3.65 ± 0.05), chloride (SI, 97.3; GI, 98.3 ± 0.4), calcium (SI, 2.36; GI, 2.29 ± 0.03), and phosphorus (SI, 1.83; GI, 1.88 ± 0.06). After withdrawal of infusions, however, there was an increase in serum phosphorus level on d 32 in the GI group (to 2.43; $P < 0.01$), but not in the SI group (to 2.11 ± 0.09 ; $P > 0.05$). The frequent liver biopsies were well tolerated because no significant increases ($P > 0.05$)

were observed in the bilirubin concentration (SI, 2.08; GI, $1.88 \pm 0.15 \mu\text{mol/L}$) and the activities of the liver enzymes γ -glutamyl transferase (SI, 34.9; GI, $38.2 \pm 4.8 \text{ U/L}$) and aspartate transaminase (SI, 77.4; GI, $86.3 \pm 8.9 \text{ U/L}$) in serum.

Feed Intake and Body Condition

Over the infusion period from d 0 to d 24, daily DMI averaged to 17.7 and $17.5 \pm 0.94 \text{ kg/d}$ for SI and GI cows ($n = 6$), respectively, and was affected by neither treatment nor dose (Table 2). Consequently, for all the following comparisons, the infused glucose can be considered the true surplus supply of glucose and energy. The surplus supply of energy to GI cows was reflected by a linear treatment $\times$ dose interaction for the EB status of the animals ($P = 0.001$; Table 2).

Linear treatment $\times$ dose interactions were detected for both BW ($P = 0.001$) and BFT ($P = 0.01$; Table 2). The interaction for BW coincided with a trend for a quadratic dose effect and was a result of cows on the GI treatment gaining more than 19 kg of BW when the infusion dose increased from 0 to 30% of the NE_L requirement, whereas cows on the SI treatment lost 13 kg of BW during the same period. The treatment $\times$ dose interaction for BFT was paralleled by a linear dose effect because of an increase in BFT of 0.46 cm in cows of the GI group, whereas BFT did not change for cows on the SI treatment.

Milk Production and Composition

Daily milk yield did not differ between treatments (Table 2). Similarly, milk energy output (MEO) and ECM were not affected by treatment. However, as cows progressed almost 1 mo in the lactation curve, MEO and ECM decreased over time regardless of treatment, as evidenced by linear dose effects ($P = 0.001$), with no treatment $\times$ dose interaction (Table 2). The reduction in MEO and ECM coincided with a linear reduction in milk fat yield ($P = 0.001$). Milk fat percentage and yield were higher for cows receiving SI than for cows receiving GI ($P = 0.002$; Table 2) but did not show a significant treatment $\times$ dose interaction. On the other hand, there was a linear treatment $\times$ dose interaction on milk protein percentage ($P = 0.01$) and yield ($P = 0.03$; Table 2) caused by a numerical increase in protein percentage (by 0.23%) for the GI group at the highest infusion dose of 30% of the NE_L requirement. The stimulating effect of GI on milk protein percentage was still evident in postinfusion samples collected 4 d after suspension of glucose infusions ($P = 0.02$). No effects of GI on lactose percentage and yield and on MUN concentrations were observed during the infusion pe-

Table 2. Dry matter intake, energy balance (EB), body condition, and milk performance of cows in the glucose-infusion (GI) and saline-infusion (SI) groups

Item	Infusion dose level, % of NE _L					P-value for contrasts					Postinfusion sample ¹		
						Treatment	Dose		Treatment × dose				
	0	10	20	30	SEM		Linear	Quadratic	Linear	Quadratic	Mean	SEM	P-value ²
DMI, kg/d						0.65	0.54	0.45	0.22	0.60			
SI	18.3	18.6	17.9	18.2	0.92						17.7	1.04	0.71
GI	17.1	18.2	17.6	18.1	0.92						18.2	1.04	0.20
EB, ³ MJ/d						0.05	0.001	0.01	0.001	0.55			
SI	−10.38	−3.72	−1.12	−1.79	6.3						−4.50	6.01	0.40
GI	−14.6	6.30	26.1	35.4	6.3						−2.25	6.01	0.001
BW, kg						0.18	0.58	0.05	0.001	0.25			
SI	614	602	591	601	28						596	20	0.12
GI	651	654	662	670	28						670	20	0.001
BFT, ⁴ cm						0.48	0.006	0.25	0.01	0.09			
SI	2.21	2.23	2.26	2.22	0.23						2.06	0.14	0.39
GI	2.31	2.34	2.41	2.77	0.23						2.89	0.14	0.02
Yield													
Milk yield, kg/d						0.20	0.37	0.23	0.81	0.48			
SI	24.8	23.9	21.7	23.5	1.20						22.3	1.7	0.22
GI	25.8	26.9	23.4	25.3	1.20						23.5	1.7	0.09
ECM, ⁵ kg/d						0.65	0.001	0.26	0.83	0.76			
SI	31.5	30.5	28.1	28.7	2.9						28.7	2.3	0.17
GI	29.4	29.5	25.7	26.7	2.9						27.5	2.3	0.14
MEO, ⁶ MJ/d						0.64	0.001	0.24	0.93	0.77			
SI	92.4	89.1	82.2	83.3	6.1						83.8	6.7	0.16
GI	85.9	85.8	75.1	78.2	6.1						79.9	6.7	0.13
Milk fat, kg/d						0.002	0.001	0.61	0.82	0.80			
SI	1.35	1.34	1.20	1.17	0.05						1.21	0.11	0.14
GI	1.16	1.10	1.05	0.93	0.05						1.05	0.11	0.13
Milk protein, kg/d						0.70	0.48	0.02	0.03	0.69			
SI	0.92	0.87	0.79	0.85	0.04						0.83	0.06	0.26
GI	0.86	0.88	0.83	0.93	0.04						0.87	0.06	0.98
Milk lactose, kg/d						0.19	0.33	0.11	0.47	1.00			
SI	1.07	1.04	1.01	1.07	0.08						1.01	0.09	0.17
GI	1.23	1.23	1.10	1.20	0.08						1.04	0.09	0.004
Milk composition													
Milk fat, %						0.002	0.001	0.08	0.30	0.48			
SI	5.64	5.75	5.69	5.06	0.29						5.36	0.19	0.26
GI	4.52	4.50	3.97	3.63	0.29						4.50	0.19	0.93
Milk protein, %						0.23	0.03	0.003	0.01	0.59			
SI	3.69	3.59	3.59	3.68	0.10						3.70	0.08	0.94
GI	3.43	3.36	3.46	3.66	0.10						3.77	0.08	0.02
Milk lactose, %						0.45	0.05	0.43	0.14	0.88			
SI	4.71	4.65	4.73	4.70	0.06						4.67	0.04	0.23
GI	4.71	4.67	4.80	4.81	0.06						4.56	0.04	0.02
MUN, mg/L						0.32	0.11	0.72	0.74	0.93			
SI	358	344	324	328	31						343	35	0.51
GI	334	319	292	287	31						300	35	0.59

¹Postinfusion samples were collected 4 d after the end of the infusion period.²Comparison of preinfusion (column 2) and postinfusion values (column 12) by paired Student's *t*-test.³Energy balance (MJ/d) = energy intake including the energy provided by infusions − energy output for maintenance, lactation, and BW gain (NRC, 2001); 1 MJ = 4.184 Mcal.⁴Backfat thickness.⁵Energy-corrected milk yield (kg/d) = (0.3246 × kg of milk) + (12.86 × kg of milk fat) + (7.04 × kg of milk protein).⁶Milk energy output (MJ/d) = 4.184 (MJ/Mcal) × milk yield (kg) × [(0.0929 × fat%) + (0.0563 × true protein%) + (0.0395 × lactose%)] (NRC, 2001).

Figure 1. Mean plasma glucose (A) and serum insulin concentrations (B), and the revised quantitative insulin-sensitivity check index (RQUICKI; C) for cows infused intravenously with glucose (■) or saline (●). Infusion doses were 0, 10, 20, 30, and 0% of the calculated daily NE_L requirement on d 0, 8, 16, 24, and 32, respectively. Values represent daily means of 4 different samples taken over 24 h. Open symbols depict postinfusion values, which were analyzed separately. Data are expressed as means $\pm$ SEM. Probability levels for statistical contrasts were as follows: treatment: 0.001 (A), 0.005 (B), 0.19 (C); dose linear: 0.001 (A), 0.02 (B), 0.04 (C); dose quadratic: 0.005 (A), 0.71 (B), 0.61 (C); treatment $\times$ dose linear: 0.001 (A), 0.006 (B), 0.001 (C); treatment $\times$ dose quadratic: 0.01 (A), 0.29 (B), 0.23 (C). Postinfusion values (d 32) were not different from preinfusion values (d 0; $P > 0.05$) for A and C, and were different in the glucose-infusion group only in B ($P = 0.03$).

riod (Table 2). However, postinfusion samples revealed decreases in milk lactose percentage ($P = 0.02$) and yield ($P = 0.004$) below the initial levels (d 0) in the GI group only.

Blood Metabolites of Glucose, Lipid, and Nitrogen Metabolism

Daily means of plasma glucose and serum insulin concentrations are shown in Figure 1, whereas Table 3 summarizes minimum, maximum, and daily AUC values. The interaction treatment $\times$ dose was significant for daily mean values (Figure 1), daily AUC, and maximum values (Table 3) of both glucose and insulin concentrations ($P < 0.05$). Glucose and insulin concentrations in GI cows increased with the dose of glucose infused, whereas there was no such effect in SI cows. However, the amplitude of this increase varied during the day. The latter was most evident at the highest infusion dose of 30% of NE_L, when maximum concentrations for plasma glucose (in 5 out of 6 GI cows) and serum insulin (in 4 out of 6 cows) were observed at 1600 h (i.e., 1 h postprandially). In contrast, daily minimum values of glucose and insulin concentrations showed no significant treatment $\times$ dose interactions (Table 3). Glucoseuria was detected in all GI cows, but exclusively at a dose of 30% of the NE_L requirement, whereas no glucosuria was observed in cows on the SI treatment (data not shown). During the postinfusion period, at d 32, plasma glucose concentration in the GI group had returned to baseline values, whereas serum insulin concentration remained (mean value in Figure 1B; $P = 0.03$) or tended to remain (AUC in Table 3; $P = 0.10$) slightly elevated compared with baseline values at d 0. The index for insulin sensitivity of peripheral tissues, RQUICKI, showed a significant treatment $\times$ dose interaction ($P = 0.001$; Figure 1C). This interaction was based on a decrease in RQUICKI in the GI group from (mean $\pm$ SEM) 0.49 to 0.36 ± 0.02 when the infusion dose increased from 0 to 30% of the NE_L requirement, whereas RQUICKI did not change in SI cows.

Daily mean (Figure 2A) and maximum values, and the daily AUC of serum BHBA concentration (Table 3) showed linear dose effects ($P = 0.001$) and linear dose $\times$ treatment interactions ($P < 0.05$). The interactions were based on a decrease in BHBA concentration in GI, but not SI, cows, occurring predominantly between dose levels of 0 to 20%. A similar trend for a linear dose $\times$ treatment interaction ($P = 0.08$), together with a linear dose effect ($P = 0.001$), was observed for the minimum serum concentration of BHBA (Table 3). Daily mean values (Figure 2B and 2C), minimum and maximum values, and daily AUC of serum NEFA and

Table 3. Daily minimum and maximum values and area under the curve (AUC) of plasma glucose, serum insulin, BUN, NEFA, and BHBA concentrations for cows in the glucose-infusion (GI) or saline-infusion (SI) group

Item	Infusion dose level, % of NE _L					P-value for contrasts					Postinfusion sample ¹		
						Treatment	Dose		Treatment × dose				
	0	10	20	30	SEM		Linear	Quadratic	Linear	Quadratic	Mean	SEM	P-value ²
Glucose													
Daily AUC, mmol/L·h						0.001	0.001	0.06	0.001	0.09			
SI	91.6	89.5	85.7	85.1	4.0						88.9	2.4	0.35
GI	91.4	98.2	100.3	130.7	4.0						86.9	2.4	0.28
Daily minimum, mmol/L						0.27	0.72	0.10	0.05	0.36			
SI	3.56	3.11	3.18	3.03	0.20						3.27	0.14	0.29
GI	3.52	3.28	3.46	4.12	0.20						3.29	0.14	0.16
Daily maximum, mmol/L						0.001	0.001	0.002	0.001	0.006			
SI	4.28	4.04	3.95	3.90	0.21						4.07	0.08	0.19
GI	4.03	4.96	5.11	9.31	0.21						3.93	0.08	0.40
Insulin													
Daily AUC, nmol/L·h						0.02	0.001	0.06	0.001	0.02			
SI	2.02	3.06	2.76	2.86	0.98						4.31	0.76	0.10
GI	1.81	4.11	8.33	19.15	0.98						2.48	0.76	0.06
Daily minimum, pmol/L						0.87	0.04	0.72	0.37	0.64			
SI	61	67	83	76	14						122	22	0.13
GI	50	63	75	90	14						53	22	0.65
Daily maximum, pmol/L						0.001	0.02	0.50	0.006	0.15			
SI	116	216	140	179	99						251	38	0.05
GI	122	409	589	2,170	99						195	38	0.07
BHBA													
Daily AUC, mmol/L·h						0.16	0.001	0.29	0.03	0.35			
SI	18.1	18.6	13.5	14.4	2.0						15.8	1.7	0.20
GI	21.3	17.5	9.9	9.7	2.0						16.4	1.7	0.02
Daily minimum, mmol/L						0.46	0.001	0.02	0.08	0.56			
SI	0.56	0.48	0.36	0.40	0.06						0.36	0.10	0.06
GI	0.63	0.40	0.29	0.25	0.06						0.42	0.10	0.02
Daily maximum, mmol/L						0.08	0.001	0.02	0.002	0.12			
SI	1.00	1.02	0.85	0.85	0.02						0.95	0.02	0.33
GI	1.19	1.26	0.58	0.58	0.02						1.00	0.02	0.07
NEFA													
Daily AUC, mmol/L·h						0.32	0.005	0.42	0.39	0.12			
SI	4.79	5.30	4.05	3.13	0.61						2.98	0.27	0.09
GI	4.92	3.64	2.41	1.99	0.61						3.27	0.27	0.16
Daily minimum, μmol/L						0.78	0.04	0.39	0.03	0.99			
SI	81	119	89	71	11						72	7	0.27
GI	101	66	62	61	11						68	7	0.01
Daily maximum, μmol/L						0.17	0.001	0.36	0.91	0.73			
SI	409	374	257	211	25						222	11	0.15
GI	326	271	149	117	25						210	11	0.18
BUN													
Daily AUC, mg/dL·h						0.31	0.004	0.003	0.29	0.26			
SI	558	523	482	499	37						507	42	0.15
GI	522	429	379	399	37						464	42	0.17
Daily minimum, mg/dL						0.31	0.01	0.02	0.40	0.59			
SI	21.7	19.6	18.4	19.0	1.6						18.7	2.1	0.31
GI	19.8	16.2	14.1	14.6	1.6						16.5	2.1	0.45
Daily maximum, mg/dL						0.35	0.01	0.003	0.46	0.16			
SI	25.2	23.8	22.4	22.9	1.7						23.6	1.6	0.13
GI	23.8	20.3	18.0	19.7	1.7						22.2	1.6	0.12

¹Postinfusion samples were taken 4 d after the end of the infusion period.²Comparison of preinfusion (column 2) and postinfusion values (column 12) by paired Student's *t*-test.

Figure 2. Mean serum concentrations of BHBA (A), NEFA (B), and BUN (C) for cows infused intravenously with glucose (■) or saline (●). Infusion doses were 0, 10, 20, 30, and 0% of the calculated daily NE_L requirement on d 0, 8, 16, 24, and 32, respectively. Values represent daily means of 4 diurnal samples. Open symbols depict the postinfusion values, which were analyzed separately. Data are expressed as means $\pm$ SEM. Probability levels for statistical contrasts were as follows: treatment: 0.51 (A), 0.32 (B), 0.31 (C); dose linear: 0.001 (A), 0.001 (B), 0.006 (C); dose quadratic: 0.20 (A), 0.70 (B), 0.005 (C); treatment $\times$ dose linear: 0.02 (A), 0.42 (B), 0.29 (C); treatment $\times$ dose quadratic: 0.36 (A), 0.12 (B), 0.32 (C). Postinfusion values (d 32) were not different from preinfusion values (d 0; $P > 0.05$).

Figure 3. Liver (A) and skeletal muscle (B) glycogen contents for cows infused intravenously with glucose (■) or saline (●). Infusion doses were 0, 10, 20, 30, and 0% of the calculated daily NE_L requirement on d 0, 8, 16, 24, and 32, respectively. Open symbols depict the postinfusion values, which were analyzed separately. Data are expressed as means $\pm$ SEM. The P -values for contrasts were as follows: treatment: 0.07 (A), 0.94 (B); dose linear: 0.04 (A), 0.02 (B); dose quadratic: 0.12 (A), 0.22 (B); treatment $\times$ dose linear: 0.001 (A), 0.06 (B); treatment $\times$ dose quadratic: 0.12 (A), 0.12 (B). Postinfusion values (d 32) were not different from preinfusion values (d 0; $P > 0.05$).

BUN concentrations decreased linearly and, in part, also quadratically ($P < 0.05$). Although a significant dose $\times$ treatment interaction was present only for the minimum values of serum NEFA concentrations, all NEFA and BUN values showed numerically larger decreases in GI cows compared with SI cows when infusion level increased from 0 to 30% of the NE_L requirement. Postinfusion values (d 32) for minimum concentrations of serum BHBA and NEFA were significantly lower compared with preinfusion values (d 0) in the GI group only ($P < 0.05$), which, in the case of BHBA, coincided with a lower postinfusion AUC ($P = 0.02$;

Table 3). Mean values, minimum and maximum values, and the AUC of serum cholesterol concentrations decreased linearly in both groups when the infusion dose increased ($P < 0.01$), without a significant treatment effect or dose $\times$ treatment interaction ($P > 0.05$; data not shown), indicating a time-dependent decrease irrespective of treatment. Accordingly, postinfusion values for all cholesterol measures were lower in both groups compared with preinfusion values ($P < 0.05$; data not shown). The mean values ($\pm$ SEM; mmol/L) of serum cholesterol concentration over the whole infusion period amounted to 5.10 and 5.19 ± 0.31 for SI and GI cows, respectively ($n = 6$).

Liver and Skeletal Muscle Glycogen Contents

Linear treatment $\times$ dose interactions were detected for liver glycogen ($P = 0.001$; Figure 3A) and, as a trend, also for skeletal muscle glycogen contents ($P = 0.06$; Figure 3B), both coinciding with linear dose effects. The treatment $\times$ dose interactions were based on increasing liver and skeletal muscle glycogen contents in GI cows with increasing glucose dose, whereas cows on the SI treatment showed no such changes throughout the study. The glycogen levels in liver and skeletal muscles of GI cows returned to preinfusion values within 4 d after the end of the infusion period (Figure 3).

DISCUSSION

The present study applied an intravenous increasing infusion protocol to study the effects of increasing the glucose supply on lactation performance, body condition, and metabolism in midlactation dairy cows. This experimental approach allowed us to measure the direct dose effects of surplus glucose that are not influenced by first-pass utilization within the portal-drained viscera (Reynolds et al., 1994) or the release of gastrointestinal hormones during glucose absorption (Holst, 2007). Diets were designed to supply a minimal amount of glucose available for direct entry from the portal-drained viscera. As such, the protocol explored the entire range of nutritionally relevant surplus glucose because temporal hyperglycemia and hyperinsulinemia, as well as glucosuria, were evident at the end of the infusion period. Further, with this protocol we avoided potentially confounding effects sometimes encountered in constant-level infusion protocols such as decreased DMI (Dhiman et al., 1993; Knowlton et al., 1998; Grünberg et al., 2006). In the present study, depression of feed intake did not occur at any level of glucose infusion, including when cows were already experiencing hyperglycemia and hyperinsulinemia. This implies, on the other hand, that glucose and insulin may not be major regulators

of feed intake in ruminants despite an obvious relationship between insulin homeostasis and the individual susceptibility to feed-depressing stimuli (Allen et al., 2005; Bradford and Allen, 2007). A second potentially confounding effect of sudden increases in glucose supply is dysregulation of phosphorus homeostasis (Grünberg et al., 2006). Decreases in serum phosphorus concentration were not evident during GI in the present study. Moreover, an increase in serum phosphorus concentration after sudden glucose withdrawal supports the view that cows in the present study maintained phosphorus homeostasis throughout the increasing infusion protocol by continuing adaptation. Consequently, the results throughout the whole infusion period should reflect the responses of well-adapted animals.

Lactation Performance

The major finding was that glucose infusion did not affect milk yield, ECM, or MEO in the present study. It may appear that the failure of GI to enhance milk production could be independent of the route of glucose administration because a similar failure has been observed previously after abomasal (Clark et al., 1977) or duodenal GI (Lemosquet et al., 1997; Hurtaud et al., 1998). Other studies with postruminal infusions of glucose, however, showed a stimulating effect on milk yield both short term (≤ 7 d; Frobish and Davis, 1977; Hurtaud et al., 2000) and long term (14 d; Knowlton et al., 1998; Rigout et al., 2002a,b). As a possible explanation for the variable effect of postruminal GI on milk yield, Hurtaud et al. (2000) developed a hypothesis from the available literature that only hypercaloric, but not isocaloric, infusions could increase milk yield. In their own experiments, however, the authors found that milk yield could also be increased by isocaloric duodenal infusions of glucose when supplied with a grass silage-based diet (Hurtaud et al., 2000), but not with a corn silage-based diet (Hurtaud et al., 1998). The latter indicated that the total availability of glucose in the duodenum from both postruminal starch and infused glucose might be decisive for the ability of glucose to increase milk yield. This suspected dose effect of duodenal glucose availability was later verified by Rigout et al. (2002b), who demonstrated that moderate amounts of duodenal glucose (443 and 963 g/d) were most efficient to increase milk yield, whereas higher amounts (2,398 g/d) blunted the response. When using moderate concentrations of glucose in intravenous GI studies, however, stimulating effects of glucose on milk yield were observed only with short infusion periods (360 to 750 g/d of glucose over approximately 5 d; Fisher and Elliot, 1966; Schlei et al., 2007), but not with longer infusion periods (342 to 737 g/d of glucose over 11 d;

Amaral et al., 1990). The latter suggests a reversal of glucose effects on milk yield by metabolic adaptation. The lack of effect on milk yield in the present study is in line with this interpretation because the present experimental protocol was intended to provide optimal day-by-day adaptation. Part of the adaptation likely lies in a day-by-day adjustment of lactose production by the mammary gland. Milk lactose yield was rather constant during GI infusion, but the sudden decline after glucose withdrawal suggests that this was due to adaptive changes. Milk lactose is considered the primary osmoregulator of milk and, hence, largely determines milk volume (Linzell and Peaker, 1971; Rigout et al., 2002b). Taken together, the results favor the view that an increase in lactose synthesis and milk production by surplus glucose is transient after intravenous GI, whereas it can be sustained by metabolic signals, hormonal signals, or both from the portal-drained viscera over longer periods. These results also demonstrate that glucose availability was not a limiting factor for milk production in the midlactation cows used in the present study.

Apart from effects on milk yield, some previous studies also showed a negative effect of glucose infusion on milk fat yield and percentage (Lemosquet et al., 1997; Hurtaud et al., 1998; Rigout et al., 2002a). The altered milk fat composition and the decreased milk fat production were related to a decrease in blood precursors for milk fat synthesis (acetate, BHBA, total triglycerides, and NEFA). The latter seemed attributable to decreased DMI (Rigout et al., 2002a) and the antilipolytic effect of insulin (McClymont and Vallance, 1962; Bauman and Griinari, 2003). In accordance with these previous findings, numerically larger decreases in milk fat percentage and yield were observed in GI cows in the present study in comparison with SI cows. However, there was no significant interaction between treatment $\times$ dose, indicating that the differences in substrate availability for milk fat synthesis (BHBA, NEFA) between GI and SI cows were likely not great enough to elicit a significant effect of glucose application on milk fat percentage and yield.

A linear treatment $\times$ dose interaction was seen on milk protein synthesis in the present study. The underlying increase in milk protein percentage and yield with increasing glucose dose is in agreement with the increases in milk protein yield (Amaral et al., 1990; Hurtaud et al., 2000; Rulquin et al., 2004) and percentage (Lemosquet et al., 1997) observed in earlier reports during intravenous or postruminal glucose administration. The increase in milk protein percentage in the present study was coupled with a numerical decrease in BUN concentration. Although the latter finding was not statistically significant, it is most logical to assume

that the provision of additional glucose spared AA from utilization in gluconeogenesis, thereby improving AA delivery to the mammary gland (Reynolds et al., 1994; Vanhatalo et al., 2003). The dose-dependent increases in insulin concentration could add to this effect by increasing the uptake of AA by the mammary gland (Mackle et al., 2000).

Body Condition

Although the GI did not result in increased milk yield, it is evident that cows directed almost all surplus energy to BW gain. Changes in BW gain have to be interpreted very carefully in ruminants because changes in rumen fill may have a large impact on actual BW. However, the parallel increases in BW and BFT with increasing glucose dose (see below) support the view that the net BW gain of 32 kg/24 d in the GI group compared with the SI group is a realistic measure for anabolism in the GI group. Part of the BW gain was attributable to glycogen storage in the liver. The saturation of liver glycogen stores with an increasing GI dose likely contributed to the occurrence of temporal hyperglycemia and hyperinsulinemia when the dose increased. The latter, in turn, were likely the cause for increased glucose uptake via insulin-insensitive (GLUT1) and insulin-sensitive glucose transporters (GLUT4) into skeletal muscle (Duhlmeier et al., 2005) with a corresponding increase in muscle glycogen storage at high glucose doses. The high prevalence of insulin-insensitive glucose transporters (GLUT1) in ruminant muscle tissue (Duhlmeier et al., 2005) ensures efficient glucose uptake during hyperglycemia, even when insulin resistance develops (see RQUICKI below). On a quantitative basis, however, the BW gain attributable to glycogen in the liver (2.2% liver weight) and skeletal muscle (0.7% muscle weight) by the end of the infusion period can explain only a very small fraction of the observed BW gain (i.e., <1.5 kg). This fraction may increase when accounting for osmotically attracted water and when accounting for muscle growth (i.e., muscle protein gain), which is usually a consequence of an increased glucose uptake into muscle (Etherton, 1982). As discussed for milk protein synthesis above, an AA-sparing effect of GI might additionally have promoted muscle protein gain.

Nonetheless, the major part of BW gain was due to increased adiposity, as indicated by the increases in BFT observed in the present study for cows provided with GI. Despite the fact that acetate is the major source of lipid in the adipose tissue of ruminants, glucose can become a quantitatively important precursor for fatty acid synthesis when glucose availability increases (Ballard et al., 1972; Prior and Scott, 1980; Ver-

non, 1980). Moreover, previous studies have shown that glucose indirectly enhances substrate (acetate, lactate) incorporation into fat by increasing the availability of NADPH₂ and glycerol-3 phosphate, which are required for fatty acid synthesis and esterification, respectively (Vernon, 1980).

According to Schröder and Staufenbiel (2006), an increase in BFT by 0.46 cm at the end of the infusion period would be equivalent to a gain of approximately 23 kg of whole-body fat. It is astonishing that an estimated gain of 23 kg of fat (38.5 MJ/kg; Panel on Macronutrients et al., 2005) could be gained by infusion of only 31 kg of glucose (15.6 MJ/kg; Panel on Macronutrients et al., 2005), especially when considering that some glucose was lost because of glucosuria at the end of the infusion period. This proves that the infused glucose did not serve merely as an energy substrate, but that it primarily enhanced the overall energetic efficiency of intermediary metabolism. Because protein utilization has the highest energetic costs (VandeHaar and St-Pierre, 2006) and because the cows in this study were on a diet high in MP, the increased energetic efficiency was likely due to positive effects on nitrogen metabolism. Supporting evidence for this hypothesis can be derived from a numerical decrease in urea load in plasma and an increased protein export via milk.

Intermediary Metabolism

The fate of intravenously infused glucose is of interest when infused glucose does not translate into higher milk production. The absence of glucosuria confirmed that all glucose had been utilized in the body at GI doses up to 20% of the NE_L requirement. Moreover, unchanged daily minimum values for plasma glucose indicated that GI cows were able to increase the glucose clearance rate to account fully for elevated glucose appearance rates during certain times of day, even at the highest infusion dose of 30% of the NE_L requirement. This is in agreement with previous studies demonstrating acceleration of glucose uptake and utilization by body tissues with increased glucose supply (Bartley and Black, 1966; Amaral et al., 1990; Rigout et al., 2002b). It is also possible that there was a corresponding decrease in endogenous glucose production for GI cows (Bartley and Black, 1966). However, it is unlikely that hepatic glucose production was markedly reduced already at low infusion dosages. When glucose was previously supplied to dairy cows as a jugular infusion of 342 or 737 g/d of glucose, there was a proportional increase in the irreversible loss of plasma glucose without a concomitant reduction in endogenous glucose production (Amaral et al., 1990).

Toward the high infusion dose of 30% of the NE_L requirement (d 24), the daily maximum values of plasma glucose concentration increased by 130%, whereas maximum serum insulin concentration increased 17-fold. The dramatic increases in blood glucose and insulin maximal concentrations were mostly due to postprandial hyperglycemia and hyperinsulinemia. These changes were, at least in part, attributable to insulin resistance, as indicated by a decreasing RQUICKI. The use of RQUICKI has been introduced as a suitable tool to monitor insulin resistance in dairy cows because glucose tolerance tests are difficult to perform and to interpret in adult ruminants (Holtenius and Holtenius, 2007). Insulin resistance was likely related to the increase in liver and skeletal muscle glycogen content with increasing GI, which could indicate a compromised ability of these organs to buffer glucose peaks. It is noteworthy that insulin sensitivity was restored within only 4 d of glucose withdrawal, as demonstrated by a complete reversal of both RQUICKI and liver and skeletal muscle glycogen content.

The main effects of GI on intermediary lipid and protein metabolism can be summarized as a diversion of lipid and protein from energy-generating pathways to synthesis pathways. The decrease in BHBA and NEFA serum concentrations as well as the increase in BFT with increasing levels of GI suggest a dose-dependent stimulation of lipid anabolism. Similar responses with decreases of blood BHBA (Amaral et al., 1990) and NEFA concentrations (Vernon, 1980; Hurtaud et al., 1998) have been described earlier during glucose infusion studies. A stimulation of protein synthesis pathways by the GI was mainly supported by an increased protein output via milk in the present experiment. As discussed above, this was likely coupled with improved body nitrogen retention. Additionally, the major blood metabolite of protein metabolism, BUN, showed numerically larger decreases in the GI group during the infusion period, even though the treatment × dose interaction was not significant for the latter. Together, this could be explained by a decrease in hepatic uptake and deamination of glucogenic AA, thereby promoting peripheral uptake of AA for protein synthesis (Obitsu et al., 2000).

CONCLUSIONS

Whole-body appearance rate of glucose is not limiting for milk yield in midlactation dairy cows on an energy-balanced diet. Cows rapidly adapt to a gradual increase in glucose supply but experience dose-dependent development of insulin resistance, with periods of increasingly severe hyperglycemia and hyperinsulinemia. Very

high glucose entry rates are additionally associated with glucosuria. Surplus glucose is transiently stored as glycogen in liver and skeletal muscle and, in the longer term, is transferred to body fat. The absence of an effect of increased glucose availability on milk yield and MEO indicates either that the mammary gland is functioning at maximum capacity or that factors other than glucose availability set the upper margins for milk production. It can be speculated that some of the latter factors could be metabolic or hormonal signals from the portal-drained viscera during high-starch feeding.

ACKNOWLEDGMENTS

We thank Carsten Benson from the Institute of Veterinary Physiology and the staff of the Clinic for Large Animal Internal Medicine, University of Leipzig, for care of the experimental animals and the support during sampling. The authors are especially grateful to Gerald F. Schusser for infrastructural support and to Anke Schmidt-Mähne for help during sampling and laboratory analyses. The work was supported by Pfizer Animal Health (Sandwich, UK).

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