Effects of profound acidemia on the dynamic glucose and insulin response and plasma potassium and phosphorus concentrations during an intravenous glucose tolerance test in neonatal calves

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ABSTRACT

Acidemia and electrolyte imbalances such as hyperkalemia are common in neonatal calves with diarrhea. Acidemia negatively affects the cellular response to insulin and may therefore result in deranged glucose, potassium, and phosphorus homeostasis. The primary aim of this study was to compare indices that characterize the dynamic glucose and insulin response between acidemic and nonacidemic neonatal diarrheic calves and a healthy control group during an intravenous glucose tolerance test (IVGTT) that consisted of i.v. administration of 0.3 g of glucose per kg of body weight. Secondary aims were to characterize the associated changes in plasma potassium and phosphorus concentrations. The effect of correction of profound acidemia with a sodium bicarbonate containing infusion on these parameters was also assessed. Thirty calves (age ≤ 21 d) were purposively assigned to one of the following groups: 10 calves with diarrhea and profound acidemia (venous blood pH < 7.20) where an IVGTT was performed before and after treatment with sodium bicarbonate, 10 calves with diarrhea and minimal acid-base disturbance (venous blood pH > 7.35), and 10 healthy control calves. Profoundly acidemic diarrheic calves (jugular venous blood pH 6.99 ± 0.10) had a similar initial increase in plasma insulin concentration to that in healthy control calves or nonacidemic calves with diarrhea. However, insulin concentrations remained relatively stable in acidemic calves between 15 and 60 min after the start of the IVGTT, whereas a marked decrease in plasma insulin concentrations occurred in all other groups during the same period of time. We conclude that acidemia does not alter cell glucose availability or the dynamic response of glucose, phosphorus, and potassium to insulin; however, acidemia markedly

prolongs plasma insulin concentrations following an IVGTT through an unidentified mechanism. Results of this study emphasize the importance of correcting acidemia and metabolic acidosis in neonatal calves with diarrhea.

Key words: calves, insulin resistance, glucose, potassium, phosphorus

INTRODUCTION

Dehydration, azotemia, and development of a hyponatremic strong ion (metabolic) acidosis with variable degrees of hyper D-lactatemia are well-known complications in neonatal calves with diarrhea (Lorenz, 2004; Constable et al., 2005; Trefz et al., 2015a). Electrolyte imbalances such as hyperkalemia are also common in diarrheic calves and can result in skeletal muscle weakness and life-threatening cardiac conduction abnormalities and arrhythmias (Lewis and Phillips, 1973; Weldon et al., 1992; Trefz et al., 2013b).

Potassium is predominantly an intracellular cation and hyperkalemia in neonatal diarrheic calves has traditionally been attributed to an acidemia-induced dysregulation of internal potassium balance. Intracellular buffering of hydrogen ions and impaired Na^+/K^+ -ATPase activity have therefore been proposed as underlying mechanisms for hyperkalemia in diarrheic calves (Lewis and Phillips, 1973; Sweeney, 1999; Constable, 2002). However, recent research indicates that the occurrence of hyperkalemia in neonatal diarrheic calves is primarily dependent on the degree of dehydration and to a lesser extent on the cause of the acidemia (Trefz et al., 2013a,b). Decreased renal perfusion and glomerular filtration rate therefore appear to play decisive roles in the development of hyperkalemia. Nevertheless, other factors still have the potential to play an important pathophysiological role in the development of hyperkalemia in diarrheic calves.

The mechanism linking acidemia and altered intracellular potassium homeostasis is not fully understood.

Received February 5, 2017.

Accepted July 21, 2017.

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Insulin is involved in the regulation of internal potassium balance by providing a net cellular uptake of potassium (Cox et al., 1978; Bia and DeFronzo, 1981). As even mild acidemia (blood pH, 7.27 ± 0.01 and 7.37 ± 0.02) has been associated with insulin resistance in humans (DeFronzo and Beckles, 1979; Mak, 1998), it is likely that insulin-dependent cellular potassium uptake is impaired in the presence of acidemia (Constable and Grünberg, 2013). Impaired cellular responsiveness to insulin provides a potential explanation for the association between acidemia and hyperkalemia. Feeding a low DCAD (-41 mEq/100 g) ration to nonlactating nonpregnant dairy cows induced acidemia (blood pH, 7.32), metabolic acidosis (plasma HCO_3^- , 17.8 mmol/L; urine pH, 5.4), and decreased insulin responsiveness, as assessed by higher peak plasma glucose and lower peak plasma insulin concentrations in response to an intravenous glucose tolerance test (IVGTT; Bigner et al., 1996). We therefore hypothesized that neonatal calves with diarrhea and profound acidemia and hyperkalemia have decreased insulin responsiveness and a slower rate of decrease in plasma potassium concentration, compared with diarrheic calves with minimal disturbances in acid-base balance and healthy calves without diarrhea.

Combined administration of insulin and glucose is a well-established treatment for hyperkalemia in humans (Weisberg, 2008). Evaluation of indices that characterize the dynamic glucose and insulin response to an IVGTT in acidemic neonatal calves should improve our understanding of the pathophysiology of potassium balance disorders in neonatal calves with diarrhea and therefore improve treatment protocols for hyperkalemia in acidemic calves. Although it is well known that restoration of potassium homeostasis in diarrheic acidemic calves can be achieved by rehydration and alkalization using intravenous solutions containing sodium bicarbonate (Koch and Kaske, 2008; Coskun et al., 2010; Trefz et al., 2015b), we hypothesized that intravenous administration of a glucose-containing solution induces endogenous insulin release and thereby exerts a potassium-lowering effect. This might be especially of relevance in the initial treatment of affected calves, where treatment objectives focus on the rapid correction of hyperkalemia, hypoglycemia, and profound acidemia (Constable and Grünberg, 2013; Lorenz and Trefz, 2016). This research question is also of interest because administration of glucose containing infusion solutions to diarrheic calves that require intravenous fluid therapy is popular in ambulatory field practice. Intravenous glucose is administered to counteract negative energy balance and provide a readily utilizable energy source when calves are housed in cold ambient conditions (Berchtold, 2009).

The major objectives of the study reported here were therefore to administer an IVGTT to characterize the dynamic glucose and insulin response and changes in plasma potassium concentrations between acidemic and nonacidemic neonatal diarrheic calves and a healthy control group. Because insulin also induces a compartmental shift of inorganic phosphorus (P_i) into insulin-responsive cells in cattle (Knochel, 1977; Grünberg et al., 2006) by upregulating Na/P_i co-transporter gene expression (Li et al., 1996), we also examined the P_i concentration-time relationship during an IVGTT. An additional objective was to determine whether correction of profound acidemia with a sodium bicarbonate-containing infusion has an immediate effect on these parameters.

MATERIALS AND METHODS

The methods of this study were approved by the Animal Welfare and Ethics Committee of the government of Upper Bavaria (permit: 55.2-1-54-2532-11-14) according to German Animal Welfare Legislation.

Calves

An IVGTT was performed in 30 neonatal calves up to 21 d of age. Calves were purposively selected to be assigned to one of the following 3 groups:

1. Ten calves with diarrhea and profound acidemia (venous blood pH <7.20 ; acidemic group; group **A**). An IVGTT was performed before treatment (**A-BT**) and after treatment (**A-AT**) with intravenous sodium bicarbonate as described below.
2. Ten calves with diarrhea and minimal acid-base disturbance (venous blood pH >7.35 ; nonacidemic group; group **NA**).
3. Ten healthy calves with normal fecal consistency and volume (control group; group **C**).

Before inclusion into the study, calves underwent a thorough physical examination that included the assessment of the general condition and examination of the respiratory, gastrointestinal, and cardiovascular system as well as palpation of navel structures and joints. Physical examination included the clinical assessment of posture/ability to stand, behavior, rectal temperature, and extent of enophthalmos (mm). Physical examination was repeated in calves of group A before start of the second IVGTT. Posture was scored as 1 = standing securely; 2 = insecurely, able to correct position; 3 = insecurely, unable to correct position; and 4 = unable to stand. Behavior was scored as 1 = adequate reaction, very bright and alert; 2 = adequate reaction;

3 = delayed reaction; 4 = calf reacts only to painful stimuli; and 5 = no reaction to painful stimuli. The severity of enophthalmos was quantified by measuring the distance (mm) between the medial canthus and the eyeball (Constable et al., 1998; Niethammer, 2007). Diarrhea was defined as fecal consistency that allowed feces to run through slightly open fingers (Trefz et al., 2015a).

Exclusion criteria for calves assigned to groups A and NA were severe hypothermia (rectal temperature $<37^{\circ}\text{C}$), severe hypoglycemia (plasma glucose concentration <2.5 mmol/L), severe clinical dehydration (degree of enophthalmos >6 mm), venous blood pH <6.80 , and severe concurrent health problems including obvious signs of septicemia, navel infections requiring surgical intervention or clinical evidence of pneumonia.

To avoid breed-specific differences on the dynamic glucose and insulin response to an IVGTT (Bossaert et al., 2009), only calves of the Simmental breed (German Fleckvieh; the most common dairy breed in Bavaria) were included in the study. All experiments were performed at the Clinic for Ruminants with Ambulatory and Herd Health Services, Ludwig-Maximilians-Universität (LMU) Munich. A total of 9 calves of group C, 3 calves of group NA, and 1 calf of group A were obtained from the teaching and research farm of LMU Munich, which is located within 500 m of the Clinic for Ruminants, and were transported to the hospital on the day of the experiment. The remaining calves were recruited from the patient pool of the Clinic for Ruminants. Written informed consent was obtained from the owners of calves before inclusion in the study.

Experimental Protocol

Calves were weighed before the trial and all experiments were performed at least 6 h after the last milk feeding, based on reports by the owners of calves. A 16-gauge, 15-cm catheter (Splittocan Infusionskatheter, Walter Veterinär Instrumente, Baruth, Germany) was placed in a jugular vein, which was introduced through a 14-gauge Splittocan cannula and used for glucose infusion and blood sampling. For this purpose, the area over the respective jugular vein was clipped, aseptically prepared, and 2 mL of a 2% procaine solution injected into and under the skin before catheterization. The catheter set contained a 13-cm elongation tube, which was used to avoid manipulation of calves during blood sampling. After catheter placement, calves were allowed to rest for at least 30 min until blood sampling began.

The IVGTT consisted of an i.v. infusion of 0.3 g of glucose/kg of BW administered over a period of 1 min (equivalent to 0.75 mL of a 40% glucose solution per kg of BW; 400 g/L glucose anhydrous, B. Braun Melsun-

gen AG, Melsungen, Germany). Lithium-heparinized blood samples were anaerobically collected at -15 , 0, 3, 6, 10, 15, 20, 30, 40, 50, 60, 75, 90, and 120 min relative to the onset of the infusion. After glucose infusion and blood sampling the catheter was flushed with 5 mL of a 0.9% NaCl solution.

At time (t) = 120 min, the experiment was finished for calves of group NA and C. In calves of group A, correction of profound acidemia was then carried out by an infusion containing an amount of sodium bicarbonate, which was calculated according to following formula: NaHCO_3 (mmol) = BW (kg) $\times$ base deficit (mmol/L) $\times$ 0.7. For this purpose, the required volume of a commercially available 8.4% sodium bicarbonate solution was diluted with sterile water (Ampuwa, Fresenius Kabi GmbH, Germany) to a volume of 2.5 L and the resulting infusion solution was administered over a period of 2.5 h as described by Lorenz and Vogt (2006). After completion of the infusion, calves were reweighed and the IVGTT repeated after an additional period of 30 min as described above.

Laboratory Analyses

Blood samples were kept at room temperature until determination of blood pH, partial pressure of carbon dioxide (pCO_2), sodium, chloride, potassium, and ionized calcium concentrations within 10 min after collection using a blood pH, gas, and electrolyte analyzer with ion selective electrodes (Rapidpoint 405, Siemens Healthcare Diagnostics, Tarrytown, NY). Blood pH and pCO_2 were corrected for rectal temperature using standard algorithms (Thomas, 1972). Actual bicarbonate concentration (cHCO_3^-) was automatically calculated by the blood gas unit using the Henderson-Hasselbalch equation with measured blood pH and pCO_2 at 37°C : $\text{cHCO}_3^- = S \times \text{pCO}_2 \times 10^{(\text{pH} - \text{pK}_1')}$. Values for the negative logarithm of the dissociation constant of carbonic acid (pK_1') and solubility of carbon dioxide (S) for plasma were 6.105 and 0.0307 mmol/L per mm of Hg, respectively. After measuring the hemoglobin concentration (Hb in g/dL) photometrically, blood base excess (in vitro base excess) was automatically calculated in units of mmol/L with measured blood pH at 37°C and the determined actual bicarbonate concentration (CLSI, 2009): $\text{base excess} = (1 - 0.014 \times \text{cHb}) \times [(\text{cHCO}_3^- - 24.8) + (1.43 \times \text{cHb} + 7.7) \times (\text{pH} - 7.4)]$.

After blood gas analysis, syringes were placed in a refrigerator and centrifuged within 30 min after collection at $1,500 \times g$ for 10 min. Harvested plasma samples were assayed for glucose (hexokinase), P_i (molybdenum), and insulin at all sampling times. Plasma concentrations of urea (urease), creatinine (picric acid), D-lactate (D-lactate dehydrogenase), L-lactate (L-lactate dehy-

drogenase), and total protein (biuret) were additionally determined at $t = -15$ min. An automatic analyzing system (Cobas c 311, Roche Diagnostics, Mannheim, Germany) was used for biochemical analysis. Plasma insulin concentrations were determined in duplicate using a commercially available ELISA kit (Bovine Insulin ELISA, EIA-4748, DRG Instruments, Marburg, Germany; provided by Mercodia, Uppsala, Sweden). For the latter, plasma samples were stored at -25°C until analysis. Reported inter- and intraassay coefficients of variation of this assay were ≤ 7.0 and $\leq 5.3\%$, respectively. Plasma insulin concentrations were determined as $\mu\text{g/L}$ and converted for the present analysis to $\mu\text{IU/mL}$ by multiplying values by 20.56 (Abuelo et al., 2012). Due to recommendations of the test provider, insulin concentrations below a concentration of $1.03 \mu\text{IU/mL}$ ($0.05 \mu\text{g/L}$) were not calculated from the calibration curve and were assigned a concentration $1.03 \mu\text{IU/mL}$. This was true for 63 out of 560 samples including 25 samples between $t = -15$ min and 3 min as well as 37 samples between $t = 30$ min and 120 min in groups A-BT, NA, and A-AT. In group C, one sample was assigned a value of $1.03 \mu\text{IU/mL}$ at $t = 90$ min.

Baseline concentrations of glucose ($\text{Baseline}_{\text{Glucose}}$), insulin ($\text{Baseline}_{\text{Insulin}}$), potassium ($\text{Baseline}_{\text{K}}$), and phosphorus ($\text{Baseline}_{\text{Pi}}$) were calculated by averaging values from samples taken at $t = -15$ min and 0 min relative to the onset of glucose injection.

The percent change of plasma volume after treatment in calves of group A was extrapolated from the change of plasma total protein concentrations measured at baseline of each IVGTT before $[(\text{total protein})_{\text{BT}}]$ and after treatment $[(\text{total protein})_{\text{AT}}]$ with sodium bicarbonate, such that (Van Beaumont et al., 1972): $\Delta \text{Plasma volume} = [(\text{total protein})_{\text{BT}} - (\text{total protein})_{\text{AT}}] \times 100 / [(\text{total protein})_{\text{AT}}]$.

Analysis of Glucose Kinetics

Peak concentrations for glucose ($\text{Peak}_{\text{Glucose}}$) were determined from individual plasma glucose concentration-time curves. The area under the plasma glucose concentration-time curve ($\text{AUC}_{\text{Glucose-60}}$) from 0 to 60 min after the start of dextrose infusion was calculated using the trapezoidal rule.

Clearance rates for glucose were calculated between $t = 10$ min and $t = 60$ min of the IVGTT by applying a one compartment monoexponential model with nonzero asymptote, such that: $\text{glucose} = (A_o - A_{\text{asym}}) \times e^{-kt} + A_{\text{asym}}$, with t = time after the start of intravenous glucose administration; k = the elimination rate constant for glucose ($\%/min$); A_o = the extrapolated plasma glucose concentration assuming instantaneous

mixing when $t = 0$; A_{asym} = asymptotic plasma glucose concentration when time $\gg 0$ min (Grünberg et al., 2011).

The apparent volume of distribution for glucose (V_d) in L was calculated from the modeled curve for each IVGTT by dividing the amount of glucose infused ($0.3 \text{ g/kg of BW} = 1.67 \text{ mmol/kg of BW}$) by the difference between A_o and $\text{Baseline}_{\text{Glucose}}$ concentration (mmol/L) using the equation: $V_d = [1.67 \times (\text{BW in kg})] / (A_o - \text{Baseline}_{\text{Glucose}})$. The apparent volume of distribution for glucose was calculated to provide an estimate for changes in the extracellular fluid volume because V_d reflects the instantaneous apparent distribution volume for glucose.

Analysis of the Dynamic Glucose and Insulin Response to an IVGTT

The dynamic glucose and insulin response to an IVGTT was characterized by calculating the peak insulin concentration ($\text{Peak}_{\text{Insulin}}$), the time interval to $\text{Peak}_{\text{Insulin}}$, the increment in plasma insulin concentration for each IVGTT ($\text{Max}_{\text{Diff Insulin}}$) by subtracting the $\text{Baseline}_{\text{Insulin}}$ from $\text{Peak}_{\text{Insulin}}$, the area under the plasma insulin concentration curve from 15 to 60 min after the start of the dextrose infusion ($\text{AUC}_{\text{Insulin-60}}$), and the ratio of $\text{Baseline}_{\text{Insulin}}$ to $\text{Baseline}_{\text{Glucose}}$ ($\text{Baseline I/G ratio}$, Grünberg et al., 2011). The area under the concentration time curve for the insulin to glucose ratio ($\text{AUC}_{\text{I/G-60}}$) between 15 to 60 min after the start of the dextrose infusion was also calculated. Fifteen and 60 min were selected as the start and end points for AUC calculations related to insulin as this period represented the time interval where insulin clearance predominated over insulin release in healthy calves in this study reported here and elsewhere (MacPherson et al., 2016).

Insulin clearance rates were not calculated using a similar monoexponential decay equation as for plasma glucose concentrations because the plasma insulin concentrations in group A-BT did not follow an exponential decline. Instead, an insulin sensitivity index (IS_i) was calculated using the following equation, which uses the change in the area under the plasma insulin concentration-time relationship ($\Delta\text{AUC}_{\text{Insulin}}$) for time = 0 to 60 min, such that $\text{IS}_i = \alpha \times k / (\Delta\text{AUC}_{\text{Insulin}}/T)$, where α is a scaling factor ($\alpha = 0.276$), k is the determined elimination rate constant for glucose ($\%/min$), $\Delta\text{AUC}_{\text{Insulin}}$ is the area under the insulin-time relationship (above basal) calculated using the trapezoid method, and T is the time interval from 0 to 60 min (= 60 min) when k and $\Delta\text{AUC}_{\text{Insulin}}$ are calculated (Tura et al., 2010; Pantophlet et al., 2016a). This index was considered to provide the primary measure of insulin

sensitivity (tissue responsiveness to insulin) as the IS_i relates glucose clearance to the prevailing plasma insulin concentration. Insulin sensitivity was also evaluated by calculating surrogate indices of insulin sensitivity from $Baseline_{Glucose}$ and $Baseline_{Insulin}$ including the reciprocal of baseline plasma insulin concentration (1/insulin), the baseline glucose to insulin ratio (G/I; glucose in mg/dL), and the quantitative insulin sensitivity check index (QUICKI; Katz et al., 2000; Muniyappa et al., 2008). The QUICKI was calculated as follows (Katz et al., 2000; Muniyappa et al., 2008): $QUICKI = 1 / \{\log_{10} [Baseline_{Insulin} (\mu IU/mL)] + \log_{10} [Baseline_{Glucose} (mg/dL)]\}$.

Analysis of the Potassium-Insulin Relationship During an IVGTT

Changes of plasma potassium concentration during the IVGTT and its association with changes of insulin concentrations were evaluated by determining the maximum decrement for plasma potassium concentration ($Max_{DiffK-60}$) measured within 60 min after glucose infusion, and the ratio between the observed decrement of plasma potassium concentration relative to baseline and insulin concentrations ($DiffK:I$) at the time point of $Peak_{Insulin}$. The net change in area under the plasma potassium concentration-time curve ($AUC_{DiffK-60}$) from 0 to 60 min after the start of glucose infusion relative to baseline was calculated using the trapezoidal rule.

Analysis of the Phosphorus-Insulin Relationship During an IVGTT

The maximal decrease in plasma P_i ($Max_{DiffPi-60}$) was calculated by subtracting the lowest P_i measured within 60 min after glucose infusion from the $Baseline_{Pi}$. Also similar to the plasma potassium concentrations, the ratio between the observed decrement of plasma P_i relative to baseline and insulin concentrations ($DiffP_i:I$) at the time point of $Peak_{Insulin}$ was calculated.

The net change in the area under the plasma P_i concentration-time curve ($AUC_{DiffPi-60}$) from 0 to 60 min after the start of glucose infusion relative to baseline was calculated using the trapezoidal rule.

Statistical Analysis

Statistical analysis was conducted using SPSS (version 23, IBM, New York, NY), SAS (version 9.3, SAS Inst. Inc., Cary, NC), and GraphPad Prism (version 7.01, GraphPad software, La Jolla, CA). P -values <0.05 were considered to be statistically significant and a statistical trend was declared in case of P -values between 0.05 and 0.10. A normal distribution of data was

assessed by the Shapiro-Wilk test and visual inspection of QQ plots. If necessary, data were log-transformed to achieve a normal distribution of respective variables. Values of nonnormally distributed variables were back-transformed after statistical analysis to facilitate comparisons of values to previous studies and are reported as geometric mean and respective 95% confidence interval of the data. Values of untransformed variables are reported as mean $\pm$ standard deviation.

A one-way ANOVA was used to determine group effects on assessed IVGTT parameters and a Bonferroni test was used as post-hoc test to compare variables between the 3 treatment groups (A-BT, NA, and C). A paired t -test was used to compare IVGTT variables in calves of group A before and after treatment. Group comparisons were not performed between calves of groups A-AT, NA, and C. Accordingly, scores of clinical parameters were compared using a nonparametric Kruskal-Wallis test (overall group effect), a Mann-Whitney U-test (comparisons between calves of groups A-BT, NA, and C), and a paired Wilcoxon test (comparisons between A-BT and A-AT).

RESULTS

Basal Conditions

Basal clinical and laboratory conditions of calves before conduction of IVGTT are presented in Tables 1 and 2. The age of group A calves did not differ from calves of group NA and C, but group NA calves were older than group C calves (Table 1). Calves of group A predominantly suffered from severe acidemia and presented with lower values for rectal temperature and higher scores for posture and behavior than calves of groups NA and C, respectively. The degree of enophthalmos and significantly higher plasma urea concentrations compared with calves of groups NA and C also indicated the presence of slight dehydration in group A calves.

Calves of group A had lower initial basal glucose concentrations than calves of group C and a hypoglycemic state was observed in most of the calves of group A after correction of metabolic acidosis. Basal insulin and P_i concentrations differed in calves of group A before and after treatment as well as between calves of groups NA and C. Initial plasma potassium concentrations were similar in groups A-BT, NA, and C, but plasma potassium concentration decreased and a hypokalemic state was observed in calves of group A after treatment.

The mean increase in plasma volume and BW at the start of the second IVGTT (3 h after the start of treatment) in group A calves was $19.0 \pm 7.9\%$ and 0.5 ± 0.8 kg, respectively.

Table 1. Age, BW, and basal clinical values in 30 neonatal calves before administration of an intravenous glucose tolerance test¹

Variable ²	Group effect				Treatment effect	
	A-BT	NA	C	P-value ³	A-AT	P-value ⁴
Age (d)	11 ± 5 ^{ab}	13 ± 4 ^a	8 ± 3 ^b	0.018	11 ± 5	N/A
Posture (score 1–4)	3 (2–3) ^a	1 (1–1) ^b	1 (1–1) ^b	<0.001	2 (1–3)	0.014
Behavior (score 1–5)	3 (3–4) ^a	1 (1–1) ^b	1 (1–2) ^b	<0.001	3 (2–3)	0.011
Enophthalmos (mm)	2.5 (1–3) ^a	2 (1–2) ^{ab}	1 (1–1) ^b	0.019	1.0 (1–2)	0.015
Rectal temperature (°C)	38.5 ± 0.7 ^a	39.3 ± 0.2 ^b	39.1 ± 0.3 ^b	0.002	39.0 ± 0.6	0.057
BW (kg)	43.7 ± 4.3 ^a	52.5 ± 8.3 ^b	49.0 ± 4.2 ^{ab}	0.009	44.2 ± 4.5	0.071

^{a,b}Values within a row with different superscripts differ significantly (Bonferroni post hoc test or Mann-Whitney U-test; $P < 0.05$).

¹Each of 10 calves belonged to 1 of 3 of the following groups: acidemic diarrheic calves with an initial venous blood pH <7.20 (group A) where an intravenous glucose tolerance test was performed before (group A-BT) and after treatment of acidemia (group A-AT), nonacidemic diarrheic calves with a measured venous blood pH >7.35 (group NA), and healthy neonatal calves serving as a control group (group C).

²Data are reported as median and interquartile ranges or means ± SD. Posture was scored as 1 = standing securely, 2 = insecurely, able to correct position; 3 = insecurely, unable to correct position; 4 = unable to stand. Behavior was scored as 1 = adequate reaction; very bright and alert; 2 = adequate reaction; 3 = delayed reaction; 4 = calf reacts only to painful stimuli; 5 = no reaction to painful stimuli. Enophthalmos was quantified by measuring the distance (mm) between the medial canthus and the eyeball.

³P-values reflect the results of the overall ANOVA analysis or a nonparametric Kruskal-Wallis test.

⁴P-values reflect the results of a pairwise comparisons of values of group A calves before and after treatment (paired *t*-test or nonparametric Wilcoxon test); N/A = not applicable.

Changes of plasma glucose and insulin concentrations during the IVGTT are displayed in Figure 1, and the observed decrement in plasma potassium and phosphorus concentrations are displayed in Figure 2. Results of the comparisons of analyzed IVGTT variables between treatment groups are presented in Table 3.

Dynamic Glucose and Insulin Response to an IVGTT

Differences were not identified for $AUC_{\text{Gluc-60}}$ between calves of groups A-BT, NA, and C, but $AUC_{\text{Gluc-60}}$ was lower in calves of group A after treatment when compared with values before treatment ($P = 0.005$). Glucose clearance rates were similar for calves in groups A-BT, NA, and C, but a trend was observed toward higher clearance rates in calves of group A after treatment when compared with values before treatment ($P = 0.061$).

The calculated apparent volume of distribution for glucose (V_d) was similar ($P = 1.0$) in calves of group NA and C, but was higher in those groups ($P \leq 0.002$) when compared with calves in group A-BT. The calculated value for V_d increased by 2.3 ± 0.7 L in group A-AT calves, compared with group A-BT.

Marked differences in the slopes and amplitudes of the plasma insulin-concentration time curves were observed. The time to $\text{Peak}_{\text{Insulin}}$ was significantly higher in calves of group A-BT than in groups NA, C, and A-AT, respectively. Also, insulin concentrations remained relatively stable in calves of group A-BT between $t = 15$ min and 60 min, whereas a marked decrease of insulin concentrations was observed in calves of groups NA, C, and A-AT during the same period of time (Figure 1). The observed values for $AUC_{\text{Insulin-60}}$ and $AUC_{\text{I/G-60}}$

were significantly higher in calves of group A-BT than in calves of groups NA, and A-AT, but not when compared with calves of group C. A statistically significant difference for calculated values of IS_i was also found between groups A-BT, NA and A-AT, but not between A-BT and C, and not between NA and C.

The calculated values for surrogate indices of insulin sensitivity at baseline including $1/\text{Insulin}$, G/I ratio, and QUICKI differed between calves of groups NA and C as well as between A-BT and A-AT.

Plasma Potassium-Insulin Relationship

Values for $AUC_{\text{DiffK-60}}$ in A-BT calves did not differ from those of groups NA, C, and A-AT, but a difference was found between calves of groups NA and C. No difference was observed in DiffK:I at the time point of $\text{Peak}_{\text{Insulin}}$ between groups.

Plasma Phosphorus-Insulin Relationship

Only minimal changes for P_i concentrations were observed during the IVGTT, with no difference between treatment groups.

DISCUSSION

The objective of the present study was to determine the magnitude of the effect of marked acidemia due to metabolic acidosis on the dynamic glucose and insulin response to an IVGTT in neonatal diarrheic calves. Central findings of this study indicate that the performed glucose challenge in profoundly acidemic diarrheic calves resulted in a comparable glucose clear-

Table 2. Basal laboratory conditions in 30 neonatal calves before conduction of an intravenous glucose tolerance test¹

Variable ²	Group effect			Treatment effect		
	A-BT	NA	C	A-AT	P-value ³	P-value ⁴
Acid-base variable						
Venous blood pH	6.987 ± 0.098 ^a	7.376 ± 0.021 ^b	7.399 ± 0.015 ^b	7.401 ± 0.032	<0.001	<0.001
pCO ₂ (mm Hg)	34.1 ± 4.8 ^a	49.5 ± 4.7 ^b	52.9 ± 3.2 ^b	45.8 ± 3.6	<0.001	<0.001
HCO ₃ ⁻ (mmol/L)	8.1 ± 2.8 ^a	27.8 ± 3.3 ^b	31.4 ± 2.0 ^c	27.3 ± 3.0	<0.001	<0.001
Base excess (mmol/L)	-22.1 ± 4.5 ^a	2.7 ± 3.0 ^b	6.2 ± 1.8 ^b	2.7 ± 2.9	<0.001	<0.001
AG (mEq/L)	23.0 ± 3.7 ^a	11.3 ± 2.4 ^b	12.1 ± 2.5 ^b	19.1 ± 4.4	<0.001	<0.001
SIG (mEq/L)	-13.8 ± 4.4 ^a	1.8 ± 1.9 ^b	1.2 ± 2.7 ^b	-7.2 ± 5.6	<0.001	<0.001
Clinical biochemistry analysis						
Glucose (mmol/L)	4.4 ± 0.5 ^a	5.2 ± 1.0 ^{ab}	6.1 ± 0.7 ^b	3.9 ± 0.7	<0.001	0.014
Insulin (μIU/mL)	4.4 (0.5-37.5) ^{ab}	2.3 (0.6-8.1) ^a	6.4 (2.5-16.3) ^b	1.3 (0.5-3.7)	0.022	0.006
D-Lactate (mmol/L)	8.5 (3.1-23.2) ^a	0.3 (0.0-6.1) ^b	0.2 (0-22.1) ^b	8.1 (2.9-22.4)	<0.001	0.19
L-Lactate (mmol/L)	0.8 ± 0.2 ^a	1.1 ± 0.2 ^b	1.1 ± 0.1 ^b	1.5 ± 0.6	0.008	0.002
Total protein (g/L)	60.5 ± 11.7	57.5 ± 5.2	57.3 ± 6.7	50.9 ± 9.2	0.63	<0.001
Urea (mmol/L)	10.4 (4.9-22.0) ^a	3.0 (1.3-6.9) ^b	2.7 (0.9-7.8) ^b	9.1 (4.1-19.9)	<0.001	<0.001
Creatinine (μmol/L)	118 (54-257) ^a	84 (58-122) ^b	101 (66-154) ^{ab}	107 (50-230)	0.038	0.001
Phosphorus (mmol/L)	2.4 ± 0.4 ^{ab}	2.2 ± 0.2 ^a	2.6 ± 0.2 ^b	2.0 ± 0.3	0.022	0.001
Electrolyte (mmol/L)						
K ⁺	4.3 ± 0.7	4.1 ± 0.3	4.3 ± 0.2	2.9 ± 0.4	0.63	<0.001
Na ⁺	134.1 ± 5.3	135.4 ± 1.6	137.7 ± 2.0	141.1 ± 4.4	0.08	<0.001
Cl ⁻	107 ± 5 ^a	100 ± 3 ^b	99 ± 3 ^b	98 ± 5	<0.001	<0.001
Ca ²⁺	1.40 ± 0.08 ^a	1.25 ± 0.03 ^b	1.26 ± 0.04 ^b	0.94 ± 0.12	<0.001	<0.001

^aValues within a row with different superscripts differ significantly (Bonferroni post hoc test $P < 0.05$).¹Each of 10 calves belonged to 1 of 3 of the following groups: acidemic diarrheic calves with an initial venous blood pH <7.20 (group A), where an intravenous glucose tolerance test was performed before (group A-BT) and after treatment of acidemia (group A-AT), nonacidemic diarrheic calves with a measured venous blood pH >7.35 (group NA), and healthy neonatal calves serving as a control group (group C).²Values are given as means ± SD or geometric means and respective 95% CI of the data. pCO₂ = partial pressure of carbon dioxide; AG = anion gap calculated as (Na⁺ + K⁺) - (Cl⁻ + HCO₃⁻); SIG = strong ion gap calculated as [(total protein) × 0.343]/(1 + 10^(7.08-pH)) - AG; Constable et al. (2005).³P-value reflects the result of the overall ANOVA.⁴P-value reflects the result of a pairwise comparisons of values of group A calves before and after treatment (paired *t*-test).

ance rate to that in nonacidemic diarrheic and healthy calves, but was associated with a markedly longer period of hyperinsulinemia and consequently a lower index of insulin sensitivity.

Previous studies have shown that even a very mild degree of acidemia is associated with the occurrence of insulin resistance in respect to glucose metabolism as observed in humans and dogs following ammonium-chloride-induced acidemia due to strong ion acidosis (Mackler et al., 1951; DeFronzo and Beckles, 1979), humans and rats with acidemia due to diabetic keto-acidosis (Walker et al., 1963; Cuthbert and Alberti,

1978), and in vitro studies on cultured adipocytes (Van Putten et al., 1985; Igarashi et al., 1993). In agreement with those studies, treatment with sodium bicarbonate increased insulin sensitivity and secretion in human patients with chronic kidney disease-related metabolic acidosis (Reaich et al., 1995; Mak, 1998), and lower serum bicarbonate concentrations and higher values for anion gap were found to be independently associated with an index of reduced insulin sensitivity in adult humans without diabetes or other chronic diseases (Farwell and Taylor, 2008). Acidemia-induced insulin resistance is caused at the cellular level by a reduced receptor bind-

Figure 1. Means $\pm$ SD of plasma glucose (linear scale) and insulin concentrations (logarithmic scale) during an intravenous glucose tolerance test (IVGTT) in 30 neonatal calves. Values were slightly offset to improve readability. A and B: Changes of IVGTT variables in 10 acidemic diarrheic calves with venous blood pH < 7.20 (○-), 10 nonacidemic diarrheic calves with a measured venous blood pH > 7.35 (□-), and 10 healthy neonatal calves (△-). C and D: Changes of IVGTT variables of the 10 diarrheic calves with an initial venous blood pH < 7.20 after correction of acidemia (●-) in comparison to values of the same calves before treatment (○-). A and C: Gray-shaded areas indicate the reference range (4.4 to 6.9 mmol/L) for plasma glucose concentrations in calves (Kraft and Dürr, 1995). Horizontal dashed lines indicate the anticipated range of values (8.3 to 9.5 mmol/L) for renal threshold in calves (Scholz and Hoppe, 1987; Hostettler-Allen et al., 1994).

ing affinity of insulin as well as by postbinding alterations (Van Putten et al., 1985; Igarashi et al., 1993). If insulin release is unaffected by acidemia, the net result of decreased plasma insulin clearance due to reduced insulin receptor binding would be sustained plasma insulin concentrations consistent with decreased entry of insulin into cells. In addition to an acidemia-induced alteration of the insulin-receptor interaction, a reduced insulin receptor concentration would represent another plausible explanation, as observed in studies on isolated adipocytes from ketoacidotic and ammonium-chloride acidotic rats (Whittaker et al., 1981, 1982). Interestingly, the number of insulin receptors in cell preparations from ketoacidotic rats was increased when the rats were treated with sodium bicarbonate, compared

with untreated acidemic rats (Whittaker et al., 1982). Although not investigated in the study reported here, a bicarbonate-induced increase in the number of insulin receptors provides an explanation as to why correction of acidemia immediately increased insulin clearance in calves of the present study.

To the best of our knowledge the existence of an acidemia-induced insulin resistance has not been previously evaluated in neonatal diarrheic calves, but an impaired glucose-induced insulin response was reported in mildly acidemic cows after feeding an acidogenic diet (Bigner et al., 1996). This finding was, however, not confirmed in a study by Grünberg et al. (2011) where a mild but fully compensated metabolic acidosis in dairy cows did not affect insulin responsiveness or indices of

Figure 2. Means $\pm$ SD of changes of plasma potassium and phosphorus concentrations relative to baseline during an intravenous glucose tolerance test in 30 neonatal calves. A and B: Changes of variables in 10 acidemic diarrheic calves with venous blood pH <7.20 (-○-), 10 non-acidemic diarrheic calves with a measured venous blood pH >7.35 (-□-), and 10 healthy neonatal calves (-Δ-). Values were slightly offset to improve readability. C and D: Changes of variables of the 10 diarrheic calves with an initial venous blood pH <7.20 after correction of acidemia (-●-) in comparison to values of the same calves before treatment (-○-).

Table 3. Outcome variables reflecting the dynamic glucose and insulin response, as well as observed changes of plasma potassium and inorganic phosphorus concentrations, in 30 neonatal calves undergoing an intravenous glucose tolerance test (IVGTT)¹

Variable ²	Group effect			Treatment effect		
	A-BT	NA	C	P-value ³	A-AT	P-value ⁴
Glucose index						
Peak _{Glucose} (mmol/L)	14.3 ± 2.0	12.8 ± 1.2	13.0 ± 0.9	0.051	11.3 ± 1.0	<0.001
AUC _{Glucose-60} (mmol × 60 min/L)	507 ± 80	476 ± 67	499 ± 72	0.62	431 ± 61	0.005
k (%/min)	2.4 ± 0.7	3.0 ± 0.9	2.9 ± 0.9	0.16	3.1 ± 1.1	0.061
Apparent V _d (L)	8.9 ± 0.7 ^a	11.9 ± 2.5 ^b	12.1 ± 1.4 ^b	<0.001	11.2 ± 0.8	<0.001
Insulin index						
Peak _{Insulin} (μIU/mL)	71.7 (33.0–155.8)	41.0 (14.5–116)	71.5 (21.1–242)	0.036	33.3 (11.1–99.8)	0.007
Time to Peak _{Insulin} (min)	45 ± 19 ^a	13 ± 4 ^b	14 ± 5 ^b	<0.001	22 ± 18	<0.001
Max _{Diff Insulin} (μIU/mL)	65.6 (31.6–136.1)	38.6 (13.7–108.9)	64.8 (18.4–229.0)	0.052	31.6 (9.9–100.7)	0.011
AUC _{Insulin-60} (mIU × 45 min/mL)	1.79 (0.47–6.82) ^a	0.65 (0.14–2.97) ^b	1.45 (0.39–5.34) ^{ab}	0.009	0.74 (0.22–2.50)	<0.001
Baseline 1/G ratio (mIU/mmol)	1.00 (0.12–7.99) ^{ab}	0.44 (0.16–1.26) ^a	1.05 (0.43–2.60) ^b	0.024	0.34 (0.13–0.94)	0.012
AUC _{1/G-60} (mIU × 45 min/mmol)	246 (63–957) ^a	86 (21–362) ^b	185 (45–764) ^{ab}	0.009	110 (37–330)	0.001
IS _i [$\times 10^{-4} \times \text{min}^{-1} \times (\mu\text{IU}/\text{mL})^{-1}$]	2.09 (0.43–10.31) ^a	5.48 (1.47–20.42) ^b	2.76 (0.62–12.22) ^{ab}	0.023	5.09 (1.05–24.77)	0.004
Baseline 1/insulin	0.23 (0.03–1.94) ^{ab}	0.44 (0.12–1.56) ^a	0.16 (0.06–0.40) ^b	0.022	0.75 (0.27–2.07)	0.006
Baseline G/I ratio	18.1 (2.3–144.8) ^{ab}	40.6 (14.2–115.7) ^a	17.1 (6.9–42.4) ^b	0.024	52.2 (19.1–142.9)	0.012
QUICKI	0.41 ± 0.08 ^{ab}	0.44 ± 0.07 ^a	0.35 ± 0.03 ^b	0.020	0.51 ± 0.05	0.003
Potassium-insulin relationship						
Max _{DiffK-60} (mmol/L)	−0.36 ± 0.12	−0.35 ± 0.08	−0.45 ± 0.11	0.088	−0.30 ± 0.09	0.11
AUC _{DiffK-60} (mmol × 60 min/L)	−15.0 ± 5.3 ^{ab}	−10.8 ± 5.2 ^a	−18.1 ± 7.1 ^b	0.035	−12.1 ± 5.8	0.23
DiffK:I (mmol/IU)	−4.5 ± 3.1	−7.0 ± 3.4	−4.6 ± 3.1	0.15	−8.4 ± 5.9	0.13
Phosphorus-insulin relationship						
Max _{DiffP-60} (mmol/L)	−0.18 ± 0.05	−0.13 ± 0.07	−0.17 ± 0.09	0.28	−0.21 ± 0.08	0.17
AUC _{DiffP-60} (mmol × 60 min/L)	−7.0 ± 3.7	−3.6 ± 3.3	−5.9 ± 3.3	0.10	−7.1 ± 3.4	0.90
DiffP:I (mmol/IU)	−2.0 ± 1.3	−1.0 ± 2.6	−0.8 ± 0.6	0.28	−2.7 ± 3.5	0.56

^{a,b}Values within a row with different superscripts differ significantly (Bonferroni post hoc test $P < 0.05$).¹Each of 10 calves was allocated to one of the following groups: acidemic diarrheic calves with an initial venous blood pH < 7.20 where an IVGTT was performed before (group A-BT) and after treatment of acidemia (group A-AT), nonacidemic diarrheic calves with a measured venous blood pH > 7.35 (group NA), and healthy calves serving as a control group (group C).²Data are reported as means ± SD or geometric mean and respective 95% CI of the data. Peak_{Glucose} = peak plasma glucose concentration; AUC_{Glucose-60} = area under the glucose concentration-time relationship during the first 60 min after the start of the IVGTT; k = elimination rate for glucose; apparent V_d = apparent volume of distribution for glucose; Peak_{Insulin} = peak plasma insulin concentration; time to Peak_{Insulin} = time to peak insulin concentration; Max_{Diff Insulin} = maximal increment of plasma insulin concentration relative to baseline; AUC_{Insulin-60} = area under the plasma insulin concentration-time relationship between time (t) = 15 and 60 min after the start of the IVGTT; baseline 1/G ratio = plasma insulin to glucose ratio at baseline; AUC_{1/G-60} = area under the insulin to glucose ratio-time relationship between t = 15 and 60 min after the start of the IVGTT; IS_i = insulin sensitivity index; 1/Insulin = reciprocal of plasma insulin concentration; G/I ratio: plasma glucose to insulin ratio; QUICKI = quantitative insulin sensitivity check index; Max_{DiffK-60} = maximal decrement of plasma potassium concentration relative to baseline during the first 60 min after the start of the IVGTT; AUC_{DiffK-60} = area under the plasma potassium concentration-time curve for the decrement of plasma potassium concentration relative to baseline and insulin concentration at the time of peak insulin concentration; Max_{DiffP-60} = maximal decrement of plasma phosphorus concentration relative to baseline and insulin concentration at the time of peak insulin concentration; AUC_{DiffP-60} = area under the plasma phosphorus concentration-time curve for the decrement of plasma phosphorus concentration relative to baseline and insulin concentration at the time of peak insulin concentration.³P-value reflects the result of the overall ANOVA analysis.⁴P-value reflects the result of a pairwise comparisons of values of group A calves before and after treatment (paired t-test).

insulin sensitivity during an IVGTT. Moreover, our finding in the study reported here of similar values for $\text{Peak}_{\text{Insulin}}$ in groups A-BT, NA, and C suggest that insulin secretion is not impaired in severely acidemic calves.

Nonsignificant differences for $\text{AUC}_{\text{Gluc-60}}$ and the observed glucose clearance rates additionally indicated that acidemic diarrheic calves were similar effective in controlling glycemia than calves of groups NA and C. The observed glucose clearance rates in the present study were comparable to those of previously reported values for neonatal Belgian Blue calves but higher than previously reported values for neonatal Holstein calves (Bossaert et al., 2009) or periparturient Holstein cows (Mann et al., 2016), although methodical aspects need to be considered when comparing glucose clearance between studies.

The numerically lower values for $\text{AUC}_{\text{Gluc-60}}$ as well as the significantly lower $\text{Peak}_{\text{Glucose}}$ in calves of group A-AT compared with A-BT in calves of the present can be at least partly explained by an increase of plasma volume and consequently an increased distribution space for glucose. The calculated values for V_d increased by a mean of 2.3 L, which is consistent with an infusion volume of 2.5 L in those calves, whereas BW only increased by 0.5 kg. The latter suggests that those calves urinated or defecated over the 2.5-h study period with a net loss of 2.0 kg.

Of importance is our finding that values for $\text{AUC}_{\text{Insulin-60}}$ and $\text{AUC}_{\text{I/G-60}}$ were significantly higher and values for IS_i were significantly lower in acidemic calves than in group NA and in the same calves after treatment with sodium bicarbonate. Also, a remarkable finding was that the plasma insulin concentration remained unchanged between $t = 15$ min and $t = 60$ min after glucose injections, whereas a marked elimination of insulin was observed in all other groups. These findings suggest that acidemic calves are insulin resistant in that acidemic calves required a prolonged period of hyperinsulinemia for controlling the induced glucose challenge. This conclusion is, however, not supported by values of surrogate indices, which were calculated from baseline plasma insulin and glucose concentration and were not found to be significantly different between calves of group A-BT and calves of groups NA and C. Surrogate indices are widely used in human medicine as indirect and reliable measures of insulin sensitivity/resistance and have subsequently been copied for use in veterinary medicine although those measures require validation for use in cattle (Muniyappa et al., 2008; De Koster and Opsomer, 2013). Also, in the present study the results of these surrogate indices should be interpreted cautiously as different levels of glycemia were evident at baseline, which were not reflected

by insulin sensitivity. Hypoglycemia was observed in calves of group A at baseline of the IVGTT before and after treatment with sodium bicarbonate, and it is therefore likely that the glucose to insulin ratio was influenced in those animals by hypoinsulinemia as well as by counter-regulatory mechanisms in an attempt to increase plasma glucose concentrations. Nevertheless, the observed significant differences for surrogate indices together with numerically (but not significantly) higher values for $\text{AUC}_{\text{Insulin-60}}$ and $\text{AUC}_{\text{I/G-60}}$ as well as numerically lower values for IS_i in calves of groups C compared with calves of group NA requires explanation. Previous studies have shown that the plane of nutrition affects insulin sensitivity in milk-fed dairy calves (Hostettler-Allen et al., 1994; Hugi et al., 1997; Bach et al., 2013). Although those observations are based on experiments in older calves and on high-intensity feeding programs performed over a period of several weeks, it is conceivable that a lower intake of milk in diarrheic calves compared with healthy control calves might have had an effect on insulin sensitivity. This would also mean that the results of the comparison of $\text{AUC}_{\text{Insulin-60}}$, $\text{AUC}_{\text{I/G-60}}$, and IS_i between calves of groups A-BT and C should be interpreted cautiously in as much that a higher level of insulin sensitivity due to malnutrition could have been evident in calves of group A (which is supported by the observed $\text{AUC}_{\text{Insulin-60}}$ and $\text{AUC}_{\text{I/G-60}}$ after treatment) but was, however, masked during an acidemic state and therefore resulted in a similar insulin response than in calves of group C. Remarkably, values for IS_i in group C calves were much lower than previously published values for neonatal Holstein Frisian and Jersey calves (Stanley et al., 2002) and only slightly higher than reported values for older milk fed veal Holstein calves (Pantophlet et al., 2016b), which might be related to methodical differences. In addition to the effect of correction of metabolic acidosis, nutritional aspects might also provide an explanation for the observed differences of IVGTT parameters between calves of groups A-BT and A-AT as there was a time period of at least 5 h without feed intake before start of the second IVGTT. This was likely reflected by the observed baseline glucose and insulin concentrations in those calves after treatment.

Aside from nutritional aspects a different level of stress susceptibility might also have affected the outcome of our analyses. Although stress was reduced as much as possible and calves appeared generally quiet during the IVGTT, it is still possible that healthy control calves were more susceptible to unavoidable stressors such as new environmental conditions or catheterization as it was the case in ill calves suffering from diarrhea and a certain level of depression. Another issue is a high inter-individual variability of IVGTT parameters that was

especially observed in calves of group A. This might be related to the presence of concurrent clinical and metabolic conditions in some calves. Although we tried to minimize the effect of concurrent problems as much as possible by definition of exclusion criteria, the effect of hypoglycemia, hypothermia, or variable degrees of dehydration could not be completely prevented as they are frequently associated with the presence of severe acidemia due to metabolic acidosis in diarrheic calves.

No statistically significant difference was found in the present study for $AUC_{DiffK-60}$ between calves of group A-BT and calves of groups NA, C, and A-AT. Also the calculated values of $DiffK:I$ at the time of $Peak_{Insulin}$ were not different between treatment groups. Collectively, it appears that the insulin-induced potassium response was not impaired by an acidemic state.

Insulin induces a cellular uptake of glucose, potassium, and phosphorus that is achieved by different mechanisms at the cellular level. Insulin-stimulated glucose uptake is mediated by the facilitative glucose transporter GLUT4, which is translocated to the plasma membrane in insulin-sensitive tissues (Gumà et al., 1995; Duhlmeier et al., 2005). Insulin-stimulated potassium uptake is mediated by activation of the Na^+/K^+ -ATPase, which was reported to be further enhanced by an increased intracellular sodium flux (Rosic et al., 1985; Hundal et al., 1992). Insulin-stimulated phosphorus uptake is mediated by insulin-dependent upregulation of Na/P_i co-transporter gene expression (Li et al., 1996). Only minimal changes of plasma phosphorus concentrations were observed during the IVGTT in the present study, which is in contrast to studies in periparturient dairy cows, where significant decreases of plasma phosphorus concentrations after bolus injections of glucose solutions has been reported (Grünberg et al., 2006, 2011). Several potential mechanisms might have contributed to this finding. The renal phosphorus threshold in ruminants is 7.0 mg/dL (2.3 mmol/L; Symonds and Manston, 1974) and neonatal calves typically excrete 40% of daily phosphorus intake in urine, compared with a very low percentage in adult ruminants (Boehncke and Tiews, 1972). Also calves have much more available skeletal phosphorus, which is different from the situation in adult ruminants (Horst, 1986). Therefore, intracellular phosphorus translocation in response to insulin release might have happened in calves of the present study, but increased mobilization of phosphorus from bone as well as increased renal reabsorption most likely occurred at a fast enough rate to keep plasma phosphorus concentrations stable. Increased renal reabsorption might have been enhanced by hyperinsulinemia, which was reported to result in increased tubular phosphorus reabsorption in dogs, thereby reducing phosphorus losses into urine (DeFron-

zo et al., 1976; Hammerman et al., 1984). A potential explanation for a marked phosphorus-lowering effect of hyperinsulinemia in response to dextrose infusions in dairy cows (Grünberg et al., 2006, 2011) is that adult ruminants are not able to decrease urinary phosphorus excretion and have very little rapidly mobilizable phosphorus from bone. It would therefore appear that the insulin-phosphorus relationship in neonatal calves is not a good method to dissect out the mechanisms of insulin dysfunction in acidemia.

Interestingly, previous studies in humans have shown that the insulin-stimulated cellular uptake of glucose, potassium, or phosphorus are not coupled and are independently regulated from each other. Selective resistance mechanisms exist such that impaired glucose disposal (as in diabetic patients) does not necessarily affect potassium uptake and vice versa (Cohen et al., 1991; Nguyen et al., 2011). An experimental study in rats has shown that even a short-term potassium deprivation due to administration of a potassium-deficient diet (which only caused a 9% reduction of plasma potassium concentration) resulted in an 80% reduction in insulin-stimulated cellular potassium uptake, whereas glucose disposal rates were not affected (Choi et al., 2001). The occurrence of this selective insulin resistance was considered as a homeostatic mechanism to maintain extracellular potassium concentration after ingestion of meals that are rich in carbohydrates but low in potassium (Youn and McDonough, 2009). A similar situation might have also been evident in the present study as calves had to handle a potassium-free glucose challenge. Furthermore, all calves in the present study were nonhyperkalemic, and marked hypokalemia was even evident in calves of group A after correction of acidemia. This finding can be interpreted as a marked depletion of body potassium stores as the plasma potassium concentration is considered to more accurately reflect body potassium stores when blood pH values are within the reference range (Burnell and Scribner, 1957). Consequently, a selective insulin resistance for cellular potassium uptake (not caused by acidemia) might explain the observed $Max_{DiffK-60}$ values in the present study, which were generally low and would be unlikely to induce a clinically relevant treatment effect in diarrheic calves suffering from hyperkalemia. The therapeutic utility of endogenous insulin release in response to administration of a glucose containing infusion solutions should therefore be assessed in diarrheic calves with hyperkalemia.

CONCLUSIONS

A prolonged period of hyperinsulinemia as well as an increased insulin to glucose ratio during an IVGTT is

suggestive for the existence of insulin resistance in respect to glucose metabolism in severely acidemic calves with diarrhea. How much those findings are relevant to intravenous fluid therapy in those animals needs to be assessed in future investigations. However, the finding that acidemic calves were similarly effective in controlling the induced glucose challenge suggests that acidemic calves could be treated with glucose if necessary. Also, our finding that acidemic calves had a similar decline of plasma potassium concentrations per unit insulin than healthy control calves or nonacidemic calves with diarrhea indicates that glucose-containing infusion solutions may have therapeutic utility in the treatment of acidemic calves with hyperkalemia. From a clinical relevance viewpoint, findings of this study again emphasize the importance of correcting acidemia due to metabolic acidosis in the treatment of neonatal diarrheic calves.

ACKNOWLEDGMENTS

This study was supported by a research grant (TR 1321/1-1) of the German Research Foundation (Deutsche Forschungsgemeinschaft, Bonn, Germany). The authors thank Stefan Nüske (Teaching and Research Farm of LMU Munich) for providing us with calves and Sandra Kirsch, Ingrid Hartmann, and Monika Altmann (Clinic for Ruminants, LMU Munich) for technical assistance.

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