Effect of dehydration and acidemia on the potassium content of muscle tissue and erythrocytes in calves with neonatal diarrhea

L. Golbeck,*¹ I. Cohrs,* S. Leonhard-Marek,† and W. Grünberg*

*Clinic for Cattle, and

†Library and Department of Physiology, University of Veterinary Medicine Hannover Foundation, D-30173 Hanover, Germany

ABSTRACT

Disturbances of extracellular potassium (K) homeostasis in calves with severe neonatal diarrhea have been studied extensively. Although total body depletion of this predominantly intracellular electrolyte is generally thought to occur in diarrheic calves, the mechanisms through which K depletion occurs are poorly understood. The aim of this study was to investigate how intracellular K homeostasis is affected by dehydration and acidemia, the 2 most important metabolic disturbances in calves with naturally occurring diarrhea. Twenty-seven calves with naturally occurring neonatal diarrhea, pronounced dehydration, and acidemia, and 2 groups of 10 healthy control calves were included in this study. Blood samples and muscle biopsies were obtained immediately before initiation of treatment (T_0) and after complete rehydration and correction of acidemia (T_1) from diarrheic calves. Blood samples were used to perform blood gas, blood biochemical, and hematological analyses and to determine K content in erythrocytes. Muscle biopsies were used to determine muscle tissue K content and tissue dry matter. Controls were used to determine values for erythrocyte and muscle tissue K content in healthy neonatal calves for comparison with diarrheic calves. As defined by the inclusion criteria, diarrheic calves showed pronounced acidemia and dehydration at T_0 . Mean muscle tissue K content and tissue dry matter remained unchanged between sampling times and did not differ from values measured in healthy control calves. Erythrocyte K content increased from 73.63 ± 13.73 to 77.64 ± 15.97 mmol/L ($\pm$ standard deviation) but was associated with a concomitant decline in erythrocyte volume. Values measured at both sampling times in diarrheic calves did not differ from erythrocyte K measured in healthy control calves. The plasma K concentration (median [interquartile range]) decreased from 5.44 [4.76–6.17] to

4.16 [3.99–4.31] mmol/L between T_0 and T_1 . Although changes in plasma [K] were associated with the degree of dehydration, neither dehydration nor acidemia was associated with changes of K content in muscle tissue or erythrocytes. In conclusion, severe dehydration and acidemia in diarrheic calves were not associated with notable changes in K content of muscle tissue or erythrocytes. These results do not support the concept of pronounced K depletion occurring in calves with neonatal diarrhea. Erythrocytes are a poor surrogate tissue in which to measure changes of intracellular K content in diarrheic calves because of concomitant changes in erythrocyte volume that complicate the interpretation of results.

Key words: calf, intracellular, potassium, depletion

INTRODUCTION

Diarrhea remains one of the most common diseases and the most important cause of calf mortality in the first month of life (USDA, 2007; Medrano-Galarza et al., 2018). Neonatal diarrhea of calves is associated with several metabolic disturbances including dehydration, acidemia, hypoglycemia, and hyperkalemia (Smith and Berchtold, 2014). Disturbances of extracellular K balance in particular and the underlying causes have been studied in considerable detail (Trefz et al., 2013a, 2015a, 2017; Trefz and Lorenz, 2017). Hyperkalemia in diarrheic calves, occurring with an incidence of up to 34%, has been recognized as a potentially life-threatening sequela associated with bradycardia, arrhythmia, and, in severe cases, death of the affected patient (Groutides and Michell, 1990; Weldon et al., 1992; Trefz et al., 2013b). Dehydration (and the resultant decreased renal perfusion) and, to a lesser extent, acidemia (and the ensuing compartmental K shifts) have been identified as the most important driving forces behind observed changes in plasma K concentrations ([K]; Trefz et al., 2013a; Trefz and Lorenz, 2017). In contrast to extracellular K homeostasis, little is known about the potential effects of dehydration and acidemia on intracellular K homeostasis of diarrheic calves. Studies of the 1970s

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¹Corresponding author: lennartgolbeck@gmx.de

reported a pronounced depletion of intracellular K content in muscle tissue that occurred within hours in calves with experimentally induced neonatal diarrhea (Lewis and Phillips, 1972, 1973). However, the underlying mechanisms causing these important losses of K from the intracellular space, which accounts for approximately 98% of the total body K pool, have not been established. To better understand intracellular K homeostasis in diarrheic calves, the aims of this study were (1) to determine the intracellular K content on the example of muscle tissue and erythrocytes in calves affected by naturally occurring neonatal diarrhea before and after correction of dehydration and acidemia, (2) to compare them to values obtained in healthy calves, and (3) to identify factors contributing to the alteration of the intracellular K content in these animals. We hypothesized that acidemia and dehydration both have important effects on intracellular K homeostasis as described for extracellular K homeostasis.

MATERIALS AND METHODS

Animals

This study was approved by the Animal Welfare and Ethics Committee of the government of Lower Saxony, Germany (permit no 33,19-42502-05-16A019). Written consent was obtained from the owners of the animals participating in this study.

A prospective clinical study over the course of 1 yr (May 2016 to May 2017) was conducted on calves presented to the Clinic for Cattle, University of Veterinary Medicine Hannover, Foundation (Hanover, Germany), for the treatment of severe neonatal diarrhea. Main inclusion criteria were naturally occurring diarrhea, age between 3 and 21 d at the time of presentation, clinical signs of severe dehydration (eyeball recession over 3 mm, prolonged skin tenting), and a base excess (BE) lower than -10 mmol/L. To be able to standardize the initial therapy, we elected to exclude animals that did not require parenteral fluid therapy. Therefore, calves that were still ambulatory, had adequate suckle reflex, and thus were suitable for exclusive oral fluid therapy were excluded. Further exclusion criteria were a rectal temperature $>40.0^{\circ}\text{C}$, clinical signs suggestive of septicemia, and concomitant diseases potentially impeding recovery (e.g., omphalitis or pneumonia) at the time of admission.

Established reference ranges for muscle tissue and erythrocyte K content in calves are not available; therefore, we included 2 groups of healthy age-matched control calves (10 animals in each group) to obtain an estimate of normal values for muscle tissue and erythrocyte K content. The sample size of the control

groups was determined arbitrarily. Control calves for the determination of muscle K content and muscle tissue DM in healthy animals were chosen from the pool of clinically healthy neonates of the Clinic for Cattle obtained by C-section. Values of erythrocyte K content were obtained from an unrelated study conducted under field conditions that investigated the development of erythrocyte electrolyte content in healthy dairy calves in the first weeks of life. Samples from this data set were selected to match the age distribution of the group of diarrheic calves. Calves serving as control animals were determined to be healthy based on thorough physical examination.

Housing and Feeding

Diarrheic calves were housed in individual calf stalls on rubber mats, copiously bedded with straw in a temperature-controlled facility. Control calves were housed in individual hutches located outside and copiously bedded with straw; calves had free access to water and were fed 2 L of whole milk 4 times daily.

Calves with diarrhea were offered 2 L of a commercial milk replacer (MR; Sprayfo Excellent, Sloten GmbH, Diepholz, Germany) from a nipple bucket 4 times per day. A custom-made oral electrolyte solution (OES) containing 20.1 g of dextrose, 3.9 g of NaCl, 3 g of KHCO_3 , and 3 g of Na-propionate per L (strong ion difference = 74 mEq/L) also offered from a nipple bucket was accessible ad libitum between meals. Consumption of MR and OES was recorded. Clean water offered from a bucket was available at all times, and calves had ad libitum access to hay and water.

Standard Procedures

At admission, calves underwent a complete physical exam, were weighed on an electronic scale, and were evaluated for inclusion and exclusion criteria. Blood was collected by venipuncture from a jugular vein (Strauss-Kanüle, 17G, Dispomed Witt oHG, Gelnhausen, Germany) in lithium heparin and $\text{K}_3\text{-EDTA}$ tubes. Blood gas capillaries were filled by directly inserting the capillaries into the conus of the needle. Blood samples were processed as described below. Blood from calves serving as controls for the erythrocyte K content was obtained by venipuncture from a jugular vein as described for diarrheic calves.

An electrocardiogram (ECG, DIMEQ EKG 601, Bosch Healthcare Solutions, Waiblingen, Germany) was recorded by base-apex derivation as described earlier in ruminants (DeRoth, 1980; Grünberg et al., 2015).

Muscle biopsies were obtained from the vastus lateralis of the musculus quadriceps femoris using a

sampling technique described for humans and cattle that was modified for the use in calves (Tarnopolsky et al., 2011; Grünberg et al., 2015). Briefly, the biopsy site (halfway between hip and stifle joint; 3 cm cranial of the femoral bone) was shaven, surgically prepared, and anesthetized subcutaneously with 2 mL of 2% procaine with adrenaline (Isocain 2%, Selectavet Dr. Otto Fischer GmbH, Weyarn-Holzolling, Germany). A small stab incision through skin and muscle fascia was then placed with a scalpel. A suction-modified Bergstrom muscle biopsy needle (6 mm in diameter, Walther Veterinärinstrumente, Baruth, Germany) was advanced into the muscle tissue through the incision to obtain muscle tissue by suction. Harvested tissue was immediately submersed into liquid nitrogen to prevent dehydration. Frozen specimens were kept at -80°C until further processing. Each time a biopsy was performed, 2 specimens of similar size were obtained to measure muscle K content and DM. Muscle biopsies in control calves were performed in the same way.

After completion of the initial sample collection, a standard intravenous treatment was initiated. For this, a 22-gauge catheter was placed aseptically in an ear vein (Vasofix Braunüle G 22, B. Braun, Melsungen, Germany) or, in one case, in a jugular vein using a 17-gauge catheter (Vasofix Braunüle G 17, B. Braun). Calves were administered an intravenous bolus infusion of hypertonic (8.4%) sodium bicarbonate solution (HSB; Natriumhydrogencarbonat 8.4% Infusionslösung, B. Braun) for correction of acidemia. The amount of HSB to be administered was calculated using the following equation (Smith and Berchtold, 2014): $8.4\% \text{ HSB (mL)} = \text{BE} \times 0.6 \times \text{body mass (kg)}$. Independently of the degree of acidemia and body mass of the calf, the volume of the bolus infusion was capped at 500 mL of 8.4% HSB. Immediately after bolus infusion, a standard drip infusion (5 L of 0.9% NaCl + 250 mL of 8.4% HSB + 60 mL of dextrose 40%; Smith and Berchtold, 2014) was administered at a variable infusion rate. The infusion rate was adjusted over time in accordance to the degree of dehydration and voluntary oral fluid consumption. All calves received a treatment with 0.5 mg/kg of meloxicam (Melosolute 20 mg/mL, CP-Pharma, Burgdorf, Germany) at the onset of treatment. The use of antimicrobial drugs, any complementary treatment, or a deviation of the treatment protocol during the study period was at the discretion of the clinician on duty when deemed necessary in a specific case. Calves included in this study only received K contained in the MR or OES. Deviations from the protocol were recorded such that retrospective elimination from the study was possible in some circumstances (e.g., administration of blood transfusion).

Follow-up examinations were performed twice daily; general demeanor, hydration, and oral intake of MR and OES were assessed. Repeated blood biochemical and gas analyses were conducted at the discretion of the clinician on duty. Treatment with parenteral rehydration was continued until a self-sustained state was reached.

A second set of samples and analyses (blood, ECG, muscular biopsy from the opposite limb) was obtained after successful correction of hydration and acidemia and discontinuation of the parenteral fluid therapy. To be eligible for the post-treatment sampling, calves had to be considered entirely rehydrated based on clinical examination, have a BE between -5 and $+10$ mmol/L, be off parenteral fluids for at least 12 h, and be capable of sustaining hydration and acid-base homeostasis exclusively by oral uptake of MR, water, and OES. Resolution of diarrhea was not a requirement for eligibility for the collection of the second set of samples. Post-treatment samples were always collected between 0600 and 0800 h after at least 8 h without consumption of MR to minimize interference of feeding on the homeostasis of electrolytes and hydration. Calves had completed the study after collection of post-treatment samples but remained in the clinic until resolution of the diarrhea and complete recovery. This protocol produced 2 data sets, designated T_0 for the acidemic and dehydrated calf before any therapeutic intervention, and T_1 for the rehydrated calf with a physiological BE.

Sample Processing and Analysis

Venous blood gas analysis was conducted calf-side using a handheld blood gas analyzer (epoc Host² and epoc Reader, Alere GmbH, Cologne, Germany). Results were corrected for rectal temperature. The analyzer measures pH, partial pressure of CO_2 , and partial pressure of O_2 by direct potentiometry, and L-lactate through amperometry; and calculates the temperature-corrected pH, BE, and actual bicarbonate using a standard algorithm (Alere North America, 2017).

The packed cell volume (PCV) was determined immediately via capillary centrifugation (Hettich Haematokrit centrifuge, Hettich, Kirchlingern, Germany). A hemolysate of whole blood was prepared by thoroughly mixing 1 mL of lithium heparinized blood with 5 mL of deionized water. It was stored at -20°C until analyzed for [K] by inductively coupled plasma optical emission spectrometry.

Plasma was obtained by centrifuging lithium heparinized blood ($3,500 \times g$ for 10 min) within 10 min of sample collection. Harvested plasma was immediately submitted to the diagnostic laboratory and analyzed

for plasma [K], plasma sodium concentration ([Na]), and plasma chloride concentration ([Cl]) by indirect potentiometry. Total protein (**TP**, biuret) and plasma creatinine ([**CREA**] picric acid) were determined on an automated analyzer (ABX Pentra 400, Horiba Europe GmbH, Langenhagen, Germany). D-Lactate was determined enzymatically (combined D-LDH and D-GPT method). K₃-EDTA blood was submitted to the diagnostic laboratory for hematological examination of mean corpuscular volume (**MCV**, V/HC-cytography), hemoglobin (cyanide method), and erythrocyte count (Flowcell technology, Advia 120 Hematology System, Siemens Healthcare GmbH, Erlangen, Germany).

One of the 2 muscle biopsy specimens was processed using a method similar to that used on muscle tissue of adult cattle (Grünberg et al., 2015). The K content in muscle [**K_{MUSC}**; wet weight (**WW**) basis] was determined by inductively coupled plasma optical emission spectrometry (Braselton et al., 1997; Grünberg et al., 2015). The other biopsy of the same sampling time was used for determination of muscle DM by drying to constant weight at 85°C.

Paper printouts of the ECG recordings were screened for the presence of arrhythmias and used to determine the heart rate; average length of P and T waves; Rs complex; PR and ST segments; PR, QT, TQ, TP, and R-R intervals.

Data Analysis

From each patient, the age at admission and duration of diarrhea (**DD**) before admission as per patient owner were obtained and included in the data set. From daily MR and OES consumption, the total amount of K consumed between both sampling times (**K_{ORAL-TOT}**) as well as the mean oral K consumption per 24 h (**K_{ORAL-24}**) were calculated.

The erythrocyte K content (**K_{ERY}**, mmol/L erythrocytes) was derived from the [K] in plasma and hemolysate (mmol/L), and PCV (L/L) using following equation:

$$K_{ERY} = \frac{[(\text{hemolysate [K]} \times 6) - (1 - \text{PCV}) \times \text{plasma [K]}]}{\text{PCV}},$$

where the factor of 6 was used to account for dilution of the hemolysate in deionized water. A similar equation has been described and used previously (Evans, 1957).

Muscle K content in DM (**K_{MUSC-DM}**, μmol/g of DM) was derived from **K_{MUSC}** and DM (%) using the following equation:

$$K_{MUSC-DM} = \frac{K_{MUSC}}{DM}.$$

The delta (Δ) of total protein (**ΔTP**) and sodium (**ΔNa**) (x) were calculated using the equation

$$\Delta x = x(T_0) - x(T_1).$$

The percentage change in total protein (**%ΔTP**) was calculated (Naylor, 1987) as follows:

$$\% \Delta TP = [\Delta TP / TP(T_1)] \times 100;$$

ΔTP and %ΔTP (rather than TP) at T₀ were used to retrospectively quantify the degree of hemoconcentration because subnormal TP values, presumably resulting from failure of transfer of passive immunity, were common at T₁. It therefore seemed reasonable to use the difference in TP between T₀ and T₁ rather than TP at T₀ as a proxy for hydration status. The ΔNa was included in the multiple stepwise regression analyses to identify possible associations between changes in extracellular [Na] and [K] in the calves studied here.

Statistical Analysis

Data are presented as mean ± standard deviation (**SD**) or as median and interquartile range (**IQR**) in the case of non-normally distributed data. Normal distribution was assessed by evaluating the Shapiro-Wilk W value and visual inspection of quantile-quantile (QQ) plots. In case of non-normal distribution, log or square root transformations were used to achieve normal distribution. Paired *t*-tests were used to determine differences between T₀ and T₁ in treated animals. Differences between diarrheic calves and control animals were explored by means of the Student *t*-tests. To identify possible differences associated with hyper-D-lactatemia, the comparisons described above were also conducted for diarrheic calves with and without hyper-D-lactatemia separately. Presence of hyper-D-lactatemia was arbitrarily defined as a plasma D-lactate concentration ≥4.0 mmol/L (**D-lact+**), in agreement with other studies in diarrheic calves (Lorenz et al., 2003). Consequently animals with plasma D-lactate concentrations <4.0 mmol/L were considered as not having hyper-D-lactatemia (**D-lact−**).

Multiple stepwise regression analyses were conducted for each sampling time separately to identify factors with the strongest associations with plasma [K], **K_{MUSC}**, and **K_{ERY}**. Parameters included as independent variables

for the analysis of plasma [K] at T_0 were DD; age at admission; parameters related to acid-base equilibrium determined at the corresponding sampling time such as BE, pH, and D-Lact; parameters of intracellular K homeostasis such as K_{MUSC} and K_{ERY} of the corresponding sampling time; ΔTP and $\% \Delta TP$ as parameters for dehydration; and MCV to identify a possible effect of changes in erythrocyte volume that were observed in this study. For analysis of plasma [K] at T_1 , parameters reflecting oral K intake since admission ($K_{ORAL-TOT}$ and $K_{ORAL-24}$) were also included.

For K_{ERY} , parameters included as independent variables were DD, age at admissions, BE, pH, and D-lact determined at the same sampling time; plasma [K] and K_{MUSC} of the corresponding sampling time; ΔTP and $\% \Delta TP$; as well as MCV as an indicator for the intracellular volume of distribution. For the analysis of K_{ERY} at T_1 , $K_{ORAL-TOT}$ and $K_{ORAL-24}$ were included to identify a possible effect of oral K intake on K_{ERY} , and D-lact- T_0 was included to identify a possible carryover effect of hyper-D-lactatemia.

For the parameters K_{MUSC} and $K_{MUSC-DM}$, independent variables included in the analysis were again DD, age at admission, BE, pH, and D-lact of the same sampling time; plasma [K] and K_{ERY} of the corresponding sampling time; ΔTP and $\% \Delta TP$; plasma [CREA], as a parameter reflecting muscle mass and muscle metabolic activity; and DM as parameter for tissue water content and thus intracellular volume of distribution. For the analysis of K_{MUSC} at T_1 and $K_{MUSC-DM}$ at T_1 , $K_{ORAL-TOT}$, $K_{ORAL-24}$, and D-lact- T_0 were also included.

To identify potential mechanisms associated with the change in MCV between sampling times observed in this study, we conducted a multiple stepwise regression analysis with MCV as dependent variable and with age at admission, DD, pH, BE, D-lact, plasma [K], and K_{ERY} measured at the corresponding sampling time, as well as ΔTP and $\% \Delta TP$ as independent variables. The analysis of MCV at T_1 again included the parameters $K_{ORAL-TOT}$ and $K_{ORAL-24}$.

Pearson correlation analyses were conducted to identify possible correlations between independent variables included in the same multiple stepwise regression analysis. In the case of suspected covariance, the parameter showing the weaker association with the dependent variable was removed from the final regression model.

Retrospective power analyses were conducted to determine the required sample size to reach significance for the observed numerical difference in K_{MUSC} between sampling times and the smallest effect size detectable with the sample size of this study; $P < 0.05$ was considered significant. The SAS software package was used for statistical analysis (version 9.3; SAS Institute Inc., Cary, NC).

RESULTS

Thirty-three calves were considered eligible for inclusion at the time of admission. Two calves died before completion of the study and 4 calves were retrospectively excluded due to administration of a blood transfusion ($n = 3$) and occurrence of an abomasal displacement ($n = 1$), both events occurring before T_1 and potentially confounding the results obtained from these animals. Thus, data from 27 calves were included in the data analysis. In some instances, results from individual samples could not be included. This was the case for 1 K_3 -EDTA sample (animal no. 1; T_0) and 1 plasma sample (animal no. 10; T_0), which were hemolytic. Muscle tissue DM was only determined in 46 (T_0 $n = 22$, missing samples no. 1, 8, 9, 13, 20; T_1 $n = 24$, missing samples no. 7, 22, 27) of 54 biopsy samples because, in several instances, the second specimen was required for a repeated biochemical analysis.

The median [IQR] age and body mass at admission were 10 [7–10] d and 39 [36.6–44.0] kg, respectively. Breed distribution was 23 Holstein-Friesian (12 male, 11 female), 3 mixed breed (all female), and 1 Charolais (female). The time interval between collection of samples at T_0 and T_1 was 59.5 [45.00–65.25] h. In control animals for muscle and erythrocyte K contents, the median age was 13 [10–14] d and 14 [9–14] d, respectively. Control animals for erythrocyte K content were all female; the control group for muscle K content consisted of 2 male and 8 female calves. All control animals were Holstein-Friesian breed.

As defined per inclusion and sampling criteria, all calves presented with profound dehydration and acidemia at T_0 and had dehydration and acid-base disturbances that were corrected at T_1 . Values of BE, pH, PCV, TP, MCV, erythrocyte counts, [CREA], [Na], actual HCO_3^- , hemoglobin, and D-lact at T_0 and T_1 are summarized in Table 1; ΔTP was 12 ± 6.1 g/L, $\% \Delta TP$ was $22 \pm 11\%$, ΔNa was -3.44 ± 8.61 mmol/L, $K_{ORAL-TOT}$ was 986 ± 355 mmol, $K_{ORAL-24}$ was 393 ± 78 mmol/d, and DD was 2 [1–3] d.

At T_0 , 5 of 27 calves showed a D-lact of < 4 mmol/L (group D-lact–) and 22 of 27 calves a D-lact > 4 mmol/L (group D-lact+, range: 6.7–20.5 mmol/L). At T_1 , 4 of 27 calves had a D-lact > 4 mmol/L (range: 4.6–7.1 mmol/L), all of which also had pronounced hyper-D-lactatemia at T_0 (range: 11.5–14.9 mmol/L). Comparison of K_{ERY} and K_{MUSC} between groups D-lact– and D-lact+ at T_0 and T_1 revealed no significant group differences at the 2 sampling times. Plasma [K] was numerically higher in calves of group D-lact– than in calves of group D-lact+; however, the difference did not reach significance (Supplemental Figure S1; <https://doi.org/10.3168/jds.2018-14790>).

Table 1. Whole-blood and plasma values of calves ($n = 27$) with neonatal diarrhea before (T_0) and after correction of acidemia and dehydration (T_1)¹

Variable ²	T_0	T_1
pH	7.098 $\pm$ 0.1	7.367 $\pm$ 0.048****
BE (mmol/L)	-17.74 $\pm$ 4.83	3.34 $\pm$ 6.15****
HCO _{3 act} (mmol/L)	11.61 $\pm$ 3.51	28.17 $\pm$ 5.55****
Erythrocytes ($10^6/\mu\text{L}$)	9.81 $\pm$ 1.76 ³	7.75 $\pm$ 1.44****
MCV (fL)	38.71 $\pm$ 4.22 ³	35.61 $\pm$ 3.31****
PCV (L/L)	0.395 $\pm$ 0.0071	0.295 $\pm$ 0.056****
Hemoglobin (g/dL)	12.33 $\pm$ 2.34 ³	9.55 $\pm$ 1.98****
D-Lactate (mmol/L)	10.7 [7.19–13.8]	0.54 [0.03–3.54] ^{3,****}
Creatinine ($\mu\text{mol/L}$)	122 [92.8–226.0]	63.4 [58.0–68.2]****
Total protein (g/dL)	66.7 $\pm$ 9.44	54.7 $\pm$ 6.85****
Plasma [Na] (mmol/L)	130.74 $\pm$ 9.44	134.18 $\pm$ 4.23*

¹Values are reported as mean $\pm$ standard deviation or median [interquartile range].

²BE = base excess; HCO_{3 act} = actual bicarbonate; MCV = mean corpuscular volume; PCV = packed cell volume.

³ $n = 26$.

**** $P < 0.0001$ and * $P < 0.05$; values at T_1 differ significantly from the corresponding value at T_0 .

The K content in muscle (K_{MUSC}) at T_0 and T_1 was 79.84 ± 11.51 and 78.62 ± 11.93 $\mu\text{mol/g}$ of WW, respectively. The difference between the 2 sampling times was only numerical (1.23 ± 13.67 $\mu\text{mol/g}$ of WW, Figure 1). A retrospective power analyses determined the sample size of this study to be adequate to identify an effect size of 10% change in K_{MUSC} between sampling times. The effect size observed here would have required inclusion of over 1,300 calves to reach a level of significance. The K_{MUSC} in 10 control calves was 76.1 ± 23.52 $\mu\text{mol/g}$ of WW, and K_{MUSC} in control animals did not differ from K_{MUSC} in diarrheic calves at T_0 or T_1 . Muscle tissue DM did not differ between sampling times with $20.9 \pm 1.5\%$ of WW at T_0 ($n = 22$) and $20.1 \pm 1.0\%$ of WW at T_1 ($n = 24$) and did not differ from muscle tissue DM of control animals ($20.6 \pm 1.5\%$ WW).

The regression analysis identified the strongest associations of K_{MUSC} at T_0 ($n = 21$) with DD (negative association, partial $R^2 = 0.245$, $P = 0.0226$) and with plasma [CREA] at T_1 ($n = 22$, negative association, partial $R^2 = 0.274$, $P = 0.0104$) followed by a positive association with ΔTP (partial $R^2 = 0.246$, $P = 0.0045$) and a negative association with $K_{\text{ORAL-TOT}}$ (partial $R^2 = 0.121$, $P = 0.0205$). Similar to K_{MUSC} , mean $K_{\text{MUSC-DM}}$ at T_0 (387.4 ± 55.49 $\mu\text{mol/g}$ of DM) did not differ from that determined at T_1 (390.33 ± 49.29 $\mu\text{mol/g}$ of DM). The stepwise regression analysis at T_0 ($n = 21$) revealed a negative association with age at admission (partial $R^2 = 0.308$, $P = 0.009$) and at T_1 ($n = 24$) with plasma [CREA] (partial $R^2 = 0.292$, $P = 0.0064$).

The K content in erythrocytes (K_{ERY}) at T_0 was 73.63 ± 13.73 ($n = 25$) and 77.64 ± 15.97 ($n = 27$) mmol/L of erythrocytes at T_1 , with an average increase of 4.63 ± 9.13 mmol/L of erythrocytes from T_0 to T_1 ,

corresponding to an increase of $>5\%$ (Figure 1). As presented in Table 1, MCV concomitantly declined by 9%, on average, between T_0 and T_1 . The multiple stepwise regression analysis identified MCV as the only parameter associated with K_{ERY} at T_0 ($n = 25$; positive association, partial $R^2 = 0.345$, $P = 0.002$, Supplemental Figure S2; <https://doi.org/10.3168/jds.2018-14790>) and T_1 ($n = 26$; positive association, partial $R^2 = 0.300$, $P = 0.0038$, Supplemental Figure S2). Conversely, MCV as a dependent variable was strongly associated with K_{ERY} (partial $R^2 = 0.345$, $P = 0.002$, $n = 25$, Supplemental Figure S2) at T_0 but not at T_1 ($n = 26$). At T_1 , age at admission (negative association, partial $R^2 = 0.283$, $P = 0.0052$, Supplemental Figure S2) was identified as the only parameter associated with MCV.

In 10 control calves, K_{ERY} was 86.91 ± 20.1 mmol/L of erythrocytes. Comparison of K_{ERY} at both sampling times and K_{ERY} determined in control animals revealed no significant differences.

Plasma [K] at T_0 was 5.44 [4.76–6.17] mmol/L and decreased markedly to 4.16 [3.99–4.31] mmol/L at T_1 (Figure 1). At T_0 , 2 calves were hypokalemic (<4.13 mmol/L), 10 calves were normokalemic (4.13–5.41 mmol/L), and 14 were hyperkalemic (>5.41 mmol/L) when applying recently published reference values for calves (Dillane et al., 2018). At T_1 , hypo-, normo-, and hyperkalemia occurred in 13, 14, and 0 instances, respectively. The multiple stepwise regression analysis revealed the strongest associations of plasma [K] at T_0 ($n = 25$) with age of admission (negative association, partial $R^2 = 0.395$, $P = 0.0008$, Supplemental Figure S3; <https://doi.org/10.3168/jds.2018-14790>) and ΔTP (positive association, partial $R^2 = 0.163$, $P = 0.0094$, Supplemental Figure S3), whereas none of the parameters included were found to be associated with plasma

Figure 1. (A) Potassium content in muscle tissue wet weight (K_{MUSC}) in diarrheic calves before (T_0) and after (T_1) correction of dehydration and acidemia; values at the 2 sampling times do not differ significantly ($n = 27$). Horizontal dotted lines indicate the upper and lower limits of the reference of plasma [K] in calves (Dillane et al., 2018). (B) Erythrocyte K content (K_{ERY}) in diarrheic calves at T_0 and T_1 ; values at the 2 sampling times differ significantly ($n = 25$; $P = 0.018$). (C) Plasma [K] in diarrheic calves at T_0 and T_1 ; values at the 2 sampling times differ significantly ($n = 26$; $P < 0.0001$). Box and whisker plots represent median (horizontal line), lower and upper quartiles (bottom and top of box respectively) as well as minimum and maximum (lower and upper end of whiskers, respectively).

[K] at T_1 ($n = 27$). A scatterplot of plasma [K] at T_0 by age at admission indicates that supraphysiological plasma [K] occurred only in diarrheic calves up to 10 d of age (Supplemental Figure S3). Dividing calves into groups from 2 to 10 d and >10 d yielded a likelihood ratio of 3.75 ($P = 0.0527$) for diarrheic calves up to 10 d of age to be hyperkalemic.

Cardiac arrhythmia associated with mild bradycardia (53 and 57 beats per min) was diagnosed in 2 calves at admission. In both cases, the presence of arrhythmia was associated with markedly elevated plasma [K] (8.68 and 9.16 mmol/L), and ECG analysis in both cases was consistent with atrial fibrillation (Supplemental Figure S4; <https://doi.org/10.3168/jds.2018-14790>; Fregin, 1992). The analysis of ECG results including all 25 animals (excluding 2 calves with cardiac arrhythmia) yielded differences between T_0 and T_1 in length of the P wave (78 ± 17.2 and 66.8 ± 9 ms at T_0 and T_1 , respectively; $P = 0.003$) and ST segment (67 ± 33.4 and 93.2 ± 26 ms, respectively $P < 0.0001$). The stepwise regression analysis identified associations of the plasma [K] with the ST segment (partial $R^2 = 0.3037$, $P < 0.0001$) and the length of the P wave (partial $R^2 = 0.0638$, $P = 0.0365$) at T_0 .

DISCUSSION

Neonatal diarrhea in calves is widely held to be associated with total body K depletion (Sweeney, 1999; Smith and Berchtold, 2014; Trefz et al., 2015a,b). Further to recent reports documenting the effect of dehydration and, to a lesser extent, acidemia on extracellular K homeostasis in diarrheic calves (Trefz et al., 2013a; Trefz and Lorenz, 2017), we proposed to study how dehydration and acidemia, the 2 most important metabolic disturbances occurring in diarrheic calves, affect intracellular K homeostasis of diarrheic calves. The most important finding of this study was that although we studied animals with the most pronounced disturbances of water and acid-base balance, we observed neither marked K depletion of muscle tissue nor an effect of dehydration or acidemia on intracellular K homeostasis.

Intracellular K homeostasis was studied in 2 distinct tissues: striated muscle and red blood cells. Striated muscle tissue was chosen as a surrogate for the intracellular space because muscle not only contains considerable amounts of K but also accounts for between 30 and 50% of total body mass (Breidenstein et al., 1968). Accordingly, it has been proposed that muscle tissue contains 75% of whole-body K (Rose, 1981; Palmer and Clegg, 2016). Thus, biologically relevant body K depletion, as reported in diarrheic calves, should be reflected by changes in muscle tissue K content (Constable et

al., 2013). Erythrocytes, in contrast, were chosen for convenience, because red blood cells are also rich in K and are easy to collect and process for analysis of intracellular K content (Schneider et al., 2016).

The K contents in striated muscle tissue measured in this study are in good agreement with previously determined values in adult cattle and healthy calves (Kowalczyk and Mayer, 1972; Lewis and Phillips, 1973; Grünberg et al., 2015; Schneider et al., 2016). The values for muscle tissue K content in this study showed a considerable degree of variation in both diarrheic and control animals, which is consistent with the degree of variation and the range of values reported in earlier studies in adult and juvenile cattle (Schnabel et al., 1987; Constable et al., 2014; Schneider et al., 2016). Low variation in muscle tissue K (in the range of 5%) has been achieved when using animals of same sex, breed, and production stage that were fed the same TMR for several weeks before sample collection (Grünberg et al., 2015). Surprisingly, mean K_{MUSC} did not differ between time points—before (T_0) and after correction (T_1) of acidemia and dehydration—and did not differ from values determined in healthy control calves. This suggests that in these diarrheic calves, pronounced changes in intracellular K homeostasis did not occur, at least in muscle tissue. This finding is in contrast to an earlier study that reported a decrease in intracellular K content of muscle tissue of approximately 12% occurring within hours of experimentally induced diarrhea in calves (Lewis and Phillips, 1973). The main difference between the current study and that of Lewis and Phillips (1973) is that our study used animals with naturally occurring diarrhea, whereas the earlier study used calves with experimentally induced diarrhea. Furthermore, Lewis and Phillips (1973) was based on a small sample size (6 animals). Experimental induction of diarrhea has an important advantage in that samples obtained at the time of diarrhea can be compared with samples obtained in a healthy state and are unbiased by treatment. Also, experimental animals serve as their own control, eliminating the issue of inter-individual variation, and animals can be sampled repeatedly during the course of disease, providing an estimate of the kinetics of changes in K homeostasis occurring as disease progresses. An important disadvantage of this approach, however, is the uncertainty about how a specific model for experimental induction of diarrhea reflects changes occurring with naturally occurring diarrhea. Animals included in this study presumably represent a more diverse range of etiological agents with potentially varying pathophysiology of diarrhea.

In addition to the unchanged muscle tissue K content, muscle tissue water content remained constant and did not differ from values determined in healthy

control calves. These results corroborate the concept that dehydration in diarrheic calves occurs primarily at the extracellular level (Fayet, 1971; Phillips and Lewis, 1973; Thornton and English, 1978; Hartmann et al., 1984) and indicate that changes in the muscle tissue K homeostasis were not masked by changes in muscle tissue water content.

The results of the multiple stepwise regression analysis conducted to explore potential associations with K content in striated muscle tissue revealed that neither dehydration nor acidemia measurably affected the muscle tissue K content at T_0 . Furthermore, K_{MUSC} did not appear to be affected by the severity of D-hyperlactatemia as has been reported for plasma [K] (Trefz et al., 2013a). Calves included in the present study covered a broad range of plasma D-lactate concentration; only a few animals in this study had plasma D-lactate concentrations <4 mmol/L and thus were considered D-lact-. Although hyper-D-lactatemia is observed in most calves with neonatal diarrhea (Omole et al., 2001; Lorenz, 2004), it may be worth exploring whether the findings of the present study can be reproduced in future studies using a larger number of calves without D-lactic acidemia.

We did identify a negative association between K_{MUSC} and days of diarrhea before admission, suggesting that muscle tissue K depletion occurred as the disease persisted. Earlier studies report a similar association between duration of disease and duration of feed intake depression with plasma [K] (Trefz et al., 2015b). It remains to be determined whether the observed association between duration of diarrhea and muscle K content is specific to diarrhea or whether it is a nonspecific effect of diseases attributable to anorexia.

In the present study, K_{MUSC} at T_1 was associated with the plasma creatinine concentration. As reported in other species, muscular contraction is associated with compartmental shifts of K from the intracellular space and increased release of creatinine (Calles-Escandon et al., 1984; Sjøgaard, 1990). The association of muscle tissue K and plasma creatinine occurred in rehydrated and nonacidemic calves, suggesting that this association is based on a physiological process and is not attributable to dehydration or acidemia (Constable et al., 2013).

A small but significant increase ($\sim 5\%$) after rehydration and correction of acidemia was observed for K_{ERY} , an effect that was accompanied by a decline in MCV of $\sim 9\%$. Lower K content in erythrocytes of dehydrated calves may not necessarily be caused by K loss from erythrocytes, but could result from K dilution in erythrocytes with larger cytosolic volume. An increase of cell volume resulting from compartmental fluid shifts toward the intracellular space due to disturbances of

the osmotic equilibrium is well established for other tissues and may be the underlying cause of increases of erythrocyte volume (Nguyen and Kurtz, 2006). Despite the significant effect over time, K_{ERY} in diarrheic calves remained in the range of values determined in control calves throughout the study, suggesting that the observed effect is of minor clinical relevance. Similar to the results for K content in muscle tissue, the findings of this study indicate that neither acid-base disturbance nor dehydration affected erythrocyte K content at T_0 .

Because of the number of factors complicating the interpretation of simple changes of erythrocyte K content, we consider red blood cells a poor surrogate tissue to estimate changes in intracellular K content, at least in neonatal calves.

Plasma [K] values and the occurrence of normo-, hyper-, and hypokalemia in dehydrated and rehydrated calves are in good agreement with previous studies and confirm that extracellular K depletion does not typically occur in calves with neonatal diarrhea (Lewis and Phillips, 1973; Groutides and Michell, 1990; Trefz et al., 2013a,b). An interesting finding of this study was that the occurrence of hyperkalemia tended to be associated with age, with pronounced hyperkalemia occurring primarily in calves up to 10 d of age, an effect that did not appear to be confounded by D-lactate (Supplemental Figure S3; <https://doi.org/10.3168/jds.2018-14790>). This finding, which does not seem to have been reported previously, deserves to be studied and confirmed on a larger data set.

Recent studies have shown that the predominant effects responsible for hyperkalemia in dehydrated calves are decreased renal perfusion (and thus reduced renal K excretion) and, to a much lesser extent, disturbances of the acid-base balance, resulting in compartmental K shifts (Trefz et al., 2013a; Trefz and Lorenz, 2017). Therefore, even marked increases of the plasma [K] of 3 mmol/L or more without a measurable decline in muscle tissue K content, as observed in the present study, are plausible.

Hypokalemia after correction of dehydration and acidemia was recorded in nearly 50% of study animals in the present study, which is higher than reported in earlier studies (Trefz et al., 2015b). We attribute this result to 2 factors. First, sample collection for T_1 occurred at standardized times in the morning and always shortly before feeding. Calves had thus not consumed milk replacer for at least 8 h and presumably were at the lowest diurnal level of the plasma [K], which is strongly and rapidly affected by feed intake. Furthermore, we applied a recently established reference range specific for calves with a higher threshold for plasma [K] (lower limit for plasma [K] of 4.13 mmol/L; Dillane et al., 2018). Earlier studies used references ranges for

cattle that typically have a lower limit of 3.9 mmol/L (Kaneko et al., 2008). In the present study, hypokalemia at T_1 was so mild in 50% of affected calves that these animals would have been classified as normokalemic when applying 3.9 instead of 4.1 mmol/L as the threshold for hypokalemia.

Considering the results of intracellular and extracellular K homeostasis obtained in this study, we found no indication of the generally accepted concept that neonatal diarrhea is associated with clinically relevant depletion of the body K pool. Furthermore, in contrast to well-established effects on extracellular K homeostasis (Trefz et al., 2013a), intracellular K homeostasis seems to be unaffected by dehydration, acidemia, or the type of acidosis involved. A study of K balance in neonatal diarrheic calves reported that these animals have a negative K balance because more K is lost than is ingested through the diet (Lewis and Phillips, 1972). A negative K balance, however, is not necessarily associated with body K depletion, particularly if it is associated with concomitant tissue atrophy and a reduction of the intracellular volume of distribution (Blaxter and Wood, 1953), as is likely to occur in states of disease and anorexia (Sweeney, 1999). Supplementation of K to correct K depletion would be warranted with a physiologically relevant reduction of the K content in one or several compartments.

Not all animals developed signs of bradycardia or arrhythmia despite showing elevated plasma [K]. As previously reported, hyperkalemia increases the risk of bradycardia and arrhythmia, but does not inevitably cause them (Fisher, 1965; Montague et al., 2008; Trefz et al., 2013b). In humans, as in cattle, plasma K levels do not correlate well with specific types of ECG derangement but more commonly result in ECG readings related to disturbances in development and distribution of the transmembrane potential in cardiac tissue and thus remain variable in manifestation (Fisher, 1965; Montague et al., 2008). As reported in earlier studies, pronounced bradycardia and arrhythmia indicate pronounced and potentially life-threatening hyperkalemia (Lewis and Phillips, 1973; Weldon et al., 1992; Trefz et al., 2013b). Virtually any arrhythmia or conduction disturbance has been reported to occur in states of hyperkalemia (Alfonzo et al., 2006).

Basing this study on cases of naturally occurring diarrhea with profound disturbances of water and acid-base balance means that results are unlikely to be confounded by artifacts of models for experimental induction of diarrhea. This approach, however, precludes the possibility of obtaining baseline samples before disease occurs. Samples obtained at the time of severe dehydration were compared with samples obtained after restoration of the water and acid-base balance. Nev-

ertheless, animals in this study were still on OES and in several instances still had diarrhea, so that a steady state of water- and acid-base balance of a healthy animal may not yet have been reached. This limitation was addressed by including age-matched healthy control animals. Importantly, the inclusion criteria preselected calves with the most severe disturbances of hydration and acid-base balance. Based on our clinical experience, we believe that most calves included in this study would have died within hours if left untreated. We assume, therefore, that if clinically relevant disturbances of intracellular K homeostasis occurred in calves with dehydration and acidemia, these would have been readily identifiable at the time of admission in this group of most severely affected calves.

Another limitation of the study presented here is the uncertainty of the effect of treatments that calves may have received before admission. In particular, the consumption of OES could not be reliably quantified from patient history and thus remained entirely unconsidered. It is conceivable that, in some cases, muscle tissue K depletion may have been corrected or alleviated by oral K supplementation before admission. It is worth noting, however, that at admission all study calves had muscle tissue K contents within a range that has been reported in healthy calves and adult cattle (Schnabel et al., 1987). Furthermore, the monitored consumption of considerable amounts of K in the form of OES during the study did not alter the—admittedly not markedly decreased—muscle tissue K content between the 2 sampling times.

CONCLUSIONS

This study on the intracellular K homeostasis in striated muscle tissue and erythrocytes indicated that diarrhea associated with profound dehydration and acidemia is not associated with total body K depletion in calves suffering from neonatal diarrhea. Furthermore, in contrast to what is reported for extracellular K balance, neither dehydration nor acidemia seemed to markedly affect intracellular K balance in muscle or erythrocytes. We conclude that red blood cells are poorly suited as a proxy for the intracellular space, particularly in neonatal calves, due to age-dependent effects that may confound results obtained in these animals.

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