Eur J Nutr (2005) 44 : 499–508
DOI 10.1007/s00394-005-0553-z

ORIGINAL CONTRIBUTION

Heidrun Kiwull-Schöne
Hermann Kalhoff
Friedrich Manz
Peter Kiwull

Food mineral composition and acid-base balance in rabbits

Summary *Background* Alkali-rich diets are often recommended in human medicine to prevent the pathological consequences of nutritional acid load in conditions of impaired renal function. *Aim of the study* This study was undertaken in rabbits as common laboratory animals for basic medical research to explore the impact of high versus low dietary alkali intake on systemic acid-base balance and renal control in a typical herbivore. *Methods* Male rabbits (2.3–4.8 kg) were kept in a metabolism cage. The 24 h urine and arterial blood samples were analysed for acid-base data. The metabolic CO_2 production was measured to calculate alveolar ventilation. Three randomized groups of animals were fed ad libitum with rabbit chow providing sufficient energy but variable alkali load, assessed by the ashes' cation-anion difference. *Results* The average daily nutritional alkali load (± SEM) was 67.1 ± 2.2 mEq · kg^{-1} (N = 58) in the group on high, 45.4 ± 2.5 mEq · kg^{-1} (N = 31) in the group on normal and 1.7 ± 0.5 mEq · kg^{-1} (N = 11) in the group on low alkali food. Respective mean arterial base excess values (BE) were 1.4 ± 0.3 mM, 0.3 ± 0.4 mM and 0.0 ± 0.3 mM, being significantly higher on high alkali food ($P < 0.05$) than in the other groups. Arterial PCO_2, alveolar ventilation and metabolic CO_2 production were not significantly different between groups. On normal and high-alkali chow, an alkaline urine ($pH_u > 8.0$) with 18–20 mmol · kg^{-1} bicarbonate/carbonate was excreted daily, typically containing an insoluble precipitate of 35–60 % carbonate. On low-alkali diet, the mean pH_u decreased to 6.26 ± 0.14, due to a strong reduction of daily excreted soluble bicarbonate and precipitated carbonate to 1.2 ± 0.6 and 0.7 ± 0.2 mmol · kg^{-1}, respectively. Thereby, nearly complete fractional base reabsorption of 97.8 ± 0.7 % was reached. *Conclusion* Herbivore nutritional alkali-load elicited large rates of renal base excretion including precipitates, to which the urinary tract of the rabbits appeared to be adapted. Dietary base variations were more accurately reflected in the urine than by the blood acid-base status. A strongly base-deficient diet exerted maximum impact on renal base saving mechanisms, implying a critical precondition for growing susceptibility to metabolic acidosis also in the rabbit.

Key words nutrition – alkali load – acid-base balance – renal base excretion – urinary precipitate – rabbits

Received: 14 July 2004
Accepted: 20 December 2004
Published online: 9 February 2005

Dr. H. Kiwull-Schöne, M.D. (✉) · P. Kiwull
Dept. of Physiology
Faculty of Medicine
Ruhr-University
44780 Bochum, Germany
Tel.: +49-234/32-24886
Fax: +49-234/32-14733
E-Mail: heidrun.kiwull-schoene@rub.de

H. Kalhoff
Paediatric Clinic
Dortmund, Germany

F. Manz
Research Institute of Child Nutrition
Dortmund, Germany

Introduction

The rabbit is a common laboratory animal for basic medical research in respiratory and renal physiology. Thus, handbook data of this species are available under comparative aspects to other species [1, 2] as well as with special regard to rabbit anatomy, physiology, biochemistry and nutrition [3, 4] or veterinary medicine [5, 6].

However, less attention has been paid in laboratory rabbits to the role of food mineral uptake for adaptive functions of acid-base balance. Since most laboratories

© Steinkopff Verlag 2005

share the feeding standards of their central animal care units, a non-intended variability in food composition may influence basic experimental conditions. In medical animal research, the importance of feed mineral composition and uptake for acid-base balance is often ignored. Many research groups use only general statements like "pelleted feed" [7], "commercial rabbit laboratory diet" [8] or "standard rabbit chow" [9–11]. There are only a few reports on the exact composition and/or name of the feed, by which rabbits are pretreated before experiments [12–16], showing marked differences in the mineral composition of the standard feed given by these research groups. Laboratory rabbit pellet chow, adapted to the herbivore nutrition habit of this species, is characterized by an alkaline ash, the sum of fixed cations exceeding that of fixed anions, providing a measure of dietary alkalinity or "potential bicarbonate" [12, 17].

Thus, the rabbit appears as a suitable animal model for strict herbivore nutrition and may help to understand adverse effects due to extreme base excretion and high urinary pH, e. g. for urinary tract infection [18] and/or carbonate stone formation [19]. This may be of special interest, when alkali-rich diets or therapies are recommended in human medicine to prevent the pathological consequences of nutritional acid load in humans in conditions with impaired renal function, e. g. due to immaturity in preterm infants [20, 21] or to regression in elderly persons [22].

Rabbits normally adapted to alkali-rich nutrition were also investigated for systemic or renal responses to chronic metabolic acidosis [12–15, 23, 24]. This was experimentally attempted by ingestion of HCl or NH_4Cl, but could be achieved only, when normal feed was withheld, implying concomitant energy deficiency.

The aim of the present study is threefold: First, to obtain representative values for the acid-base status in blood and urine under the influence of spontaneous variations in normal nutritional alkali load without invasive instrumentation in conscious rabbits; second, to examine the role of high alkali load for urinary pH, bicarbonate excretion and formation of precipitated carbonates, and third to investigate the possible role of strongly reduced dietary alkali load (at normal energy intake) for the development of metabolic acidosis.

Materials and methods

A total of 92 healthy adult male conscious rabbits (Chinchilla Bastard) were investigated, provided by a commercial breeder (Charles River, Germany). The "Principles of laboratory animal care" (NIH 1985) were followed, and experiments were approved on March 14, 2000 and January 31, 2003 by the regional government Arnsberg, according to the "German Law on the Protection of Animals". The animals were kept in the central animal care unit of the department on two different types of standard rabbit chow. On unchanged type of food, they were randomly selected for measurements and individually accustomed to a metabolism cage (EBECO, Castrop-Rauxel, Germany) for at least 5 days. Animals were kept in natural light-dark cycles on food and water ad libitum, 75 % of the studies being performed in September to March. Daily food consumption, water intake and urine excretion were supervised.

For the study, 60 rabbits received high-energy, alkali (Ca^{++})-rich pellets (*Matador* Kanin 4, Matador Mischfutter GmbH, Recklinghausen, Germany), as usually given by local rabbit breeders, while 32 rabbits were fed lower calorie pellets with normal alkali content (*Altromin* 2123, Altromin GmbH, Lage, Germany), primarily to control bodyweight in laboratory animals restricted from their natural mobility. In addition, a subgroup of 11 randomly selected animals received a commercial high-calorie/low-alkali mixture for five days, to assess the role of alkali and energy content separately. This mixture, also being used in private rabbit husbandry, consisted of peanuts, cornflakes and carob-tree-fruit-skin (PCC) for choice ad libitum. The electrolyte composition of the feed pellets and the diet was determined by analysis of ashes in co-operation with the Research Institute of Child Nutrition, Dortmund, and the Institute for Animal Health and Food Quality, Kiel (Table 1).

Blood analysis

Arterial blood samples were taken from the central ear artery under superficial local anaesthesia, without any stress to the animal [25]. During blood sampling, most animals inhaled O_2-enriched air (F_IO_2: 0.4–0.6), to minimize peripheral chemoreflex responses. Determination of blood gases and acid-base status was performed at 38 °C. Arterial pH (pHa) and partial pressures of oxygen or carbon dioxide (PaO_2, $PaCO_2$) were measured by conventional electrodes (ABL 5 Radiometer, Copenhagen, Denmark). To assess the whole blood CO_2-buffering capacity ($\beta = \Delta PaCO_2/\Delta pHa$), standard bicarbonate concentration (HCO_3^-st) and base excess (BE), the relationship between $PaCO_2$ and pHa was determined directly by means of the two-gas equilibration method [26], using the BMS2 Mk2 blood microsystem and PHM 84 Research pH meter (Radiometer, Copenhagen, Denmark) together with a precision gas mixing pump (Wösthoff, Bochum, Germany). The actual bicarbonate concentration was calculated by the Henderson-Hasselbalch equation (38 °C, $pK_1' = 6.102$, solubility of CO_2 $S = 0.030$ $mM \cdot mmHg^{-1}$).

Concentrations of lactate (Lac^-) and haemoglobin (Hb) were determined photometrically by commercial test combinations (Labor + Technik Eberhard Lehmann,

Table 1 Electrolyte composition and energy content of rabbit feed

Electrolytes		Matador High alkali	Altromin Normal alkali	PCC Low alkali
Sodium (Na^+)	[mEq/100 g]	5.79	9.38	0.61
Potassium (K^+)	[mEq/100 g]	39.55	51.31	15.34
Magnesium (Mg^{++})	[mEq/100 g]	18.63	20.01	8.30
Calcium (Ca^{++})	[mEq/100 g]	151.24	78.26	7.10
Total fixed cations	[mEq/100 g]	215.21	158.96	31.35
Phosphorus (P_i)*	[mEq/100 g]	32.85	31.57	12.91
Chloride (Cl^-)	[mEq/100 g]	9.46	9.60	2.31
Sulfate (SO_4^{--})	[mEq/100 g]	2.91	4.44	1.04
Total fixed anions	[mEq/100 g]	45.22	45.61	16.26
Cations – Anions	[mEq/100 g]	170.00	113.35	15.09
Energy	[kJ/100 g]	1026	628	1746
Protein	[% of energy]	26	38	17
Fat	[% of energy]	4	9	22
Carbohydrates	[% of energy]	70	53	60

The table shows the average electrolyte and energy content of two different standard rabbit feed pellets with high and normal alkali load, as well as that of a particularly alkali-poor diet, consisting of **p**eanuts, **c**ornflakes and **c**arob-tree-fruit-skin (cellulose). The alkalinity of the feed was estimated by "fixed cations" exceeding "fixed anions" in the ash [12, 17].
* P_i [mEq] = 1.8 mmol

Berlin, and Merkotest®, E. Merck, Darmstadt, Germany; photometer Hitachi-100–10, Japan). From centrifugates of the same blood samples, serum electrolyte and creatinine concentrations were determined in cooperation with the laboratory of the Paediatric Clinic, Dortmund.

Assessment of metabolic rate and alveolar ventilation

The CO_2 production ($\dot{V}CO_2$) was determined by placing the animals for 25 min (10 min adaptation, 15 min measurement) into a plexiglas box, sufficiently flooded with room-air. The expired CO_2 was bound to barium hydroxide $Ba(OH)_2$, thereby being changed to barium carbonate $BaCO_3$. $Ba(OH)_2$ concentrations before and after this reaction were determined titrimetrically by adding 0.1 N HCl (pH electrode and Titrator DL 70 ES, Mettler-Toledo, Gießen, Germany). The difference equivalent to mmoles of CO_2 produced during the observation time was converted to [ml · min^{-1}] under standard conditions for pressure and temperature (STPD) giving alveolar ventilation ($\dot{V}_A$) at body temperature and saturated water vapour (BTPS) as $\dot{V}_{A\ BTPS} = 863 \cdot (\dot{V}CO_{2\ STPD}/PaCO_2)$.

Urine analysis

The excreted 24 h urine was collected anaerobically under a (30 mm) paraffin oil layer, in order to prevent the loss of carbon dioxide. Anaerobic conditions were verified by measurements in equally treated bicarbonate/ammonium standard solutions. Contamination by feed or faeces particles was avoided by the wire-mesh bottom of the metabolism cage. Microbial contamination was prevented by every day cleaning and disinfecting the cage and by performing the analyses immediately after sampling. Supervision was accomplished by screening for nitrite (Medi-Test, Combi 7, Macherey-Nagel, Düren, Germany). All measurements in urine samples were determined in duplicate at room temperature. Unless duplicate determinations revealed differences below 3 %, measurements were repeated.

Since the urine contained a considerable amount of precipitate, it was stirred and an aliquot of 9 ml was centrifuged anaerobically for separate analysis of supernatant and precipitate. The acid-base status of the clear *supernatant* urine was determined titrimetrically [27], the initial pH value of the urine (pH_u) was measured, before adding 1 N hydrochloric acid to the urine. The mixture was boiled to drive off carbon dioxide and then titrated automatically with 0.1 N NaOH back to pH_u as the end-point, to obtain the equivalent of the total amount of bicarbonate and carbonate (pH electrode and Titrator DL 70 ES, Mettler-Toledo, Gießen, Germany). The titration difference for the range between 7.4 and actual pH_u above was addressed as titrable base (TB) and taken as an estimate of the bivalent carbonate portion, correspondingly that for the range between 7.4 and actual pH_u below was addressed as titrable acid (TA). The ammonium concentration was determined by adding 5 ml of 8 % formaldehyde, the ensuing drop in

© Steinkopff Verlag 2005

pH being back-titrated with 0.1 N NaOH [28]. The remaining supernatant urine was used for colorimetric analysis of inorganic phosphate [29] and creatinine concentrations (Labor + Technik Eberhard Lehmann, Berlin, Germany). The creatinine clearance served as an estimate of glomerular filtration rate (GFR).

The *precipitate* of two aliquots was dried for several hours at 60 °C, weighed and analysed for carbonate and phosphate. Carbonate was determined as the loss of CO_2 after adding 10–15 ml 1 N HCl and back-titrating with 1 N NaOH to equivalence. Phosphate was determined by colorimetry [29] after completely dissolving the remainder of the precipitate in 1 N HCl.

Statistical analysis

Data obtained from urine and blood samples were first averaged for each animal before calculating group mean values, standard deviations (SD), and standard errors of the mean (SEM). Variables were tested for normal distribution by the one-sample Kolmogorov-Smirnov test. Differences of mean values between groups were tested for significance by independent samples t-tests. The level of significance was taken as at least $P \leq 0.05$. The degree of correlation between variables was determined by linear regression analysis, regression lines being characterized by slopes, intercepts and 95 % mean confidence intervals. Statistical analysis was in part carried out by using SPSS 8.0 for Windows software (SPSS Inc. Chicago, IL, USA).

Results

Food intake and fluid balance

The energy content and the electrolyte composition of the different types of food are shown in Table 1. The usable energy of the mixed diet *(PCC)* was highest, followed by that of the high-calorie pellets *(Matador)*, being nearly twice that of the low-calorie pellets *(Altromin)*. The difference between "fixed cations" and "fixed anions" from the ash analysis provides a measure of dietary alkalinity or of "potential bicarbonate" [12, 17]. Considering this cation-anion difference, *Matador* provided the highest dietary alkali load (mainly due to the great amount of Ca^{++}), *Altromin* was about two thirds and *PCC* was only about one tenth. Besides food mineral composition, daily consumed "potential bicarbonate" is also determined by spontaneous food intake ad libitum. Two animals on high and one on normal alkali food spontaneously refused food intake for the 24 hours of observation and were excluded from the calculations in Fig. 3 and Tables 2–4.

The two groups of rabbits on the different types of standard pellet feed (*Matador* and *Altromin*) did not differ with respect to body weight, food and water intake or daily urine volume excretion (Table 2). The group on the low-alkali feed showed lower daily food intake than the others, but no difference in mean body weight and daily energy uptake, due to the high energy content of the diet. Although urinary volume excretion was not different, daily water intake and glomerular filtration rate (GFR) were significantly lower than in the other groups.

Metabolic and respiratory acid-base status in the arterial blood

The acid-base status in the arterial blood is shown by Table 3. Non-respiratory acid-base data tended towards alkalosis in the group on high-alkali food, values for HCO_3^-st and BE being significantly higher than those of the other groups. Only in the group on high-alkali food could significantly positive BE values be discerned, whereas in the groups on normal and low alimentary alkali load, metabolic acid-base conditions were balanced at zero BE. Individual BE values ranged between –6.5 and + 6.5 mM and those of HCO_3^-st between 19 and 29

Table 2 Daily food intake and fluid balance in rabbits on different types of feed

		Matador (N = 58) High alkali	Altromin (N = 31) Normal alkali	PCC (N = 11) Low alkali
Body weight	[kg]	3.45 ± 0.07	3.26 ± 0.10	3.34 ± 0.18
Food intake	$[g \cdot kg^{-1} \cdot d^{-1}]$	39.5 ± 1.3	39.5 ± 2.0	12.1 ± 1.2***
Energy intake	$[kJ \cdot kg^{-1} \cdot d^{-1}]$	405.3 ± 13.5***	248.5 ± 12.8	209.4 ± 47.1
Potential HCO_3^-	$[mEq \cdot kg^{-1} \cdot d^{-1}]$	67.1 ± 2.2***	45.4 ± 2.5	1.7 ± 0.5***
Water intake	$[ml \cdot kg^{-1} \cdot d^{-1}]$	79.8 ± 3.5	76.7 ± 4.7	51.9 ± 9.3*
Urine volume	$[ml \cdot kg^{-1} \cdot d^{-1}]$	43.7 ± 2.7	41.3 ± 3.3	39.0 ± 9.7
GFR	$[ml \cdot min^{-1}]$	12.6 ± 0.9	10.7 ± 0.5	8.4 ± 0.8*

Data are means ± SEM of N rabbits in each group, with the exception of N = 23 for glomerular filtration rate (GFR) in the high alkali group. Differences compared to the control group on normal- alkali food were significant at levels of * $P \leq 0.05$; ** $P \leq 0.01$ and *** $P \leq 0.001$, calculated by independent samples t-test

Table 3 Arterial acid-base status and serum electrolytes in rabbits on different dietary alkali load

		Matador (N = 58) High Alkali	Altromin (N = 31) Normal Alkali	PCC (N = 11) Low Alkali
Arterial blood				
pHa		7.446±0.005*	7.425±0.006	7.421±0.012
HCO_3^-a	[mM]	24.3±0.3	23.4±0.5	23.3±0.9
$PaCO_2$	[mmHg]	36.6±0.5	37.0±0.8	37.3±1.5
$\dot{V}CO_2$	$[ml \cdot min^{-1} \cdot kg^{-1}]_{STPD}$	10.4±0.7	11.6±0.6	8.7±0.9
$\dot{V}_A$	$[ml \cdot min^{-1} \cdot kg^{-1}]_{BTPS}$	247.8±21.6	273.5±15.4	199.0±22.2
HCO_3^-st	[mM]	25.0±0.3*	24.1±0.3	23.9±0.7
Base excess (BE)	[mM]	1.4±0.3*	0.3±0.4	0.0±0.8
PaO_2	[mmHg]	140.1±9.5***	212.4±10.7	203.6±18.0
Hb	$[g \cdot dl^{-1}]$	13.2±0.1	13.0±0.2	13.8±0.3*
Blood serum				
Sodium (Na^+)	[mM]	145.3±0.9	142.8±1.1	145.0±3.5
Potassium (K^+)	[mM]	4.68±0.10**	4.26±0.11	3.79±0.18*
Calcium (Ca^{++})	[mM]	3.74±0.05*	3.57±0.05	3.25±0.12**
Chloride (Cl^-)	[mM]	100.9±0.9***	106.8±1.1	101.8±3.3
Lactate (Lac^-)	[mM]	2.9±0.2**	2.1±0.2	2.0±0.3
$Na^+ + K^+ - Cl^- - Lac^-$ (SID)	[mM]	46.4±1.1***	38.1±1.1	44.9±3.6*
Osmolality (Osm)	[mM]	281.4±1.3	283.0±1.9	279.1±5.7

Data are means ± SEM of N rabbits in each group, with the exception of N = 14 for metabolic CO_2 production ($\dot{V}CO_2$) and alveolar ventilation ($\dot{V}_A$) in the high alkali group. Means were compared to the control group on normal alkali food. Differences were significant at levels of * $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$, when calculated by independent samples t-test.
Animals inhaled oxygen-enriched air, when blood samples were taken, except of some animals in the high alkali group, showing lower mean values of PaO_2

mM. Despite considerable variations in nutritional alkali load, the inter-individual correlation also revealed only small (but significant) effects on arterial acid-base conditions, the average rise of individual BE values amounting to about 0.2 mM per 10 mEq · kg^{-1} increase in daily dietary alkali load (Fig. 1).

Table 3 shows furthermore that alimentary alkali load was significantly reflected by serum potassium and calcium levels. Due to equivocal behaviour of sodium, lactate and chloride, mean values for serum strong ion difference ($SID = Na^+ + K^+ - Cl^- - Lac^-$) were not predictable from the food alkali content. Serum osmolality was unaffected by food mineral composition and food intake.

Respiratory variables did not differ significantly between the groups on different types of food (Table 3). Mean values for $PaCO_2$ were about 36.8 mmHg (4.9 kPa), those for alveolar ventilation ($\dot{V}_A$) about 260 $[ml \cdot kg^{-1} \cdot min^{-1}]_{BTPS}$ and those for the average CO_2 production ($\dot{V}CO_2$) about 11.0 $[ml \cdot kg^{-1} \cdot min^{-1}]_{STPD}$. Individual $PaCO_2$ values for all groups ranged between 27 and 50 mmHg (3.6 and 6.7 kPa) and, besides dietary alkali load or daily food intake, were also independent of metabolic CO_2 production ($r = -0.001$, $P = 0.99$, $n = 82$).

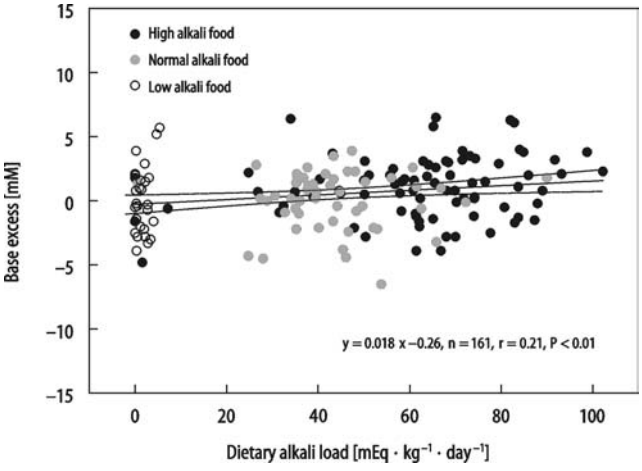

Fig. 1 Effect of alimentary alkali load on arterial base excess. Ordinate: Arterial base excess (BE) determined in 161 blood samples from rabbits on different types of food. Abscissa: Dietary alkali load calculated from feed's ash cation-anion difference and daily food intake ad libitum. Linear regression analysis includes the 95 % mean confidence interval. Note small but significant correlation between arterial base excess and dietary alkali load

Renal acid-base excretion and reabsorption

Urinary acid-base values are shown by Table 4. The two groups of rabbits on standard food pellets excreted highly alkaline urines ($pH_u > 8.0$). Considering soluble and insoluble compounds, the total daily base excretion

European Journal of Nutrition (2005) Vol. 44, Number 8
© Steinkopff Verlag 2005

Table 4 Acid-base status in 24 h urine from rabbits on different dietary alkali load

		Matador (N = 58) High alkali	Altromin (N = 31) Normal alkali	PCC (N = 11) Low alkali
Supernatant				
pHu		8.09 ± 0.04*	8.21 ± 0.03	6.27 ± 0.14***
HCO_3^-	[mM]	197.5 ± 10.2***	313.7 ± 15.5	28.3 ± 13.7***
TB	[mM]	8.2 ± 0.8*	11.2 ± 1.0	
TA	[mM]			18.9 ± 5.5
HCO_3^-	[mmol · kg^{-1} · d^{-1}]	7.7 ± 0.3***	11.8 ± 0.8	1.2 ± 0.6***
NH_4^+	[mmol · kg^{-1} · d^{-1}]	0.97 ± 0.10	0.70 ± 0.13	0.30 ± 0.06*
$HPO_4^{--}/H_2PO_4^-$	[mmol · kg^{-1} · d^{-1}]	0.013 ± 0.004	0.021 ± 0.002	0.234 ± 0.078*
Precipitate				
Amount	[g · kg^{-1} · d^{-1}]	1.63 ± 0.13***	1.04 ± 0.08	0.28 ± 0.07***
CO_3^{--}	[mmol · kg^{-1} · d^{-1}]	12.3 ± 1.0***	6.3 ± 0.5	0.7 ± 0.2***
PO_4^{---}	[mmol · kg^{-1} · d^{-1}]	0.080 ± 0.027***	0.374 ± 0.050	0.300 ± 0.110
Fluid and Precipitate				
Total base	[mmol · kg^{-1} · d^{-1}]	20.2 ± 1.1	18.1 ± 1.1	1.9 ± 0.6***
Total phosphate	[mmol · kg^{-1} · d^{-1}]	0.093 ± 0.031***	0.395 ± 0.051	0.534 ± 0.147
NAE	[mmol · kg^{-1} · d^{-1}]	−6.7 ± 0.3***	−11.1 ± 0.7	−0.4 ± 0.6***

Data are means ± SEM of N rabbits in each group, with the exception of N = 43 for the phosphate values of the high alkali group. Means were compared to the control group on normal alkali food (Altromin), differences being significant at levels of * $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$, when calculated by independent samples t-test.

TB titrable base; *TA* titrable acid; *NAE* = TA + NH_4^+ − HCO_3^-, net acid excretion

(HCO_3^- plus CO_3^-) was at about equal averages of 18–20 mEq · kg^{-1} · d^{-1} in both groups. Compared to that, daily excretion of ammonium was only 4–5 %, and that of phosphate was at the limit of detection (< 1 %) in the group with high Ca^{++} intake, rising to about 2 % in the control group on lower Ca^{++} consumption. In the group on alkali-(and Ca^{++})-rich food, a vast portion of total CO_2 (about 60 %) was excreted as insoluble carbonate with the precipitate, compared to only about 35 % in the group on normal-alkali food with lower Ca^{++} content. In the group consuming the alkali-poor diet, total base excretion was only ~10 % of control and ammonium excretion was about half that of the control group. Soluble phosphate was elevated to 9.0 ± 4.0 mM, corresponding to ~16 mEq/L primary/secondary buffer pair, thus contributing to most of the titrable acid (TA). Total (soluble and precipitated) phosphate excretion was not significantly higher than in the group on normal alkali food. Even on the alkali-poor diet no net acid excretion (NAE) occurred.

The creatinine clearance was determined to estimate glomerular filtration rate (GFR) and to assess renal base excretion in relation to GFR. Of the 23–24 mEq plasma HCO_3^- filtered per litre GFR, on an average 19–20 mEq/L (~83 %) were reabsorbed upon consuming the two standard feeds, irrespective of differences in mineral composition. When the low-alkali diet PCC was consumed, the fractional reabsorption of HCO_3^- rose to 97.8 ± 0.7 %.

Over the whole investigated range, Fig. 2A shows significant proportionality in a ratio of about 1:3 between individual renal base excretion and dietary alkali load, and Fig. 2B shows the corresponding inverse linear correlation of dietary alkali load and fractional renal base reabsorption. Fig. 3 gives a summarizing overview of dietary alkali load, renal base excretion and blood buffer base values for the three types of food investigated.

Discussion

Metabolic acid-base conditions in the arterial blood

Large differences in dietary alkali load by the three types of investigated rabbit food were responded to by proportional renal base excretion rates, but were only weakly reflected by the metabolic acid-base status of the arterial blood. The mean base excess value on high alkali load was slightly but significantly higher than control, pointing to a lower limit of renal fractional base reabsorption (at about 70–80 %), whereas BE values on low-alkali load were maintained within the control range by the effectively enhanced fractional base reabsorption up to ~98 %. Another factor of acid-base balance under these conditions may be extracellular volume contraction [21], a view being supported by the reduced water intake and slightly elevated haemoglobin concentrations in the group on low-alkali diet.

Interestingly, recalculation from literature data for rabbits revealed large differences in mean BE values between 0 mM [9, 30] and –5 to –8 mM [2, 11]. Since, however, the feed was not specified in these studies, data can-

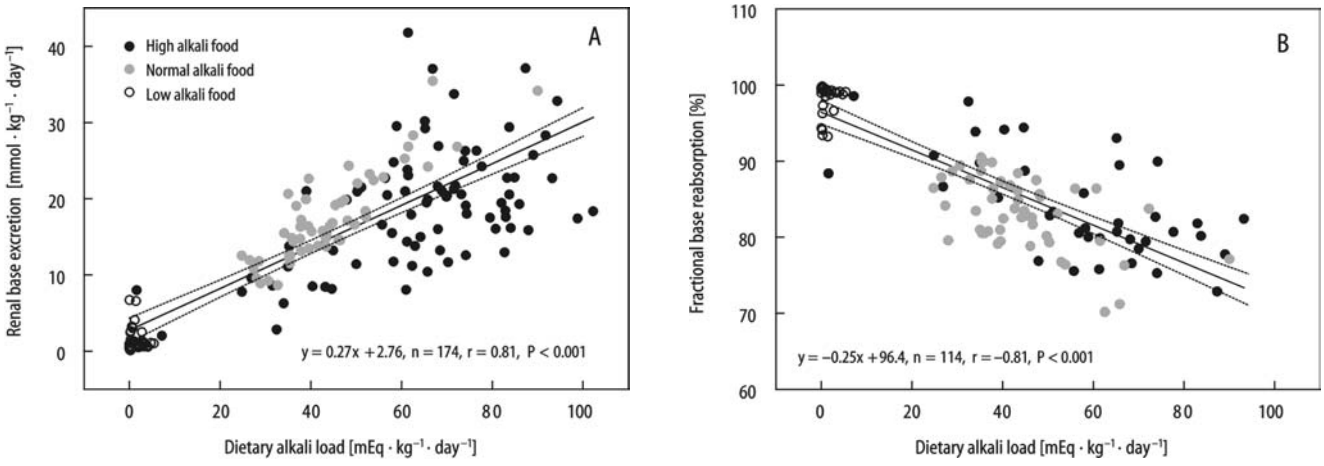

Fig. 2 Effect of dietary alkali load on renal base excretion and fractional reabsorption. Ordinate: **A** Daily renal base excretion (soluble HCO_3^- plus precipitated CO_3^{--}) determined in 174 centrifuged urine samples of rabbits on different types of food. **B** Fractional base reabsorption calculated in a subgroup from total base excretion and bicarbonate filtration rate. Abscissa: Dietary alkali load calculated from feed's ash cation-anion difference and daily food intake ad libitum. Linear regression analysis includes the 95 % mean confidence interval. Note highly significant proportionality between dietary alkali load and renal base excretion and inverse correlation with fractional base reabsorption

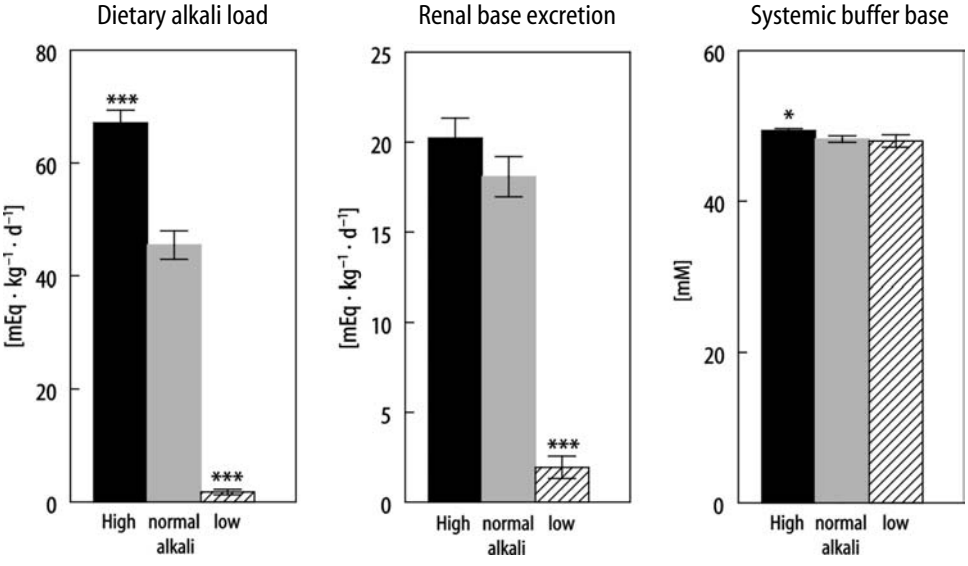

Fig. 3 Distribution of dietary alkali load, renal base excretion and systemic buffer base in three groups of rabbits on food with high (N = 58), normal (N = 31) and low (N = 11) alkali content. Bars are means ± SEM. Significant differences compared to the control group on normal-alkali food are indicated as * P < 0.05 and *** P < 0.001 (independent samples t-test). Distinctly reduced renal base excretion upon low alkali food effectively maintains systemic buffer base (BE + 48 mM) within the normal range

not be judged by dietary acid-base load. On the other hand, data for urine and blood acid-base status in normal awake rabbits on a well-defined commercial rabbit chow "Purina" were provided by Halperin and his colleagues [12, 13]. Although the cation-anion difference was much lower (80 mEq/100 g) than in the standard feed we used, blood acid-base data did not greatly differ from ours. Thus, metabolic acidosis reported elsewhere for rabbits on undefined nutritional conditions may also be secondary to hyperventilation and renal compensation of respiratory alkalosis.

Recently, chronic metabolic acidosis has been attempted in rabbits to explore adaptive responses of functional renal subsystems, e. g. regulation of kidney medullary carbonic anhydrase IV [24]. This was achieved by ingesting NH_4Cl with the drinking water, when standard chow was restricted to 2–3 % of body weight. As stated above, our rabbits reached the limit of maximal renal base reabsorption upon the special alkali deficient diet PCC, although systemic acid-base balance was still maintained. We have evidence, however, that this situation rendered the animals highly susceptible to metabolic acidosis, since additional uptake of ammonium chloride elicited profound reduction in blood base excess (unpublished data). This implies also that under these experimental conditions, chronic metabolic acidosis may be induced in rabbits without food and (hence energy) withdrawal.

© Steinkopff Verlag 2005

Respiratory acid-base conditions in the arterial blood

Although an important process by which the pH is regulated in body fluids by air-breathers is excretion of CO_2 through the lungs [31], mean values of $PaCO_2$ and alveolar ventilation were not significantly different in our three study groups, pointing to negligible roles being played by respiratory control functions, when nutritional acid-base load was varied in the investigated range.

Literature data on arterial PCO_2 values for awake rabbits [2] show inter-individual variability comparable to ours, but strikingly differing mean values within and among laboratories [9–11, 30, 32]. In our experiments several factors were excluded that could influence the arterial PCO_2 in an unpredictable manner: 1) Animals were breathing oxygen-enriched air during blood sampling, to avoid secondary reflex effects from peripheral chemoreceptors. 2) We always analysed arterial instead of venous blood [13]. 3) We exclusively studied male rabbits to exclude respiratory effects of the female hormonal cycle, in contrast to Barzago et al. [30], and 4) compared to others [e. g. 9, 11], we did not perform transient anaesthesia or surgical instrumentation before blood sampling, but only used superficial skin anaesthesia [25]. Our mean values for $PaCO_2$ may thus represent "normal values" for conscious undisturbed rabbits. Accordingly, the mean alveolar ventilation we found was about 80% of pulmonary ventilation ($300–350\ ml \cdot kg^{-1} \cdot min^{-1}$) that was reported elsewhere for awake rabbits [1, 3, 32].

Urinary acid-base conditions in herbivores

Rabbits on herbivore standard feed with high alkali content have to deal with a vast base excretion, the excretion of ammonia and phosphate being small. Another peculiarity is the high urinary alkalinity ($pH_u > 8.0$) caused by high concentrations of bicarbonate/carbonate, whereby a considerable portion of the primarily filtered HCO_3^- is precipitated as carbonate. Therefore we attempted higher accuracy by analysing the supernatant clear urine and the precipitate separately.

In the past, titrimetrically determined CO_2 content of alkaline urine was generally overestimated compared to gasometrical methods [33, 34], the difference being particularly high (> 10 mEq/L), when animals were made alkalotic by sodium bicarbonate infusion and apparently suspended calcium carbonate compounds were present [34]. Theoretically, the ratio of monovalent bicarbonate and divalent carbonate is given by the second (pK_2) dissociation constant of carbonic acid [35]. Whereas for a pH_u below 7.4 more than 99% of the titrated anions is the monovalent HCO_3^-, this portion is progressively replaced by divalent CO_3^- with rising pH, so that the titration difference between 7.4 and actual pH_u can be considered as titrable base (TB), in our case coming to about 8–12 mM. Thus, in the clear supernatant of centrifuged urine an average of ~4% of the titrated soluble components was determined twofold as bivalent CO_3^{--} anions and was corrected accordingly.

Flatt and Carpenter [7] have visualized calcium carbonate and calcium carbonate monohydrate crystals ($CaCO_3$, $CaCO_3 \cdot H_2O$) by microscopy in rabbit urinary precipitate, together with crystals of ammonium magnesium phosphate (NH_4MgPO_4). Considering equal amounts of calcium carbonate compounds, between 85% and 65% of the excreted precipitate should be calcium carbonates in our rabbits, when consuming standard feed with high and normal alkali (Ca^{++}) content, respectively. Correspondingly, when also considering the molecular weight of 137.3 g/mol for NH_4MgPO_4, the precipitate should contain only small (~5%) or negligible (~0.5%) amounts of phosphate. Interestingly, although rabbit urine does contain more or less carbonate "gravel", the herbivore urinary system appears to be adapted to this situation without damage [31].

Renal control functions

The daily nutritional alkali load of our rabbits on the two types of standard pellet feed ranged between 44 and 66 $mEq \cdot kg^{-1} \cdot d^{-1}$, about one third of which being proportionally excreted as carbonic acid anions. Rabbits on a standard pellet chow different from ours, providing lower daily alkali uptake [12], exhibited also lower total CO_2 excretion rates, as predicted from our Fig. 2. Since blood acid-base data of rabbits on this type of feed were not greatly different from ours [13], again priority of renal control functions in acid-base balance becomes evident.

Considering the high Ca^{++} content of the *Matador* pellets as well as differences in intestinal reabsorption for different cations and anions, the nutritional alkali-load may have been overestimated by about 20%, since occasional faeces analysis on that food revealed 88% fractional intestinal uptake for K^+ and 61–68% uptake for Na^+, Mg^{++} and Ca^{++}. Consuming the low-alkali diet (PCC) for five days with strongly reduced calcium, the total amount of precipitate was reduced and the relative portion of calcium carbonates decreased to 25%, whereas that of the phosphates amounted to about 20%. This agrees qualitatively with the observations of Barr et al. [16] and Bourdeau et al. [36] in young rabbits on a calcium-free diet for up to several weeks that renal adaptation to dietary Ca^{++} deprivation consisted in tubular reabsorption accelerated for Ca and inhibited for P. Likewise, low dietary phosphorus concentrations were shown to reduce kidney calcification in growing rabbits [37].

Independent of last details in mineral composition, on an average 83 % of the filtered plasma HCO_3^- was reabsorbed upon consuming the two standard feeds. The lower limit for fractional base reabsorption in rabbits may be reached at ~70 %, e. g. during strong alkali loading by intravenous sodium bicarbonate infusion [13], whereas on nutritional alkali deficiency, the fractional reabsorption of HCO_3^- amounted to nearly 100 %.

Although HCO_3^- disposal in the liver along with urea genesis may not be neglected as an important mechanism for regulation of the systemic acid-base status [31], it has to be considered that nitrogen is limiting in a herbivore diet. In line with this, urinary bicarbonate excretion in rabbits already starts at much lower plasma HCO_3^- concentrations (of 23–24 mM) than in humans (at 26–28 mM). Likewise, rabbits on alkali-poor diet did not perform net acid excretion (NAE), denoting the sum of titrable acid (TA) and NH_4^+ minus HCO_3^- [23, 27, 28, 34, 38], although they achieved acid urinary pH-values distinctly below 7.4. Rather, the rabbit kidney seems to possess flexible mechanisms for base retention or secretion in cortical collecting ducts [39, 40], dependent on prior acid or base load.

There is general agreement that HCO_3^- reabsorption and hence renal acid-base regulation is primarily performed by proximal tubules [41]. HCO_3^- reabsorption of rabbit S2 proximal tubules perfused *in vitro* showed differential responses, being reduced upon isohydric basolateral rises in bicarbonate, but enhanced upon isohydric rises in basolateral PCO_2 [42].

At the molecular level, the sodium-proton exchanger subtype NHE3 is profoundly involved in proximal tubular bicarbonate retention; $NaHCO_3$ enriched diets leading to considerable decreases in NHE3 protein abundance [43]. Vice versa, cortical carbonic anhydrase IV mRNA expression, presumably localized to proximal tubules, is enhanced during chronic metabolic acidosis [24]. It remains to be clarified, which key molecules are responsible for the adaptive responses of fractional base reabsorption in rabbits to high and low nutritional alkali load.

Conclusion

Different types of herbivore rabbit feed distinctly differ with respect to mineral composition and together with voluntary food intake provide considerable variations in dietary alkali load. Due to effective renal compensation, no notable acid-base disturbances were detected in the arterial blood, respiratory control functions being negligible. This implies that nutritional acid-base challenges to the organism can be judged much earlier and more safely by urinary rather than by blood acid-base analysis. The usual strong base load of the herbivore feed elicited a highly alkaline urine excretion containing a considerable portion of carbonate precipitates, which however does not appear to be harmful to the rabbit urinary tract. The strong nutritional base deficiency induced by a special low-alkali diet with normal energy supply turned out to have a maximum impact on renal base saving mechanisms, implying a possible promoting factor for metabolic acidosis in the rabbit.

References

1. Spector S (1956) Handbook of Biological Data. WB Saunders Comp, Philadelphia
2. Altmann PL, Dittmer DS (1974) Biology Data Book, 2nd edn, Vol 3. Federation of American Societies for Experimental Biology, Bethesda
3. Kozma C, Macklin W, Cummins LM, Mauer R (1974) The anatomy, physiology and the biochemistry of the rabbit. In: Weisbroth StH, Flatt RE, Kraus AL (eds) The Biology of the Laboratory Rabbit. Academic Press, New York, pp 50–72
4. Hunt ChE, Harrington DD (1974) Nutrition and nutritional diseases of the rabbit. In: Weisbroth StH, Flatt RE, Kraus AL (eds) The Biology of the Laboratory Rabbit. Academic Press, New York, pp 403–433
5. Kaplan HM, Timmons EH (1979) The Rabbit. A Model for the Principles of Mammalian Physiology and Surgery. Academic Press, New York
6. Harkness JE, Wagner JE (1983) The Biology and Medicine of Rabbits and Rodents, 2nd edn. Lea and Febiger, Philadelphia
7. Flatt RE, Carpenter AB (1971) Identification of crystalline material in urine of rabbits. Am J Vet Res 32:655–658
8. Krapf R (1989) Mechanisms of adaptation to chronic respiratory acidosis in the rabbit proximal tubule. J Clin Invest 83:890–896
9. Nattie EE, Reeder R (1983) Ventilation and CSF ions during isocapnic HCl and HNO_3 acidosis in conscious rabbits. J Appl Physiol 55:1758–1766
10. Nattie EE, Moore Ph (1986) CSF acid-base regulation and ventilation in iso- and hypocapnic Hacetate acidosis. J Appl Physiol 61:851–858
11. Nattie EE (1987) Central vs. peripheral chemoreceptors in ventilatory stimulation by Hacetate. J Appl Physiol 62: 2154–2159
12. Richardson RMA, Goldstein MB, Stinebaugh BJ, Halperin ML (1979) Influence of diet and metabolism on urinary acid excretion in the rat and the rabbit. J Lab Clin Med 94:510–518
13. Giammarco RA, Goldstein MB, Halperin JS, Hammeke MD, Richardson RMA, Robson WLM, Stinebaugh BJ, Halperin ML (1978) Collecting duct hydrogen ion secretion in the rabbit: role of potassium. J Lab Clin Med 91: 948–959
14. Hannaford MC, Goldstein MB, Josse RG, Halperin ML (1982) Role of acidosis in the protein wasting of fasting in the rat and in the rabbit. Can J Physiol Pharmacol 60:331–334
15. Cruz-Soto M, Batlle D, Sabatini S, Arruda JAL, Kurtzman NA (1982) Distal acidification in the rabbit: role of diet and blood pH. Am J Physiol 243: F364–F371

© Steinkopff Verlag 2005

16. Barr DR, Sadowski DL, Hu J, Bourdeau JE (1991) Characterization of the renal and intestinal adaptations to dietary calcium deprivation in growing female rabbits. Mineral Electrolyte Metabolism 17:32–40
17. Emmett M, Alpern RJ, Seldin DW (1992) Metabolic acidosis. In: Seldin DW, Giebisch G (eds) The Kidney: Physiology and Pathophysiology Raven Press, Ltd., New York, pp 2759–2836
18. Kalhoff H (2004) The effect of dietary factors on the risk of developing urinary tract infection. Pediatr Nephrol 19:1303
19. Hesse A, Heimbach D (1999) Causes of phosphate stone formation and the importance of metaphylaxis by urinary acidification: a review. World J Urol 17: 308–315
20. Kalhoff H, Manz F (2001) Nutrition, acid-base status and growth in early childhood. Eur J Nutr 40:221–230
21. Manz F (2001) History of nutrition and acid-base physiology. Eur J Nutr 40: 189–199
22. Frassetto L, Morris Jr RC, Sellmeyer DE, Todd K, Sebastian A (2001) Diet, evolution and aging. The pathophysiologic effects of the post-agricultural inversion of the potassium-to-sodium and base-to-chloride ratios in the human diet. Eur J Nutr 40:200–213
23. Bungart S (1996) Strategien der Säure-Basen-Regulation beim Pflanzenfresser am Beispiel des Kaninchens (Oryctolagus cuniculus) Ph. D. Thesis, Faculty of Biology, Ruhr-University Bochum, Germany
24. Winkler CA, Kittelberger AM, Schwartz GJ (1997) Expression of carbonic anhydrase IV mRNA in rabbit kidney: stimulation by metabolic acidosis. Am J Physiol 272:F551–F560
25. Kiwull-Schöne H, Kalhoff H, Manz F, Diekmann L, Kiwull P (2001) Minimal-invasive approach to study pulmonary, metabolic and renal responses to alimentary acid-base changes in conscious rabbits. Eur J Nutr 40:255–259
26. Siggaard-Andersen O (1974) The acid-base status of the blood, 4th edition. Munksgaard, Copenhagen
27. Lin SL, Chan JCM (1973) Urinary bicarbonate: A titrimetric method for determination. Clinical Biochemistry 6: 207–210
28. Chan JCM (1972) The rapid determination of urinary titrable acid and ammonium and evaluation of freezing as a method of preservation. Clinical Biochemistry 5:94–98
29. Fiske CH, Subbarow Y (1925) The colorimetric determination of phosphorus. J Biological Chemistry 66:375–400
30. Barzago MM, Bortolotti A, Omarini D, Aramayona JJ, Bonati M (1992) Monitoring of blood gas parameters and acid-base balance of pregnant and non-pregnant rabbits (Oryctolagus cuniculus) in routine experimental conditions. Laboratory Animals 26:73–79
31. Atkinson DE, Bourke E (1987) Metabolic aspects of the regulation of systemic pH. Am J Physiol 252 (Renal Fluid Electrolyte Physiol 21) F947–F956
32. Honda Y (1968) Ventilatory response to CO_2 during hypoxia and hyperoxia in awake and anesthetized rabbits. Respir Physiol 5:279–287
33. Lüthy Ch, Moser C, Oetliker O (1977) Dreistufige Säure-Basen-Titration im Urin. Medizinisches Labor 30:174–181
34. Tam S-C, Goldstein MB, Chen CB, Halperin ML, Stinebaugh BJ (1981) Implications of the finding of calcium carbonate in rabbit urine with respect to renal acidification. Can J Pharmacol 59: 800–805
35. Maren ThH (1978) Carbon dioxide equilibria in the kidney: The problems of elevated carbon dioxide tension, delayed dehydration, and disequilibrium pH. Kidney Int 14:395–405
36. Bourdeau JE, Bouillon R, Zikos D, Langman CB (1988) Renal responses to calcium deprivation in young rabbits. Mineral Electrolyte Metab 14:150–157
37. Ritskes-Hoitinga J, Grooten HNA, Wienk KJH, Peters M, Lemmens AG, Beynen AC (2004) Lowering dietary phosphorus concentrations reduces kidney calcification, but does not adversely affect growth, mineral metabolism, and bone development in growing rabbits. Br J Nutr 91:367–376
38. Lin SH, Cheema-Dhali S, Chayaraks S, Chen CB, Gowrishankar M, Halperin ML (1998) Physiological disposal of the potential alkali load in diet of the rat: steps to achieve acid-base balance. Am J Physiol 274 (Renal Physiol 43): F1037–F1044
39. McKinney TD, Burg MB (1977) Bicarbonate transport by rabbit cortical collecting tubules. Effect of acid and alkali loads in vivo on transport in vitro. J Clin Invest 60:766–768
40. Marin-Grez M, Vallés P (1994) Evidence for an inhibitory effect of kallikrein on collecting duct bicarbonate secretion in rats and rabbits. Renal Physiology and Biochemistry 17:301–306
41. Giebisch G, Windhager E (2003) Transport of acids and bases. In: Boron WF, Boulpaep EL (eds) Medical Physiology. WB Saunders Comp. Philadelphia, pp 845–860
42. Zhao J, Zhou Y, Boron W (2003) Effect of isolated removal of either basolateral HCO_3^- or basolateral CO_2 on HCO_3^- reabsorption by rabbit S2 proximal tubule. Am J Physiol Renal Physiol 285: F359–F369
43. Eladari D, Leviel F, Pezy F, Paillard M, Chambrey R (2002) Rat proximal NHE3 adapts to chronic acid-base disorders but not to chronic changes in dietary NaCl intake. Am J Physiol Renal Physiol 282:F835–F843