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ORIGINAL CONTRIBUTION

H. Kiwull-Schöne
H. Kalhoff
F. Manz
L. Diekmann
P. Kiwull

Minimal-invasive approach to study pulmonary, metabolic and renal responses to alimentary acid-base changes in conscious rabbits

Summary *Background* Systemic acid-base balance is maintained by the complex interplay of renal and pulmonary control functions and metabolic adaptations, whereby intake and mineral composition of feed are important factors. *Aim of the study* It was intended to explore the role of alimentary acid-base load and carbonic anhydrase activity for regulatory responses of renal, pulmonary or metabolic origin in rabbits as typical herbivores. *Methods* Sixty-eight conscious male rabbits (about 3.5 kg) were kept in a metabolic cage, to determine daily water intake, urine excretion and food consumption. Different groups were fed either alkali-rich rabbit standard pellets, or modified rabbit chow with low Ca^{++}-content, or a special diet with very low alkali content, or standard food together with a low oral dose (about 20 mg · kg^{-1} · d^{-1}) acetazolamide. Samples from the central ear artery were analyzed for blood gases (PaO_2, $PaCO_2$), pH_a, base excess (BE) and actual bicarbonate (HCO^-_{3a}). The metabolic CO_2 production ($\dot{V}CO_{2\ STPD}$) was determined, to calculate alveolar ventilation ($\dot{V}_{A\ BTPS}$). Anaerobically collected urine was analyzed for pH_u and for concentrations of bicarbonate/carbonate (HCO^-_3/CO^{--}_3), ammonium (NH_4^+), and phosphate. *Results* 1) Systemic BE was not affected by alimentary alkali load, either varied spontaneously by standard food intake or by the low-Ca^{++} diet, and decreased only slightly on the low-alkali diet, but distinctly upon carbonic anhydrase inhibition. 2) Under all conditions of alimentation, $PaCO_2$ was closely correlated with BE without a detectable set-point, the normal-range variability of BE being sufficient to elicit corresponding changes in $\dot{V}_A$. In contrast, acetazolamide led to much lower values of $PaCO_2$ than predicted by the reference PCO_2/BE relationship, being primarily caused by significant reductions in $\dot{V}CO_2$ (> 20 %). 3) Prior to other systems, renal base excretion, normally being high on species-adapted standard chow, closely followed any variation of alimentary alkali load and approached zero upon the low-alkali diet. It was, however, not significantly influenced by carbonic anhydrase (CA) inhibition on alkali-rich alimentation. *Conclusions* Blood acid-base balance in rabbits is maintained over a wide range of alimentary alkali load by effective adaptation of renal base excretion, independent of CA activity. Ventilatory pH control is perpetuated even in the normal range of BE, provided metabolic rate is not impaired, e. g., by CA inhibition. These results may help one understand the different manifestations of acid-base disorders in body fluids under clinical conditions.

Key words Acid-base balance – Food mineral content – Respiratory control – Renal base excretion – Acetazolamide – Rabbits

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H. Kiwull-Schöne (✉) · P. Kiwull
Dept. of Physiology
Ruhr-University
44780 Bochum, Germany
Tel.: +49-2 34/3 22 48 86
Fax: +49-2 34/3 21 47 33
E-Mail: Heidrun.F.Kiwull-Schoene@ruhr-uni-bochum.de

H. Kalhoff · L. Diekmann
Paediatric Clinic
44137 Dortmund, Germany

F. Manz
Research Institute of Child Nutrition
44225 Dortmund, Germany

Introduction

Acid-base balance is known to be maintained by the complex interplay of renal and pulmonary control functions, as well as of metabolic adaptations [1–3]. In most acute animal experiments to study acid-base regulation, systemic homoeostasis is overridden by intravenous application of strong mineral acids and bases. Therefore, these investigations, besides requiring anesthesia [4], involve a variety of side effects, among which physicochemical release of CO_2 seriously complicates any ventilatory response [5]. Much less is known about the relative role and significance of pulmonary ventilation compared to renal control for acid-base balance during alimentary acid-base load. Observations in preterm infants, investigated for the diagnosis of "incipient late metabolic acidosis" revealed that alimentary acid load is much earlier and more precisely indicated by acid-base changes in the urine than in the arterial blood [3, 6]. The aim of this study was to explore, if or to which extent changes in alimentary base load within the physiological range of herbivore nutrition may elicit regulatory responses. The relative significance of renal and pulmonary responses, or possible adaptations of metabolism was investigated by variations of food intake and/or food mineral composition, as well as during carbonic anhydrase inhibition in conscious rabbits by a minimally invasive experimental approach.

Materials and methods

Sixty-eight conscious male rabbits (3.52 ± 0.05 kg) were kept in metabolic cages, to determine daily water intake and urine excretion as well as daily food consumption. The control group of 58 animals was fed with a rabbit standard pellet chow of normal energy content, but relatively rich in Ca^{++} (Kanin 4, Matador GmbH, Recklinghausen, Germany). One subgroup of them (N=7) received for three days a low oral dose (about $20\ mg \cdot kg^{-1} \cdot d^{-1}$) acetazolamide with the drinking water, another subgroup (N=7) was fed for three days with a diet of low Ca^{++} and low alkali content (PCC). The PCC diet consisted of *p*eanuts, *c*orn-flakes and *c*arob-tree-fruit-skin (cellulose). A separate group of rabbits (N=10) was continuously fed a modified rabbit pellet chow of low Ca^{++} but high alkali content (Altromin 2123, Altromin GmbH, Lage, Germany). The electrolyte content of the feed pellets and the diets was determined in duplicate by ash analysis (Table 1).

Table 1 Electrolyte content of standard rabbit laboratory feed and modified diet. Mineral composition of different rabbit feed pellets and a modified diet. Results from ash analysis [mEq/100 g] determined in duplicate. The Ca^{++} rich standard pellet chow (Kanin) was fed to the control group. For comparison, a feed with reduced Ca^{++} (Altromin) and a special diet with reduced Ca^{++} and alkali content of peanuts, cornflakes and cellulose (PCC) were given. The composition of a feed with intermediate Ca^{++} and alkali content (Purina), used by Richardson et al. [11] is also shown. The daily uptaken potential bicarbonate (Figs. 1 and 3) is based on the difference of fixed cations and fixed anions [2, 11]

	Kanin control	Altromin low Ca^{++}	Purina Ref. [11]	PCC low alkali
Sodium (Na^+)	5.79	7.92	80.00	0.80
Potassium (K^+)	39.55	51.63	24.00	16.80
Magnesium (Mg^{++})	18.63	20.63	33.00	8.28
Calcium (Ca^{++})	151.24	89.57	47.00	7.67
Total fixed cations	215.21	169.75	112.00	33.55
Phosphorus (P_i)*	32.85	31.76	20.00	13.21
Chloride (Cl^-)	9.46	9.05	11.00	2.26
Sulfate (SO_4^-)	2.91	5.41	10.00	
Total fixed anions	45.22	46.22	41.00	15.47
Fixed cations – fixed anions	170.00	123.52	71.00	18.08
Energy (kcal/100 g)	296	150		379
$Na^+ - K^+ - Cl^-$	35.88	50.50	21.00	15.14

* P_i [mEq] = 1.8 [mmol], according to [11].

Measurements

Blood samples from the central ear artery were analyzed. Besides concentrations of lactate (Lac-) and hemoglobin (Hb), the oxygen partial pressure (PaO_2) and plasma pH (pHa) were measured by conventional electrodes (Radiometer). Arterial CO_2 partial pressure ($PaCO_2$), base excess (BE) as well as actual and standard bicarbonate concentrations (HCO_{3a}^-, HCO_{3st}^-) were determined by the two-gas equilibration method [7]. Serum electrolytes and creatinine concentrations were assayed by standard methods. The metabolic CO_2 production ($\dot{V}CO_2$) was determined from the expired CO_2 bound to barium hydroxide $Ba(OH)_2$ through back-titration of $Ba(OH)_2$ that was not converted to barium carbonate $BaCO_3$, whereby $V_{A\ BTPS} = VCO_{2\ STPD} \cdot 863/PaCO_2$ gives alveolar ventilation. The excreted urine was collected under paraffin oil, to prevent the loss of carbon dioxide. Since the urine contained a considerable amount of precipitate, an aliquot was centrifuged and the supernatant clear urine was analyzed separately from the precipitate. The acid-base status of the supernatant was investigated titrimetrically [8, 9] for actual pH (pH_u) and concentrations of bicarbonate (HCO_3^-), titrable acid or base (TA, TB) and ammonium (NH_4^+), as well as for concentration of inorganic phosphate by colorimetry [9, 10]. The precipitate was dried for several hours at 60 °C, weighed and analyzed for carbonate and phosphate. Carbonate (CO_3^{--}) was determined as the loss of CO_2 after adding HCl and back-titration with NaOH to pH 7.0. Colorimetric phosphate determination was accomplished after completely dissolving the precipitate in HCl. Total base excretion was defined as the sum of

soluble HCO_3^-/CO_3^{--} in the supernatant and of CO_3^{--} in the precipitate, considering each monovalent and bivalent carbonic acid anion to originate from filtered HCO_3^-.

Statistical analysis

Data from urine and blood analyses were averaged to obtain group mean values, standard deviations (SD), and standard errors of the mean (SEM), whereby n indicates the number of observations in each group. For the control group, correlations between selected variables were determined by linear regression analysis, yielding reference lines with 95 % confidence prediction limits. After corroborating normal distribution (one-sample Kolmogorow-Smirnov test), group mean values were tested for significant differences by unpaired t-tests. The level of significance was taken as $P < 0.05$. Statistical analysis was in part carried out by using SPSS 8.0 for Windows software (SPSS Inc. Chicago, IL, USA).

Results

The role of food intake and composition to determine blood base excess

Spontaneous variations in food intake ad libitum and thereby induced variations in alimentary alkali load and/or "potential bicarbonate" [2, 11] had no effect on the acid base status of the arterial blood (Fig. 1). Furthermore, feeding solely low Ca^{++} diet was not sufficient to cause significant lowering of the blood base excess (BE). However, a slight but significant metabolic base deficit could be elicited by the diet with pronounced alkali reduction (PCC). The most considerable decrease in BE occurred upon carbonic anhydrase inhibition, despite nearly normal alimentary alkali load.

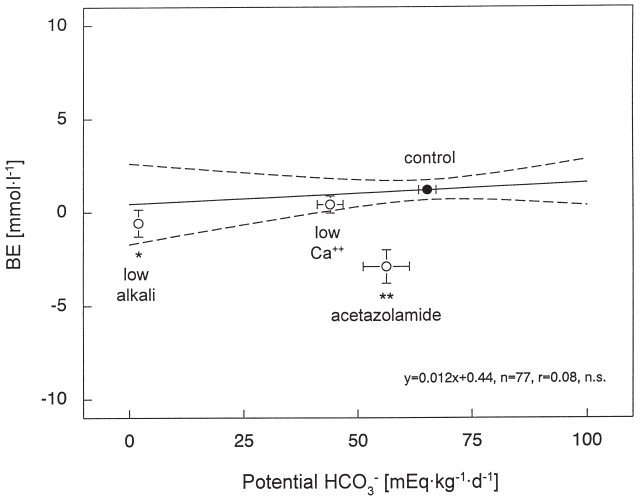

Fig. 1 Effect of alimentary alkali load on blood acid-base status. Base excess (BE) as function of daily intake of "potential" bicarbonate (HCO_3^-), calculated from food intake and mineral composition (Table 1). The regression line with 95 % confidence range refers to the control group, changes of alimentary alkali load being caused by spontaneous variations in food intake ad libitum. Mean values (± SEM) are shown for the control group (n=77) and the group upon carbonic anhydrase inhibition (n=9), as well as for groups on diets with reduced Ca^{++} (n=19) or strongly reduced alkali content (n=13). Note the slight reduction in BE with the low alkali diet and distinct reduction upon carbonic anhydrase inhibition. Significant BE-difference from control: *$P < 0.05$ and **$P < 0.01$.

The role of respiratory compensation during metabolic acid-base variations

Although independent of food intake, even in the otherwise untreated control group, a considerable variation of base excess could be seen, ranging from –4 to +6.5 mM. These variations in BE were accompanied by proportional changes in $PaCO_2$ (Fig. 2), the corresponding reference regression line showing a significant linear relationship between both variables. The 95 % confidence range is matched by the mean values (±SEM) resulting from feeding either low Ca^{++} or low alkali diet. On the other hand, oral treatment with acetazolamide led to much lower values of $PaCO_2$ than predicted by the reference $PaCO_2$/BE relationship. To decide whether the observed adaptations of arterial PCO_2 indicate unequivocally respiratory compensation of metabolic acid-base deviations changes, CO_2 production ($\dot{V}CO_2$) was determined additionally in part of the animals, assuming $\dot{V}CO_2$ to be equal to O_2 consumption (and hence meta-

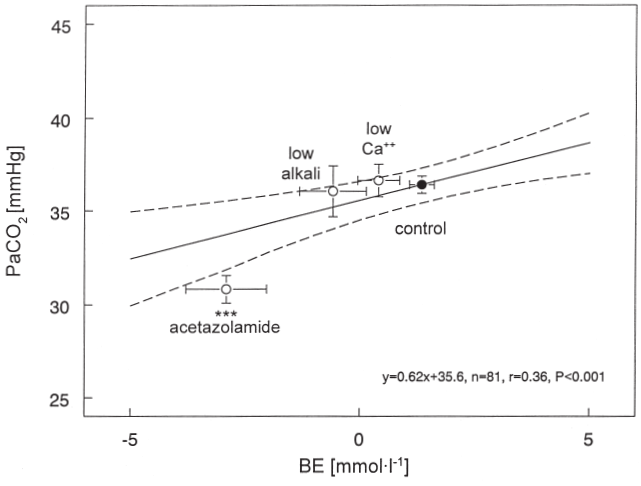

Fig. 2 Respiratory compensation of metabolic acid-base changes. Arterial CO_2 partial pressure ($PaCO_2$) as a function of blood base excess (BE). The regression line with the 95 % confidence range refers to the control group. Mean values (± SEM) are shown for the control group (n = 81) and for the group upon carbonic anhydrase inhibition (n=9), as well as for groups on diets with reduced Ca^{++} (n=19) or strongly reduced alkali (n=13). Note stronger reduction in $PaCO_2$ than predicted by the reference line upon carbonic anhydrase inhibition. Significant $PaCO_2$-difference from control: ***$P < 0.001$.

bolic rate) in herbivores. Under control conditions, the mean $\dot{V}CO_{2\,STPD}$ (± SEM) was 10.04 ± 0.44 ml · min^{-1} · kg^{-1} (n=36), low Ca^{++} diet had no influence on the average metabolic rate (10.50 ± 0.99 ml · min^{-1} · kg^{-1}, n=19). Linear regression analysis (not shown) revealed a significant correlation between alveolar ventilation ($\dot{V}_A$) and BE (r=0.31, n=42, P < 0.05), $\dot{V}_A$ being 254 ± 15.0 ml · min^{-1} · kg^{-1} at 0 mM BE and increasing by about 6% per mM BE-reduction. On the other hand, significant reductions in $\dot{V}CO_2$ occurred upon carbonic anhydrase inhibition (by 21.3 ± 7.4%, n=9, P < 0.05). This implies that the fine adjustment of PCO_2 in response to acid-base variations over the predicted range (Fig. 2) is mostly mediated by respiratory control. However, the lowered $PaCO_2$ in response to metabolic acidosis caused by acetazolamide is mainly due to a reduction in metabolic rate and not to enhanced alveolar ventilation.

The role of renal base excretion in response to alimentary alkali load

As a typical feature of herbivores, rabbits on species-adapted standard chow excreted a highly alkaline urine (pH_u > 8.0) and a considerable daily amount of precipitate (about 1.5 g · kg^{-1} · d^{-1}), presumably calcium carbonate and calcium carbonate monohydrate [12]. Excreted amounts of NH_4^+ and phosphate were negligible. On the low Ca^{++} diet, pH_u remained high and total excretion of bicarbonate/carbonate was nearly unchanged, at the expense of insoluble CO_3^{--}, which was reduced by about one third. Consuming the very low alkali diet PCC for three days, the rabbits excreted an acidic urine (pH_u < 6.5) with reduced precipitate by about 80%, the latter containing a considerable amount of phosphate instead of carbonate. In general, any degree of alimentary alkali load, either varied spontaneously by food intake or experimentally by food mineral composition, uniquely led to proportional responses of renal base excretion. Regression analysis of these data (Fig. 3) shows that even under control conditions on standard food rich in alkali and Ca^{++}, a significant linear relationship between spontaneous variations in potential HCO_3^- intake and urinary HCO_3^-/CO_3^{--} excretion can be discerned. The 95% confidence range of the reference curve was matched by all data, including those resulting from feeding only a low Ca^{++} diet, those from feeding a moderately [11] or strongly reduced alkali diet and those upon treatment with acetazolamide. Whereas base excretion is close to zero on the very poor alkali diet, selectively reduced alimentary Ca^{++} only slightly contributes to renal base saving. Surprisingly, base excretion is not significantly influenced by carbonic anhydrase inhibition on alkali-rich alimentation. Considering endogenous creatinine clearance as a measure of glomerular filtration rate (GFR), about 80% of the filtered HCO_3^- load was reabsorbed in the reference group and in the low Ca^{++} group. This portion remained rather uninfluenced by carbonic anhydrase inhibition, but amounted to 98% on the poor alkali diet.

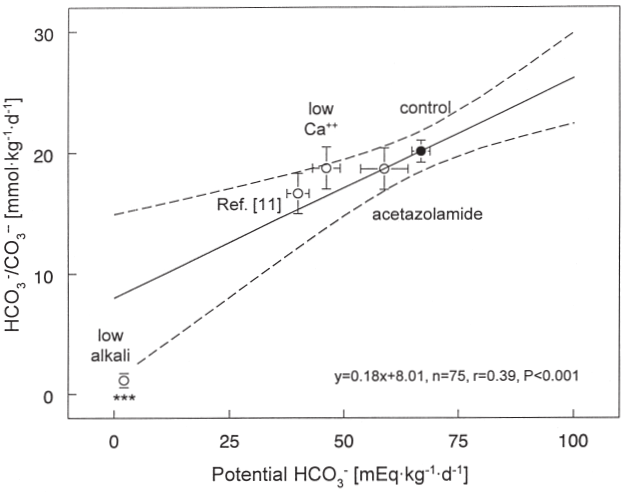

Fig. 3 Adjustment of renal base excretion to alimentary alkali load. Totally excreted soluble and insoluble bicarbonate/carbonate (HCO_3^-/CO_3^{--}) as function of daily intake of "potential" bicarbonate (HCO_3^-), calculated from food intake and mineral composition (Table 1). The regression line with 95% confidence range refers to the control group, whereby changes in alimentary alkali load were solely caused by spontaneous variations in food intake ad libitum. Mean values (± SEM) are shown for the control group (n=75) and for the group upon carbonic anhydrase inhibition (n=10), as well as and for groups on diets with reduced Ca^{++} (n=16) and with strongly reduced alkali content (n=14). For comparison, data from [11] refer to rabbits on moderately low-alkali feed (Table 1). Note that base excretion approaches zero upon diet with strongly reduced alkali content. Significant difference of base excretion from control: ***P < 0.001.

Discussion

The interplay of renal, pulmonary and metabolic responses to variations of food intake and/or food alkali composition, as well as during carbonic anhydrase inhibition, was studied in rabbits as a typical herbivore animal. One of the most important observations was that considerable variations in food intake and food mineral composition, including a wide range of alimentary alkali load, had only small influences on blood acid-base status. In contrast to this relative stability of systemic acid-base balance, even spontaneous variations in daily food intake of constant mineral composition caused distinct changes in the acid-base status of the urine. On the other hand, in the nearly "normal" range of blood base excess, adjustment of $PaCO_2$ took place, showing "permanent" regulation without a detectable threshold or set-point. Analogously, Dempsey and Forster [13] reviewed unique correlations between $PaCO_2$ and actual bicarbonate (HCO_{3a}^-) over a wide range of metabolic acid-base disturbances for many species, including man. This correlation is usually taken to quantify respiratory com-

pensation of metabolic acid-base disturbances, although $PaCO_2$ and HCO^-_{3a} are biochemically linked by cellular buffering of CO_2. To circumvent this inherent methodological difficulty, we chose the function between $PaCO_2$ and (the CO_2-independent variable) BE as a more appropriate measure of the respiratory response (Fig. 2). Furthermore, since $PaCO_2$ is a target variable to both alveolar ventilation and CO_2 production, we also attempted to differentiate between these possible influences. Indeed, despite variations in food intake and mineral composition the average $\dot{V}CO_2$ remained unchanged, and $PaCO_2$ adjustments to BE were mostly attributable to changes in alveolar ventilation, except for carbonic anhydrase inhibition. Low dose acetazolamide was shown to attenuate (respiratory) muscle function in anesthetized rabbits [4], at some level of electro-mechanical coupling. This would agree with the present finding in conscious rabbits that the distinctly lowered $PaCO_2$ upon carbonic anhydrase inhibition was not caused by increased ventilation, but by reduced metabolic rate. On the other hand, renal base excretion was independent of carbonic anhydrase activity during herbivore alkali-rich alimentation, which is rather compatible with *in vitro* findings on rabbit renal proximal tubule S2 segments at elevated ambient HCO^-_3 concentrations [14].

Generally, the relationship between intake of "potential" bicarbonate varied by mineral composition and the total base excretion was rather predictable over a wide range of experimental changes in alimentary alkali load (Fig. 3). This implies a leading role for renal regulatory processes, considerably prior to any manifestation of systemic acid-base changes (Fig. 1). Analogously, during alimentary acid load, the early phase of a systemic retention acidosis in human preterms is characterized by maximal renal acid stimulation, but widely normal blood acid-base status, before final clinical manifestation of "late metabolic acidosis". In other words, "incipient late metabolic acidosis" is characterized by widely normal systemic acid-base conditions during maximal renal acid stimulation [6].

To summarize, the presented minimally invasive experiments in conscious rabbits permit specific variations of food mineral composition combined with experimental restrictions of renal (and pulmonary) functions to study their complex interplay for adjusting systemic acid-base balance in a typical herbivore animal. Likewise, this approach opens perspectives for long-term studies on ion exchange proteins responsible for acid-base homeostasis in kidney and brainstem, which (analogous to the observed kidney responses) may exhibit early adaptations to challenges, before systemic disturbances become manifest. Such results from comparative physiology may also help to understand the range of patho-physiological acid-base deviations underlying clinical conditions from early infancy [3, 6] to progressive aging in adults [15].

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