REVIEW

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Renal function during metabolic acidosis

Anna K. Kurpińska, Wiesław F. Skrzypczak

Department of Animal Physiology and Cytobiology, Faculty of Biotechnology and Animal Husbandry, West Pomeranian University of Technology, Szczecin, Poland

Abstract: Metabolic acidosis influences renal structural and functional changes that occur to restore acid-base homeostasis. In this review selected aspects of these changes are discussed, focusing especially on alternations in tubular reabsorption and excretion, changes in water homeostasis and induction of hypertrophy. Also highlighted is the usage of proteomic techniques and gene expression analysis as useful tools which facilitate the obtaining of a wider view on changes in the kidneys during metabolic acidosis.

Key words: metabolic acidosis, acid-base imbalance, renal function, renal tubules, excretion, reabsorption, proteomics

For normal body function it is vital to maintain acid-base homeostasis. The average blood pH ranges between 7.35-7.45 [1]. Metabolic acidosis occurrence is indicated by pH under 7.35, decrease of HCO_3^- concentration, increase of H^+ concentration and nonvolatile acid production exceeding excretion of acids from the body [2]. Metabolic acidosis can occur either as a result of increased acid production or decreased base production. Production of acidic compounds – e.g. lactic acid (lactic acidosis), ketone bodies (ketosis), compounds produced during protein degradation, disorders in ion transport, decreased H^+ excretion in kidney, decreased base concentration in blood as a result of intestinal juice lost – can conduce acidosis [3]. Metabolic acidosis can also be induced by administration of acidic compounds e.g. NH_4Cl [4].

There exists a feedback between acid-base balance and kidney function [5]. On the one hand, kidneys are important organs maintaining acid-base balance. For that purpose buffering compounds/systems such as HCO_3^-:CO_2, HPO_4^{2-}:$H_2PO_4^-$, NH_3:NH_4^+ and citrate are used [2, 6]. On the other hand, acid-base imbalance influences structural and functional changes in the kidneys.

In sheep with lactic acidosis, systemic arterial blood pressure reduction was observed which resulted in about 40% decrease in renal blood flow (for a reduction in blood pH of 0.55). Glomerular filtration rate was also decreased [7]. Also in rats with increasing metabolic acidosis, a progressive decrease in estimated renal blood flow was observed. The reduction of renal blood flow and glomerular filtration rate was likewise observed in humans with chronic metabolic acidosis. It should be stressed that in humans the differences between acidotic subjects and control subjects were statistically non-significant [8, 9]. On the contrary, according to a study conducted by Cheval et al. [4] on mice and Faroqui et al. [10] on rats, there were no changes in the glomerular filtration rate induced by acidosis.

A lot of studies about the metabolic acidosis influence on kidney function focus on alternations in compounds/substances reabsorption and excretion.

In rats with chronic metabolic acidosis there were no changes in Na^+ filtered load, but an increase in Na^+ proximal and post-proximal excretion was observed [11]. Faroqui et al. [10] observed in rats an increased natriuresis after 24h of NH_4Cl treatment, which was connected with decreased Na^+ reabsorption in the proximal tubule, distal tubule and collecting duct. According to Herbert et al. [12], decrease in water and salt reabsorption in the proximal tubule is correlated with a reduced bicarbonate concentration in the peritubular capillaries. It should be emphasized that as the chronic metabolic acidosis grows, the progressive increase of sodium reabsorption is observed. This could be combined with a progressive increase in NHE3 *(Na^+/H^+ Exchanger 3 isoform)* protein abundance observed in the apical membranes along the convoluted proximal tubule and thick ascending limb [13]. Early decreased sodium reabsorption in the distal tubule and collecting duct, observed at the beginning of chronic metabolic acidosis, results from decreased Sgk-1 expression (*serum- and glucocorticoid-regulated kinase).* Sgk-1 mediates trafficking of the renal ENaC to the cell membrane, consequently these changes lead to decreased ENaC (*epithelial Na^+ channel*) activity in the distal tubule and collecting duct [10, 14]. It should be emphasized that after 5 days of chronic metabolic acidosis, aldosterone-induced upregulation of Sgk1, ENaC, NCC *(sodium-chloride cotransporter)* was observed, which resulted in the return of the urinary sodium excretion rate to below baseline level [10]. Amlal et al. [15] reported that enhanced sodium reabsorption in the distal tubule is a compensatory response to impaired proximal tubule function. As a result, in rats and mice with acidosis, urine Na^+ excretion was comparable to control animals [4, 15]. Menegon et al. [11] reported that in rats with chronic metabolic acidosis, urinary K^+ excretion increased. Scandling et al. [16] claim that a high aldosterone level during chronic metabolic acidosis stimulates this process. In children with acute metabolic acidosis, the urine flow rate, urinary sodium, potassium and chloride excretion increased [17]. During acidosis in humans, urinary calcium excretion also increased, but urinary magnesium excretion remained unchanged [18]. Blumberg et al. [19] reported that ammonium chloride administration induced acute metabolic acidosis and enlarged the plasma free magnesium fraction and the excretion of urinary magnesium.

Increased H^+ concentration during acidosis requires its increased removal. This is possible through a progressive increase of NHE3 protein abundance, observed during chronic metabolic acidosis in the apical membranes along the convoluted proximal tubule and thick ascending

Corresponding author: Anna K. Kurpińska, MSc. in Biology, Department of Animal Physiology and Cytobiology, Faculty of Biotechnology and Animal Husbandry, West Pomeranian University of Technology, Doktora Judyma 6, 71-466 Szczecin, Poland.
E-mail: anna.kurpinska@zut.edu.pl

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limb [13]. Additionally, acid-base imbalance and high aldosterone concentration stimulate proton-translocating ATPases activity: H-ATPase present in almost all nephron segments and H-K-ATPase present in the connecting tubule and collecting duct [20]. Tsuruoka et al. [21] reported that increased synthesis and exocytosis of these proton pumps to the collecting duct apical membranes, stimulate bicarbonate absorption (H^+ secretion) in rabbits with metabolic acidosis. Langhmani et al. [22] observed in rats with chronic metabolic acidosis, an additionally enhanced bicarbonate absorptive capacity in the thick ascending limb.

In animals with metabolic acidosis, the buffering process and acid excess removal is connected with increased glutamine intake and increased glutamine metabolism (glutamine is an ammonium precursor) [23, 24]. In mice and rats with metabolic acidosis, expression of glutamine transporter SNAT3 (SN1) (*system N amino acid transporter 3*) to the basolateral membrane of the proximal tubule increased [24, 25, 26]. In rats, the expression of SN1 protein in the kidney during chronic metabolic acidosis, increased five- to six-fold. The increased expression and activity of phosphate-activated glutaminase was also observed [23]. In rats with acidosis, a 10-fold increase of SN1 mRNA level in the whole kidney was observed, whereas in the cortex, a 100-fold increase of SN1 mRNA level was observed, in comparison to control animals. Moreover, during chronic metabolic acidosis, Na-dependent glutamine uptake into BLMV *(basolatelar membrane vesicles)* and BBMV *(brush – border membrane vesicles)* in the cortex was induced [26]. In rats, increased glutamine metabolism generates additionally 2 bicarbonate ions (glutamine is converted through α-ketoglutarate into glucose). Bicarbonate ions are reabsorbed into the venous blood, which partially compensate chronic metabolic acidosis [27]. In rats, after NH_4Cl administration, a 20 % reduction of pendrin abundance (pendrin is involved in bicarbonate excretion) was observed in the apical plasma membrane of type B and non-A-non-B intercalated cells of the cortical collecting duct and connecting tubule [28]. Petrovic et al. [29] observed a 60% decrease in pendrin activity in rats with metabolic acidosis, a 68% mRNA expression decrease in the kidney cortex was also observed. Hafner et al. [30] noticed a reduced abundance of pendrin in the apical side of renal non-type A intercalated cells in mice during metabolic acidosis.

Curthoys [27] reported that in rats with chronic metabolic acidosis, ammonium ions excreted in the urine, derived from glutamine, facilitate the acid excretion (as expendable cations). Garibotto et al. [9] reported that in humans, during chronic metabolic acidosis, ammonium production and ammonium urine excretion is significantly augmented. In rats with chronic metabolic acidosis, in both the outer and inner medulla collecting duct, increased Rhcg (Rh C Glycoprotein) protein expression was observed [31]. Increased ammonia production and excretion in mice with metabolic acidosis is mediated in part by angiotensin receptor dependent enhancement of NHE3 expression, therefore angiotensin II signalling is necessary for adaptive enhancements of ammonia excretion by the kidney and ammonia production and secretion in the convoluted part of proximal tubule [32].

In rats, increased NaDC-1 (*sodium/dicarboxylate-1 cotransporter*) protein abundance, especially in the S2 segment of the proximal tubule, and increased NaDC-1 mRNA abundance during chronic metabolic acidosis, allowed an increased citrate absorption [33].

An increased phosphate excretion rate was also observed during metabolic acidosis. Ambühl et al. [34] proved that decreased phosphate reabsorption was connected with decreased proximal tubular apical brush border membrane Na/Pi cotransport activity. Na/Pi cotransport activity was reduced by 61% after 10 days of metabolic acidosis. Nowik et al. [35] reported that in mice increased phosphate excretion was a result of the inhibitory effect of low pH on cotransporters, but this cannot be explained by decreased protein expression of NaPi-IIa i NaPi-IIc, located in the brush border membrane of the proximal tubule. The authors suggested that Pit1 and Pit2 (*Na^+- dependent P_i transporters*) may be involved in the process of compensation.

In rats with metabolic acidosis, a significant increase in inorganic sulfate excretion in the proximal tubule was observed. This was associated with decreased Na-S_i cotransport activity, decreased NaS_i-1 protein abundance in the brush border membrane and decreased cortical NaS_i-1 mRNA abundance [36].

In acid-base imbalance it is vital to maintain water homeostasis. Decreased proximal tubular water reabsorption may be balanced by regulation of water reabsorption through AQP-2 and AQP-3 along the collecting duct. Increased mRNA expression of these water channels was observed during metabolic acidosis in mice [4]. In rats with metabolic acidosis, in the kidney cortex and medulla AQP-2 protein abundance and AQP-2 mRNA increased along the collecting duct, whereas AQP-1 protein abundance remained unchanged [15, 37]. It is worth emphasizing that Mouri et al. [37] observed in rats a 92% decrease of urinary AQP2 excretion.

In rats with metabolic acidosis no changes in urine volume were observed; however, acidosis lead to a decrease in urine pH, even to 5.5, and an increase of urine osmolality [15]. In contrast, Mouri et al. [37], in rats with metabolic acidosis observed lower urine osmolality.

During metabolic acidosis a number of hormones involved in kidney function are regulated. Observation in children denote that during acute metabolic acidosis, RAA system activity increased. Natriuresis and diuresis-related plasma rennin activity increase, hyperkalaemia, and direct stimulation of adrenal steroidogenesis are involved in this process [17]. In mice, besides the above-mentioned RAA system activity stimulation, vasopresine (involved in water balance regulation) stimulation was observed [4, 5]. In rats, a 4-fold plasma vasopresine concentration increase was observed [15]. During chronic metabolic acidosis no significant changes in the plasma concentration of cortisol, corticosterone, or deoxycorticosterone and in the urinary excretion of 17-hydroxycorticoids were observed [38].

Many authors highlight the metabolic acidosis influence on kidney hypertrophy. In rats with metabolic acidosis, despite lower body weigh gain, higher renal weight was observed [11]. Lotspeich [39] reported that NH_4Cl induced chronic metabolic acidosis in rats, increased kidney hypertrophy, wet and dry kidney weight and nitrogen content of the kidney tissue. *In vitro* research showed that ammonia induces kidney hypertrophy by increased protein synthesis and decreased rate of protein degradation [40]. Apart from that, kidney enlargement in rats with acidosis was observed. The authors suggested that IGF-I may play a role in initiating kidney growth [41]. In humans with chronic metabolic acidosis, with the exception of the suppression of protein degradation, an increased kidney uptake of amino acids was observed while

glutamine and glycyne uptake increased, and a decreased release of glutamine, serine, tyrosine, leucine into the renal vein was observed [9]. Cheval et al. [4] observed increased kidney cell proliferation in mice with metabolic acidosis. In cultured opossum kidney cells exposed to NH_4Cl, the cell number remained unchanged, although hypertrophy and an 18% increase in cell protein content (caused by decreased protein degradation rate) was observed [42]. Fawcett et al. [41] reported that in rats with acidosis, depressed renal proteolysis may be related to reduced cathepsin B and L gene expression and reduced renal cathepsin activity. In lambs with induced metabolic acidosis, no alteration in mRNA of the proteins involved in proteasome protein degradation was observed, which could indicate that primarily in protein degradation in acidosis, mechanisms are involved which are different from the ubiquitin-mediated proteolysis mechanisms [43].

Observation of gene expression changes in the kidney, induced by metabolic acidosis, gives wider view of changes in this organ. Changes in the expression of genes involved in cell proliferation, water, sodium, acid-base transport, urine acidification in the mouse outer medullary collecting duct were observed. It was demonstrated that genes involved in acid-base balance regulation are regulated during acidosis, e.g. subunits of H-ATPase, aldolase, kidney anion exchanger, chloride channel Clcka, carbonic anhydrase-2 [4]. Nowik et al. [44] showed that about 4,000 transcripts in mouse kidney were differentially expressed in control *vs.* acidosis subjects. The changes of expression concern genes involved in transport, cell cycle, water homeostasis and ammoniagenesis. The authors highlighted changes in oxidative phosphorylation gene expression. A large number of genes, after early increased expression, reach base level after a few days of metabolic acidosis, which suggests kidney adaptation.

The development of proteomic techniques enables multifaceted and multigradual analysis of changes in kidney during metabolic acidosis [45]. Proteomic studies revealed changes in the expression of proteins involved in different processes, such as: oxidative phosphorylation, gluconeogenesis, ammoniagenesis and ribosome function in mouse proximal tubule. Additionally, changes in actin cytoskeleton machinery protein and focal adhesion complexes protein expression suggested that in the remodeling of proximal tubule during metabolic acidosis, an important role may be played by the modulation of endocytosis and/or exocytosis. The wide range of changes induced by metabolic acidosis provides the fact that 172 proteins in convoluted and 164 proteins in strait proximal tubule revealed variations in expression [46]. In rats, proteomic proximal tubule analysis enabled the selection of 120 proteins differentially expressed among 2,000 proteins resolved on the gel. Out these, in animals with acidosis, there were identified: 21 proteins that were increased 1.5-8.7- fold, e.g. PEPCK, mitochondrial glutaminase (66 i 68 kDa subunits), glutathione S-transferase, glutamate dehydrogenase; 16 proteins that were decreased 0.03-0.67-fold, eg.: argininosuccinate synthetase, Mu-crystallin, pyruvate carboxylase, transglutaminase. The majority of the proteins with increased expression revealed gradual kinetic changes during acidosis. As an example, calmodulin increased 5.6-fold 2 hours after onset of acidosis, and after 7 days of acidosis returned to control levels [47].

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