Clinical Role of Dietary Thiamine on Regulation of Renal Response to Metabolic Acidosis in Adult Male Rats

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Abstract: The kidney plays a central role in maintaining the composition of body fluids by regulating water, NaCl, acid base, and solute reabsorption and excretion, respectively. The present study was done to investigate the physiological role of thiamine in regulation of renal response to metabolic acidosis induced by NH_4Cl in adult male rats. For this experiment, fifty rats were used. They were divided into five groups. Control rats received basal diet; rats fed on basal diet mixed with NH_4Cl (4g NH_4Cl/100g diet) to induce severe metabolic acidosis, rats fed on basal supplemented diet with thiamine (600 mg/kg diet), and rats fed on basal supplemented diet with thiamine before and after induction of metabolic acidosis by NH_4Cl for 14 days. The results showed that the plasma levels of chloride, urea, and creatinine were significantly elevated in metabolic acidosis induced by NH_4Cl. Thiamine supplementation at high dose before or after induction improved the chloride values. Feeding diets supplemented with thiamine modulated the plasma sodium and bicarbonate values. Supplementation with vitamin B1 as preventive agent significantly restored these changes to near control value. Also, feeding of thiamine as curative agent improved plasma creatinine and urea levels. Urinary pH and potassium levels were decreased significantly in metabolic acidotic rats when compared to all experimental groups. Urinary ammonia and aldosterone levels were decreased by thiamine supplementation as protective agent.
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Introduction

Metabolic acidosis (MA) refers to the condition where the acid base balance of the body is disrupted due to an increased production of acid or the reduced excretion and decreased production of bicarbonates **(Jones And Walter, 2007)**. Sometimes, metabolic acidosis can occur even without the excessive production of acids, when the kidney fails to excrete them through urine, which can be a symptom of renal failure **(Nowik et al., 2010).** The kidney plays a central role in maintaining the composition of body fluids by regulating water, NaCl, acid base, and solute reabsorption and excretion, respectively **(Quentin et al., 2004)**. The standard protocol to induce metabolic acidosis in experimental animals involves application of ammonium chloride (NH_4Cl), which is supplied most frequently in diet **(Ingrid and Arcangelo, 2014)**. Metabolic acidosis induced by NH_4Cl has been associated with decreased reabsorption of NaCl and water in the proximal tubule of rat **(Faroqui et al., 2006).** Conditions like malnutrition are also associated with metabolic acidosis. The importance of vitamins, or micronutrients, in healthy individuals has been well established **(Shradha et al., 2007**). Thiamine is an essential cofactor for aerobic metabolism and facilitates the entry of pyruvate into the tricarboxylic acid cycle **(Abdoulaye, 2008).** Deficiency of thiamine impairs pyruvate utilization and thus results in a rise in serum lactate level **(Hung et al., 2001).** Thiamine is the precursor of thiamine pyrophosphate (TPP), a coenzyme in the cleavage of carbon-to-carbon bonds and the oxidative decarboxylation of keto acids of the citric-acid cycle **(Parvesh et al., 2004).** As a result, thiamine is essential for producing energy from glucose. Thiamine is an important co-factor required for multiple enzymes involved in carbohydrate metabolism **(Derrick, 2006)**. To maintain acid-base homeostasis the kidney increases its excretion of the acid load, and any failure on the part of the kidney to excrete the acid will lead to metabolic acidosis **(Appel and Downs, 2008).**

Materials And Methods

Materials

Thiamine (Vitamin B1) and ammonium chloride (NH_4Cl) were purchased from EL-Nasr Pharmaceutical Chemicals Co., Cairo, Egypt.

Experimental animals

Five homogenous groups each of ten adult male Albino rats (Sprague-Dawely) strain, mean weight varied between 125.4 to 135.9 g were used. The animals were 6 weeks old at the beginning of the experiment. They were supplied from breeding unit of the Egyptian Organization for Biological Products and Vaccines (Helwan breeding farm, Cairo, Egypt). Animals received human care, housed individually in metabolic stainless steel cages with wire mesh bottoms and maintained at temperature 25°C±5°C,

humidity 50% ± 5% and light dark cycle held constant 12/12 h. During the experiment, food and water were provided ad libitum.

Groups were classified as follows:

Group1: Normal control untreated rats [Control group]

Group2: Rats were fed on basal diet mixed with NH_4Cl for 14 days (4g NH_4Cl/100g diet) to induce severe metabolic acidosis [MA group)

Group3: Rats were fed on basal diet supplemented with high dose of thiamine for 14 days (600 mg/kg diet) [B1S group]

Group 4: Rats were fed on basal diet supplemented with high dose of thiamine for 14 days before and 14 days after mixed with NH_4Cl as a protective agent against metabolic acidosis [B1S-MA group]

Group 5: Rats were fed on basal diet mixed with NH_4Cl for 14 days before and 14 days after supplemented with high dose of thiamine as a curative agent against metabolic acidosis [MA-B1S group]

Plasma Analysis

At the end of the experiment (6 weeks), rats were fasted for 12 hrs, then the animals were anesthetized by ether and blood samples were taken from hepatic portal vein by syringe and transferred into heparinized centrifuge tubes. The tubes centrifuged at 600 x g for 30 minutes at 25°C to provide plasma needed for the biochemical analysis. Plasma samples were taken and kept in dry clean plastic and stored at –20°C till used for the different analysis Fresh plasma was tested for content of bicarbonate **(Belding and James, 1954)**. Plasma chloride was determined according to the colorimetric method **(Schoenfeld and Lewellen, 1964)**, urea **(Patton and Crouch, 1977),** creatinine **(Bartels et al., 1972)**, albumin **(Doumas, 1971)**, and sodium **(Henry, 1974)** Anion gap was calculated theoretically by the equation: Anion gap (AG) = ($[Na^+]$) - ($[Cl^-]$ + $[HCO_3^-]$) **(Jeffrey and Nicolaos, 2007).**

Urine Analysis

All animals were kept in individual metabolic cages and 24h urine samples were collected at the end of experimental period. The preparation of samples was carried out immediately after urine collection and analyzed for urinary parameters. Urine samples were acidified with 10% hydrochloric acid to block the growth of bacteria and molds and stored below 4°C for subsequent analysis. Urinary pH was measured using a pH meter (Beckman coulter). Urinary pH was measured using a pH meter (Beckman coulter). Urine was also analyzed for calcium **(Tietz, 1970)**, phosphate **(Drewes, 1972)** potassium **(Henry, 1974)** and ammonia **(Gips *et al*., 1970)** Urinary aldosterone in urine was measured with a radioimmunoassay method according to **(Philip et al., 1979).**

Statistical Analysis

The data were statistically analyzed by SPSS version 10.0 statistical packages. Data were presented as the means ± SD; statistical differences between groups were performed using t-test. Differences were considered significant when $p<0.01$.

Results

Effect of NH_4Cl and thiamine (Vitamin B1) supplementation on plasma sodium, bicarbonate, chloride, and anion gap values:

Table 1: Effect of different experimental diet on plasma sodium, bicarbonate, chloride, and anion gap values

	Sodium (Na^+) (mmol/L)	**Bicarbonate (HCO_3^-)** (mmol/L)	**Chloride (Cl^-)** (mmol/L)	**Anion Gap (AG)** (mmol/L)
G1 Basal Control	147.8±11.6	30.5±2.8	104.8±8.1	12.5±2.6
G2 Basal + NH_4Cl [MA]	135.6±6.6	17.8±3.6	113.7±3.2	4.10±0.8
G3 Thiamine Supplemented (B_1S)	145.3±9.3	29.7±3.0	100.1±4.6	15.6±1.7
G4 B_1S-MA	141.6±6.4	22.9±5.8	107.3±9.3	11.4±0.75
G5 MA-B_1S	139.2±12.1	23.3±5.5	103.1±3.2	12.8±2.5

Values are means ± SD, n = 10 Significant difference at $p< 0.01$

The concentration of plasma chloride was significantly ($p<0.01$) elevated in metabolic acidosis induced by NH_4Cl group (MA) at 14 days when compared to the control group. Addition of thiamine at high dose before (B1S-MA) or after (MA-B1S) induction improved the chloride values when compared with MA rats. Induction of metabolic acidotic state by NH_4Cl resulted in significant

reduction of bicarbonate levels as compared to control rats. Feeding diets supplemented with thiamine (G4 and G5) were modulate the plasma sodium and bicarbonate values than that MA rats (G2) fed diets mixed with NH_4Cl alone without any thiamine treatments. Plasma values of anion gap was reduced in the metabolic acidotic rats when compared to control group and then gradually improved by thiamine supplementation (Table 1).

Effect of NH_4Cl and thiamine (Vitamin B1) supplementation on plasma creatinine, urea and albumin levels:

Plasma albumin concentration was reduced significantly ($p<0.01$) in the metabolic acidotic rats (MA) when compared to control group. Plasma creatinine and urea concentrations were significantly higher in MA group and gradually decreased in other groups by adding thiamine at high level. Supplementation with vitamin B1 as preventive agent in G4 (B_1S-MA) significantly modulated these changes and restored it to near control value. Also, feeding of thiamine as curative agent after metabolic acidosis induction improved plasma creatinine and urea levels as compared to MA group (Table 2).

Table 2: Effect of different experimental diet on plasma creatinine, urea, and albumin levels

	Creatinine (mg/dl)	**Urea** (mg/dl)	**Albumin** (g/dl)
G1 Basal Control	1.03±0.08	33.5±2.8	5.1±1.1
G2 Basal + NH_4Cl [MA]	1.60±0.12	60.9±4.4	2.4±0.8
G3 Thiamine Supplemented (B_1S)	0.95±0.06	31.5±2.5	4.8±0.9
G4 B_1S-MA	1.25±0.09	39.2±6.7	2.6±0.5
G5 MA-B_1S	1.32±0.09	45.2±3.1	2.7±0.9

Values are means ± SD, n = 10 Significant difference at $p< 0.01$

Effect of NH_4Cl and thiamine (Vitamin B1) supplementation on urinary pH, ammonia, and aldosterone values:

Urinary pH value was decreased significantly ($p<0.01$) in metabolic acidotic (G2) when compared to all experimental groups. The results of the present study showed that the urinary ammonia excretion was increased significantly ($p<0.01$) in NH_4Cl induced group (G2) and gradually decreased by thiamine supplementation as preventive agent (B_1S-MA). Also, the urinary aldosterone hormone concentration was increased in metabolic acidosis induced group (G2) when compared to control group.

Supplementations with vitamin B1 before induction (B_1S-MA) prevented these changes and regulate renal response against any changes induced by NH_4Cl load. Addition of thiamine at high dose mixed with basal diet alone without metabolic acidosis induction (B_1S) maintains theses urinary parameters as control rats (Table 3).

Table 3: Effect of different experimental diet on urinary pH, ammonia, and aldosterone values

	Urine pH	**Ammonia (NH_3)** (µmol/24h)	**Aldosterone** (nmol/24h)
G1 Basal Control	7.1±0.1	15.4±1.6	0.99±0.06
G2 Basal + NH_4Cl [MA]	6.8±0.2	45.2±3.9	2.21±0.35
G3 Thiamine Supplemented (B_1S)	7.2±0.1	14.2±2.3	0.86±0.15
G4 B_1S-MA	7.0±0.2	19.8±1.8	1.25±0.09
G5 MA-B_1S	7.0±0.2	28.3±2.7	1.98±0.07

Values are means ± SD, n = 10 Significant difference at $p< 0.01$

Effect of NH_4Cl and thiamine (Vitamin B1) supplementation on urinary potassium, calcium, and phosphate levels:

NH_4Cl feeding with basal diet resulted in a significant increase in urinary calcium and phosphate excretion as compared to control (G1). Feeding of thiamine before or after metabolic acidosis induction had no improvement effect on urinary calcium or phosphate levels as compared with control group. Whereas, supplementation of thiamine before metabolic acidosis induction improved urinary potassium concentrations as compared with MA rats (Table 4). There are no significant differences in the value of urinary potassium, calcium and phosphate in rats supplemented with thiamine after induction as curative agent as compared to G1 and G3 groups (Table 4).

Table 4: Effect of different experimental diet on urinary potassium, calcium, and phosphate levels

	Potassium (K^+) (mg/24h)	**Calcium (Ca^{+2})** (mg/24h)	**Phosphate (Pi)** (mg/24h)
G1 Basal Control	28.2±1.9	0.322±0.04	0.150±0.03
G2 Basal + NH_4Cl [MA]	25.1±3.5	0.638±0.08	3.55±0.45
G3 Thiamine Supplemented (B_1S)	27.6±1.2	0.352±0.02	0.091±0.001
G4 B_1S-MA	29.1±1.7	0.611±0.03	2.34±0.02
G5 MA-B_1S	25.3±4.2	0.623±0.05	3.10±0.1

Values are means ± SD, n = 10 Significant difference at $p< 0.01$

Discussion

Urinary excretion of ammonia is essential for maintenance of normal acid-base homeostasis in several species including rat, and it was demonstrated that an increase in urinary ammonia is a typical response to metabolic acidosis **(Tom And Andrew, 2012)**. The early renal metabolic response was studied in rats made acidotic by oral feeding of ammonium chloride **(Nowik et al., 2010).** The data show that after NH_4Cl, rats already have a severe metabolic acidosis, and in response to this, there is increased urinary excretion of ammonia. The rise in urinary ammonia at acidotic rats induced by NH_4Cl indicates an increase in renal ammonia production. It has been shown that glutamine is the major source of urinary ammonia. There are two possible pathways by which glutamine may contribute to renal ammonia. Glutamine may be deaminated to form glutamate and ammonia or alternatively, the amino group may be removed by transamination with the resultant formation of α-ketoglutarate and an amino acid **(Nowik et al., 2010).**

The results of the present study showed that, animals fed on diets supplemented with high dose of thiamine were less acidotic compared to the group receiving NH_4Cl in diet without any treatment. The results found higher urea excretion in metabolic acidotic rats (MA) compared to the control group. Rats on the acid diet tended to receive a higher NH_4Cl load than the respective thiamine supplemented groups and therefore an overall increased nitrogen load that might explain the observation **(Nowik et al., 2010).** Moreover, the higher urinary urea excretion might also reflect increased catabolism of proteins **(Maria et al., 2012).** The excretion of chloride was lower in rats receiving thiamine as protective agent before metabolic acidosis induction by NH_4Cl. Since urinary chloride excretion might reflect net chloride intake and ammonium absorption, it might indicate a lower absorbed NH_4Cl load in metabolic acidotic rats receiving NH_4Cl mixed with basal diet without thiamine supplementation (Table 1). However, interpretation of urinary chloride excretion is difficult since it may not directly correlate with intestinal chloride or even ammonium absorption. The results of the present study showed that the urinary aldosterone was elevated in metabolic acidotic rats. Metabolic acidosis state activates the renin-angiotensin-aldosterone system and stimulates synthesis of angiotensin II and secretion of aldosterone (**Gabriel and Geetha, 2014**). Induction of MA followed by feeding thiamine supplemented diet (MAB_1S) resulted in an elevation of urine aldosterone excretion as compared with control. Elevated aldosterone levels were not reflected by changes in plasma sodium and urinary potassium excretion. This might be explained by direct effects of acidosis on aldosterone targets such as potassium channel or sodium channel. Urinary calcium excretion was increased in all acidotic rats. Hypercalciuria is thought to develop at least in part

due to inhibition of calcium channel in the distal convoluted tubule and connecting tubule **(Watts, 2005).**The results of the present study showed that the value of plasma bicarbonate, albumin, sodium, and anion gap were reduced in metabolic acidotic rats as compared with other experimental groups. Anion gap represents unmeasured anions in plasma. The unmeasured anions include–anionic proteins, phosphate, sulphate and organic anions **(Figge et al., 1998)**. An increase in AG is due to increase in unmeasured anions and less commonly due to decrease in unmeasured cations (Ca^{+2}, Mg^{+2}, K^{+}). The results of the present study showed that the urinary calcium was increased significantly in metabolic acidotic rats and the supplementation of thiamine had no protective or curative effect as compared with control group (Table 4). On the other hand, urinary potassium concentration reduced by feeding NH_4Cl in MA rats. Addition of thiamine modulates renal response, and resulted in an elevation of urinary potassium levels as protective agent (B_1S-MA). The AG may decrease with a decrease in anionic albumin, either due to decreased albumin concentration or alkalosis which alters albumin charge **(Figge et al., 1998)**. Supplementation with vitamin B1 as preventive agent in G4 (B_1S-MA) significantly modulated these changes and restored it to near control value. Also, feeding of thiamine as curative agent after metabolic acidosis induction improved plasma creatinine and urea levels as compared to MA group (Table 2). The present study concluded that, thiamine had a regulatory role in maintaining renal acid base balance. High thiamine diet improved the renal function and response to metabolic acidosis induced byNH_4Cl in adult male rats.

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