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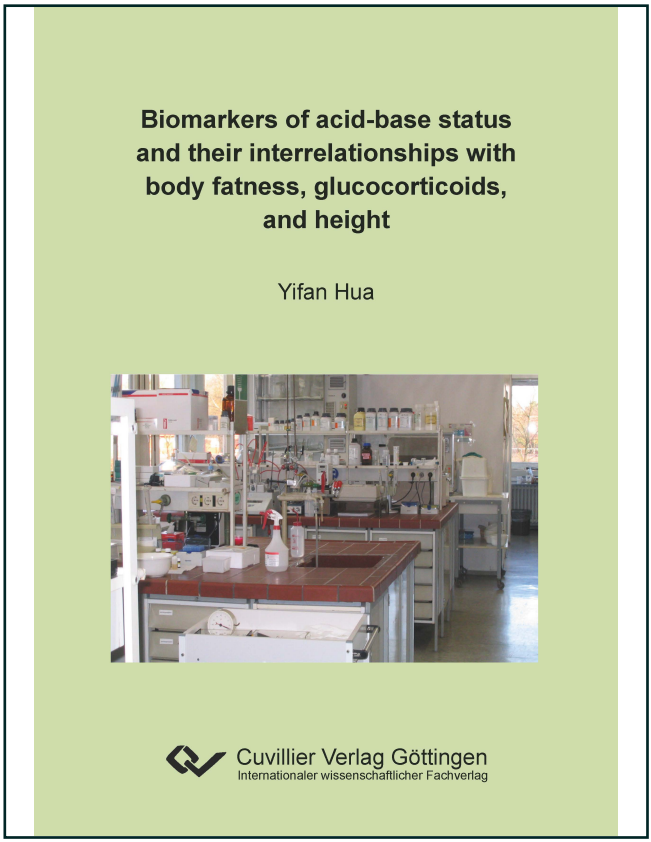
Biomarkers of acid-base status and their interrelationships with body fatness, glucocorticoids, and height



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1 INTRODUCTION

Acid-base homeostasis is critical for normal physiology and health. Abnormal acid-base homeostasis and disorders lead to clinical problems such as reduced bone strength, muscle atrophy, nephrolithiasis, growth retardation in children, and increased mortality in patients with or without chronic kidney disease (CKD) [1]. As the central organ of the urinary system, the kidneys excrete metabolites and body wastes as well as regulate electrolyte balance and acid-base balance [2]. Acid excretion by the kidneys consists predominantly of buffered protons and to a small part of free protons quantified as urine pH. A low urine pH is one primary determinant of kidney stone formation indicating an impaired renal tubular net acid excretion function, in case of adjusted average daily acid loads. The daily amount of acids that need to be renally excreted is relevantly influenced by dietary intakes of acid and base precursors. Our research does concentrate on nutritive influences of human acid-base status and its potential relevance to the prevention of nutrition-related health problems.

In periods of childhood and adolescent growth, overweight has become a growing health concern. In the last decades, studies on childhood obesity mainly focussed on relationships with, e.g., metabolic syndrome, diabetes, and cardiovascular diseases later in adulthood. Among others, obesity is directly related to an increased risk of kidney stones and obesity-related insulin resistance, which is a known independent risk factor of nephrolithiasis in adults as well as children and adolescents [3–5]. In line with this, Zhu and Scherer [6] reported, in detail, an interaction of adipose tissue with the kidney — referred to as adipo-renal axis — and the immunological and endocrinological functions of adipose tissue with regard to kidney diseases. Additionally, calcium supersaturation with calcium salts in urine and a reduced citrate excretion, i.e., hypocitraturia, increase the prevalence of pediatric nephrolithiasis as well [7]. Interestingly, a reduction of renal citrate output has been observed in patients with Cushing's syndrome [8], i.e., in conditions with excess levels of cortisol. However, this high cortisol–low citrate output relationship has not yet been examined in healthy individuals. Against this background, the objectives of this thesis were to examine, firstly, the relationship of body fatness to 24-h urine pH, a biomarker of kidney's acid excretion function, in healthy children and adolescents participating in the DOrtmund Nutritional and Anthropometric Longitudinally Designed (DONALD) Study; secondly, the possible mediation roles of adipose tissue-related inflammatory markers in the body fat–urine pH relation in healthy DONALD adults; and, thirdly, whether higher glucocorticoid activity still within the physiological range already shows an association with reduced 24-h urinary citrate excretion in healthy children.

In addition to the renal function of maintaining acid-base homeostasis, acid-base-related anabolic effects on growth, particularly on adult hight were further examined. Poor nutrition and illness are the main non-genetic environmental factors limiting children's growth [9]. Sufficient protein intake and alkaline supplementation (correction of metabolic acidosis) have known growth-promoting effects in

malnourished and/or stunted children [9–11]. Although a relation of childhood protein intake with adult height has been described for healthy male individuals [12], no data for both sexes with both detailed 3-d dietary records and 24-h urinary measurements of biomarkers of protein intake over almost the whole growth period are available. Thus, lastly, we aimed to examine whether a protein intake higher than the daily recommendations may enhance adult height independent of the potential endogenous acid load in healthy, free-living DONALD children and adolescents and whether a low dietary acid load might additionally add to the growth potential.

2 THEORETICAL BACKGROUNDS

2.1 Acid-base balance

Acid-base balance is a homeostatic physiological regulation of blood pH by many organs including intestine, liver, lung, the kidney, and the buffer system. Blood pH usually varies within a narrow range in healthy humans from 7.36 to 7.44, however, the urine pH can vary within a wider range due to the alterations of endogenous acid-base status caused by, e.g., an acidic diet [2]. In this section, the physiological background of acid-bases status and the mechanisms in maintaining acid-base status in human beings as well as the potential modulations of body fatness and glucocorticoids to acid-base homeostasis will be described. Furthermore, protein intake and acid-base status in relation to childhood growth will also be introduced.

2.1.1 Endogenous buffer system

An overview of the major organs and their interactions in influencing systemic acid-base balance is given in **Figure 1** [13]. As shown in this figure, there are four major organs involved in regulating acid-base homeostasis apart from the nervous system. The initial organ intestine regulates the endogenous levels of acids and alkali depending on diet composition and absorption rates rather than generate acid or base equivalents itself [13]. Generation of acid or alkali loads in the intestine is based on (i) the different bioavailability of cations and anions and (ii) the principle of electroneutrality [13]. After consuming a diet with $MgCl_2$, for example, ~32% of Mg^{2+} and ~95% of Cl^- are absorbed into the blood. Thus, the unabsorbed Mg^{2+} (~68%) not compensating the almost totally absorbed Cl^- needs to be replaced by stoichiometric amounts of bicarbonate (HCO_3^-) from the most important buffer system (H_2CO_3/Na^+-HCO_3^- buffer system) supplied with the pancreatic fluid. The sodium (Na^+) having also a bioavailability of around 95% from the pancreatic buffer gets absorbed along with the excess Cl^-. So that secretion of $NaHCO_3$ and (re)absorption of anions and cations in the intestine eventually result in a loss of systemic buffer Na^+-HCO_3^- which corresponds to a generation of an acid load. Later on, a real production of acids (H^+) takes place in the liver and/or other metabolic active tissues in dependency of metabolism of nutrients, particularly sulfur-containing amino acids [13].

The oxidation of sulfur-containing amino acids (AA-S), especially methionine and cysteine being essential constituents of protein [14], in the liver yields sulfuric acid along with urea (AA-S → $2H^+$-SO_4^{2-} + urea + CO_2). The sulfuric acid is then immediately buffered with Na^+-HCO_3^- from the circulating of cellular buffer system ($2H^+$-SO_4^{2-} + $2Na^+$-HCO_3^- → $2Na^+$-SO_4^{2-} + $2H_2O$ + $2CO_2$). In human beings, around 15,000 mEq/d volatile acids are exhaled as CO_2 and have no long-lasting impact on acid-base status. However, depending on daily food intake and body weight, around 70 to 100 mEq/d (1.0 to 1.5 mEq/kg/d H^+) fixed acids or non-volatile acids such as sulfuric or various inorganic acids are generated in the body. These acids directly impact the acid-base status and have

to be excreted via the kidneys. Therefore, the kidney is the major acid-excreting organ and importantly, during the acid excretion process, reabsorbs Na^+-HCO_3^- and thus "generates" new HCO^- to compensate the lost buffer. In this thesis, our focus is laid on non-volatile acids and their physiological implications among free-living subjects.

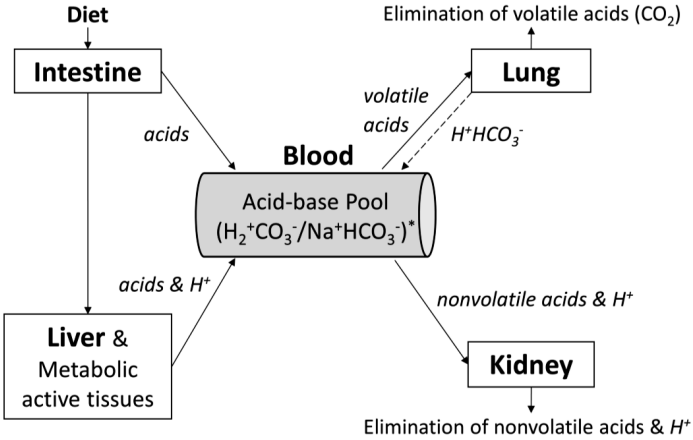

Figure 1 Interaction of organs in acid-base metabolism. *The most important buffer system in the blood especially for buffering free protons. Figure created according to Remer et al. [13].

2.1.2 The kidney

The kidneys have a predominant role in regulating systemic bicarbonate concentration and, thus, are responsible for acid-base homeostasis [2, 13]. Under the renal control of HCO_3^-, the blood pH maintains in a narrow and stable range (arterial pH between 7.36 and 7.44, intracellular pH at around 7.2 [2]).

One primary function of the kidney is the reabsorption of filtered bicarbonate. Those reabsorbed HCO_3^- in the kidney is returned to the blood and fills the buffer gap resulted from acid neutralization. On average, about 4500 mmol filtered HCO_3^- is reabsorbed in renal tubules [2, 15]. However, it is not sufficient to maintain acid-base balance even in case of a complete reabsorption of filtered bicarbonate [1]. Therefore, another important function of the kidney is the so-called "new bicarbonate" generation, which replenishes this bicarbonate utilized for buffering acid loads.

The "new bicarbonate" is generated as by-product during the processes of (i) ammonia production and (ii) titratable acid excretion [1]. Ammonia metabolism includes net ammoniagenesis and renal epithelial cell ammonia/ammonium transport. In the following sections, ammoniagenesis, ammonia/ammonium transport, and titratable acid excretion will be discussed. **Figure 2** briefly shows

the HCO_3^- generation and its reabsorption along with Na^+ as well as the excretion of H^+ as titratable acids and/or ammonium.

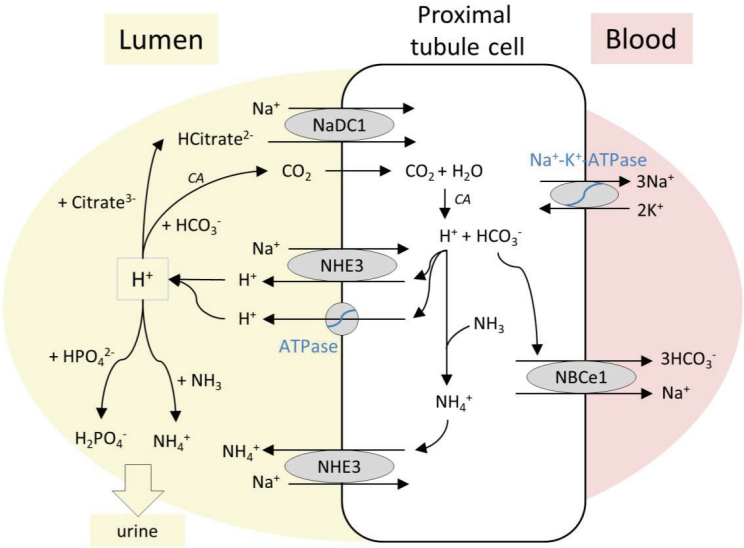

Figure 2 Proximal tubule HCO_3^- reabsorption and H^+ secretion. Unlike NaCl reabsorption, luminal acidification is mediated by dedicated acid-base transporters. CA, carbonic anhydrase. Figure created according to Curthoys and Moe [16].

2.1.2.1 Function of proximal tubule and its response to acid loads

The kidney produces around 160 to 170 L ultrafiltrate per day and the proximal tubule is responsible for reabsorbing water, NaCl, $NaHCO_3$, and nearly all nutrients in the ultrafiltrate to regulate the fluid, electrolyte, and nutrient homeostasis [16].

Ammoniagenesis

Proximal tubule is the major site of renal ammoniagenesis and glutamine is the primary substrate for ammonia generation [17]. The Na^+-dependent apical AA transporter B^0AT-1 is responsible for the reabsorption of the filtered glutamine and LAT2, a neutral AA exchanger in the basolateral membrane, for returning largely the reabsorbed glutamine to the blood (**Figure 3**). Under acidic conditions, one molecule glutamine — after entering mitochondria — is completely metabolized through phosphate-dependent glutaminase (GA) and glutamate dehydrogenase (GDH) to 2 molecules of NH_3, which further react with water and carbon dioxide in the cellular environment and eventually generate 2 molecules of NH_4^+ as well as HCO_3^- ions ($2NH_3 + 2H_2O + 2CO_2 \rightarrow 2NH_4^+ + 2HCO_3^-$) [1, 17]. Apical NH_4^+ is secreted preferentially into the luminal fluid through NHE3 transporter in parallel with

Na^+/NH_4^+ exchange; while the bicarbonate is then transported across the basolateral membrane via the electrogenic Na^+-HCO_3^- cotransporter NBCe1 (NBCe-1A), and serves as "new bicarbonate" generated by the kidney. Filtered bicarbonate reabsorption accompanied by "new bicarbonate" generation as well as excretion of non-volatile acids are the central functions of the kidney for acid-base homeostasis [17]. For every milliequivalent of NH_4^+ excreted, one milliequivalent of new HCO_3^- is returned to the blood along with reabsorbed Na^+ replenishing the initial buffer loss through the acid load.

As shown in **Figure 3**, in order to efficiently excrete net acids from the body, the amount of plasma glutamine extracted within the proximal tubule via SNAT3 (a basolateral Na^+-coupled glutamine transporter) increases from < 3% to ~35% under an acid load. Renal catabolism of glutamine raises with increased expression of mitochondrial glutamine transporter and NBCe1. As a result, the α-ketoglutarate generated from glutamine catabolism is primarily converted to glucose (gluconeogenesis in the kidney [18, 19]), and during this process, one net HCO_3^- is synthesized and transported into the renal venous blood to restore acid-base balance [16].

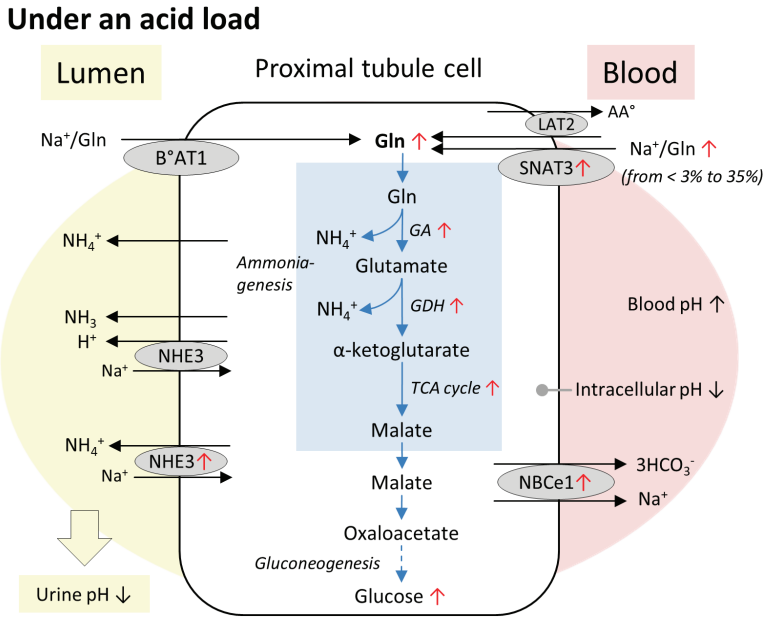

Figure 3 Renal ammoniagenesis under an acid load with more glutamine transported into the mitochondria (blue square). CA, carbonic anhydrase; GA, glutaminase; GDH, glutamate dehydrogenase; Gln, glutamine; TCA cycle, tricarboxylic acid cycle. ↑, increased expression of the genes and/or increased activities of transporters. Figure created according to Curthoys, Weiner, and Koeppen et al. [1, 15, 16, 20].

Ammonia/ammonium transport

Approximately 50% (increasing to 70 – 80% in response to metabolic acidosis) of generated ammonia will be excreted while the remaining percentage enters the systemic circulation being completely metabolized in the liver to urea [15, 17]. Renal ammonia secretion occurs between the end of the proximal tubule and the bend of the loop of Henle [1, 2]. In the thick ascending limb, a major transport of ammonia takes place via the apical transporter NKCC2 primarily and the proportion of ammonia reduced to 20 – 40% at the early distal tubule [17]. Afterwards, ammonia is then secreted by the collecting duct and account for the remaining 60 to 80% of total urinary ammonia [1, 17].

Taking AA-S as an example again, AA-S is oxidized in the liver and yields H_2SO_4, which then is immediately buffered by systemic buffer to Na_2SO_4 (Section 2.1.1). During the process of the sulfuric acid ($2Na^+$-SO_4^{2-}) excretion in the kidney, for example, reabsorption of the systemic buffer $2Na^+$ and secretion of $2H^+$ happens simultaneously ($2Na^+$-$SO_4^{2-} \rightarrow 2H^+$-$SO_4^{2-}$; $2Na^+$-$SO_4^{2-} + 2H_2O + 2CO_2 \rightarrow 2H^+$-$SO_4^{2-} + 2Na^+$-$2HCO_3^-$). The $2H^+$ secreted into renal tubules are excreted in parallel with $2NH_3$ in the urine as $2NH_4^+$ ($2NH_3 + 2H^+$-$SO_4^{2-} \rightarrow 2NH_4^+$-$SO_4^{2-}$). Correspondingly, ammoniagenesis and ammonia/ammonium transport provide ammonia buffer for daily net acid excretion and constitute the major quantity (50 to 70%) of acids buffered and excreted.

Phosphate transport and titratable acid excretion

Phosphate homeostasis is regulated by different organs, such as bone, intestine, the kidney, and their crosstalk via fibroblast growth factor-23 and α-klotho. In the kidneys, three apical transporters, i.e., NaPi-2a, NaPi-2c, and PiT-2 [21] mediate renal inorganic phosphate entry to the proximal tubule cells with different preferred valence of P_i and Na^+/K^+-ATPase. Na^+/K^+-ATPase creates an inward negative membrane potential and Na^+-gradient [16], which provides primarily the driving force for apical phosphate entry.

The phosphate functions as an important urinary buffer in acid excretion as titratable acid (TA, $HPO_4^{2-} + H^+ \rightarrow H_2PO_4^-$). Although the TA accounts for the minor quantity of acid excretion and reaches its peak usually on the first day of acid loading, a renal increase of phosphate in the lumen is the first response in case of excreting a higher acid load [17]. Phosphate reabsorption is almost accomplished by the proximal tubule [22] and the regulation of phosphate transport is relatively strict, because the proximal tubule apical membrane is the only and final site of determination of extracellular phosphate balance by the kidney [16]. Although the overall regulation of phosphate excretion under an acidotic situation is complex and not fully understood, a direct down-regulation of phosphate transporters by reduction of urine pH level and a decrease of apical membrane phosphate transport take place. The reduction of phosphate reabsorption guarantees enough phosphate buffer for free proton binding and buffered excretion in the first place.

2.1.2.2 Net acid excretion and urine pH

Net acid excretion (NAE) represents the total amount of buffered protons that are renally eliminated during a certain time period. Almost one-third to one-half of NAE is excreted in form of TA (mEq/d), largely represented by protons buffered with phosphate, while about one-half to two thirds of urinary protons are accepted and buffered by ammonia (mmol/d) provided by the kidney. Because the kidney is responsible for the reabsorption of almost all of the filtered bicarbonate and generation of "new bicarbonate" during acid excretion, thereby restocking the systemic amount of bicarbonate (mmol/d) consumed by endogenously produced acids (Section 2.1.2.1), NAE can be determined as (**Figure 4**):

NAE (mEq/d) = NH_4^+ + TA – HCO_3^-

A reduced urine pH — displaying higher amounts of free protons — represents an increased obligatory stimulus to the kidney to expand urinary buffer capacity, primarily by stimulation of ammonia production. The amount of protons secreted from tubular cells into the lumen determines the amount of NH_3/NH_4^+ transported from the medulla to the collecting duct [23], thus, higher concentration of free protons (the lower the pH value) triggers more ammonia physiologically generated in the kidney.

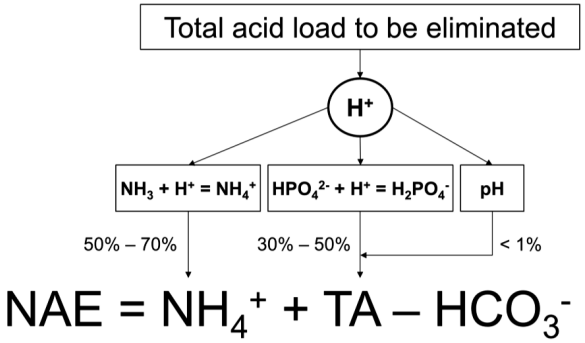

Figure 4 Acid excreted in the urine measuring as net acid excretion (NAE). TA, titratable acid. Figure created according to Hamm et al. [2].

2.1.2.3 Net acid excretion capacity

As has been discussed, one important function of the kidneys is to excrete acid loads, thereby maintaining systemic acid-base balance within a narrow range [24]. Studies have shown that even a mild impairment of renal function might lead to a low degree of acid retention although a manifest

metabolic acidosis is not always discernible [25]. To identify disturbances in acid excretion in cases in which a systemic acidosis is not yet apparent, experimental testing, e.g., with ammonium chloride might be needed [26]. When experimental testing is not possible such as in observational studies in healthy pediatric populations, acid excretion capacity can also be non-invasively estimated from 24-h urine samples collected under normal living conditions using information on urinary pH and NAE [27, 28].

Since the urine pH represents the most important stimulus for renal acid and ammonia excretion [29], when renal acid excretion capacity is impaired, a lower urine pH is needed to excrete the same amount of acids. In other words, the NAE at a given urine pH can be used as an indirect estimate of renal net acid excretion capacity (NAEC) [27, 28]. To illustrate the concept of NAEC, the relationship between 24-h NAE and 24-h urine pH is shown in **Figure 5** for 1121 urine samples of 374 participants characterized in detail in our previous study [30].

As has already been shown in a similar population [27], there is an inverse association between urine pH and NAE which is steeper at higher urine pH values. NAEC can now be determined by calculating the distance between each individual NAE measurement and the regression line of the NAE–urine pH relationship. One participant with a low NAEC is given as example 1 (in red) in **Figure 5**. This person's actual NAE is below the regression line, i.e., below the mean NAE predicted for a given urine pH. In contrast, example 2 (**Figure 5**) shows an individual who's actual NAE-value is above the predicted NAE with a high NAEC.

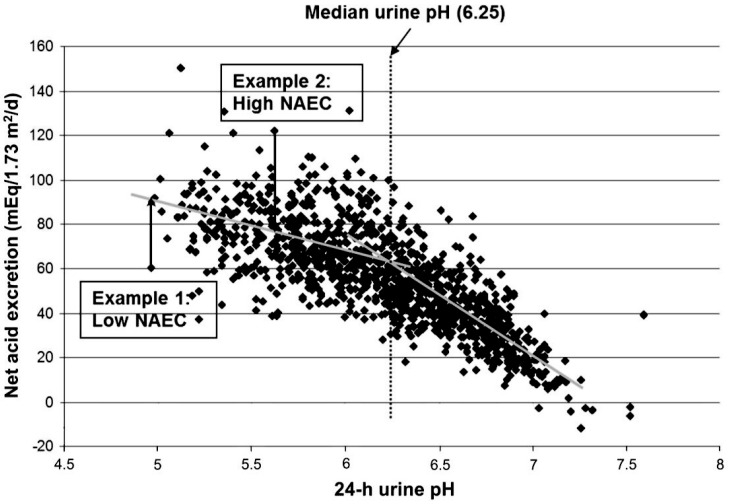

Figure 5 Relationship of net acid excretion (NAE, adjusted to a body surface area of 1.73 m^2) to 24-h urine pH in 1211 urine samples of 374 adolescent DONALD participants. Figure used with permission [30].

2.1.3 Dietary influences on acid-base status

As described in Section 2.1.1, endogenous non-volatile acids are produced by the liver and/or other metabolic active tissues in dependency of the daily diet and body's metabolic status. Plant foods and products mostly contribute to alkali loads, while the acid loads result rather from animal foods, e.g., meat, eggs, and cheese [13, 23, 31], but can also be derived from plant foods like rice and bread [23].

2.1.3.1 Protein- *vs.* fruits and vegetables-rich diet

A diet experimental study from Remer and Manz in 1994 showed that with an increased protein intake, 24-h NAE increases along with a fall of 24-h urine pH [32]. Higher protein intake stimulates glomerular filtration rate (GFR), which causes an increased renal energy requirement. Consequently, the renal energy fuel glutamine is metabolized at a higher rate and more ammonia is produced for proton acceptance. Thus, the urinary NH_4^+ rises along with a decrease of urine pH caused by higher protein intake [13]. In healthy humans, urine pH values below ~5.5 indicate that the "normal" physiological level of acid excretion reserve is left and the range of maximum acid stimulation reached, which implies the occurrence of adverse metabolic changes in circulation. [13, 32, 33].

Sulfur-containing amino acids (i.e., cysteine and methionine) and phosphoproteins are acid forming. During their metabolization, the oxidation of AA-S, for instance, yields sulfuric acids along with urea and CO_2 (Section 2.1.1). These non-volatile sulfuric acids are the endogenously produced acid loads which must be excreted renally. Although plant foods can also produce fixed acids (e.g., polyphenols) and increase diet-dependent acid load, most fruits and vegetables (FV) contain an excess of alkali equivalents like inorganic alkali cations (e.g., magnesium and potassium) and organic anions (e.g., citrate and malate). The oxidation of organic alkali salts, for instance, $3Na^+Citrate_{3^-}$ to water and CO_2, produces the respective $Na^+HCO_3^-$, which increases the circulating alkali reserve or make up blood base pool [13].

An ionogram with protein- and FV-rich diet is given in **Figure 6**. According to this figure, daily NAE of a protein-rich diet is obviously higher than the NAE of a FV-rich diet (formula in Section 2.1.2.2). In addition to NAE, the endogenously produced acid load dependent on dietary intake can also be estimated as potential renal acid load. Explanations of potential renal acid load and **Figure 6** will be given in detail in the next section.