Higher diet-dependent renal acid load associates with higher glucocorticoid secretion and potentially bioactive free glucocorticoids in healthy children

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Metabolic acidosis induces elevated glucocorticoid (GC) levels. However, the influence of less strong daily acid loads on GCs is largely unexplored. To investigate this, we studied whether higher acid loads in children, fully within the normal range of habitual diets, associate with endogenous GCs. In a specific quasi-experimental design, we examined 200 6- to 10-year-old healthy participants of the Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD) Study equally divided to either high or low 24-hour renal net acid excretion. Major urinary GC metabolites were analyzed by gas chromatography–mass spectrometry to assess daily adrenal GC secretion and metabolites of tissue cortisol catabolism (6β-hydroxycortisol and 20α-dihydrocortisol). Liquid chromatography–mass spectrometry was used to quantify urinary free cortisol and cortisone. After confounder adjustment, significant positive associations were unmasked for urinary potential renal acid load and net acid excretion with adrenal GC secretion, free cortisone, free cortisone plus cortisol, 6β-hydroxycortisol, and 20α-dihydrocortisol. An inverse association emerged for an enzymatic marker (5β-reductase) of irreversible GC inactivation. Our data suggest that existing moderate elevations in diet-dependent acid loads suffice to raise GCs and affect cortisol metabolism. Thus, potential detrimental effects of high acid loading appear to be mediated, in part, by increased GC activity via increased GC secretion and/or reduced GC inactivation. Higher cortisone levels, directly available for intracrine activation to cortisol may play a special role.

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Glucocorticoids (GCs) secreted by the adrenal gland are endogenous steroid hormones that play a major role in human metabolism by influencing functions of almost all tissues and organs. GCs are involved in the regulation of physiological processes, such as bone remodeling and modeling, immune response, carbohydrate metabolism (gluconeogenesis), fat cell growth, or blood pressure adjustment. A GC excess caused by endogenous overproduction leads to a cluster of clinically relevant symptoms defined as Cushing syndrome, including central obesity, insulin resistance, blood pressure elevation, myopathy, and osteoporosis.[1,2] Impaired bone function is also an unwanted side effect of treatment with therapeutic GCs.[1] Apart from skeletal symptoms induced by pathologically risen GC levels, several studies suggest that already slightly elevated GC levels can affect bone status. Increased, but still fully within the normal physiological range lying cortisol levels, have been reported to be inversely associated with bone status for example in young healthy adults,[3,4] elderly,[5–7] and even healthy children.[8]

In accord with their regulatory functions in metabolism and maintenance of overall stress preparedness, GCs do also essentially influence the capacity of the kidney to excrete acid loads. This can be seen in various forms of metabolic acidosis whether caused by illnesses[9,10] or induced experimentally. Higher GC levels induce an increased ammonia production in the kidney via an increased muscular protein degradation, eventually resulting in enhanced provision of glutamine to the kidney's tubular cells as primary energy substrate.[11,12] Ammonia stemming from glutamine oxidation then serves as the immediate vehicle for effective renal H^+-elimination.[9] GC increases during metabolic acidosis are mediated at least partly via adrenocorticotropic hormone stimulation.[11,13,14] All in all, the GC increase in response to an acidosis represents a physiological mechanism to allow a more efficient renal elimination of hydrogen ions.[9]

To assess the human body's acid base status, different methods can be used. Renal net acid excretion (NAE) is a precise and specific surrogate parameter to assess total acid load comprising the sum of both endogenously produced and diet-derived acid equivalents.[15,16] Another noninvasive approach to more specifically determine dietary mineral– and protein intake–dependent acid loads to the body is to measure urinary potential renal acid load (uPRAL), reflecting the ionic

difference between urinary strong anions and strong cations in 24-hour urine samples.[16]

More than a decade ago, Maurer *et al.*[17] demonstrated that not only strong forms of metabolic acidosis impact on endogenous GC levels. The investigators observed significantly reduced plasma cortisol levels and reduced 24-hour excretion rates of both cortisol and major urinary GC metabolites after an oral alkalization with potassium and sodium bicarbonate in healthy male subjects on a moderately acidifying usual Western diet. Similar results have been recently reported by Buehlmeier *et al.*[18]

Against this background, the present study examined whether healthy children on ordinary diets with higher 24-hour NAE levels may already demonstrate increases in GC secretion, potentially bioactive free GCs (i.e., the sum of urinary free cortisol and cortisone), and further particular cortisol metabolites, when compared with same-aged children on usual diets characterized by low NAEs in their 24-hour urine samples. The relationship between direct diet-dependent uPRAL and GC outcomes was additionally examined.

RESULTS

A general description of the study sample with regard to anthropometric and urinary characteristics within the 2 NAE groups is given in Table 1. Most of the examined characteristics, especially age, body mass index, and body surface area (BSA) were comparable between the 2 groups. Only urine volume and NAE differed significantly. The median of 24-hour NAE was, on average, 27 mEq/d higher in the high-NAE group than in the low-NAE group. Corrected for adult BSA, average absolute daily NAE in the high- versus the low-NAE group was 75 mEq/d/1.73 m^2 and 34 mEq/d/1.73 m^2, respectively, yielding a moderate to medium strong difference of 40 mEq/d in daily acid load between both groups for a notional adult with a BSA of 1.73 m^2.

Figure 1 shows that the adjusted means of GC secretion and its tissue-derived metabolites 6β-hydroxycortisol, and 20α-dihydrocortisol as well as urinary free cortisone were higher in the high-NAE group than in the low-NAE group. Multiple linear regression analyses, in addition, revealed that in contrast to total GC secretion, urinary free cortisol was not elevated in the high-NAE group (Figure 2). However, in accordance with the elevated GC secretion, the sum of urinary free cortisol and cortisone, that is, overall potentially bioactive free glucocorticoids, was increased under the metabolically more acidic conditions. Renal activity of 11β-hydroxysteroid dehydrogenase type-2 (11β-HSD2), which intrarenally converts cortisol to cortisone, was also higher in children of the high-NAE group (Figure 2). Additionally, the low-NAE group showed a higher activity of 5β-reductase, reflecting a higher overall GC catabolism under metabolically more alkaline conditions.

Cortisol secretion, tissue-derived hydroxylated cortisol metabolites, and cortisone were all positively associated with uPRAL and stronger slopes were discernible for the regressions of the single GC metabolites with uPRAL as compared to ∑C21 (Figure 3).

Table 1 | Crude characteristics of the study population (n = 200)[a]

	Low-NAE group (n = 100, 50 boys)	High-NAE group (n = 100, 50 boys)
Anthropometric measurements		
Age (yr)	8.0 (7.0, 9.1)	8.5 (7.0, 9.9)
Height (cm)	131 ± 8.6	133 ± 10.3
Weight (kg)	27.8 (24.1, 32.3)	29.2 (24.6, 34.4)
BMI (kg/m^2)	16.2 (15.2, 17.4)	16.4 (15.4, 18.0)
BSA (m^2)	1.0 (0.9, 1.1)	1.1 (1.0, 1.2)
24-h urinary nonhormonal data		
Duration of storage [urine] (yr)	12.0 (9.0, 19.0)	16.5 (10.0, 20.5)
Urine volume (ml)	772 (573, 1023)	582 (458, 736)[d]
NAE (mEq/d)	19.7 (15.6, 22.5)	47.1 (40.5, 54.0)[d]
NAE (mEq/d/1.73 m^2)[b]	34.0 (28.3, 37.3)	75.4 (69.9, 82.4)[d]
uPRAL (mEq/d)	−7.5 ± 10.8	17.4 ± 10.1[d]
Urinary organic acids (mEq/d)	28.6 ± 5.6	26.5 ± 5.4[e]
Nitrogen (mmol/d)[c]	445 (410, 521)	480 (425, 540)
Urea (mmol/d)	214 (185, 245)	212 (186, 248)
Creatinine (mmol/d)	4.6 (3.9, 5.5)	4.7 (3.9, 5.7)
Sodium (mmol/d)	88.9 (64.0, 111)	66.8 (51.6, 88.3)[e]
Potassium (mmol/d)	45.9 (38.5, 53.2)	35.1 (28.5, 44.4)[d]
24-h urinary glucocorticoid parameter (outcomes)		
Urinary free cortisol (μg/d)	7.5 (5.8, 10.0)	8.0 (5.3, 10.9)
Urinary free cortisone (μg/d)	18.2 ± 8.5	19.7 ± 7.8
Urinary free cortisol + cortisone (μg/d)	26.3 ± 11.0	28.1 ± 10.2
6β-Hydroxycortisol (μg/d)	45.6 (29.2, 64.0)	48.6 (38.0, 76.3)
20α-Dihydrocortisol (μg/d)	20.1 (15.7, 26.2)	22.2 (16.0, 27.4)
11β-HSD2 (relative activity)	2.3 (1.7, 3.0)	2.3 (2.0, 3.0)
11β-HSD1 (relative activity)	0.63 (0.54, 0.73)	0.57 (0.49, 0.70)
∑C21 (μg/d)	3491 (2812, 4263)	3514 (2873, 4791)

BSA, body surface area; BMI, body mass index; NAE, renal net acid excretion; uPRAL, urinary potential renal acid load; 11β-HSD1, 11β-hydroxysteroid dehydrogenase.
Urinary free cortisol + urinary free cortisone, reflecting potentially bioactive free glucocorticoids; 11β-HSD2 activity, calculated from the ratio of free cortisone/free cortisol; 11β-HSD1 activity, assessed by using the urinary ratio of A-ring-reduced cortisol metabolites tetrahydrocortisol and 5α-tetrahydrocortisol to the tetrahydrometabolite of cortisone; ∑C21, sum of the 7 quantitatively most important urinary glucocorticoid metabolites, representing total daily adrenal cortisol (overall glucocorticoid) secretion.
[a]Values are presented as median (25th, 75th percentiles) if not normally distributed or as arithmetic mean ± SD if normally distributed.
[b]Average mean NAE/BSA difference between low- and high-NAE groups was 41.4 mEq/d/1.73 m^2.
[c]Average urinary nitrogen excretion corresponded to median protein intakes of 1.74 (1.49, 1.99) and 1.74 (1.54, 1.96) g/kg/d for low- and high-NAE groups, respectively.
[d]$P < 0.0001$.
[e]$P < 0.05$. Differences were tested with unpaired t-tests for normally distributed variables and nonparametric Wilcoxon tests for nonnormally distributed variables.

DISCUSSION

Our findings in healthy young 6- to 10-year-old children reveal that already moderate elevations in diet-dependent acid loads, completely within the normal physiological range, will be sufficient to influence cortisol secretion (∑C21), potentially bioactive free glucocorticoids, as well as glucocorticoid catabolism.

The results additionally suggest that higher cortisone levels, being the immediate substrate for intracrine cortisol production, may play an important role in mediating GC