UNIVERZITA KARLOVA V PRAZE
LÉKAŘSKÁ FAKULTA V HRADCI KRÁLOVÉ

MUDr. Sylva Skálová

THE DIAGNOSTIC ROLE OF URINARY N-ACETYL-BETA-D-GLUCOSAMINIDASE AS A MARKER OF RENAL TUBULAR IMPAIRMENT IN CHILDREN

DIAGNOSTICKÝ VÝZNAM N-ACETYL-BETA-D-GLUCOSAMINIDÁZY V MOČI JAKO UKAZATELE TUBULÁRNÍHO POŠKOZENÍ V DĚTSKÉM VĚKU

AUTOREFERÁT DIZERTAČNÍ PRÁCE

Doktorský studijní program
PEDIATRIE
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Dizertační práce byla vypracována v rámci kombinovaného studia v doktorském studijním programu pediatrie na Dětské klinice Fakultní nemocnice a Lékařské fakulty UK v Hradci Králové.

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Stanovisko k dizertaci bylo vypracováno na Dětské klinice Fakultní nemocnice a Lékařské fakulty UK v Hradci Králové.

Obhajoba dizertace se koná dne .....v..... hod.

před komisí pro obhajoby dizertačních prací v doktorském studijním programu obor pediatrie

S dizertační prací je možno se seznámit na děkanátu Lékařské fakulty UK v Hradci Králové, Šimkova 870, 500 38 Hradec Králové

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Obsah /Contents:

1. General introduction

Basic anatomy and physiology of the kidney

The kidneys regulate the volume and concentration of fluids in the body by producing urine in a process called glomerular filtration, which is the removal of waste products, minerals, and water from the blood. The kidneys maintain the volume and concentration of urine by filtering waste products and reabsorbing certain substances and water from the blood.

The major functions of the kidney are:

- to maintain body fluid and electrolyte homeostasis
- to remove waste products of metabolism
- to provide endocrine functions (vitamin D activation, erythropoetin production)
- to perform metabolic functions (gluconeogenesis)
- to regulate regional blood flow (via angiotensin II)
- to regulate blood pressure and electrolyte balance (via renin-angiotensin axis).

The major homeostatic functions of the kidney are carried out by the processes of glomerular ultrafiltration, tubular reabsorption, and secretion. The renal tubular system consists of proximal tubule, the loop of Henle, distal tubules, and collecting ducts. Extensions of the cortex project into the medulla and divide the tissue into renal pyramids. The renal pyramids extend into funnel-like extensions (calyces), where the collection of urine occurs. Minor calyces merge to form major calyces and major calyces merge to form the renal pelvis, the upper portion of the ureter. Each section of the renal tubule performs a different function. The proximal tubule is characterized by iso-osmotic reabsorption of the glomerular ultrafiltrate. Under physiologic conditions, two thirds of the glomerular ultrafiltrate is reabsorbed from the proximal tubule; a number of solutes, such as glucose and aminoacids, are completely reabsorbed, and potassium is nearly completely reabsorbed. Most phosphate is also reabsorbed from the proximal tubule, and calcium is reabsorbed in parallel with sodium reabsorption. The straight portion of the proximal tubule is responsible for secreting organic acids, including drugs such as penicillin The loop of Henle (sometimes known as the nephron loop) is a U-shaped tube that consists of a descending limb and ascending limb. It begins in the cortex, receiving filtrate from the proximal convoluted tubule, extends into the medulla, and then returns to the cortex to empty into the distal convoluted tubule. Its primary role is to concentrate the salt in the interstitium, the tissue surrounding the loop. The distal tubule is made up of the distal convoluted tubule, which is not permeable for water and continues to carry out the dilution of luminal fluid by way of active sodium chloride absorption, and the collecting ducts, which are the primary sites of antidiuretic hormone activity. The distal convoluted tubule is similar to the proximal convoluted tubule in structure and function. These distal segments are the site of potassium secretion, which is virtually all the potassium excreted within the nephron system. Hydrogen ion secretion, which is responsible for the final acidification of the urine, also occurs at the distal convolution.

Evaluation of renal tubular functions

Tubular function tests involve evaluation of functions of the proximal tubule (*i.e.* tubular handling of sodium, glucose, phosphate, calcium, bicarbonate and aminoacids) and distal tubule (urinary acidification and concentration). The evaluation of renal tubular functions can be assessed by examining the:

- renal concentration capacity
- urinary electrolyte excretion in relation to glomerular filtration rate
- tests for urinary acidification
- tubular proteins
- tubular enzymes

Unfortunately, there is not one single reliable test to detect the degree of renal tubular impairment.

- **The renal concentration capacity** can be evaluated by means of **water deprivation test**, or **vasopressin/adiuretin test**. This test examines renal concentrating ability by the administration of a synthetic ADH analogue (desmopressin) and subsequent measurement of urine osmolality.

- **Urinary electrolyte excretion in relation to glomerular filtration rate** can be assessed by means of the fractional excretion of sodium, potassium, calcium and phosphate, respectively.

- **Tests for urinary acidification** include assessment of: plasma anion gap, urine anion gap, urine pH, urine to blood CO_2 difference and fractional excretion of bicarbonate.

- **Tubular proteins** can be detected in cases of renal tubular impairment. The most frequently used renal tubular proteins are alpha-1-microglobulin, beta-2-microglobulin and cystatin C.

- **Tubular enzymes**

The assessment of urinary enzymes is a relatively simple, cheap, fast and non-invasive method in the detection and follow-up of renal disorders. Brush border enzymes, cytosolic enzymes and lysosomal enzymes can be detected in urine. However, the activities of enzymes in urine are, even under physiological conditions, affected by a large number of parameters, such as hormonal influences, volume depletion, diuresis, urine concentration, age and sex. Furthermore, in normal urinary enzyme activity there is a wide variation which makes the interpretation of a laboratory result rather difficult. For these reasons, enzymuria is not being used as routine method in comparison with the use of certain serum enzymes. However, it is important to select a few urinary enzymes that can be used as reliable markers of renal damage. In addition, it is important to point out that enzymuria is associated with the acute and not chronic effects of toxins, because urine is an open system. Elevated levels of urinary enzymes are considered more sensitive methods of detecting renal damage than changes in serum creatinine or a drop in creatinine clearance. The enzymes such as alkaline phosphatase, aminopeptidases, beta-galactosidase, beta-glucosidase, lactic dehydrogenase, glutathione-S-transferase and N-acetyl-beta-D-glucosaminidase have been at various times assessed as markers of nephrotoxicity, however only the last two of them are currently used for this purpose. **It should be clearly pointed out that the evaluation of enzymuria is really scarce in routine clinical practice when compared to the omnipresent evaluation of enzymes in serum or plasma.**

Ligandin (glutathione-S-transferase)

Ligandin is a cytosolic enzyme, originating predominantly from the proximal tubule, and has been considered as a marker of proximal tubular injury of various origin.

N-acetyl-beta-D-glucosaminidase

N-acetyl-beta-D-glucosaminidase (NAG) is a lysosomal enzyme which is present in high concentrations in renal proximal tubules. Its high molecular weight of 130 000 - 140 000 daltons does not permit its filtration through the glomerular basal membrane, and its urinary excretion is relatively constant with minimal diurnal changes. Increased urinary excretion of NAG has been observed to correlate well with tubular dysfunction or damage in patients with a variety of diseases (diabetes mellitus, nephrotic syndrome, vesicoureteric reflux, urinary tract infection, hypercalciuria, urolithiasis, perinatal asphyxia, heavy metals poisoning, treatment with aminoglycosides or valproate). Urinary NAG (U-NAG) activity is the most frequently used method in the assessment of renal tubular disorders when it comes to the assessment of enzymuria. Furthermore, detection of U-NAG is considered a better sensitive method of detecting renal damage than changes in serum creatinine or a drop in creatinine clearance, as changes in U-NAG clearly precede changes in other parameters of kidney function.

Out of several promising methods, only the evaluation of two urinary enzymes(U-NAG and U-glutathione-S-transferase) should be currently considered as a helpful tool in the diagnosis of renal disorders.

As the evaluation and monitoring of urinary enzymes is not generally implemented method in the detection of renal tubular impairment, this thesis is focused on the assessment of U-NAG in various disease states, in order to underline the important role of this enzyme in patients with kidney disorders. The aims of the thesis are delineated in Chapter 2.

2. Aims of the studies and outline of the thesis

2.1. Aims

The ***first aim*** of the thesis was to assess reference data for urinary N-acetyl-β-D-glucosaminidase (U-NAG) activity for all paediatric age groups in order to expand our reference values, because knowledge of normal physiological variation is necessary to identify pathological changes.

The ***second aim*** was to study the changes in U-NAG activity in various disease states and to look for relationship between U-NAG activity and relevant clinical and laboratory data, this by means of original results and primary findings.

The specific research questions were:

- What are the values of U-NAG activity in healthy children and how do these values relate to age?
- Is there any relationship between U-NAG activity and degree of calciuria and skeletal status in children with idiopathic hypercalciuria?
- Are there any changes in U-NAG and calciuria in children with nocturnal enuresis?
- What are the U-NAG levels in children with vesicoureteral reflux and is there any relationship between U-NAG and the grading of vesicoureteral reflux (VUR)?
- Is there any increase of U-NAG in children with hydronephrosis, and if yes, are these changes related to the grading of hydronephrosis and to the surgical prognosis ?

2.2. Outline of the thesis

This thesis gives a detailed account of various studies, not necessarily in the sequence in which these were carried out.

- **Chapter 3** is a review article concerning the diagnostic role of U-NAG activity in the detection of renal tubular impairment, summarizing current knowledge on NAG in renal disease.
- **Chapter 4** is an original article presenting reference data of U-NAG activity for all paediatric age groups. The reference data are based on statistical analysis of U-NAG/Cr evaluation in 262 healthy children aged 0-18 years
- **Chapter 5** is an original article dealing with U-NAG values in 20 paediatric patients with idiopathic hypercalciuria (IH) and U-NAG relationship to urinary calcium (U-Ca).
- **Chapter 6** is an original article evaluating bone mineral density (BMD) and U-NAG values in 15 children with IH, and looking for mutual relationship among U-Ca, U-NAG and BMD, respectively .
- **Chapter 7** is a Letter to the Editor concerning the values of U-Ca and U-NAG and their relationship in 14 children with nocturnal enuresis (NE).
- **Chapter 8** is an extended abstract presenting U-NAG in 22 children with various grades of VUR and evaluating relationship betwen U-NAG and grading of VUR.
- **Chapter 9** is an original article on U-NAG in 31 paediatric patients with hydronephrosis. Relationship between grading of hydronephrosis and U-NAG is sought.
- **Chapter 10** summarizes this thesis.

3. The diagnostic role of urinary N-acetyl-beta-D-glucosaminidase (NAG) activity in the detection of renal tubular impairment.

Acta Medica 2005;48:75

NAG is a lysosomal enzyme which is abundantly present in cells of the proximal kidney tubule. The NAG has a relative high molecular weight of 130 000 to 140 000 daltons which does not permit its filtration through the glomerular basal membrane. Therefore, its urinary excretion is relatively constant with minimal diurnal changes. NAG is stable against changes in pH and temperature. The NAG consists of several isoenzymes. The two principal izoenzymes which are present in the kidney and liver, respectively, are the acidic form A and basic form B, together with small amount of intermediate forms I_1 and I_2. In the serum, NAG is represented predominantly by the A^s form which is also the only NAG form in the cerebrospinal and synovial fluid. Serum of the pregnant women contains P form of NAG which is similar to the I_2 form. C form of NAG is present in the nervous tissue. The urine of healthy human subjects contains small amount of NAG, with the A isoenzyme:B isoenzyme ratio of 4:1 to 10:1, while the intermediary forms are not detectable. In patients with tubular and interstitial renal impairment, the total activity of urinary NAG is elevated, in particular its B form, resulting in changes of the A:B ratio. The intermediary forms of NAG are increased as well, but their activity seldom exceeds 5% of the total urinary NAG. In diseases affecting the glomerular membrane, the A^s isoenzyme is usually detectable in the urine. Currently, there are several methods of assaying the U-NAG catalytic activity. The fluorimetric assay based on the fluorescent 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide substrate was introduced in the late sixties and has been followed by more user-friendly colorimetric and spectrophotometric methods The fluorescent method is sensitive enough to determine very low enzyme activities in urinary specimen diluted 20 – 50-fold to eliminate the influence of endogenous low molecular weight effectors. Moreover, each laboratory had to establish its own normal reference intervals as a consequence of the activity arisen in the interlaboratory standardization of the procedure.However, fluorimetry still remains a useful method in most routine laboratories, as it is cheap, simple and relatively user-friendly. The spectrophotometric method is based on highly soluble and stable 4-nitrophenyl-N-acetyl-β-D-glucosaminide as substrate. However, the sensitivity of the assay could only be kept at an acceptable level by the addition of large aliquots of the urine samples to the reaction mixtures. Highly sophisticated and powerful colorimetric procedures are based on the use of 2-methoxy-4-(2′-nitrovinyl)-phenyl-N-acetyl-β-D-glucosaminide and m-cresolsulphonphthaleinyl-N-acetyl-β-D-glucosaminide as substrate, respectively. In both these cases the colour of the urine does not disturb the assays. The U-NAG values should be expressed as a ratio to urinary creatinine concentration, as this relationship shows less variability than the urinary enzyme excretions related to volume or time. When evaluating the U-NAG activity in various disease states, most authors used control groups for comparison. However, especially in children, it seems more appropriate to obtain and use age-dependent U-NAG reference values from sufficiently large healthy population for the proper evaluation of kidney function. Due to the fact that the urinary NAG/creatinine ratio tends do decrease with age in children as a result of a concomitant rise in the urinary creatinine concentration, and as there is a great interindividual variability of those values in children, as reflected by the standard deviation, the use of proper pediatric reference

values is quite reasonable. The determination of U-NAG is a non-invasive test and provides a very sensitive and reliable indicator of renal damage, such as injury or dysfunction due to diabetes mellitus, nephrotic syndrome, inflammation, urinary tract infection, hypercalciuria, urolithiasis, nephrocalcinosis, perinatal asphyxia, heavy metals poisoning, treatment with aminoglycosides, valproate or other nephrotoxic drugs, vesicoureteral reflux, hypoxia, hypertension. U-NAG is used as a routine marker of renal tubular impairment in the above mentioned disease states. As of November 20, 2004, there were 1165 publications on U-NAG indexed in the Medline/PubMed. Siginificant changes were found in patients with ***developmental kidney abnormalities, vesicoureteral reflux, obstructive uropathy, urinary tract infection (UTI), nephrotic syndrome, hypercalciuria, urolithiasis, nephrocalcinosis, hypertension, vasculitis (Henoch-Schonlein purpura), diabetes mellitus***, in patients using ***nephrotoxic drugs***, in patients after ***heavy metals poisoning,*** and in patients with ***kidney transplants,*** and after ***cardiac surgery.*** The assessment of U-NAG should be considered as a useful marker of renal tubular impairment in various disease states. Other urinary enzymes (such as alanin aminopeptidase, alkaline phosphatase) are also sensitive indicators of kidney parenchymal damage compared to functional measurements. However, U-NAG remains the most widely used marker of renal tubular impairment.

4. Urinary N-acetyl-beta-D-glucosaminidase activity in healthy children

Nephrology 2004;9:19-21

Aim: The principal aim was to establish pediatric reference data for the urinary N-acetyl-β-D-glucosaminidase (U-NAG) activity.

Subjects, Methods: 262 healthy children aged 0-18 years (0-1 month, n=38; 1 mo-1 year, n=50; 1-3 y, n=50; 3-6 y, n=46; 6-10 y, n=29; 10-18 y, n=49) had a urine sample collected and the U-NAG activity was evaluated by using fluorimetry and related to urinary creatinine as nkat/mmol ratio.

Results: A strong age dependence of the U-NAG/creatinine ratio and its high interindividual variability in children was observed; the highest values of upper reference range being in the 0-1 mo and 1 mo-1y group (134.8 and 50, respectively), which dropped gradually to 7.25 in the oldest age group (10-18 years).

Table 1 Reference values of the urinary NAG/creatinine excretion

Age (years)	Number of subjects	Mean value (nkat/mmol)	Standard deviation	95 centile (non-parametric)	95 centile (parametric)
0-0.08	38	53.44	35.69	134.80	148.55
0.08-1	50	20.28	13.06	50.0	51.71
1-3	50	6.19	3.75	14.02	16.63
3-6	46	4.98	3.33	13.21	15.41
6-10	29	4.53	2.22	7.5	11.5
10-18	49	3.32	1.96	7.25	8.82

Conclusion: The establishment of U-NAG reference pediatric values is a potentially useful tool for proper evaluation of renal tubular function in childhood.

5. Renal tubular impairment in children with idiopathic hypercalciuria.

Acta Medica 2006;49:109-111

Background: Idiopathic hypercalciuria (IH) is defined as hypercalciuria that persists after correction of dietary inbalances and has no detectable cause. Renal tubular dysfunction has been described in patients with IH. The excretion of U-NAG, a marker of proximal tubular damage, has been previously reported as either increased or normal in children with IH.

Patients, Materials, Methods: We evaluated U-NAG in 20 children (13 boys and 7 girls, mean age 10.3 years ± 5.7 SD) with IH (urinary calcium excretion above 0.1 mmol/kg/24 hours, with no detectable cause) and with otherwise normal renal function tests. Ultrasound examination revealed urolithiasis (n = 4) and nephrocalcinosis (n = 1). The U-NAG values were evaluated in the spot urine collected from the second morning void and calculated as the urinary NAG/creatinine ratio (U-NAG/Cr) and expressed in nkat/mmol. The 24-hour urinary calcium excretion (U-Ca/24h) was assessed in a urinary sample from 24 – hour collected urine and calculated in mmol/kg. The obtained results of U-Ca/24h and U-NAG/Cr were expressed as Z-scores.

Results: When compared to the reference data, the U-Ca/24h and U-NAG/Cr were significantly higher ($p<0.0004$ and $p<0.006$, respectively). There was no correlation between the U-NAG/Cr and U-Ca/24h ($r = 0.18$, $p = 0.20$). The U-NAG/Cr values were significantly higher in the 5 patients with urolithiasis/nephrocalcinosis, whether compared to the rest of the group ($p<0.02$), or to the reference data ($p<0.01$). The U-NAG/Cr activity was higher in 15 children without urolithiasis/nephrocalcinosis when compared to reference data ($p < 0.01$). There was no difference in U-Ca/24h between the children with and without urolithiasis/nephrocalcinosis ($p = 0.58$).

Conclusions: These findings suggest that tubular impairment, as reflected by U-NAG/Cr, might occur in children with IH, especially in patients with urolithiasis/nephrocalcinosis. There doesn't seem to be a direct relationship between the U-NAG/Cr activity and the degree of calcium leakage.

6. Bone mineral density and urinary N-acetyl-beta-D-glucosaminidase activity in paediatric patients with idiopathic hypercalciuria.

Nephrology2005;10:99-102

Background: Idiopathic hypercalciuria (IH) is defined as hypercalciuria that persists after correction of dietary inbalances and has no detectable causes. Patients with IH have a higher prevalence of osteoporosis. Defective reabsorption of calcium by the renal tubule is considered a likely mechanism of IH. NAG is a lysosomal enzyme that is a very sensitive marker of renal tubular impairment. Our objectives were to assess the tubular function by means of urinary U-NAG evaluation and BMD by dual energy X-ray absorptiometry (DXA) in paediatric patients with IH, and to look for a possible relationship between calciuria, BMD and U-NAG

Methods: Fifteen patients (nine boys and six girls, mean age 12.4 ± 4.0 years) with IH (urinary calcium excretion >0.1 mmol/kg per 24 h) had their bodyweight, height, body mass index (BMI), urinary NAG/creatinine ratio (U-NAG/Cr) and 24-h urinary calcium excretion (U-Ca/24 h) assessed. L1–L4 bone mineral density (BMD) was measured by dual energy X-ray absorptiometry and volumetric BMD (BMDvol) was calculated. The obtained results were expressed as Z-scores.

Results: The values of basic anthropometric parameters did not differ significantly from the values of the reference population and there was a tendency to short stature, which did not reach statistical significance ($p = 0.08$). The values of calciuria and U-NAG/Cr were significantly higher while BMD was significantly lower when compared to the reference values ($p < 0.0006$, $p < 0.006$ and $p < 0.001$, respectively). Inverse and significant correlations were found between U-Ca/24 h and BMD, U-Ca/24 h and body height, and U-Ca/24 h and BMDvol ($r = -0.64$ and -0.70, respectively, $p < 0.01$; $r = -0.55$, $p < 0.05$), while there was no correlation between U-NAG/Cr and U-Ca/24 h, nor between BMD and weight or BMD and BMI.

Conclusions: Tubular impairment is highly probable in children with IH, but there is a poor relationship with the degree of calcium leakage. Idiopathic hypercalciuria should be considered as a risk factor for stunted growth and low bone mass.

7. High urinary N-acetyl-beta-D-glucosaminidase activity (NAG) and normal calciuria in children with nocturnal enuresis.

Indian Pediatrics 2006;43:655-656

Background, Aims: As hypercalciuria has been encountered in patients with nocturnal enuresis (NE) and increased urinary NAG activity has been previously reported in patients with hypercalciuria, our objective was to evaluate the urinary NAG and calciuria in patients with NE.
Patients, Methods: Fourteen patients (11 boys and 3 girls, mean age 6.8 yr ± 1.6 SD, range 5-10 yr) with primary NE were and nad their urinary calcium/creatinine (U-Ca/Cr; mmol/L:mmol/L) and urinary NAG/creatinine ratios (U-NAG/Cr; nkat/L:mmol/L) assessed in urine collected after the first morning void. To eliminate the influence of age, the obtained results of U-Ca/Cr and UNAG/Cr were expressed as Z-scores by the equation Z-score = (actual of individual value – mean reference value for age)/standard deviation for age. The reference values were based on previously published data on healthy Czech children.
Results: U-Ca/Cr values were within the reference range in 13 children, and in only 1 patient the value exceeded the 95th percentile. The values of U-Ca/Cr did not differ significantly from the reference data (p=0.77). In 4 patients the U-NAG/Cr values exceeded the age-related 95th percentile range In the entire group of 14 patients, the U-NAG/Cr values were significantly higher compared to reference values ($p = 0.003$). There was no correlation between U-NAG/Cr and U-Ca/Cr ($r = 0.13$, $P = 0.55$).
Conclusion: Hypercalciuria was not found in children with NE. The presence of elevated urinary levels of UNAG/Cr suggest tubular dysfunction in patients with NE.

8. Urinary N-acetyl-beta-D-glucosaminidase activity in children with vesicoureteral reflux.

Pediatric Nephrology 2006;21:1528

Objectives: As U-NAG activity is considered a sensitive marker of renal tubular impairment, the aim was to measure U-NAG in children with vesicoureteral reflux (VUR) and look for relationship among selected clinical parameters.
Materials and Methods: 22 children (10 boys and 12 girls, mean age 2.83 ± 2.42 years) with VUR had U-NAG/Cr ratio measured in the spot urine, collected after the first morning void. To eliminate the influence of age, the obtained results of U-NAG/Cr were expressed as Z-scores. The reference values were based on previously published data on healthy Czech children.
Results: The U-NAG/Cr values were significantly higher in the VUR patients in comparison to the reference data (p= 0.0001). There was no difference in U-NAG/Cr between children with unilateral (n=8) and bilateral (n=14) VUR (p= 0.66). There was no difference in U-NAG/Cr between patients with VUR grade I-III and VUR grade IV-V (p=0.67). The U-NAG/Cr activity was high in patients with reflux nephropathy (RN; n=9) when compared to reference data (p= 0.0001), however there was no difference in comparison to children with VUR and without RN ($p = 0.84$). We found a weak correlation between U-NAG/Cr and grade of VUR, which didn't reach statistical significance ($r = 0.38$, $p = 0.08$).

Conclusions: U-NAG is a useful marker of renal tubular impairment, as it is increased in children with VUR grade I-V, however there is only a very weak relationship with the grade of VUR. This might further support more recent observations that VUR grade doesn't fully correspond with the degree of kidney damage.

9. Increased urinary N-acetyl-beta-D-glucosaminidase activity in children with hydronephrosis.

International Brazilian Journal of Urology 2007; 33:80-86

Background, Aims: Hydronephrosis leads to deterioration of renal function. As urinary N-acetyl-beta-D-glucosaminidase (U-NAG) activity is considered a sensitive marker of renal tubular impairment, our aim was to measure U-NAG in children with hydronephrosis and to look for relationship among selected clinical parameters.

Materials and Methods: 31 children (22 boys and 9 girls, mean age 2.3 ± 2.5 years) with hydronephrosis grade 1-4 had U-NAG/Cr measured. To eliminate the influence of age, the obtained results of U-NAG/Cr were expressed as Z-scores. The reference values were based on previously published data on healthy Czech children.

Results: The U-NAG/Cr was significantly higher in the patients with hydronephrosis compared to reference data ($p = 0.002$). There was no difference in U-NAG/Cr between children with unilateral and bilateral hydronephrosis ($p= 0.51$). There was no significant difference in U-NAG/Cr between children with grades 1-3 (pooled data) and grade 4, respectively ($p=0.89$). There was no correlation between U-NAG/Cr and the grade of hydronephrosis ($r = 0.01$). The renal functions, as assessed by the ^{99m}Tc MAG3 renography, were not severely impaired.

Conclusions: U-NAG/Cr is increased in children with hydronephrosis grade 1-4, and there is no relationship with the grade of hydronephrosis. These results might suggest that the renal function, as assessed by ^{99m}Tc MAG3 renography might not be solely related to the grade of hydronephrosis, and that U-NAG in hydronephrosis is not dependent on the amount of affected renal tissue. We can't rule out that the U-NAG can reflect even very mild changes in renal tubular function, which might occur even in low-grade non-obstructive hydronephrosis.

U-NAG is a useful marker of renal tubular dysfunction, however its relationship with the degree of kidney damage in patients with hydronephrosis should be considered as doubtful.

10. Summary and Conclusions, Recommendations

10.1. Summary and conclusions

Chapter 1

Chapter 1 gives brief information on renal anatomy and physiology, and in particular on anatomy, physiology and pathophysiology of renal tubules, including diagnostic procedures. This introduction is essential for the integrity of the thesis.

Chapter 2

Chapter 2 gives basic information concerning this thesis, its primary and secondary aims.

Chapter 3

Chapter 3 gives a state-of-art overview of the diagnostic role of U-NAG in the detection of renal tubular impairment and its clinical applicability. 92 articles are evaluated in this review in a critical way. U-NAG activity is a useful marker of renal tubular impairment in various disease states. When compared to other urinary enzymes, U-NAG is the most frequently used urinary enzymatic marker when it comes to the evaluation of tubular function. However, the routine evaluation of enzymuria is much less frequently used when compared to the routine evaluation of enzymes in serum or plasma.

Chapter 4

Chapter 4 presents reference data of U-NAG for all paediatric age groups. In conclusion, the U-NAG/Cr values of 262 healthy children aged 0-18 years, obtained with fluorimetric assay, are strongly age-dependent. The establishment of U-NAG/Cr reference paediatric values is a potentially useful tool for proper evaluation of renal tubular impairment in childhood, because knowledge of normal physiological variation is necessary to identify pathological changes.

Chapter 5

Chapter 5 gives information on U-NAG/Cr values in 20 paediatric patients with idiopathic hypercalciuria. High U-NAG suggests renal tubular injury, however the absence of correlation between U-NAG/Cr and U-Ca/24h also suggests that increased urinary concentration of calcium might lead to damage of tubular cells, even in the absence of lithiasis. In conclusion, children with IH have some degree of secondary renal tubular impairment. The tubular impairment is most probably aggravated by the increased urinary concentration of calcium, and, in particular, by the cell-crystal interactions. There doesn't seem to be a direct relationship between this tubular impairment and the degree of calcium leakage.

Chapter 6

Chapter 6 analyses the relationship among bone mineral density, body height and U-NAG/Cr values in 15 children with idiopathic hypercalciuria (IH). High U-NAG/Cr, low BMD and a tendency to short stature were observed in children with IH. We found inverse and significant correlation between BMD and U-Ca/24 h, and a similar correlation between U-Ca/24 h and body height. There was no correlation between U-NAG/Cr and U-Ca/24 h. It seems likely that hypercalciuria can contribute to the skeletal demineralization and to the development of nephrocalcinosis, both resulting in stunted growth. In conclusion, tubular impairment is highly probable in children with IH, as high U-NAG/Cr was observed, but there seems to be a poor relationship with the degree of calcium leakage. Idiopathic hypercalciuria should be considered as a risk factor for stunted growth and low bone mass, however it doesn't correlate with U-NAG/Cr..

Chapter 7

Chapter 7 presents data on calciuria and U-NAG/Cr activity in 14 children with NE. The values of U-Ca/Cr did not differ significantly from the reference data, however U-NAG/Cr values were significantly higher compared to reference values. There was no correlation between U-NAG/Cr and U-Ca/Cr. In conclusion, hypercalciuria was not found in children with NE. The presence of elevated urinary levels of U-NAG/Cr suggest that tubular dysfunction might be present in patients with NE.

Chapter 8

Chapter 8 presents U-NAG activity in 22 children with various grades of vesicoureteral reflux (VUR). The U-NAG/Cr values were significantly higher in the VUR patients in comparison to the reference data. There was no difference in U-NAG/Cr between children with unilateral and bilateral VUR and no significant difference between VUR I-III and VUR IV-V subgroups. The U-NAG/Cr activity was high in patients with RN when compared to reference data, but there was no difference in comparison to children with VUR but without RN. We found no correlation between U-NAG/Cr and grade of VUR, without statistical significance. Number of pyelonephritic episodes in patients' personal history was not related to U-NAG/Cr or VUR grade. In conclusion, tubular dysfunction is common in children with VUR. U-NAG/Cr should be considered as a useful marker of renal tubular impairment in patients with VUR, however there is a very weak relationship with the grade of VUR. This might further support more recent observations that severity of VUR doesn't always fully correspond with the degree of kidney damage.

Chapter 9

Chapter 9 gives data on U-NAG activity in 31 patients with hydronephrosis (HN). The U-NAG/Cr values were significantly higher in the patients with HN in comparison to the reference data. There was no difference in U-NAG/Cr between children with unilateral and bilateral HN. There was no significant difference in U-NAG/Cr between children with HN grade 1-3 and grade 4, respectively No correlations were observed between U-NAG/Cr and the grade of hydronephrosis. The renal functions, as assessed by the ^{99m}Tc MAG3 renography, were not severely impaired. These results might suggest that the renal function, as assessed by ^{99m}Tc MAG3 renography might not be solely related to the grade of hydronephrosis, and that U-NAG in hydronephrosis is not dependent on the amount of affected renal tissue. We can't rule out that the U-NAG can reflect even very mild changes in renal tubular function, which might occur even in low-grade non-obstructive hydronephrosis. In conclusion, U-NAG/Cr is increased in children with hydronephrosis grade 1-4, however there is no relationship with the grade of hydronephrosis or with the amount of affected renal tissue. U-NAG/Cr is a useful marker of renal tubular impairment, however there is no relationship with the degree of kidney damage in patients with hydronephrosis.

10.General conclusions and recommendations

1. U-NAG/Cr is an important marker of renal tubular dysfunction.
2. U-NAG/Cr can reflect even very mild changes in renal tubular function.
3. U-NAG/Cr is significantly increased in the following urinary tract disorders: idiopathic hypercalciuria, nocturnal enuresis, vesicoureteral reflux and hydronephrosis.
4. In the above mentioned disease states affecting the uropoetic system, the U-NAG/Cr is elevated, but not correlated with the severity of the disease.

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12.Author´s relevant publications (i.e. concerning U-NAG)

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7. **Skálová S,** Rejtar P, Kutílek S. Increased urinary N-acetyl-beta-D-glucosaminidase activity in children with hydronephrosis Int Braz J Urol 2007;33:80-86.

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1. **Skálová S,** Palička V, Kutílek Š. Bone density and renal tubular function in paediatric patients with idiopathic hypercalciuria. J Bone Miner Res 2003;18:Suppl 2:S287:SU445.
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6. **Skálová S,** Rejtar P. Kutílek Š. Urinary N-acetyl-beta-D-glucosaminidase activity in children with vesicoureteral reflux. Pediatr Nephrol 2006;21:1528.
7. **Skálová S,** Rejtar P, Kutílek S. Detection of renal tubular impairment in children with hydronephrosis. Pediatr Nephrol 2006 21:1559.

13. Summary

13.1. English Summary

This thesis deals with the evaluation of U-NAG in various disease states, in order to underline the important role of this enzyme in patients with kidney disorders.

Chapter 1

Chapter 1 gives brief information on renal anatomy and physiology, and in particular on anatomy, physiology and pathophysiology of renal tubules, including diagnostic procedures. This introduction is essential for the integrity of the thesis.

Chapter 2

Chapter 2 gives basic information concerning this thesis, its primary and secondary aims. This thesis gives a detailed account of various studies, not necessarily in the sequence in which these were carried out.

Chapter 3

Chapter 3 gives a state-of-art overview of the diagnostic role of U-NAG in the detection of renal tubular impairment and its clinical applicability. 92 articles are evaluated in this review in a critical way. U-NAG activity is a useful marker of renal tubular impairment in various disease states. When compared to other urinary enzymes, U-NAG is the most frequently used urinary enzymatic marker when it comes to the evaluation of tubular function. However, the routine evaluation of enzymuria is much less frequently used when compared to the routine evaluation of enzymes in serum or plasma.

Chapter 4

Chapter 4 presents reference data of U-NAG for all paediatric age groups. In conclusion, the U-NAG/Cr values of 262 healthy children aged 0-18 years, obtained with fluorimetric assay, are strongly age-dependent. The establishment of U-NAG/Cr reference paediatric values is a potentially useful tool for proper evaluation of renal tubular impairment in childhood, because knowledge of normal physiological variation is necessary to identify pathological changes.

Chapter 5

Chapter 5 gives information on U-NAG/Cr values in 20 paediatric patients with idiopathic hypercalciuria. High U-NAG suggests renal tubular injury, however the absence of correlation between U-NAG/Cr and U-Ca/24h also suggests that increased urinary concentration of calcium might lead to damage of tubular cells, even in the absence of lithiasis. In conclusion, children with IH have some degree of secondary renal tubular impairment. The tubular impairment is most probably aggravated by the increased urinary concentration of calcium, and, in particular, by the cell-crystal interactions. There doesn't seem to be a direct relationship between this tubular impairment and the degree of calcium leakage.

Chapter 6

Chapter 6 analyses the relationship among bone mineral density, body height and U-NAG/Cr values in 15 children with idiopathic hypercalciuria (IH). High U-NAG/Cr, low BMD and a tendency to short stature were observed in children with IH. We found inverse and significant correlation between BMD and U-Ca/24 h, and a similar correlation between U-Ca/24 h and body height. There was no correlation between U-NAG/Cr and U-Ca/24 h. It seems likely that hypercalciuria can contribute to the skeletal demineralization and to the development of nephrocalcinosis, both resulting in stunted growth. In conclusion, tubular impairment is highly probable in children with IH, as high U-NAG/Cr was observed, but there seems to be a poor relationship with the degree of calcium leakage. Idiopathic hypercalciuria should be considered

as a risk factor for stunted growth and low bone mass, however it doesn't correlate with U-NAG/Cr.

Chapter 7

Chapter 7 presents data on calciuria and U-NAG/Cr activity in 14 children with NE. The values of U-Ca/Cr did not differ significantly from the reference data, however U-NAG/Cr values were significantly higher compared to reference values. There was no correlation between U-NAG/Cr and U-Ca/Cr. In conclusion, hypercalciuria was not found in children with NE. The presence of elevated urinary levels of U-NAG/Cr suggest that tubular dysfunction might be present in patients with NE.

Chapter 8

Chapter 8 presents U-NAG activity in 22 children with various grades of vesicoureteral reflux (VUR). The U-NAG/Cr values were significantly higher in the VUR patients in comparison to the reference data. There was no difference in U-NAG/Cr between children with unilateral and bilateral VUR and no significant difference between VUR I-III and VUR IV-V subgroups. The U-NAG/Cr activity was high in patients with RN when compared to reference data, but there was no difference in comparison to children with VUR but without RN. We found no correlation between U-NAG/Cr and grade of VUR, without statistical significance. Number of pyelonephritic episodes in patients' personal history was not related to U-NAG/Cr or VUR grade. In conclusion, tubular dysfunction is common in children with VUR. U-NAG/Cr should be considered as a useful marker of renal tubular impairment in patients with VUR, however there is a very weak relationship with the grade of VUR. This might further support more recent observations that severity of VUR doesn't always fully correspond with the degree of kidney damage.

Chapter 9

Chapter 9 gives data on U-NAG activity in 31 patients with hydronephrosis (HN). The U-NAG/Cr values were significantly higher in the patients with HN in comparison to the reference data. There was no difference in U-NAG/Cr between children with unilateral and bilateral HN. There was no significant difference in U-NAG/Cr between children with HN grade 1-3 and grade 4, respectively No correlations were observed between U-NAG/Cr and the grade of hydronephrosis. The renal functions, as assessed by the ^{99m}Tc MAG3 renography, were not severely impaired. These results might suggest that the renal function, as assessed by ^{99m}Tc MAG3 renography might not be solely related to the grade of hydronephrosis, and that U-NAG in hydronephrosis is not dependent on the amount of affected renal tissue. We can't rule out that the U-NAG can reflect even very mild changes in renal tubular function, which might occur even in low-grade non-obstructive hydronephrosis. In conclusion, U-NAG/Cr is increased in children with hydronephrosis grade 1-4, however there is no relationship with the grade of hydronephrosis or with the amount of affected renal tissue. U-NAG/Cr is a useful marker of renal tubular impairment, however there is no relationship with the degree of kidney damage in patients with hydronephrosis.

10.General conclusions and recommendations

5. U-NAG/Cr is an important marker of renal tubular dysfunction.
6. U-NAG/Cr can reflect even very mild changes in renal tubular function.
7. U-NAG/Cr is significantly increased in the following urinary tract disorders: idiopathic hypercalciuria, nocturnal enuresis, vesicoureteral reflux and hydronephrosis.
8. In the above mentioned disease states affecting the uropoetic system, the U-NAG/Cr is elevated, but not correlated with the severity of the disease.

13.2. Czech Summary/Souhrn

Práce je věnována problematice tubulárního postižení a jeho detekci pomocí enzymurie, konkrétně vyšetření enzymu N-acetyl-β-D-glukosaminidázy v moči.

Kapitola 1

Kapitola 1 podává základní informace o renální anatomii a fyziologii, zvláště pak se zaměřením na renální tubuly, včetně diagnostických postupů při vyhodnocování tubulárních funkcí. Tato úvodní kapitola je nezbytná pro celistvost předkládané dizertace.

Kapitola 2

Kapitola 2 předkládá základní koncepci dizertace a její cíle. Prvním cílem dizertace bylo získat referenční hodnoty ukazatele tubulárního počkození U-NAG/Cr v dětském a dorostovém věku. Znalost fyziologických hodnot je nezbytná pro detekci patologických změn. Druhým cílem bylo vyhodnotit změny U-NAG/Cr u dětských pacientů s idiopatickou hyperkalciurií (IH), noční enurézou (NE), hydronefrózou (HN) a vezikoureterálním refluxem (VUR) v porovnání s hodnotami zdravých dětí a korelovat U-NAG/Cr s vybranými klinickými parametry. Dizertace sestává z prací publikovaných v anglickém jazyce, které prošly recenzním řízením.

Kapitola 3

Kapitola 3 je souborným referátem týkajícím se diagnostické úlohy U-NAG při detekci postižení renálních tubulů. V této kapitole je kriticky zhodnoceno 92 publikovaných prací na uvedené téma. U-NAG/Cr je nejčastěji používaým tubulárním enzymatickým markerem. V porovnání s běžně užívanými enzymatickými ukazateli vyšetřovanými v plazmě či séru je však využití enzymurie minimální.

Kapitola 4

Kapitola 4 přináší referenční hodnoty U-NAG pro všechny věkové skupiny v období dětství a dospívání. Hodnoty U-NAG/Cr získané od 262 zdravých dětí ve věku 0-18 let jsou výrazně závislé na věku. Se stoupajícím věkem klesají hodnoty U-NAG/Cr. Referenční hodnoty pro dětský věk jsou důležitým přínosem, neboť znalost normální fyziologické variace je nezbytná pro určení patologických změn.

Kapitola 5

Kapitola 5 se týká hodnot U-NAG/Cr u 20 dětí s idiopatickou hyperkalciurií (IH). Byly zjištěny zvýšené hodnoty U-NAG/Cr, které svědčí pro poškození ledvinných tubulů. Nebyla zjištěna korelace mezi odpadem kalcia v moči za 24 hodin (U-Ca/24h) a U-NAG/Cr. Poškození ledvinných tubulů u pacientů s IH je nejspíše důsledkem iritace tubulárních buněk zvýšenou koncentrací kalcia v moči a též traumatizace tubulů krystaly vápníku. Není vztah mezi stupněm poškození tubulárních buněk a mírou kalciurie.

Kapitola 6

Kapitola 6 analyzuje vztah mezi denzitou kostního minerálu, tělesnou výškou a U-NAG/Cr u 15 dětí s IH. U dětských pacientů s IH byly zjištěny vysoké hodnoty U-NAG/Cr, snížená BMD a tendence k malému vzrůstu. Byla nalezena inverzní a významná korelace mezi BMD a U-Ca/24h a obdobná korelace mezi U-Ca/24 h a tělesnou výškou. Nebyl zjištěn vztah mezi U-Ca/24h a U-NAG/Cr. Nelze vyloučit, že IH přispívá k demineralizaci skeletu a rozvoji nefrokalcinózy, což obojí vede k zpomalení růstu. U pacientů s IH je přítomno poškození ledvinných tubulů, které nesouvisí s mírou kalciurie. IH může být považována za rizikový faktor osteoporózy a malého vzrůstu.

Kapitola 7

Kapitola 7 přináší údaje o kalciurii a U-NAG/Cr u 14 dětí s noční enurézou (EN). Hodnoty U-Ca/Cr se významně nelišily od referenčních hodnot pro pediatrickou českou populaci. Byly zjištěny vysoké hodnoty U-NAG/Cr. Nebyla nalezena korelace mezi U-Ca/Cr a U-NAG/Cr. U dětí s EN nebyla zjištěna hyperkalciurie, vzhledem k vyšším hodnotám U-NAG/Cr je pravděpodobná přítomnost renální tubulární dysfunkce.

Kapitola 8

V kapitole 8 je vyhodnocen U-NAG/Cr u 22 dětí s různým stupněm vezikoureterálního refluxu (VUR). Hodnoty U-NAG/Cr byly u pacientů s VUR výrazně zvýšeny oproti hodnotám referenčním. Mezi pacienty s obustranným a jednostranným VUR nebyl zjištěn rozdíl v hodnotách U-NAG/Cr. Rovněž tak nebyl zjištěn rozdíl U-NAG/Cr mezi 2 podskupinami pacientů s VUR I.-III. a IV.-V. stupně. Hodnoty U-NAG/Cr u pacientů s refluxovou nefropatií (RN) se nelišily od hodnot pacientů bez RN. Nebyla zjištěna korelace mezi U-NAG/Cr a stupněm VUR, stejně jako nebyl nalezen vztah mezi počtem prodělaných pyelonefritid a U-NAG/Cr či stupněm VUR. U-NAG/Cr je zvýšen u pacientů s VUR, neexistuje ovšem vztah mezi stupněm VUR a aktivitou U-NAG/Cr.

Kapitola 9

Kapitola 9 přináší údaje o U-NAG/Cr u 31 pacientů s hydronefrózou (HN). Hodnoty U-NAG/Cr byly signifikantně zvýšeny u dětí s HN v porovnání s referenčními daty. Nebyl shledán rozdíl v hodnotách U-NAG/Cr mezi dětmi s jednostrannou či oboustrannou HN. Nebyl zjištěn rozdíl U-NAG/Cr mezi 2 podskupinami pacientů s HN 1.-3. stupně a HN 4. stupně. Nebyla zjištěna korelace mezi U-NAG/Cr a stupněm hydronefrózy. Renální funkce, které byly vyšetřeny pomocí ^{99m}Tc MAG3 nebyly významně sníženy. Nelze vyloučit, že pomocí U-NAG/Cr jsme schopni detekovat i velmi diskrétní poruchu tubulárních funkcí. U-NAG/Cr je zvýšen u dětí s HN, toto zvýšení však nesouvisí se stupněm HN či s rozsahem postižení renálního parenchymu.

Kapitola 10. Závěry a doporučení

1. U-NAG/Cr je důležitým ukazatelem poškození či dysfunkce renálních tubulů.
2. U-NAG/Cr detekuje již velmi mírnou dysfunkci či poškození renálních tubulů.
3. U-NAG/Cr je signifikantně zvýšen u následujících onemocnění uropoetického traktu: idiopatické hyperkalciurie, enuresis nocturna, vezikoureterálního refluxu a hydronefrózy.
4. U výše uvedených chorobných stavů je U-NAG/Cr zvýšen, nicméně uvedené zvýšení nesouvisí se stupněm závažnosti příslušného onemocnění.

14. Acknowledgments

I would like to express my thanks to my tutors Eliška Marklová, MD, PhD, and Alexander Kolský, MD, PhD, as well as to my superiors Assoc. Prof. Eva Pařízková, MD, PhD, and Assoc. Prof. Oldřich Pozler, MD, PhD for their support. Furthermore, I am very grateful to Prof. Vladimír Palička, MD, PhD, and Assoc. Prof. Pavel Živný, MD, PhD, for the evaluation of U-NAG/Cr in their laboratory, and to my co-authors, Jaroslav Chládek, PhD, and Assoc. Prof. Štěpán Kutílek, MD, PhD for their assistance, advice and support in the preparation of the manuscripts. Many thanks to Pavel Rejtar, MD, for ultrasound examinations of the urinary tract in the presented patients.