Normal values for urinary *N*-acetyl-beta-glucosaminidase excretion in preterm and term babies

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SUMMARY Urinary *N*-acetyl-beta-glucosaminidase (NAG) excretion was measured in 14 healthy, preterm, male neonates with gestational ages between 32 and 35 weeks. Daily NAG excretion increased significantly during the first four weeks of life. No correlation was observed between urinary NAG:creatinine ratio and postnatal age regardless of whether measurements were taken from the whole 24 hour urine collection or from an isolated urine spot sample at the same time on each day. When the preterm infants were compared with a group of 20 healthy, full term, male infants at a postnatal age of 7 days the NAG:creatinine ratio was significantly higher in the preterm group, the measurements having been taken from single urine spot samples. We suggest that this variable be used in the evaluation of renal tubular integrity during the neonatal period.

Renal anatomic integrity can be reflected in the output of urinary enzymes.¹ Of these enzymes, *N*-acetyl-beta-glucosaminidase (NAG) may be of greatest interest as it is present in appreciable amounts in the proximal tubular cells.² The molecular weight of NAG is about 140 000 daltons.³ Enzymes with molecular weights that exceed 70 000 daltons are not normally filtered into the urine.⁴

Increased urinary excretion of NAG has been found to be correlated with tubular damage in children with a variety of renal diseases.^{5–6} In adults urinary excretion of NAG has been shown to be a good predictor of aminoglycoside toxicity to the tubular epithelium.⁷ Few data are available on the urinary excretion of NAG in neonates who present with renal damage.^{5–6–8–9} Parini *et al* used NAG excretion in the neonatal period as a predictor of possible renal toxicity during treatment with amikacin. They gave no information, however, about the normal excretion of this enzyme in growing preterm newborns.¹⁰

The purpose of this study was to measure the urinary excretion of this lysosomal enzyme in healthy preterm babies and to investigate whether the urinary NAG excretion is influenced by postnatal age.

Patients and methods

Renal function and urinary NAG excretion were evaluated in 14 healthy neonates whose gestational

ages were between 32 and 35 weeks.¹¹ Gestational age was assessed according to the Finnström score.¹² Only male infants were recruited due to the difficulties in obtaining precise 24 hour urine collections in girls. Infants were excluded if they had any of the following conditions: nephrotoxic drugs administered to the mother during the final trimester, Apgar score below 7 at one minute, respiratory distress syndrome, infectious diseases, any drugs in the neonatal period, episodes of hypotension or hypertension according to Bucci's criteria, serum bilirubin concentration >206 µmol/l, and patent ductus arteriosus. Fluids were administered as follows: 60 ml/kg/day on day 1, 80 ml/kg/day on day 2, 100 ml/kg/day on day 4, 130 ml/kg/day on day 6, and 150–180 ml/kg/day from day 8 onwards. Parenteral fluids (10% dextrose and electrolytes) were given from day 1 to day 5. A formula adapted for preterm neonates was introduced as soon as intestinal tolerance was observed.

A 24 hour urine collection was obtained on days 1, 7, 14, 21, and 28, using an adapted urine collection bag. The collection period was carefully timed. Determinations of creatinuria and enzymuria were calculated in terms of absolute rate of excretion (total amount excreted/collection time).

NAG was measured in the whole 24 hour urine collection as well as in a single urine spot sample taken at the same time on each day and immediately frozen at –20°C. NAG activity does not deteriorate

when frozen and is not affected by either bacteriological colonisation or storage for several hours at 4–5°C.¹³ The urine NAG activity was determined by the method of Maruhn.¹⁴ Plasma and urine concentrations of creatinine were determined by Jaffe's reaction after deproteinisation (Boehringer).¹⁵ NAG excretion was expressed as $\mu\text{mol}/24\text{h}$ for the 24 hour collection and in $\mu\text{mol}/\text{l}$ for the single urine spot sample. Creatininuria was determined from the same whole 24 hour urine collection ($\text{mmol}/24\text{h}$) and urinary creatinine concentration measured from each urinary spot sample (mmol/l).

We also compared the NAG excretion at a postnatal age of 7 days between these preterm babies and a group of 20 healthy, appropriate of gestational age, full term male neonates, the measurement being taken this time in both groups from single urine spot samples only. These term infants were breast or bottle (infant formula) fed by their mothers in the nursery.

Glomerular filtration rate, as estimated by the creatinine clearance in the 14 preterm infants and by the Schwartz formula in the 20 full term infants, was within the normal range of published values for all infants,^{16–18} confirming that our subjects had normal renal function.

Statistical analysis was performed using Student's *t* test, comparison between two independent correlations test, comparison between two mean values

with standard deviation, and χ^2 test with Yates's correction.

Results

Longitudinal study in preterm infants showed a significant increase in urinary NAG excretion/24h during the first four weeks of life ($r=0.59$; $p<0.001$) (Fig. 1). Similarly, the daily creatininuria also showed a significant increase with postnatal age ($r=0.38$; $0.02<p<0.05$), so that when expressed as daily NAG excreted/unit of creatinine excreted ($\mu\text{mol}/\text{mmol}$) there was no change with postnatal age ($r=0.27$; $p>0.05$) (Fig. 2).

Moreover, the NAG:creatinine ratio ($\mu\text{mol}/\text{mmol}$) was the same in preterm infants whether measured in urine spot samples or 24 hour speci-

Fig. 1 Urinary N-acetyl-beta-glucosaminidase (NAG) excretion over 24 hours in relation to postnatal age in preterm neonates with a gestational age between 32 and 35 weeks. (Standard deviation $y=0.728+1.14 \cdot 10^{-2} \times x$; $r=0.59$; $p<0.001$).

Fig. 2 Urinary N-acetyl-beta-glucosaminidase (NAG) creatinine ratio in relation to the first four weeks of life in preterm neonates with gestational age between 32 and 35 weeks and in full term babies at a postnatal age of 7 days. Measurements were taken in the preterm group from the whole 24 hour collection (○) (total amount excreted/ collection time) ($r=0.27$; $p>0.05$) and from a single urine spot sample (●) ($r=-0.066$; $p>0.1$). The measurements in the term group were taken from a single urine spot sample only.

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Table Measurements of urinary NAG excretion taken from 24 hour collection or from spot sample in preterm and term infants

Urinary NAG excretion ($\mu\text{mol}/\text{mmol}$ creatinine)	Preterm group		Term group
	24 Hour diuresis (n=35)	Spot sample (n=39)	Spot sample (n=20)
Mean (SD)	3.6 (2.39)	4.43 (2.41)	1.75 (1.1)
5th-95th Centile limits	0.9-9.7	1.2-9.8	0.4-4.3

NAG=N-Acetyl-beta-glucosaminidase; NS=not significant.

mens ($p>0.05$), and this ratio calculated on a single urine spot sample also remained stable during the four weeks of the study ($r=-0.066$; $p>0.1$) (Fig. 2).

The NAG:creatinine ratio measured in a single urine spot sample on the seventh day of life was significantly higher in preterm infants than in term infants (mean (SD) ratios 4.9 (2.9) $\mu\text{mol}/\text{mmol}$ and 1.75 (1.1) $\mu\text{mol}/\text{mmol}$, respectively; $p<0.001$).

The 95th centile limits for the NAG:creatinine ratios in the preterm babies during the four weeks were 9.8 μmol NAG:mmol creatinine and in the term week old infants were 4.3 μmol NAG:mmol creatinine (Table).

Discussion

Urinary excretion of N-acetyl-beta-glucosaminidase has been studied extensively in adults suffering from renal diseases.^{1 3 7 17} This enzyme meets all the criteria of Gonick *et al* for selecting enzymes that may be useful in detecting renal tubular damage and seems to be a good indicator of renal tubular cell integrity.¹⁷ In particular, increased urinary excretion of NAG can be a good predictor of aminoglycoside toxicity to the proximal tubular epithelium.⁷

In fact, Morin *et al* considered that the site of the toxic action of an aminoglycoside such as gentamicin was exclusively the lysosome of the proximal cell.² Lysosomal overloading can lead to disruption of its membrane and subsequently to cell death.² Increased NAG excretion has been observed in children who present with well characterised tubulointerstitial disease. This phenomenon seems to be more important in girls aged under 2 years.⁵

Few data are currently available on the normal excretion of NAG in healthy preterm and full term infants.^{1 6} All infants enrolled into this study were healthy and had a normal glomerular filtration rate.^{16 18}

Our data indicate that NAG excretion in neonates reaches much higher values than in adults. Our results also indicate that NAG excretion at a postnatal age of 7 days is lower in full term babies than

in preterm babies. This agrees with previous reports.⁶ We have shown a rise in absolute urinary NAG excretion/24 hours with postnatal age.

The NAG:creatinine ratio showed no correlation with postnatal age in the preterm babies. Similarly, no correlation was observed between the NAG:creatinine ratio and increasing postnatal age when the measurements were taken from an isolated single urine spot sample in the same group of preterm infants. Many authors have routinely used creatininuria as a denominator to avoid urine collection.^{6 7 14 17} This mode of expression has been criticised with regard to the neonatal period because of the age dependent changes in the creatininuria.¹⁹ Nevertheless, our data suggest this determination to be accurate for routine use.

The value of using an increase in urinary NAG excretion as an indicator of renal tubular damage in the neonatal period, as has been done for adults and older children, remains to be tested. This is presently under investigation in newborn infants who have suffered severe episodes of asphyxia as well as in those who have received potentially nephrotoxic drugs.

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