ACTA PÆDIATRICA
NURTURING THE CHILD

Urinary ammonium: paediatric reference values

Journal:	*Acta Paediatrica*
Manuscript ID	SPAE-2020-0831.R1
Manuscript Type:	Brief Report
Date Submitted by the Author:	n/a
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Keywords:	Urine ammonium, metabolic acidosis, reference values

Urinary ammonium: paediatric reference values

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Urinary ammonium excretion plays a central role in the renal regulation of acid-based homeostasis, since it represents approximately a half to two-thirds (40-50 mmol/day) of the net acid excreted by the kidneys. In normal anion gap metabolic acidosis, quantifying the ammonium in urine provides information about the origin of the defect in urinary acid excretion. Urinary ammonium measurement is not usually available in clinical laboratories and is estimated by indirect methods, such as urinary anion gap or urinary osmolal gap. These are considered inaccurate when it comes to assessing ammonium concentration (1). In fact, most clinical guidelines do not include direct ammonium measurement and those that do usually refer to 24-hour urine collection.

Despite the benefits of urinary ammonium direct quantification in metabolic acidosis, there are a lack of paediatric reference values, which are mandatory to implement its use in clinical practice.

Our study evaluated urinary ammonium reference values using an automated method developed in our laboratory (2). We used simple urine samples, as collecting urine from children is not easy and 24-hour urine collection is even more difficult.

Diet is known to influence acid-based balance and some studies have shown that ammonium excretion generally increased after protein intake rises (3). We examined whether fasting could influence urinary ammonium concentrations, in order to indicate the best pre-analytical requirements.

We prospectively observed 180 children without concurrent renal, metabolic or systemic disease who attended the Hospital Universitario Central de Asturias, Oviedo, Spain, from February to June 2017 for a check-up before minor scheduled surgery. The study was approved by the hospital Ethics Committee (approval number 26/17). Data about fasting and whether first morning urine was collected were registered.

Samples were collected in urine Vacuette tubes with no additives, centrifuged at 1500 rpm for 10 minutes and immediately stored at -80ºC until assayed. Ammonium measurements were performed on a Roche Cobas c501 analyser (Roche Diagnostics International AG, Basel, Switzerland) by adapting an automated plasma ammonium assay (2). The results were expressed as the ammonium/creatinine ratio.

Reference values were calculated following the Clinical and Laboratory Standards Institute guideline C28-A3, using SPSS 15.0 (SPSS Inc, Chicago, USA) and MedCalc, version 12.5.0.0 (MedCalc Software Ltd, Ostend, Belgium), after excluding outliers using Tukey's test. Non-Gaussian distributions of urinary ammonium concentrations were obtained using the D'Agostino-Pearson test and non-parametric methods were applied. A p value of ≤ 0.05 was considered statistically significant.

We analysed the ammonium/creatinine ratio reference values of 171 children after nine outliers were excluded: 27 children were under five years of age (mean age 3.4 ±0.4 years) and 144 children were five years or more (mean 9.3 ±0.2 years). A significant negative correlation was observed between age and ammonium/creatinine ratio (Spearman correlation Rho -0.352, $p<0.01$). Significant differences were observed between the younger and older age groups: median ammonium/creatinine ratio 5369 µmol/mmol versus 3861 µmol/mmol, $p<0.01$. However, there were no significant differences in gender (78% boys versus 74% girls), providing first urine samples (11% versus 16%) or fasting before testing (44% versus 65%). The urinary ammonium reference values were only calculated for children over five years of age because the younger sample was not representative. No significant differences were observed between the ammonium concentrations of first morning and subsequent urine or fasting and non-fasting urine samples, or between children aged 5-10 years and those older than 10 years.

The reference interval was calculated using the non-parametric method, ranging from 776 µmol/mmol (90% confidence interval 562-1317) to 8217 µmol/mmol (90% confidence interval 7246-9877) (Figure 1).

Our study provides reference values for urinary ammonium expressed as ammonium/creatinine ratio in isolated morning urine samples of healthy children. References values have been reported in paediatric populations after 24-hour urine collections and expressed as µmol/min/1.73 m^2 (4). Urinary collections over 24 hours have proved inaccurate, as they need a bladder catheter and preservatives to avoid increases in urinary ammonium concentrations until analysis. Thus, a single urine sample is more appropriate because the variations in urine flow and concentration are avoided by measuring creatinine in the same sample and expressing the results as ammonium/creatinine ratio. Traditionally, single urine samples for ammonium quantification were collected under paraffin, but we previously showed no significant differences between samples collected with or without paraffin (2).

Urine ammonium excretion in healthy individuals can be moderately influenced by diet (3). We found that fasting and using first morning urine samples had no significant differences on the outcomes.

The concentrations found in our children (4000-73175 µmol/L) were higher than adult samples (3000-65000 µmol/L) (5), due to higher net acid excretion (ammonium + tritable acid - HCO_3^-) in children.

To optimise results, samples should be centrifuged, separated and analysed as soon as possible or frozen if the analysis is delayed. Such results are unreliable if there are urinary infections by urea-splitting bacteria. One study limitation was that the samples

were not tested for possible urinary infections, but they were collected from healthy children with no clinical signs of these.

Our study was the first to describe robust reference values for ammonium/creatinine ratio in healthy children older than five years, measured by a direct automated quantification method.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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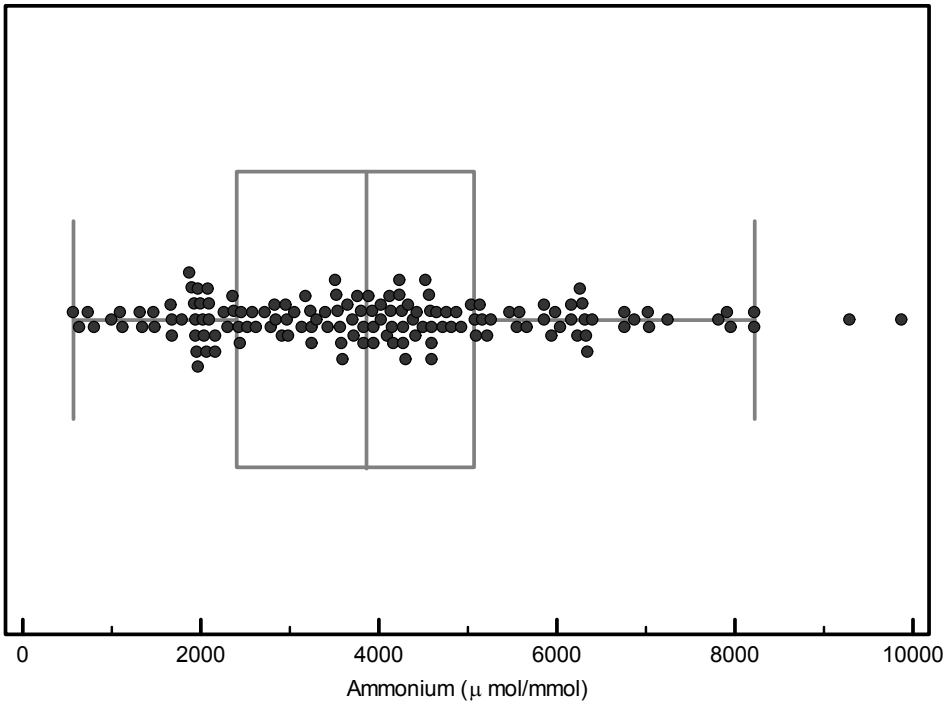
FIGURES

Figure 1. Ammonium/creatinine ratio reference values in isolated morning urine samples of 144 healthy children aged 5-17 years. Box lines represent median, 5th, 25th, 75th, and 95th percentiles.



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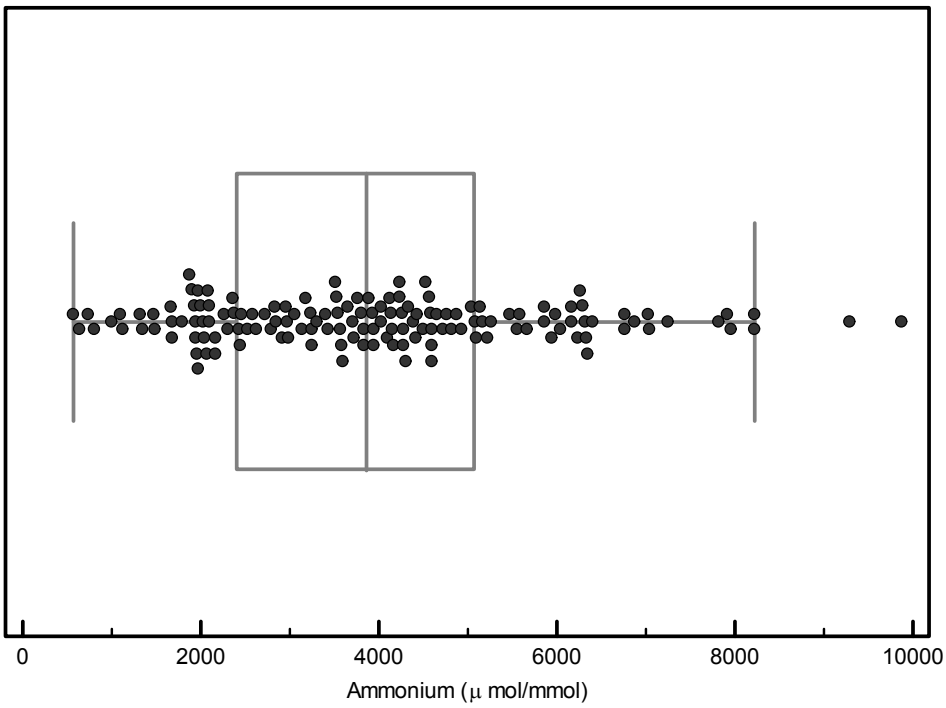

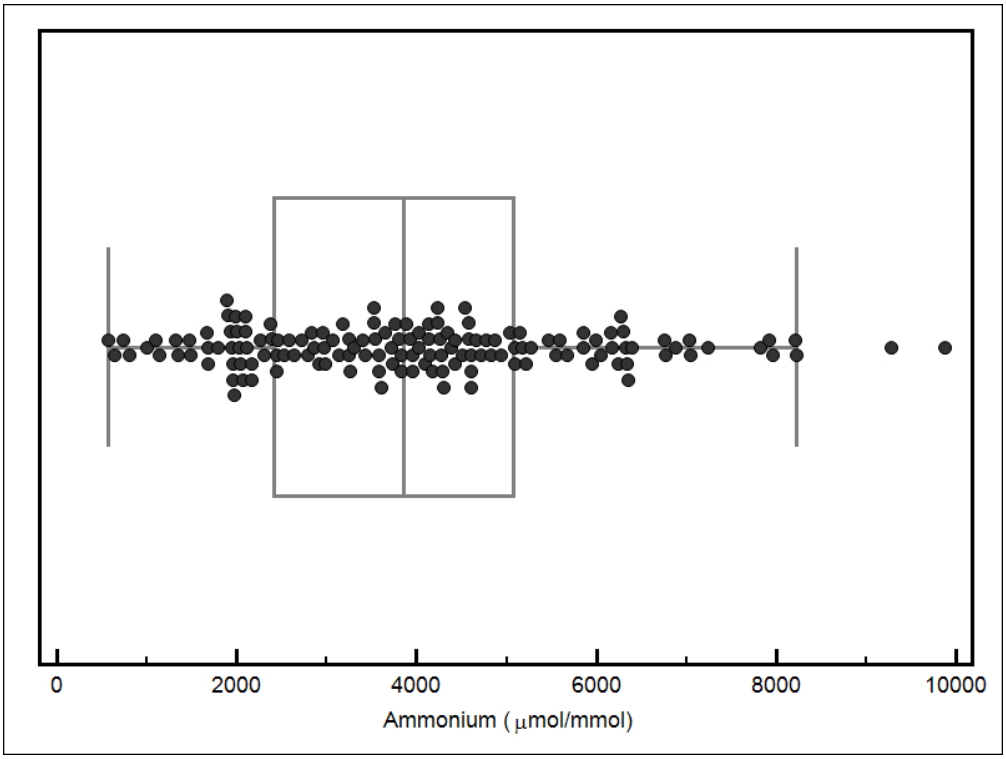

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