Development of human renal function: reference intervals for 10 biochemical markers in fetal urine

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Evaluation of fetal renal function by analysis of fetal urine sampled in utero may improve perinatal care after a prenatal diagnosis of bilateral obstructive uropathy. We provide reference intervals for 10 fetal urinary compounds and examine their variation with gestational age. Forty-one fetuses with bilateral obstructive uropathy (urine sampled between 20 and 38 weeks of gestational age) had normal, healthy values for serum creatinine (≤50 μmol/L) at ages 1–2 years. These cases were thus assumed to represent a reasonable approximation to healthy values. Sodium and β₂-microglobulin concentrations significantly decreased with gestational age; calcium, ammonia, and creatinine significantly increased; glucose, phosphorus, chloride, urea, and total protein concentrations did not vary. Our results provide reference values for prenatal evaluation of fetal renal function and suggest that glomerular filtration of macromolecules and tubular reabsorption of glucose and phosphorus are mature by 20 weeks of gestation, whereas tubular reabsorption of sodium and β₂-microglobulin increases progressively during the second half of gestation.

INDEXING TERMS: prenatal diagnosis • renal function • bilateral obstructive uropathy • fetal status • sodium • β₂-microglobulin • calcium • ammonia • creatinine • glucose • phosphorus • chloride • urea • protein • age-related effects

Obstructive uropathies are the most frequent fetal anomalies diagnosed by prenatal ultrasound. In cases such as those involving posterior urethral valves, the obstruction is potentially curable but may induce a wide spectrum of renal damage. In the most severe cases, major renal dysplasia results in perinatal death, or survival with renal failure. In the mildest forms of obstructive uropathy, the infants survive with normal renal function.

On the basis of their experimental work with fetal lambs, Adzick et al. [1] studied human renal function by analysis of fetal urine sampled in utero and showed that fetal urine sodium was predictive of short-term perinatal outcome. More recently, we and our colleagues have shown that prenatal assessment of various fetal urinary analytes, including sodium and β₂-microglobulin, can predict postnatal renal function later in childhood [2, 3]. However, the clinical value of these prognostic tests has been questioned [4, 5] because of the lack of precise reference values, which may hamper the interpretation of fetal urinalysis, and discrepancies in the definitions of normal renal function.

In this study, we have derived reference intervals (mean ± SD) for fetal urinalysis of 10 compounds from results for a group of 41 fetuses with isolated bilateral uropathy, in whom renal function was subsequently (postnatal age 1–2 years) found to be normal according to stringent criteria.

**Materials and Methods**

Between 1986 and 1992, 167 pregnant women underwent fetal urine sampling because of diagnosis of bilateral dilatation of the fetal urinary tract at routine ultrasonography. In all cases, the following investigations were performed: (a) detailed sonography specifying the location of obstruction, the amniotic fluid volume, and the renal parenchymal structure; (b) fetal karyotyping by amniocentesis or fetal blood sampling; and (c) transabdominal fetal urine sampling through an ultrasound-guided 20-gauge needle.

Urine was obtained from a renal pelvis or a ureter whenever possible, or from the fetal bladder when pelves or ureters were inaccessible. When pelvic dilatations were not symmetrical, urine was obtained either from the least-enlarged pelvis or from both sides; in the case of bilateral sampling, results from the least-enlarged pelvis were used in the analysis. To keep the procedure minimally invasive, we made no attempt to decom-
press the urinary tract, and repeat urine samplings were not performed routinely.

None of the fetuses underwent intrauterine uroamniotic shunting, and the results of fetal urinalysis were not taken into account to alter postnatal management. Complete follow-up was achieved for 157 of the 167 cases. There were 63 terminations of pregnancy: 25 for associated anomalies (7 chromosome anomalies, 18 multiple malformations with normal karyotype) and 38 for marked oligohydramnios with severe bilateral renal damage. Two fetuses with multiple organ malformations died in utero. There were also 21 neonatal deaths: 3 from associated malformations not diagnosed prenatally (2 VACTEL associations, 1 severe hydrocephalus), 2 from neonatal sepsis, and 16 from acute respiratory distress, pulmonary hypoplasia, and renal failure. Two children died before age 6 months, one from sudden infant death syndrome and one from megalycystis-microcolon. By 1 year postpartum, 69 children were still alive. Two of these (1 male, 1 female) had megalycystis-microcolon; the remaining 67 survivors all had isolated obstructive uropathies, without any associated malformation. In all survivors, follow-up included clinical examination in a pediatric nephrology unit, radiology and ultrasonography of the urinary tract, serial biochemical analysis, and bacteriological urinalysis. All these infants were followed until at least age 1 year (range of follow-up, 1–7 years). Postnatal renal function was not considered normal in the 26 cases with postnatal serum creatinine concentrations >50 μmol/L (5.6 mg/L) at 1–2 years. In contrast, 41 children were considered to have normal renal function, and these 41 form the database of this study. Twenty-two of these 41 cases were previously included in a study investigating the ability of fetal urinalysis to predict the renal function of children with bilateral obstructive uropathy [2].

The criterion we used to define normal postnatal renal function was serum creatinine ≤50 μmol/L (≤5.6 mg/L) at age 1–2 years. This threshold represents 2SD above the mean in healthy children [6, 7]. The height and weight of all children whose serum creatinine was ≤50 μmol/L were within the respective normal ranges. In addition, none of these children had any clinical symptom related to renal function. Renal function also remained normal in those followed for >2 years.

In these 41 cases, bilateral urinary tract dilatation was diagnosed between 20 and 38 weeks since the mother’s last menstrual period. Fetal urine was sampled from a renal pelvis in 29 cases and from the bladder in 12. When several samples were collected from the same fetus at different gestational ages, only the first sample was used in the calculations.

Before performing biochemical urinalysis, we checked for the absence of contamination with blood by making red and white blood cell counts. Lack of contamination with amniotic fluid was noted by the absence of intestinal enzymes, namely, γ-glutamyltransferase, aminopeptidase M, and alkaline phosphatase isoenzymes. The following analytes were studied: sodium, chloride, calcium, phosphorus, ammonium, urea, creatinine, glucose, total proteins, and β2-microglobulin. Sodium was determined by a spectrophotometric method, urea by glutamic dehydrogenase enzymatic assay (Abbott, N. Chicago, IL), creatinine by the Jaffe method (Beckman, Galway, Ireland), proteins by sulfosalicyclic acid precipitation, chloride by a direct thiocyanate colorimetric method, calcium by o-cresolphthalein colorimetric method (Abbott), phosphorus by molybdate colorimetry (Abbott), ammonia by an l-glutamyl dehydrogenase enzymatic method (Boehringer, Mannheim, Germany), glucose by enzymatic colorimetry (Merck, Paris, France), and β2-microglobulin by RIA (Pharmacia, Uppsala, Sweden).

The urinary concentration of each compound was expressed as mean ± SD and was studied as a function of gestational age through the construction of age-related reference centiles by using absolute residuals [8].

Results

Reference intervals were established from results for 41 fetuses with bilateral obstructive uropathy whose postnatal renal function at age 1 year was normal (defined as postnatal serum creatinine <50 μmol/L). For these infants, their mean ± SD serum creatinine concentration was 39 ± 6 μmol/L.

Of these 41 cases, prenatal dilatation of the urinary tract involved only the renal pelvis in 14 cases; in 3 cases, the ureters were also dilated but not the bladder. The bladder was involved in 24 cases. Amniotic fluid was normal in 38 cases, moderately oligohydramniotic in 3 cases; none had severe oligohydramnios.

Postnatal diagnoses were as follows: posterior urethral valves (n = 12), megabladder (n = 2), prune belly syndrome (n = 3), bilateral megaureter (n = 6), and bilateral pyeloureteral junction syndrome (n = 18). Thirty-two infants (78%) underwent surgery, with the interval between birth and surgical treatment depending on diagnosis. For posterior urethral valves, the interval was 5 ± 2.6 days; for other diseases, it was 68 ± 20 days. In the remaining 9 cases, surgical treatment was unnecessary: 3 cases of prune belly syndrome, 3 pyeloureteral junction syndromes, 2 megabladders, and 1 megaureter.

Table 1 presents the reference values for 10 biochemical analytes. The mean gestational age at sampling was 32 weeks (range 20–38).

We also studied the correlation of each analyte with gestational age between 20 and 38 weeks. β2-Microglobulin (Fig. 1A) and sodium (Fig. 1B) decreased significantly (P <0.001) throughout gestation. Three analytes increased with gestational age: calcium (Fig. 1C), ammonium (Fig. 1D), and creatinine (Fig. 1E). Three compounds were virtually absent from fetal urine during the second half of the gestational period (20–38 weeks): phosphorus, glucose, and total protein; and there was no statistically significant change in chloride or urea. These five analytes are therefore not presented in Fig. 1.

The validity of the reference intervals was confirmed by superimposing on the centile plots the data points for 70 cases with poor outcome (Fig. 2). These 70 cases consisted of the 38 terminated pregnancies performed because of renal damage with severe oligohydramnios; the 16 neonates who died because of acute respiratory distress, pulmonary hypoplasia, and renal failure; and the 26 survivors with serum creatinine ≥50 μmol/L at age 1–2 years. The cases in which termination of pregnancy or postnatal death was related to extrarenal anomalies, such as multiple malformations or sepsis, were not taken into account in this analysis.
Table 1. Reference values for analytes in fetal urine (41 cases).

<table>
<thead>
<tr>
<th>Reference values</th>
<th>95% confidence interval</th>
</tr>
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<tbody>
<tr>
<td><strong>Gestational age</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>32 ± 4</td>
<td>32 ± 4</td>
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<tr>
<td><strong>Protein, g/L</strong></td>
<td>0.04 ± 0.07</td>
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<tr>
<td><strong>β₂-Microglobulin, mg/L</strong></td>
<td>0.96 ± 1.2</td>
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<tr>
<td><strong>Urea, mmol/L</strong></td>
<td>8.7 ± 2.9</td>
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<tr>
<td><strong>Creatinine, μmol/L</strong></td>
<td>216 ± 68</td>
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<tr>
<td><strong>Ammonia, μmol/L</strong></td>
<td>695 ± 410</td>
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<tr>
<td><strong>Sodium, mmol/L</strong></td>
<td>50 ± 9</td>
</tr>
<tr>
<td><strong>Chloride, mmol/L</strong></td>
<td>50 ± 7</td>
</tr>
<tr>
<td><strong>Glucose, mmol/L</strong></td>
<td>0.15 ± 0.19</td>
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<tr>
<td><strong>Calcium, mmol/L</strong></td>
<td>0.65 ± 0.36</td>
</tr>
<tr>
<td><strong>Phosphate, mmol/L</strong></td>
<td>0.15 ± 0.25</td>
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* Weeks of amenorrhea.

**Discussion**

This study establishes reference intervals for the concentrations of 10 urinary compounds in fetuses with bilateral uropathy who survived free of clinical symptoms of renal failure in infancy and whose serum creatinine was <50 μmol/L at ages 1-2 years.

The prognosis of bilateral obstructive uropathies diagnosed in utero depends on the expected postnatal renal function. Prenatal sonographic evaluation of renal parenchymal structure and of amniotic fluid volume provides an indirect evaluation of renal function and can predict postnatal outcome only in the most severe cases [9-13]. In contrast, fetal urinalysis is a direct indicator of renal function. Although lethal cases can be identified by sonography alone, fetal urinalysis is required to recognize the cases at risk for survival with renal failure [2, 14]. Prenatal evaluation of renal function is crucial in cases with obstructive uropathies such as posterior urethral valves. In fetuses with altered renal function, intrauterine therapy may be required to prevent further renal damage by restoring a normal pressure in the urinary tract, e.g., by percutaneous uroamniotic drainage [15].

In the human fetus, fetal urinalysis is probably the most accurate approach to quantitative evaluation of renal function. This is because during fetal life the mother supplies the fetus through the placenta with balanced nutrients, and fetal homeostasis is ensured without the intervention of fetal kidneys. Therefore, the composition of fetal urine depends only on fetal renal function and reflects renal potential. However, the biochemical evaluation of fetal renal function is hampered by the lack of precise reference values. Moreover, to allow for a more accurate prognostic evaluation in utero, we needed to define normal urinalysis values as a function of gestational age.

Although sodium is the most widely used fetal urinary marker, we had shown previously that other compounds are also of interest [2]. Notably, we found that for prediction of above-normal concentrations of serum creatinine during the second postnatal year, the fetal urinary concentration of β₂-microglobulin was both specific (0.80) and sensitive (0.83), whereas sodium chloride and urea concentrations were sensitive (≥0.70) but lacked specificity (<0.65). In contrast, fetal urinary glucose, phosphorus, calcium, ammonium, and total protein were specific (≥0.70) but lacked sensitivity (<0.65). Therefore, we believed that a combination of biochemical markers could be useful for the prenatal evaluation of renal function, underscoring the need for more extensive reference data.

Given the technical difficulty and questions of ethical propriety in sampling fetal urine in normal pregnancies, one cannot establish true "normal" values for the composition of fetal urine. Nicolini et al. [16] studied urine from 26 fetuses, sampled either before termination of pregnancy for a nonrenal anomaly or at the time of intrauterine transfusion for Rh alloimmunization. However, this only approximated normal fetal urine, because renal function may have been altered in severely anemic fetuses undergoing intrauterine transfusions. An alternative approach would be to establish reference intervals based on the biochemical data obtained from those fetuses with obstructive uropathy who underwent intrauterine urine sampling and who subsequently were found to have a good outcome. Unfortunately, the literature shows major discrepancies in how investigators have defined uropathies that have a good functional outcome. In 1985, the earliest report on human fetal urinalysis established that urinary sodium >100 mmol/L was predictive of perinatal death or severe bilateral dysplasia [15]. In a 40-case series, Crombleholme et al. reported a higher survival rate in fetuses with urinary sodium <100 mmol/L [14]. Lipitz et al. showed that fetal urinary concentrations of sodium, calcium, and β₂-microglobulin are predictors of neonatal survival [17]. However, we think it likely that infants who survived with renal failure were overlooked in early reports because perinatal survival was the only outcome criterion. This is illustrated by a series of nine cases of fetal uropathies reported by Wilkins et al. [4], in which four infants with fetal urinary sodium values <100 mmol/L survived, three of whom subsequently developed renal failure in infancy.

More recently, Nicolaides et al. established reference ranges for fetal urinary sodium, calcium, urea, and creatinine, based on a series of 20 fetuses with obstructive uropathy without renal dysplasia [18]. In this series, however, only 13 infants survived, and their renal function was considered normal if their serum creatinine in infancy was <70 μmol/L.

In contrast, in the present study, all 41 patients included actually did survive. In utero ultrasound examination demonstrated normal renal parenchymal structure in 38 cases (93%) and normal amniotic fluid volume in 37 cases (90%). In no case was the association of oligohydramnios and abnormal renal structure observed. Even the 7 cases with a low amniotic fluid volume or thin renal parenchyma in utero had normal serum creatinine after age 1 year. Moreover, serum creatinine was considered normal only if <50 μmol/L by age 1-2 years, which represents 2SD above the mean for healthy children [6, 7]. Because we used more stringent criteria to define a homogeneous reference group, we believe that our reference intervals could be a more appropriate approximation to normal fetal development. We are aware that, given the presence of an obstructive uropathy, our cases were not, strictly speaking, "normal" fetuses. From a clinical point of view, however,
survivors with normal postnatal renal function are adequate controls. For sodium, β₂-microglobulin, and calcium, data points from fetuses with poor outcome overlapped little with our reference intervals, underscoring the potential clinical usefulness of these markers.

In addition to this potential clinical application, our results provide new insights into human kidney development. Fetal urine production can be documented as early as 12 weeks after conception. The morphological development of human kidney has been extensively described. Briefly, the branching process of the collecting system is completed by 20 weeks. The induction of the metanephric blastema by the distal portion of the branches results in the formation of 8–12 generations of nephrons. This process of nephrogenesis is complete by 32–36 weeks of gestation, at which time each kidney has ~800 000 nephrons [19]. Little, however, is known about the functional aspects of renal development. Our results suggest that the various functions of the kidney do not develop at the same time.

![Graphs](image)

**Fig. 1.** Variation of reference ranges for fetal urinalysis with gestational age: (A) β₂-microglobulin (n = 41), (B) sodium (n = 41), (C) calcium (n = 41), (D) ammonia (n = 39), and (E) creatinine (n = 39).

Regression curves (5th, 50th, and 95th centiles) are superimposed on data obtained by fetal urine sampling in cases resulting in the birth of children whose serum creatinine was >50 μmol/L at ages 1–2 years.
Fig. 2. Fetal urinalysis: abnormal cases.

Data points for 70 cases with poor outcome are superimposed on the 5th, 50th, and 95th centiles reference regression curves. ▲, Termination of pregnancy because of renal damage with severe oligohydramnios (n = 38); ■, Neonatal death caused by renal failure (n = 16). □, Survivors with serum creatinine >50 μmol/L at ages 1–2 years (n = 26). In some cases, urine sample volume was too small to allow measurement of all analytes.
For instance, at 20 weeks of gestation, the low protein content of fetal urine suggests that glomerular renal function, analyzed in terms of protein filtration, is mature. The virtual absence of urinary glucose and phosphorus at this gestational age suggests that the maturation of tubular reabsorption of these compounds is also achieved by then. Earlier reports of higher fetal urinary concentrations of phosphate (0.91 mmol/L) at 16 weeks [14] suggest immature tubular reabsorption of phosphate at this stage of development. In contrast, our results are consistent with progressive maturation of tubular reabsorption of sodium and β2-microglobulin and tubular secretion of calcium after 20 weeks. These changes in biological markers of tubular maturation parallel the increase in kidney mass and the number of nephrons and confirm previous reports (based on smaller series) showing a decreased sodium concentration throughout gestation [16, 18]. In addition, our data indicate that fetal urinary calcium increases significantly (P < 0.001) with gestational age. This had been overlooked in previous series, probably because of their small study populations. From 30 weeks onwards, we observed a clear progressive increase in the elimination of nitrogen compounds (urea, creatinine, and ammonia); this could be related to the increase in fetal muscular mass.

In conclusion, although fetal urine sampling is increasingly used for prenatal evaluation of obstructive uropathies, the interpretation of fetal urinalysis is hampered by the lack of precise normal values. We provide reference values for 10 fetal urinary biochemical analytes for gestational ages between 22 and 38 weeks. We are aware that our cases represent only an approximation to normal fetal urine. However, in our series the number of cases is greater, and the definition of the normality of renal function is more stringent, than in previous reports. In addition, we analyzed a greater number of fetal urinary compounds, allowing for new insights into the physiological development of human renal function. Therefore, we believe that our results contribute to a more accurate evaluation of fetal urinary biochemical findings, allowing for more rational selection of those fetuses that could benefit from intrauterine intervention.

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References