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Both extrauterine and intrauterine growth restriction impair renal function in children born very preterm

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A single-center prospective cohort study was designed to identify alterations of renal function during childhood in children born prematurely. A cohort of 143 such babies born over a 4-year period (birth weight less than 1000 g and/or less than 30 weeks of gestation) was prospectively included at birth. A mailing was sent to all parents to propose renal evaluation. Among the 50 included children, 23 had intrauterine and 16 had extrauterine growth retardation. When comparing both of these groups to 11 children with appropriate pre- and postnatal growth at a mean follow-up of 7.6 years, both groups of growth-restricted children had slightly but significantly lower glomerular filtration rates, measured by inulin clearance, although both groups were still within the normal range for their ages. There were no differences for other renal parameters, neonatal therapies or complications, except for postnatal corticosteroid exposure. Children with extrauterine growth restriction were found to have significantly lower protein-energy intake during their first week of life than the intrauterine growth–restricted or the normotrophic children. Our study found that children with either intra- or extrauterine growth retardation are at risk of decreased glomerular filtration rates during childhood. Extrauterine growth restriction represents a new risk factor for long-term renal impairment in premature children.

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Long-term renal outcome of children born preterm remains uncertain.[1] Intermediate-term [2–4] and long-term [5–7] evaluations during childhood and early adulthood have shown subtle changes in the renal function of premature children, mainly in blood pressure (BP), kidney size, tubular function, and glomerular filtration rate (GFR). In most of these studies, GFR is estimated by using Schwartz formula in children or Cockcroft/MDRD formula in young adults. However, GFR measurement using exogenous markers, such as inulin, iothalamate, or iohexol, is recommended despite technical and financial limitations.[8] Moreover, several studies did not distinguish children according to the presence or absence of intrauterine growth retardation (IUGR).

In humans, approximately 60% of the nephron population develops during the third trimester of gestation.[9] Renal development ends between 35 and 36 weeks of gestational age (GA) and is influenced by prematurity, leading to 'oligonephropathy' with quantitative and qualitative alteration of nephron formation.[10] Brenner and Anderson[11] suggested an inverse correlation between the total renal filtration surface area and the risk of arterial hypertension. This concept of 'reduction in nephron number' leads to a progressive renal impairment: increase of single-nephron glomerular filtration, compensatory nephron hypertrophy, decrease of functional reserve, microalbuminuria, arterial hypertension, glomerulosclerosis, overt proteinuria, progressive fibrosis, chronic kidney disease, and a reduction in nephron number. Further studies have shown a strong correlation between birth weight (BW), glomerular number, and glomerular size.[12]

Barker *et al.*[13] showed that children with IUGR were at risk of developing metabolic syndrome and cardiovascular disease in adulthood. Moreover, the clinical course of some glomerular diseases (for example, IgA nephropathy, membranous nephropathy, and minimal change nephrotic syndrome) is influenced by IUGR.[14,15]

Recent studies have also emphasized the importance of extrauterine growth retardation (EUGR), usually defined as a

height or weight below the 10th percentile at discharge from the neonatal intensive care unit, in children born with an appropriate BW for GA.[16] The pathophysiology of EUGR remains unclear, involving both genetic and environmental factors. However, EUGR is linked to early protein-caloric deficiency,[17] BW,[18] gender, need for mechanical ventilation, necrotizing enterocolitis, and exposure to neonatal corticosteroids.[16] It may also be associated with impaired long-term growth and with lower psychomotor development at 2 years of age.[19,20] Recent animal studies have suggested that restriction of both perinatal and early postnatal growth may increase BP in male rats,[21] but renal consequences of EUGR in humans remain unknown.

The aim of this study was to investigate the renal outcome of children born preterm in a single-center cohort and to identify risk factors predictive of intermediate-term renal impairment.

RESULTS

The flowchart of the study is summarized in Figure 1. Fifty children underwent the renal exploration. There were no significant differences between included children and those from the cohort whose parents did not answer (Table 1), particularly in terms of BW, GA, proportion of IUGR, proportion of morbidities (bronchopulmonary dysplasia, patent ductus arteriosus, sepsis, periventricular leucomalacia, and necrotizing enterocolitis), perinatal nutrition, and drug exposure.

In the cohort of 50 children undergoing the renal exploration, that is, BP assessment, inulin clearance, tubular explorations, and renal ultrasounds (Table 2), the mean age at renal assessment was 7.6 ± 1.3 years (5.8–10.3). During the neonatal period, no child experienced severe oliguria or required renal replacement therapy. There were 23 (46%) children with severe IUGR and 16 (32%) with severe EUGR; only 44 inulin clearance tests were available because of technical problems (failure of venous puncture or timed collection of urine). No child had significant arterial hypertension. The 95% confidence interval for mean diastolic BP percentile was above the 50th percentile (51–65), whereas the 95% confidence interval for mean systolic BP percentile included the 50th percentile (38–53). The true GFR was above 90 ml/min per 1.73 m^2 in all cases, with a mean inulin clearance of 112 ml/min per 1.73 m^2 (91–158). The mean urine calcium-to-creatinine ratio was 0.3 mmol/mmol (0.02–1.28). Two children had hypercalciuria (a urine calcium-to-creatinine ratio > 0.9 mmol/mmol): one child received acetazolamide because of hydrocephalus and the other suffered from post-necrotizing enterocolitis chronic malabsorption with hyperoxaluria and hypercalciuria. Two children had moderate microalbuminuria. There was no obvious tubular dysfunction but β2-microglobulinuria (> 0.20 mg/l) was present in eight patients (17%). Ultrasounds revealed more small-sized kidneys than expected, when expressing kidney size in percentile according to statural height: the 95% confidence interval for the mean standard deviation score (SDS) of kidney size according to height was (−1.3; −1.9) for the right kidney and (−1.1; −1.6) for the left kidney. In addition, ultrasounds revealed moderate cortical nephrocalcinosis in a 7-year-old girl with renal hypodysplasia and three undiagnosed silent malformations.

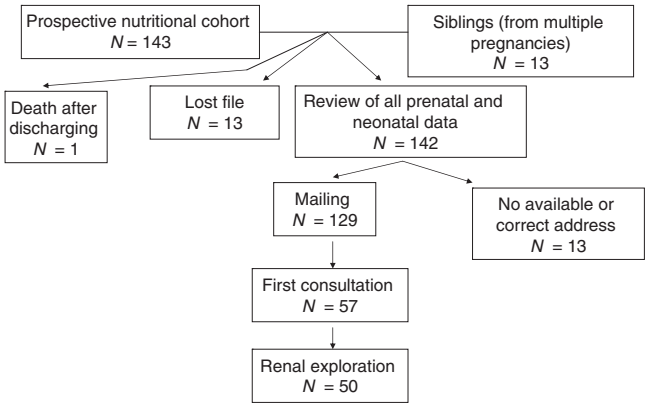

Figure 1 | Flowchart of the study.

A comparison between IUGR ($n = 23$), EUGR ($n = 16$), and children with normal pre- and postnatal growth ($n = 11$) was performed (Tables 2 and 3). There were no differences among these three groups in terms of prenatal corticosteroids, oligohydramnios, umbilical venous and arterial catheters, neonatal drug exposure, and neonatal complications, except bronchopulmonary dysplasia (EUGR 63%, IUGR 35%, and normotrophic 18%, $P = 0.054$) and postnatal corticosteroid exposure (EUGR 50%, IUGR 17%, and normotrophic 9%, $P = 0.026$). Moreover, maximal blood urea nitrogen in EUGR children was greater (EUGR 13.3 ± 5.1, IUGR 10.2 ± 8.2, and normotrophic 11.8 ± 3.4 mmol/l, $P = 0.011$), whereas they received less protein-calorie intakes at 7 days of life. This difference was not present at 14, 21, and 28 days. The final results of inulin clearance were available in 12 children with EUGR, in 21 children with IUGR, and in 11 normotrophic children with normal postnatal growth. IUGR and EUGR children experienced a comparable decreased GFR (however, within the normal range) without any other differences for BP, renal size, urine calcium-to-creatinine ratio, and microalbuminuria. Figure 2 represents the relation between growth retardation and GFR.

Univariate analysis of intermediate-term renal abnormalities (BP, GFR, microalbuminuria, urine calcium-to-creatinine ratio, and kidney size) according to different neonatal conditions, mainly morbidities (bronchopulmonary dysplasia, patent ductus arteriosus, and bacterial-proven sepsis) and therapies, was performed. Children with bronchopulmonary dysplasia were at risk of impaired intermediate-term renal function (higher microalbuminuria: 2.1 ± 1.5 vs 1.2 ± 0.8 mg/mmol, $P = 0.013$), without any difference for GFR (108 ± 8 vs 115 ± 17 ml/min per 1.73 m^2, $P =$ NS). There were no differences for intermediate-term renal parameters between children with and without patent

Table 1 | Comparison of neonatal parameters among 50 children with renal assessment and 92 children whose parents did not answer

	Patients with renal assessment (mean ± s.d. or %)	Children without renal assessment (mean ± s.d. or %)	*P*-value	*N*
Number of patients	50	92		142
Birth				
Gestational age (weeks)	27.3 ± 1.97	27.3 ± 1.64	0.98	142
Birth weight (g)	855 ± 210	847 ± 201	0.69	142
Birth size (cm)	34 ± 3	34 ± 3	0.98	142
Birth head circumference (cm)	24 ± 2	24 ± 2	0.88	142
Oligohydramnios (%)	21	24	0.63	138
IUGR (%)	46	42	0.68	142
Cesarean (%)	76	76	0.95	140
Antenatal corticosteroids (%)	78	74	0.61	139
First days				
Patients with umbilical venous catheter (%)	66	77	0.15	142
Patients with umbilical arterial catheter (%)	66	59	0.39	142
Therapies received during the neonatal period				
Ibuprofen (%)	16	14	0.76	142
Indomethacin (%)	36	50	0.11	142
Vasoactive drugs (%)	8	9	0.89	142
Postnatal corticosteroids (%)	26	33	0.41	142
Furosemide more than 5 consecutive days (%)	8	13	0.36	142
Spironolactone+HCT (%)	12	15	0.6	142
Vancomycin (%)	36	50	0.11	142
Amikacin after the first 48 h of life (%)	38	47	0.32	142
Neonatal morbidities				
Duration of oxygene therapy (h)	461 ± 562	527 ± 734	0.87	140
Duration of ventilation (h)	198 ± 295	218 ± 281	0.31	141
Leucomalacia (%)	2	2	0.95	142
Stage III or IV intraventricular hemorrhage (%)	0	4	0.14	142
Necrotizing enterocolitis (%)	2	4	0.47	142
Bacterial-proven infection (%)	22	21	0.85	142
Bronchopulmonary dysplasia (%)	40	52	0.17	142
Patent ductus arteriosus (%)	26	39	0.12	142
Maximal blood urea nitrogen (mmol/l)	11.6 ± 6.5	12.6 ± 6.5	0.2	141
Discharge				
Postnatal age at discharge (days)	79 ± 29	85 ± 32	0.4	140
Weight (g)	2334 ± 463	2411 ± 597	0.39	140
Size (cm)	43.7 ± 2.9	44.2 ± 3	0.42	138
Head circumference (cm)	32.7 ± 1.8	32.8 ± 4.1	0.24	133
Nutritional data				
Maximal neonatal weight loss (%)	15.1 ± 6.2	12.8 ± 6.7	0.039	142
Duration of initial parenteral nutrition (days)	22 ± 18	25 ± 19	0.89	139
Proportion of patients receiving secondary parenteral nutrition (%)	10	10	0.97	142
Insulin therapy (%)	44	45	0.95	142
Day 7				
Daily total protein intake (g/kg/day)	2.65 ± 0.62	2.64 ± 0.51	0.55	141
Daily total caloric intake (kcal/kg/day)	95.5 ± 25.7	93.7 ± 20.2	0.76	141
Weight (g)	776 ± 196	786 ± 152	0.29	141

HCT, hydrochlorothiazide; IUGR, intrauterine growth retardation.

ductus arteriosus (for example, GFR: 109 ± 9 vs 113 ± 16 ml/min per 1.73 m^2, P = NS) and between children with and without neonatal bacterial-proven sepsis (for example, GFR: 110 ± 13 vs 113 ± 15 ml/min per 1.73 m^2, P = NS). There were no differences for intermediate-term renal parameters between children who were and were not exposed to corticosteroids (antenatal and postnatal), non-steroidal anti-inflammatory drugs, diuretics, vancomycine, and aminoglycosides (during the neonatal period and after the first 48 h of life).

After adjustment for calorie intake at day 7, the presence of main comorbidities (that is, patent ductus arteriosus, bacterial-proven sepsis, and bronchopulmonary dysplasia) and therapies (that is, ibuprofen and early postnatal corticosteroids), the multiple linear regression analysis showed that GFR remained significantly lower both in IUGR and in EUGR children, in comparison with normotrophic children (−19.5 and −15.4 ml/min per 1.73 m^2, with absolute 95% confidence intervals of 8.5–30.5 and 2.4–28.3,

Table 2 | Renal evaluation in the total cohort and in IUGR, EUGR, and normotrophic children

Parameters	N	Normal values	Total cohort N=50	Normotrophic children, N=11	EUGR N=16	IUGR N=23	P
			Mean ± s.d.	Mean ± s.d.	Mean ± s.d.	Mean ± s.d.	
Age (years)	50		7.6 ± 1.3	6.8 ± 0.9	7.9 ± 1.3^	7.8 ± 1.3*	<0.05
Weight (kg)	50		22.9 ± 5.2	21 ± 5.1	24.7 ± 4.3	22.6 ± 5.7	NS
SDS for weight	50		−0.2 ± 2.2	−0.2 ± 2.9	0.35 ± 1.7	−0.6 ± 2.1	NS
Height (cm)	50		121 ± 8	117 ± 8	124 ± 6	121 ± 9	NS
SDS for height	50		−0.2 ± 1.2	−0.1 ± 1.4	0.2 ± 1.2	−0.5 ± 1.1	NS
Systolic BP (mm Hg)	50		100 ± 9	98 ± 9	102 ± 6	99 ± 11	NS
Percentile for systolic BP	50		46 ± 26	47 ± 27	49 ± 20	43 ± 31	NS
Diastolic BP (mm Hg)	50		58 ± 7	59 ± 8	60 ± 6	58 ± 8	NS
Percentile for diastolic BP	50		58 ± 24	59 ± 28	62 ± 18	54 ± 26	NS
Inulin clearance (ml/min/1.73 m^2)	44	117 ± 16	112 ± 14	125 ± 17	110 ± 13^	107 ± 9*	<0.05
Chloride RR (%)	46	98.47 ± 0.27	98.56 ± 0.55	98.56 ± 0.56	98.61 ± 0.66	98.52 ± 0.48	NS
Sodium RR (%)	46	99.13 ± 0.54	99.14 ± 0.37	99.0 ± 0.41	99.23 ± 0.41	99.14 ± 0.31	NS
Potassium RR (%)	46	83.61 ± 7.17	90.56 ± 4.51	93.18 ± 3.48	89.54 ± 4.60	90.04 ± 4.58	NS
Phosphate RR (%)	46	90.3 ± 5.1	91.86 ± 2.94	92.29 ± 3.82	92.99 ± 2.36	90.85 ± 2.61	NS
TmP/GFR (mmol/l)	46	1.05–1.72	1.42 ± 0.18	1.35 ± 0.13	1.48 ± 0.22	1.41 ± 0.17	NS
Uric acid clearance (ml/min/1.73 m^2)	47	12.4 ± 3.2	12.6 ± 4.4	14.4 ± 4.9	13.4 ± 5.0	11.0 ± 2.9	NS
Urine maximal osmolality (mOsm/kg)	47	>700	799 ± 219	901 ± 188	774 ± 256	765 ± 197	NS
Urine calcium:creatinine ratio (mmol/mmol)	47	0.04–0.7	0.33 ± 0.24	0.44 ± 0.36	0.31 ± 0.22	0.28 ± 0.17	NS
Urine albumin:creatinine ratio ratio (mg/mmol)	47	<2	1.58 ± 1.25	1.9 ± 1.6	1.6 ± 1.1	1.5 ± 1.2	NS
Urine magnesium:creatinine ratio (mmol/mmol)	47	0.3–0.9	0.59 ± 0.21	0.70 ± 0.22	0.53 ± 0.20	0.58 ± 0.19	NS
Serum cystatin C (mg/l)	48	0.5–1.3	0.74 ± 0.1	0.71 ± 0.08	0.74 ± 0.10	0.75 ± 0.11	NS
Serum uric acid (μmol/l)	48	120–400	226 ± 47	216 ± 49	214 ± 48	240 ± 44	NS
Right/left renal length (mm)	44		75 ± 8/77 ± 8	75 ± 7/76 ± 8	76 ± 5/79 ± 6	74 ± 10/78 ± 7	NS
SDS for right/left renal length	44		−1.6 ± 1/−1.4 ± 0.9	−1.2 ± 1.1/−1.3 ± 0.8	−1.7 ± 0.9/−1.4 ± 1	−1.7 ± 1/−1.4 ± 0.9	NS
Proportion of uropathies (%)	44		8	9	12	4	NS

ANOVA, analysis of variance; BP, blood pressure; EUGR, extrauterine growth retardation; IUGR, intrauterine growth retardation; RR, reabsorption rate; SDS, standard deviation score; TmP/GFR, maximal tubular phosphate reabsorption/glomerular filtration rate.
P, ANOVA or Kruskall Wallis tests when comparing normotrophic, EUGR, and IUGR children.
*$P<0.05$ normotrophic vs IUGR.
^$P<0.05$ normotrophic vs EUGR.
#$P<0.05$ IUGR vs EUGR.
Normal values according to Hatos' references[29] and local normal references.

and $P=0.001$ and 0.02, respectively). For microalbuminuria, the unique determinant that remained significant in a multivariable model was bronchopulmonary dysplasia ($P=0.007$).

DISCUSSION

This study population is based on only 50 children; however, the reference standard for GFR (inulin clearance) was performed in a prospective single-center cohort with neonatal exhaustive and accurate parameters. A selection bias regarding included and non-included children is unlikely as there is no significant difference in the neonatal period in terms of general characteristics, anthropometric items, morbidities, and drug exposure. However, there was a trend toward a higher prevalence of bronchopulmonary dysplasia and patent ductus arteriosus in the children who did not vs those who did have renal evaluation. These two entities could represent significant risk factors for adverse renal outcomes, mainly bronchopulmonary dysplasia. Although not statistically different, the possibility that the final cohort studied underestimates intermediate-term renal dysfunction in children born preterm should nevertheless be hypothesized, reinforcing the need of a renal follow-up of all children born very preterm.

In the cohort of the 50 included children, all had a true GFR (inulin clearance) above 90 ml/min per 1.73 m^2. However, as illustrated in Figure 1, seven children declined to participate between the first and the second visit, corresponding respectively to the 'inclusion' visit and the renal exploration, and did not want to undergo inulin clearance anymore: among them, three children had an impaired estimated GFR (eGFR) (two bilateral hypodysplasia, one chronic renal insufficiency following neonatal acute renal insufficiency; respective eGFR 59, 69, and 76 ml/min per 1.73 m^2); all three nevertheless required a specific medical follow-up. It would have been interesting to have age-matched controls to

Table 3 | Neonatal parameters according to EUGR, IUGR, or normotrophic status

Parameters	*N*	Normotrophic children, *N*=11 Mean ± s.d.	EUGR, *N*=16 Mean ± s.d.	IUGR, *N*=23 Mean ± s.d.	*P*-value
Birth weight (g)	50	1039 ± 278	845 ± 146^	773 ± 155*	<0.05
Gestational age (weeks)	50	27.1 ± 1.8	26.2 ± 1.8#	28.2 ± 1.8	<0.05
Proportion of boys (%)	50	46	63	35	NS
Children with venous UC (%)	50	46	63	78	NS
Children with arterial UC (%)	50	64	81	57	NS
Protein intake day 7 (g/kg/day)	50	2.9 ± 0.6	2.2 ± 0.5^,#	2.8 ± 0.5	<0.05
Calorie intake day 7 (kcal/kg/day)	50	111 ± 21	79 ± 19^,#	99 ± 26	<0.05
Parenteral water intake day 7 (ml/kg/day)	50	77 ± 48	125 ± 33^,#	100 ± 32	<0.05
Total water intake day 7 (ml/kg/day)	50	147 ± 13	150 ± 19	141 ± 21	NS
Protein intake day 14 (g/kg/day)	50	3.2 ± 0.8	2.9 ± 0.7	3.0 ± 0.5	NS
Calorie intake day 14 (kcal/kg/day)	50	109 ± 30	105 ± 27	117 ± 37	NS
Protein intake day 21 (g/kg/day)	50	3.1 ± 0.5	3.0 ± 0.7	3.0 ± 0.7	NS
Calorie intake day 21 (kcal/kg/day)	50	124 ± 24	111 ± 24	122 ± 29	NS
Age at discharge (days)	50	72 ± 33	88 ± 23	76 ± 31	NS
Weight at discharge (g)	50	2523 ± 636	2408 ± 379	2191 ± 391	NS
SDS for weight at discharge	50	−0.9 ± 0.6	−1.8 ± 1^,#	−2.5 ± 1*	<0.05
SDS for height at discharge	50	−1.2 ± 0.5	−2.7 ± 0.5^,#	−3.6 ± 0.7*	<0.05

ANOVA, analysis of variance; EUGR, extrauterine growth retardation; IUGR, intrauterine growth retardation; SDS, standard deviation score; UC, umbilical catheter.
P, ANOVA or Kruskall–Wallis tests when comparing normotrophic, EUGR, and IUGR children.
*$P<0.05$, normotrophic vs IUGR.
^$P<0.05$, normotrophic vs EUGR.
#$P<0.05$, IUGR vs EUGR.

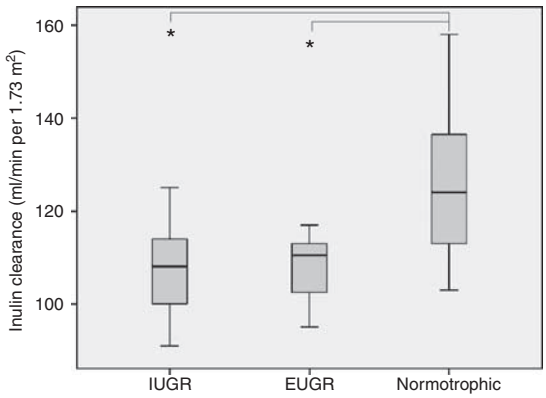

Figure 2 | Relationship between perinatal growth and intermediate-term glomerular filtration rate. IUGR: intrauterine growth retardation. EUGR: extrauterine growth retardation. *$P<0.05$.

compare inulin clearance in children born preterm and full term. However, it was ethically not possible to propose inulin clearance to healthy pediatric controls.

The mean diastolic BP is significantly higher in comparison with expected values. The clinical relevance of such a difference can be discussed (for a height of 120 cm in male individuals, the difference between the 50th percentile and the 58th percentile of diastolic BP is only 4 mm Hg). However, this may be amplified along with age and may lead to a further impact during adulthood. Moreover, in adults, data from observational studies and randomized controlled trials have suggested that lowering population-wide diastolic BP by only 2 mm Hg could result in a 17% decrease in the prevalence of arterial hypertension, as well as a 6% decrease in the risk of coronary heart disease, and a 15% reduction in the risk of stroke and transient ischemic attacks.[22]

The combination of a diastolic BP within the upper normal range and kidney size within the lower normal range after adjustment on height indicates the presence of subtle abnormalities, possibly secondary to a reduction in nephron number. The absence of change in GFR cannot preclude reduced nephron number in any or all of the groups, as it is well known both in animal models and in humans that a nephron deficit of moderate degree often yields normal GFR during childhood. Overall, our results are consistent with previous studies showing a discrete but early renal impairment in children born preterm. However, they cannot provide direct information on the true nephron number in these children, as there were no available data on renal shape and there were no direct glomerular counts. Moreover, all these children were born very preterm and mostly likely all had a lower number of nephrons than did full-term children. As their GFRs were all in the normal range, relative to a likely low number of nephrons, all these children may have a high single-nephron GFR, thus putting all of them at risk for long-term renal impairment, according to Brenner's hyperfiltration theory.[11]

The high proportion of children with IUGR (46%) is similar as that observed in other studies; in the literature, 30–50% of extremely preterm neonates are IUGR.[23]. A comparable alteration of GFR in both EUGR and IUGR patients is indeed remarkable. Nevertheless, the pathophysiology of such intermediate-term renal impairment is

unclear. In IUGR children, the reduction in nephron number is at least partially explained by *in utero* mechanisms involving vascular changes. It would have been interesting to individualize IUGR children with early catch-up growth before discharge from the neonatal intensive care unit from others (that is, to study four different groups: normotrophic children, EUGR children, IUGR with early neonatal catch-up growth, and IUGR with additional EUGR); however, the population was too small to perform such an analysis.

Having only 11 children in the control group of very preterm children with normal BW for GA and with normal postnatal growth represents a limitation for this study. Results are nevertheless statistically significant, with a comparable decrease of GFR in IUGR and EUGR children, however, within the normal range. This slight decrease of GFR could correspond to a moderate reduced nephron number, clinically asymptomatic during childhood. This moderate decrease could nevertheless be of importance during adulthood as GFR decreases every year by 1 ml/min from the age of 40 years. Moreover, even if a reduced nephron number is present in these children, it could reflect either impaired primary nephrogenesis or secondary loss of nephrons due to toxic or hemodynamic injuries during the neonatal period. Another hypothesis could be a relative greater degree of hyperfiltration in normotrophic children; however, mild hyperfiltration was found in two normotrophic children (GFR 143 and 158 ml/min per 1.73 m^2) and in one IUGR child (GFR 147 ml/min per 1.73 m^2). Furthermore, this hypothesis seems unlikely as IUGR is a well-known risk factor of long-term renal impairment.

EUGR and IUGR groups at the time of the study were older (by a full year on average) than the normotrophic group. However, inulin clearance adjusted for average adult body surface area is stable over the age range studied.[24] Given the age differences between the three groups, body weight and height were expressed in SDS. For the same reason, BP and renal size were adjusted on statural height.

In this study, EUGR children had a lower GA and a lower BW than babies with normal pre- and postnatal growth. Even if total water intake was not different on day 7, parenteral water intake in EUGR children was significantly higher, suggesting that these children were more severely ill during the first week of life. Moreover, they also received postnatal corticosteroids more often and suffered more often from bronchopulmonary dysplasia, as previously reported.[16] An early deficiency of protein-calorie intake (during the first 7 days of life) may have a role in the pathophysiology of both EUGR and intermediate-term renal impairment of EUGR and could explain the reduced nephron number in these patients. This early proteinocaloric deficiency would not influence long-term growth and catch-up in these children. Furthermore, it could be an early neonatal explanation for the reduced nephron number as Rodriguez *et al.*[10] showed on histopathological studies that glomerulogenesis occurred only in the first 40 days after birth in very preterm children, suggesting the importance of early postnatal factors in the late nephrogenesis of preterm children.

Moreover, these results of a long-term renal impairment in case of neonatal growth retardation are consistent with the hypothesis of Yeung[9] who speculated in 2006 that, in very preterm newborns with prolonged early postnatal growth deceleration as a consequence of undernutrition, oligonephropathy could occur due to a protein-calorie deficiency. Most neonatal intensivists have recently increased protein-calorie intake during the first days of life; thus, in the future, the comparison of this cohort with more recent cohorts will confirm the true impact of protein-calorie intake on renal development in children born preterm.

In univariate and multivariate analysis, despite the population size, bronchopulmonary dysplasia seems to be associated with intermediate-term subtle renal abnormalities, partly due to EUGR. No drug seems to be associated with renal impairment during childhood, whereas Rodriguez-Soriano *et al.*[2] discussed the role of early exposure to aminoglycosides in the genesis of intermediate-term hypercalciuria. In our study, all but one child received aminoglycosides during the first 48 h of life (due to local protocol antibiotic therapy at birth in premature children between 1998 and 2001); there were no differences for intermediate-term renal parameters between children who were and were not exposed to aminoglycosides during the neonatal period and after the first 48 h of life.

In conclusion, although GFR is normal during childhood in children born preterm, borderline BP and reduced kidney size can be regarded as markers of the reduced nephron number. This hypothesis is reinforced by a lower GFR in both IUGR and EUGR children. In addition to IUGR, EUGR may represent a new risk factor for long-term renal impairment in children born preterm. The pathophysiology of such an impairment may be attributed to an early relative deficit in protein-calorie intake. As the reduction in nephron number may have long-term consequences, three conclusions can be drawn. First, pediatricians may perform an early prevention focused on cardiovascular risks, nutrition (mainly protein and salt intakes), and obesity. Nephrotoxic drugs may be avoided, particularly non-steroidal anti-inflammatory therapies. A follow-up of renal parameters (BP yearly, serum creatinine and microalbuminuria every 5 years) may be performed in these children who should be referred when abnormalities have been highlighted. Second, adult nephrologists may keep in mind that children born very preterm in the 1980s have become young adults; thus, in case of chronic kidney disease without any evident etiology, BW and GA should be recorded. Finally, large prospective studies are required: even if comparisons of different early neonatal protein-calorie intakes will be difficult to drive, randomized controlled trials aiming at evaluating the role of early nephroprotection may be performed.

MATERIALS AND METHODS

A single-center cohort of 143 premature babies born between 1998 and 2001 (<1000 g and/or <30 weeks GA) was prospectively included at birth in a database of nutritional parameters. All

Prenatal data	General parameters	Antenatal corticosteroids method of delivery Presence of oligohydramnios or polyhydramnios
Neonatal data	General parameters	Anthropometric parameters (body weight, height, head circumference from birth to discharge, once a week) Maximal neonatal weight loss
	Nutritional parameters	Oral and intravenous intakes of protein, lipid, carbohydrate, energy, sodium, calcium, and phosphate Daily collection during 3 weeks, followed by weekly collection during 4–11 weeks Duration of initial parenteral nutrition and insulin therapy Beginning of oral nutrition and weaning
	Biological parameters	Maximal blood urea nitrogen Hemoglobin level at birth and discharge Plasma bicarbonate during the second week of life Plasma protein concentration at discharge Calcium and phosphate serum levels at 14 days of age
	Drug exposure	Vasoactive drugs Ibuprofen/indomethacin Corticosteroids Diuretics (furosemide, combination of spironolactone and hydrochlorothiazide) Antibiotics (vancomycin, amikacin, and penicillin) Maximal caffeine plasma level Insulin requirement
	Morbidities	Bronchopulmonary dysplasia Patent ductus arteriosus Sepsis Neurological damage (periventricular leucomalacia, stage III/IV intraventricular hemorrhage) Necrotizing enterocolitis
	Others	Apgar score Use and duration of umbilical arterial and venous catheters Duration of oxygen therapy, mechanical ventilation, and continuous positive airway pressure
Renal exploration	Clinical data	Weight, height, body mass index, and blood pressure
	Biological data	Inulin clearance, cystatin C Tubular tests, β2-microglobulinuria Microalbuminuria
	Ultrasounds	Renal ultrasounds: kidney size

Figure 3 | Parameters assessed in children.

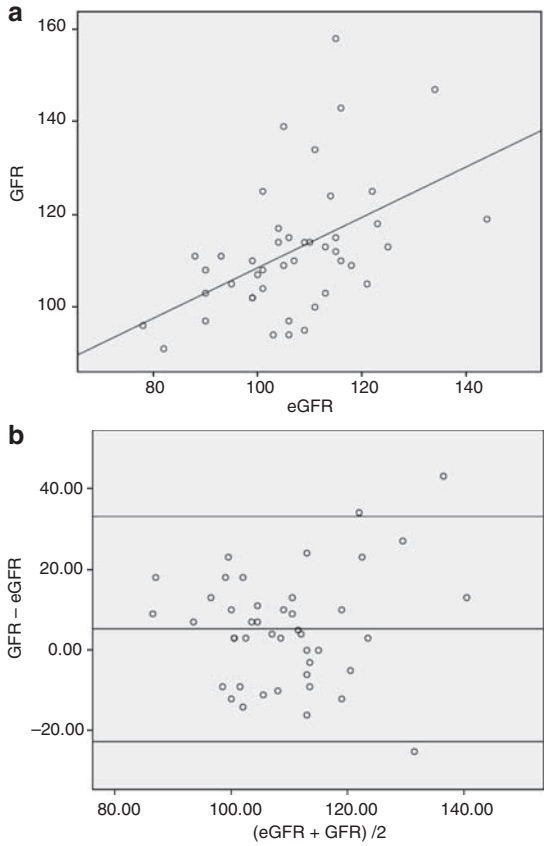

Figure 4 | Relation between glomerular filtration rate measured with inulin clearance (GFR) and that calculated with locally adapted Schwartz formula (eGFR). (**a**) Spearman correlation ($r = 0.526$, $P < 0.001$). (**b**) Bland and Altman plot, results for (GFR−eGFR): 5.3 ± 13.9 ml/min per 1.73 m^2 (mean ± s.d.). eGFR, estimated GFR; GFR, glomerular filtration rate.

recorded parameters are summarized in Figure 3. SDS for neonatal anthropometric parameters were assessed according to Usher and McLean charts.[25] Severe IUGR was defined as a BW $\leqslant -2$ SDS for GA and severe EUGR as a normal BW for GA and a weight or height at discharge $\leqslant -2$ SDS for the corrected GA. Normotrophic children corresponded to premature children from this cohort with a BW above the limit of −2 s.d. for GA and a birth and a height at discharge from the neonatal intensive care unit above the limit of −2 s.d. for their corrected GA. The 'corrected GA' was defined as the 'age the child would be if the pregnancy had actually gone to term.'

A mail was sent in 2007 to all parents to propose an evaluation of kidney function, including BP assessment, renal ultrasonography, tubular tests, and GFR measurement using inulin clearance. When parents agreed to enroll their child, a first visit was proposed to explain the study. A second visit was then scheduled for renal evaluation. The study was approved by an independent ethical committee (Comité de Protection des Personnes Lyon Sud Est II) and informed consent was obtained after written information had been given.

Anthropometric parameters during childhood were evaluated according to national French growth charts. BP was measured three consecutive times with an automatic device (Dynamap, GE Healthcare Dinamap, Procare Monitor, Velizy, France). The lower diastolic BP and the lower systolic BP were recorded. Arterial hypertension was defined as a BP above the 97.5th percentile. Percentiles for BP were determined according to statural height and gender, and SDS for renal length were determined according to statural height.[26,27] Serum creatinine was measured with a colorimetric compensated method (kinetic Jaffe, Modular, Roche Diagnosis, Meylan, France). Tubular assessment included phosphate and uric acid clearance, electrolyte reabsorption rate (chloride, sodium, potassium, and phosphate), urine magnesium-to-creatinine

ratio, and urine calcium-to-creatinine ratio. Microalbuminuria (nephelemetry, BM2; Behring Siemens, Paris, France) and β2-microglobulin (nephelemetry, BM2; Behring Siemens) concentration were obtained. Plasma cystatin C (nephelemetry, BM2; Behring Siemens) and plasma osmolality were also recorded. The true GFR was measured by the clearance of inulin (polyfructosan infusion, Inutest; Fresenius Kagi, Graz, Austria). A standard technique was used by a trained staff, with a continuous infusion after a priming dose of polyfructosan, 30 mg/kg. Water diuresis was induced by oral administration of 5 ml/kg of water followed by 3 ml/kg every 30 min combined with an intravenous infusion of 0.9% sodium chloride. This enabled the patients to spontaneously empty their bladder every 30 min. Three to four urine samples were collected and a blood sample was drawn mid-way through each collection period. Clearance values were obtained from the mean values of three to four clearance periods. Measurements of plasma and urine polyfructosan were performed using an enzymatic method. In children with technical problems with inulin clearance (failure of venous puncture or timed collection of urine), eGFR was obtained from a locally adapted Schwartz formula (eGFR = $33 \times$ height (cm)/plasma creatinine (μmol/l)).[28] Figure 4 illustrates the relationship between inulin clearance and eGFR in the cohort (Spearman correlation, $r = 0.526$, $P < 0.001$; Bland and Altman plot). Normal ranges were obtained from local normal values and Matos' values for tubular parameters.[29]

Data are presented as mean ± s.d. for continuous variables and as percentage for categorical variables. Univariate comparisons of continuous variables were performed using an unpaired *t*-test or the non-parametric Wilcoxon rank-sum test when covariates were not normally distributed. Multiple comparisons were performed using analysis of variance test or Kruskall–Wallis test. Dichotomized variables were compared using the Pearson χ^2-test. The Pearson correlation test was used when data were normally distributed and the Spearman correlation test was used when they were not. A multiple linear regression was performed to estimate the relationship between baseline parameters and renal outcomes (inulin clearance and microalbuminuria). To evaluate the impact of IUGR and EUGR on GFR, a multiple linear regression analysis was performed after adjustment for main neonatal morbidities and received therapies. All statistical tests were performed at the two-sided 0.05 level of significance. Statistical analysis was performed using the SPSS software 15.0 for Windows.

DISCLOSURE

All the authors declared no competing interests.

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