Urinary saturation and nephrocalcinosis in preterm infants: effect of parenteral nutrition

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Abstract

Urinary lithogenic and inhibitory factors were studied in 27 preterm infants; 16 had total parenteral nutrition (TPN) and 11 had breastmilk with an additional glucose-sodium chloride infusion. Urines were collected for 24 hours on day 2 (period A), day 3 (B), and once between days 4 and 10 (C). Urinary calcium oxalate saturation was calculated by the computer program EQUIL 2. Renal ultrasonography was performed every second week until discharge.

The calcium/creatinine ratio increased in infants on TPN (A 0.91; C 1.68 mol/mol) and was significantly higher at period C than that in infants on breastmilk/infusion (A 0.52; C 0.36). The oxalate/creatinine ratio was persistently higher with TPN (203 mmol/mol) than with breastmilk/infusion (98; 137). The citrate/creatinine remained constant with TPN (0.44 mol/mol), whereas it increased significantly with breastmilk/infusion (0.26; 0.49). Calcium/citrate rose considerably with TPN, but decreased with breastmilk/infusion to a significantly lower level than with TPN. The urinary calcium oxalate saturation increased with TPN (2.4; 4.5) and decreased with breastmilk/infusion (2.1; 1.5) to a significantly lower value than with TPN. Nephrocalcinosis developed in two infants on TPN. Mean daily calcium intake was similar in both groups, whereas protein, sodium, and phosphorus intake were significantly higher on TPN.

It is concluded that the increase in urinary calcium oxalate saturation observed with TPN is due to the combined effect of an increased urinary calcium excretion and higher urinary oxalate/creatinine and calcium/citrate ratios. The changes observed are likely to be caused by TPN itself, which differs in several respects from breastmilk feeding.

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calcinosis by raising urinary calcium excretion.⁷ However, a correlation was noted only between the duration of ventilation or oxygen requirement and the development of nephrocalcinosis by Short and Cooke.⁸ Finally Campfield and Braden found a rising urinary oxalate excretion in very low birthweight (VLBW) infants receiving total parenteral nutrition (TPN) and hence suggested that the risk of calcium oxalate precipitation and nephrocalcinosis was increased.^{9 10}

In order to understand better the underlying mechanisms and the effect, if any, of TPN on the risk of renal calcification, we studied urinary lithogenic and inhibitory substances (for example citrate) and the urinary calcium oxalate saturation in preterm infants receiving either TPN or breastmilk with an additional glucose-sodium chloride infusion. In addition, renal ultrasound examinations were performed repeatedly.

Patients and methods

Preterm infants <35 weeks' gestation were studied. We excluded infants who were small for date and those with renal or intestinal disease, complex malformations, or cardiac anomalies. Thirty five infants entered the study; eight patients (six on TPN and two on breastmilk/infusion) were excluded because of an incomplete urine collection. Of the remaining 27 infants 16 had TPN and 11 received breastmilk/infusion. The decision about the form of nutrition was based on clinical grounds as randomisation was not acceptable for ethical reasons. Accordingly, infants on TPN were more critically ill and had a lower mean birth weight (table 1). For infants on TPN, Vamin glucose 7% containing 50 mmol/l sodium chloride, 20 mmol/l potassium, 2.5 mmol/l calcium, and 1.5 mmol/l magnesium and Intralipid 20% (Kabi Pharmacia, Stockholm, Sweden) were used with added vitamins and trace elements. TPN was started on the second day of life with one third of the final amount. The complete intake consisted of 35 ml amino acid solution, 10 ml fat emulsion, 60 ml glucose 10%, and 20 ml glucose 50% (expressed per kg and day) and was usually reached by the seventh day. Infants receiving breastmilk had an additional glucose-sodium-chloride infusion. No infant received dexamethasone or high doses (>1.5 mg/kg/day) of frusemide. The acid-base status was determined daily and was corrected as needed to keep the base excess to ± 3 mmol/l.

Urines were collected in plastic bags for 24 hours on day 2 (period A), day 3 (B), and once

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Nephrocalcinosis is a well known complication in critically ill preterm infants^{1–6} and its aetiology is still a matter of debate. Hypercalciuria induced by frusemide administration was at first considered to be the main cause of nephrocalcinosis.^{1–3} Indeed 20 to 64% of all premature infants receiving frusemide develop nephrocalcinosis.^{4–6} Long term dexamethasone treatment in bronchopulmonary dysplasia has also been incriminated to cause renal

Table 1 Clinical data of infants studied; values are mean (SD)

	TPN (n=16)		Breastmilk/infusion (n=11)	
No of infants ventilated	14		10	
Birth weight (g)	1526 (806)		2085 (523)	
Gestational age (weeks)	30.7 (3.4)		33.1 (2.1)	
Vitamin D ₃ (IU/day)	350 intravenous		700 by mouth	
	Period A	Period C	Period A	Period C
Frusemide dose (mg/kg/day)	0.44 (0.49)	0.55 (0.49)	0.43 (0.62)	0.40 (0.40)
Ascorbic acid (mg/day)	16.5 (10.8)	16.5 (10.8)	2.8 (1.2)	10.6 (5.6)
Total urine volume (ml/24 hours)	97 (54)	105 (55)	121 (74)	104 (55)

*p<0.05 (breastmilk/infusion v TPN); **p<0.0001 (period A: breastmilk/infusion v TPN).

Table 2 Urinary calcium: total excretion, concentration, and Ca/Cr ratio; values are mean (SEM)

Study period (days of life)	Calcium excretion (mmol/kg/24 hours)		Calcium concentration (mmol/l)		Ca/Cr (mol/mol)	
	TPN	Breastmilk/infusion	TPN	Breastmilk/infusion	TPN	Breastmilk/infusion
A (2)	0.057 (0.019)	0.045 (0.022)	0.88 (0.16)	0.59 (0.16)	0.91 (0.21)	0.52 (0.20)
B (3)	0.087 (0.024)	0.038 (0.012)	*1.37 (0.21)	0.64 (0.22)	1.23 (0.25)	0.70 (0.21)
C (4-10)	*0.117 (0.022) **	0.020 (0.006)	*1.49 (0.25) **	0.44 (0.15)	*1.68 (0.28) ***	0.36 (0.11)

*p<0.05 (TPN: B and/or C v A; **p<0.005 (breastmilk/infusion v TPN); ***p<0.001 (breastmilk/infusion v TPN).

Table 3 Urinary oxalate excretion, Ox/Cr ratio and calcium oxalate saturation; values are mean (SEM)

Study period (days of life)	Oxalate excretion (μmol/kg/24 hours)		Ox/Cr (mmol/mol)		Calcium oxalate saturation	
	TPN	Breastmilk/infusion	TPN	Breastmilk/infusion	TPN	Breastmilk/infusion
A (2)	10.0 (2.0)	7.5 (1.1)	202 (41)	98 (5)	2.41 (0.48)	2.12 (0.60)
B (3)	11.8 (1.6)	7.6 (0.96)	199 (32)	119 (15)	*4.05 (0.88)	1.68 (0.36)
C (4-10)	11.4 (1.8)	8.4 (1.6)	203 (25)	137 (22)	*4.47 (0.89) †	1.52 (0.36)

*p<0.05 (TPN: B and C v A); †p<0.05 (breastmilk/infusion v TPN).

between days 4 and 10 (C) after full nutrition was reached, which was the case on day 7 (± 1.8) in infants on TPN and day 6 (± 1.3) in those on breastmilk/infusion. The bags were emptied at two hour intervals into bottles containing 6 M hydrochloric acid to acidify the urine to pH <2. Urines were frozen at the end of the collection and analysed within one month. Separation and quantification of oxalate was performed using a Dionex Series 4000i gradient ion chromatography system (Dionex Co, Sunnyvale, CA, USA). The background conductivity was minimised with 25 mM sulphuric acid regenerant.¹¹ Citrate was determined using the citrate lyase method.¹² The following analyses were done on a Technicon RA-1000 Analyzer (Technicon Instrumental Corporation, Tarrytown, NY, USA): creatinine by a standard kinetic Jaffé procedure, sodium and potassium by ion selective electrodes, calcium with a colorimetric cresolphthalein-complexon method, magnesium with a xylydyl blue reaction, and inorganic phosphorus using an ultraviolet molybdate complex method. The pH of the urine was determined by indicator sticks (Boehringer Mannheim, Germany) in freshly voided morning specimens after 24 hour pH profiles had shown small fluctuations only (± 0.5). From these parameters and from the urinary volume per 24 hours the urinary calcium oxalate saturation was calculated by the computer program EQUIL 2 according to Werness *et al.*¹³ Renal ultrasonography was performed every second week until discharge using a real time sector scanner with a 7.5 or 5 MHz transducer (Sonolayer, V-SSA 100 A, Toshiba Medical Services, Zurich, Switzerland).

Data were expressed as mean (SEM). Significance was calculated using the Wilcoxon signed rank test or the Wilcoxon rank sum test, respectively; p values less than 0.05 were considered significant.

The study was approved by the ethical committee of the children's hospital.

Results

The relevant clinical data of the 27 preterm infants are summarised in table 1. No significant differences between the two groups were observed except for the mean birth weight, which was significantly ($p<0.05$) lower in the infants on TPN. Relative weight loss was similar in both groups reaching -4.5% (average) at period C.

The urinary calcium/creatinine ratio (Ca/Cr, mol/mol) increased significantly ($p<0.05$) in those on TPN from 0.91 in period A to 1.68 in period C (table 2; figure). The total urinary calcium concentration and excretion increased similarly. In contrast calcium excretion did not rise in infants on breastmilk/infusion. As a result their Ca/Cr ratio in period C was significantly ($p<0.001$) lower (0.36) than in those on TPN (figure), as were the urinary calcium concentration and excretion ($p<0.005$; table 2).

The oxalate/creatinine (Ox/Cr, mmol/mol) ratio in infants receiving TPN remained constant but was consistently higher (A 202; C203) than that in those on breastmilk/infusion (98; 137, table 3 and figure). This difference was significant ($p<0.05$) at period B only. Urinary oxalate excretion ($\mu\text{mol/kg/24 hours}$) increased slightly in both groups, being persistently higher in TPN than in breastmilk/infusion.

Mean (SEM) Ca/Cr, Ox/Cr, and Ca/Cit ratios and calcium oxalate saturation in 24 hour urines of preterm infants receiving either TPN or breastmilk/infusion at periods A, B, and C; *significant difference between TPN and breastmilk/infusion.

The mean urinary citrate (Cit) excretion (table 4) increased in both groups to a comparable level at period C (0.035 mmol/kg/day in TPN, 0.031 in breastmilk/infusion). In those on breastmilk/infusion the Cit/Cr ratio was initially (A) considerably lower, but increased significantly ($p < 0.01$) to a slightly higher level (C) than in those on TPN. An important difference was observed in the Ca/Cit ratio, which increased twofold in infants on TPN, yet steadily decreased in those on breastmilk/infusion to a significantly ($p < 0.005$) lower value at period C (table 4; figure), than in TPN.

The urinary calcium oxalate saturation (table 3, figure) was similar in both groups at period A (2.41 in TPN; 2.12 in breastmilk/

infusion). A significant ($p < 0.05$) increase was then observed in TPN to 4.47 in period C. In contrast a steady decrease was noted in infants on breastmilk/infusion (table 3), the calcium oxalate saturation being significantly ($p < 0.05$) lower than in TPN at period C (1.52).

Slight medullary nephrocalcinosis was diagnosed ultrasonographically on days 31 and 18, respectively, in two infants on TPN. Nephrocalcinosis remained unchanged at the time of transfer to other hospitals on days 45 and 60. Ionised serum calcium was comparable at period A (0.99 mmol/l in TPN; 0.98 mmol/l in breastmilk/infusion), but slightly higher at period C in TPN (1.12 in TPN v 1.02 in breastmilk/infusion). Serum sodium

Table 4 Urinary citrate excretion, Cit/Cr and Ca/Cit ratios; values are mean (SEM)

Study period (days of life)	Citrate excretion (mmol/kg/24 hours)		Cit/Cr (mol/mol)		Ca/Cit (mol/mol)	
	TPN	Breastmilk/infusion	TPN	Breastmilk/infusion	TPN	Breastmilk/infusion
A (2)	0.024 (0.004)	0.020 (0.003)	0.45 (0.06)	0.26 (0.05)	3.20 (0.74)	2.98 (1.11)
B (3)	0.024 (0.003)	0.025 (0.003)	0.42 (0.06)	0.40* (0.07)	3.44 (0.67)	2.33 (0.96)
C (4-10)	0.035 (0.011)	0.031 (0.006)	0.44 (0.05)	0.49* (0.09)	6.16 (2.25)	** 1.14 (0.50)

* $p < 0.05$ (breastmilk/infusion: B and C v A); ** $p < 0.005$ (breastmilk/infusion v TPN).

Table 5 Dietary intake: preterm infants receiving TPN or breastmilk/infusion; values are mean (SEM)

Study period (days of life)	Protein (g/kg/24 hours)		Sodium (mmol/kg/24 hours)		Phosphorus (mmol/kg/24 hours)		Calcium (mmol/kg/24 hours)	
	TPN	Breastmilk/infusion	TPN	Breastmilk/infusion	TPN	Breastmilk/infusion	TPN	Breastmilk/infusion
A (2)	1.33 (0.17)	** 0.32 (0.04)	0.94 (0.12)	* 0.61 (0.05)	0.85 (0.06)	** 0.14 (0.02)	0.72 (0.11)	* 0.43 (0.39)
B (3)	1.81 (0.20)	** 0.65 (0.09)	1.28 (0.14)	* 0.71 (0.05)	0.78 (0.06)	** 0.29 (0.05)	0.84 (0.06)	0.63 (0.11)
C (4-10)	2.48 (0.17)	** 1.33 (0.22)	1.74 (0.11)	** 0.96 (0.09)	0.83 (0.06)	* 0.55 (0.09)	0.92 (0.07)	1.00 (0.16)

* $p < 0.05$ (breastmilk/infusion v TPN); ** $p < 0.001$ (breastmilk/infusion v TPN).

and creatinine concentrations did not differ between the two study groups.

The amount of protein, sodium, and phosphorus administered per day (per kg body weight) increased in both groups, but remained significantly ($p < 0.05$) lower for those on breastmilk/infusion during the whole study period (table 5). In contrast, calcium intake was similar in both groups at periods B and C. The ascorbic acid dosage given (per kg and day) was lower in those on breastmilk/infusion, particularly at period A ($p < 0.0001$; table 1).

Discussion

A steady increase of the urinary calcium oxalate saturation in infants receiving TPN was the main finding. It is the result of several factors, that is a rising urinary calcium excretion, an Ox/Cr ratio remaining constantly higher than that in infants on breastmilk/infusion, and an increasing Ca/Cit ratio. In contrast, the calcium oxalate saturation in infants on breastmilk/infusion decreased further because of lower ratios for Ca/Cr, Ox/Cr, and Ca/Cit. These results clearly show that the infants on TPN were at increased risk of calcium oxalate precipitation. This is further substantiated by the finding of medullary nephrocalcinosis in two infants on TPN.

Could this difference be due to the lower mean birth weight of the infants on TPN as compared with those on breastmilk/infusion? This appears unlikely as the excretion rates, if expressed per kg body weight, showed the same tendency. Furthermore, the initial Ca/Cr, Ox/Cr, and Ca/Cit ratios, as well as the urinary calcium oxalate saturation were similar in both groups at period A. However, an effect of different renal maturation between the two groups is not formally excluded.¹⁴

It is noteworthy that both groups had the same frusemide dosage and a comparable daily calcium intake, yet only infants on TPN showed an increase of the urinary calcium excretion and of the Ca/Cr ratio. Thus we cannot incriminate frusemide for the rising urinary calcium, as was done in other studies where higher dosages of frusemide were administered.¹⁻⁶

Contrary to our expectations and to the results of Campfield and Braden,^{9 10} no significant increase of the urinary oxalate excretion was found. However, the Ox/Cr ratio in those on TPN was persistently higher than that in those on breastmilk/infusion. Campfield and Braden observed a rise of the oxalate excretion and of the Ox/Cr ratio in VLBW infants on TPN after the protein intake was increased from 0.5 to 1.5 g/kg/day.⁹ These authors used a standard amino acid solution (Travasol, Travenol Laboratories) and a paediatric amino acid solution (Trophamine, Kendall McGraw Lab). The difference between their data and our results is thus not explained by the protein content of TPN *per se*, as the mean protein supply in our study was consistently higher. However, it might be related to differences in the amino acid composition in TPN or to another dosage of supplements. The amount of

ascorbic acid (a precursor of oxalate) administered by Campfield and Braden⁹ was considerably higher (35 mg/day) than in our study (16.5 mg/day). The studies are not entirely comparable for other reasons, for example urine specimens were collected during eight hour periods and oxalate was analysed by oxalate oxidase.⁹ Nevertheless, the role of the urinary oxalate excretion cannot easily be dismissed. Oxalate is 10–15 times more potent than an equimolar amount of calcium in increasing the risk of calcium oxalate precipitation.¹⁵ Hence, the higher Ox/Cr ratio found in TPN also contributed to the increase in urinary calcium oxalate saturation.

Hardly any data exist about urinary citrate excretion in preterm infants.^{16 17} Citrate is a potent inhibitor of calcium oxalate aggregation.^{18 19} We found a similar increase of the citrate excretion in both groups. However, the Cit/Cr ratio increased only in the infants receiving breastmilk/infusion and remained stable in those on TPN. Due to the rise of the urinary calcium excretion, there was a twofold increase of the Ca/Cit ratio in TPN as opposed to a decrease in those on breastmilk/infusion. The higher the Ca/Cit ratio, the greater is the risk of nephrocalcinosis and nephrolithiasis.²⁰ These changes, together with the higher urinary oxalate excretion, contributed primarily to the increasing calcium oxalate saturation in infants on TPN. These infants clearly are at risk for renal calcifications. As a corollary, two of the infants on TPN, but none of those on breastmilk/infusion, developed nephrocalcinosis as demonstrated by ultrasonography.

Could these results be explained by differences in nutrition? Indeed the mean daily intake (per kg of body weight) of protein, sodium, and phosphorus was significantly higher in those TPN than in those on breastmilk/infusion. The higher protein content and a different amino acid composition of the parenteral solution used as compared with breastmilk may have resulted in a higher urinary oxalate excretion.⁹ The infants on TPN also had a higher dosage of ascorbic acid, a (potential) precursor of oxalate.¹⁵ Furthermore, the higher sodium intake may have increased calcium excretion as renal sodium and calcium handling are closely linked.²¹ Additional studies in preterm infants are needed to evaluate the influence of nutritional factors on urinary lithogenic and inhibitory parameters and on the risk of nephrocalcinosis.

In conclusion, we observed a considerably higher risk of calcium oxalate precipitation and of nephrocalcinosis in preterm infants on TPN than in those on breastmilk/infusion. The higher calcium oxalate saturation was due to increased calcium excretion, higher Ca/Cit ratios, and persistently higher Ox/Cr ratios. Both groups studied had similar (low) doses of frusemide, but the infants on TPN had a considerably higher daily intake of protein, sodium, phosphorus, and ascorbic acid.

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