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ORIGINAL ARTICLE

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Increased kidney growth in formula-fed versus breast-fed healthy infants

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Abstract A high protein intake results in increased kidney growth and glomerular filtration rate in human adults and young rats. It is unknown whether kidney size in young infants is influenced by increased protein intake in formula-fed compared with breast-fed infants. We investigated the effect of formula versus breast feeding on kidney growth in a cohort of 631 healthy children examined at birth, and at 3 and 18 months of age. Kidney size was determined by ultrasonography and related to gender, age, body size, and feeding category (fully breast fed, partially breast fed, or fully formula fed at 3 months). Serum urea nitrogen, serum creatinine, and estimated creatinine clearance were measured at 3 months of age. Kidney growth and serum urea nitrogen were significantly increased in partially or fully formula-fed 3-month-old infants. This effect was more pronounced in boys than in girls. The changes in relative kidney size were temporary, as they did not persist at 18 months of age, when all children received a normal mixed diet. The immediate renal effects of formula feeding should be taken into consideration for recommendations concerning infant feeding. Whether there are any long-term effects of early increased protein intake on later kidney function remains to be seen.

Keywords Protein · Breast milk · Formula · Kidney growth · Infant · Gender

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Introduction

A high protein intake after weaning in young rats produces increased kidney growth and glomerular filtration rate (GFR) exceeding the concomitant increase in body weight [1]. These changes are reversible on discontinuation of a high-protein diet [1].

Infants who are exclusively formula fed have a significantly higher daily protein intake per kilogram body weight compared with breast-fed infants [2, 3]. This results in an elevated serum urea nitrogen level [2, 3, 4, 5, 6, 7, 8, 9] and an overall increase in the serum amino acid concentration [3, 8, 10]. In human adults a high-protein intake is associated with increased kidney size [11, 12]. The consequences of increased protein intake for kidney growth in healthy infants, however, are as yet unknown.

We therefore investigated the effect of breast feeding versus standard formula feeding on kidney growth, serum urea nitrogen, serum creatinine, and estimated creatinine clearance (C_{Cr}) in a cohort of 631 healthy children followed from birth to 18 months of age.

Materials and methods

Design

A prospective, longitudinal, observational cohort study was performed from 1997 to 2002 at Rigshospitalet and Hvidovre Hospital, University of Copenhagen. All pregnant women of Danish origin, geographically belonging to the hospital referral area, were consecutively asked to join the study in their first trimester. The children were part of an ongoing cohort study establishing reference materials for kidney size and malformations, genital development and malformations, and general growth [13, 14, 15, 16].

The children were examined within the first 5 days of life (0 months) and again at 3 and 18 months of age. At all examinations bilateral kidney ultrasonography and anthropometrical measurements were performed. At 3 months of age information on infant diet was collected and a venous blood sample was taken. At each visit the child was examined by one of a team of eight doctors. All methods of measurement were standardized at workshops.

Of 1,270 eligible children, 631 children were included; 81 dropped out prior to birth (loss of interest, moving, miscarriage, or fetal death). In total of 548 were excluded according to the inclusion criteria and 10 due to technical problems with ultrasound scanning of one or both kidneys at more than one examination.

Inclusion and exclusion criteria

In the present study exclusively healthy, mature (gestational age 37–42 weeks, 259–294 days) singletons with a birth weight appropriate for gestational age were included. If any renal abnormality was found at any time during the study, the child was excluded. Additionally, children with other congenital diseases, severe perinatal asphyxia, severe infections, or missing data at the 3-month examination were excluded. The cohort has previously been described in more detail elsewhere [13].

Gestational age and anthropometry

Gestational age was determined by routine ultrasonography in pregnancy week 18–20. In 2.8% of cases gestational age was determined from the last menstrual period. Birth weight, length, and head circumference were obtained from birth records. Weight for gestational age (WGA) was expressed as the percentage deviation from the expected mean WGA. For this calculation for each gender a fourth-degree polynomial equation was used [17]. Birth weight appropriate for gestational age was defined as WGA between –22% and +22% of expected WGA approximate to the interval –2 SD to +2 SD.

Body weight was measured on a digital scale (Solotop Oy, Baby-Scale Model, Helsinki, Finland) to the nearest 0.005 kg. Body length was measured supine with a Kiddimeter (Raven Equipment, Essex, UK) to the nearest 0.1 cm. All anthropometrical measurements were registered as a mean of three measurements. Body surface area (BSA) was determined using the equation of Du Bois and Du Bois [18] BSA=body weight$^{0.425}$* body height$^{0.725}$*0.007184.

Ultrasonography

Kidney size was determined by ultrasonography using a 5-MHz sector probe with an accuracy of 0.1 mm (Aloka SSD 500, Aloka, Tokyo, Japan). Three longitudinal and three cross-sectional scans of each kidney were obtained with the probe placed on the back of the child. All dimensions were measured to nearest 0.1 cm and mean length, depth, and width were calculated. Maximum pelvic anteroposterior diameter was measured to the nearest 0.1 mm. More details on the scanning technique have been reported previously [13].

Kidney volume was calculated in cubic centimeters using the equation of an ellipsoid: volume=mean length*mean width*mean depth*0.523 [19]. Left and right kidney volumes were added for the combined kidney volume (cm^3). Relative kidney volume was calculated as combined kidney volume/body weight (cm^3/kg) or combined kidney volume/BSA (cm^3/m^2). Relative kidney growth was calculated as volume increase/weight increase (cm^3/kg) over the two intervals 0–3 months and 3–18 months of age. Body weight and combined kidney volume are linearly correlated at this age [13].

Feeding classification

No intervention was made concerning early nutrition. If the child had received any kind of infant formula, the name of the product was registered, as was the age (weeks) at which formula feeding had started and, if relevant, the age at which breast feeding had stopped. The children were stratified into three feeding categories according to the type of feeding during the last months prior to the 3-month examination (1) fully breast fed (nothing but breast feeding since birth), (2) partially breast fed (combined breast and formula feeding), and (3) fully formula fed (no breast milk since 2 months of age).

Milk sampling and analysis

The mothers were asked to collect small amounts of breast milk at home from postnatal week 4 to 12 over consecutive meals to a maximum volume of 250 ml. The milk was stored in a glass bottle in the household freezer until the 3-month hospital visit and thereafter at −20°C until analysis. The protein concentration (exclusively protein bound nitrogen) was determined by infrared analysis to the nearest 0.01 g/100 ml [coefficient of variation (CV) 0.4%] using a Milko-scan 104 IR Analyzer (A/S N. Foss Electric, Woodstock, Vt., USA) [20]. The protein concentration of the different infant formulas used was derived from the product information.

Blood sampling and analysis

At 3 months of age a non-fasting venous blood sample was drawn from the cubital vein. One sampling attempt was allowed, resulting in an overall success rate of 71%. Serum was stored at −20°C until analysis. All available samples from partially breast-fed and formula-fed infants plus the available samples from 200 randomly selected breast-fed infants (100 boys, 100 girls) were chosen for serum analyses. The resulting number of samples for analysis was 129 in boys (81/32/16) and 136 in girls (83/37/16) (fully breast fed, partially breast fed, and fully formula fed, respectively). Serum creatinine was measured with the Jaffé method by kinetic colorimetric assay (Roche Diagnostics, Mannheim, Germany) with a detection limit of 0.0088 mmol/l and intra- and interassay CVs of 0.7% and 2.3%, respectively. Serum urea nitrogen was measured by kinetic UV assay (Roche Diagnostics) with a detection limit of 0.83 mmol/l and intra- and interassay CVs of 0.8% and 3.4%, respectively. C_{Cr} was estimated using the Schwartz equation: C_{Cr} (ml/min per 1.73 m^2)=[0.45*height (cm)]/[serum creatinine (mg/dl)] [21], recommended by the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative [22].

Statistics

Descriptive statistics are given as mean (SD) or geometric mean (95% confidence interval). Parameters that were not normally distributed were log transformed prior to analysis or non-parametric statistical methods were applied. Log-transformed combined and relative kidney volumes were converted into z-scores [(Xn−Xmean)/SD] (Xn=the value of the individual child, Xmean=the mean value for age and gender, SD=the standard deviation at the same age). Differences between two feeding categories were tested with independent sample *t*-test or Mann-Whitney U test, and between three feeding categories with one-way ANOVA or Kruskal Wallis test, depending on the distribution of data. Results were considered significant if P <0.05. The analyses were performed in SPSS 11.0 for Windows.

Ethics

The study was performed according to the Helsinki II declaration and approved by the local ethics committee [j.no. (KF) 01–030/97] and the Danish Data Protection Agency (no. 1997–1200–074). Informed written consent was obtained from the parents.

Results

The number of children in each feeding category available for analysis is given in Table 1. Children who re-

Table 1 Number of infants and examinations performed at 0, 3, and 18 months of age stratified according to gender and feeding category at 3 months of age

	Boys			Girls		
	Fully breast fed	Partially breast fed	Fully formula fed	Fully breast fed	Partially breast fed	Fully formula fed
Total number	200	43	25	283	52	28
Age at examination						
0 months	199	40	24	275	51	25
3 months	200	43	25	283	52	28
18 months	172	36	20	242	44	21

Table 2 Antropometric measurements, gestational age (GA), and Apgar score in boys and girls from birth to 18 months stratified by feeding category at 3 months of age (*BSA* body surface area)

	Boys			Girls		
	Mean (SD)			Mean (SD)		
	Fully breast fed	Partially breast fed	Fully formula fed	Fully breast fed	Partially breast fed	Fully formula fed
Birth record						
Birth weight (kg)	3.64 (0.40)	3.60 (0.40)	3.62 (0.43)	3.57 (0.39)	3.51 (0.42)	3.55 (0.40)
Birth length (cm)	52.6 (2.0)	52.4 (2.0)	52.4 (2.0)	51.9 (2.0)	51.5 (2.0)	52.1 (2.2)
GA (days)	281 (8)	281 (8)	280 (7)	282 (8)	280 (8)	282 (8)
Weight for GA (%)	−0.34 (9.45)	−1.45 (11.0)	0.15 (10.9)	0.30 (10.4)	−0.37 (10.6)	−0.21 (10.6)
Apgar score at 1 min[a]	10.0 (5.0–10.0)	10.0 (4.2–10.0)	9.5 (7.0–10.0)	10.0 (6.0–10.0)	10.0 (5.3–10.0)	9.5 (4.0–10.0)
0 months examination						
Weight (kg)	3.54 (0.44)	3.56 (0.44)	3.56 (0.51)	3.48 (0.45)	3.34 (0.43)	3.42 (0.36)
Height (cm)	51.3 (1.9)	51.3 (1.9)	50.9 (1.9)	50.9 (2.2)	50.7 (1.4)	50.7 (1.4)
BSA (m^2)	0.21 (0.02)	0.21 (0.02)	0.21 (0.02)	0.21 (0.02)	0.21 (0.01)	0.21 (0.01)
3 months examination						
Weight (kg)	6.57 (0.69)	6.35 (0.89	6.76 (0.68)	6.05 (0.63)	5.82 (0.62)	6.08 (0.72)
Height (cm)	62.3 (1.8)	61.8 (1.9)	62.7 (2.3)	60.8 (2.0)	60.2 (2.7)	60.4 (1.9)
BSA (m^2)*	0.32 (0.02)	0.31 (0.02)	0.33 (0.02)	0.30 (0.02)	0.30 (0.02)	0.30 (0.02)
18 months examination						
Weight (kg)	11.89 (1.16)	11.95 (1.15)	12.08 (1.22)	11.09 (1.14)	11.42 (1.11)	10.95 (1.01)
Height (cm)**	83.9 (2.8)	83.8 (2.9)	84.6 (3.1)	81.9 (2.8)	82.8 (3.2)	81.0 (2.7)
BSA (m^2)	0.51 (0.03)	0.51 (0.03)	0.52 (0.03)	0.49 (0.03)	0.50 (0.03)	0.48 (0.03)

* Difference between feeding category among boys P=0.036

** Difference between feeding category among girls P=0.051

[a] Apgar score: results are given as median (95% confidence interval)

ceived both breast milk and formula were a heterogeneous group, as the number of formula meals ranged from one to all but one meal per day. At birth all children were exclusively breast fed, except for 2 girls from the later formula-fed group who were partially breast fed at the time of the neonatal examination. At 18 months of age all received a normal mixed diet.

There were no significant differences between feeding categories in birth weight and length, Apgar score, gestational age and WGA, as well as weight, height, and BSA at 0, 3, and 18 months of age, except for minor differences in BSA in boys at 3 months of age and height in girls at 18 months (Table 2).

The mean protein concentration in breast milk was significantly lower than in the formulas used: boys 1.0 (0.7–1.6) versus 1.5 (1.4–1.6) g/100 ml (P<0.001) and girls 1.1 (0.7–1.6) versus 1.5 (1.4–1.6) g/100 ml (P<0.001). Six different types of formula were registered. No gender difference in the dietary protein concentration was found.

In boys both absolute and relative kidney size at 3 months of age was significantly larger in the fully formula-fed compared with the fully breast-fed group: combined kidney volume (P=0.004), kidney volume/weight (P=0.011), and kidney volume/BSA (P=0.006). When including the more heterogeneous group of partially breast-fed babies, this correlation weakened (Table 3). No differences in absolute or relative kidney size were found between feeding categories at 0 and 18 months of age.

In girls the influence of feeding on kidney size at 3 months of age, both absolute and relative, showed the same tendency as in boys, but did not reach significance between fully breast-fed and fully formula-fed babies. However, when including the partially breast-fed group, there was a significant difference in kidney volume/weight (Table 3). As in boys, no differences in absolute or

Table 3 Absolute and relative kidney size in boys and girls from birth to 18 months by feeding category at 3 months of age

	Boys				Girls			
	Geometric mean (95% prediction interval)			Difference*	Geometric mean (95% prediction interval)			Difference*
	Fully breast fed	Partially breast fed	Fully formula fed	*P*	Fully breast fed	Partially breast fed	Fully formula fed	*P*
0 months examination								
Combined kidney volume (cm^3)	26.6 (16.8–42.1)	25.2 (16.2–37.5)	26.8 (16.0–45.2)	NS	24.3 (15.6–37.7)	24.2 (15.7–36.3)	23.1 (14.3–37.3)	NS
Combined kidney volume/weight (cm^3/kg)	7.6 (5.0–11.6)	7.1 (4.5–10.5)	7.6 (4.5–12.8)	NS	7.0 (4.6–10.6)	7.4 (4.5–10.5)	6.8 (4.2–11.0)	NS
Combined kidney volume/BSA (cm^3/m^2)	125.2 (82.3–190.6)	118.3 (78.5–173.6)	126.5 (76.7–208.5)	NS	114.4 (76.7–170.7)	118.1 (76.7–168.3)	111.1 (70.1–175.9)	NS
3 months examination								
Combined kidney volume (cm^3)	38.5 (26.3–56.3)	36.3 (24.5–56.0)	45.5 (32.1–56.3)	0.014	33.8 (22.6–50.4)	34.0 (21.3–54.0)	35.9 (23.8–54.6)	NS
Combined kidney volume/weight (cm^3/kg)	5.9 (4.1–8.5)	5.8 (3.8–9.8)	6.4 (5.0–8.1)	NS	5.6 (4.0–7.9)	5.9 (3.9–8.6)	5.9 (4.1–8.5)	0.051
Combined kidney volume/BSA (cm^3/m^2)	120.3 (83.9–172.4)	117.4 (77.2–178.7)	131.6 (101.5–170.7)	0.055	111.1 (77.5–159.2)	118.9 (75.8–176.9)	119.1 (83.1–170.7)	0.079
18 months examination								
Combined kidney volume (cm^3)	66.0 (48.0–90.9)	65.0 (47.7–94.2)	66.0 (45.2–96.5)	NS	60.3 (40.4–90.0)	62.7 (48.0–76.6)	62.2 (48.9–79.0)	NS
Combined kidney volume/weight (cm^3/kg)	5.6 (4.3–7.5)	5.4 (4.0–7.3)	5.5 (4.1–7.4)	NS	5.5 (4.0–7.5)	5.6 (4.7–8.0)	5.7 (4.2–7.7)	NS
Combined kidney volume/BSA (cm^3/m^2)	130.3 (98.5–172.4)	125.8 (96.9–169.4)	127.7 (92.8–175.9)	NS	124.0 (88.2–174.2)	126.8 (104.2–175.5)	129.0 (101.5–164.0)	NS

* Differences between feeding categories were assessed with one-way ANOVA of the log-transformed values

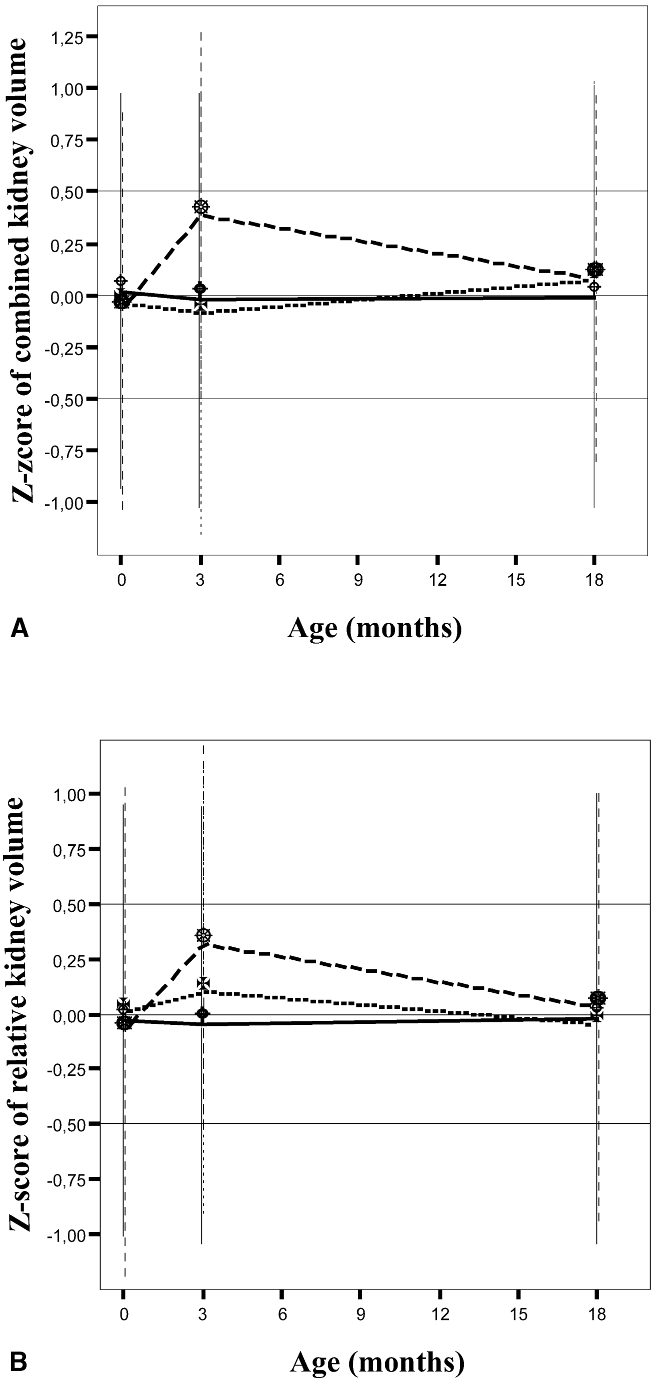

Fig. 1 Combined data on boys and girls. **A** Z-score of combined kidney volume (cm^3) according to diet at 3 months of age. **B** Z-score of relative kidney volume (cm^3/kg) according to diet at 3 months of age. Fully breast fed: *square*—, partially breast fed: *cross*···, fully formula fed: *circle*—. *Dots*: mean values, *bars*: ±1 SD

relative kidney size were found at 0 and 18 months of age (Table 3).

In both sexes no differences in mean renal pelvic diameter (mm) were seen between any feeding categories at any age: boys 1.7 (0.6), 1.9 (0.7), and 2.0 (0.6) mm at 0, 3, and 18 months and girls 1.5 (0.6), 1.8 (0.7), and 2.0 (0.6) mm, respectively.

Z-scores of absolute and relative kidney size (cm^3/kg) at 3 months of age were significantly correlated with feeding category (Fig. 1A and B), combined kidney volume (P=0.014) and relative kidney volume (P=0.024). No differences were seen at 0 and 18 months of age.

Relative kidney growth (kidney volume increase/weight increase) from 0 to 3 months of age was significantly influenced in both sexes by feeding category, with increasing relative kidney growth with increasing dietary proportion of formula (Table 4). From 3 to 18 months of age, the pattern of relative kidney growth was the opposite, borderline significant in boys and non-significant in girls (Table 4).

Serum urea nitrogen was significantly influenced by feeding category (fully breast fed, partially breast fed, fully formula fed): boys 2.6 (0.6) versus 3.1 (0.8) versus 3.8 (0.5) mmol/l, P<0.001 and girls 2.9 (0.6) versus 3.1 (0.8) versus 3.9 (0.7) mmol/l, P<0.001. Serum creatinine and estimated C_{Cr} were not different between feeding categories in boys or girls: boys serum creatinine 0.050 (0.007) mmol/l and C_{Cr} 50.2 (7.0) ml/min per 1.73 m^2 and girls serum creatinine 0.049 (0.007) mmol/l and C_{Cr} 50.5 (8.4) ml/min per 1.73 m^2.

Discussion

In the present study formula feeding had a significant impact on kidney growth in human infants. A significant association between increasing proportion of formula in the diet and increasing combined and relative kidney size in healthy 3-month-old infants was found for the first time. An obvious dietary difference between the feeding categories is the protein concentration. Studies in human adults [11, 12] and animals [1, 23, 24, 25] have demonstrated that increased protein intake leads to enlarged kidney size. In the present study these differences were no longer visible at the age of 18 months when all children received a normal mixed diet. A similar temporary increase in kidney size induced by increased protein intake has previously been found in both humans and rats [1, 11]. The increased kidney size may reflect an adaptive response to hyperfiltration induced by the increased protein intake. However, other dietary differences between breast milk and formula might have an additional impact on kidney growth.

The protein concentration of the commercial formulas used was significantly higher than that of breast milk. We did not have detailed information on the volume (breast milk or formula) consumed by the individual child, thus we were not able to determine the exact daily protein intake. It is, however, reasonable to assume that the fully formula-fed and, to some extent, the partially breast-fed infants had a substantially higher daily protein intake compared with the breast-fed group. This is substantiated by the significantly increased serum urea nitrogen with

Table 4 Relative kidney growth from 0 to 3 and 3 to 18 months of age according to gender and feeding category at 3 months of age

	Geometric mean (95% prediction interval)			Difference*
	Fully breast fed	Partially breast fed	Fully formula fed	*P*
Boys				
Relative kidney growth 0–3 months (cm^3/kg)	4.0 (-0.5–9.9)	3.9 (-0.6–14.5)	5.7 (0.1–10.7)	0.021
Relative kidney growth 3–18 months (cm^3/kg)	5.3 (1.3–9.2)	4.9 (1.7–8.0)	4.2 (2.0–7.6)	0.054
Girls				
Relative kidney growth 0–3 months (cm^3/kg)	3.8 (0.1–10.0)	4.1 (-0.4–8.5)	4.4 (1.7–11.1)	0.049
Relative kidney growth 3–18 months (cm^3/kg)	5.5 (1.5–9.6)	5.3 (0.7–9.0)	4.9 (3.4–10.1)	NS

* Differences between feeding categories were assessed with Kruskal Wallis test. Level of significance $P<0.05$

increasing proportion of formula in the diet. Previous studies confirm this assumption [2, 8, 26].

The percentage of children in each feeding category at 3 months of age (76.4% fully breast fed, 15.1% partially breast fed, and 8.4% fully formula fed) was representative of the Danish population. In a contemporary cohort study (The Danish National Birth Cohort), the frequency of exclusive breast feeding at 12 weeks of age among 12,500 infants was 70.2%, partial breast feeding 16.9%, and exclusive formula feeding 12.9% (J. Baker and T.I.A. Sorensen, personal communication).

From both human and animal studies it is well known that an increased protein intake is associated with a rise in GFR [1, 12, 24, 25, 27, 28, 29] as a result of increased glomerular plasma flow and transglomerular pressure [27, 30]. In the present study no significant difference in the estimated C_{Cr} was found between the three feeding categories. This may be due to a relatively low number of infants in the partially breast and fully formula-fed groups or the uncertainty of the method of clearance estimation. Other studies have shown that GFR is positively associated with kidney size [31, 32, 33, 34], and both increased GFR and kidney size have been found in young rats on a high-protein diet [1].

It is well known that formula-fed infants have increased serum urea nitrogen [2, 3, 4, 5, 6, 7, 8, 9] and an overall increase of the amino acid concentration compared with breast-fed infants [3, 8, 10]. A recent in vitro study has demonstrated that an increased amino acid concentration may induce injury in rat glomerular mesangial cell cultures [35]. Whether similar changes occur in human subjects and whether such changes are reversible is currently unknown. However, avoiding a high-protein intake does provide long-term beneficial effects in patients and animals with pre-existing renal disease [36, 37, 38].

There is increasing evidence of the long-term beneficial effect of breast feeding with regard to blood pressure, ischemic heart disease, and non-insulin-dependent-diabetes (NIDDM) [39, 40]. Two large cohort studies including term, healthy infants showed that systolic blood pressure was significantly higher in the children and young adults who were exclusively formula fed in infancy compared with those that were breast fed [41, 42]. Additionally, infant feeding and age of weaning influence the adult serum lipid concentration and mortality from ischemic heart disease [40]. In a high-risk population, exclusive breast feeding during the first 2 months of life was associated with a significantly lower rate of NIDDM at age 10–39 years compared with exclusive formula feeding [43]. The possible mechanisms by which early nutrition influence later morbidity and mortality are still largely unknown. Whether renal size and function in adulthood are influenced by early nutrition has to our knowledge not yet been addressed.

A specific gender difference in response to formula feeding at 3 months of age was disclosed as the effects were more pronounced in boys than in girls. This gender difference was not due to differences in dietary protein concentration between boys and girls. In animal studies, gender differences in renal morphology [44], response to a high-protein intake [24, 45], changes following unilateral nephrectomy [46], and renal response to dyslipidemia and hypercholesterolemia [47] have been described. In all these studies, male animals were more sensitive to the imposed actions and the induced changes were more profound than in females. In healthy infants, kidney size is significantly larger in boys compared with girls at 3 months of age but not at 18 months [13]. The timing of this gender difference coincides with the childhood "mini-puberty", indicating that kidney size may be influenced by sex steroids.

In conclusion, a significant increase in kidney growth associated with increasing proportion of formula compared with breast feeding in healthy 3-month-old infants has been shown. The effect of formula feeding on kidney size was more pronounced in boys than in girls. The differences in relative kidney size were temporary, as they did not persist at 18 months of age when all children received a normal mixed diet. The immediate renal effects of formula feeding should be taken into consideration for recommendations concerning infant feeding. Whether there are any long-term effects of early increased protein intake on later kidney function remains to be seen.

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