Does insulin-like growth factor-1 mediate protein-induced kidney growth in infants?: A secondary analysis from a randomized controlled trial

Veronica Luque[1], Joaquin Escribano[1], Veit Grote[2], Natalia Ferre[1], Berthold Koletzko[2], Dariusz Gruszfeld[3], Piotr Socha[3], Jean-Paul Langhendries[4], Philippe Goyens[5] and Ricardo Closa-Monasterolo[1]; for the European Childhood Obesity Project

BACKGROUND: Animal models have shown that insulin-like growth factor I (IGF-I) may mediate protein-induced kidney growth. Our aim was to analyze the effect of IGF-I on protein-induced kidney growth in healthy infants.

METHODS: This is a secondary analysis of a randomized trial that compared growth of infants fed with a higher-protein (HP) (n = 169) vs. lower-protein (LP) (n = 182) formula (in the first year of life). Outcome measures were anthropometric parameters, kidney volume (cm^3), and total and free IGF-I (ng/ml).

RESULTS: The highest levels of total and free IGF-I were found in the HP group. Both parameters correlated significantly with BMI z-score ($r = 0.229$, $P < 0.001$ and $r = 0.223$, $P < 0.001$, respectively), kidney volume ($r = 0.115$, $P = 0.006$ and $r = 0.208$, $P < 0.001$, respectively), and kidney volume/body length ($r = 0.109$, $P = 0.010$ and $r = 0.194$, $P < 0.001$, respectively) at 6 mo. Linear regression analyses showed a significant effect of free IGF-I on kidney volume in models, including significant effects of HP formula and anthropometry. The structural equation model revealed a significant direct effect of the HP formula on kidney volume and an indirect effect mediated by free IGF-I.

CONCLUSION: This study suggests that IGF-I partly mediates protein-induced kidney growth in healthy infants. IGF-I could be involved in a pathway for the programming of the renal system.

Recent research on the early origins of adult diseases has highlighted the importance of nutrition early in life. Early nutrition could affect body structures and tissue development, which could be expressed as a permanent effect on a function that could affect health in adulthood (1). This is what is known as nutritional programming. Nutritional factors could act in different tissues and systems during a critical period of development affecting growth (2). Programming through early growth mechanisms may affect different body tissues and organs, such as bone, muscle, adipose tissue, the heart, and the kidney. Different nutritional interventions early in life have been shown to produce permanent effects on kidney function and structure. For instance, it has been proposed that poor nutritional status during gestation may affect nephrogenesis in the offspring, which could lead to impaired kidney development that could induce hypertension risk as well as poor kidney function in adulthood (3,4). Conversely, increased protein supply has been reported to stimulate kidney growth not only prenatally but also in postnatal life, both in animal models (5) and in humans (6). One of the mechanisms that may induce compensatory kidney growth in response to an increase in protein supply is the increased renal workload in response to urea and other compounds derived from protein metabolism (7,8). Furthermore, animal models have shown that insulin-like growth factor I (IGF-I) may also mediate protein-induced kidney growth. However, there is little evidence suggesting a direct effect of IGF-I on kidney size or function in humans. The relationship between IGF-I and kidney volume in humans has been postulated in different scenarios. In patients with unilateral nephrectomies, compensatory growth of the remaining kidney is accompanied by an increase in IGF-I levels (9). Human recombinant IGF-I treatment also promoted an increase in kidney size and function in a series of patients with chronic renal failure (10) and in a series of healthy patients (11).

In healthy infants, it has been demonstrated that increased protein intake stimulates kidney growth (6) and is also directly related to higher serum IGF-I concentrations (12,13). However, the possible mediation of kidney growth by IGF-I has not been tested. Deciphering this mechanism could lead to a better understanding of the possible pathways for programming kidney and cardiovascular disease, such as renal insufficiency or hypertension. The aim of this study was to analyze the effects of IGF-I on protein-induced kidney growth in healthy infants.

RESULTS

Study Sample

Of the 652 formula-fed infants remaining in the study at 6 mo (324 higher-protein (HP) formula-fed and 328 lower-protein

The first two authors contributed equally to this work.
[1]Pediatrics Research Unit, Universitat Rovira i Virgili, IISPV, Reus, Spain; [2]Division of Nutritional Medicine and Metabolism, Hauner Children's Hospital, University of Munich Medical Centre, Munich, Germany; [3]Neonatal Intensive Care Unit and Department of Gastroenterology, Hepatology and Immunology, Children's Memorial Health Institute, Warsaw, Poland; [4]Department of Paediatrics, CHC St Vincent, Liège-Rocourt, Belgium; [5]Department of Pediatrics, University Children's Hospital Queen Fabiola, Brussels, Belgium. Correspondence: Ricardo Closa-Monasterolo (ricardo.closa@urv.cat)

Received 13 June 2012; accepted 29 December 2012; advance online publication 10 July 2013. doi:10.1038/pr.2013.87

(LP) formula-fed), 601 (92%) returned to the study centers for the kidney ultrasound assessment. Of those, 50 (8.3%) were not included in the analyses due to measurement errors or kidney anomalies. Of the remaining 551 participants in the formula-fed groups, 46 (8.3%) declined to perform the blood sample analysis. A blood sample was drawn from 506 formula-fed infants, and 438 IGF-I measurements were valid (68 blood samples did not have enough serum or were excluded for other reasons) (**Figure 1**). There were no differences in anthropometric and socioeconomic baseline characteristics of children randomized to the formula groups. We did not find any effect of gender, country, or feeding group in withdrawals or reasons for sample exclusion (**Figure 1**).

Of the 236 breastfed (BF) infants remaining in the study at 6 mo, 204 had a kidney ultrasound, and 186 were analyzed for comparison as well (18 (10%) were excluded for incorrect measurements or kidney anomalies). Of those, 185 participated in the blood sample analysis. IGF-I concentrations were obtained from 165 BF infants.

Effect of Protein Intake on Body and Kidney Growth

Feeding groups exhibited significant differences in anthropometrical parameters and kidney volume at 6 mo. Infants fed with the HP formula had significantly higher body weight *z*-scores ($P = 0.009$) than did the infants fed with the LP formula (**Figure 2a**), whereas no differences existed for body length *z*-score (0.38 (±0.96) vs. 0.33 (0.90)). Kidney volume was also significantly greater among the infants fed with the HP formula ($P < 0.001$) (**Figure 2b**).

Effect of Protein Intake on Total and Free IGF-I

The highest levels of total and free IGF-I were found in the HP group, which exhibited an ~35% increase in median levels as compared with the LP group ($P < 0.001$ for total and free IGF-I between formula groups) (**Figure 3a**,**b**).

Relationship Among the IGF-I Axis, Anthropometry, and the Kidney

Total and free IGF-I were significantly correlated with all anthropometric parameters (weight, length, BMI, and body surface area) at 6 mo (**Table 1**) among formula-fed infants.

IGF-I was also correlated with kidney volume; the correlation was slightly stronger for free IGF-I in formula-fed infants (**Table 1**). Similarly, free IGF-I was significantly correlated with estimated glomerular filtration rate ($r = 0.22$, $P < 0.001$).

Effect of the IGF-I Axis on Protein-Induced Kidney Growth

Linear regression analyses revealed a slight effect of total IGF-I concentrations on kidney volume ($\beta = 0.025$ (0.005, 0.046), $P = 0.016$) in a linear regression model including the infant

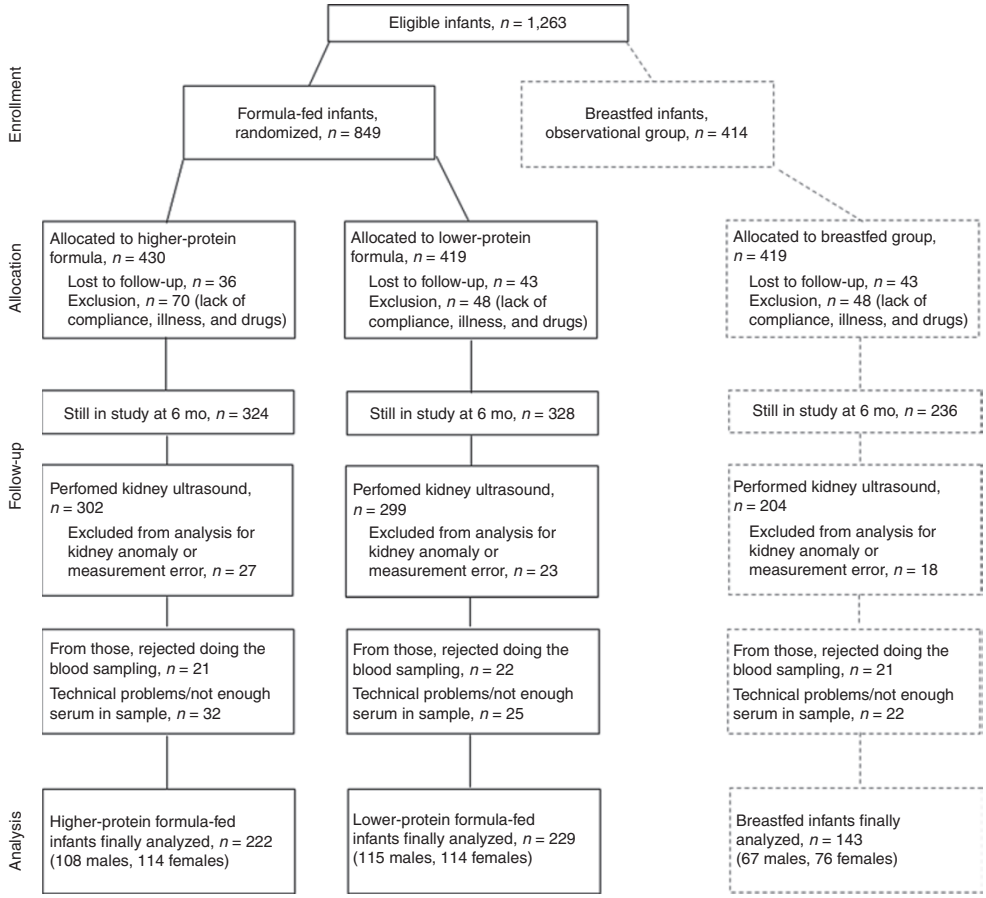

Figure 1. Flow diagram of randomization, allocation, follow-up, and data analysis. Solid line: formula groups (randomized intervention trial), dashed line: observational group of breastfed infants as a reference.

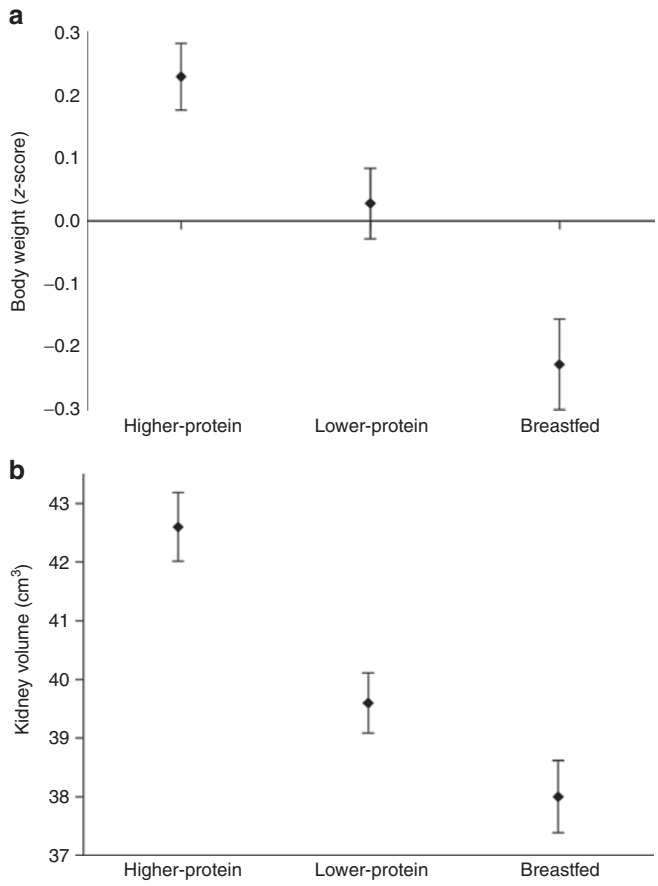

Figure 2. Effect of feeding type on (**a**) body growth and (**b**) kidney volume (means (SEM)). Higher-protein formula–fed infants had higher body weight (*z*-score) and kidney volume (cm^3) than lower-protein formula–fed infants ($P = 0.009$ and $P < 0.001$, respectively). Breastfed infants had lower body weight than both formula-fed groups ($P < 0.001$ and $P = 0.005$, as compared with higher- and lower-protein formulas, respectively) and lower kidney volume than infants fed with the higher-protein formula ($P < 0.001$). Breastfed infants did not differ from lower-protein formula–fed infants in kidney volume.

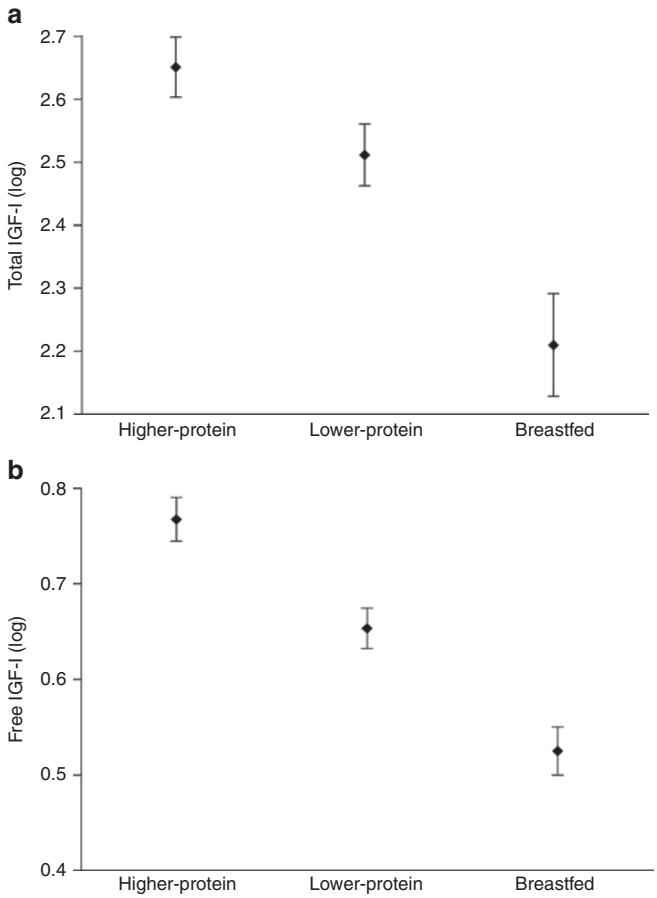

Figure 3. Effect of feeding type on (**a**) total and (**b**) free IGF-I serum levels (mean (SEM)). Lower-protein formula–fed infants had lower total and free IGF-I concentrations than infants fed with the higher-protein formula ($P < 0.001$, both parameters). Breastfed infants had lower concentrations than both formula groups of total IGF-I ($P < 0.001$, both comparisons) and free IGF-I ($P < 0.001$ and $P = 0.004$, as compared with higher- and lower-protein formula–fed infants, respectively). IGF-I, insulin-like growth factor I.

formula; however, this effect disappeared after adjusting for current anthropometrical variables.

Free IGF-I concentrations showed a stronger effect on kidney volume in a linear regression model including the type of formula and current anthropometrical parameters, and adjusted for country and gender, explaining up to 22.3% of its variability ($P < 0.001$) (**Table 2**).

Breastfeeding showed no effect on kidney volume as compared with the lower-protein formula. The effect of gender on kidney volume disappeared after adjustment for anthropometrical variables.

The direct and indirect effects of the formula and IGF-I on kidney volume were calculated in a structural equation model. The strongest effect of the intervention on kidney volume was direct (the HP formula increased the standardized kidney volume by 0.12 (95% confidence interval 0.19, 0.45) as compared with the LP formula). In addition, the HP formula had a significant indirect effect through increasing free IGF-I, which in turn had a direct and an indirect (through weight) positive effect on kidney volume. The total of all indirect effects can be derived by adding the product results along each path (i.e., 0.16×0.14 (formula effect directly through free IGF-I) + $0.16 \times 0.18 \times 0.17$ (formula effect through free IGF-I and weight) = 0.029). Thus, the indirect effects of formula on kidney volume through free IGF-I make up ~24.3% of its total effect (**Figure 4**). The statistical power calculation *a posteriori* shows that the direct and

Table 1. Correlations of IGF-I with body measures and kidney volume at 6 mo of age among formula-fed infants ($n = 451$)

	Total IGF-I (ng/ml)	Free IGF-I (ng/ml)
Weight *z*-score	0.29***	0.29***
Length *z*-score	0.19***	0.19***
BMI *z*-score	0.23***	0.22***
Body surface area (m^2)	0.23***	0.22***
Kidney volume (cm^3)	0.123**	0.19***
Kidney volume/cm	0.11*	0.18***

IGF-I, insulin-like growth factor I.

Spearman's ρ correlations, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 2. Effect of dietary protein content and IGF-I on kidney volume at 6 mo of age ($n = 440$)

Variable affecting kidney volume (cm^3)	β Estimate	95% CI (upper, lower)	P value	R^2
Formula (lower- vs. higher-protein formula)	2.289	(0.824, 3.755)	0.002	0.22
Free IGF-I (ng/ml)	1.608	(0.239, 2.976)	0.021	
Length (cm)	1.031	(0.586, 1.476)	<0.001	
Body weight (kg)	1.784	(0.657, 2.912)	0.002	

CI, confidence interval; HP, higher protein; IGF-I, insulin-like growth factor I; LP, lower protein.

Formula codes were LP = 1 vs. HP = 2. Regression adjusted by gender (1 = male, 2 = female), which showed no effect, and study country (1 = Germany, 2 = Belgium, 4= Poland, and 5= Spain), which had a significant effect on kidney volume ($\beta = -0.898$, $P < 0.001$).

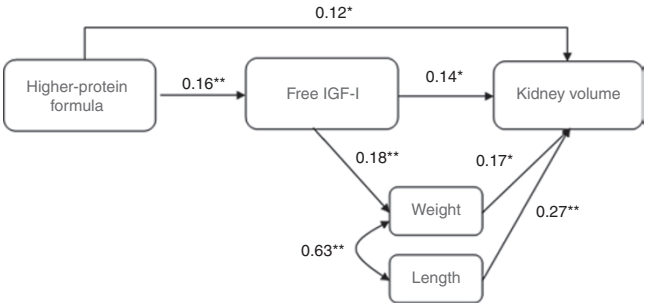

Figure 4. The results from a structural equation model. This figure shows the effect of each factor through each path (represented by arrows). Numbers are standardized coefficients (SCs); one change in SC of a factor leads to x SC change in the next factor, e.g., as compared with the lower-protein formula, the higher-protein formula increases the kidney volume directly by 0.12 (95% CI: 0.19, 0.45). In addition, we see an indirect effect mediated through free IGF-I by 0.029 (addition of product results along each path). The indirect effects of formula on kidney volume through free IGF-I explain 24.3% of the kidney volume variability. For clearer depiction, the paths included in the model of country of origin on free IGF-I, weight, height, and kidney volume are not shown. *$P < 0.01$, **$P < 0.005$. CI, confidence interval; IGF-I, insulin-like growth factor I.

indirect effects of the formula on kidney volume had a power of 100%, with a confidence interval of 95%.

DISCUSSION

IGF-I and the Kidney

This is the first clinical trial to show that the IGF-I axis may affect kidney size and possibly kidney function in healthy infants.

Our results are consistent with those published in the literature, mainly based on animal models. Rats treated with human recombinant IGF-I exhibit kidney growth and an increase in glomerular filtration rate (14). Models for compensatory kidney growth after partial nephrectomy have shown a relationship among the operation, an increase in IGF-I levels, and subsequent compensatory kidney growth (9). The possible direct effect of the IGF-I axis on kidney growth is supported by the IGF-I receptor patterns found in both rat and human kidneys.

In our study, we found significant direct associations between the IGF-I axis and kidney volume and a slight but significant correlation between the free IGF-I axis and kidney function. It is possible that the limitations of the estimation of glomerular filtration using creatinine plasma values (through the Schwartz equation) instead of a 24-h urine evaluation could partially mask these results (7).

Animal models had previously shown a direct relation between HP intake and higher total and free IGF-I levels in the serum (15–17). This effect has also been shown for infants and children in observational studies (18–20) and, more recently, in the randomized Childhood Obesity Project (CHOP) trial (12).

A possible limitation of the study is the number of subjects available for analysis by feeding group (<50% of the infants originally recruited at birth). However, we do not expect that the effect of IGF-I on the kidney could be biased by the number of subjects available, given that we did not find differences in baseline characteristics of remaining subjects or in reasons to withdraw from the study.

IGF-I and Body Growth

The IGF-I axis has been shown to be closely related to body growth in infants (21,22) and children (22). It has been reported that IGF-I concentrations in infancy are predictive of early postnatal growth rates (22), differential length gain, and adiposity (23). In our sample, consistent with the literature, total and free IGF-I were both directly correlated with weight, length, BMI, and body surface area.

IGF-I as a Mediator of Protein-Induced Kidney Growth

Our research team has already reported a significant effect of protein intake during the first months of life on kidney size (6). Infants fed a HP diet exhibited increased levels of urea and other renal workload parameters. In turn, these metabolites were correlated with kidney volume.

Research in animal models has shown that increased renal workload (by a HP supply) could induce adaptive kidney overgrowth. In addition, HP intake stimulates IGF-I secretion, which in turn could promote kidney growth directly (17). The present work reveals that the main effect on kidney size is the one produced directly by the HP formula through yet unknown mechanisms, as shown by the mediation model analyses. Furthermore, consistent with our hypothesis, this study suggests that IGF-I may partly mediate the protein-induced kidney growth in healthy infants. IGF-I may have a direct effect on kidney growth and on body weight gain. In turn, the resultant higher body mass may cause a renal work overload through an increased production of nitrogenous products such as creatinine (7).

Therefore, a triple mechanism may induce increased kidney growth upon protein intake: compensatory kidney growth induced by the increase in nitrogen substances derived from protein metabolism (the main mechanism); hypertrophy produced by increased IGF-I secretion, which may act directly on the kidney; and protein-induced body growth (also promoted by an increase in IGF-I secretion), which may also be accompanied by kidney growth. **Figure 5** shows the different pathways that may participate in protein-induced kidney growth.

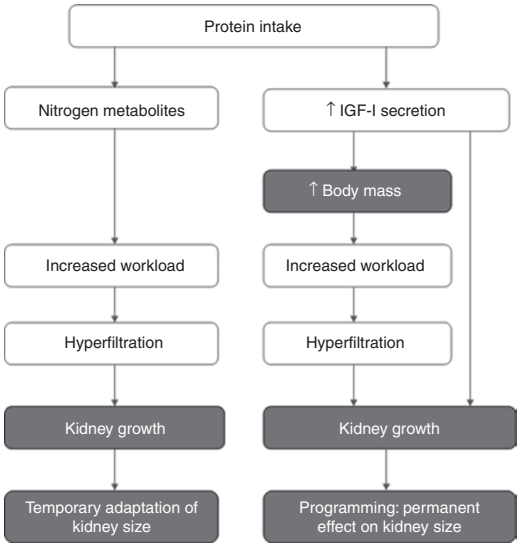

Figure 5. Dietary protein and kidney size and function: deciphering the possible mechanism. IGF-I, insulin-like growth factor I.

However, these different pathways could have different consequences. It has been suggested that protein intake early in life may be correlated with later obesity risk (24–26). Published results from this randomized clinical trial show an increased weight and BMI among infants fed with the HP formula, at 2 y of life, 1 y after the end of nutritional intervention. These findings support a possible role for protein intake in nutritional programming, the proposed mechanism of which is the stimulation of the IGF-I axis. Considering that this increased body growth (programmed by IGF-I modulation early in life) could be permanent, we foresee a slight long-term effect on hyperfiltration that in turn may program kidney volume at later stages.

We hypothesize that compensatory hypertrophy through an increase in protein intake may be a reversible process that could cease with the nutritional intervention (as shown in previous work) (27). However, kidney growth accompanying body weight gain early in life and mediated by the IGF-I axis could be a permanent effect. We do not expect that this increase in kidney volume may have any health effect in healthy children during infancy, but the long-term health implications of such overgrowth, if overweight is permanently established, remain to be determined. Higher kidney volume, if promoted after birth, is not expected to cause a higher renal functional capacity (because nephrogenesis in humans finishes before the end of gestation) (28). Although the differences in kidney growth in our study sample might not be clinically relevant in the long term, on the basis of the observed results, we could hypothesize that kidney hypertrophy due to excessive body weight gain early in life could result in a permanent functional overload, with a possible increasing risk of renal disease and hypertension (29,30), resulting in kidney programming. Further follow-up of these infants may help to elucidate the possible role of IGF-I on programming the renal system, by studying the relationship among weight, kidney volume, kidney function, and hypertension later in life.

In conclusion, this randomized clinical trial suggests that the IGF-I axis affects kidney size and partly mediates protein-induced kidney growth in healthy infants. The IGF-I axis could be involved in the nutritional programming of the renal system.

METHODS

Study Design

The data presented were collected in a double-blind randomized controlled trial; this is a secondary analysis of a study designed to detect differences in body growth. The details of the study have been published previously (24), in addition to other secondary analyses (12,13). Term healthy infants fed an HP formula (infant formula: 2.05 g protein/100 ml; follow-on formula: 3.2 g protein/100 ml) or an LP formula (infant formula: 1.25 g protein/100 ml; follow-on formula: 1.6 g protein/100 ml) during the first year of life were compared. The composition of HP and LP study formulas fulfilled the 1991 EU Directive on Infant and Follow-on Formulae (31) (European Commission directive 91/321), and the energy contents of the two formulas were identical. Further details of these formulas have been published elsewhere (24). Families were provided with the formulas during the first year of life and did not receive any other nutritional intervention from the study team.

In addition, as a reference for growth and development, a control group of BF infants (exclusive breastfeeding for a minimum of 3 mo) was also recruited and observed in parallel during the same period, as recommended by the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (32). The results of this BF infant group are shown as a gold standard reference.

Subjects

Our target population was a sample of 652 formula-fed and 236 BF 6-mo-old infants from four European countries (Germany, Belgium, Poland, and Spain) who had been recruited during the first 8 wk of life (median age = 14 d) to participate in the EU Childhood Obesity Project. **Figure 1** illustrates the recruitment, randomization, and follow-up until the age of 6 mo of studied infants.

Measurements

Anthropometry. The nude weight and the length of the infant were determined at recruitment and at 6 mo of life. BMI (weight (kg)/length (m^2)) and body surface area ((m^2) = ((weight (kg) × length (cm))/3,600)^2) were calculated. Weight, length, and BMI were expressed as *z*-scores relative to the World Health Organization growth standards for BF infants (33).

Blood and urine sampling and analysis. At 6 mo of age, a venous blood sample was drawn, and a urine sample was collected using an adhesive urine collection bag. Efforts were undertaken to draw blood no less than 2 h after the last feed. Total and free IGF-I (ng/dl) were determined using a highly specific commercially available radioimmunoassay test in a single laboratory (Children's Memorial Health Institute, Warsaw, Poland). Serum creatinine and urea (both in mg/dl), as well as urine urinary creatinine (mg/dl) and osmolarity (mmol/l), were determined at local laboratories. The estimated glomerular filtration rate (ml/(min × 1.73 m^2)) was calculated according to the Schwartz equation (estimated glomerular filtration rate = (0.45 × length (cm))/(serum creatinine) (mg/dl)).

Kidney measures. Ultrasonographic kidney measurements were taken by 17 trained and blinded radiologists using a linear or sector ultrasound (5–7.5 MHz) by a posterior approach or lateral approach in the prone position to measure the longest length possible (34).

Infants who exhibited anomalous kidney development, urinary tract disease (such as hydronephrosis), or kidney asymmetry (considered as a >15% length difference between kidneys) were excluded from the analyses. Measures of length, width, maximum depth in the longitudinal section (D1), and maximum depth in the transverse section (perpendicular to the hilar region) (D2) of both kidneys were measured (cm).

Kidney volume (hereafter also "kidney size") was calculated according to the equation for an ellipsoid (kidney volume (ml) = length × width × 0.5 (D1 + D2) × 0.523) (35) and presented as the absolute value (cm^3) and corrected by body length (cm^3/cm). The analysis was based on the sum of the right and left kidney volumes.

Data Analyses

Z-scores for anthropometry variables were calculated using the World Health Organization Anthro for personal computers software, version 3.2.2, 2011 (World Health Organization, Geneva, Switzerland). Descriptive results were expressed as means and SDs. Skewed variables were transformed to their logarithmic form; *t*-test for normally distributed variables and Mann–Whitney *U*-test for skewed variables were used for statistical comparisons between the feeding groups. The BF group of infants was used as a reference in descriptive analyses, but not included in analyses to relate feeding with IGF-I and kidney volume (because this was a not randomized group). Pearson's or Spearman's correlations were used to test for linear associations among continuous variables, as appropriate. Linear regression analysis was applied to assess the effect of feeding type (HP vs. LP formula, encoded as LP = 1 and HP = 2), total and free IGF-I, body length, and body weight on kidney volume, adjusting for the potential confounders gender (encoded as male = 1, female = 2), and country (1 = Germany, 2 = Belgium, 4 = Poland, and 5 = Spain).

Structural equation modeling (36) was performed to separate the direct and indirect effects of the type of formula and IGF-I, including the additional effects of weight, height, and study country. Structural equation modeling is a straightforward extension of multiple regression that provides estimates of the magnitude and significance of hypothesized causal connections between variables. This is performed by the decomposition of observed correlations between the analyzed variables and is best depicted in a path diagram. Because this was not the primary hypothesis of the project, we did not perform the calculation for the sample size needed *a priori*. Therefore, we calculated the statistical power *a posteriori*. The linear regression model and the structural equation model considered significant effect values in 95% confidence intervals.

Data management and statistical analyses were conducted with SAS version 9.2 (SAS Institute, Cary, NC), Stata version 12.0 (Stata, College Station, TX), and SPSS Statistics version 17.0 (SPSS, Chicago, IL).

Ethical Issues

The project was approved by the ethical committees of all study centers. Informed consent was obtained from all of the families that participated in the study.

The study followed the Declaration of Helsinki (37) and the CONSORT Statement (38).

The clinical trial was registered at http://www.clinicaltrials.gov as the EU Childhood Obesity Project (NCT00338689).

ACKNOWLEDGMENTS

We acknowledge the European Childhood Obesity Project group and the laboratory personnel for their hard work. We also gratefully acknowledge statistical advice from Pilar Hernández (from the Institut d'Investigació Sanitària Pere Virgili) and Joan Fernández-Ballart (from the Universitat Rovira i Virgili). European Childhood Obesity Project group: J. Beyer, M. Fritsch, G. Haile, U. Handel, I. Hannibal, S. Kreichauf, I. Pawellek, S. Schiess, S. Verwied-Jorky, R. von Kries, and M. Weber (Children's University Hospital, University of Munich Medical Centre, Munich, Germany); M. Zaragoza-Jordana (Pediatrics Unit, Universitat Rovira i Virgili, Reus, Spain); A. Dobrzańska, R. Janas, A. Wierzbicka, A. Stolarczyk, and J. Socha (Children's Memorial Health Institute, Warsaw, Poland); C. Carlier, E. Dain, J.N. Van Hees, J. Hoyos, F. Martin, P. Poncelet, and A. Xhonneux (ULB Bruxelles and CHC, St Vincent Liege); E. Perrin (Danone Research Centre for Specialised Nutrition, Schiphol, The Netherlands); C. Agostoni, M. Giovannini, A. Re Dionigi, E. Riva, S. Scaglioni, F. Vecchi, and E. Verducci (University of Milan).

STATEMENT OF FINANCIAL SUPPORT

The studies reported herein have been conducted with partial financial support from the Commission of the European Communities, the specific RTD Programme "Quality of Life and Management of Living Resources," within the 5th Framework Programme, research grant nos. QLRT–2001–00389 and QLK1-CT-2002-30582, and the 6th Framework Programme, contract no. 007036. This article does not necessarily reflect the views of the Commission and in no way anticipates the future policy in this area. B. K. is the recipient of a Freedom to Discover Award of the Bristol-Myers Squibb Foundation, New York, NY.

Disclosure: The authors declare no conflict of interest.

REFERENCES

1. Lucas A. Programming by early nutrition: an experimental approach. J Nutr 1998;128:Suppl 2:401S–6S.
2. Mathers JC. Early nutrition: impact on epigenetics. Forum Nutr 2007;60:42–8.
3. Langley-Evans SC. Nutritional programming of disease: unravelling the mechanism. J Anat 2009;215:36–51.
4. Langley-Evans SC, Langley-Evans AJ, Marchand MC. Nutritional programming of blood pressure and renal morphology. Arch Physiol Biochem 2003;111:8–16.
5. Gopalakrishnan GS, Gardner DS, Dandrea J, et al. Influence of maternal pre-pregnancy body composition and diet during early-mid pregnancy on cardiovascular function and nephron number in juvenile sheep. Br J Nutr 2005;94:938–47.
6. Escribano J, Luque V, Ferre N, et al. Increased protein intake augments kidney volume and function in healthy infants. Kidney Int 2011;79:783–90.
7. King AJ, Levey AS. Dietary protein and renal function. J Am Soc Nephrol 1993;3:1723–37.
8. Woods LL. Intrarenal mechanisms of renal reserve. Semin Nephrol 1995;15:386–95.
9. Yildiz B, Kural N, Colak O, Ak I, Akcar N. IGF-1, IGFBP-3, VEGF and MMP-9 levels and their potential relationship with renal functions in patients with compensatory renal growth. Clin Physiol Funct Imaging 2008;28:107–12.
10. O'Shea MH, Miller SB, Hammerman MR. Effects of IGF-I on renal function in patients with chronic renal failure. Am J Physiol 1993;264(5 Pt 2):F917–22.
11. Hirschberg R, Brunori G, Kopple JD, Guler HP. Effects of insulin-like growth factor I on renal function in normal men. Kidney Int 1993;43:387–97.
12. Socha P, Grote V, Gruszfeld D, et al.; European Childhood Obesity Trial Study Group. Milk protein intake, the metabolic-endocrine response, and growth in infancy: data from a randomized clinical trial. Am J Clin Nutr 2011;94:Suppl 6:1776S–84S.
13. Closa-Monasterolo R, Ferré N, Luque V, et al.; Childhood Obesity Project Study Group. Sex differences in the endocrine system in response to protein intake early in life. Am J Clin Nutr 2011;94:Suppl 6:1920S–7S.
14. Hirschberg R, Kopple JD, Blantz RC, Tucker BJ. Effects of recombinant human insulin-like growth factor I on glomerular dynamics in the rat. J Clin Invest 1991;87:1200–6.
15. Chin E, Bondy C. Insulin-like growth factor system gene expression in the human kidney. J Clin Endocrinol Metab 1992;75:962–8.
16. Hirschberg R, Kopple JD. Response of insulin-like growth factor I and renal hemodynamics to a high- and low-protein diet in the rat. J Am Soc Nephrol 1991;1:1034–40.
17. Murray BM, Campos SP, Schoenl M, MacGillivray MH. Effect of dietary protein intake on renal growth: possible role of insulin-like growth factor-I. J Lab Clin Med 1993;122:677–85.
18. Hoppe C, Udam TR, Lauritzen L, Mølgaard C, Juul A, Michaelsen KF. Animal protein intake, serum insulin-like growth factor I, and growth in healthy 2.5-y-old Danish children. Am J Clin Nutr 2004;80:447–52.
19. Rogers I, Emmett P, Gunnell D, Dunger D, Holly J; ALSPAC Study Tteam. Milk as a food for growth? The insulin-like growth factors link. Public Health Nutr 2006;9:359–68.
20. Cadogan J, Eastell R, Jones N, Barker ME. Milk intake and bone mineral acquisition in adolescent girls: randomised, controlled intervention trial. BMJ 1997;315:1255–60.

21. Chellakooty M, Juul A, Boisen KA, et al. A prospective study of serum insulin-like growth factor I (IGF-I) and IGF-binding protein-3 in 942 healthy infants: associations with birth weight, gender, growth velocity, and breastfeeding. J Clin Endocrinol Metab 2006;91:820–6.
22. Ong K, Kratzsch J, Kiess W, Dunger D; ALSPAC Study Team. Circulating IGF-I levels in childhood are related to both current body composition and early postnatal growth rate. J Clin Endocrinol Metab 2002;87:1041–4.
23. Ong KK, Langkamp M, Ranke MB, et al. Insulin-like growth factor I concentrations in infancy predict differential gains in body length and adiposity: the Cambridge Baby Growth Study. Am J Clin Nutr 2009;90:156–61.
24. Koletzko B, von Kries R, Closa R, et al.; European Childhood Obesity Trial Study Group. Lower protein in infant formula is associated with lower weight up to age 2 y: a randomized clinical trial. Am J Clin Nutr 2009;89:1836–45.
25. Koletzko B, von Kries R, Closa R et al. Can infant feeding choices modulate later obesity risk? Am J Clin Nutr 2009;89:Suppl:1502S–8S.
26. Koletzko B, Broekaert I, Demmelmair H, et al.; EU Childhood Obesity Project. Protein intake in the first year of life: a risk factor for later obesity? The E.U. childhood obesity project. Adv Exp Med Biol 2005;569:69–79.
27. Schmidt IM, Main KM, Damgaard IN, et al. Kidney growth in 717 healthy children aged 0-18 months: a longitudinal cohort study. Pediatr Nephrol 2004;19:992–1003.
28. Hinchliffe SA, Sargent PH, Howard CV, Chan YF, van Velzen D. Human intrauterine renal growth expressed in absolute number of glomeruli assessed by the disector method and Cavalieri principle. Lab Invest 1991;64:777–84.
29. Bagby SP. Maternal nutrition, low nephron number, and hypertension in later life: pathways of nutritional programming. J Nutr 2007;137:1066–72.
30. Escribano J, Luque V, Koletzko B, Closa-Monasterolo R. The authors reply. Kidney Int 2011;80:318–9.
31. European Commission. Commission Directive 91/321/EEC of 14 May 1991 on infant and follow-on formulae. Off J Eur Comm 1991;175:35–49.
32. Aggett PJ, Agostini C, Goulet O, et al.; European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition. The nutritional and safety assessment of breast milk substitutes and other dietary products for infants: a commentary by the ESPGHAN Committee on Nutrition. J Pediatr Gastroenterol Nutr 2001;32:256–8.
33. WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards based on length/height, weight and age. Acta Paediatr Suppl 2006;450:S76–S85.
34. Nakamura M, Itoh K. Renal length changes with position. Pediatr Radiol 1998;28:197.
35. Dinkel E, Ertel M, Dittrich M, Peters H, Berres M, Schulte-Wissermann H. Kidney size in childhood. Sonographical growth charts for kidney length and volume. Pediatr Radiol 1985;15:38–43.
36. Kline RB. Principles and Practice of Structural Equation Modeling, 3rd edn. New York: Guilford Press, 2011.
37. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 2000;284:3043–5.
38. Schulz KF, Altman DG, Moher D; CONSORT Group. CONSORT 2010 Statement: Updated guidelines for reporting parallel group randomised trials. J Clin Epidemiol 2010;63:834–40.