

Milk protein intake, the metabolic-endocrine response, and growth in infancy: data from a randomized clinical trial^{1–7}

Piotr Socha, Veit Grote, Dariusz Gruszfeld, Roman Janas, Hans Demmelmair, Ricardo Closa-Monasterolo, Joaquín Escribano Subías, Silvia Scaglioni, Elvira Verduci, Elena Dain, Jean-Paul Langhendries, Emmanuel Perrin, and Berthold Koletzko for the European Childhood Obesity Trial Study Group

ABSTRACT

Background: Protein intake in early infancy has been suggested to be an important risk factor for later obesity, but information on potential mechanisms is very limited.

Objective: This study examined the influence of protein intake in infancy on serum amino acids, insulin, and the insulin-like growth factor I (IGF-I) axis and its possible relation to growth in the first 2 y of life.

Design: In a multicenter European study, 1138 healthy, formula-fed infants were randomly assigned to receive cow-milk-based infant and follow-on formulas with lower protein (LP; 1.77 and 2.2 g protein/100 kcal) or higher protein (HP; 2.9 and 4.4 g protein/100 kcal) contents for the first year. Biochemical variables were measured at age 6 mo in 339 infants receiving LP formula and 333 infants receiving HP formula and in 237 breastfed infants.

Results: Essential amino acids, especially branched-chain amino acids, IGF-I, and urinary C-peptide:creatinine ratio, were significantly ($P < 0.001$) higher in the HP group than in the LP group, whereas IGF-binding protein (IGF-BP) 2 was lower and IGF-BP3 did not differ significantly. The median IGF-I total serum concentration was 48.4 ng/mL (25th, 75th percentile: 27.2, 81.8 ng/mL) in the HP group and 34.7 ng/mL (17.7, 57.5 ng/mL) in the LP group; the urine C-peptide:creatinine ratios were 140.6 ng/mg (80.0, 203.8 ng/mg) and 107.3 ng/mg (65.2, 194.7 ng/mg), respectively. Most essential amino acids, IGF-I, C-peptide, and urea increased significantly in both the LP and HP groups compared with the breastfed group. Total IGF-I was significantly associated with growth until 6 mo but not thereafter.

Conclusions: HP intake stimulates the IGF-I axis and insulin release in infancy. IGF-I enhances growth during the first 6 mo of life. This trial was registered at clinicaltrials.gov as NCT00338689. *Am J Clin Nutr* 2011;94(suppl):1776S–84S.

INTRODUCTION

A high milk protein intake with infant formula provided during the first year of life was shown to induce excessive weight gain in early childhood in a double-blind, randomized controlled trial (1). Animal and observational studies in humans, as well as some small controlled trials in infants (2–4), showed diet, and especially protein intake, to modulate blood concentrations of insulin-like growth factor (IGF)-I (5). The IGF axis is known to regulate early growth and was also shown to influence adipose tissue differentiation and early adipogenesis in animals and in humans (6–8).

IGF-I may exert effects in adipose tissue in an autocrine–paracrine and an endocrine way. It shows a strong structural homology to insulin, which is also reflected in the binding motifs of the IGF binding proteins, which suggests a role of IGF-I and its binding proteins in glucose homeostasis. Furthermore, amino acids, and in particular the branched-chained amino acids (BCAAs) leucine, isoleucine, and valine, are physiologic stimulators of insulin secretion (9).

Because of the different quality of cow-milk protein, formula-fed infants have higher protein intakes than breastfed infants (10). They are reported to have higher concentrations of many plasma amino acids (11) and higher IGF-I concentrations (12, 13), along with different growth patterns in the first 2 y of life (14, 15). Therefore, the hypothesis was raised that the higher protein in formula causes higher BCAA, IGF-I, and insulin concentrations

¹ From the Children's Memorial Health Institute, Warsaw, Poland (PS, DG, and RJ); Dr von Hauner Children's Hospital, University of Munich Medical Centre, Munich, Germany (VG, HD, and BK); the Institute of Social Pediatrics and Adolescent Medicine, University of Munich, Munich, Germany (VG); Universitat Rovira i Virgili, IISPV, Tarragona, Spain (RC-M and JES); the Department of Pediatrics, University of Milano, Milan, Italy (SS and EV); the Department of Pediatrics, Université Libre de Bruxelles, Brussels, Belgium (ED); CHC St Vincent, Liège-Rocourt, Belgium (J-PL); and the Danone Research Centre for Specialized Nutrition, Schiphol, Netherlands (EP).

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³ PS and VG contributed equally to this manuscript.

⁴ Members of the European Childhood Obesity Trial Study Group are listed before the References.

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⁷ Address correspondence to P Socha, Al. Dzieci Polskich 20, 04-730 Warszawa, Poland. E-mail: p.socha@czd.pl.

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and thereby leads to accelerated growth, increased adipose tissue, and increased risk of later obesity (16, 17). We explored the effects of protein intake on biochemical and endocrine markers at the age of 6 mo in healthy term infants who participated in the European Childhood Obesity Project (1). Furthermore, we looked into the effect of IGF-I and C-peptide on growth during the first 2 y of life. During this period we have seen a differential growth between the higher and lower protein groups in the same study (1).

SUBJECTS AND METHODS

Inclusion criteria and dietary intervention

The subjects were participants of a double-blind, randomized, multicentric intervention trial conducted in Germany, Belgium, Italy, Poland, and Spain. The formula-fed infants were randomly assigned to receive formulas with higher and lower protein content, which were given to the children until 12 mo of life. In addition, a nonrandomized reference group of breastfed infants was followed (1). Briefly, eligible for study participation were apparently healthy, singleton, term infants who were born from uncomplicated, singleton pregnancies. Infants were enrolled during the first 8 wk of life (median age at study entry: 14 d). Formula-fed infants had to be exclusively formula fed at the end

of the eighth week of life. Breastfed children had to be exclusively breastfed for the first 3 mo.

Infant formulas were replaced by follow-on formulas from the fifth month of age onwards, as has been suggested by the European Union Directive of 1991 (18). The lower protein (LP) and higher protein (HP) infant and follow-on formulas (manufactured by Bledina, Steenvoorde, France, and provided free of charge to families) differed in the content of cow-milk protein but had an identical energy density because the difference in protein content was adjusted through the fat content (Table 1). The composition of all study formulas complied with the 1991 European Union Directive on Infant and Follow-on Formulae (18), and protein contents represented approximately the lowest and highest amounts, respectively, of the range accepted in this Directive. The relative contents of amino acids did not differ between all 4 formulas (eg, BCAAs made up ≈23% of the protein content in all infant and follow-on formulas). An exception was the LP infant formula, which was supplemented with small amounts of arginine and tryptophan.

Recruitment procedures in all centers were designed to promote and support breastfeeding. Introduction of any food other than study formula or breast milk before the age of 4 completed months was discouraged, but no other attempts were made to influence the local and family traditions of the introduction of solids into the infants' diets.

TABLE 1
Macronutrient and amino acid content of study formulas compared with human milk¹

	Infant formula		Follow-on formula		Human milk
	Lower	Higher	Lower	Higher	
Whey:casein ratio	1:4	1:4	1:4	1:4	3:2
Energy (g/100 mL)	69.9	69.8	72.7	72.5	70
Proteins (g/100 mL)	1.25	2.05	1.6	3.2	1.2
Protein (% of energy)	7.1	11.7	8.8	17.6	—
Nonprotein nitrogen (g/100 mL)	0.07	0.1	0.08	0.15	—
Nonprotein nitrogen (% of energy)	0.4	0.6	0.45	0.9	—
Lipids (g/100 mL)	3.9	3.5	4.0	3.27	3.6
Carbohydrates (g/100 mL)	7.5	7.5	7.6	7.6	7.4
Essential amino acids (dL/100 mL)					
Cysteine + methionine	40	66	52	103	37
Histidine	32	53	41	82	25
Isoleucine	77	128	100	200	57
Leucine	119	197	154	308	104
Lysine	94	155	121	243	69
Phenylalanine	58	97	75	151	39
Threonine	56	92	72	144	46
Tryptophan	22	29	23	46	19
Valine	84	139	108	216	58
Total essential amino acids	582	956	746	1493	432
Nonessential amino acids (dL/100 mL)					
Alanine	42	69	54	108	37
Arginine	50	74	57	115	38
Asparagine	89	147	115	230	83
Glutamic acid	286	473	369	738	179
Glycine	24	40	31	62	23
Proline	135	223	174	348	88
Serine	71	118	92	184	45
Tyrosine	62	103	80	161	43
Total nonessential amino acids	759	1247	972	1946	536

¹ Human milk values were taken from the Davis Area Research on Lactation, Infant Nutrition, and Growth (DARLING) study (19); amino acid concentrations in human milk were taken from reference 20.

The study followed the recommendations made in the CONSORT (Consolidated Standards of Reporting Trials) guidelines (21). It was approved by the ethics committees of all study centers, and written informed parental consent was obtained for each infant.

Study population

The allocation, demographic, dietary, anthropometric, and clinical characteristics of all 1678 originally enrolled infants has been described in detail previously (1). A total of 1200 children were still participating in the study at the age of 6 mo. In all 312 children from Italy, no blood was collected. In 812 (91%) of the remaining 888 children, blood or urine samples were available [606 children in the intervention groups and 206 in the breastfed group (**Figure 1**)]. For 588 children, both blood and urine samples were collected at the age of 6 completed months. Breastfed infants, as well as girls, abstained significantly more often from blood or urine testing than formula-fed infants or boys, respectively. There were also some differences between countries in the frequency of blood or urine sampling: whereas all the Polish children's samples were available, only 78% of the Belgium children's samples were available. The characteristics of the population of the LP and HP groups with blood and/or urine samples did not differ significantly from the original 1678 children or between both groups at 6 mo of age with regard to gender, mother's education, smoking in the family, mother's age, and birth weight, which indicates that the randomization was not substantially disturbed in this subsample.

The intakes of energy and protein as measured by 3-d food protocols, and their difference between the HP group and the LP group, were approximately the same in our subsample as in the whole study group (1), showing the same energy intake in both groups except at 6 mo (when the LP group had slightly higher intakes), and higher protein intakes up to age 12 mo in the HP group. Weight and length were measured in accordance with standardized procedures at baseline (ie, inclusion in the study) and at 6, 12, and 24 mo. Anthropometric measures were expressed

as z scores relative to the growth standards of the World Health Organization for breastfed children (22).

Laboratory procedures

At 6 mo of age, a venous blood sample was drawn and a urine sample was collected with the use of a baby urine collection bag. Efforts were undertaken to draw blood ≥ 2 h after the last feed. Urine and serum samples were stored at -70°C and transported on dry ice to one central laboratory (The Children's Memorial Health Institute, Warsaw, Poland) for analysis of serum amino acids, total IGF-I, free IGF-I, IGF-BP2, IGF-BP3, and urinary C-peptide and creatinine. Glucose and urea were analyzed in the respective laboratories of the local study centers.

Quantitative amino acids analysis was performed by HPLC with the use of the Pico-Tag method (Waters, Milford, MA). The samples were deproteinized by ultra filtration (Ultrafree; Millipore, Billerica, MA). Precolumn derivatization of the amino acids with phenylisothiocyanate was followed by separation of the derivatized amino acids by reversed HPLC. Amino acids were detected by measurement of the absorbance of the column eluate at 254 nm. The computer program Millennium 32 (Waters) was used for the identification of amino acids and the calculation of the results (23).

The variables of the IGF axis were measured with the use of immunoradiometric assay kits, and urinary C-peptide with a radioimmunoassay kit (all from Diagnostic Systems Laboratories Inc, Webster, TX). Urine creatinine was analyzed with a kinetic assay based on the Jaffe reaction in an automated ADVIA 1650/Mega (Bayer Healthcare AG, Leverkusen, Germany). The urine samples were centrifuged at $2000 \times g$ for 10 min at 4°C before measurement. Urinary C-peptide was expressed per mg urinary creatinine/dL, as suggested by others (24, 25).

Amino acids, variables of the IGF axis, and C-peptide were not normally distributed and are presented as median values with interquartile ranges (25th and 75th percentile). Group comparisons were done with a Kruskal-Wallis rank test as appropriate.

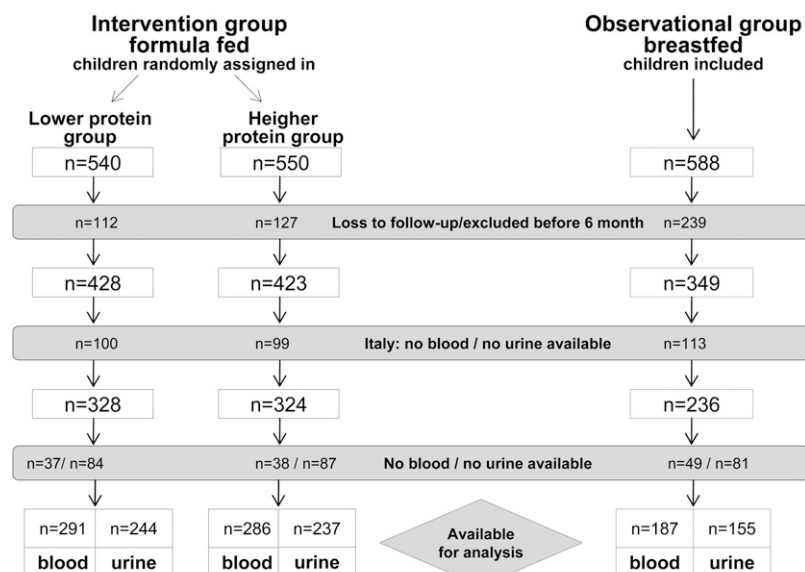


FIGURE 1. Numbers of participants and of samples available for analysis at 6 mo of age.

Spearman rank correlations with Bonferroni correction for multiple testing were calculated to look for correlations between weight-for-length at baseline and at 6 and 12 mo of age, the difference between consecutive time points, and IGF-I total and C-peptide. Significance was assumed at $P < 0.05$. All statistical analyses were performed with Stata 9.2 (StataCorp, College Station, TX).

RESULTS

Serum amino acids and urea

The comparison of serum concentrations of the 18 amino acids assessed is summarized in **Table 2**. The totals of these free amino acids were 2841 (interquartile range: 2523, 3186) $\mu\text{mol/L}$ in the LP and 3041 (interquartile range: 2679, 3394) $\mu\text{mol/L}$ in the HP group ($P < 0.001$). The biggest differences between the LP group and the HP group were seen for the BCAAs valine (+42%), leucine (+37%), and isoleucine (+32%). Concentrations for all other essential amino acids were $\geq 10\%$ higher in the HP group than in the LP group, and the sum of all essential amino acids was significantly higher ($P < 0.001$) in the HP group than in the LP group (**Figure 2**). In contrast, most nonessential amino acids were either not different or even lower in the HP group; only tyrosine and asparagine were significantly higher in the HP group. Total nonessential amino acids had lower concentrations in the HP group ($P = 0.001$). We observed no differences in amino acid concentrations between males and females in both formula groups. Serum urea concentration was significantly higher in infants fed HP formula (**Table 3**).

IGF-I, IGF-BP2, and IGF-BP3

In the HP formula group, serum concentrations of total IGF-I and free IGF-I were $\approx 40\%$ higher than in the LP formula group, whereas IGF-BP2 concentrations were $\approx 30\%$ lower (Table 3). There was no significant group difference for IGF-BP3.

Urinary C-peptide and serum glucose

Infants receiving the HP formula showed a higher urinary C-peptide concentration and C-peptide:creatinine ratio and a lower serum glucose concentration than did those receiving the LP formula (Table 3).

IGF-I total, C-peptide, and anthropometric data at baseline and at 6, 12, and 24 mo

Total IGF-I was positively associated with weight-for-length at 6, 12, and 24 mo (**Figure 3**). However, looking at change in weight-for-length between baseline and 6 mo, 6 and 12 mo, and 12 and 24 mo, only the first was significantly associated with total IGF-I concentrations (Figure 3). This association persisted after adjustment for the baseline measurement of weight-for-length. C-peptide showed no association with weight-for-length or change in weight-for-length (data not shown).

Breastfed children

There were remarkable differences between both formula groups and the breast milk group. Compared with the breastfed group, variables of the IGF axis and C-peptide were increased in the formula groups. Total IGF-I, free IGF-I, and IGF-BP3 concentrations were all significantly, $\leq \approx 60\%$, lower in the breastfed

TABLE 2

Serum amino acid (AA) concentrations in infants at 6 mo of age fed higher-protein (HP) or lower-protein (LP) formula and in breastfed (BF) infants¹

	LP	HP	P value (HP compared with LP)	BF
Essential amino acids ($\mu\text{mol/L}$)				
Isoleucine	64 (50, 80) ²	85 (62, 114) ³	<0.001	58 (46, 74)
Leucine	120 (98, 143) ⁴	165 (124, 212) ³	<0.001	106 (90, 133)
Lysine	166 (134, 197) ³	197 (156, 248) ³	<0.001	145 (121, 184)
Methionine	31 (26, 39) ³	35 (26, 46) ³	<0.001	27 (22, 35)
Phenylalanine	72 (61, 83) ³	84 (70, 100) ³	<0.001	61 (48, 74)
Threonine	126 (101, 154)	142 (118, 173) ³	<0.001	119 (92, 150)
Tryptophan	56 (47, 67) ⁴	67 (54, 82) ³	<0.001	60 (50, 74)
Valine	214 (182, 247) ³	304 (241, 376) ³	<0.001	172 (143, 208)
Nonessential amino acids ($\mu\text{mol/L}$)				
Alanine	440 (346, 526)	420 (349, 517)	0.304	430 (355, 495)
Arginine	115 (97, 137)	110 (91, 128)	0.038	113 (91, 129)
Asparagine	54 (45, 64)	58 (47, 68) ⁴	0.015	52 (45, 64)
Aspartic acid	25 (17, 35)	27 (19, 35)	0.143	26 (18, 38)
Glutamine	605 (542, 683) ³	556 (490, 613) ³	<0.001	664 (573, 748)
Glutamic acid	122 (95, 168)	115 (88, 172)	0.179	130 (90, 193)
Glycine	267 (217, 319) ³	230 (199, 273) ²	<0.001	220 (185, 264)
Histidine	105 (88, 123) ³	107 (93, 124) ³	0.215	88 (74, 105)
Serine	161 (138, 194) ³	159 (140, 189) ³	0.750	187 (156, 207)
Tyrosine	83 (70, 103) ³	101 (76, 125) ³	<0.001	66 (54, 80)

¹ All values are medians; interquartile ranges in parentheses. P values were computed with the use of the Kruskal-Wallis rank test.

²⁻⁴ Significantly different from the BF group: ² $P < 0.05$, ³ $P < 0.001$, ⁴ $P < 0.01$.

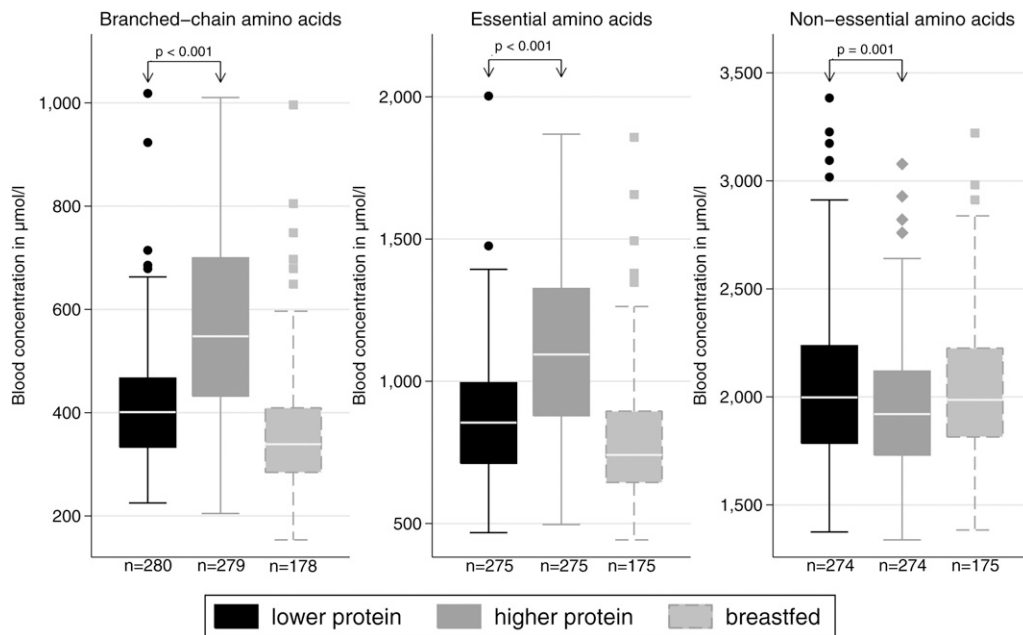


FIGURE 2. Serum amino acid concentrations in formula-fed and breastfed infants at 6 mo of age.

than in the formula groups (Table 2). IGF-BP2 was markedly higher in the breastfed group than in both formula groups. Serum glucose, urinary C-peptide, and the C-peptide:creatinine ratio were all significantly lower in the breastfed than in the formula groups (Table 2). Essential amino acids, especially BCAAs, were lower in the breastfed group than in the LP group, whereas nonessential amino acids had approximately the same concentrations (Figure 2).

DISCUSSION

This randomized controlled trial shows that the endocrine and metabolic response of infants is significantly affected by dietary protein supply. HP compared with LP formula increased plasma concentrations of essential amino acids, especially BCAAs, whereas nonessential amino acids were lowered. Higher protein intakes increased concentrations of total and free IGF-I, whereas IGF-BP2 was decreased and IGF-BP3 was unaffected by the protein intake; urinary C-peptide concentrations, which repre-

sented endogenous insulin secretion, increased and serum glucose concentrations decreased. Breastfed children had generally lower plasma amino acid concentrations and a less active IGF-I axis and lower insulin production than did formula-fed children. Lowering the protein intake with infant formula resulted in a metabolic and endocrine response in formula-fed infants more similar to that of breastfed infants. In addition, we have shown that IGF-I concentrations at 6 mo of age are associated with weight gain in the first 6 mo of life, but not thereafter.

Serum amino acids

Changes in plasma concentrations of amino acids due to nutritional intake, and their significance, have not been studied extensively (26). Therefore, most conclusions from observations in this study can only be speculative. Whereas the HP group had a $\geq 50\%$ higher protein supply than did the LP group, with identical amino acid composition, total serum free amino acids were only marginally higher in the HP group. The serum amino acid pattern was significantly shifted toward essential amino

TABLE 3

Serum concentrations of insulin-like growth factor (IGF)-I free, IGF-I total, IGF-binding protein (IGF-BP) 2, IGF-BP3, glucose, urea, and urinary C-peptide in infants receiving lower-protein (LP) and higher-protein (HP) formula and in breastfed (BF) infants¹

Variable	LP	HP	P value (HP compared with LP)	BF
IGF-I free (ng/mL)	0.43 (0.27, -0.77)	0.60 (0.34, 1.11)	<0.001	0.31 (0.21, 0.48)
IGF-I total (ng/mL)	34.7 (17.7, 57.5)	48.4 (27.2, 81.8)	<0.001	14.1 (5.1, 33.2)
IGF-BP2 (ng/mL)	1090 (865, 1438)	765 (575, 1013)	<0.001	1370 (1055, 1740)
IGF-BP3 (ng/mL)	2908 (2449, 3440)	2969 (2538, 3483)	0.248	2454 (1984, 2794)
C-peptide:creatinine (ng/mg)	107.3 (65.2, 194.7)	140.6 (80.0, 203.8)	0.030	57.0 (27.3, 119.3)
C-peptide (ng/mL)	19.5 (9.4, 34.6)	26.9 (13.3, 45.6)	0.002	9.3 (3.5, 20.1)
Glucose (mg/dL)	85 (77, 93)	83 (77, 89)	0.022	86 (79, 93)
Urea (mg/dL)	18 (14, 21)	29 (20, 36)	<0.001	11 (8, 16)

¹ All values are medians; interquartile ranges in parentheses. P values were computed with the use of the Kruskal-Wallis rank test. P < 0.001 for comparison of LP and BF groups (except for glucose). P < 0.001 for comparison of HP and BF groups.

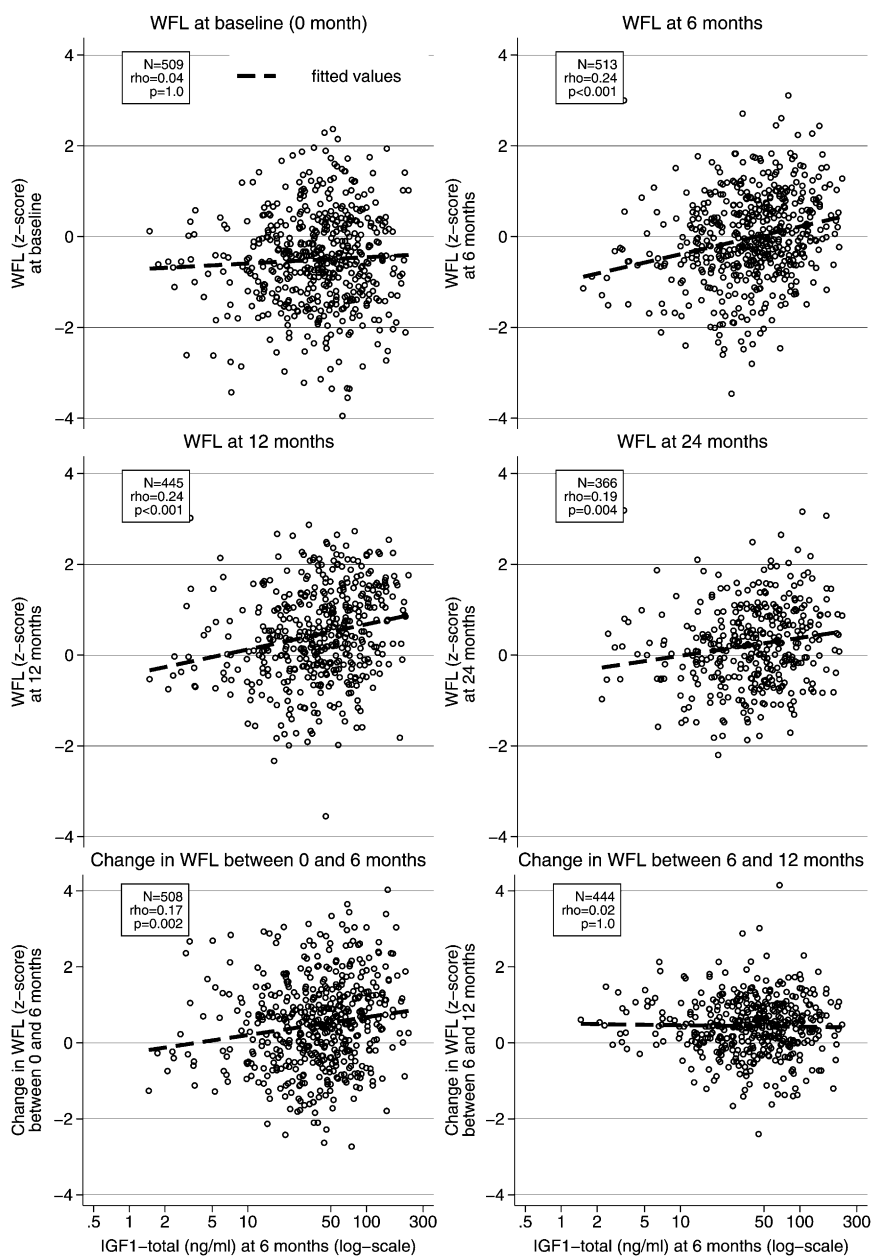


FIGURE 3. Insulin-like growth factor I (IGF1) total at 6 mo of age and weight-for-length (WFL) at baseline (median 14 d) and at 3, 6, and 12 mo, as well as difference in WFL between 0 and 6 mo and 6 and 12 mo with number of children, Spearman correlation coefficient, and *P* value.

acids, an effect that was also seen in other small randomized trials that compared a higher and lower protein intake with infant formula (2, 27). Another study (28) that compared groups with higher and lower protein intake showed at 3 mo of age a difference in plasma concentrations of leucine, isoleucine, tyrosine, phenylalanine, lysine, and threonine but not for histidine, methionine, cysteine, tryptophan, or valine. In agreement with our results, the same authors observed that even a formula with 1.1 g/100-mL protein content did not produce amino acid concentrations similar to those of breastfed children, which emphasizes that not only the amount of protein but also the type of protein, for instance the whey-to-casein ratio, and corresponding differences in amino acid composition, are of importance (29).

Formula-fed infants tend to have higher postprandial (27) and fasting (28, 39, 31) concentrations of BCAAs compared with

breastfed infants. The strong effect of protein intake on BCAAs seen in this study may be important for biological and functional effects. The lower concentrations of nonessential amino acids with higher protein are not fully understood but may be related to the extensive catabolism of absorbed nonessential amino acids in the intestine, as well as the major contribution of their de novo synthesis on serum concentrations. Nonessential amino acids are more extensively catabolized in the intestine than are essential amino acids (32) and thus are less affected by an increased protein intake.

Like other authors (30), we interpret the significant differences in plasma urea concentrations between the HP group and the LP group as well as between both formula groups and the breastfed group as a reflection of enhanced amino acid catabolism. However, when formula-fed and breastfed infants are compared, several



other studies did not find differences (28, 33, 34), which highlights the limitation of this comparison because formula differs in many aspects (eg, composition and kinetics of digestion).

Insulin and IGF axis

The increased urinary C-peptide:creatinine ratio is a reflection of enhanced insulin secretion. Higher urinary C-peptide concentrations and postprandial insulin secretion were reported previously in formula-fed compared with breastfed or between LP and HP formula-fed children (35–38).

The observed effect of formula protein contents on total and free IGF-I agrees with earlier observations of lower IGF-I concentrations in breastfed compared with formula-fed infants (12, 13, 39) as well as observations of higher concentrations of IGF-I associated with higher protein or milk intakes in infants and children (40–42), adolescents (39), and adults (43–45). Several animal and human studies have reported reduced IGF-I concentrations caused by underweight or protein-caloric malnutrition (46). Amino acid supply seems to play an important role in the insulin-related metabolic activity. BCAAs, especially leucine, have been reported to stimulate insulin release (47, 48) but not necessarily IGF-I (49).

The observed lower IGF-BP2 concentrations agree with the increased IGF-I total and free concentrations, which indicates a marked modulation of the IGF axis by higher dietary protein intake (5). IGF-BP2 acts mainly by reduction of the IGF-I bioavailability and was shown to inhibit adipogenesis by modulation of IGF-I activity (50). Overexpression of IGF-BP2 in a transgenic mouse model led to a significant reduction in fat cell size in both chow-fed and high-fat-fed mice, which further supports an important function of IGF-BP2 in adipocyte biology and a protective role IGF-BP2 against obesity development (51).

Contrary to IGF-I and IGF-BP2, the concentrations of IGF-BP3 did not differ between the formula-fed groups. This is somewhat surprising because IGF-BP3 is the major binding protein of IGF-I, and binds >90% of IGF-I (52). However, IGF-BP3 is relatively stable and is only depressed after prolonged periods of severe malnutrition (5). Factors other than protein intake have to explain the difference in IGF-BP3 between formula-fed and breastfed infants. Some experimental data suggest an inhibitory effect of IGF-BP3 on adipogenesis (53). IGF-BP3 has also been shown to inhibit insulin action independently of IGF-I and was reported to induce insulin resistance (51).

Long-term effects of elevated IGF-I concentrations in infancy have been proposed. Formula feeding, higher protein intake, and higher IGF-I concentrations in infancy have been associated with lower IGF-I concentrations in later life (39, 54–56), whereas breastfeeding is associated with lower IGF-I in infancy but higher IGF-I in later childhood (54). In otherwise healthy adults, a lower IGF-I concentration is associated with an increased risk of both ischemic heart disease and diabetes (57) as well as with neoplasia of the prostate and the breast (58). Therefore, early programming of the IGF-I axis may have a relevant effect on the later risk of adult diseases (54).

IGF-I, C-peptide, and growth

In the present study, IGF-I at 6 mo of age was associated with weight at 6, 12, and 24 mo but with weight gain only in the first

6 mo of age and not thereafter. This might indicate that IGF-I concentrations are especially important in early growth. IGF-I has been shown to be positively related to fetal growth and birth weight (59–61). Savino et al (62) also showed that IGF-I was directly correlated with the weight, body mass index, and tricipital skinfold thickness during the first 5 mo of age. Whereas one study showed that IGF-I was associated with immediate postnatal growth (63), another study, which looked at IGF-I concentrations at 3 mo of age and later growth, showed an association only with length gain until 12 mo of age but not with weight gain (64). Our observation agrees with our previous observation (1) that higher protein intake led to a differential weight gain during this period with a persistent weight-for-length difference until 24 mo of age.

Strengths and limitations

A strength of this study is the double-blind randomized design and the large number of infants included, which provides convincing evidence for a causal relation between the dietary intervention and the observed metabolic and endocrine response. In contrast to some previous studies that evaluated the effects of formula feeding on plasma amino acid patterns, in our study only the total protein content, but not the amino acid composition, of the study formulas was modified, which is of importance because some plasma amino acid concentrations were shown to be higher when whey-dominant rather than casein-dominant protein sources were supplied (29). A limitation of the study was that, to limit the burden of the healthy infants who participated, only blood samples from one time point were available. The chosen time point of 6 mo, however, is considered to be within the relevant time window when diet may affect growth, body composition, and long-term health. There was considerable attrition but we did not have any indication of a differential drop-out between intervention groups, or of a relevant introduction of bias.

Potential relevance

Our results indicate that protein intake of infants modulates the IGF axis and insulin release, which is associated with a higher weight-for-length and body mass index at the age of 2 y (1). These effects have all been shown to influence later health. A reduced protein content of formulas, more similar to the protein content of human milk, supports an endocrine and metabolic profile of formula-fed infants that is closer to that of breastfed infants. However, considerable differences between formula-fed and breastfed infants remain that are not explained by the level of protein supply.

The European Childhood Obesity Trial Study Group members are as follows: Annick Xhonneux (CHC St Vincent, Liège-Rocourt, Belgium); Anna Stolarczyk, Jerzy Socha, Roman Janas, and Ewa Pietraszek (Children's Memorial Health Institute, Warsaw, Poland); Sabine Verwied-Jorky, Sonia Schiess, Ingrid Pawellek, Uschi Handel, Iris Hannibal, and Michaela Fritsch (Dr von Hauner Childrens Hospital, Ludwig Maximilians University of Munich, Germany); Helfried Groebe, Anna Reith, and Renate Hofmann (Klinikum Numberg Sued, Nurnberg, Germany); Pascale Poncelet (Université Libre de Bruxelles, Brussels, Belgium); Verónica Luque Moreno, Georgina Méndez Riera, Marta Zaragoza-Jordana, and Natalia Ferré (Universitat Rovira i Virgili, IISPV, Tarragona, Spain); and Carlo Agostoni, Fiammetta Vecchi (University of Milan, Italy).



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