Glutamate Supply and Metabolism in Infants

Berthold Koletzko

Ludwig-Maximilians-University Munich, Dr. von Hauner Children's Hospital, University of Munich Medical Center, Munich, Germany

Keywords

Amino acid metabolism · Breastfeeding · Infant formula feeding · Glutamic acid · Dispensable amino acids · Splanchnic circulation

Abstract

Glutamate (Glu) is the most abundant amino acid (AA) in human milk. Available information on Glu supply and metabolism was reviewed, based on an electronic literature search. In 22 cross-sectional global studies with 4,747 women, free Glu increases with lactation duration and contributes to about 38% of total and 63% of dispensable milk AA. Based on these data, a typical breastfed infant obtains a mean free Glu intake of 35.7 mg/kg body weight (bw; upper end of the normal range 134.6 mg/kg), while precisely collected longitudinal data indicate mean intakes of 40 mg/kg and upper range intakes of 70.2 mg/kg. Mean intakes with cows' milk formula at median concentrations are 0.7 mg/kg (upper range 1.0 mg/kg) and with extensive protein hydrolysate formula 171.7 mg/kg bw (upper range 249.8 mg/kg bw), with no indication of untoward effects. Infants almost entirely utilize dietary Glu in splanchnic organs, with no appreciable effect on plasma Glu concentrations. Estimated free Glu in-

takes in breastfed infants are up to 4.5-fold higher than the acceptable daily intake (ADI) set by the European Food Safety Authority for the total population including infants, and the respective intakes of infants fed a formula based on an extensive protein hydrolysate are up to 8.3-fold higher. There is no reason to assume that feeding human milk or an extensive protein hydrolysate infant formula would be unsuitable or unsafe for infants. Setting an ADI below the range of the normal intake with a safe diet in generally healthy individuals, that is, below 250 mg/kg per day, seems inappropriate.

© 2018 The Author(s)

Published by S. Karger AG, Basel

Introduction

An adequate supply and metabolism of amino acids (AA) is central to infant and child growth, structural integrity of all body cells and tissues, and the normal function of enzymes, transport molecules, and hormones [1–3]. Protein is formed from 20 α -AA that are classified as indispensable (or essential) and dispensable (non-essential) AA. Recommendations on reference nutrient intakes (also referred to as population reference intakes or rec-

ommended dietary allowances) for infants and children usually provide guidance on adequate intakes for protein but not for individual AA [4]. For infant formula and for follow-on formula for infants, requirements for minimum contents of indispensable AA have been defined based on typical amounts provided with breastfeeding, but the contents of dispensable AA are generally not subjected to regulatory requirements [5–9].

Breastfeeding is considered the optimal choice of infant feeding [10, 11], and the quantitative assessment of the normal range of nutrient supply in breastfed infants can provide guidance on suitable and safe intakes in infancy [12]. The quantitatively dominant AA in human milk is the dispensable AA glutamic acid (glutamate [Glu]). Therefore, available information on Glu supply and metabolism in infants are reviewed here, including information on the feeding of infant formulae which have a history of an apparently safe use.

Methods

The database PubMed of the US National Library of Medicine was searched in May 2018 and for an update again on September 15, 2018, with the search terms “(glutamic acid OR Glu) AND infant.” All hits were screened by the author for publications providing quantitative information on Glu supply with human milk and Glu metabolism in infants. Infant Glu intakes were calculated based on a reference infant with a weight of 5 kg consuming per day an energy content of 500 kcal (100 kcal/kg body weight [bw] per day), as defined by the Scientific Committee on Food of the European Commission and the European Food Safety Authority [13, 14]. Glu values expressed as $\mu\text{mol/L}$ were converted into mg/dL by multiplication with 0.014713. Results are presented here in the form of a narrative review.

Results

Nitrogen Supply with Breastfeeding

Lonnerdal et al. [15] performed a systematic review compiling the results of 43 original articles published between 1953 and 2011 that provided data on nitrogen supply with human milk. Of the nitrogen in human milk, about 75–80% is comprised of true protein which provides 90–95% of the total AA (TAA). Some 20–25% of the human milk nitrogen is provided as non-protein nitrogen comprising about 8–22% free AA (FAA; about 5–10% of TAA) as well as peptides, urea, ammonia, creatinine, creatine, nucleic acids, carnitine, amino sugars and other components. The true protein content in human milk continuously declines with increasing duration of lactation.

Median contents of true protein in milk are 2.06 g/100 mL at 0–5 days after delivery, 1.57 g/100 mL at 16 and 30 days, and 1.10 g/100 mL at 90–360 days [15].

Glu Supply with Breastfeeding

Zhang et al. [16] published a systematic review of all data on both TAA and FAA in term and preterm human milk globally that were published until February 2010. The authors provide a collection of cross-sectional data based on studies of apparently healthy women on free-living diets who delivered healthy term infants, or preterm infants with a birth weight appropriate for gestational age. Included were only AA data derived from ion exchange chromatography or an automatic AA analyzer with quantitative data on FAA or on TAA, the latter measured in samples treated for protein hydrolysis. Most studies were from Asia, Europe, and North America, while there was a paucity of data from Africa. Some 26 studies including 3,774 subjects provided results on human milk TAA, whereas 22 studies with 4,747 subjects reported FAA data. Only 6 of the studies reported on preterm human milk.

In these cross-sectional data, Glu was the quantitatively most abundant AA in human milk. For example, in mature human milk collected between 21 and 58 days of lactation, the mean Glu content of 201.3 mg/dL contributed to 30.5% of the total DAA (639.7 mg/dL) and 12.1% of the TAA (1,669.3 mg/100 dL). Along with the decrease of the mean total milk nitrogen content with increasing duration of lactation from 3.5 g/dL in colostrum (100%) to 2.6 g/dL (74.3%) in transitional and 1.9 g/dL (54.3%) in mature milk at 4 months, milk total Glu decreased proportionally from 332.7 mg/dL in colostrum (100%) to 241.9 mg/dL (72.7%) in transitional and 189.2 mg/dL (56.9%) in mature milk at 4 months (Fig. 1).

Among the FAA, again Glu was quantitatively most abundant, contributing at 2 months of lactation a mean 1,175 $\mu\text{mol/L}$, equivalent to 63.4% of the free DAA (1,883.3 $\mu\text{mol/L}$) and 38.2% of the total FAA (3,079.9 $\mu\text{mol/L}$). In contrast to TAA, free Glu concentrations in milk increased during the first months after birth (Fig. 2), along with an increase of Glu, whereas there was a marked decrease of the concentrations of all other FAA except for alanine, glycine, serine, and cysteine.

Transitional preterm human milk showed a trend to slightly higher total Glu concentrations than transitional term milk (305.3 vs. 252.9 mg/100 mL) [16], reflecting the known higher total nitrogen and protein contents in preterm compared to term milk during the early stages of

Fig. 1. Mean total glutamate (Glu) in human milk (mg/100 mL) based on cross-sectional global data at different stages of lactation (colostrum = 0–5 days, transitional milk = 6–20 days, 2 months = 21–58 days, 4 months = 59–135 days, 18 months = 136–540 days). Drawn from data of reference [16].

Fig. 2. Mean free glutamate (Glu) in human milk (μmol/L) based on cross-sectional global data at different stages of lactation (colostrum = 0–5 days, transitional milk = 6–20 days, 2 months = 21–60 days, >2 months = >60 days). Drawn from data of reference [16].

lactation [17]. Of interest, the mean TAA contents in human milk reported by Zhang et al. [16] are rather similar to those that were proposed in 2005 [5] and adopted by Codex Alimentarius [6] for setting the global regulatory standard for infant formula composition.

Recently, van Sadelhoff et al. [18] published a methodologically impressive study on the longitudinal evolution of TAA and FAA contents in milk samples collected monthly from 1 to 6 months of lactation in the same group of European women, which provides important additional and presumably more precise information on the change of AA content with duration of lactation [18]. Again Glu was quantitatively clearly dominant among the FAA. Free Glu milk concentration increased from 1 to 4 months of lactation and then reached a stable plateau (Fig. 3). In addition, the contribution of Glu to the sum of FAA increased from 53.7% at one month of lactation to 56.9% at 6 months.

The daily Glu intake of breastfed infants can be estimated for a reference infant with a weight of 5 kg, consuming a daily energy intake of 500 kcal (100 kcal/kg bw per day) [13, 14]. Based on an energy content of 63 kcal/dL of human milk at 3–6 months of lactation [12], the mean daily human milk volume intake can be calculated as 158.7 mL/kg bw. At the age of 4 months, the SD of milk intake of healthy breastfed infants was recorded as 32.5%

of the mean (221/680 mL) [12], thus the upper end of the normal range of human milk intake of infants (mean plus 2 SD) can be estimated as 261.9 mL/kg bw (158.7 mL/kg * 1.65).

The total Glu intake was calculated based on cross-sectional global data on Glu milk concentration at 39–135 days and free Glu intake based on milk concentrations from the same data set at >60 days [16].

The assumed variation of free Glu in human milk was based on the data of Agostoni et al. [19] who measured the free Glu concentration in human milk of 40 healthy, well-nourished mothers of term infants at the end of the first month of lactation as 284.8 ± 182.9 μmol/L (mean ± SD), that is, the SD equaled 64.2% of the mean concentration. Thus, the mean +2SD can be calculated as 228.4% of the mean (Table 1).

Accordingly, the mean daily intake of a breastfed reference infant is 300.3 mg total Glu/kg bw and 35.7 mg free Glu/kg bw, based on cross-sectional global data [16] (Table 1). At the upper range of the normal distribution of high milk intakes (mean + 2SD, expected to cover about 95% of the infant population), the free Glu intake is 81.5 mg/kg bw with a mean milk Glu concentration and 134.6 mg/kg bw with a high milk Glu concentration (mean + assumed 2SD) and high milk volume (mean + 2SD), again based on cross-sectional global data [16] and

Fig. 3. Mean free glutamate (Glu) in human milk ($\mu\text{mol/L}$) based on longitudinal, monthly collected data from 1 to 6 months of lactation in a cohort of European women. Drawn from data of reference [18].

variation of free Glu as reported by Agostoni et al. [19] (Table 1). Based on the presumably more precise recent data obtained at 3 months of lactation in a longitudinal European study, the mean intake of free Glu is 40 mg/kg bw with a mean milk Glu concentration and 70.2 mg/kg bw with both a high milk Glu concentration and a high milk volume (mean + 2SD; Table 1).

Free Glu Supply with Infant Formula

Agostoni et al. [19] and Ventura et al. [20] reported free Glu contents in 12 and 6, respectively, standard infant formula products based on intact cows' milk protein, partial protein hydrolysate, and soy protein isolate. Compared to human milk, free Glu concentrations were lower in powdered and liquid formulae based on intact or partially hydrolyzed cows' protein (about 0.15–1.9 mg/dL), and also in formula based on soy protein isolate (undetectable to 0.15 mg/dL). In contrast, very high free Glu concentrations of about 110–120 mg/dL were measured in formulae based on extensive protein hydrolysates [20].

Based on an average formula energy density of 67 kcal/dL and a daily energy intake of the reference infant of 100 kcal/kg, the resulting mean daily formula intake is 149.3 mL/kg. Thus, the average daily free Glu intake with a cows' milk based formula with a median free Glu concentration of 0.41 mg/dL is 0.71 mg/kg bw. Accordingly, average daily free Glu intake with an extensive protein hydrolysate with a median free Glu concentration of

115 mg/dL is 179.2 mg/kg bw (Table 1). In a clinical trial published by Fleddermann et al. [2], the mean $\pm$ 2SD daily energy intake of healthy infants aged 60 days fed a standard infant formula with 67 kcal/dL was 145.5 kcal/kg bw, which is equivalent to 217.2 mL formula/kg bw. At this volume intake, a cows' milk-based formula (median free Glu 0.41 mg/dL) provides a free Glu intake of 1.03 mg/kg bw, and an extensive protein hydrolysate formula (median free Glu 115 mg/dL) 249.78 mg/kg bw (Table 1).

Metabolism of Enteral Glu Supply in Infants

Hays et al. [21] studied the metabolism of enterally supplied Glu in premature infants using stable isotope methodology. Five enterally fed preterm infants with a gestational age of 31 ± 0 weeks and a weight of $1,555 \pm 131$ g (mean $\pm$ SE) were studied twice at the postnatal ages of 10 ± 1 and 17 ± 1 days, respectively, and received an intragastric infusion of Glu labeled uniformly with ^{13}C . Plasma Glu remained unaffected by the Glu infusion. Plasma ^{13}C glucose enrichment was only 0.3% after 5 h ingestion of Glu labeled to 4%, indicating an only minor and insignificant contribution of dietary Glu carbon to glucose. The authors conclude that splanchnic extraction is the major fate of dietary Glu, and Glu is not a significant gluconeogenic substrate in these infants.

Riedijk et al. [22] used a dual tracer technique in 5 preterm infants with a gestational age of 29 ± 1 weeks and a birth weight of 1.2 ± 0.2 kg studied at the postnatal age of 14–19 days. Fractional first-pass Glu uptake was $77 \pm 18\%$ on day 1 and $70 \pm 7\%$ on day 2 (mean $74 \pm 13\%$). Some $86 \pm 7\%$ of the Glu used in the first pass was directed towards oxidation to carbon dioxide. The authors conclude that there is a high splanchnic fractional first-pass uptake and a high oxidation rate of Glu in enterally fed preterm infants, for whom Glu is an important source of energy for the splanchnic tissues.

Socha et al. [23] reported plasma AA concentrations in healthy full-term infants aged 6 months who were fed human milk or were randomized to receive isocaloric infant formula with contents of the same protein of 1.25 or 2.05 g/dL, respectively, resulting in a 1.65-fold difference in Glu supply, followed by follow-on formula with 1.6 or 3.2 g protein/dL, with a 2-fold difference in Glu supply. Plasma Glu concentrations were not different in 339 infants fed the lower protein formulae (median 122 $\mu\text{mol/L}$, interquartile range 95, 168 $\mu\text{mol/L}$), 333 infants fed the higher protein formulae (115 [88, 172] $\mu\text{mol/L}$), and 237 breastfed infants (130 [90, 193] $\mu\text{mol/L}$). Thus, in term infants – similar to preterm infants – marked variation in dietary Glu intake has no

Table 1. Estimated daily intake of total and free glutamate (Glu) of a breastfed reference infant (weight 5 kg, daily energy intake 100 kcal/kg body weight), based on Glu contents in mature human milk at about 4 months of lactation, as reported in a systematic review of global data [16], and free Glu intake of a similar reference infant fed formula based on cows' milk protein [19, 31] or on an extensive protein hydrolysate [31] (see text)

	Milk Glu, mg/dL	Mean daily intake (mg/kg) at a mean milk intake (158.7 mL/kg)	Upper range daily intake (mg/kg) at high milk intake (mean +2 SD 261.9 mL/kg)
<i>Breastfed infant, based on cross-sectional global data at >60 days of lactation [16]</i>			
Total Glu			
Based on mean milk concentration	189.2	300.3	353.6
Free Glu			
Based on mean milk concentration	22.5	35.7	58.9
Based on mean + assumed 2SD of milk concentration [19]	51.4	81.5	134.6
<i>Breastfed infant, based on longitudinally collected data at 3 months of lactation in European women [18]</i>			
Free Glu			
Based on mean milk concentration	25.2	40.0	66.0
Based on mean + measured 2SD of milk concentration [18]	26.8	42.6	70.2
	Formula Glu, mg/dL	Mean daily intake (mg/kg) at an average formula intake (149.3 mL/kg)	Upper range daily intake (mg/kg) at a high formula intake (217.2 mL/kg)
<i>Infant fed formula</i>			
Free Glu, cows' milk formula			
Based on median formula content [19, 20]	0.4	0.7	1.0
Free Glu, extensive protein hydrolysate formula			
Based on median formula content [20]	115.0	171.7	249.8

appreciable effect on plasma Glu concentrations, which suggest that enteral Glu supply is predominantly utilized by splanchnic tissues.

Discussion

The data reviewed indicate that Glu is by far the most abundant AA in human milk, both among TAA and among FAA. With longer duration of lactation, the mean TAA Glu concentration in human milk declines in proportion to the decline in true protein content, with a particularly marked reduction during the first 4 months after birth. In contrast, the mean FAA Glu increases considerably during the first 4 months after birth and then appears to reach a plateau.

Based on cross-sectional global data, the mean daily free Glu intake of a breastfed reference infant, as defined by the Scientific Committee on Food of the European

Commission and the European Food Safety Authority [13, 14], is 35.7 mg/kg bw and reaches up to 134.6 mg/kg bw at the upper end of the normal range, defined by mean + 2SD of both milk volume intake and milk-free Glu contents (Table 1). Based on recently published longitudinal data on milk composition in European women [18], which we regard to be based on even better methodology, the estimated mean daily free Glu intake of a breastfed reference infant is 40 mg/kg bw and the intake at the upper end of the normal range is 70.2 mg/kg bw.

In contrast, the mean daily free Glu intake of a reference infant fed cows' milk-based formula is much lower with only 0.7 mg/kg bw (upper range 1.0 mg/kg bw), whereas an infant fed a formula based on an extensive protein hydrolysate has a high mean intake of 171.7 mg/kg bw (upper range 249.8 mg/kg bw; Table 1).

Both the mean and upper range of intakes of free Glu in healthy breastfed infants, and also in the many infants fed formula based on an extensive protein hydrolysate, are

clearly higher than the acceptable daily intake (ADI) of 30 mg/kg bw per day set by the European Food Safety Authority for the whole population, including infants and children [24]. The European Food Safety Authority panel based this ADI value on concerns raised regarding potential neurotoxicity, based on their interpretation of data from an animal study [24]. However, it is universally agreed that breastfeeding is the optimal form of infant feeding that supports health and superior neurodevelopment, as compared to infants fed regular cows' milk-based infant formula (with a lower free Glu supply) [10, 11, 14]. It is unreasonable and seems unacceptable to set an ADI for a nutrient that is lower than the normal intake of this nutrient in breastfed infants, thereby implying that the nutrient supply with breastfeeding would not be safe, for which there is no accountable evidence. While estimated mean and upper range free Glu intakes of a breastfed infant are up to 1.3- and 4.5-fold higher than the ADI value set by the European Food Safety Authority, the respective intakes of an infant fed a formula based on an extensive protein hydrolysate are 5.7- and 8.3-fold higher than this ADI value. There is no basis to assume that feeding infant formula based on an extensive protein hydrolysate would not be suitable and safe for infants. Dietary Glu supplied to infants is almost entirely utilized by the splanchnic organs and has no appreciable effect on plasma Glu concentrations [21–23]. In accordance with these results in infants, studies in animal models have confirmed that very little Glu of dietary origin escapes metabolism in the small intestinal mucosa [25–28]. Therefore, it seems unlikely that Glu not reaching the systemic circulation would have untoward systemic or neurotoxic effects.

Infant formulae based on extensive protein hydrolysates are widely used in infants with cows' milk protein allergy and some other disorders, and they have been evaluated for their suitability and safety in numerous preclinical and clinical studies, including studies evaluating long-term outcome until school age and adolescence in large numbers of exposed children [29, 30]. There are no indications that infant formulae based on extensive protein hydrolysates would be unsafe or would induce alterations in neurodevelopment. Since many decades, numerous extensive protein hydrolysate formulae are accepted for infant feeding in the European Union by the respective competent authorities, for which safety is an essential prerequisite. Infants who are successfully treated for a cows' milk protein allergy by exclusion of cows' milk from their diet and by feeding an extensive protein hydrolysate formula are generally healthy, grow normally, and have a normal metabolism and neurodevelopment. Therefore, setting an

ADI below the range of their normal intake with a safe diet in these generally healthy individuals, that is, below 250 mg/kg bw per day, must be regarded as inappropriate.

Funding Source

The work of B.K. is supported in part by the European Commission's funding in the Early Nutrition (FP7-289346), DYNAHEALTH (H2020-633595) and LIFECYCLE (H2020-SC1-2016-RTD) projects, the European Research Council (Advanced Grant META-GROWTH, ERC-2012 AdG 322605), the Erasmus Plus Programs Early Nutrition eAcademy Southeast Asia (573651-EPP-1-2016-1-EN-EPPKA2-CBHE-P) and Capacity Building to Improve Early Nutrition and Health in South Africa – 598488-EPP-1-2018-1-DE-EPPKA2-CBHE-JP, and the EU Interreg Program Focus in CD (CE111). Additional support was provided by Federal Ministry of Education and Research (No. 01 GI 0825), German Research Foundation (KO912/10-1), and McHealth Innovation Initiative of the Ludwig-Maximilians-University Munich. LMU and its employees have received support for scientific and educational projects of various commercial actors, mainly in the context of publicly funded research projects supported by the European Commission, the European Research Council, and the Federal Ministry of Education and Research.

Disclosure Statement

The Workshop preparation, setting, and attendance have been supported by the International Glutamate Technical Committee (IGTC), Brussels. The views of the author are his own, and do not necessarily reflect those of the IGTC. As a member of the Nutrition Commission of the German Association of Pediatrics and Adolescent Medicine, the National Nursing Commission, the Scientific Advisory Board of the Young Family Network, and President-Elect of the International Society for Research on Human Milk and Lactation B.K. tends to be biased towards breastfeeding.

Author Contributions

B.K.: initiated and conducted the literature survey, performed the data analysis and interpretation, and wrote the manuscript. He agrees to be accountable for the work.

References

- 1 Koletzko B, Bhatia J, Bhutta Z, Cooper P, Makrides M, Uauy R, et al: *Pediatric Nutrition in Practice*, ed 2. Basel, Karger, 2015.
- 2 Fleddermann M, Demmelmair H, Grote V, Nikolic T, Trisic B, Koletzko B: Infant formula composition affects energetic efficiency for growth: the BeMIM study, a randomized controlled trial. *Clin Nutr* 2014;33:588–595.
- 3 Duggan CP, Walker WA, Koletzko B, Watkins JB: *Nutrition in Pediatrics*, ed 5. Raleigh, People's Medical Publishing House, 2016, vol. 1–2.

- 4 Koletzko B, Dokoupil K: Reference nutrient intakes of infants, children and adolescents. *World Rev Nutr Diet* 2015;113:308–315.
- 5 Koletzko B, Baker S, Cleghorn G, Neto UF, Gopalan S, Hernell O, et al: Global standard for the composition of infant formula: recommendations of an ESPGHAN coordinated international expert group. *J Pediatr Gastroenterol Nutr* 2005;41:584–599.
- 6 Codex-Alimentarius-Commission: Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants. Codex Stan 72-1981. Rome, Codex-Alimentarius-Commission, 2007, pp 1–21.
- 7 European-Commission: Commission Delegated Regulation (EU) 2016/127 of 25 September 2015 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for infant formula and follow-on formula and as regards requirements on information relating to infant and young child feeding. *OJL* 25, 2.2.2016, pp 1–29.
- 8 Suthuvoravut U, Abiodun P, Chomtho S, Chongviriyaphan N, Cruchet S, Davies P, et al: Composition of follow-up formula for young children aged 12–36 months: recommendations of an international expert group coordinated by the nutrition association of Thailand and the early nutrition academy. *Ann Nutr Metab* 2015;67:119–132.
- 9 Codex-Alimentarius-Commission: Codex Standard for Follow-Up Formula. Codex Stan 156-1987. Rome, Codex Alimentarius Commission, 1987, pp 1–9.
- 10 Prell C, Koletzko B: Breastfeeding and complementary feeding – recommendations on infant nutrition. *Dtsch Arztebl Int* 2016;113:435–444.
- 11 ESPGHAN Committee on Nutrition, Agostoni C, Braegger C, Decsi T, Kolacek S, Koletzko B, et al: Breast-feeding: a commentary by the ESPGHAN committee on nutrition. *J Pediatr Gastroenterol Nutr* 2009;49:112–125.
- 12 Grote V, Verduci E, Scaglioni S, Vecchi F, Contarini G, Giovannini M, et al: Breast milk composition and infant nutrient intakes during the first 12 months of life. *Eur J Clin Nutr* 2016;70:250–256.
- 13 European Commission, Scientific Committee on Food, Koletzko B, Saris WH, Flynn A, Palou A, Wal JM, et al: Report of the Scientific Committee on Food on the Revision of Essential Requirements of Infant Formulae and Follow-on Formulae. Brussels, European Commission, 2003.
- 14 EFSA-Panel-on-Dietetic-Products: Scientific opinion on the essential composition of infant and follow-on formulae. *EFSA J* 2014;12:106.
- 15 Lonnerdal B, Erdmann P, Thakkar SK, Sauser J, Destaillets F: Longitudinal evolution of true protein, amino acids and bioactive proteins in breast milk: a developmental perspective. *J Nutr Biochem* 2017;41:1–11.
- 16 Zhang Z, Adelman AS, Rai D, Boettcher J, Lonnerdal B: Amino acid profiles in term and preterm human milk through lactation: a systematic review. *Nutrients* 2013;5:4800–4821.
- 17 Gidrewicz DA, Fenton TR: A systematic review and meta-analysis of the nutrient content of preterm and term breast milk. *BMC Pediatr* 2014;14:216.
- 18 van Sadelhoff JHJ, van de Heijning BJM, Stahl B, Amodio S, Rings EHHM, Mearin ML, et al: Longitudinal variation of amino acid levels in human milk and their associations with infant gender. *Nutrients* 2018;10:pii:E1233.
- 19 Agostoni C, Carratu B, Boniglia C, Riva E, Sanzini E: Free amino acid content in standard infant formulas: comparison with human milk. *J Am Coll Nutr* 2000;19:434–438.
- 20 Ventura AK, Gabriel AS, Hirota M, Mennella JA: Free amino acid content in infant formulas. *Nutr Food Sci* 2012;42:271–279.
- 21 Hays SP, Ordóñez JM, Burrin DG, Sunehag AL: Dietary glutamate is almost entirely removed in its first pass through the splanchnic bed in premature infants. *Pediatr Res* 2007;62:353–356.
- 22 Riedijk MA, de Gast-Bakker DA, Wattimena JL, van Goudoever JB: Splanchnic oxidation is the major metabolic fate of dietary glutamate in enterally fed preterm infants. *Pediatr Res* 2007;62:468–473.
- 23 Socha P, Grote V, Gruszfeld D, Janas R, Demelmair H, Closa-Monasterolo R, et al: Milk protein intake, the metabolic-endocrine response, and growth in infancy: data from a randomized clinical trial. *Am J Clin Nutr* 2011;94(6 suppl):1776S–1784S.
- 24 EFSA Panel on Food Additives and Nutrient Sources added to Food: Re-evaluation of glutamic acid (E 620), sodium glutamate (E 621), potassium glutamate (E 622), calcium glutamate (E 623), ammonium glutamate (E 624) and magnesium glutamate (E 625) as food additives. *EFSA J* 2017;15.
- 25 Watford M, Reeds PJ: Glutamate metabolism in the gut. *Forum Nutr* 2003;56:81–82.
- 26 Reeds PJ, Burrin DG, Stoll B, Jahoor F: Intestinal glutamate metabolism. *J Nutr* 2000;130(4S suppl):978S–982S.
- 27 Reeds PJ, Burrin DG, Jahoor F, Wykes L, Henry J, Frazer EM: Enteral glutamate is almost completely metabolized in first pass by the gastrointestinal tract of infant pigs. *Am J Physiol* 1996;270(3 pt 1):E413–E418.
- 28 Burrin DG, Stoll B: Metabolic fate and function of dietary glutamate in the gut. *Am J Clin Nutr* 2009;90:850S–856S.
- 29 von Berg A, Filipiak-Pittroff B, Schulz H, Hoffmann U, Link E, Sussmann M, et al: Allergic manifestation 15 years after early intervention with hydrolyzed formulas – the GINI study. *Allergy* 2016;71:210–219.
- 30 Rzehak P, Sausenthaler S, Koletzko S, Reinhardt D, von Berg A, Kramer U, et al: Long-term effects of hydrolyzed protein infant formulas on growth – extended follow-up to 10 y of age: results from the German Infant Nutritional Intervention (GINI) study. *Am J Clin Nutr* 2011;94(6 suppl):1803S–1807S.
- 31 ESPGHAN Working Group on Coeliac Disease Diagnosis: ESPGHAN Gastroenterology Committee, Husby S, Koletzko S, Korpány-Szabo IR, Mearin ML, Phillips A, et al: European society for pediatric gastroenterology, hepatology, and nutrition guidelines for the diagnosis of coeliac disease. *J Pediatr Gastroenterol Nutr* 2012;54:136–160.