



Free amino acid content in infant formulas

FAA content in
infant formulas

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Abstract

Purpose – Infant formula is the sole nutrition and food source for many infants. Information on the free amino acid (FAA) content of formulas, particularly those that are protein hydrolysate based, is limited, despite emerging evidence for the role of FAAs in regulating eating behaviour. The purpose of this paper is to measure levels of essential, semi-essential, and nonessential FAAs in commercially available infant formulas to provide a foundation for future research examining the influence of FAAs on infant development.

Design/methodology/approach – Using an automatic amino acid analyzer, we measured the concentrations of FAAs in four types of formula: two cow milk (CMF); three soy protein (SPF); and three protein hydrolysate (PHF), one of which was a partial hydrolysate (pPHF) and two of which were extensive hydrolysate (ePHF).

Findings – It was found that the amount and number of FAAs varied significantly across formula types: for CMF, total FAAs ranged from 523-864 $\mu\text{mol/L}$, with taurine being the most prominent; SPF, 1933-2450, methionine; pPHF, 2329, taurine; and ePHF, 80375-85445, leucine. ePHF had the highest levels and the most diversified profiles of FAAs.

Research limitations/implications – Striking discrepancies exist for FAA profiles of infant formulas. Comparison of these data to published psychophysical data on the taste qualities of individual FAAs provides insights into the unique flavor profiles of infant formulas. Overall, the data from this study provide a necessary foundation for future research examining the influence of FAAs in formulas on infant growth and development.

Originality/value – Published data on the FAA content of PHF is limited, despite their increased availability and use. This research is the first to report the FAA content of partial and extensive PHF, and to compare these values to CMF and SPF.

Keywords Free amino acids, Infant formulas, Protein hydrolysate formula

Paper type Research paper



The project described was supported by awards R01HD37119, R01HD072307-01 and F32 HD063343-01A1 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development. Dr Ventura was a postdoctoral trainee supported by NIH grant T32-DC00014. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Eunice Kennedy Shriver National Institute of Child Health and Human Development or the National Institutes of Health. The authors would also like to thank Dr Toshimi Mizukoshi for his help and critical advice in the interpretation of the data analysis.

1. Introduction

During early life, the protein in human milk and infant formulas supports infant growth, immune function, and behavioral development by supplying nitrogen and essential and semi-essential amino acids (Dewey *et al.*, 1996). Because of rapid rates of growth during infancy, and because the metabolic pathways needed to create semi-essential amino acids are not fully developed (Imura and Okada, 1998), the protein content and levels of the nine essential and four semi-essential amino acids for infant formulas are specified by the World Health Organization (WHO) (FAO/WHO, 2007).

Current formulas for term infants vary in protein source and/or degree of protein hydrolysis (see American Academy of Pediatrics Committee on Nutrition (2000), Koletzko *et al.* (2005) for review). Cow milk formula (CMF), which accounted for 76 percent of all US infant formula sales in 2000 (ERS/USDA, 2005), has a protein source that includes casein or whey proteins. Soy protein formula (SPF) contains soy protein isolate, usually intact but sometimes partially digested. Less prevalent is extensive protein hydrolysate formula (ePHF), which is typically fed to infants who have cow milk protein allergy or intolerance to intact protein (American Academy of Pediatrics Committee on Nutrition, 2000). The milk proteins in ePHF are treated with enzymes to break down the protein structure to reduce allergenicity and facilitate digestion (Cook and Sarett, 1982; Lee, 1992). Partial protein hydrolysate formulas (pPHF) are also available, containing a combination of intact and partially digested proteins.

Differences in protein source, processing, and degree of hydrolysis create large differences in the non-protein nitrogen content of formulas (Cook and Sarett, 1982; Lee, 1992), which includes free amino acids (FAAs), urea, small peptides, and any other nitrogen-containing compounds that are not bound to intact proteins (Rudloff and Kunz, 1997). Despite the significant contribution of non-protein nitrogen to the total nitrogen content of infant formulas (Donovan and Lonnerdal, 1989), WHO standards do not specify FAA content in infant formulas (FAO/WHO, 2007; Koletzko *et al.*, 2005). Further, the exact levels of FAAs present in infant formulas are not typically provided on manufacturers' web sites and research documenting the FAA content of CMF and SPF is limited (Agostoni *et al.*, 2000; Caja *et al.*, 2006; Chuang *et al.*, 2005; Ferreira, 2003; Yeung *et al.*, 2006; Zunin and Evangelisti, 1999). Additionally, no studies have described the FAA content of pPHFs or ePHFs.

The paucity of information on the FAAs in infant formulas, particularly PHF, is significant for two reasons. First, the consumption of both pPHF and ePHF has been on the rise among healthy formula-fed infants. Second, formula-fed infants are not a homogeneous group. For example, we recently reported that, when compared to breastfed infants, CMF-fed infants weight gain was accelerated, whereas the weight gain of ePHF-fed infants was normative (Mennella *et al.*, 2011). The more normative growth may be attributed to the finding that ePHF-fed infants consumed less formula to satiation during monthly laboratory-based, infant-led feeding sessions than did CMF-fed infants.

Based on these data, we hypothesize that higher levels of FAAs in ePHF compared to CMF contribute, in part, to these differences in satiation and growth. However, this hypothesis, and others like it, cannot be tested until data on the exact levels of FAA in formulas are available. To this end, we measured levels of essential, semi-essential, and nonessential FAAs in commercially available infant formulas to provide a foundation for future research examining the influence of FAAs on infant development.

2. Materials and methods

A representative sample of the formulas most commonly consumed by US infants (ERS/USDA, 2005) was selected at random from a supermarket in Philadelphia (PA, USA). To this end, eight different formulas were studied: two CMF, three SPF, one pPHF, and two ePHF (Table I). We chose the liquid-ready-to-feed versions, rather than powdered or liquid concentrate, to minimize errors in preparation (FAO/WHO, 2007). We also analyzed two powdered versions (CMF-1, PHF-1) to compare powdered versus liquid-ready-to-feed formulations.

For each formula, 20 mL was deproteinized with 10 mL sulfosalicylic acid (6 percent), mixed on vortex type mixer, and centrifuged immediately (4°C, 15 min, 1,200 *g*). The supernatant was purified with a 0.45 μm filter, and molecules > 10 kDa were removed with an Amicon[®] Ultra centrifugal filter (Millipore, Tokyo, Japan). We measured amino acid concentrations with an automatic amino acid analyzer (model L-8900; Hitachi, Tokyo, Japan) that separates amino acids by cation-exchange chromatography. The analyzer system has a high-reproducibility retention time with coefficient of variation (CV) 0.3 percent (Arg), peak area with CV 1.0 percent (Gly, His), and limit of detection (LOD) of 3 pmol (signal-to-noise ratio (S:N) = 2; Asp). Reproducibility was verified by measurements of standard preparations.

Each amino acid was detected spectrophotometrically after postcolumn reaction with ninhydrin reagent (Noguchi *et al.*, 2006). We diluted SPF-3 (six-fold) and ePHF-1 (7.5-fold) because initial analyses revealed that amino acid levels were out of range. Samples were analyzed in duplicate; data were adjusted for dilution factor (if applicable) and are presented as average micromoles per liter. Amino acid standards were intercalated with each sample to ensure reproducibility \geq 98 percent or CV < 2 percent. LOD varied from 2 to 10 $\mu\text{mol/L}$ and was specific to each amino acid. Values < three-fold the S:N were considered to be in the LOD.

3. Results and discussion

Table I presents FAA levels for each formula. ePHF had the highest levels of FAAs, averaging 120-fold higher than CMF, 39-fold higher than SPF, and 36-fold higher than pPHF. Many FAAs were undetectable or present at the LOD (i.e. present in trace amounts), meaning these amino acids are present only within intact proteins in these formulas. In contrast, all FAAs were detected in ePHF (with the exception of glutamine in ePHF-1).

ePHF was most abundant in free leucine (14-15 percent), glutamic acid (9-10 percent), lysine (8-10 percent), and valine (9 percent), which accounted for approximately 42 percent of total FAA content. ePHF also differed within formula type: ePHF-2 contained more FAAs than did ePHF-1 (85,445 versus 80,375 $\mu\text{mol/L}$), almost two-fold the proline of ePHF-1, and a small amount of glutamine, versus none in ePHF-1. Levels of FAAs were substantially lower in pPHF than in ePHF, and some amino acids (isoleucine, leucine, valine, cystine, glutamine) found at high levels in ePHF were not detectable in pPHF.

Overall, the total FAA content for SPF, ranging from 1,933 to 2,450 $\mu\text{mol/L}$, was lower than for ePHF. Only seven to 12 of the FAAs found in SPF exceeded the LOD in free form. SPF was most abundant in methionine, which contributed 65-75 percent of FAA content. Taurine was the next most abundant, contributing 21-22 percent of FAA content. The other FAAs each contributed <2 percent of the FAA content.

Table I.
FAA content ($\mu\text{mol/L}$)
of different types of infant
formulas

Type Formula	CMF			SPF		SPF-3		pPHE-1		PHF		ePHE-2	
	CMF-1 Enfamil LJPII ^a	CMF-2 Similac Advance ^b	SPF-1 Enfamil ProSobee ^c	SPF-2 Similac Isomil Advance ^b	Gerber Good Start Plus ^c	Gerber Good Start Gentle Plus ^c	Gerber Good Start Gentle Plus ^c	Nutrigen LJPII ^a	Nutrigen LJPII ^a	Similac Alimentum ^b			
Degree of hydrolysis	None	None	None	None	Partial	Partial	Partial	Extensive	Extensive	Extensive	Extensive	Extensive	Extensive
Calories (kcal/100 mL)	67.7	67.6	67.7	67.6	67.0	67.0	67.0	67.7	67.7	67.6	67.6	67.6	67.6
Protein (g/100 mL)	1.4	1.4	1.7	1.8	1.7	1.5	1.5	1.9	1.9	1.9	1.9	1.9	1.9
<i>Essential FAAs</i>													
Histidine	9	tr	tr	nd	tr	25	tr	1,880	1,880	1,739	1,739	1,739	1,739
Isoleucine	tr	tr	tr	nd	tr	nd	tr	5,327	5,327	4,953	4,953	4,953	4,953
Leucine	nd	nd	12	tr	42	nd	42	11,886	11,886	12,315	12,315	12,315	12,315
Lysine	22	11	9	tr	43	607	tr	8,254	8,254	7,226	7,226	7,226	7,226
Methionine	nd	nd	1,446	1,441	1,598	24	1,598	2,854	2,854	2,776	2,776	2,776	2,776
Phenylalanine	11	nd	6	nd	nd	157	nd	4,283	4,283	4,763	4,763	4,763	4,763
Threonine	5	tr	22	23	24	226	24	4,653	4,653	4,486	4,486	4,486	4,486
Tryptophan	nd	nd	nd	nd	nd	nd	nd	1,348	1,348	1,273	1,273	1,273	1,273
Valine	10	tr	nd	nd	nd	nd	nd	7,038	7,038	7,485	7,485	7,485	7,485
<i>Semi-essential FAAs</i>													
Arginine	10	tr	18	16	50	129	16	3,489	3,489	3,613	3,613	3,613	3,613
Cystine	nd	nd	nd	nd	nd	nd	nd	542	542	695	695	695	695
Taurine	529	385	425	426	526	806	426	496	496	734	734	734	734
Tyrosine	9	nd	tr	nd	tr	26	nd	1,170	1,170	1,049	1,049	1,049	1,049
<i>Non-essential FAAs</i>													
Alanine	31	13	15	tr	43	35	tr	4,905	4,905	5,571	5,571	5,571	5,571
Asparagine	tr	nd	tr	tr	45	52	tr	3,705	3,705	3,717	3,717	3,717	3,717
Aspartic acid	9	nd	18	11	tr	15	tr	1,535	1,535	1,846	1,846	1,846	1,846
Glutamic acid	109	86	11	9	tr	113	9	7,472	7,472	8,226	8,226	8,226	8,226
Glutamine	nd	nd	nd	nd	nd	nd	nd	nd	nd	234	234	234	234
Glycine	38	28	10	7	26	26	7	1,668	1,668	1,907	1,907	1,907	1,907
Proline	63	nd	nd	nd	nd	24	nd	2,667	2,667	5,606	5,606	5,606	5,606
Serine	9	tr	10	nd	53	15	nd	5,231	5,231	5,231	5,231	5,231	5,231
Total essential FAAs	57	11	1,495	1,464	1,707	1,088	1,464	47,523	47,523	47,016	47,016	47,016	47,016
Total semi- and nonessential FAAs	807	512	507	469	743	1,241	469	32,852	32,852	38,429	38,429	38,429	38,429
Total FAAs	864	523	2,002	1,933	2,450	2,329	1,933	80,375	80,375	85,445	85,445	85,445	85,445

Notes: ^aManufactured by Mead-Johnson Nutrition, Glenview, IL; data obtained 9 April 2012 from manufacturer's web site: www.mjn.com/app/fwp/hcp2/content2.do?dm = mj&id = /HCP_Home2/ProductInformation/hcpProducts&wpsst = MJN&ls = 0&csred = 1&r = 344924179; ^bManufactured by Abbott Laboratories, Abbott Park, IL; data obtained 9 April 2012 from manufacturer's web site: <http://abbottnutrition.com/Infant-And-New-Mother/Infant-And-Baby-Formula.aspx>; ^cManufactured by Société des Produits Nestlé, S.A. Vevey, Switzerland; data obtained 9 April 2012 from manufacturer's web site: www.gerber.com/Birth/Products/GoodStart_Formulas.aspx?PL_inelid = cc27fb48-a094-4015-b29c-f8d861415f88&PCatId = 06a3314b-3aea-47c4-b79e-089ec09efc3; tr – trace amount; defined as values which are at or slightly below the respective detection limit; nd – not detected; CMF – cow milk formula; SPF – soy protein formula; pPHF – partial protein hydrolysate formula; ePHF – extensive protein hydrolysate formula

The total FAA content for CMF (523-864 $\mu\text{mol/L}$) was considerably lower than for SPF and PHF. While the most abundant FAAs in CMF were taurine (61-74 percent) and glutamic acid (13-17 percent), the two CMFs differed most in proline content: 7 percent in CMF-1 versus none in CMF-2. CMF-1 also contained 1.7-fold the amount of FAAs found in CMF-2.

Comparison of powdered and liquid-ready-to-feed versions of CMF-1 and ePHF-1 showed little variation (data not shown). For example, CMF-1 as powder contained 125 $\mu\text{mol/L}$ free glutamate, versus 109 $\mu\text{mol/L}$ in the liquid-ready-to-feed version. Similarly, ePHF-1 as powder contained 7,238 $\mu\text{mol/L}$ free glutamate, versus 7,472 $\mu\text{mol/L}$ in the liquid-ready-to-feed version. The CV was 1.7 between the CMF-1 formulations and 0.8 between the ePHF-1 formulations.

It remains unknown why the levels of FAAs are so high in some formulas and what impact FAA content may have on infant development (see commentary in Hernell and Lonnerdal (2003)). In the present study, we found that total levels of FAAs in ePHF were substantially higher than in human breast milk (3,019 $\mu\text{mol/L}$ (Agostoni *et al.*, 2000)). In contrast, FAA levels in CMF or SPF were two- to five-fold lower than in breast milk (Agostoni *et al.*, 2000). Several studies have documented that infants who feed ePHF have much higher serum FAAs than do infants fed breast milk or non-hydrolyzed formulas (Giovannini *et al.*, 1994; Hernell and Lonnerdal, 2003; Rigo *et al.*, 1995), the consequence of which is still unclear. The effects, if any, that the relative and absolute levels of FAAs in breast milk or formulas have on the establishment of long-term flavor and food preferences and understanding the impact of their effects on infant feeding, growth, and development should be a priority for future research.

Infants can detect flavor differences among various types of formulas and grow to prefer the brand that they experience early in life (Mennella and Beauchamp, 2005). Because these flavors are complex, psychophysical data for formulas will not provide information on how their varying FAA levels contribute to overall flavor. As a preliminary examination of the potential relation between formula feeding and flavor preferences, we compared published psychophysical data on the taste qualities of individual FAAs (Schiffman *et al.*, 1979; Schiffman and Dackis, 1975) with the FAA profiles of individual formulas from the present study (Table II). For each formula under study, FAAs that comprised greater than 1 percent of the total FAA content were ranked based on the amount present in the formula (1 = highest amount).

By combining the findings of the present study with the information provided by Schiffman and Dackis (1975), one may predict the impact FAA content on the flavor of infant formula. For example, sensory panel work has shown that SPF tastes more sweet, sour, and bitter than CMF (Cook and Sarett, 1982). SPF is enriched with both taurine and methionine, as reflected in the relative abundance of these two amino acids in the soy formulas analyzed in the present study. Methionine has been reported to taste “repulsive, metallic, minerally [. . .] bitter” (Schiffman and Dackis, 1975), which is may be one contributor to the bitterness of SPF. Sweet flavors of SPF may come from, in part, the relatively higher levels of taurine (Schiffman and Dackis, 1975).

The savory, sour, and bitter flavors of ePHF may be attributable to the high levels of glutamic acid (meaty or savory), valine (slightly bitter with sour and sweet aspects), and lysine (salty and bitter) (Schiffman and Dackis, 1975). Additionally, differences in FAA profiles between ePHFs may underlie reports that ePHF-2 is sweeter than ePHF-1 (Mennella and Beauchamp, 2005): ePHF-2 contains almost twice as much proline,

Table II.
Psychophysical taste
qualities of FAAs^a and
FAA ranking^b according
to amount detected in a
variety of infant formulas

Type Formula	Degree of hydrolysis	Type	CMF			SPF			pPHF-1		PHF	
			None	None	None	None	None	None	Partial	Partial	Extensive	Extensive
FAA		Psychophysical taste qualities ^a										
Histidine		Flat to bitter, sometimes salty, sour, or mineral									14 ^c	16
Isoleucine		Flat to bitter									5	8
Leucine		Flat to bitter					8				1	1
Lysine		Flat to bitter, complex and salty					6		2		2	4
Methionine		Flat to bitter, sometimes sulfurous, meaty, or sweet					1		1		12	13
Phenylalanine		Mineral, bitter, and metallic							4		9	9
Threonine		Flat to sweet; sometimes bitter, sour, or fatty							8		8	10
Tryptophan		Flat to bitter							3		17	
Valine		Flat to bitter, with slightly sweet and sour							5		4	3
Arginine		Flat to bitter, alkaline, complex					4		5		11	12
Cystine		Sulfurous										
Taurine ^d		Slightly sweet	1	1	2	2	2	2	1			
Tyrosine ^d		No taste										
Alanine		Sweet, sometimes complex with bitter aftertaste							7		7	6
Asparagine		Flat to bitter	5	4					5		10	11
Aspartic acid		Flat, sour, slightly bitter							7		16	15
Glutamic acid		Meaty, salty, bitter, complex								6	3	2
Glutamine		Flat, sweet, meaty, somewhat unpleasant	2	2								
Glycine		Sweet	4	3							15	14
Proline		Sweet; sometimes complex with salty or sour	3								13	5
Serine		Flat to sweet; sometimes complex or sour								3	6	7

Notes: ^aPsychophysical taste qualities data from Schiffman and Dackis (1975) and Schiffman *et al.* (1979); ^bFAA content ranking was tabulated for FAAs that exceeded 1 percent of the total FAA content of the particular formula (1 = highest amount); ^cbolded rankings indicate that the amount of FAA present in the formula exceeded the adult taste threshold identified by Schiffman *et al.* (1979); ^dno adult taste threshold information was identified in Schiffman *et al.* (1979); CMF – cow milk formula; SPF – soy protein formula; pPHF – partial protein hydrolysate formula; ePHF – extensive protein hydrolysate formula

a sweet tasting amino acid, as ePHF-1. However, it is also important to note that the difference in sweetness may also be due to differences in carbohydrate source, as ePHF-2 contains sucrose and corn maltodextrin while ePHF-1 contains corn syrup solids and modified corn starches. Overall, the higher levels of FAAs in ePHF may create a more powerful flavor experience compared with other formula types and thus underlie strong and distinctive bitter, sour, and savory taste characteristics of ePHF.

4. Conclusions

Striking differences exist both among and within different types of infant formulas in total amounts and numbers of FAAs. Differences between CMF and SPF centered on taurine and methionine, as previously reported (Agostoni *et al.*, 2000), whereas differences among non-hydrolyzed (CMF, SPF), partially hydrolyzed (pPHF), and extensively hydrolyzed (ePHF) formulas were considerable. These findings provide insights into the unique flavor profiles of infant formulas and a foundation for examining the mechanisms underlying the differential impact of infant formulas on infant satiation and growth.

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