The nutritional value of protein-hydrolysed formulae

CARLO AGOSTONI[1], LUIGI TERRACCIANO[2], ELENA VARIN[1] AND ALESSANDRO FIOCCHI[2]

[1]Department of Pediatrics, Fondazione IRCCS C´a Granda–Ospedale Maggiore Policlinico, University of Milan, Italy

[2]Department of Pediatrics, Melloni Hospital, Milan, Italy

Correspondence to:

Carlo Agostoni, MD,

Department of Pediatrics – Dipartimento di Scienze Cliniche e di Comunità,

Fondazione IRCCS C´a Granda – Ospedale Maggiore Policlinico,

University of Milan,

Via della Commenda, 9,

I–20122 Milano,

Italy

E-mail: agostoc@tin.it, carlo.agostoni@unimi.it

Conflict of Interest: Nothing to declare

ABSTRACT

Allergy to cow's milk proteins is a challenging condition in early infancy. Allergic infants may be predisposed to impairments of growth for either the disease itself or the nutritional constraints of the exclusion diet they should follow. Formulae based on extensively hydrolysed cow's milk proteins are widely used, representing therapy, and constituting 100% nutrient source in the first 4 to 6 months of life and half the daily nutrient intake in the second semester of life. In some cases these products are used also for preventive purposes. Some impairments in growth have been reported for infants using these products , even if mostly limited to the first year of life, with no apparent consequences at either medium- or long-term. The macronutrient content of infant formulae based on protein hydrolysates, whichever the source, should carefully be tested not only as far optimal utilization of nitrogenous sources but also the nature and metabolic fate of non-nitrogen caloric sources, represented by carbohydrates and fats, and micronutrients, particularly iron. It is recommended that studies aimed at the allergologic effects of these products include also an appropriate nutritional evaluation to conclude on their efficiency.

Key-words: cow's milk allergy, infant growth, growth rates of allergic infants, free amino acids

Treating cow's milk allergy (CMA) entails a nutritional risk, as milk is a staple food in particular under two years of age. When a replacement formula is needed, allergists may choose among different types of formulae, including formulae based on extensively hydrolised proteins (mostly cow's milk proteins, casein and whey, or vegetal proteins, mostly rice and soy) (eHF), amino acid formulae (AAF), soy formulae and milks from other mammals. There is therefore a relatively wide choice of safe and nutritionally adequate formulae to use as cow's milk substitutes in children affected by CMA.

Whichever the choice, the impact on the nutritional status of the allergic infant should always be considered. Indeed, although allergic disorders are widespread, few studies have evaluated so far the influence of the isolated allergic status on nutrient management (including absorption and metabolic rates) and growth. Indeed, allergy-associated conditions could impair growth processes, particularly in the first months of life, as detailed in the next section. Therefore, the additional effects of the products chosen for the replacement should reasonably be taken into account, particularly in the case of hydrolysed proteins, that are mostly recommended for therapy.

Allergy status and growth

A reduced growth in infants with allergy to cow's milk has been shown during the first year of life (Isolauri et al., 1998). The fall in relative length coincided with the onset of the symptoms suggestive of cow's milk allergy and the start of the elimination diet. No catch-up was seen by 24 months of age. The relative weight in patients continued to fall compared with that in the control group. In an observational study (Agostoni et al. 2000), follow-up data at the age of 12

months were available for 55 infants with atopic dermatitis, AD, and for 114 controls. Infants affected by AD showed a progressive impairment of growth both in weight-for-age, WA, and length-for-age, LA, z-scores. The growth indices drastically worsened after the onset of disease, even if they had already shown a significantly negative mean LA z-score. Differences between AD infants and controls were significant from the second month of age onwards, more markedly in the second 6 months of life. Only the severity of disease was associated with a more pronounced WA growth impairment in the second 6 months of life. Accordingly, infants with severe dermatitis had a lower WA z-score in the 6- to 12-month period than did those with non-severe disease. The difference remained significant at 6 months of age also after adjustment for confounders. Different factors, beyond therapeutic elimination diets, may negatively influence the growth pattern of allergic children, although the primary cause has not been clearly identified yet. An impaired ability to absorb and utilize nutrients, possibly caused by an allergy-induced inflammation of the gut mucosa, together with increased nutritional demands, as in cases of AD, considering the higher metabolic requirements of the accelerated skin turnover, or even restless-related itching conditions, may represent plausible explanations.

Even breastfeeding and the use of human milk has been questioned in the therapy of CMA. The switch from human milk to a special formula, with a parallel increase of energy intake, has been associated with growth improvement in some allergic infants (Isolauri et al., 1999). Cessation of breast-feeding was shown to benefit serum prealbumin, urea, and zinc concentrations. It has been observed (Agostoni et al. 2000) that atopic infants who had been breastfed showed impairment in growth parameters earlier than in formula-fed counterparts. It is possible that the delicate balance of nutrients supplied by human milk in some breastfed infants may fail to meet the

higher requirements dictated by the atopic status and possibly increased intestinal losses. This observation has raised discussions on the opportunity to maintain breastfeeding, in selected cases with CMA and poor growth, putting on the balance the other well-known benefits of prolonged breastfeeding. Quite recently the presence of endogenous human milk epitopes recognized by specific IgE from infants and children with CMA has been demonstrated, with functional properties at least in vitro. Even if their role in provoking allergic symptoms in infants exclusively breastfed by mothers strictly avoiding dietary milk remains unclear, these findings add more uncertainty to the scenario previously described (Jarvinen et al., 2012). Within this context the role of diet and single nutrients still needs elucidation. Individually tailored elimination diets considering the pros and cons of some food avoidances (for instance, foods presumably allergenic but rich in the immune-modulating n-3 fatty acids) may be required to sustain adequate growth rates in this population.

Hydrolysed formulas : definitions

When breast-feeding is not possible or not desired, cow's milk-based infant formulae should be replaced by a substitute, mostly represented by an extensively hydrolysed formula (eHF) based on the extensive hydrolysis of a source protein, usually from milk, in order to considerably reduce allergenicity. The antigenicity and allergenicity of HF is partially dependent on the molecular weight of their constituent proteins, but even small fragments of 700 to 1400 Dalton can be immunogenic. In 1998 it was shown that the minimal molecular mass for IgE binding in vitro is between 1400 and 970 Dalton, whereas peptides with a molecular mass >1400 Dalton are needed for skin reactivity (Terracciano et al., 2002). If this is so, all commercially available HF

will contain peptide fragments that can induce clinical reactions in allergic infants and children, On an operational standpoint an eHF should be tolerated by more than 90% of infants presenting with hypersensitivity to the proteins from which the hydrolysate product is manufactured and the content of immunoreactive proteins in the hydrolysates should be limited to <1% of the total content of N-containing substances. These eHF are distinct from partially hydrolysed formulae (pHF, referred to as 'hypoallergenic' in some countries), not suitable for the treatment of CMA, and to be used only to feed nonbreast-fed infants considered to be at risk for allergy (Dupont et al. 2011) . The lower degrees of hydrolysis put pHF within a safe margin as far nutritional value, according to an accepted principle connecting a higher degree of hydrolysis with a lower antigenic potential but also a lower nutritional efficiency (Vandenplas et al. 1993). However there are no physical, chemical or immunological criteria that allow any regulatory distinction between a pHF and an eHF, and any formula may provoke a reaction among infants who are allergic to CM.

From the recommendations of official scientific institutions such as ESPACI/ESPGHAN (Host et al., 1999) and AAP (AAP, 2000) a consensus emerges (with some differences on the use of soy formulae) that, although eHF may retain a detectable and clinically significant amount of antigenicity, they can be deemed generally safe for most children with CMA. In this context the reactivity to any specific product must be carefully evaluated in every single patient before a CM hydrolysate is adopted as a substitute formula. It has been proposed that when an allergy to cow's milk and soy is diagnosed, hydrolysed formulae based on rice proteins (eHF-R) might constitute a safe alternative to cow's milk hydrolysates while offering a safe and nutritionally

adequate alternative feed which avoids unnecessary dietary restrictions (Terracciano et al., 2002).

Nutrient composition of hydrolysed formulae

Besides definitions for allergic purposes, several nutritional issues concerning the macronutrient composition of hydrolysed formulae, including both nitrogen (that is, protein-equivalents) and non-nitrogen containing compounds (represented by fats and carbohydrates) have been raised. These issues may be crucial to catch-up the own growth trajectory in infants who are exposed to early growth impairments due to their allergic status. According to an ESPGHAN coordinated International Group (Koletzko et al, 2005) a variety of different cows' milk protein hydrolysates are used in special infant formulae, which may differ in total content, relative composition and bioavailability of amino acids. The Group acknowledged that major differences have been reported for different protein hydrolysate formulae with respect to nitrogen retention and growth in the recipient infants, which point to a potentially significant variation in the biologic value of different hydrolysates. For optimal utilization the hydrolyzed protein source should respond to a precise pattern of indispensable amino acids with the branched chain amino acids and valine representing around 50% the essential amino acid quote. The Group recommended in particular that infant formulae based on cows' milk protein hydrolysates with a content of protein hydrolysate less than 2.25 g/100 kcal had to be clinically tested: *such products should only be accepted if the results have been evaluated by an independent scientific body before introduction into the market*. Accordingly, the lowest protein content of infant formulae based on cows' milk protein hydrolysates should not be less than 1.8 g/100 kcal and not be greater than 3.0 g/100

kcal. Finally, also the different rates of digestion, absorption and metabolism of free amino acids, should be taken into account. Indeed, eHF contain more than 100 times the concentrations of free amino acids compared to a standard infant formulae (Ventura AK et al., 2012, and Ventura AK et al., in press). Free amino acids are also diversified within the different type of formulae, taurine practically being the only amino acid in standard infant formulae (Agostoni et al., 2000a), while branched chain amino acids and glutamate are the major free amino acids in eHF. In human milk only free glutamate presents with significant concentrations, progressively increasing in the first three months of lactation (Agostoni et al., 2000b). The issue of the safety aspects of free amino acid absorption and utilization from eHF has been extensively reviewed (Schaafsma, 2009), with the proposal of a *decision tree* useful for safety assessment of protein hydrolysates. The intriguing observation that the rate of entry into the circulation of amino acids from protein hydrolysates is faster than that from intact dietary proteins and may even be faster than that from free amino acids (Bilsborough and Mann, 2006) has been mentioned, but the biologic meaning and its consequences remain actually obscure. Nonetheless, this observation could partly explain the different observations on metabolic and growth patterns, respectively, observed in infants fed eHF.

Besides the nitrogen-containing compounds, a further issue is represented by the non-nitrogen components. Due to the multiuse availability of hydrolysed formulae (for gastrointestinal disorders, malabsorptive states, chronic diarrhoeal disorders, diseases of the liver and billiary tract, even beyond the indications for their clinical use) the list of nutrients may include different carbohydrate sources other than lactose, and medium-chain triglycerides. While the roles of these compounds in a diseased mucosa may be helpful in decreasing symptoms and improving

absorption (Koletzko et al, 2005; Klenoff-Brumberg and Genen, 2003), in undefined states of disease the final effect could be at the best non-physiologic on digestion, absorption and energy expenditure, given the complex dynamic changes in body composition and macronutrient oxidation occurring during infant growth (Jordan and Hall, 2008). Finally, the micronutrient-related status is almost unknown, particularly iron, theoretically more exposed to intestinal losses in allergic infants.

The use of hydrolysates in preterm and term infants

The effects of protein hydrolysates should be separated between preterm infants and term infants. In spite of the development of protein hydrolysate formulas specifically addressed for preterm infants, the indications for the use of such formulas are unclear (Szajewska, 2007). It has been hypothesized that infant formulas containing hydrolyzed cow's milk protein may be used to reduce feeding intolerance and to improve gastric emptying, positively affecting gastrointestinal motility. Accordingly, some trials suggest clinical advantages of using protein hydrolysate formula in premature low birth weight infants resulting in a shorter time to achieve full feedings. However, whether this strategy should be adopted as routine practice is still debated because of limited information regarding the nutritional adequacy in this population with higher requirements. Indeed, the dietary needs of preterms are different according to both gestational age and appropriateness of weight to gestational age, consequently a single formulation could not meet the requirements for catch-up in some infants, or be even excessive for others. Finally, the few available data do not allow also for definitive conclusions on the potential benefit of using

eHF or pHF for preterms to prevent allergic reactions in preterm infants with an atopic predisposition.

On the contrary, indications for the use of eHF in term infants are better defined, including the feeding of infants intolerant to cow's milk and soy protein, and fall between standardized ranges of requirements even considering the multiuse availability of extensively hydrolysed formulae previously mentioned. Therefore we will focus on observations of the use of hydrolysed formulas in term infants for the major homogeneity of dietary needs and indications.

Feeding Hydrolyzed Protein Infant Formulae in the first months of life: Short- and Long-Term Effects on Growth

We have data on the development of body mass index (BMI) from birth up to the age of 10 years for the four formula-feeding groups, pHF-whey (pHF-W), eHF-whey (eHF-W), eHF-casein (eHF-C), or cow's milk (CM)F, and a group of infants exclusively breastfed for the first 16 weeks of life (Rzehak et al, 2011). This was the longest prospective, randomized, double-blind trial of full-term neonates with family history for atopic disease. Infants fed an eHF-C formula showed a significantly lower gain in weight and BMI than the other formula groups in the first year of life and up to the first half year of life for breastfed infants. No significant differences in weight and absolute or WHO-standardized BMI trajectories were found among the other formula groups (pHF-W, eHF-W, and CMF) or the breastfed group over the entire period. These results are consistent and largely expand previous findings (Giovannini et al, 1994) on anthropometric indexes of infants randomized to eHF-C, eHF from soy/ collagen, a pHF-W, or a soy formula, and compared with those breastfed through the first 6 months of life. Infants who

received the eHF-C showed a lower BMI at 3 months of age despite a comparable or even higher caloric and protein intake and higher blood nitrogen concentrations. This observations might indicate a lower nitrogen utilization for the eHF-C in the very early stages, most likely because of amino acid imbalances, as observed also in term infants for different types of eHF-W (Rigo et al., 1994). The amino acid imbalance in infant's blood is a constant observation, with the use of eHF, no matter the protein sources, as well as higher levels of urea in plasma and urines, consistent with the hypothesis of a lower efficiency of nitrogen sources (Vandenplas, 1993).

The possible inefficient use of nutrients with an eHF-C has been shown in a recent trial on healthy, non allergic infants. In spite of a higher protein equivalent content, those fed the eHF-C had lower growth rates compared with those fed the standard formula, in association with lower quantities ingested, suggesting an additional role for the taste and odor characteristics of these products (Mennella et al., 2011). Videotape recording of infants fed the hydrolysate products confirmed a different acceptance of these formulae. The Authors have then challenged the effects of equivalent amounts of free glutamate, as found in hydrolysates, when added to standard formulae derived from cow's milk proteins (Ventura AK et al., 2012). Accordingly, the enrichment of the formula with free glutamate produced a limitation in the amounts ingested, raising the point that glutamate may represent a key signal for satiation in humans, as also shown in animals (Niijima , 2000). The added amounts of glutamate were in any case quite higher compared to those found in human milk (Agostoni et al. 2000).

Feeding Hydrolyzed Protein Infant Formulae in the complementary feeding period

Some clinical trials have focused whether the type of formula used in the complementary feeding period (6-12 months of age) might differently affect growth in infants with CMA. One prospective randomized trial has evaluated growth by comparing the effects of special formulae (soy and eHF-W, respectively) in infants affected by CMA from 7 months through 48 months of age (Seppo et al., 2005). Both nutritional status and growth were within reference values in the two groups. Another study (Agostoni et al., 2007) looked at the changes of growth parameters in four groups of infants with CMA, differing for the type of milk consumed in the 6- to 12-month period. Infants with CMA breastfed at least 4 months and progressively weaned in the 5- to 6-month period were randomly assigned to eHF-C, eHF-R or soy formula. A fourth, non-randomized group was made up by allergic infants still breastfed up to 12 months. The median age at CMA diagnosis was at around 5 months. All the four groups showed quite negative values for both WA and LA at 6 months. In the 6- to 12-month period, when solids were introduced according to the individual allergic response, all CMA groups showed positive values of LA z-score progression, while only infants fed either eHF-C or eHF-R showed a trend of positive WA z-score gain. The recorded amounts of formula ingested was similar in the three artificially fed groups. Accordingly, hydrolyzed proteins could have a qualitative advantage from a nutritional standpoint compared with complete protein sources (such as soy formulae or human milk) in infants with diagnosis of IgE-mediated allergy posssibly improving also the absorption of nutrients from solids. Therefore, the quality and type, more than quantity, by which nitrogenous compounds are supplied to allergic infants could be relevant not just for the treatment of the specific symptoms, but also to improve growth.The association with the introduction of solids suggests a possible widespread effect on mucosal physiology.

Conclusions

CMA is a major determinant of growth rate in the first year of life. Infant formulae based on hydrolysate proteins, eHF-C in particular, may be associated to some growth impairments, apparently limited to the first year of life, when started in early stages, before the introduction of solids. Once solids are introduced, few data suggest the eHF utilization may even improve nutrient utilization. Interventions may be required to limit firstly the negative effects of the allergic status on daily nutrient intakes and growth. To this aim, individually tailored diets may be required. The macronutrient content of infant formulae based on protein hydrolysates should be carefully tested , not only as far as the optimal utilization of nitrogen sources but also the nature and metabolic fate of non-nitrogen caloric nutrients (carbohydrates and fats) and the major micronutrients (particularly iron). In planning RCTs of dietary interventions on allergy, criteria for the correct evaluation of the nutritional efficiency should also be taken into account and prosecuted also beyond the intervention period, to make sure that not only allergic symptoms are treated, but also requirements for optimal growth are fulfilled.

References

Agostoni, C., Carratu, B., Boniglia, C., Riva, E. and Sanzini, E. (2000a) Free amino acid content in standard infant formulas: comparison with human milk. *J. Am. Coll. Nutr*,19:434–8.

Agostoni, C., Carratu, B., Boniglia, C., Lammardo, A.M., Riva, E. and Sanzini, E. (2000b) Free glutamine and glutamic acid increase in human milk through a three-month lactation period. *J. Pediatr. Gastroenterol. Nutr*, 31:508–12.

Agostoni, C., Grandi, F., Scaglioni, S., Giannı`, M.L., Torcoletti, M., Radaelli, G., Fiocchi, A and Riva, E. (2000) Growth pattern of breastfed and nonbreastfed infants with atopic dermatitis in the first year of life. *Pediatrics*,106:E73.

Agostoni, C., Fiocchi, A., Riva, E., Terracciano, L., Sarratud, T., Martelli, A., Lodi, F., D'Auria, E., Zuccotti, G., and Giovannini, M. (2007) Growth of infants with IgE-mediated cow's milk allergy fed different formulas in the complementary feeding period. *Pediatr. Allergy Immunol*,18:599-606.

American Academy of Pediatrics. (1999) Committee on Nutrition. Hypoallergenic infant formulae. *Pediatrics*, 81:80-4

Bilsborough, S., Mann, N. (2006) A review of issues of dietary protein intake in humans. *Int J Sport Nutr Exerc Metab* 16:129–152.

Dupont, C., Chouraqui , J.P., de Boissieu, D., Bocquet, A., Bresson, J.L., Briend, A.; Darmaun, D., Frelut, M.L., Ghisolfi, J., Girardet, J.P., Goulet, O., Hankard, R., Rieu, D., Vidailhet ,M., Turck, D. (2011) Dietary treatment of cows' milk protein allergy in childhood: a commentary by the Committee on Nutrition of the French Society of Paediatrics.*Br J Nutr*, 107:325-38

Host, A., Koletzko, B., Dreborg, S., Muraro, A., Wahn, U., Aggett, P., Bresson, J.L., Hernell, O., Lafeber, H., Michaelsen, K.F., Micheli, J.L., Rigo, J., Weaver, L., Heymans, H., Strobel, S. and Vandenplas, Y. (1999) Dietary products used in infants for treatment and prevention of food allergy. Joint Statement of the European Society for Paediatric Allergology and Clinical Immunology (ESPACI) Committee on Hypoallergenic Formulae and the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition. *Arch. Dis. Child*, 81:80-4

Giovannini, M., Agostoni, C., Fiocchi, A., Bellu, R., Trojan, S., and Riva, E. (1994) Antigen-reduced infant formulas versus human milk: growth and metabolic parameters in the first 6 months of life. *J. Am. Coll. Nutr*,13:357-63.

Isolauri, E., Sutas, Y., Salo, M.K., Isosomppi, R. and Kaila, M.(1998) Elimination diet in cow's milk allergy: risk for impaired growth in young children. *J. Pediatr,*132:1004-9.

Isolauri, E., Tahvanainen, A.; Peltola, T. and Arvola, T. (1999) Breast-feeding of allergic infants. *J. Pediatr*, 134:27-32.

Järvinen, K.M., Geller, L., Bencharitiwong, R., and Sampson, H.A. (2012) Presence of functional,autoreactive human milk-specific IgE in infants with cow's milk allergy. *Clin. Exp. Allergy*, 42:238-47.

Jordan, P.N. and Hall, K.D. (2008) Dynamic coordination of macronutrient balance during infant growth: insights from a mathematical model. *Am. J. Clin. Nutr,* 87:692-703.

Klenoff-Brumberg, H.L. and Genen, L.H. (2003) High versus low medium chain triglyceride content of formula for promoting short term growth of preterm infants. *Cochrane Database Syst. Rev*, CD002777

Koletzko, B., Baker, S., Cleghorn, G., Fagundes Neto, U., Gopalan, S., Hernell, O., Seng Hock, Q., Jirapinyo, P., Lonnerdal, B., Pencharz, P., Pzyrembel, H., Ramirez-Mayans, J., Shamir, R., Turck, D., Yamashiro, Y., and Zong-Yi D. (2005) Global standard for the composition of infant formula: recommendations of an ESPGHAN coordinated international expert group. *J. Pediatr. Gastroenterol. Nutr*, 41:584-99

Mennella, J.A., Ventura, A.K. and Beauchamp, G.K. (2011) Differential growth patterns among healthy infants fed protein hydrolysate or cow-milk formulas. *Pediatrics* 127:110-8

Niijima, A. (2000) Reflex effects of oral, gastrointestinal and hepatoportal glutamate sensors on vagal nerve activity. *J. Nutr,* 130:971S–3S.

Rigo, J., Salle, B., Putet, G. and Senterre, J. (1994) Nutritional evaluation of various protein hydrolysate formulae in term infants during the first month of life. Acta Paediatr, Suppl. 402:100-4.

Rzehak, P., Sausenthaler, S., Koletzko, S., Reinhardt, D., von Berg, A., Krämer, U., Berdel, D., Bollrath, C., Grübl, A., Bauer, C.P., Wichmann, H.E., Heinrich, J. and the GINI-plus Study Group. (2011) 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,* 94(6 Suppl):1803-7S

Schaafsma, G. (2009) Safety of protein hydrolysates, fractions thereof and bioactive peptides in human nutrition. *Eur J Clin Nutr* 63:1161-8

Seppo, L., Korpela, R., Lonnerdal B, Metsaniitty, L., Juntunen-Backman, K., Klemola, T., Paganus, A., Vanto, T. (2005) A follow-up study of nutrient intake, nutritional status, and growth in infants with cow milk allergy fed either a soy formula or an extensively hydrolyzed whey formula. *Am. J. Clin. Nutr,*82:140-5.

Szajewska, H. (2007) Extensive and partial protein hydrolysate preterm formulas.(2007) J Pediatr Gastroenterol Nutr 45 Suppl 3:S183-7

Terracciano, L., Isoardi, P., Arrigoni, S., Zoja, A. and Martelli, A. (2002) Use of hydrolysates in the treatment of cow's milk allergy. *Ann. Allergy Asthma Immunol* , 89(Suppl 1):86-90

Vandenplas, Y., Hauser, B., Blecker, U., Suys, B., Peeters, S., Keymolen, K., Loeb, H. (1993) The nutritional value of a whey hydrolysate formula compared with a whey-predominant formula in healthy infants.*J Pediatr Gastroenterol Nutr* 17:92-6

Ventura, A.K., Beauchamp, G.K. and Mennella, J.A. (2012) Infant regulation of intake: the effect of free glutamate content in infant formulae. *Am. J. Clin. Nutr*, 95:875-81

Ventura, A.K., San Gabriel, A., Hirota, M.and Mennella, J.A. Determination of free amino acid content in infant formulae. *Nutr. Food Sci* (*in press*)

