



Extensively hydrolysed infant formulas: Need for aligned definition of peptide size characteristics and standardisation of analytical methods

To the Editor: We read with interest the publication by Levin *et al.*^[1] on the peptide composition and potential residual allergenicity of a range of speciality infant formulas. This publication is topical, as current cow's milk protein allergy guidelines have provided relatively little detail on the molecular specifications and technical requirements of extensively hydrolysed formulas (EHFs).^[2-5] Currently, significant differences exist in the peptide composition of marketed EHFs which are thought to be the basis for observed differences in their clinical tolerability.^[6,7] Some EHFs with a comparatively high residual allergen content may be associated with a significant rate of allergic reactions; however, their exact allergen contents and peptide profiles are generally not publicly disclosed. For this reason, providing published information on the degree of hydrolysis and residual allergen content in EHFs is welcome and will assist clinicians in selecting a hypoallergenic formula with a low residual allergen content.

Levin *et al.*^[1] evaluated several EHFs and amino acid-based formulas (AAFs) available in South Africa (SA) for their residual allergen content, peptide size profile and amino acid content. EHFs are manufactured by extensive enzymatic hydrolysis, heat denaturation and sometimes ultrafiltration, which eliminates the vast majority of allergenic peptides. Conversely, AAFs are not prepared by hydrolysis and contain free amino acids, but no residual cow's milk-derived peptides. We were intrigued to see some large differences and high values obtained for peptide compounds between 1.5 and 3 kDa in a range of EHFs and AAFs, as summarised in Table 2 of the publication. Levin *et al.*^[1] report values for Alfaré, a whey-based EHF, which are in stark contradiction to our own analytical results. Based on area under the curve (AUC) data provided, they found 14% peptides of 1.5 - 3 kDa in size for Alfaré, compared with <2% of peptides >1.2 kDa found by our internal analyses.^[8] In addition, several AAFs were reported as having a significant content of larger peptides, which is not plausible given the manufacturing process from single amino acids. We speculate that the detection of larger peptides in EHFs and AAFs may be due to an analysis artefact, possibly relating to residual starches or fat in formula samples.

In conclusion, we congratulate the authors on addressing the heterogeneity of EHFs in terms of residual allergen content and peptide composition. However, we question the validity of the reported content of larger peptides (1.5 - 3 kDa) in EHFs and AAFs. The study highlights the need for standardisation of analytical methods in the quantification of the residual allergen content in hypoallergenic formulas. Furthermore, formal consensus is required on the definition and technical requirements of EHFs with regard to peptide molecular weight profile and proportion of peptides >1.2 kDa. Importantly, while molecular peptide characteristics of EHFs may suggest hypoallergenicity, these findings need to be assessed by rigorous clinical trials demonstrating tolerance in at least 90% of cow's milk-allergic children by double-blind, placebo-controlled food challenge, in line with the guidance provided by the American Academy of Pediatrics.^[2]

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1. Levin ME, Blackhurst DM, Kirstein F, Kok D, van der Watt GF, Marais AD. Residual allergenicity of amino acid-based and extensively hydrolysed cow's milk formulas. *S Afr Med J* 2017;107(9):763-767. <https://doi.org/10.7196/SAMJ.2017.v107i9.12137>
2. American Academy of Pediatrics, Committee on Nutrition. Hypoallergenic infant formulas. *Pediatrics* 2000;106(2 Pt 1):346-349. <https://doi.org/10.1542/peds.106.2.346>
3. Fioocchi A, Schünemann H, Ansotegui I, et al. World Allergy Organization (WAO) diagnosis and rationale for action against cow's milk allergy (DRACMA) guidelines. *World Allergy Organ J* 2010;3(4):57-161. <https://doi.org/10.1097/WOX.0b013e3181dfeeb9>
4. Koletzko S, Niggemann B, Arato A; European Society of Pediatric Gastroenterology, Hepatology, and Nutrition. Diagnostic approach and management of cow's-milk protein allergy in infants and children: ESPGHAN GI Committee practical guidelines. *J Pediatr Gastroenterol Nutr* 2012;55(2):221-229. <https://doi.org/10.1097/MPG.0b013e31825c9482>
5. Muraro A, Werfel T, Hoffmann-Sommergruber K; EAACI Food Allergy and Anaphylaxis Guidelines Group. EAACI food allergy and anaphylaxis guidelines: Diagnosis and management of food allergy. *Allergy* 2014;69(8):1008-1025. <https://doi.org/10.1111/all.12429>
6. Lambers TT, Gloerich J, van Hoffen E, Alkema W, Hondmann DH, van Tol EA. Clustering analyses in peptidomics revealed that peptide profiles of infant formulae are descriptive. *Food Sci Nutr* 2015;3(1):81-90. <https://doi.org/10.1002/fsn3.196>
7. Towards optimized management of cow's milk allergy. *EMJ Allergy Immunol* 2018;3(1):50-59. <http://emj.europanmedical-group.com/wp-content/uploads/sites/2/2018/07/Towards-Optimised-Management....pdf> (accessed 5 October 2018).
8. Johns PW, Jacobs WA, Phillips RR, McKenna RJ, O'Kane KA, McEwen JW. Characterisation of peptide molecular mass distribution in commercial hydrolysates and hydrolysate-based nutritional products. *Food Chem* 2011;125(3):1041-1050. <https://doi.org/10.1016/j.foodchem.2010.10.015>

Levin *et al.* respond: We welcome the interest in and comments on the analysis we performed on the various milk formulas available in SA. We agree that nutrition, especially in the young, is most important and that standardisation of methods is very important. As dedicated laboratories are not available in SA, the analysis was done in a research laboratory setting and with such methods as were available to answer the question about the suitability of the various milk formulas for patients. As indicated in the results section for high-performance liquid chromatography, the ultraviolet absorption wavelength is not necessarily specific. The AUC for the molecular size range of peptides lacked specific peaks that might have prompted us to investigate this region in more detail. This was corroborated by the lack of fluorescent derivatives of carboxylic acid moieties on the gel. Since we were confident that all products appeared satisfactory, we did not investigate the difference in the AUC in the small-peptide region any further. We agree that the additional AUC may be due to other substances.

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