Correlation of the allergenicity and tolerogenicity of two cow's milk protein products with intestinal uptake

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Introduction: It remains largely unknown which features of food proteins that render them allergenic versus tolerogenic. However, it has been suggested that the protein-chemical features affects protein uptake in the intestine, and that protein uptake route may impact on the risk of sensitisation.

Objectives: The aim of this study was to investigate the interplay between protein-chemical characteristics, the allergenic versus tolerogenic properties and the intestinal uptake of two protein products. The allergenic versus tolerogenic capacity of a heat-treated whey product, consisting of partly denatured and aggregated proteins, was compared to an unmodified whey product in: 1) an i.p. and 2) a gavage sensitisation model, 3) an oral primary prevention model and 4) an oral desensitisation model in Brown Norway (BN) rats. Elicitation was measured by *in vivo* tests and antibody responses by various ELISAs. The intestinal uptake was investigated by quantifying β-lactoglobulin levels in serum and various small intestinal tissues at different time points after dosing naïve BN rats by oral gavage. The *in vivo* measures were supplemented by *in vitro* uptake experiments in cell lines and primary cells.

Results: Though this study showed that both unmodified and heat-treated whey had immunogenic, sensitising and eliciting capacities as well as tolerance inducing capacity, significant differences between the two products were observed. The heat-treated product was found to have a lower allergenicity combined with high tolerogenicity compared to the unmodified product. Competitive IgG1 ELISAs indicated that heat-treatment of whey induced *de novo* epitopes while the original epitopes were maintained.

Newly established methods to study *in vivo* intestinal uptake were successfully applied to compare the uptake kinetics of the two products in different small intestinal tissues and serum. Collectively the *in vivo* and *in vitro* uptake experiments suggested that uptake kinetics and the major intestinal uptake route differed between the heat-treated and unmodified product.

Conclusion: This study showed that heat-treatment, which induces partly protein denaturation and aggregation, changes the immunological properties and intestinal uptake of a whey protein product. The heat-treated product was found to have a lower allergenicity combined with high tolerogenicity compared to the unmodified product, which highlights this products promising potential for induction of cow's milk tolerance.