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Mild protein hydrolysation of lactose-free milk further reduces milk-related gastrointestinal symptoms

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Gastrointestinal symptoms associated with milk are common. Besides lactose, milk proteins may cause symptoms in sensitive individuals. We have developed a method for mild enzymatic hydrolysation of milk proteins and studied the effects of hydrolysed milk on gastrointestinal symptoms in adults with a self-diagnosed sensitive stomach. In a double blind, randomised placebo-controlled study, 97 subjects consumed protein-hydrolysed lactose-free milk or commercially available lactose-free milk for 10 d. Frequency of gastrointestinal symptoms during the study period was reported and a symptom score was calculated. Rumbling and flatulence decreased significantly in the hydrolysed milk group ($P < 0{\cdot}05$). Also, the total symptom score was lower in subjects who consumed hydrolysed milk ($P < 0{\cdot}05$). No difference between groups was seen in abdominal pain ($P = 0{\cdot}47$) or bloating ($P = 0{\cdot}076$). The results suggest that mild enzymatic protein hydrolysation may decrease gastrointestinal symptoms in adults with a sensitive stomach.

Keywords: Milk protein, enzymatic hydrolysation, gastrointestinal symptoms, human.

According to clinical experience, food-related reasons are commonly suspected as being the cause of various gastrointestinal symptoms. These symptoms often induce significant inconvenience, reduce working capacity and generate healthcare costs.

Cow's milk is the single most common food item considered to induce gastrointestinal discomfort. In a Finnish study with 1900 subjects attending outpatient clinics for gastrointestinal symptoms, about 40% proposed milk as the cause (Anthoni, 2009). The prevalence of gastrointestinal symptoms associated with milk consumption is higher than the prevalence of lactose intolerance, cow's milk allergy, coeliac disease, inflammatory bowel diseases as well as irritable bowel syndrome (IBS), which may also be associated with reduced tolerability of cow's milk. The manifestation of stomach symptoms in healthy subjects is affected by meal composition, which has an important effect on gastric emptying and thereby on the lactose load in the gut (Martini et al. 1988; Vesa et al. 1997). Also, differences in individual sensitivity and gut microflora may affect the tolerability of milk.

Milk proteins are often suspected to be the cause of non-specific, undiagnosed gastrointestinal symptoms in adults. Although IgE-mediated cow's milk allergy in adults is rare, studies have shown high serum reactivity and delayed hypersensitivity reactions to milk in healthy subjects (Bengtsson et al. 1997; Pelto et al. 1998, 1999; Ulanova et al. 2000). Hydrolysis of milk proteins may enhance the tolerability of milk. We investigated the hypothesis that gastrointestinal problems could be reduced by hydrolysation of milk protein to smaller peptides.

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Materials and methods

Subjects

Subjects were recruited via advertisements posted on popular Finnish health-related internet pages (www.tohtori.fi, www.klinikka.fi). The inclusion criteria were: self-diagnosed sensitivity to milk, ability to consume 2–3 dl lactose-free milk daily during the study and age between 18 and 65 years. Subjects were excluded if they had a diagnosis of inflammatory bowel disease, milk allergy, cancer, IBS, they had received antimicrobials during the last month or had diagnosed depression. Also, pregnant and lactating women were not eligible.

Study design

The study was a randomised, placebo-controlled, parallel-group intervention of 10 d. Subjects were randomised to

two groups adjusted for age, gender and history of gastrointestinal symptoms from lactose-free milk. All subjects provided informed consent before entering the study.

During the intervention, subjects consumed at least 2 dl of mildly hydrolysed, lactose-free milk (Valio Ltd, Helsinki, Finland) or commercially available lactose-free milk (Valio Ltd, Helsinki, Finland) daily. The product could be used at any time of day and could simply be drunk or be included in tea or coffee, added to cereals or used in cooking. Use of other fresh and/or liquid milk products (milks, sour milks, cream, yoghurt, quark, sour cream etc.) was prohibited during the study. Otherwise, the subjects continued to follow their habitual diet.

Symptoms

Data on the occurrence of gastrointestinal symptoms – abdominal pain, flatulence, rumbling and bloating – was collected with a questionnaire before entering the study (on the subjects' habitual diet) and after the study was completed. The frequency of symptoms was evaluated on a scale of 1 (absence of symptoms) to 7 (constant symptoms).

Study products

Lactose free milk was produced according to EP1503630B1. The process involves ultrafiltration and nanofiltration of milk to remove lactose, followed by enzymatic hydrolysis of the remaining lactose. The lactose content of the milk was <0·01%. Carbohydrate content in lactose free milk was 3·1% instead of 4·8% in normal milk. Otherwise, the composition of lactose free milk was the same as that of normal milk.

Protein hydrolysed milk was produced according to FI123201. Lactose free milk was produced as described above and was then subjected to hydrolysis of proteins in a controlled way so that the degree of hydrolysis was 150 µg free tyrosine/ml as analysed according to the modified method of Matsubara et al. (1958). Analysis was performed for samples which were boiled for 4 min at 100 °C and centrifuged. Soluble tyrosine was determined for the supernatant after centrifugation (3000 rcf, 15 min).

According to capillary electrophoresis (Miralles et al. 2001), hydrolysis was directed predominantly on β-casein and κ-casein. According to the anion exchange gel filtration chromatographic method (Korbes et al. 1994), concentration of β-casein was reduced by 60–90% as compared to the concentration in normal lactose free milk. According to SDS-PAGE, whey proteins were not hydrolysed significantly (Laemmli, 1970) (Fig. 1). SDS-PAGE analyses were carried out using ready made 18% Tris-HCL polyacrylamide gels (Bio-Rad, Hercules, USA). The amount of protein added to each sample well was about 5 µg. Protein bands were stained with Coomassie Brilliant Blue R-250 (Bio-Rad, UK) and compared with molecular weight markers Precision Plus Protein All Blue Standards (Bio-Rad Laboratories, USA).

The energy and macronutrient content of the study products was similar. Both products contained 164 KJ (39 kcal), 3,3 g protein, 1,5 g fat and 3,1 g carbohydrates per 100 g milk.

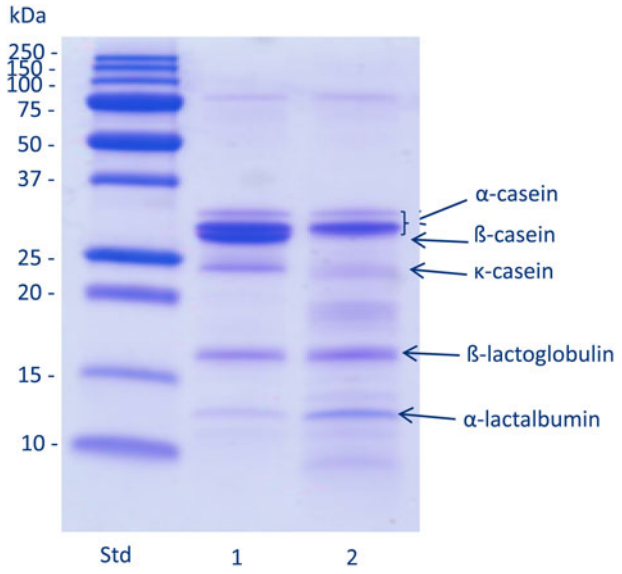

Fig. 1. SDS-PAGE of the study products. Std (Bio-rad 161-0373); (1) Lactose-free milk; (2) Protein-hydrolysed, lactose-free milk. Major milk proteins are identified.

Statistics

The primary outcome measure was total symptom score (abdominal pain + flatulence + bloating + rumbling). Secondary outcome scores were the scores of individual symptoms. Differences between groups in the occurrence of symptoms were compared by analysis of covariance with baseline as covariate using SPSS (version 19.0, SPSS Inc. Chicago, IL, USA).

Results

Subjects

Of the 97 subjects who entered the study, 94 subjects completed the study. Four subjects had used the study products during less than half of the days and were excluded from the analyses. Thus, the results are presented from 90 subjects (72 women and 18 men).

The mean age of subjects was 38 years and the majority had experienced symptoms from milk for more than 5 years. Of the subjects, 70% considered their symptoms rather or very disturbing. Baseline characteristics of subjects are shown in Table 1.

Products

Both milks were well accepted. The taste of both products was considered good, with average scores for the lactose-free milk and hydrolysed milk being 5,4 and 5,5,

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Table 1. Baseline characteristics of study subjects

	Lactose-free milk ($N = 42$)	Protein hydrolysed milk ($N = 48$)
Age range (years)	21–64	21–61
Mean age (years)	37	39
Sex		
Female (%)	86	75
Male (%)	14	25
History of stomach symptoms		
>5 years (%)	74	65
1–5 years (%)	26	27
<1 year (%)	0	8

respectively on a scale from 1 to 7. The difference between groups was not significant.

Compliance was good; 96% of subjects consumed at least 2 dl of the products daily on at least eight of the 10 study days.

Gastrointestinal symptoms

The mean pre-study total symptom scores (abdominal pain + bloating + flatulence + rumbling) did not differ between groups and were 20,4 points and 21,4 points, for the lactose-free and hydrolysed milk groups, respectively. In general, flatulence and bloating were the most common symptoms, both before and after the study, affecting over 70% of the subjects frequently or continuously.

The total symptom score decreased in both groups during the study, with a significant difference between groups ($P = 0{\cdot}039$) (Fig. 2). The decrease was 9,8 points in the hydrolysed milk group (from 21,4 to 11,7 points) and 7,1 points in the lactose-free milk group (from 20,3 to 13,2 points). Also, flatulence ($P = 0{\cdot}014$) and rumbling ($P = 0{\cdot}039$) decreased more in the hydrolysed milk group. No difference between groups was seen in abdominal pain ($P = 0{\cdot}47$) or bloating ($P = 0{\cdot}076$). Results for eight recently-affected subjects (<1 year history of stomach symptoms) in the hydrolysed milk group did not differ from those of the whole group.

Discussion

This study investigated the effects of mild enzymatic hydrolysation of milk proteins on gastrointestinal symptoms in adults with a self-diagnosed sensitive stomach. The total symptom score decreased in both groups, but was significantly lower in the hydrolysed milk group. Also, less flatulence and rumbling were reported in the group consuming hydrolysed milk. While a major part of individuals with milk-related gastrointestinal symptoms benefit from removal of lactose, even mild hydrolysis of milk proteins seems to provide some additional benefit.

In our subjects, the habitual consumption of milk products and lactose was low as 73% of the subjects followed a low-lactose or lactose-free diet. Therefore, the prohibition of use of other milk products during the study did not significantly change their dairy intake and does not explain the general improvement observed in both groups. Before the study, over 80% of the subjects reported experiencing adverse symptoms even after using low-lactose or lactose-free products. In the blinded design, symptoms significantly decreased also in the group using standard lactose-free milk. A placebo response has been shown to be common in interventions where gastrointestinal symptoms are studied (Patel et al. 2005) and was probably, at least to some extent, involved also in this study. The response was not associated with the history of symptoms (length of duration) and was similar in both sexes.

Mild enzymatic hydrolysis of proteins produces peptides of different length. The digestion and absorption of protein hydrolysates is faster and postprandial amino acid availability is augmented compared to intact protein (Koopman et al. 2009). Also the immunogenic properties may be modified, which could affect local immune responses in the gastrointestinal tract.

In children, cow's milk allergy is the main cause of milk-related gastrointestinal symptoms, affecting 2–3% of children under 10 years of age (Høst, 2002). In adults, IgE-mediated milk allergy is rare (see Crittenden & Bennett, 2005). However, in healthy adults, delayed hypersensitivity reactions to cow's milk have been detected and have been associated with gastrointestinal and general symptoms (fatigue, itching, fever) (Bengtsson et al. 1997; Ulanova et al. 2000). Also young Finnish adults with normal lactose tolerance and without IgE-mediated allergy, but who got symptoms from milk, were shown to have high serum reactivity to milk proteins (Pelto et al. 1998, 1999). In these subjects, cow's milk sensitisation results in local antibody production in the intestine and immunological activation (Ulanova et al. 2000). As hydrolysis of milk proteins renders milk suitable for allergic children, it could reduce reactivity to milk proteins and enhance the tolerability of milk also in adults.

Of the various milk proteins, β-casein, the second most abundant protein in cow's milk and specifically the A1 allele of β-casein, has been associated with gastrointestinal problems and milk intolerance (Pal et al. 2015). Proteolytic hydrolysis of A1 β-casein produces β-casomorphin 7 (BCM7), whereas it is not released from A2 β-casein (De Noni, 2008).

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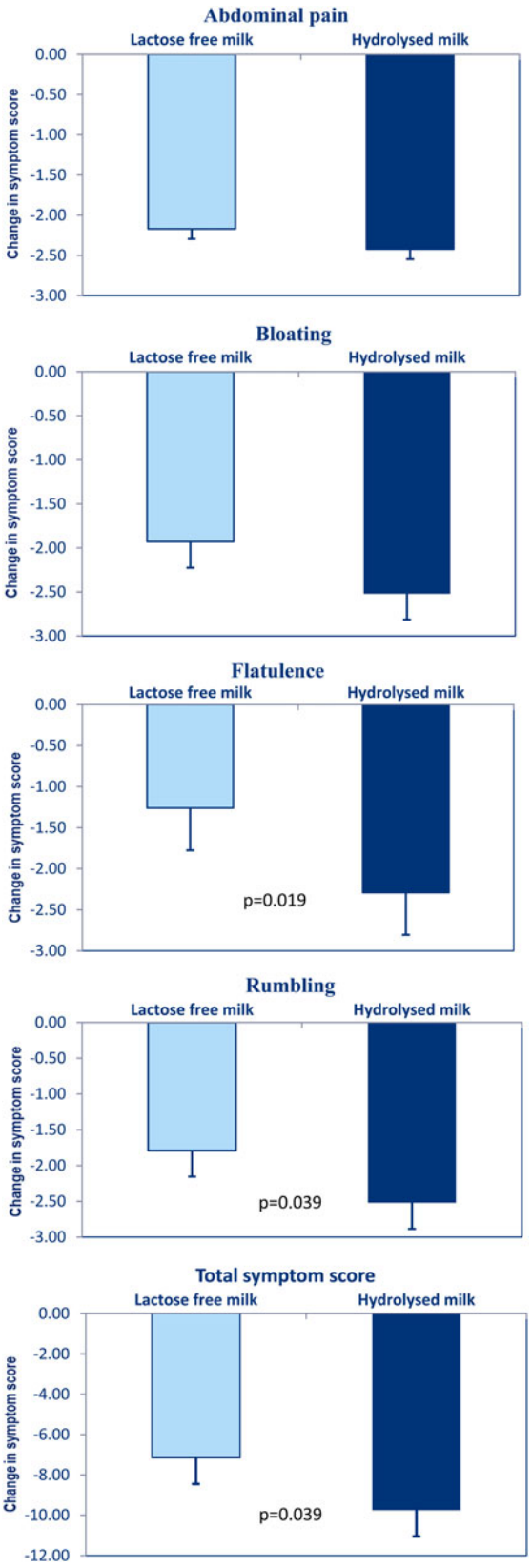

Fig. 2. Changes in gastrointestinal symptoms during the study.

In animals, BCMs have been demonstrated to prolong gastrointestinal transit time by modulating water and electrolyte absorption (Becker et al. 1990; Daniel et al. 1990) and to induce mucus production in the intestine (Claustre et al. 2002).

A recent clinical study is the first to report effects of milk containing A1 or A2 β-casein on gastrointestinal wellbeing in adults (Ho et al. 2014). Subjects consumed milk that contained β-casein of either A1 or A2 type (750 ml/d; ~7.5 g A1 or A2 β-casein) for two weeks followed by two weeks of the alternative A1 or A2 type milk after a washout period (Ho et al. 2014). A1 β-casein milk led to significantly higher stool consistency values compared with the A2 β-casein milk. There was also a significant positive association between abdominal pain and stool consistency on the A1 milk. Higher values of faecal calprotectin, a marker of intestinal inflammation, and associated intolerance measures were seen in some individuals, suggesting that some individuals may be susceptible to A1 β-casein. In the present study, the majority of β-casein was degraded in the hydrolysed milk. In our subjects with a sensitive stomach, this could, at least partly, explain the decrease in gastrointestinal symptoms.

Adverse symptoms associated with milk often lead to restricted consumption or total elimination of milk from the diet. Yet, milk is a significant source of nutrients also for adults. Consuming more than three servings of dairy per day leads to better nutrient status, improved bone health and is associated with lower blood pressure, reduced risk of cardiovascular disease and type 2 diabetes (Rice et al. 2013). Thus technologies, which allow modification of milk proteins to increase tolerability of milk provide potential to develop new dairy products also for adults with milk sensitivity. Results of the present study suggest that in addition to the removal of lactose, enzymatic hydrolysation of β-casein may also decrease gastrointestinal symptoms in subjects with a sensitive stomach.

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