

No difference in symptoms during challenges with homogenized and unhomogenized cow's milk in subjects with subjective hypersensitivity to homogenized milk

Laura Paajanen^{1,4}, Tuula Tuure², Tuija Poussa³ and Riitta Korpela^{2,4,5*}

¹ Division of Nutrition, Dept. of Applied Chemistry and Microbiology, FIN-00014 University of Helsinki, Finland (Present position is 4)

² Valio, Ltd., Research and Development, P.O. Box 30, FIN-00039 Valio, Finland

³ STAT-Consulting, Pynikintie 25, FIN-33230 Tampere, Finland

⁴ Foundation for Nutrition Research, P.O. Box 30, FIN-00039 Helsinki, Finland

⁵ Institute of Biomedicine, FIN-00014 University of Helsinki, Finland

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It has been hypothesized that certain consumers tolerate untreated cow's milk, but react to processed (i.e. homogenized and pasteurized) cow's milk although they do not suffer from IgE-mediated cow's milk allergy or lactose intolerance. The aim of the study was to compare the tolerance of unhomogenized and homogenized cow's milk in lactose tolerant adults who had repeatedly experienced better tolerance of unhomogenized than homogenized milk. Forty-four subjects were challenged with homogenized and unhomogenized cow's milk for five days in a randomized, double-blind, cross-over study. No differences in the symptoms during the challenges were found. Roughly half the subjects tolerated the homogenized milk better and the other half tolerated the unhomogenized milk better. The results of this study show no difference in the tolerance of homogenized and unhomogenized milk in adults with self-reported symptoms suggestive of hypersensitivity to homogenized milk.

Keywords: Milk homogenization, milk hypersensitivity, milk intolerance.

In Nordic countries a number of consumers claim that they tolerate raw untreated cow's milk and unhomogenized pasteurized cow's milk but show reactions of intolerance to homogenized and pasteurized commercial cow's milk and other dairy products. The parents of certain children who are allergic to cow's milk report the same phenomenon.

The hypothesis, which could explain how milk homogenization could cause symptoms, is based on the observation that the composition of allergic milk proteins is changed during homogenization. Raw cow's milk contains lipid globules of different sizes ($\varnothing$ 0.1–15 μ m), which are covered by a phospholipid layer and by membrane proteins. Around 80% of milk proteins are grouped in micelles in the raw milk. During homogenization large lipid globules are disrupted into smaller globules ($\varnothing$ <2 μ m). As a consequence, the surface area of the milk lipid globules expands and the membrane layers are no longer able to cover the fat globules and are thus partially replaced by milk proteins. In raw milk the majority of the antigenic

determinants are inside the casein micelles, but in homogenized milk the concentration of surface-exposed antigenic determinants is higher (Poulsen et al. 1987). This change should not be of physiological significance, since the concentration of antigenic determinants in raw milk is high enough to produce the reactions to persons with cow's milk protein allergy and on the other hand, antigenic determinants are not harmful to non-allergic people. For a review on homogenization see Keenan & Patton (1995).

There is some evidence from animal experiments that homogenization of milk causes hypersensitivity reactions to study animals. In animal experiments, homogenized cow's milk given orally to milk hypersensitive mice induced anaphylactic shocks (Poulsen et al. 1987), increased IgE production (Nielsen et al. 1989), increased production of milk specific immunoglobulins, increased the mass of the gut segment and induced degranulation of mast-cells (Poulsen et al. 1990), while unhomogenized cow's milk induced remarkably fewer symptoms and immunological responses, or none at all.

However, there is no evidence that homogenization of milk could cause milk hypersensitivity to humans. In

*For correspondence; e-mail: Riitta.Korpela@valio.fi

clinical studies, no difference in tolerance between homogenized and unhomogenized cow's milk has been observed, either in children with cow's milk allergy (Hansen et al. 1987; Høst & Samuelsson, 1988) or in adults with lactose intolerance (Vesa & Korpela, 1994) or milk hypersensitivity (Pelto et al. 2000). On the other hand, no studies have been made to determine whether individuals who report better tolerance of unhomogenized than homogenized milk react to processed milk.

The aim of the present study was to compare the tolerance of unhomogenized and homogenized cow's milk in lactose tolerant adults with subjective tolerance of unhomogenized milk but subjective experience of an intolerance to homogenized milk.

Materials and Methods

Subjects

Volunteers, who had experienced abdominal symptoms from homogenized milk but were able to consume unhomogenized milk with no or remarkably fewer symptoms, were recruited for the study. Of the 130 volunteers who initially showed an interest in participating in the study, 51 were included and 79 excluded. Only those who reported definite symptoms from a glass of homogenized milk and seemed likely to have enough time and interest to fully follow the study protocol were included. Patients with inflammatory bowel disease, celiac disease, extensive surgery in the gastrointestinal tract or serious chronic diseases (e.g. cancer) were excluded from the study. Three subjects had to be excluded as they did not return the study diaries.

Digestion of lactose was determined in all the subjects after an overnight fast by a three-hour hydrogen breath test described in detail elsewhere (Peuhkuri et al. 1998). Four subjects were revealed to be lactose intolerant (3 male, 1 female) and were excluded from the study. Thus the final study group consisted of 44 subjects (14 male, 30 female). The subjects were defined as having subjective hypersensitivity to homogenized milk because they reported gastrointestinal reactions such as abdominal bloating, pain, flatulence or diarrhoea following the intake of homogenized cow's milk although they did not suffer from lactose intolerance.

The subjects were interviewed with a pre-tested form (Table 1). The mean age of the subjects was 38 years (range 18–64). Of the study subjects 17 (39%) suffered from an allergic disease diagnosed by a doctor and additionally 5 (11%) reported self diagnosed allergic reactions. Thirteen (30%) subjects suffered from some other chronic disease diagnosed by a doctor, e.g. high blood pressure, according to subjects own answers.

Study products

The two milks studied were a) a homogenized, pasteurized commercial milk with fat content of 1.5% (Valio Ltd.,

Table 1. Baseline characteristics of subjects (*n*=44)

Mean age, years (range)	38 (18–64)
Male/Female	14/30
Allergic disease diagnosed by a doctor	39%
Food allergy	16%
Asthma, pollinosis, animal or dust allergy	36%
Other allergic disease	27%
Symptoms after consumption of homogenized milk†	
Flatulence	91%
Diarrhoea	70%
Abdominal pain	66%
Bloating	66%
Mean onset of symptoms (range)†	5 h (<1–24 h)
Mean recovery of symptoms (range)†	17 h (<1–72 h)

† Symptoms reported by subjects in the initial interview

Finland), and b) an unhomogenized and pasteurized commercial organic milk with a fat content of 1.5% (Valio Ltd.). Unhomogenized milk separates when left standing and therefore 20 ml whipping cream (fat content 38%) was added per litre of homogenized milk to induce an apparently similar separation in homogenized milk. In a paired comparison test a test group was not able to identify the study products, as they looked, tasted and smelt the same. The milks were contained in identical 200-ml bottles.

Study design

The study was conducted using a randomized, double-blind, cross-over design. Each subject was challenged with the two test milks for 5 d. The order of the challenges was randomized using a block randomization method. There was a run-in period of 7 d before the first milk challenge and a wash-out period of 9 d between the challenges. During the run-in and the wash-out periods the subjects followed an otherwise milk-free diet, but in order to maintain study compliance and to keep the diet as constant as possible they were given permission to take unhomogenized milk in their tea or coffee. During the challenges the subjects followed an otherwise strictly milk-free diet, but consumed one 200-ml quota of test milk every morning with their normal breakfast, and another quota later during the day. The subjects were advised to drink only the morning quota if they felt adverse reactions and to give up drinking the milks if there was any severe reaction. The subjects received detailed guidance on how to follow the strict milk-free diet, and were asked to indicate, in their diaries, if they accidentally consumed milk-containing food, or if there was a possibility that they had done so.

During the study the subjects kept a daily diary of the consistency and number of their bowel movements and of any abdominal symptoms (abdominal pain, flatulence, bloating or nausea). The consistency of bowel movements was graded from 1 (hard) to 5 (watery) and abdominal

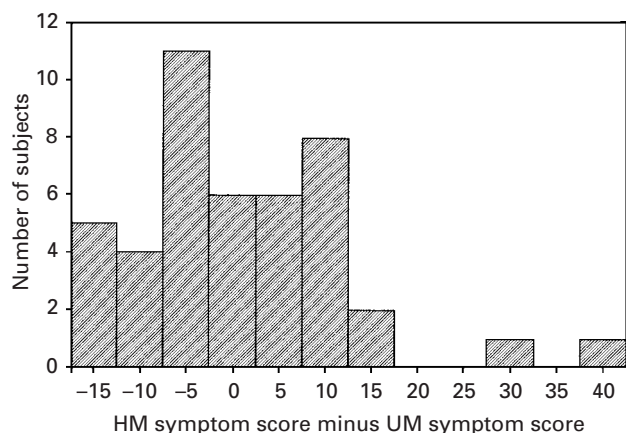


Fig. 1. The sum of abdominal symptoms (abdominal pain, flatulence, bloating or nausea) during 5 d challenge with homogenized milk (HM) minus the sum of symptoms during 5 d challenge with unhomogenized milk (UM) recorded by each study subject ($n=44$). Positive values (maximum possible=60) indicate more symptoms and negative values indicate fewer symptoms from homogenized milk compared with unhomogenized milk. Only the challenge days, when subjects ingested the study milks, are included.

symptoms were graded from 0 (no symptoms) to 3 (severe symptoms). The number of the quotas of the test milk consumed was also recorded daily.

The study protocol was approved by the Ethics Committee of the Hospital District of Helsinki and Uusimaa (HUS). Informed consent was obtained from all the study subjects.

Statistics

The sample size calculations were based on the total symptom scores (sum of abdominal symptoms). It was estimated that the clinically significant difference in symptom score between the challenges would be 4 points. It was calculated that the necessary sample size in a cross-over study would be 38 subjects in order to show the difference between challenges statistically significant at the significance level of 5% and power of 80%. Allowing for any possible withdrawals, the final number of 44 participants was considered to be sufficient.

The primary variable was the sum of abdominal symptoms (abdominal pain+flatulence+bloating+diarrhoea). This was calculated for the 5 d of the milk challenges and for the corresponding milk-free periods. The possible range was 0–12 symptom scores per d and 0–60 over the 5 d. The sum of the symptom scores for the 5 d was calculated using two approaches, with and without those days when consumption of the study milks was given up. The sum of symptoms during the homogenized and unhomogenized milk periods was analysed using the conventional ANOVA for cross-over analysis. ANOVA for repeated measures was applied to study treatment difference, period effect and the

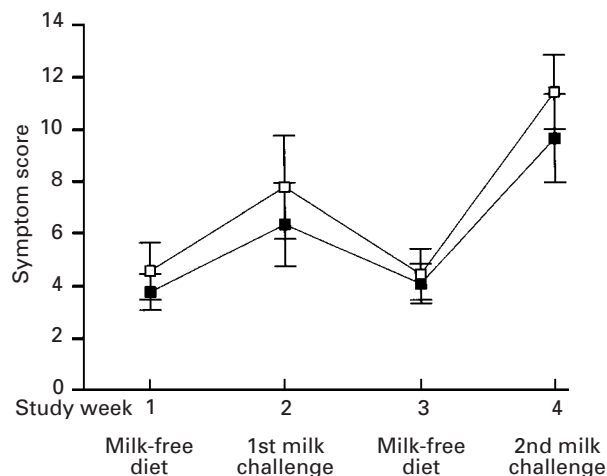


Fig. 2. The mean severity of symptoms during the cross-over study, starting with the milk-free period, during the challenges with homogenized and unhomogenized milk, and during the milk-free wash-out period between the challenges. The symptom score is the sum of 5 d (possible range 0–60). The symptom score of subjects who first ingested homogenized milk (1st challenge) and continued with unhomogenized milk (2nd challenge) is marked with black squares (■) and the symptoms score of subjects who first ingested unhomogenized milk (1st challenge) and continued with homogenized milk (2nd challenge) is marked with white squares (□). The results are given as means $\pm$ SEM. All challenge days are included, even if the subject gave up drinking the test milk.

interaction between treatment and period. The McNemar test was used to compare the prevalence of individual symptoms between homogenized and unhomogenized milks, and between milk-free period and milk challenges. Challenges with homogenized and unhomogenized milk were combined to 'milk periods' when compared to milk-free period. Results are given with mean $\pm$ SEM.

Results

Of the 44 subjects, 22 (50%) experienced more symptoms (abdominal pain, flatulence, bloating and nausea) during the challenge with homogenized milk, 21 (48%) experienced more symptoms during the challenge with unhomogenized milk and one subject (2%) had the same amount of symptoms during both challenges. In Fig. 1 the frequencies of the different symptom scores during the milk challenges are compared. Two subjects tolerated the unhomogenized milk markedly better than the homogenized milk (Fig. 1). Two subjects gave up the homogenized milk and one subject gave up the unhomogenized milk, because of severe reactions.

There was no difference between the homogenized and the unhomogenized milk on the severity (Fig. 2, Fig. 3) or the prevalence (Table 2) of symptoms. However, the severity (Fig. 2, Fig. 3) and the prevalence (Table 2) of

Table 2. Prevalence of abdominal symptoms during the baseline milk-free diet ($n=40$) and during the challenges with homogenized milk (HM) and unhomogenized milk (UM), and when the milk periods have been combined (Milk periods) in 44 subjects. Figures represent numbers of subjects (%). McNemar test was used for all comparisons

Symptom	Milk-free diet	Milk challenges			HM v. UM	Milk-free period v. Milk periods
		HM	UM	Milk periods		
Abdominal pain	17 (43%)	22 (50%)	20 (45%)	28 (70%)	$P=0.82$	$P=0.013$
Flatulence	28 (70%)	36 (82%)	34 (77%)	36 (90%)	$P=0.75$	$P=0.039$
Bloating	14 (35%)	28 (64%)	26 (59%)	33 (83%)	$P=0.81$	$P<0.0001$
Nausea	5 (13%)	9 (20%)	9 (20%)	14 (35%)	$P=1.00$	$P<0.022$

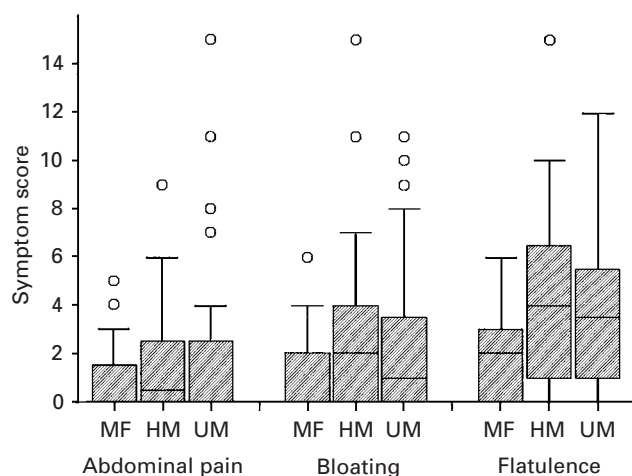


Fig. 3. Box-plot figures for the severity of abdominal pain, bloating and flatulence during the milk-free diet (MF), homogenized milk (HM) and unhomogenized milk (UM) challenges in 44 subjects. The symptom score is the sum of 5 d (possible range 0–15). The boxes indicate the lower and upper quartiles and the central line is the median. The upper end of the bar is the non-outlier maximum value. Outliers (>1.5 box length from the end of the box) are given with circles. All challenge days are included, even if the subject gave up drinking the test milk.

symptoms were higher during the milk challenges than during the milk-free period. During the milk-free period before the challenges the mean value for the sum of symptoms was 4.2 ± 0.6 (SEM). When discontinuation due to severe reactions was taken into account, the mean symptom scores were 9.4 ± 1.3 for the homogenized milk and 8.5 ± 1.2 for the unhomogenized milk ($P=0.58$). If all the challenge days are included, the symptom scores were 8.8 ± 1.1 and 8.6 ± 1.3 , during intake of homogenized milk and unhomogenized milk respectively ($P=0.91$). The difference between the scores of the homogenized and the unhomogenized milk during the 5 d milk challenges was 0.9 points (95% CI -2.4 to 4.2) or 0.2 points (-2.8 to 3.1) including only the days when subjects ingested the study the milks or all the challenge days respectively.

The subjects experienced more symptoms during the second challenge than during the first challenge, regardless of the order in which they received the different types

of milk (period effect $P=0.02$; Fig. 2). The sum of symptoms was the same during the run-in period and the wash-out period between the challenges.

There was no difference in the consistency of stools and the frequency of defecation during the challenges: the median consistency of stools was 2.4 in the milk-free diet, 2.6 in the homogenized milk period and 2.9 in the unhomogenized milk period.

Discussion

In the present study, the tolerance of homogenized and unhomogenized cow's milk was compared in subjects, all of whom, when interviewed, reported better tolerance of unhomogenized than homogenized milk. No differences in the symptoms during the challenges with the homogenized and the unhomogenized milk were discerned. Our findings are in agreement with the data of previous double-blind studies: in double-blind, placebo-controlled trials no difference between the homogenized and the unhomogenized milk has been shown in the symptoms of cow's milk allergic children (Høst & Samuelsson, 1988) or of lactose intolerant adults (Vesa & Korpela, 1994) or in the symptoms and complement receptor expression of milk hypersensitive, lactose intolerant or control subjects (Pelto et al. 2000).

To minimize non-compliance with the milk-free diet, the subjects received detailed guidance on how to follow a strictly milk-free diet and were encouraged to keep their diet as constant as possible during the study. According to the interviews with the subjects, 5 d challenges should be long enough to produce the symptoms and a 9 d wash-out period in the middle of the challenges long enough to prevent any carry-over effect. Most of the subjects experienced more symptoms during the second challenge than during the first, regardless of which milk they were consuming. The carry-over effect did not cause this, because during the wash-out period between the challenges, the subjects experienced no more symptoms than during the run-in period.

In the present study the homogenized test milk was blinded with whipping cream. The fat content of the homogenized test milk was 2.2% and of the unhomogenized test milk 1.5%. Fat-free homogenized milk mixed with the

whipping cream would have constituted a more equal fat content in both milks, but fat-free milk was not used because the fat-phase of homogenized milk is hypothesized to mediate adverse symptoms (Poulsen et al. 1987). We assume that the higher fat content of the homogenized milk did not affect the symptoms. According to the interviews, the subjects did not tolerate dairy products with high fat content better than the fat-free food.

None of the subjects had lactose intolerance, but all of them experienced adverse gastrointestinal reactions during the milk challenges. Therefore it is possible that some subjects may have had delayed cow's milk protein allergy. Until recently cow's milk allergy has been considered only a disorder of early childhood (Høst & Halken, 1990), but some recent studies suggest that delayed gastrointestinal cow's milk allergy might also be a disease of school-age children (Kokkonen et al. 2001) and young adults (Pelto et al. 1998, 1999). Unfortunately, there is no reliable diagnostic method for determining delayed reactions to cow's milk protein, and therefore delayed cow's milk allergy could not be diagnosed in the present study. Further studies are needed on the diagnosis, prevalence and treatment of delayed cow's milk allergy.

In conclusion, these results do not show any significant difference in the tolerance of homogenized or unhomogenized cow's milk in subjects with self-reported symptoms suggestive of hypersensitivity to homogenized milk.

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