Received: 15 June 2022 | First decision: 9 July 2022 | Accepted: 20 July 2022

DOI: 10.1111/apt.17171

AP&T Alimentary Pharmacology & Therapeutics WILEY

Tolerance to sterilised cow's milk in patients with eosinophilic oesophagitis triggered by milk

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Funding information
Instituto de Salud Carlos III, Grant/Award Number: CD19/00102; European Social Fund

Summary

Background: Cow's milk protein is the main food trigger for eosinophilic oesophagitis (EoE) in children and adults and should be continuously avoided once identified as such.

Aims: To evaluate tolerance of sterilised cow's milk (boiled instead of UHT processing) with regard to maintenance of EoE remission, health-related quality of life (HRQoL), nutritional intake and allergic sensitisation in patients of all ages with milk-triggered EoE

Methods: We prospectively recruited patients in whom cow's milk was demonstrated to trigger EoE after an empirical food elimination diet-based study. They were given 200 ml of sterilised cow's milk twice daily for 8 weeks. Endoscopic assessment, peak eosinophil counts, oesophageal-related symptoms, HRQoL, blood eosinophils, eosinophil cationic protein (ECP), skin prick test and serum total and specific immunoglobulin E (IgE) to major milk proteins were monitored before and after sterilised milk intake.

Results: Eighteen patients (13 male) in EoE remission underwent a sterilised milk challenge. Twelve maintained EoE remission (<15 eos/hpf) while EoE recurred in the remainder. Endoscopic appearances deteriorated in non-tolerant patients. HRQoL scored well at baseline and was maintained among patients tolerant to sterilised milk, but deteriorated in reactive ones. No significant changes in blood eosinophil count, ECP, tryptase or total and milk-specific IgE serum levels were observed from baseline. However, cow's milk-specific IgE increased slightly in non-tolerant patients. Clinical and histological remission were maintained in patients who regularly consumed sterilised milk for 1 year.

Conclusion: Sterilised milk did not trigger EoE in two-thirds of patients with documented milk-induced EoE, in either the short or long term.

The Handling Editor for this article was Doctor Colin Howden, and it was accepted for publication after full peer-review.

1 | INTRODUCTION

Eosinophilic oesophagitis (EoE) is a chronic inflammatory disease limited to the oesophagus, characterised by symptoms of oesophageal dysfunction and an eosinophil-predominant inflammation demonstrated in oesophageal biopsies.[1] From the first descriptions of the disease, EoE was characterised as a particular form of non-IgE-mediated[2] food allergy,[3] which is triggered and maintained by food allergens. Empirical avoidance of the six most common food allergens resolves oesophageal symptoms and eosinophilic inflammation in up to three quarters of all patients[4–6]; sequential food reintroduction under endoscopic and bioptic monitoring allows identification of food triggers in each patient.[5–7] Less restrictive and more convenient alternatives are the 4- or 2-food empirical elimination diets.[8–10] Cow's milk has been repeatedly identified as the most common food causing EoE in children and adults,[11,12] and its continuous avoidance is an effective non-pharmacological treatment,[13] just as in other cow's milk-mediated food allergies.[14]

Milk is composed of several proteins with allergenic potential if consumed including ß-lactoglobulin, caseins and α-lactalbumin. These are mediated by immunoglobulin E (IgE) in the majority of cases. However, a subgroup of patients has a non-IgE-mediated, cell-mediated, delayed allergy to milk, presenting primarily with gastrointestinal signs and milk ingestion-related symptoms, including eosinophilic oesophagitis.[15] Cow's milk is cross-reactive with that of other bovines due to the existing homology between its proteins. Most patients allergic to cow's milk do not, therefore, tolerate their milk either.[16,17]

In Europe, the prevalence of milk allergy varies regionally between 0.54% and 4.9%[18] tending to disappear with age. The best first-line treatment is the elimination of any food-containing milk. However, the difficulties in maintaining adherence to a milk-free diet, and its long-term effects on patients' quality of life (QoL)[19] and nutritional status,[20,21] have led to interventions allowing certain levels of milk tolerance among patients with various forms of allergy.[22]

Baked milk (in foods prepared by extensive heating) has proved to be well tolerated by up to 75% of children with IgE-mediated milk allergy,[23,24] increasing with long-term use.[25] In addition, a retrospective case series[26] has shown that baked milk is tolerated by some patients with milk-triggered EoE, as is baked cheese.[27] However, none of the EoE patients from the aforementioned studies included sterilised milk in their diets (i.e., boiled for at least 20 min); this could potentially provide a solution for patients who must avoid liquid milk. As no data are currently available on the potential tolerance and usefulness of sterilised cow's milk for such patients, this research aimed to prospectively evaluate just that in maintaining symptomatic and histological remission of the disease. Assessing changes in blood and allergy skin markers (as additional proof of tolerance) and effects on patients' HR QoL and dietary/nutritional status were additional goals.

2 | PATIENTS AND METHODS

2.1 | Study design and participants

In this quasi-experimental study, we recruited adolescent and adult patients with EoE diagnosed according to current criteria,[1] who were consecutively attending our EoE clinic and in whom cow's milk had been a demonstrable trigger for the disease. Previously treated with an empirical food elimination approach to identify causative food/s, once histopathological remission was confirmed, specific EoE food triggers were identified by sequential reintroduction. Four to 6 weeks after reintroducing each food previously withdrawn from the diet, all patients underwent endoscopy with oesophageal biopsies to document histological remission or recurrence, regardless of the presence of symptoms or endoscopy appearance. A six-food elimination diet approach[4,6] was used in 6 patients; 7 patients underwent a four-food elimination diet[8,9] and 3 a two-food elimination diet.[10] Finally, a single-food elimination (or milk-free) diet[28] was used in the remaining 2 patients.

Milk was identified as the only food triggering and maintaining EoE in 10 patients (55.6%), with the remaining having an additional food trigger. Disease remission was achieved and maintained in all study subjects without the aid of medication by avoiding those foods identified as EoE triggers. Strict avoidance of any type of product containing mammalian milk was prescribed for patients with milk-triggered EoE; baked milk was also not allowed.

All subjects were given the option of receiving readily available sterilised cow's milk, which was subjected to temperatures in excess of 100°C for 20 min, rather than ultra-high temperature (UHT) processing (milk heated to a temperature of 138–145°C for 2–10 seconds). Once the research team verified that they met the requirements for the study, different options were offered to patients, including Hacendado, RAM esterilizada, whole sterilised milk DIA and Sterilised milk Finca Cantarranas. Samples of sterilised milk were compared to UHT processed milk by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), providing evidence of extensive attenuation of the bands corresponding to 50–150 kDa, while the casein bands (19–25 kDa) were preserved intact (Figure S1). Patients were instructed to consume at least 200 ml of sterilised milk twice daily for an 8-week period.

Prior to starting, baseline symptoms and oesophageal eosinophil counts were determined for all participants. Patients were included in the study if endoscopy and biopsies were normal, with a peak eosinophil count below 15 cells per high-power field (hpf) at both the upper and the lower oesophageal halves.

Physical examinations and endoscopies with oesophageal biopsies were performed on each patient before and after sterilised milk consumption. Other dietary changes were avoided (A telephone number and e-mail address were provided in case of doubt).

Whenever possible, oral, nasal and airway steroids treatment was withdrawn during the 8-week study period. In cases of exacerbated rhinitis or asthma, anti-H1 or inhaled b2-agonists and anti-cholinergic bronchodilator drugs were allowed. Proton pump

inhibitors (PPIs) were not taken during the trial, as all patients recruited were proven non-responders to PPI.

2.2 | Endoscopy and biopsies

All endoscopic examinations were carried out by a single board-certified gastroenterologist (AJL) under sedation with propofol and using a flexible 9.2-mm-calibre Olympus GIF-H185 gastroscope (Olympus Medical Systems, Hamburg, Germany) with a 2.8-mm work channel. At least 3 biopsy samples from either distal and proximal oesophageal halves were taken with the aid of standard needle biopsy forceps (Endo Jaw FB-220U; Olympus Medical Systems). These were then fixed in 4% formalin and routinely processed for histopathological analysis.

2.3 | Histological study

Two expert pathologists (JMO and RJT) experienced in studying EoE biopsy samples and blinded to the patient biopsy identity, performed all pathological assessments. Oesophageal mucosa samples fixed in formalin were routinely processed: sections (5-μm thick) were cut from formalin-fixed, paraffin-embedded blocks and then placed on microscope slides and stained with haematoxylin and eosin. The peak number of eosinophils was counted in the most densely inflamed areas using a Nikon Eclipse 50i (Nikon Corporation, Tokyo, Japan) light microscopy at 400x (the hpf area measured 0.212 mm^2). Peak eosinophil counts were determined in the area with the highest density, independent of where the biopsy was taken or the biopsy examination site.

2.4 | Symptoms and quality of life assessment

EoE symptoms were structurally assessed with the Dysphagia Symptoms Score (DSS), one proposed by Alex Straumann in 2010[29] repeatedly used in research on EoE[5,10,30,31] and demonstrated to provide sufficient responsiveness. Briefly, the score assessed the frequency of dysphagia, ranging from none (0) to several times per day (5); the intensity of dysphagia, ranging from unhindered swallowing (0) to long-lasting complete obstruction requiring endoscopic intervention (5) and duration of dysphagia, ranging from no attacks (0) to lasting up to endoscopic removal of impacted food (5). The DSS was applied both at baseline and after 8 weeks of regular consumption of sterilised milk (on the day the endoscopic examinations were performed).

A translated, validated Spanish version of the EoE-QoL-A questionnaire[32] was used to evaluate HR QoL in all participants. The EoE-QoL-A is a disease-specific, validated instrument measuring QoL, developed by Taft et al., specifically for adult patients with EoE.[33] For the purposes of this study, 30 items applicable for all patients were scored, falling into five subscales: eating/diet impact (four items), social impact (four items), emotional impact (eight items), disease anxiety (five items), swallowing anxiety (three items), and eating/diet impact subscale (six items). The EoE-QoL-A score ranges from 0 (very good) to 4 (very poor) for every item. The final score was the weighted average of the aforementioned subscales.[34]

2.5 | Analytical study and food allergy skin test

Before beginning with sterilised milk and after the 8-week study period, blood analyses were carried out (using the venous line placed for sedation during endoscopy). These included eosinophil count, eosinophilic cationic protein (ECP), tryptase, and total serum IgE. In addition, specific IgE against the major allergenic proteins in cow's milk was also determined with the aid of the ImmunoCAP test (Pharmacia Diagnostics AB, Uppsala, Sweden). Values ranged from 0.0 UI/ml (absent or undetectable allergen-specific IgE) to >100 KU/l (very high level of allergen-specific IgE).

All subjects underwent skin prick tests (SPT) with extracted substances from commercial solutions of ALK Abelló (Madrid, Spain). The tests were performed at our hospital Allergy clinic according to the manufacturer's recommended protocol. Histamine chloride 10 mg/ml and sodium chloride 0.9% were respectively used as positive and negative controls. Test responses were read after 15 min. A wheel diameter caused by tested allergens more than 3 mm compared to the negative control was considered a positive response and sensitisation to that allergen.

2.6 | Dietary and nutritional assessment

Data on the participants' eating habits were collected by patients or parents using weekly food consumption frequency questionnaires, detailing all food and servings over a week. Adequate reproducibility and validity in assessing the consumption of groups of foods, energy and macronutrients in our environment have been demonstrated for these questionnaires.[35] Grams per day per item were calculated in relation to participant age, with expert assessment from our researcher nutritionist (MC-P). Four-week menus were to be analysed in terms of distribution of rations, energy, macro and micronutrients, before and during sterilised milk intake.

To accurately assess the nutritional composition of each patient's diet, the Organiser Metabolic Dietetic (Odimet) platform (www.odimet.es),[36] developed by the Diagnosis and Treatment of Metabolic Diseases Unit of Santiago de Compostela University Hospital, was used.[37]

2.7 | Outcome measures

Primary outcome measures were based on the histopathological response to sterilised cow's milk intake for 8 weeks. Maintenance of histological remission was identified as a peak eosinophil count of <15/

hpf in both upper and lower oesophageal halves after the challenge. Secondary outcome measures were maintaining baseline symptoms, lack of impairment in endoscopic features and changes in HR QoL and in dietary/nutritional assessment. Finally, changes in blood eosinophilia, serum ECP and tryptase, total and specific IgE levels along with SPT results before and after the challenge were evaluated. Seasonal variations regarding the point of sterilised milk introduction, endoscopic examination or eosinophil counts were not taken into account.

2.8 | Ethics

The study was conducted in accordance with the Declaration of Helsinki principles and approved by our hospital's institutional review board (code 092016). Informed consent was obtained from all patients or their legal guardians prior to all study procedures.

2.9 | Statistical analysis

Data are shown as mean ± SD for eosinophils and as a median with an interquartile range (IQR) for scoring clinical symptoms. Comparisons between groups (tolerant/intolerant) were performed with the Mann–Whitney *U* test or Student's *t* test for quantitative variables and the chi-squared test (or Fisher's exact test, where appropriate) for qualitative variables. The Wilcoxon signed rank test was used to compare values before and after sterilised milk consumption. A 0.05 level of significance was used throughout. Statistical analyses were performed with the aid of PASW 18.0 statistical analysis software (SPSS Inc).

3 | RESULTS

Between 2018 and 2021, 20 patients with milk-triggered EoE were recruited for tolerance to sterilised milk and underwent initial endoscopy; two were then excluded as eosinophilic inflammation >15 eos/hpf persisted in biopsies despite no evident symptoms of EoE activity. Eighteen patients (13 male) in EoE remission underwent the sterilised milk challenge therefore. The mean ± SD participant age was 32 ± 13.4 years (range: 13–59 years) and the average disease duration from diagnosis was 8 ± 7 years (range: 2–12). Major demographic and clinical characteristics of participants are shown in Table 1.

TABLE 1 Demographic, clinical and allergic characteristics of the cohort of EoE patients evaluated in the study

		All patients (*n* = 18)	Sterilised milk tolerant (*n* = 12)	Milk reactive (*n* = 6)	*p*
Age (years); mean ± SD (rank)		32 ± 13.4 (13–59)	31.7 ± 14.8	32.7 ± 11.3	0.887
Mean age at EoE diagnosis ± SD (rank)		24.8 ± 11.6 (8–48)	24.3 ± 13.2	25.8 ± 7.8	0.802
Gender	Male (%)	13 (72.2%)	9 (69.2%)	4 (30.8%)	0.999
	Female (%)	5 (27.8%)	3 (60%)	2 (40%)	
Atopy background, *n* (%)		13 (72.2%)	7 (58.3%)	6 (100%)	0.114
Asthma, *n* (%)		12 (66.7%)	7 (58.3%)	5 (83.3%)	0.600
Rinitis/rinoconjuntivitis, *n* (%)		12 (66.7%)	6 (50%)	6 (100%)	0.054
Atopic dermatitis, *n* (%)		3 (16.7%)	3 (25%)	0	0.515
Food allergy sensitisation, *n* (%)		5 (27.8%)	5 (41.7%)	0	0.114
EoE Phenotype	Inflammatory, *n* (%)	14 (77.8%)	9 (64.3%)	5 (35.7%)	0.526
	Stricturing, *n* (%)	2 (11.1%)	1 (50%)	1 (50%)	
	Mixed, *n* (%)	2 (11.1%)	2 (100%)	0	
Years from EoE diagnosis, median ± IQR (rank)		8 ± 7 (2–12)	8 ± 5.2	7 ± 9.2	0.925
Dietary treatment strategy	D1A	2 (11.1%)	2 (100%)	0	0.463
	D2A	3 (16.7%)	1 (33.3%)	2 (66.7%)	
	D4A	7 (38.9%)	5 (71.4%)	2 (28.6%)	
	D6A	6 (33.3%)	4 (66.7%)	2 (33.3%)	
Identified food triggers for EoE	Only milk	10 (55.6%)	6 (50%)	4 (66.7%)	0.615
	Milk and wheat	2 (11.1%)	2 (16.7%)	0	
	Milk and legumes	5 (27.8%)	3 (25%)	2 (33.3%)	
	Milk and fish/seafood	1 (5.6%)	1 (8.3%)	0	
Months on dietary therapy before sterilised milk challenge, median ± IQR (rank)		24.1 ± 36.6	14.7 ± 36.4	34.9 ± 44.4	0.049

Abbreviations: EoE, eosinophilic oesophagitis; IQR, interquartile range.

3.1 | Endoscopic and histopathological findings and symptoms before and after sterilised milk feeding

At the point of inclusion, peak eosinophil counts at any oesophageal level were <15 in all participants, with a mean ± SD of 2 ± 5 cell/hpf at distal and 0 ± 1 cell/hpf at proximal oesophageal halves. After 8 weeks of daily sterilised milk intake, 12 patients (66.7%) maintained remission, all exhibiting <15 eos/hpf in oesophageal biopsies from the upper and lower halves (<5 eos/hpf in six study subjects). EoE recurrence (demonstrated by a peak eosinophil count ≥15/hpf) occurred in the remaining six patients (Table 2 and Figure 1).

The median ± IQR DSS score was 4 ± 6 at baseline (range: 0–9) for all patients, with no significant differences between sterilised milk tolerance/intolerance (4 ± 5.8 vs. 7 ± 7.5, respectively). After the challenge, symptoms remained unchanged in tolerant patients but increased up to 9 ± 3.8 among reactive ones.

The oesophageal appearance was completely normal in all patients at baseline (EREFS score of 0) and remained unchanged in patients who tolerated sterilised milk and maintained histological remission. In contrast, scores increased up to a median ± IQR of 2.5 ± 2.5 (range: 0–4) in patients who had EoE recurrence after the challenge. This increase was evidenced by inflammatory findings (oedema, furrows and exudates), as no fibrosis features were observed.

No differences in age, gender, symptoms score, endoscopic appearance or personal/family allergic background were observed between patients who tolerated sterilised milk and who did not.

3.2 | Blood and skin allergy test results

Blood eosinophilia were within normal values at baseline, increasing after the challenge in the reactive patient subgroup with EoE recurrence. Regarding milk sensitization evaluation, total and milk-specific IgE serum levels did not change after sterilised milk consumption, excepting cow's milk-specific IgE in reactive–EoE patients (median ± IQR increasing from 0.07 ± 0.08 to 0.12 ± 0.11, $p = 0.041$ after the challenge). Changes in eosinophilic cationic protein and tryptase from baseline were not found in either sterilised milk-tolerant or reactive patients. SPT to milk was negative in all but one participant at baseline; this patient had EoE recurrence after sterilised milk exposure. No patient with a negative test at baseline became positive after the challenge. Table 3 lists overall milk sensitisation rates.

3.3 | Dietary and nutritional effects of sterilised milk consumption

Dietary questionnaires were completed by 16 patients; 14 provided information throughout the 8-week study period. Compared to baseline, sterilised cow's milk intake significantly increased saturated fatty acids and medium chain triglycerides in the overall patient cohort (at the last registered week). Weekly calcium intake also increased from baseline but achieved statistical significance only among patients who tolerated sterilised milk (Figure 2 and Table S1).

3.4 | Quality of life

The median ± IQR overall HR QoL baseline level, as assessed with the EoE-QoL-A questionnaire, was 0.87 ± 1.2 (range: 0.07–1.6), which represents a good level for EoE patients who are in clinical and histological remission. No differences in baseline HR QoL were noted among tolerant/intolerant to sterilised milk patients. As for the five dimensions in the EoE-QoL-A scale, diet/eating impact showed the highest median ± IQR score (1.3 ± 1.4), followed by disease anxiety (1 ± 1.7). The social impact was the only

TABLE 2 Baseline clinical and endoscopic characteristics of EoE patients included in our study and changes observed after sterilised milk challenge

	All patients ($n = 18$)		Sterilised milk tolerant patients ($n = 12$)			Sterilised milk reactive patients ($n = 6$)		
	Baseline	After challenge	Baseline	After challenge	p	Baseline	After challenge	p
Dysphagia Symptom Score, median ± IQR	4 ± 6	4.5 ± 7	4 ± 5.7	2.5 ± 6.5	0.131	7 ± 7.5	9 ± 3.7	0.109
EREFS total score, median ± IQR	0	0.5 ± 2	0 ± 0	0 ± 0	—	0 ± 0	2.5 ± 2.5	0.042
ERERS fibrosis subscore, median ± IQR	0	0 ± 0	0 ± 0	0 ± 0	—	0 ± 0	0 ± 0	—
Peak eosinophil count at upper oesophageal third (cells/hpf), median ± IQR	2 ± 5	0 ± 1	0 ± 3.3	0 ± 3.8	0.686	0 ± 0	5 ± 57	0.109
Peak eosinophil count at lower oesophageal third (cells/hpf), median ± IQR	5.5 ± 31	0 ± 10	2.5 ± 5.8	0 ± 6	0.553	1 ± 4.8	38 ± 27.3	0.027

Abbreviations: EoE, eosinophilic oesophagitis; EREFS, endoscopic reference score; IQR, interquartile range.

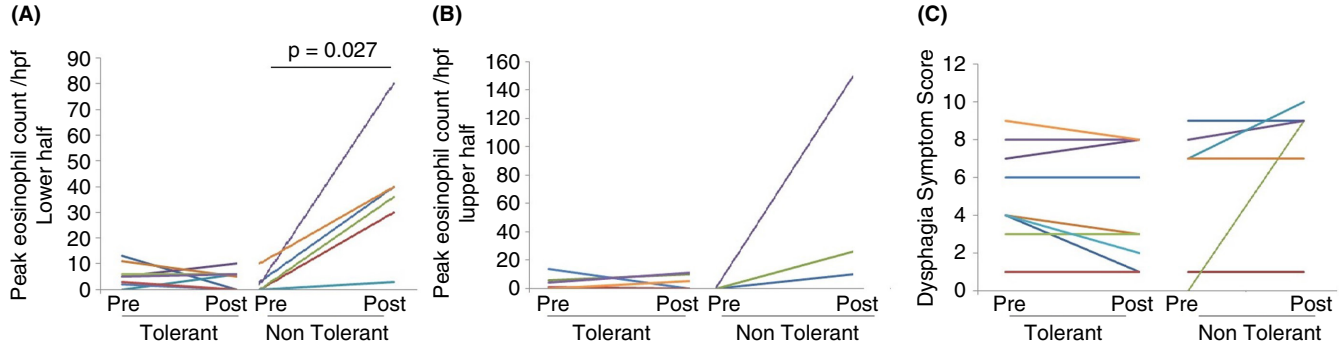

FIGURE 1 Intra-epithelial peak eosinophil counts in biopsies taken at lower (A) and upper (B) oesophageal third levels in 18 paediatric and adult patients with eosinophilic oesophagitis triggered by milk with histological and clinical remission at baseline and after 8 weeks of sterilised cow's milk challenge. Changes in symptoms before and after sterilised milk challenge (measured by dysphagia symptom score) (C). Colour lines represent individual patients who tolerated or reacted to sterilised milk.

dimension with differences at baseline in patients tolerant to the diet and not. Following 8 weeks of daily sterilised milk consumption, EoE-QoL-A scores did not change significantly in sterilised milk-tolerant patients, neither the overall score nor any dimension. However, in non-tolerant patients, HR QoL scores were significantly worse, both in the global score and in the subscores corresponding to Diet/eating Impact, Social Impact and Emotional Impact dimensions. The changes from baseline when tolerant and intolerant patients were compared revealed significant differences in the EoE-QoL-A overall score and the same three dimensions (Figure 3 and Table S2).

3.5 | Patient follow-up

One-year follow-up data were available for 10 patients who successfully tolerated sterilised milk and maintained EoE remission. They continued uninhibited consumption of sterilised milk along with products using baked milk providing these did not contain other potential EoE triggers for the patient. All were asymptomatic, with no pathological eosinophilic oesophageal inflammation (median ± IQR peak eosinophil count being 4.5 ± 7 eosinophils/hpf) after endoscopy. Furthermore, four out of these 10 successfully consumed sterilised milk and baked milk-containing products for a total of 2 years. All remained asymptomatic, with no signs of eosinophilic inflammation in a subsequent endoscopic examination (peak eosinophil count was 1 ± 6.5 cells/hpf).

4 | DISCUSSION

This work is, to our knowledge, the largest and most rigorous systematic evaluation of tolerance to sterilised cow's milk in patients with EoE triggered and maintained by milk and represents a potential opportunity for EoE patients to have HR QoL and adhere to a diet, without putting their health at risk. These results add to the available evidence that heat treatment of milk proteins reduces their immunogenicity and improves their tolerance. It is well documented that most children with IgE-mediated cow's milk allergy can tolerate baked milk,[38,39] and that cow's milk allergy resolution occurs more rapidly in cases of regular baked milk consumption,[23,38] according to cohort and retrospective studies. Our study extends this evidence to a completely different patient population, both by type of disease and by age of presentation.

According to our results, 2/3 of patients with EoE triggered and maintained by milk proteins are able to tolerate commercially available milk sterilised by cooking, instead of pasteurisation or UHT treatment, without experiencing a recurrence of oesophageal eosinophilic inflammation and related symptoms, or changes in serum profile or skin sensitization to milk proteins. We also observed that the introduction of sterilised milk produced a documented improvement in the diet's nutritional profile. QoL was maintained in sterilised milk-tolerant patients but deteriorated in non-tolerant patients. In addition, once verified, tolerance to sterilised milk patients could also tolerate products containing baked milk. Long-term EoE remission was maintained in these patients, so our study provided a significant advance in EoE practical management by improving the amount and variety of foods that milk-triggered EoE patients consume and reducing the dietary therapy burden. We previously demonstrated that the vast majority of milk-triggered EoE patients tolerated an extensively hydrolysed cow's milk-based formula,[40] however, sterilised milk is cheaper, widely available and better-tasting.

A 2013 study by Leung et al. prospectively identified 15 paediatric and adolescent patients with milk-triggered EoE, 11 (73%) of whom were tolerant to baked milk after at least 6 weeks of formal nutritional advice.[26] Our research now validates, prospectively, previous observations and provides proof of long-term remission maintenance among those patients who were tolerant to sterilised milk. Patients who tolerated sterilised milk and those who were reactive were clinically indistinguishable at baseline. In addition, peripheral eosinophilic counts, total IgE level, milk-specific IgE or milk skin testing results were not predictive of sterilised milk tolerance or reactivity. Sterilised milk undertaken for an 8-week period proved safe for all patients, with no allergic reactions identified; clinical and

TABLE 3 Analytical serum values of EoE patients before and after intake of sterilised cow's milk at baseline and differences between tolerant and reactive patients to sterilised milk

	All patients at baseline ($n = 18$)		Sterilised milk-tolerant patients ($n = 12$)			Sterilised milk-reactive patients ($n = 6$)			Baseline–after the challenge differences		
	Baseline	After challenge	Baseline	After challenge	*p*	Baseline	After challenge	*p*	Tolerant	Reactive	*p*
Blood eosinophils/mm^3	200 ± 100	250 ± 100	200 ± 175	200 ± 125	0.891	100 ± 100	350 ± 225	0.066	0 ± 175	200 ± 200	0.055
Median ± IQR (rank)	(100–600)	(100–700)									
Total serum IgE total (UI/ml)	78.2 ± 156.7	86.5 ± 187.5	70.9 ± 179	71.6 ± 212.1	0.374	139 ± 115	173.4 ± 161.8	0.593	0.9 ± 14	1 ± 49	0.781
Median ± IQR (rank)	(6.9–1056)	(7.8–1130)									
Serum-specific IgE cow milk (U/ml)	0.04 ± 0.13	0.06 ± 0.14	0.01 ± 0.22	0.02 ± 0.12	0.608	0.07 ± 0.08	0.12 ± 0.11	0.041	0 ± 0.03	0.03 ± 0.04	0.180
Median ± IQR (rank)	(0–0.3)	(0–0.44)									
IgE casein (U/ml)	0.02 ± 0.08	0.02 ± 0.12	0.02 ± 0.01	0.02 ± 0.10	0.766	0.02 ± 0.07	0.05 ± 0.13	0.131	0 ± 0.03	0.1 ± 0.06	0.298
Median ± IQR (rank)	(0–0.28)	(0–0.38)									
IgE α-lacto albumin (U/ml)	0 ± 0.01	0 ± 0.02	0 ± 0.01	0 ± 0.02	0.223	0.01 ± 0.01	0 ± 0.03	0.285	0 ± 0.1	0 ± 0.02	0.180
Median ± IQR (rank)	(0–0.21)	(0–0.09)									
IgE ß-lacto albumin (U/ml)	0 ± 0.04	0 ± 0.05	0 ± 0.04	0 ± 0.04	0.716	0.02 ± 0.04	0.02 ± 0.06	0.197	0 ± 0.01	0.01 ± 0.02	0.139
Median ± IQR (rank)	(0–0.08)	(0–0.06)									
Eosinophil cationic protein (µg/l)	48.1 ± 41.8	76.2 ± 79	49.1 ± 25	76.2 ± 60	0.260	39.8 ± 102	92.4 ± 100	0.138	37.8 ± 82	43.1 ± 84	0.549
Median ± IQR (rank)	(11.8–159)	(9.5–176)									
Tryptase (µg/l)	4.5 ± 5.9	4.7 ± 1.3	4.5 ± 0.6	4.5 ± 1.9	0.715	11.5 ± 9.1	5.6 ± 6	0.655	0.15 ± 2.1	0.9 ± 3	0.643
Median ± IQR (rank)	(2.7–11.8)	(2.6–10.7)									

Abbreviations: EoE, eosinophilic oesophagitis; IgE, immunoglobulin E; IQR, interquartile range.

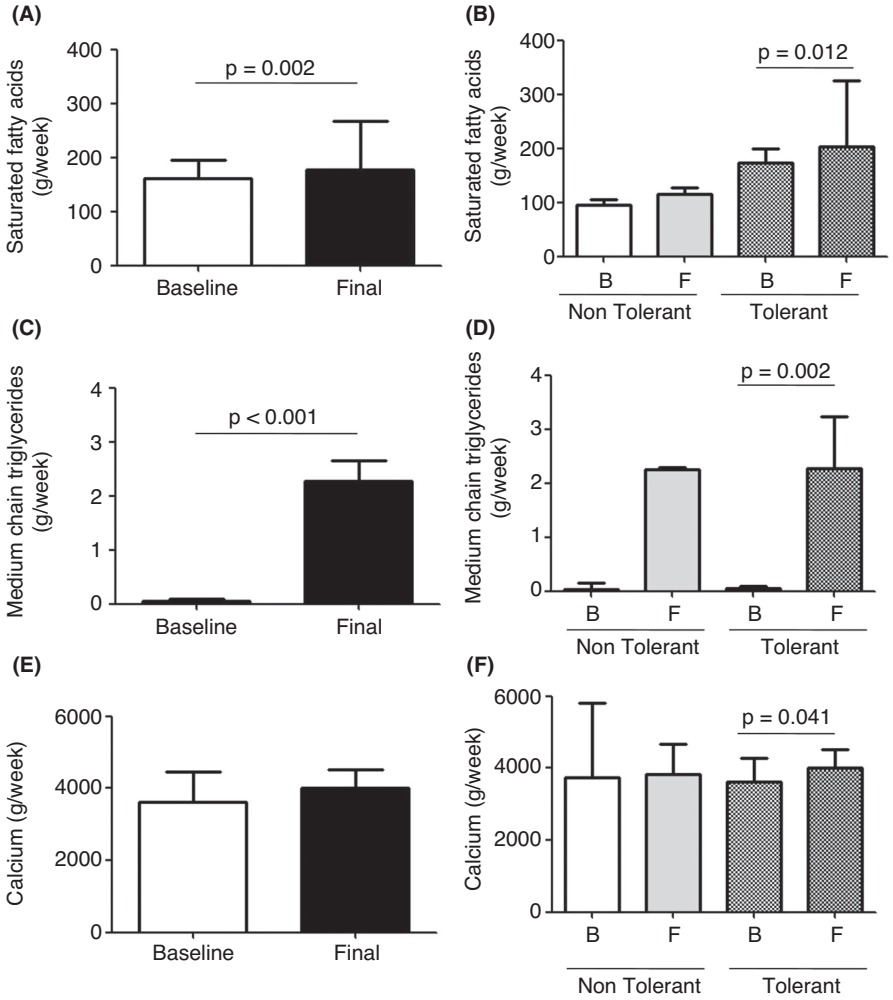

FIGURE 2 Significant changes from baseline in weekly intake of different nutrients in a cohort of patients with milk-triggered eosinophilic oesophagitis who were fed with sterilised milk. Changes in saturated fatty acids (A), medium chain triglycerides (C) and calcium (E) weekly intake before and after sterilized milk consumption. Differences in the weekly intake of saturated fatty acids (B), triglycerides (D) and calcium (F) in patients with EoE, at Baseline and at the Final week of the study period, according to patient' status of tolerance to sterilized milk.

histological EoE recurrence being the only drawback, but resolved after resuming a milk-free diet.

Direct clinical implications aside, our study provides some clues that may help us understand the sharp rise in incidence and prevalence of EoE in recent decades, currently affecting up to 1 in 850 patients approximately.[41,42] Milk proteins have been recognised as the main food allergen involved in EoE,[43] their allergenic potential being altered by such manufacturing processes as heating, chemical or enzymatic treatment, high-pressure processes or fermentation.[44] Heating milk to boiling point only modifies conformational epitopes, which could lose their binding capacity for specific IgE, and slightly reduces allergenic potential.[45] However, heating produces a negligible effect on structural epitopes that predominate in ß-lactoglobulin and, especially, casein, which maintains its allergenic capacity.[46,47] In addition, it has also been documented that intense heating during milk UHT processing can increase some allergenic characteristics[48] by exposing linear and conformational hydrophobic epitopes buried inside the protein on the protein surface following the unfolding of the structure. This could promote the allergenic properties by triggering the intestinal immune responses, as demonstrated in murine models.[49] Milk proteins can also oxidise during industrial treatment, resulting in the formation of modified amino acid residues that tend to increase the natural allergenicity of milk proteins.[50] Thus, this research provides an initial clue to the hypothetical relationship between the development of EoE and industrial food processing,[51] although it is not applicable to other potential allergens capable of causing EoE, such as wheat or eggs.

Our study has the limitation of being carried out on a small series of patients, recruited at a single centre specialised in the treatment of EoE. Symptoms were assessed with an unvalidated instrument, with limited responsiveness to short-term changes. Since participants presented a good level of HR QoL at baseline, being in EoE remission, the introduction of sterilised milk was not a significant improvement. We did not assess the long-term impact that sterilised milk tolerance (and consuming cooked milk-containing products) might have on these patients. Finally, the results of this research could not be directly applicable to small children, who show the highest rate of milk-induced EoE as a single trigger. Therefore, our results need further prospective validation in a paediatric population. However, this study has the strength of managing all patients using a common protocol that included evaluation with endoscopy and biopsies, and allergic and nutritional assessment carried out before and after the sterilised

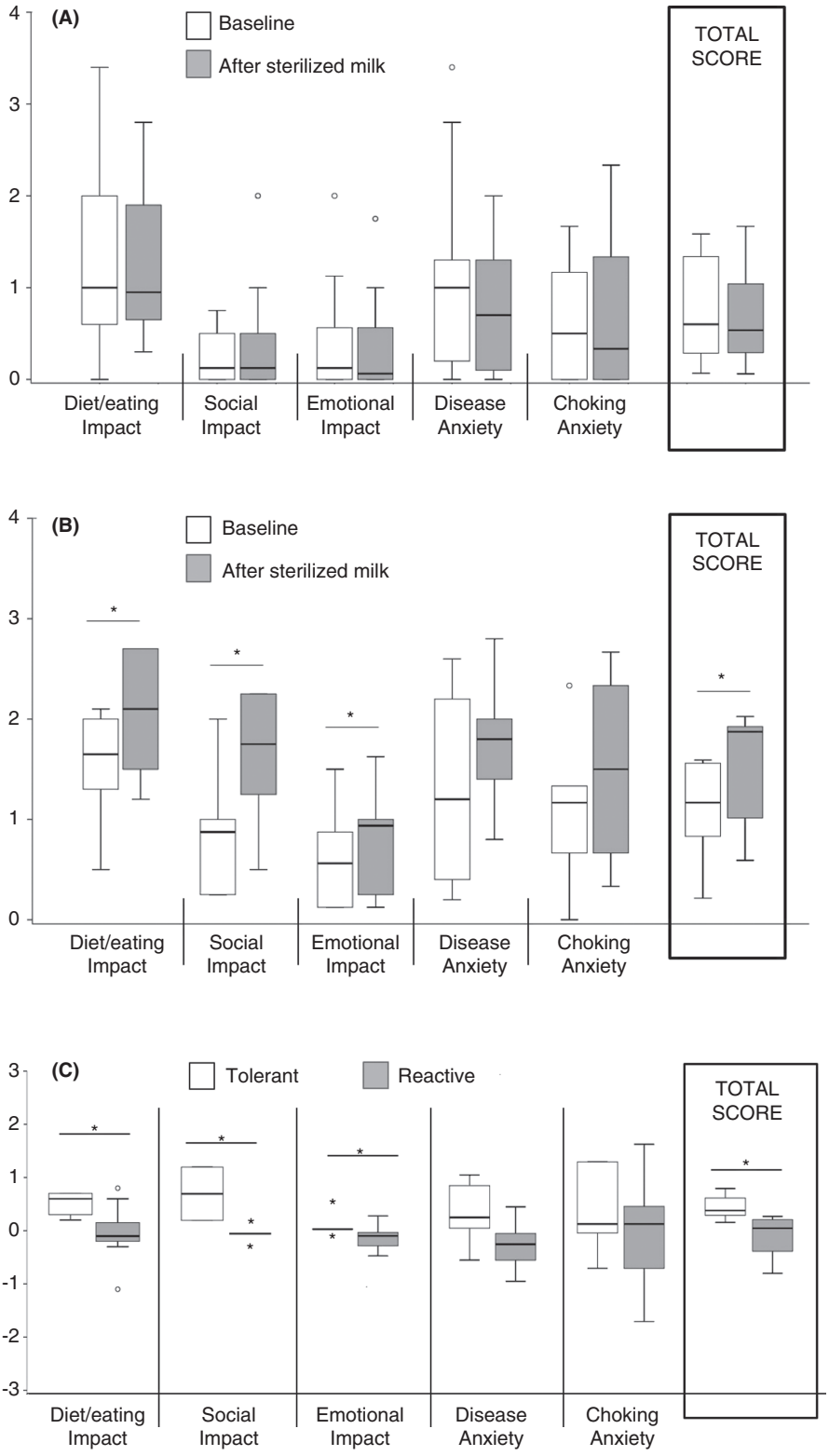

FIGURE 3 Health-related quality of life (QoL) measured by the eosinophilic oesophagitis (EoE)-QoL-A questionnaire in patients with EoE triggered by milk at baseline (while on disease remission) and at 8 weeks after challenge with sterilised cow's milk. Median and interquartile ranges (IQR) are represented in the boxes, with whiskers (vertical lines) extending to a limit of ±1.5 IQRs. Changes in the five dimensions that compose the total EoE-QoL-A score are shown by patients who tolerated (A) and were reactive (B) to sterilised milk. In addition, changes from baseline scores are shown for patients who were tolerant and reactive to sterilised cow's milk (C).

milk challenge. Most patients tolerant to sterilised milk underwent long-term evaluation with endoscopy and biopsies while continuing to consume it and baked milk foods.

In conclusion, our study shows that most patients of all ages with milk protein-triggered EoE are able to tolerate sterilised milk, without showing signs of disease recurrence. This allows for an improved diet and a greater variety of permissible foods while maintaining an adequate control of EoE in the long term. Sterilised milk consumption is safe and well tolerated.

AUTHORSHIP

Guarantor of the article: Alfredo J. Lucendo.

AUTHOR CONTRIBUTIONS

Jesus Gonzalez-Cervera: Conceptualization (equal); formal analysis (equal); investigation (equal); methodology (equal); writing – review and editing (supporting). **Angel Arias:** Formal analysis (equal); investigation (equal); methodology (equal); writing – review and editing (supporting). **Pilar Navarro:** Data curation (equal); formal analysis (equal); validation (supporting); writing – review and editing (supporting). **Maria Cobo-Palacios:** Investigation (equal); methodology (equal); resources (equal); writing – review and editing (supporting). **Rocio Juarez-Tosina:** Investigation (equal); writing – review and editing (supporting). **Jose Maria Olalla:** Investigation (equal); writing – review and editing (supporting). **Teresa Angueira:** Investigation (equal); writing – review and editing (supporting). **Alfredo J Lucendo:** Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); project administration (equal); supervision (equal); writing – review and editing (equal).

ACKNOWLEDGEMENT

Declaration of personal interests: The authors have no conflict of interest exists for the current manuscript.

FUNDING INFORMATION

PN is a recipient of a Sara Borrell (grant CD19/00102), from the Instituto de Salud Carlos III (ISCIII), Spanish Ministry of Health – Social Services and Equality, which is partly funded by the European Social Fund (period 2014–2020).

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study is available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information will be found online in the Supporting Information section.

How to cite this article: González-Cervera J, Arias Á, Navarro P, Juárez-Tosina R, Cobo-Palacios M, Olalla JM, et al. Tolerance to sterilised cow's milk in patients with eosinophilic oesophagitis triggered by milk. Aliment Pharmacol Ther. 2022;56:957–967. https://doi.org/10.1111/apt.17171