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Milk coagulation and gastric emptying in women experiencing gastrointestinal symptoms after ingestion of cow's milk

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Abstract

Background: Gastrointestinal symptoms after drinking milk are often attributed to lactose intolerance or cow's milk allergy. However, some individuals without either condition still report gastrointestinal symptoms after drinking milk. This may be caused by gastric emptying (GE) rate or gastric protein coagulation. This study aimed to compare GE rate and protein coagulation after milk consumption between individuals reporting gastrointestinal symptoms and those without symptoms using a novel gastric MRI approach.

Methods: Thirty women were included in this case–control study, of whom 15 reported gastrointestinal symptoms after drinking milk and 15 were controls. Participants underwent gastric MRI before and up to 90 min after consumption of 250 mL cow's milk. Gastric content volume and image texture of the stomach contents were used to determine GE and changes in the degree of coagulation.

Key Results: GE half-time did not differ between the groups (gastrointestinal symptom group 66 ± 18 min; control group 61 ± 14 min, $p = 0.845$). The gastrointestinal symptom group reported symptoms from 30 min onwards and rated pain highest at 90 min. The control group reported no symptoms. Image texture analyses showed a significantly higher percentage of coagulum and lower percentage of liquid in the group in the GI symptom group (MD 11%, 95% CI [3.9, 17], $p = 0.003$). In vitro data suggests that pH and proteolytic enzyme activity influence the coagulum structure.

Conclusions and Inferences: Gastric milk coagulation and emptied fraction of stomach content may differ between individuals experiencing symptoms after milk consumption, possibly due to differences in pH and proteolytic enzyme activity.

KEYWORDS
dairy, dyspepsia, imaging, protein, stomach

Abbreviations: 95% CI, 95% confidence interval; BMI, body mass index; GE, gastric emptying; GE-t50, gastric emptying half time; MD, mean difference; MRI, magnetic resonance imaging; UHT, ultra high temperature processed.

Author names in bold designate shared co-first authorship.

1 | INTRODUCTION

Cow's milk is a large part of the diet of Northern and Western European countries[1] and a relatively cheap source of essential nutrients such as protein and calcium.[2] Dairy protein is a high-quality protein as determined by the Digestible Indispensable Amino Acid Score (DIAAS), which means that it contains essential amino acids and is relatively easily digested and absorbed by the body.[3] Despite its nutritional value, the consumption of milk in Western countries is decreasing. This is due to many factors; one of them being individuals experiencing gastrointestinal (GI) symptoms after drinking milk and thus refraining from its consumption.[4]

GI symptoms after milk consumption are ascribed to various causes. The first is lactose intolerance, which is maldigestion of lactose, due to a lactase deficiency.[5] However, most of the individuals who report GI symptoms after milk consumption were not diagnosed as lactose intolerant.[6,7] In fact, they did not experience less symptoms after consumption of lactose-free milk compared to regular milk.[8] A second possible mechanism driving GI symptoms is cow's milk protein allergy, but this is rarely seen in adults, with an estimated prevalence below 0.5%, and the self-declared symptoms are not those typical of an allergic reaction.[5,9,10] A third cause that has been investigated is the effect of processing of cow's milk on digestion based on anecdotical evidence about some individuals reporting less GI symptoms after consumption of raw milk compared to pasteurized or homogenized milk.[5] Accurate studies could neither show significant differences in GI symptoms between different processing types nor immune-related mechanisms behind it.[11–14] In conclusion, the mechanism underlying GI symptoms and a general discomfort after milk consumption by a relevant part of the adult population remains unknown.

We hypothesized that the origin of GI symptoms might lie in differences in gastric emptying (GE). In individuals with digestive diseases, such as functional abdominal pain or dyspepsia, GE has already been recognized as an important factor modulating the degree of GI symptoms.[15,16] On the one hand, when the voluminous and hyperosmolar gastric content enters the small intestines too fast, it can cause symptoms such as nausea and cramping, also known as dumping syndrome.[17] On the other hand, when the gastric content is retained for a longer time, it can cause a bloated feeling.[18,19] This means that both a delayed and an accelerated GE could give rise to the GI symptoms commonly reported by some individuals after milk consumption.

One of the physical properties of milk that can influence GE rate is its protein structure. Cow's milk generally contains about 3.5% protein, of which caseins represent around 80% and whey proteins around 20%.[20] Caseins form a semi-solid network during digestion in the stomach because casein micelles are destabilized by pepsin proteolysis combined with the low gastric pH, that is around its isoelectric point, a process known as coagulation. This causes the formation of a coagulum containing protein and possibly fat globules.[21] The physical properties of this casein coagulum can affect the dynamics of gastric protein digestion and delay GE,[22] which could in turn drive the experience of GI symptoms after milk consumption. The coagulum could delay GE since the stomach only passes particles on to the duodenum if they are sized below 1–2 mm.[23]

Key points

1. Gastrointestinal symptoms after drinking milk in adults are often incorrectly attributed to lactose intolerance or cow's milk allergy, while the underlying mechanism is still unknown.
2. Gastric milk coagulation and emptied fraction of stomach content may differ between individuals experiencing symptoms after milk consumption, possibly due to differences in pH and proteolytic enzyme activity.

Gastric in vivo studies in humans are necessary to confirm the formation of coagulum and whether this indeed delays GE. Magnetic resonance imaging (MRI) provides a direct and non-harmful method to visualize the stomach contents. Currently, the main use of MRI in gastric research is measuring GE rate,[24,25] but it can also be used to visualize intragastric processes, such as changes from liquid to solid phases, gastric sieving, and phase separation.[26,27] Since gastric protein coagulation involves a change from a liquid to a solid state, MRI could potentially be used to quantify the degree of coagulation. So far, gastric coagulation has only been visually assessed using MRI,[28] however we showed image texture analysis may provide a more objective and accurate quantification.[25] The physical properties of the coagulum formed, can be studied in more detail using in vitro gastric digestion to link the mechanistic understanding of the digestive processes to the texture differences visible on MRI images during milk protein coagulation.

The objective of this study was to compare gastric digestion of cow's milk between subjects with or without GI symptoms after milk ingestion. This in vivo MRI assessment of gastric digestion was compared to in vitro measurements of coagulation, to better understand the underlying mechanisms.

2 | MATERIALS AND METHODS

2.1 | Design

This study was a parallel intervention study with two groups: women with and women without GI symptoms after milk consumption. The primary outcome of this study was GE over time. The secondary outcome was gastric coagulation as measured by image texture metrics and the tertiary outcomes were subjective ratings (pain, nausea, bloating, fullness, and discomfort).

2.2 | Participants

Two groups of healthy females were recruited using inclusion criteria: between 18 and 60 years and a BMI between 18.5 and

30 kg/m.[2] Inclusion criteria for the GI symptom group were drinking a maximum of 200 mL cow milk/week and self-reported GI discomfort after cow milk consumption and. Inclusion criteria for the control group were drinking a minimum of 700 mL cow milk/week and no GI discomfort after milk consumption. Participants were excluded if they had a history of medical or surgical events related to the GI tract, used medical drugs that influence the GI tract's normal function or microbiota, were diagnosed with lactose intolerance or cow milk allergy or if they reported GI symptoms of "vomiting" or "loose, mushy, or watery stools", adapted from Rome II[29] criteria at any level of severity following milk consumption. Only women were included since GE and GI symptoms are influenced by sex.[30] Participants were recruited via the Wageningen University website, using flyers, and on social media. First, the GI symptom group was recruited. Subsequently, the control group was recruited in order to match the groups on age and BMI with a maximum deviation of 5 years and 2 kg/m^2. This resulted in the inclusion of 15 women with (age 23 ± 1.9 years, BMI 26 ± 6.6 kg/m^2) and 15 without (22 ± 1.7 years 24 ± 3.2 kg/m^2) GI symptoms after drinking milk; see Figure 1.

Participants with GI symptoms were screened for lactose intolerance with a hydrogen breath test using 20 g of lactose, which is a physiologically relevant amount.[31] On the night before the test, they consumed a standardized meal containing rice and meat according to the guidelines of Gasbarrini et al.[31] Inclusion criteria related to the lactose breath test are shown in Table 1.

The study was conducted according to the principles of the Declaration of Helsinki (October 2013) and was approved by the ethical committee of Wageningen University. It was registered with the Dutch Trial Registry under number NTR7531 (now CCMO-register NL66536.081.18). All participants signed informed consent. All authors had access to the study data and reviewed and approved the final manuscript.

2.3 | Test session

Dairy (all dairy) and milk (whole, semi-skimmed and skimmed milk, flavored and unflavored) consumption was measured using a food frequency questionnaire.[32] All participants, either using the contraceptive pill or not, were scanned in the first 2 weeks of their menstrual cycle to mitigate hormonal influences. Participants arrived at the Gelderse Vallei hospital after an overnight fast starting at 8 PM. They were allowed to drink water and herbal tea up to 1.5 h before the visit. After arrival, participants verbally rated baseline feeling of fullness, wellbeing, bloating, and nausea on a 100 unit scale[33] and an abdominal MRI scan was made to assess baseline stomach contents. After this, participants ingested 250 mL UHT cow milk, containing 113 kcal, 3.8 g (1.5%) fat, 12 g (4.8%) carbohydrates, and 8.0 g (3.2%) protein, of the brand Bridel provided by Lactalis Research and Development, Vitré. Milk was served cooled at 4–7°C. Participants were instructed to finish the milk within 5 min but they all finished it within 2 min. Subsequently, abdominal MRI scans were performed every 10 min up until 90 min after the start of ingestion. After each scan participants verbally rated pain, nausea, bloating, fullness, and discomfort on a VAS scale from 0 to 100.

2.4 | MRI

Participants were scanned in a supine position with the use of a 3 Tesla Siemens Verio MRI scanner (Siemens AG, Munich, Germany)

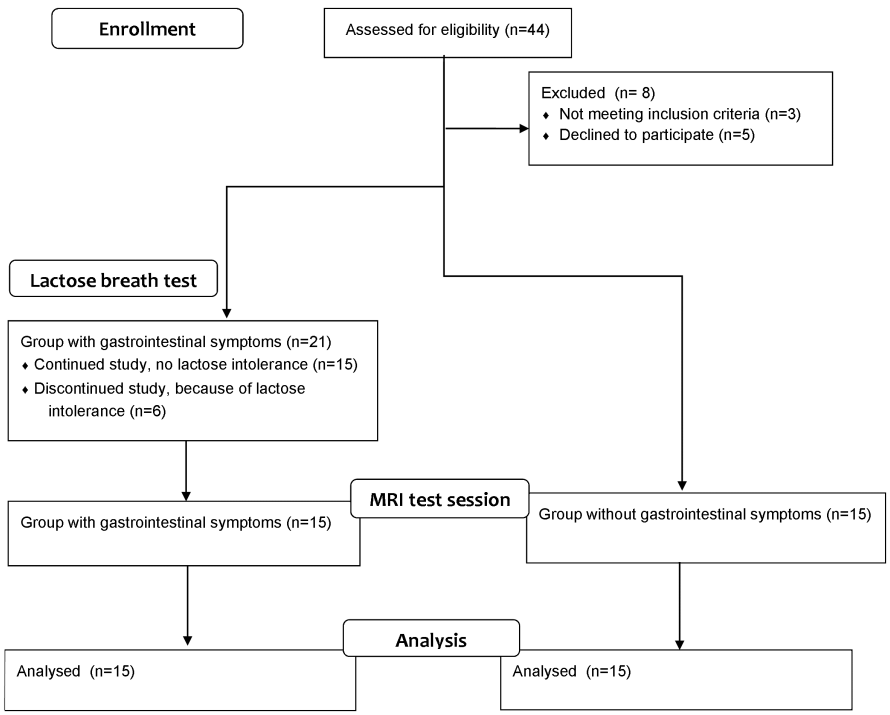

FIGURE 1 Flow diagram of participants.

TABLE 1 Lactose tolerance classification of participants after lactose breath test.[a]

H_2 (above baseline)	Symptoms	Classification	Included
<20 ppm	No	Lactose absorber	Yes
<20 ppm	Yes, but without vomiting or/and loose, mushy, or watery stools	Lactose absorber	Yes
<20 ppm	Yes, but with vomiting or/and loose, mushy, or watery stools	Lactose intolerant	No
>20 ppm	No	Lactose malabsorber	Yes
>20 ppm	Yes	Lactose intolerant	No

[a]With a 10% lactose solution in water containing 20 g lactose based on the procedure proposed by Gasbarrini et al.[31]

using a T_2-weighted spin echo sequence (HASTE, 24 6-mm axial slices, 2.4 mm gap, 1.19 × 1.19 mm in-plane resolution), with breath hold command on expiration to fixate the position of the diaphragm and the stomach. The duration of a scan was approximately 18 s. The software MIPAV (Medical Imaging Processing And Visualization, version 11.0.3, National Institutes of Health, Bethesda, MD, USA) was used to manually delineate gastric content on every slice. Volumes were calculated by multiplying the number of gastric content voxels with the voxel volume (11.9 mm^3). To quantify the (relative) volume of liquid and coagulum in stomach contents the number of lighter (liquid) and darker (coagulum) voxels was calculated by determining intensity thresholds with the use of Otsu's method[34] in Matlab (version R2023a, multifresh function), an approach previously used on in vitro MRI images of milk digestion.[35] Texture analysis of the stomach content was performed using the software LIFEx (version 7.2.0, Institut national de la santé et de la recherche médicale, France).[36] Homogeneity, coarseness, contrast, and busyness were calculated. These image texture metrics provide information on the spatial patterns of voxel intensity.[37] The Gray-Level Co-occurrence Matrix (GLCM) method was used for homogeneity (degree of similarity between voxels) and neighborhood gray-level difference matrix (NGLDM) difference of gray-levels between one voxel and its 26 neighbors in eight dimensions was used for contrast (local variations), coarseness (spatial rate of change in intensity), and busyness (spatial frequency of changes in intensity). The number of gray-levels for texture metric calculation was set at 64, intensity rescaling relative (ROI: min/max) and dimension processing 2D. In the context of this paper we interpret changes in image texture metrics as reflecting changes in the degree of coagulation. An example of two stomachs with and without coagulation and their corresponding image texture metrics can be found in Data S1. On each time point after ingestion, texture metrics were calculated per slice for the stomach content. Subsequently, a weighted average texture metric was calculated based on the gastric content volume in each slice. For the empty stomach (baseline) no texture metrics were calculated. Image texture measures at $T = 30$ min were used for correlations, since coagulation was visible at MRI scans at that time.

2.5 | Statistical analysis

Gastric emptying half time (GE-t50) is a commonly used summary measure. To estimate GE-t50, a curve was fitted for each scan session to the data of gastric volume over time using R statistical software according to an established linear-exponential model as developed on the basis of earlier models of GE.[38–41] Further analyses were performed in SPSS (version 22, IBM, Armonk, USA). GE-t50 was compared between the groups with an two-sample *t*-test. Gastric volume, image texture metrics, and subjective ratings were tested between groups using linear mixed models with time, group and interaction time*group as fixed factors, participants as random factor and baseline levels as a covariate. Normality was confirmed with Shapiro–Wilks test. Milk consumption was tested using the Mann–Whitney *U*-test, since there was no normal distribution. Missing data were handled using a Maximum Likelihood estimation. For subjective ratings and image texture metrics areas under the curve (AUC) over 90 min were calculated using Graphpad Prism 5 (Graphpad Software) following the trapezoidal rule. In addition, exploratory Pearson correlation coefficients were calculated for the association between selected gastric measures (GE-t50, image texture metrics at 30 min and initial gastric content volume) and AUC of subjective ratings (pain, nausea, bloating, fullness, and discomfort).

2.6 | In vitro gastric digestion

Alongside the human trial, static in vitro digestions of the milk were performed. As pH and pepsin concentration are the main parameters affecting gastric coagulation, these two parameters were investigated, using in vitro gastric digestion. The same UHT semi-skimmed, sterilized milk that was used for the in vivo trial was digested according to the INFOGEST protocol of Minekus et al. with adaptations of the pH and pepsin concentration to simulate in vitro stomach conditions which might occur in humans experiencing difficulties in milk digestion, resulting in three conditions: pH 3 with 100% pepsin (control), pH 4 with 100% pepsin, and pH 4 with 50% pepsin.[42] The adaptations comprised the following: (1) no amylase was added due to the absence of starch, (2) the pH of the simulated gastric fluid was prepared at pH 3 as well as pH 4; the juice at pH 4 was intended to mimic natural variation in gastric pH between healthy adults and those taking antacids/proton pump inhibitors, which leads to an ~1 point higher pH, so we went in the INFOGEST protocol from 3 to 4[43,44] (3) Pepsin show a standard deviation of average individual pepsin activity between 50% and 100%.[45,46] The pepsin concentration in one experimental

condition was decreased to 50% of the value recommended by Infogest to mimic the digestion of a person with a reduced pepsin activity to determine how much it would impact gastric digestibility. The absolute pH values were of less importance than showing that small pH variation induce significant changes the physical characteristics of the coagulum. For the structural analysis of the coagulum, photos were taken, the wet weight, and dry matter content were measured and a compression test was performed with a texture analyzer. To analyze the proteolysis, SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) was performed for the gastric samples to separate proteins based on molecular weight and the OPA (o-phthalaldehyde) method was used for to measure the degree of hydrolysis of gastric samples. A more detailed description of these methods and their results is given in Data S1.

3 | RESULTS

The FFQ data showed that mean dairy intake was 1341 ± 774 g/week in the control group and 824 ± 459 g/week in the GI symptom group (mean difference (MD) = 518 g, $p = 0.034$). Mean milk intake was 361 ± 370 g/week in the control group and 166 ± 216 g/week in the GI symptom group (MD = 195, $p = 0.137$). GE half time (GE-t50) was 60 ± 23 min for the GI symptom group and 61 ± 14 min for the control group ($p = 0.845$). Gastric volume over time did not differ between groups (MD 5.3 min, 95% confidence interval (CI) [−30, 19], $p = 0.53$) and there was no interaction between time and group; see Figure 2. However, a threshold analysis showed a significantly higher percentage of coagulum and a lower percentage of liquid in the GI symptom group (MD 11%, 95% confidence interval (CI) [3.9, 17], $p = 0.003$ and an interaction between time and group ($p = 0.017$), see Figure 3 for a visual representation of thresholded stomach and the thresholding graphs in Data S1.

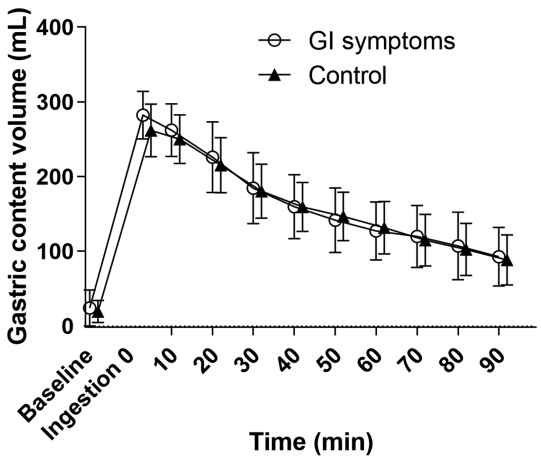

FIGURE 2 Mean ± SEM gastric content volume over time in the two groups after consumption of 250 mL milk. $T = -10$ indicates the baseline scan. $T = 3$ min is the first scan after milk ingestion. The two groups did not differ in gastric content overall and on individual time points.

3.1 | Coagulation in vivo

The formation of coagulum in the stomach was visible in all participants and formation increased over time. An example of the formation of coagulation of stomach content of a person with GI problems is shown in Figure 4 and an example of a person in the control group is shown in Data S1. This was confirmed by the image texture measures: homogeneity and busyness decreased over time and coarseness and contrast increased over time (all $p < 0.001$). The image analysis of the MR scans provided information on four parameters that reflect changes in the structure of the stomach contents (homogeneity, coarseness, contrast, and busyness). All these measures differed between the groups (homogeneity MD = 0.012, 95% CI [0.003, 0.021], $p = 0.009$, coarseness MD = −0.002, 95% CI [−0.002, −0.001], $p < 0.001$, contrast MD = −0.028, 95% CI [−0.043, −0.014], $p < 0.001$) and busyness MD = 0.006, 95% CI [0.003, 0.09], $p < 0.001$). There was no interaction effect of time*group for any texture measure. Figure 5 shows the image texture metrics of stomach content over time for the two groups.

GE-t50 correlated positively with busyness AUC ($r = 0.44$, $p = 0.017$) and negatively with coarseness AUC ($r = -0.37$, $p = 0.047$). Moreover, busyness AUC correlated positively with bloating AUC ($r = 0.52$, $p = 0.003$) and coarseness AUC correlated positively with discomfort AUC ($r = 0.42$, $p = 0.024$).

3.2 | Subjective ratings

Fullness was generally rated highest at $T = 10$ min and decreased over time (main effect time, $p < 0.001$). It did not differ between the groups ($p = 0.121$). All participants in the GI symptom group reported either pain, bloating, nausea, or discomfort, whereas these symptoms were mostly absent in the control group. These group differences were significant for all symptoms (all $p < 0.001$). In the GI symptoms group, the feeling of discomfort increased from $T = 0$ to $T = 40$ and then remained stable until $T = 90$ min. Pain was mostly absent until $T = 20$ and afterwards increased and was generally rated highest at $T = 90$ min. Bloating increased, starting at $T = 0$, increasing up to $T = 30$ and after that remained stable. Overall, nausea was low with mean ratings around 10 out of 100 and remained stable from $T = 10$ to $T = 90$ min. Graphs of fullness, bloating, pain, nausea, and discomfort can be found in Figure 6.

3.3 | Coagulation in vitro

No coagulum was formed during in vitro gastric digestion of the milk at pH 3, whereas there was visible coagulum formation at pH 4 with

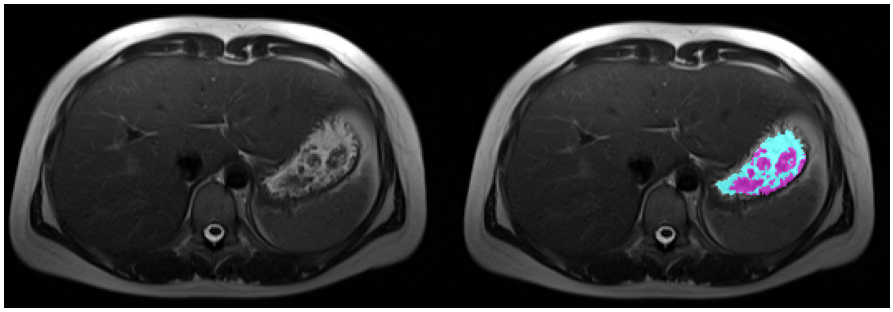

FIGURE 3 (Right) Where blue represents the liquid (lighter voxels) and purple the coagulum (darker voxels).

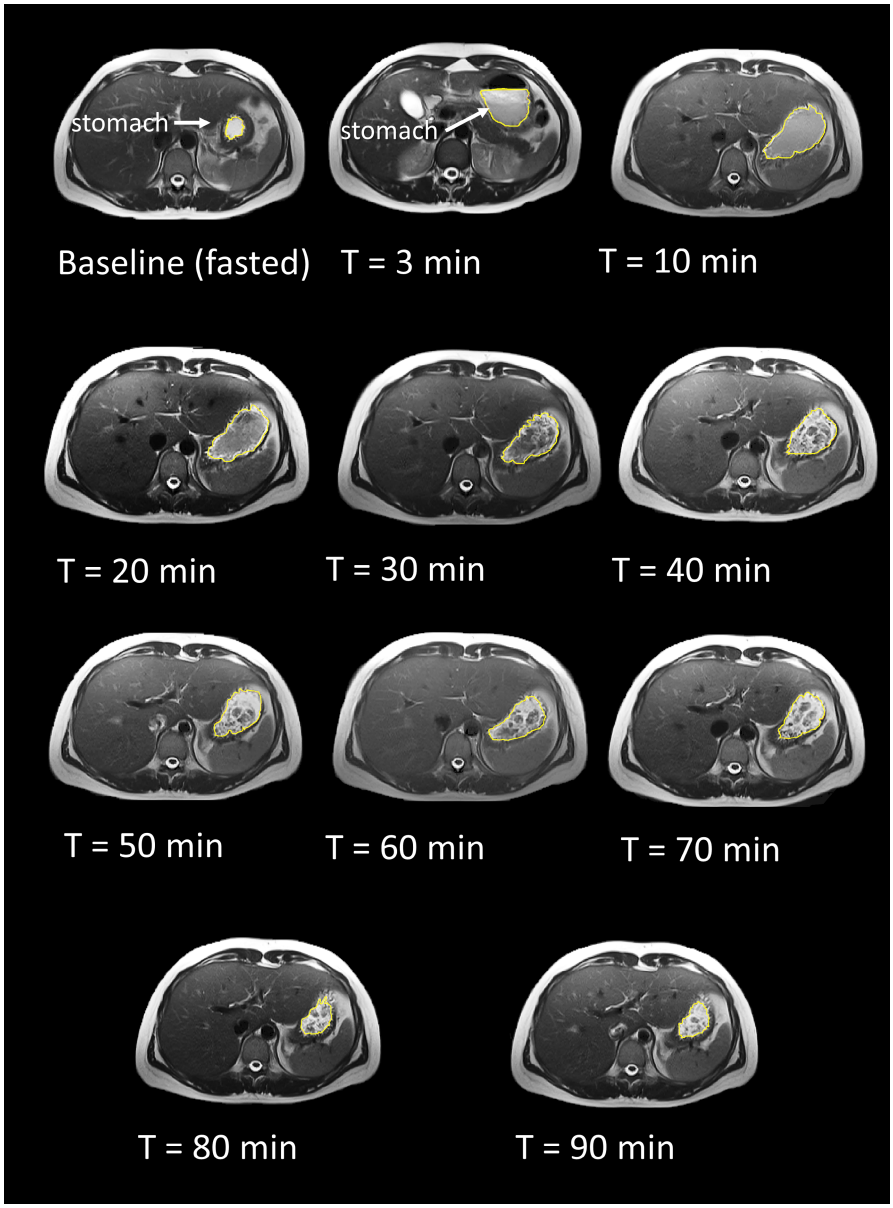

FIGURE 4 Examples of MR images of a subject with GI problems after drinking milk with panels showing the empty stomach (baseline), the stomach just after milk consumption ($T = 3$ min), a homogenous filled stomach ($T = 10$ min), the start of coagulation ($T = 10$–20 min), and the formation of a strong coagulum ($T = 30$–90 min).

more coagulum at pH 4 with 50% pepsin. The samples at pH 4 with 50% pepsin showed the largest amount of coagulum and in absolute sense the largest decrease between 5 and 30 min. The absolute values of pepsin added were 2000 U/mL for the 100% samples and 1000 U/mL for the 50% pepsin sample (both in the final gastric digestion mixture). Over time, wet weight decreased and dry matter content increased, leading to a firmer coagulum as confirmed by compression tests. The amount of coagulum at pH 4 with 100% pepsin did not clearly increase, but its firmness increased. Disappearance of intact casein according to SDS-PAGE from the supernatant was fastest at pH 3, then pH 4 followed by pH 4 with 50% less pepsin. At pH 3 all intact caseins in the liquid/soluble phase disappeared within the first 5 min of gastric digestion. Some intact caseins were still detectable after 5 min for pH 4 and pH 4 with 50% pepsin. Similar

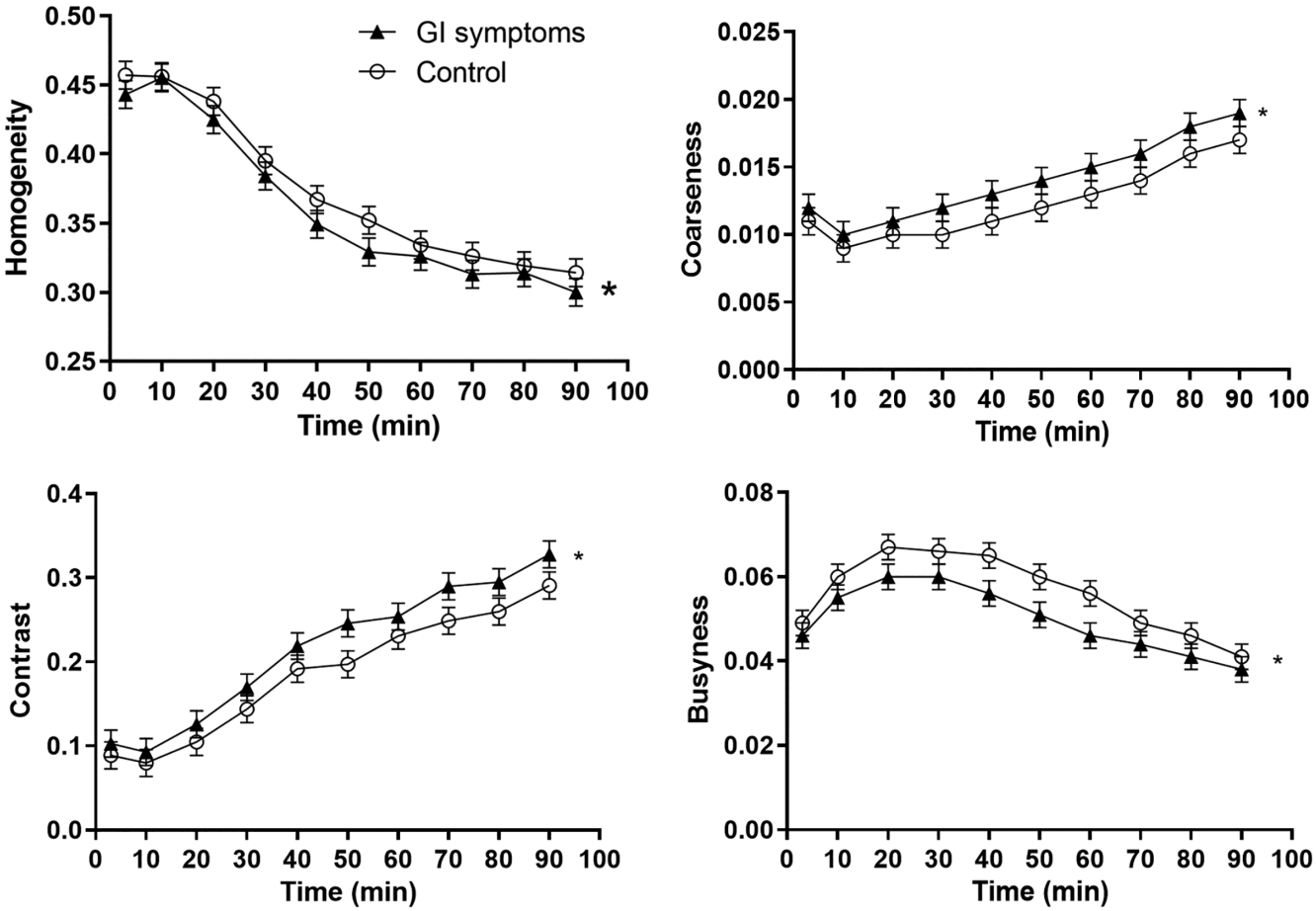

FIGURE 5 Mean ± SEM homogeneity (degree of similarity), coarseness (spatial rate of change in intensity), busyness (spatial frequency of changes in intensity), and contrast (local variations) of stomach content for individuals with GI symptom and control group. * denotes a significant difference of treatment effect.

results were found for the degree of hydrolysis, which was highest after digestion at pH 3, followed by pH 4 and pH 4 with 50% pepsin. Table 2 shows composition and texture of the gastric coagulum during in vitro digestion.

4 | DISCUSSION

This is the first study to compare gastric digestive processes of milk between individuals with and without milk-related GI symptoms using MRI. Contrary to our hypothesis, GE did not differ between participants who report GI symptoms after drinking milk and controls, although the milk did induce symptoms. This means that the GI symptoms were not driven by gastric volume, since amount of gastric content did not differ between the groups while symptoms did. This is not in line with previous studies who administered a solid caloric meal and found that bloating was associated with either rapid[47] or delayed GE.[19,48,49]

Our MRI findings suggested that GI symptoms are associated with the degree of coagulation, since our most striking result is that the image texture of the stomach contents was significantly different between the two groups. Image texture metrics showed that the degree of coagulation was higher in the GI symptom group. Image texture metrics have not been previously used to quantify coagulation on MRI images in vivo, although they have been widely used on MRI images in other areas, such as tumor differentiation and multiple sclersosis.[50–52] Immediately after consumption, milk is seen in the stomach as an homogeneous dark mass: after some minutes, the coagulation becomes visible on MRI images as grouped, darker voxels surrounded by liquid seen as whiter voxels. The progressing of the coagulation phenomena over time is clearly visible and it can be quantified as shown in Figure 4. In this study, homogeneity and busyness of gastric content were lower and coarseness and contrast were higher in the GI symptom group, which would imply a higher degree of coagulation. The in vitro data suggest that this results in a firmer coagulum. However, the exact interpretation of the structure of the coagulum and the corresponding image texture metrics should be further investigated in follow-up research.

Casein coagulation is strongly affected by gastric pH changes and pepsin concentration, which vary between individuals.[53] This variation may thus underlie the difference in GI symptoms, which should be further investigated in future human studies. In line with this, our in vitro tests clearly show that pH conditions and differential pepsin activity well account for the differences in coagulation.

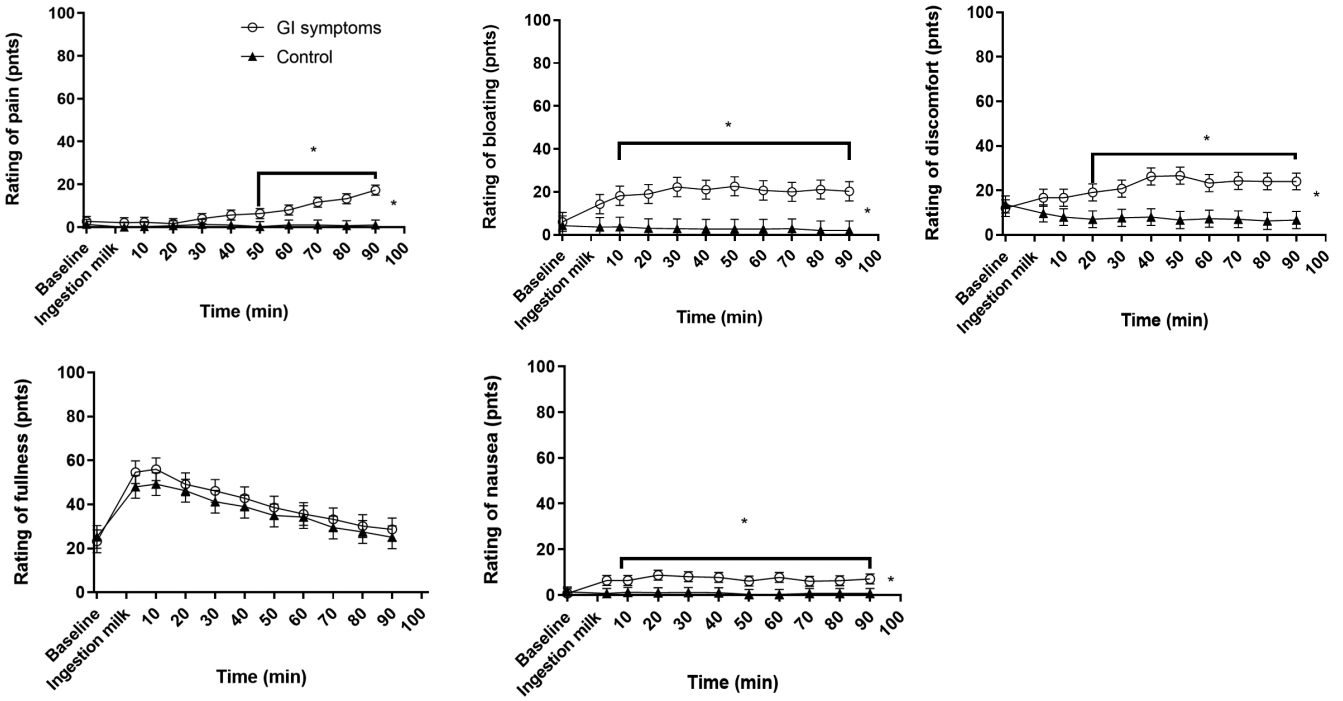

FIGURE 6 Mean ± SEM subjective ratings of fullness, pain, discomfort, nausea, and bloating over time for the two groups. * Denotes a significant difference at individual time points and treatment effect.

TABLE 2 Composition and texture of the gastric coagulum after 5 and 30 min of in vitro gastric digestion at pH 4.

	pH 4/pepsin 100%	pH 4/pepsin 50%
Coagulum composition		
Wet weight (g/100 mL gastric digesta)		
5 min	15.2	21.7
30 min	4.5	4.4
Dry weight (g/100 mL gastric digesta)		
5 min	2.4	3.1
30 min	1.8	1.8
Dry matter content (g/100 g gastric digesta)		
5 min	15.6	14.1
30 min	15.9	16.1
Coagulum texture		
Firmness (N*s)		
5 min	9.9	11.7
30 min	14.9	12.1

*N.s

Casein coagulates in the stomach due to the combined effect of pepsin and acidic precipitation reaching its maximum at the isoelectric point of the caseins (4.6), which means the closer the pH is to this value, the more easily they form a coagulum.[54] After drinking milk, which is a potent buffering liquid, gastric pH can surpass 6.0.[55] As seen in our in vitro data, if gastric pH is around 4, more coagulum formation occurs, which might induce GI symptoms. The link between coagulation and GI symptoms has been made and studied before in infants. In case of gastric complaints, babies on infant formula receive a formula that is partly predigested.[56] This leads to a softer coagulum that disappears quicker.[57] Our data suggest that something similar may occur in adults. A study on digestive discomfort in females self-reporting dairy intolerance found a decrease in GI symptoms after ingestion of milk that only contained A2 β-casein.[58] Since their intervention was based on two types of casein which are known to coagulate differently (A2 milk gives a softer coagulum or may not coagulate),[59] this supports the idea that the degree of casein coagulation in the stomach might contribute to GI symptoms. Thus, a higher degree of coagulation might be the key underlying mechanism behind discomfort experienced after milk consumption. This hypothesis is supported by our observation that two of the four image texture metrics correlate well with discomfort and bloating ($r = 0.52$ and $r = 0.42$). However, not all symptoms correlated with the image texture metrics. One thing to consider is that different texture measures capture different aspects of coagulation. For instance, smaller coagulates can be heterogeneous, but when a large coagulate is formed, it could appear more homogenous and would possibly be better quantified by another texture measure, such as contrast. Therefore it is important to analyze MRI images for multiple parameters to get a good overview of all aspects of coagulation.

Surprisingly, the apparent differences in coagulation between groups did not have an influence on overall GE. Several studies in vitro and in vivo in pigs show that amino acid absorption after milk ingestion is more rapid in the absence of coagulation,[22,60,61] which could indirectly imply a difference in GE. In this study, overall GE was not affected by coagulation, but the liquid phase emptied

quicker while the solid phase was retained longer, as there was a significantly higher percentage of coagulum and a lower percentage of liquid in the group of people with gastrointestinal problems. This is in line with previous animal in vivo research.[62] Future research should include blood sampling to track amino acid uptake, to see whether this is the case. Indeed, a recent study with similar inclusion criteria showed that less efficient digestion of milk proteins, leading to a different pattern of peptides reaching the lower gut which might explain GI problems in healthy people after milk consumption.[63] This may be preceded by a difference in gastric digestion. The possible underlying mechanism for the gastrointestinal symptoms may be the remaining coarser coagulum in the stomach. This has been previously seen in infants.[56]

A potential limitation of the study was that MRI requires a supine position for scanning, which affects the orientation of the stomach. This means that fluid dispersion throughout the stomach is different and therefore GE may be slower,[64] although relative differences are expected to remain the same.[38] A second limitation is that inclusion on milk consumption was based on self-reported data from a questionnaire and the FFQ showed slightly different results, which is probably due to differences between estimated and actual consumption of the participants. A third limitation would be that a dynamic in vitro model would be more informative than the currently used static model. In conclusion, this study demonstrated that casein coagulation was well visible with MRI and quantifiable by image texture measures. MRI could therefore play an important role in future in vivo coagulation research on milk and other foods. Future research should first focus on further calibrating this analysis approach with the help of a series of in vitro experiments with a wider range of pH values or dynamic in vitro studies. Variation in image texture metrics should be linked to coagulating attributes, such as the size of coagulates and curd firmness.[65] This could be used to optimize scan parameters in vivo to most accurately measure food matrix changes of dairy products. Another area of future research would be relating image texture metrics to instrumental texture metrics.

In conclusion, GE of individuals who report GI symptoms after drinking milk was similar to that of individuals without symptoms. This suggests that the rate of delivery of milk to the small intestine is not driving GI symptoms. Instead, our data support the idea that GI symptoms may occur due to differences in gastric casein coagulation between the two groups.

AUTHOR CONTRIBUTIONS

Paul A.M. Smeets, Mathilde Guerville, Kasper Hettinga, and Vincenzo Fogliano designed the research; Elise J.M. van Eijnatten conducted the research. Elise J.M. van Eijnatten analyzed the data and drafted the paper. Guido Camps, Mathilde Guerville, Vincenzo Fogliano, Kasper Hettinga, and Paul A.M. Smeets revised the manuscript critically for important intellectual content. Paul A.M. Smeets had primary responsibility for final content. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

Elise J.M. van Eijnatten, Guido Camps, Vincenzo Fogliano, Kasper Hettinga, Paul A.M. Smeets: no conflicts of interest. Mathilde Guerville: employed at Lactalis Research and Development.

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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