

Cow's Milk Challenge Increases Weakly Acidic Reflux in Children with Cow's Milk Allergy and Gastroesophageal Reflux Disease

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Objective To assess and compare the pattern of reflux in a selected population of infants with cow's milk (CM) allergy (CMA) and suspected gastroesophageal reflux disease (GERD) while on dietary exclusion and following challenge with CM.

Study design Seventeen children (median age: 14 months) with a proven diagnosis of CMA and suspected GERD underwent 48-hour multichannel intraluminal impedance-pH monitoring. For the first 24 hours, the infants were kept on amino acid-based formula, and for the subsequent 24 hours, they were challenged with CM.

Results The total reflux episodes and the number of weakly acidic episodes were higher during CM challenge compared with the amino acid-based formula period [total reflux episodes: 105 (58-127.5) vs 65 (39-87.5), $P < .001$; weakly acidic episodes: 53 (38.5-60.5) vs 19 (13-26.5), $P < .001$; median (25th-75th)]. No differences were found for either acid or weakly alkaline episodes (not significant). The number of weakly acidic episodes reaching the proximal, mid, and distal esophagus was higher during CM challenge ($P < .001$). No differences were found in either acid exposure time or number of long-lasting episodes (not significant).

Conclusions In children with CMA and suspected GERD, CM exposure increases the number of weakly acidic reflux episodes. CM challenge during 48-hour multichannel intraluminal impedance-pH monitoring identifies a subgroup of patients with allergen-induced reflux, and in selected cases of children with CMA in whom GERD is suspected, its use could be considered as part of diagnostic work-up. (*J Pediatr* 2012; ■: ■-■).

Cow's milk (CM) allergy (CMA) and gastroesophageal reflux disease (GERD) are both common complaints in infancy. Several studies support the hypothesis of a causal relationship between them, suggesting that at least in a subgroup of infants GERD is attributable to CMA.¹

Contrasting results have been published on the role of 24-hour pH-monitoring in differentiating between primary GERD from GERD secondary to CMA.²⁻⁴ Although 24-hour pH monitoring is one of the current standard methods for gastroesophageal reflux (GER) assessment in children,⁵ it will fail to detect some types of reflux, especially when little or no acid is present in the refluxate. Multichannel intraluminal impedance (MII) and pH monitoring combined allows better characterization of the reflux episodes as acid, weakly acidic, and weakly alkaline and also determines the height reached by the refluxate.⁶

In this prospective study, we aimed to investigate the effect of CM's challenge on the type and physical characteristics of reflux episodes during 48-hour MII-pH monitoring.

Methods

The children included in the study required a definitive diagnosis of CMA and suspected GERD and had been on amino acid-based formula (AAF) as milk substitute for at least 2 months. Exclusion criteria were known infectious and systemic disorders, structural abnormalities of the gastrointestinal tract, neurologic impairment, previous esophageal or gastrointestinal surgery, and immunodeficiency. The initial diagnosis of CMA in infants with suspected symptoms and/or signs was based on positive oral food challenge following an initial 4-week elimination diet. In all patients, a CM-free diet was started using an AAF (Neocate LCP; Nutricia UK, Wiltshire, United Kingdom) as milk substitute, and the open oral challenge was performed using a formula based on whole CM protein. The suspected diagnosis was then confirmed if the symptoms reoccurred.⁷ The diagnosis of suspected GERD was based on clinical history and symptom assessment by using the Infant GER Questionnaire Revised.⁸

AAF	Amino acid-based formula
CM	Cow's milk
CMA	Cow's milk allergy
GER	Gastroesophageal reflux
GERD	Gastroesophageal reflux disease
MI	Multichannel intraluminal impedance
PAR-2	Proteinase activated receptor 2
SAP	Symptom association probability

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Forty-Eight-Hour MII-pH Monitoring and Study Protocol

All children underwent 48-hour MII-pH monitoring using an ambulatory system (Sleuth; Sandhill Scientific, Highland Ranch, Colorado). The system included a portable data logger with impedance-pH amplifiers and an MII-pH catheter with an outer diameter of 2.1 mm (6.4-French), containing 1 pH-measuring electrode and 7 impedance sensors, in the form of 4-mm cylindrical ring electrodes. Each pair of ring electrodes represented an impedance measuring segment corresponding to 1 recording channel. The distance between each impedance sensor was 1.5 cm, resulting in a total measuring length of 9 cm. The pH-measuring electrode was localized within the distal impedance recording channel, 1 cm from the catheter tip.

Before the study, none of the children had received acid suppression therapy or drugs affecting lower esophageal sphincter function (ie, β -adrenergic agonist and prokinetics) for at least 72 hours. On the day of MII-pH monitoring, infants were fasted for at least 4 hours before the procedure. The pH electrode of the MII-pH catheter was calibrated using pH 4 and pH 7 buffer solutions. Each catheter was used once only and none of them malfunctioned. The MII-pH catheter was introduced through the nose and fluoroscopically positioned so that the pH-measuring electrode overlaid the third vertebral body above the diaphragm throughout the respiratory cycle.

All participants underwent a recording period of 48 hours. During the first 24 hours, the infant was kept on AAF (AAF period) (Neocate LCP; Nutricia, UK), and during the second recording day, an open milk challenge was performed (CM period) (SMA follow-up, SMA Nutrition, Berkshire, United Kingdom). The 2 formulas have similar osmolality (Neocate LCP: 360 mOsm/kg; SMA follow-up: 310 mOsm/kg) and composition (Neocate: fat 3.4 g/100 mL, carbohydrate 7.9 g/100 mL, protein 1.9 g/100 mL, energy: 67 Kcal/100 mL; SMA follow-up: fat 3.5 g/100 mL, carbohydrate 7.4 g/100 mL, protein 1.5 g/100 mL, energy: 70 Kcal/100 mL). The amount of milk to be ingested during both 24-hour periods was calculated by a member of the dietetic staff. During monitoring, to avoid erroneous interpretation of MII-pH data, none of the participants were given acidic beverages (pH < 5.0). Parents were instructed to keep a careful diary by recording meal times, body position (upright or recumbent), daily activities, and timing of any symptoms. When the study ended, one of the authors reviewed the diaries to verify accurate completion.

The signals from the impedance and pH channels were digitized at 50 Hz, stored in the battery-powered datalogger. At the end of the first 24 hours of the recording time, the batteries were changed by switching the datalogger to stand-by mode. At the end of the recording time the signals were downloaded into a personal computer and the MII-pH tracings were analyzed with dedicated software (BioVIEW Analysis, version 5.1.2; Sandhill Scientific). The data for each study were also manually reviewed. All data from the diaries were manually

entered into the impedance tracings. Impedance and pH signals collected during meals were excluded from the analysis.

Reflux was characterized by impedance as liquid, gas, and mixed (combined liquid and gas). Liquid reflux was defined as a retrograde 50% fall in impedance on at least the 2 more distal impedance channels. Gas reflux was defined as a rapid increase in impedance >5000 Ω , occurring simultaneously in any 2 consecutive impedance channels. Mixed liquid-gas reflux was defined as gas reflux occurring during or immediately before a liquid reflux. According to the corresponding pH changes, impedance detected reflux episodes were classified as acid (pH < 4.0), weakly acidic (pH 4-7), and weakly alkaline (pH > 7.0). Reflux episodes detected only by pH electrodes were defined as pH-only reflux. The height of a reflux event was defined as proximal, if it reached 1 or both of the 2 most proximal impedance channels; intermediate, if it reached 1 or both of the two middle impedance channels; and distal, if it reached the 2 distal impedance channels.

For each of 24-hour period the following variables were analyzed: (1) total number of reflux episodes; (2) number of acid reflux episodes; (3) number of weakly acidic episodes; (4) number of weakly alkaline episodes; (5) number of pH-only reflux; (6) height of reflux episodes; (7) percentage of the recording time during which pH was <4.0 (ie, esophageal acid exposure time); pH monitoring was deemed abnormal if the esophageal acid exposure during 24 hours exceeded 10% in infants <1 year and 5% in children >1 year; and (8) number of long-lasting reflux, defined as the number of reflux episodes lasting more than 5 minutes per 24 hours.

The association between MII-detected liquid-containing reflux and symptoms was evaluated using the symptom association probability (SAP). A 2-minute time interval preceding or following the onset of an MII-detected reflux episode was used to delimitate the time window for reflux-symptom association, and SAP values >95% were considered statistically significant.⁹

Statistical Analysis

The baseline characteristics of the patients were evaluated by means of simple descriptive analysis. The Wilcoxon signed rank test was used for relative samples. The χ^2 test or Fisher exact test was used when appropriate. All statistical tests were 2-tailed using a .05 level of significance. Analysis was performed using Prism software version 4.00 (GraphPad, San Diego, California). Data were expressed as median (25th-75th percentile) and mean $\pm$ SD, unless otherwise stated.

Results

Twenty-four patients were considered for the trial over a recruitment period of 12 months. Five infants were excluded either because they met exclusion criteria or because the parents declined to participate. Two patients were excluded from the study due to technical problems in the MII-pH

Table I. Demographics and disease characteristics of patients at diagnosis

No. of cases	17
Age (months; median and ranges)	11 (6-24)
M:F	10:7
Disease duration (weeks; median and ranges)	17 (12-24)
Symptoms and signs (number and percentage)	
Regurgitation/vomiting	16/17 (94%)
Irritability	12/17 (71%)
Food refusal	9/17 (53%)
Failure to thrive	6/17 (35%)
Diarrhea	5/17 (29%)
Constipation	3/17 (18%)
Atopic dermatitis	5/17 (29%)
Wheezing/asthma	4/17 (23%)
Previous treatment	
Proton pump inhibitors	14/17 (82%)
H2RA	7/17 (41%)
Alginic acid	5/17 (29%)
Positive total IgE	5/17 (29%)
Positive specific IgE to CM	3/17 (18%)
Family history of atopy	10/17 (59%)

H2RA, H2-receptor antagonist.

monitoring. Analysis was therefore completed in 17 children (median age 11 months; range 5-24 months) (Table I). At the time of the study, all patients were asymptomatic and only 4 were still on antireflux therapy. Of those, 3 patients were on proton pump inhibitors and the remaining 1 was on H2-receptor antagonist. At the end of recording time, in 3 children, due to the reoccurrence of symptoms, the total amount of milk intake during the CM challenge was slightly lower; however, the mean volume average among the group in the 2 recording periods was similar.

Table II summarizes the results of the 24-hour MII-pH monitoring during AAF and CM challenge. The median number of total reflux episodes was significantly higher during the CM periods compared to the AAF periods (Figure 1; available at www.jpeds.com). No difference between the 2 recording periods was found in the median number of acid, weakly alkaline reflux episodes, and pH-only events. Conversely, a significant difference between the

2 groups was found in the median number of weakly acidic reflux episodes, which was significantly higher during the CM period. Furthermore, the milk challenge significantly decreased the proportion of acid (AAF: 501 of 1142 [45%], CM: 511 of 1632 [31%], $P < .001$) and pH-only reflux episodes (AAF: 193 of 1142 [17%], CM: 176 of 1632 [11%], $P < .001$), which paralleled a significant increase in the proportion of the weakly acidic reflux episodes detected during the second 24 hours of monitoring (AAF: 325 of 1142 [28%], CM: 888 of 1632 [54%], $P < .001$) (Figure 2). No difference in the proportion of weakly alkaline reflux episodes was found between the AAF periods and CM periods (AAF: 112 of 1142 [10%], CM: 147 of 1632 [11%], NS). Finally, although in both periods the majority of weakly acidic reflux episodes occurred in the postprandial period (AAF: range 80%-100%; CM: 91%-97%), the milk challenge significantly increase the proportion of postprandial weakly acidic reflux episodes (AAF: 276 of 325, CM: 728 of 788, $P < .001$).

With regard to reflux migration, the median number of total reflux episodes reaching the proximal, mid, and distal esophagus was significantly higher in the CM period compared with the AAF period (Table III). Contingency analysis, however, showed no difference between the 2 recording periods in the proportion of total reflux episodes reaching the proximal, mid, and distal esophagus (AAF: proximal 777 of 938 [82%], mid 120 of 938 [13%], distal 41 of 938 [5%]; CM: proximal 1187 of 1456 [81%], mid 192 of 1456 [13%], distal 71 of 1456 [6%]; NS). The subgroup analysis revealed a significant increase in the median number of weakly acidic reflux episodes reaching the proximal, mid, and distal esophagus during the CM period compared with the AAF period, whereas no differences were found in the different heights of both acid and weakly alkaline reflux episodes (NS) (Table III).

In both recording periods, in all except 1 patient the esophageal acid exposure time was within the normal values. No differences between the 2 recording periods were found in the percentage of esophageal acid exposure time as well as in

Table II. Reflux characteristics during AAF and CM administration

	AAF period	CM period
Total number of reflux episodes [median (25 th to 75 th)]	65 (39-87.5)	105 (58-127.5)*
Chemical composition of refluxate [median (25 th -75 th)]		
Acid episodes	31 (9.5-44)	34 (14-41)
Weakly acidic episodes	19 (13-26.5)	53 (38.5-60.5)*
Weakly alkaline episodes	5 (3.5-10)	10 (2.5-15)
pH-only reflux episodes	9 (7-16)	11 (5.5-15)
Reflux composition		
Liquid	71%	78%
Mixed	27%	21%
Gas	2%	1%
Esophageal acid exposure time (mean $\pm$ SD)	3.4 $\pm$ 2.6	3.6 $\pm$ 2.7
Number of long-lasting episodes (>5 min) [median (25 th -75 th)]	3 (1-3)	2 (1.5-2.5)

* $P < .001$ by Wilcoxon signed rank test.

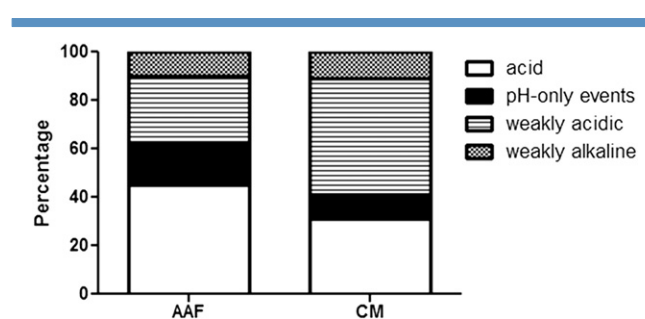


Figure 2. Proportion of the type of reflux episodes during the 2 recording periods. Note the significant decrease in proportion of acid and pH-only reflux episodes, and the significant increase in the proportion of the weakly acidic reflux episodes. Data are expressed as percentage of the total number of reflux episodes.

Table III. Height of reflux episodes during AAF and CM administration

	CM period	AAF period
Total reflux episodes		
Proximal	50 (24.5-61.5)	72 (47-94.5)*
Intermediate	6 (5-10)	10 (6.5-16)†
Distal	3 (0-3.5)	4 (3-6)‡
Acid reflux episodes		
Proximal	25 (6-38)	25 (10-34)
Intermediate	4 (3-7)	5 (2-7.5)
Distal	0 (0-2)	1 (0-2.5)
Weakly acidic reflux episodes		
Proximal	16 (12-20.5)	43 (33-51.5)*
Intermediate	2 (0.5-2)	3 (2-5.5)†
Distal	0 (0-2)	1 (0-3)‡
Weakly alkaline reflux episodes		
Proximal	5 (0-10)	5 (0-10)
Intermediate	0 (0-1)	0 (0-5)
Distal	0 (0-0)	0 (0-2)

Values given as median (25th-75th).

* $P < .001$ by Wilcoxon signed rank test.

† $P < .01$ by Wilcoxon signed rank test.

‡ $P < .05$ by Wilcoxon signed rank test.

the number of long-lasting reflux episodes (NS, [Table II](#)). Symptoms were reported during CM period in 11 patients. In 4 of them, 30 symptoms were reported but no single symptom was associated with a reflux episode. At least 1 symptom was associated with a reflux episode in the remaining 7 patients. Of a total of 126 symptoms analyzed, 92 of them were considered temporally correlated with reflux episodes, of which 68% involved weakly acid, 32% involved acid, and none involved weakly alkaline ([Table IV](#); available at www.jpeds.com). Two patients showed positive SAP for acid, and 1 had abnormal esophageal acid exposure time. The remaining 5 had positive SAP for weakly acid, and all of them showed normal esophageal acid exposure time. In the remaining 6 children who had been asymptomatic during CM challenge, gastrointestinal symptoms reoccurred within 2 weeks of the reintroduction of whole CM formula.

Discussion

CMA and GERD are both common causes of foregut symptoms in infancy, and several studies in the past few years have shown that at least in a subset of patients, GERD is not only CMA associated but also CMA induced. Although it has been estimated that the prevalence of GERD attributable to CMA is as high as 56%, such an association has not been proved in a scientifically robust manner.²⁻⁴ CM challenge significantly increased the total number of reflux episodes in a population of CMA patients. During the milk challenge there was a significant increase in weakly acidic reflux. The milk challenge does not affect the number of acid reflux episodes, the esophageal acid exposure time, and the number of long-lasting reflux episodes.

Previous studies have advocated the role of esophageal pH-monitoring in distinguishing between primary GERD and GERD secondary to CMA. It has been suggested that a partic-

ular phasic pH pattern characterized by a slow and progressive decrease in esophageal pH after each milk ingestion toward the following feeding was a sensitive and specific index for identifying patients with CMA-induced GERD.^{10,11} However, these findings have not been confirmed by other investigators.^{4,12} Nielsen et al performed 48-hour pH-monitoring in 10 children with a severe GERD and CMA, with CM elimination diet at day 1 and CM challenge at day 2. Interestingly, they failed to find any difference in the reflux parameters between the 2 recording days.⁴ Thus far, the majority of studies addressing the relationship between CMA and GERD have been performed by using esophageal pH-monitoring, the diagnostic value of which is limited by its inability to detect nonacidic reflux episodes mainly in infants who are frequently fed milk and/or milk-based formulas. In the present study, we used MII-pH monitoring that allows detection of acid, weakly acidic, and weakly alkaline reflux, as well as detection of the height of the refluxate. We confirmed previous findings that the milk challenge is not associated with an increase in the esophageal acid exposure time, supporting the hypothesis that the parameter "reflux index" as used in the analysis of esophageal pH studies is not suitable for evaluating CMA-induced GERD. Conversely, we found during the CM challenge in our CMA patient population a significant increase in the total number of reflux episodes, as well as an increase in the total number of weakly acidic reflux episodes. The diagnostic strategy in infants and young children who are suspected of having GERD and/or CMA should involve a 2- to 4-week trial of exclusion diet followed by a CM challenge to confirm or exclude a CMA.⁵ Based on our results, CM challenge during pH-impedance monitoring is potentially valuable as an objective test to differentiate whether the reoccurrence of both early and late symptoms are really related to a change in reflux pattern.

All except 1 of the patients in our study exhibited a normal esophageal acid exposure time, and 7 of them showed a positive correlation between symptoms and acid and weakly acidic reflux episodes. We identified 2 patients for whom the SAP was positive for acid reflux, and 1 had an abnormal esophageal acid exposure time. In the remaining 5, who were positive for weakly acidic reflux, all showed a normal esophageal acid exposure time. Hence, esophageal acid exposure time is a poor correlate of symptom-related reflux. In addition, >70% of the patient group showing a positive SAP involving weakly acidic reflux episodes would have been missed by 24-hour pH monitoring alone. Thus, as shown in several studies MII-pH monitoring increases the probability of documenting an association between symptoms and reflux compared with pH monitoring.¹³⁻¹⁵ Our findings are also in agreement with previous studies that support the role of nonacid reflux in pathogenesis of typical and atypical symptoms of GERD.¹⁶⁻²⁰

The mechanisms by which food allergy induces GER are still poorly understood. Data in animal models of immediate-type hypersensitivity reactions have shown that antigen challenge in vivo results in neurally mediated gastrointestinal dysmotility, including delayed gastric emptying

and abnormal gastric acid secretion.²¹ In infants with vomiting due to CMA, a milk challenge induces gastric electrical dysrhythmias and delayed gastric emptying.²² We have previously showed that early-onset neuroimmune interactions induced by CM challenge in the gastric mucosa of atopic children are associated with rapid derangement of gastric myoelectrical activity.²³ Notably, in this study, CM challenge induced rapid degranulation of mast cells and eosinophils. Activated mast cells were closely associated with mucosal nerve fibers, and released mast cell tryptase was colocalized with proteinase activated receptors 2 (PAR-2) on mucosal nerve fibers. In the same timeframe as these morphologic changes occurred, there was a rapid (within 2 minutes) induction of electrogastrographic myoelectrical abnormalities. Intriguingly, in experimental animals, gastric activation of PAR-2 induces neurally mediated motor and secretory responses represented by a fundic biphasic contractile response, which involves relaxation followed by contraction, and suppression of acid production.^{24,25} Furthermore, it has been shown that a reduced prevalence of normal electrical rhythm and an increased rate of episodes of dysrhythmia are associated with antral hypocontractility, which in turn promotes a gastric emptying delay.^{26,27} Delayed gastric emptying may increase GER by increasing the availability of material to reflux or by inducing prolonged gastric distention and more transient lower esophageal sphincter relaxations, which are the major mechanism of reflux in normal subjects and GERD patients.^{28,29} Using simultaneous gastric emptying and pH-MII, Sifrim et al showed that the slower the gastric emptying, the higher are the pH and proximal extent of the reflux episodes.³⁰ Finally, as confirmed in our study, it is well known that non-acid reflux episodes are more likely to occur during feeding and during the first postprandial hours. Thus, we are tempted to suggest that neuroimmune interactions induced during CM challenge by activating gastric PAR-2 suppress the acid gastric production and derange the gastric motor activity, which in turn delay the gastric emptying and increase the rate of transient lower esophageal sphincter relaxations, resulting in an increase in the number of weakly acidic reflux episodes. This theory needs corroboration.

Our study has some limitations. First, the diagnosis of GERD was performed using the "Infant" GER Questionnaire Revised, and we are aware that no symptom or cluster of symptoms have been shown to reliably predict the diagnosis of GERD.⁵ Second, because the efficacy of empiric trial with AAF, no patients underwent upper gastrointestinal endoscopy. Thus, we cannot rule out that some infants in our study population had an eosinophilic infiltration of the esophageal mucosa. However, all patients had an excellent clinical response to CM withdrawal and, based on international guidelines, they are classified as having CMA. Finally, although we have used 2 formulas with similar osmolality and composition, in the absence of a gastric emptying time assessment we cannot rule out differences in gastric emptying time between the 2 recording periods. A simultaneous study with pH-impedance and gastric emptying time is advocated to

rule out the influence of gastric emptying time on the type of reflux.

In conclusion, our data provide evidence that at least in a subgroup of children with CMA and suspected GERD, exposure to CM increases the number of weakly acidic reflux episodes. CM challenge during 48-hour MII-pH monitoring increases the yield in identifying this subgroup of patients, and in selected cases of children with CMA in whom GERD is suspected its use should be considered as part of diagnostic work-up. Further studies for delineating the role of TLESRs in the pathogenesis of CMA-induced GERD are warranted. ■

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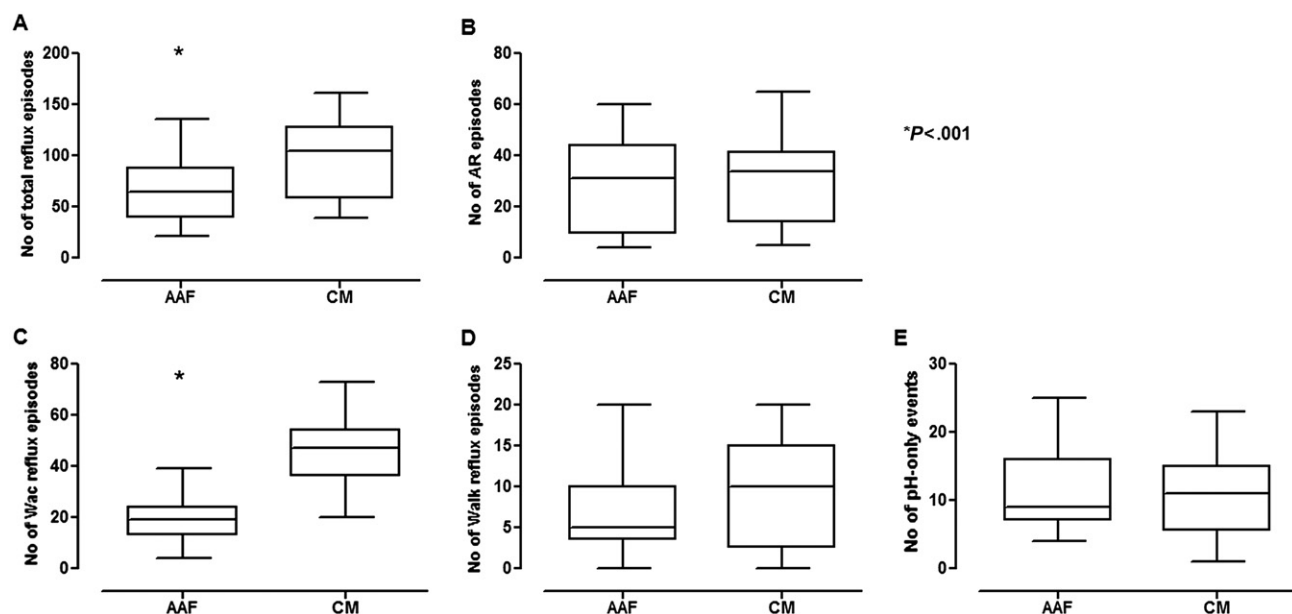


Figure 1. Number and types of reflux episodes during the 2 recording periods. **A**, The total number of reflux episodes as well as **C**, the number of weakly acidic reflux episodes were significantly higher during the CM challenge. No difference was found in number of **B**, acid and **D**, weakly alkaline reflux episodes and **E**, pH-only events. The bars indicate the medians, the lines the 25th and 75th percentile values. * $P < .001$ vs AAF. AR, acid reflux; Wac, weakly acidic; Walk, weakly alkaline.

Table IV. Symptoms associated with reflux episodes during CM challenge

Symptom type	No. of patients	No. of symptoms	GER related	Acid reflux episodes related	Weakly acidic reflux episodes-related	Weakly alkaline reflux episodes-related
Crying/pain	6	57	41 (72%)	17 (41%)	24 (59%)	0 (0%)
Vomiting/regurgitation	3	34	29 (85%)	3 (10%)	26 (90%)	0 (0%)
Cough	2	21	13 (62%)	7 (54%)	6 (46%)	0 (0%)
Arching/fussiness	2	14	9 (64%)	2 (22%)	7 (78%)	0 (0%)
Total	7	126	92 (73%)	29 (32%)	63 (68%)	0 (0%)