The motilin receptor agonist erythromycin stimulates hunger and food intake through a cholinergic pathway[1]

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ABSTRACT

Background: Motilin-induced phase III contractions have been identified as a hunger signal. These phase III contractions occur as part of the migrating motor complex (MMC), a contractility pattern of the gastrointestinal tract during fasting. The mechanism involved in this association between subjective hunger feelings and gastrointestinal motility during the MMC is largely unknown, however, as is its ability to stimulate food intake.

Objectives: We sought to *1*) investigate the occurrence of hunger peaks and their relation to phase III contractions, *2*) evaluate whether this relation was cholinergically driven, and *3*) assess the ability of the motilin receptor agonist erythromycin to induce food intake.

Design: An algorithm was developed to detect hunger peaks. The association with phase III contractions was studied in 14 healthy volunteers [50% men; mean ± SEM age: 25 ± 2 y; mean ± SEM body mass index (BMI; in kg/m^2): 23 ± 1]. The impact of pharmacologically induced phase III contractions on the occurrence of hunger peaks and the involvement of a cholinergic pathway were assessed in 14 healthy volunteers (43% men; age: 29 ± 3 y; BMI: 23 ± 1). Last, the effect of erythromycin administration on food intake was examined in 15 healthy volunteers (40% men; age: 28 ± 3 y; BMI: 22 ± 1).

Results: The occurrence of hunger peaks and their significant association with phase III contractions was confirmed ($P < 0.0001$). Pharmacologically induced phase III contractions were also significantly associated with hunger peaks ($P < 0.05$), and this association involved a cholinergic pathway. Administering erythromycin significantly stimulated food intake compared with placebo (53% ± 13% compared with 10% ± 5%; $P < 0.05$).

Conclusions: Motilin-induced phase III contractions induced hunger feelings through a cholinergic pathway. Moreover, erythromycin stimulated food intake, suggesting a physiologic role of motilin as an orexigenic signal from the gastrointestinal tract. This trial was registered at www.clinicaltrials.gov as NCT02633579. *Am J Clin Nutr* 2016;103:730–7.

Keywords: hunger, food intake, motilin, migrating motor complex, erythromycin

INTRODUCTION

During the fasting state, the gastrointestinal tract (GIT)[4] exhibits a specific contractility period called the migrating motor complex (MMC) (1). This highly organized system of migrating contractions can be divided into 3 phases of activity. Phase I is a quiescent phase with no apparent contractions. Phase II is a period with high-amplitude contractions in the stomach and low-amplitude contractions in the small intestine but with an irregular interval between consecutive contractions. As phase II progresses, the interval between contractions becomes shorter and will eventually increase to the maximum frequency of 3 contractions/min in the stomach and 11 contractions/min in the duodenum—defined as phase III of the MMC. Although phase III is the shortest of all phases, it is the one with the highest contractile activity, and it is believed that these contractions clean the stomach and small intestine of all food remnants, which is why the MMC is referred to as "the housekeeper of the GIT" (2). The approximate duration of one cycle is ~130 min, and eating interrupts the complex (3, 4). Thompson et al. (5) reported a median value of 13 complexes/24 h in the fasting state. One meal of 492 kcal reduced the value to 10 complexes/24 h.

Motilin, a peptide hormone produced by the proximal small intestine, is considered the physiologic regulator of gastric phase III contractile activity. Indeed, in humans, only phase III contractions with a gastric but not a small-intestinal origin are preceded by a motilin plasma peak (6, 7). In addition, administering exogenous motilin to dogs and humans induces gastric phase III contractions (8, 9). A pharmacologic compound known to stimulate gastric phase III contractions is erythromycin, a macrolide antibiotic that acts as a motilin receptor agonist (10–15).

Simultaneous measurement of the MMC and auscultation of the abdominal region have shown that phase III contractions with a gastric origin create the rumbling noise or borborygmus that is

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[4] Abbreviations used: GIT, gastrointestinal tract; HRM, high-resolution manometry; MMC, migrating motor complex; VAS, visual analog scale.

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considered to coincide with a sensation of hunger; however, hunger was not scored during these measurements (16). In a recent study that used water-perfused manometry catheters, we observed that hunger ratings increased during gastric phase III contractions in humans and seemed to generate hunger peaks, but the existence of hunger peaks has never been systematically analyzed and reported (17).

The aims of this study were to *1*) confirm the occurrence of hunger peaks based on an objective peak-detecting algorithm; *2*) correlate physiologically occurring or erythromycin-induced antroduodenal motility patterns, as measured with high-resolution manometry (HRM) during the fasting state, with simultaneously assessed hunger peaks; *3*) evaluate the role of a cholinergic pathway in this hunger mechanism; and *4*) investigate whether food intake can be triggered by erythromycin administration.

METHODS

This study was approved by the Medical Ethics Committee of the Leuven University Hospital, Leuven, Belgium, and performed in full accordance with the Declaration of Helsinki.

Study design

The study consisted of 4 independent protocols: algorithmic, physiologic, cholinergic, and food intake. Each protocol was performed on 1 single test day. In protocols involving drug administration, medication was given in a randomized single-blind placebo-controlled crossover manner.

Subjects

Volunteers were eligible to participate if they were healthy, aged between 18 and 60 y, had a BMI (in kg/m^2) between 18 and 25, and were recruited from an existing volunteer database in our group. Exclusion criteria were gastrointestinal diseases, abdominal surgery (appendectomy allowed), psychiatric illnesses, and usage of drugs affecting the GIT (including motilin-analogue antibiotics, opiates, or anticholinergic agents) or central nervous system. Volunteers allergic to macrolide antibiotics or atropine were not included in the food intake or cholinergic protocol, respectively. Written informed consent was obtained from all volunteers before the start of the study. Twenty-seven volunteers (48% men; mean ± SEM age: 31 ± 8 y; mean ± SEM BMI: 23 ± 1) participated in the algorithmic protocol; 14 (50% men; age: 25 ± 2 y; BMI: 23 ± 1) participated in the physiologic protocol; 14 (43% men; age: 29 ± 3 y; BMI: 23 ± 1) participated in the cholinergic protocol; and 15 (40% men; age: 28 ± 3 y; BMI: 22 ± 1) participated in the food intake protocol. One volunteer participated in both the physiologic and cholinergic protocol, 3 volunteers participated in both the cholinergic and food intake protocol, and 1 volunteer participated in the physiologic, cholinergic, and food intake protocol. All volunteers participated after an overnight fast of 12 h and were asked to refrain from alcohol, tea, and coffee for at least 12 h and from smoking for at least 1 h before the start of the study (0800). During the entire duration of the study, the subject was in the room together with the experimenter, but no one else was allowed in the room. Communication between the experimenter and the subject was minimal, and subjects were instructed to watch nature documentary movies provided by the experimenter to pass the time.

Study protocols

Preparation of the volunteers

MMC activity was measured with use of a ManoScan 360 HRM catheter and ManoView analysis software version 2.0 (Sierra Scientific Instruments). The total length of the catheter was ~140 cm. The measuring sensors of the catheter expanded ~36 cm, and each sensor was spaced 1 cm apart. The catheter was inserted through a nostril and placed until the lower esophageal sphincter was visible on the recording. The most distal recording point was placed as distally as possible in each subject. In most volunteers it reached until the third part of the duodenum; in others it just passed the corner of Treitz. Placement of the catheter was done under fluoroscopic guidance to position the catheter in the right position because it can curl up in the stomach and yield false pressure measurements. Moreover, visual guidance is also warranted when placing the catheter through the pylorus (**Figure 1**A). Once the catheter was in position it was secured to the subject's nose with adhesive tape. Intravenous administration of saline (placebo), erythromycin lactobionate (Amdipharm Limited), or atropine sulfate (Sterop) was done through a cannula placed in a forearm vein at the start of both the cholinergic and food intake protocol.

Algorithmic protocol

This protocol was aimed at developing an objective method to detect hunger peaks in hunger ratings. Volunteers were asked to score their hunger at 5-min intervals with use of a 10-cm visual analog scale (VAS) (0 cm: not at all hungry; 10 cm: as hungry as I have ever felt) for a period of 4–5 h (18).

Physiologic protocol

This protocol was aimed at establishing the occurrence of hunger peaks based on an objective peak-detecting algorithm and elucidating their relation to gastrointestinal motility patterns. Volunteers were asked to score their hunger at 5-min intervals with use of a 10-cm VAS. Pressure measurement and hunger ratings were recorded for a period of 7 h.

Cholinergic protocol

This protocol was aimed at evaluating the involvement of a cholinergic pathway in erythromycin-induced hunger peaks and gastric phase III contractions. It followed the same outline as the physiologic protocol, but at fixed time points (90, 180, 270, and 360 min) an intravenous infusion of placebo or 40 mg erythromycin was administered over a 20-min period in a volume of 100 mL 0.9% NaCl (Figure 1B). Administration was done in a single-blind randomized fashion, with 2 infusions of erythromycin and 2 infusions of saline. Either placebo or atropine sulfate was given 10 min before the administration of erythromycin. Atropine (15 μg/kg) was given as an intravenous bolus followed by a continuous infusion of 15 $\mu g \cdot kg^{-1} \cdot h^{-1}$ over 30 min while arterial pulse frequency was continuously monitored. A placebo pretreatment was always given when saline was administered instead of erythromycin. In total, each participant received 8 infusions during the measuring period.

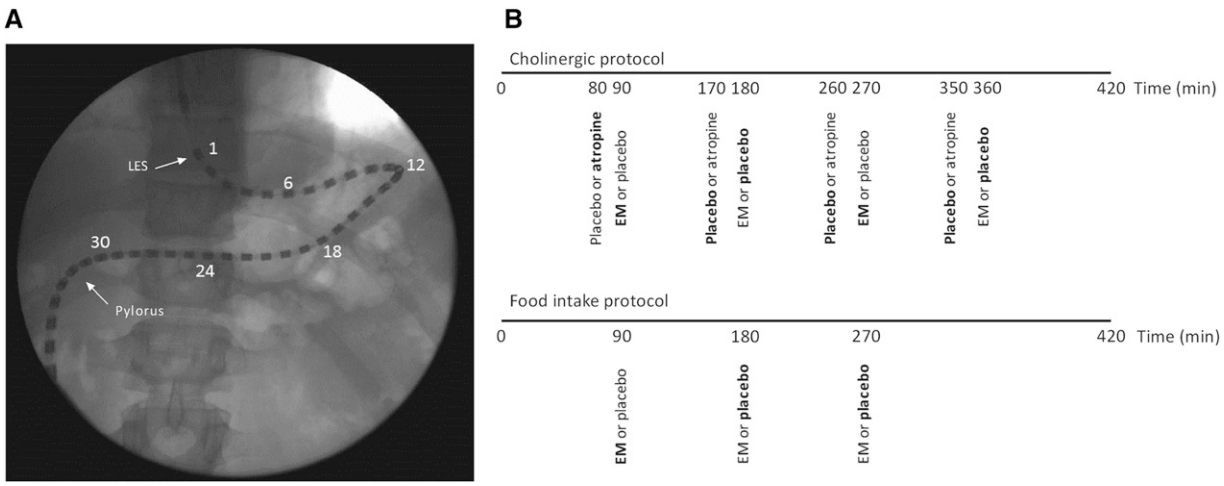

FIGURE 1 X-ray image of catheter position and schematic representation of the protocol. (A) Placement of solid-state high-resolution manometry catheter with measuring points in the stomach and proximal part of the duodenum. Channels 1, 6, 12, 18, 24, and 30 and the approximate location of the LES and pylorus are indicated. (B) Study design of cholinergic and food intake protocol. Both protocols are single-blinded randomized placebo-controlled trials. A possible administration protocol is indicated in bold for both protocols. EM, erythromycin; LES, lower esophageal sphincter.

Food intake protocol

This protocol studied the association between erythromycin administration and food intake. It followed the same outline as the physiologic protocol, but at fixed time points (90, 180, and 270 min) an intravenous infusion of placebo or 40 mg erythromycin was administered over a 20-min period in a volume of 100 mL 0.9% NaCl (Figure 1B). Administration was done in a single-blind randomized fashion. Each subject received 3 infusions during the measuring period, 2 of which were saline and 1 of which was an infusion of erythromycin. Subjects were instructed that they could eat a small soup meal at any time of their choice during the experiment but with a maximum of 2 meal intakes and without obligation to take the meal. They were also instructed to inform the experimenter when they wanted a meal. The soup meal consisted of a 160-mL low-caloric broth (Maggi Opkikker Tuinkruiden; Nestlé) that contained 9 kcal (0.7 g protein, 1.3 g carbohydrates, 0 g fat, 0.1 g fiber, and 0.55 g Na). Soup was chosen because it did not cause any swallowing problems with the catheter in place. A low-caloric broth was chosen together with the limitation of a maximum of 2 meal intakes to limit the duration of the MMC interruption (19). Antroduodenal motility was monitored continuously during the measurement to record the shift from fasting to a fed motility pattern during the intake. Before the start of the experiment and at time points of ingestion, subjects had to score visual appeal, desire to eat, and smell of the soup on a 10-cm VAS. Volunteers were also asked to rate the palatability at the time of consumption. Time points of meal request were recorded by the examiner.

Measurements

Motility indexes

The activity of the antroduodenal motility was analyzed as described previously (20). In brief, a motility index was calculated with use of the following formula: (number of contractions × average amplitude contractions × average duration contractions) ÷ 5 min. A mean motility index was calculated from 6 antral and 6 duodenal channels.

Algorithm for hunger peak detection

Visual inspection of hunger ratings over time indicated the occurrence of sharp increases (i.e., hunger peaks). All individual hunger score traces were presented to 10 investigators who were asked to indicate the occurrence of hunger peaks, if present. A peak was considered to be present when 7 of 10 assessors identified it as a hunger peak. An exponentially weighted moving average was used to calculate a baseline through the original data (21). A hunger peak was considered to be present if the difference between the original hunger scores and the calculated baseline was greater than a predefined threshold. To determine which combination of smoothing constant and threshold showed the best agreement with the subjective identification of hunger peaks by the panel, smoothing constants ranging from 0.1 to 0.4 and thresholds ranging from 4 to 6 were tested. A threshold of 6 mm with a smoothing constant of 0.3 generated a κ coefficient of 0.50, a sensitivity and specificity of 84, and a statistically significant ($P < 0.0004$) McNemar's test. Based on this analysis, a hunger peak was defined as a difference between the original data and the corresponding baseline value >6 mm. A second condition to define a hunger peak was a minimum duration of 10 min.

Statistical analyses

Comparison of hunger ratings from hunger peaks detected by the algorithm and assessors

Hunger ratings were screened for peaks by the assessors and by the algorithm. The hunger ratings from hunger peaks detected by both methods were compared with use of mixed-model analysis (SAS 9.3; SAS Institute) with hunger ratings as the dependent variable. Method (algorithm and assessors) and time were entered as categorical variables and were included as main effects together with an interaction effect between them. Intercept and time were included as random effects.

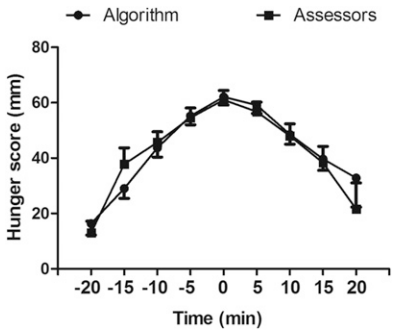

FIGURE 2 Comparison of hunger peaks detected by the assessors and the algorithm. Hunger was scored every 5 min on a 10-cm visual analog scale. Hunger ratings of 27 volunteers were screened for hunger peaks by the algorithm and by 10 independent assessors. The hunger scores were analyzed with use of mixed-model analysis, with hunger as the dependent variable and method of hunger peak detection and time as independent variables. There was no significant difference between hunger peaks detected by the algorithm or by the assessors ($P = 0.79$). The graph represents the means and SEMs of the hunger peaks detected by both methods. Time point 0 indicates the summit of the peak.

Analysis of physiologic phase III contractions and hunger peaks

Phase III contractions were visually identified in the manometry tracing according to standard definitions (22). Associations between phase III contractions and hunger peaks were analyzed with use of a generalized linear mixed model (SAS 9.3). The presence of both a hunger peak and phase III contractions in each 10-min time interval was used as a binary variable, with the presence of a hunger peak as the dependent variable (logit link function) and presence of phase III contractions as the independent variable of interest. Time was included as an additional continuous independent variable. Intercept and time were entered as random effects.

Analysis of pharmacologically induced phase III contractions and hunger peaks

Associations between drug administration (placebo, erythromycin, atropine), phase III contractions, and hunger peaks were also analyzed with use of generalized linear mixed models (SAS 9.3). Administration of the drugs was used as a binary independent variable of interest. Administration duration was set at four 10-min time intervals (or 40 min). In all, 2 analyses were done, one with phase III as the dependent variable and the other with hunger peaks as the dependent variable. Both included time as a continuous independent variable. We conducted a mediation analysis to test whether the association between drug administration and hunger peak was mediated through an effect of phase III contractions. Administration was the independent variable, hunger peak was the dependent variable, and phase III contractions were the mediator variable (23).

Occurrence of food intake during erythromycin administration

Soup intake was associated with the infusion if it occurred during the 20 min of infusion. For each subject, the percentage of erythromycin and placebo infusion associated with soup intake was calculated and compared with use of Wilcoxon's signed rank test.

Assessment of the meal

Data were analyzed with use of mixed-model analysis (SAS 9.3) with either smell, visual appeal, desire to eat, or palatability as the dependent variable and intake (baseline, first soup, second soup) as the independent repeated variable. Post hoc *t* tests were corrected for multiple testing with use of Bonferroni correction. Significance was set at $\alpha < 0.05$. Data are represented as means ± SEMs.

RESULTS

Hunger peaks can be detected with use of an automated algorithm

The hunger ratings of 27 healthy volunteers were analyzed for hunger peaks by both the assessors and the hunger peak–detecting algorithm. The automated method detected all of the hunger peaks that were recognized by a minimal 70% of the assessors, but it also recognized 48% of the hunger peaks that were recognized by <70% of the assessors. **Figure 2** shows that there was no significant ($P = 0.79$) difference between the

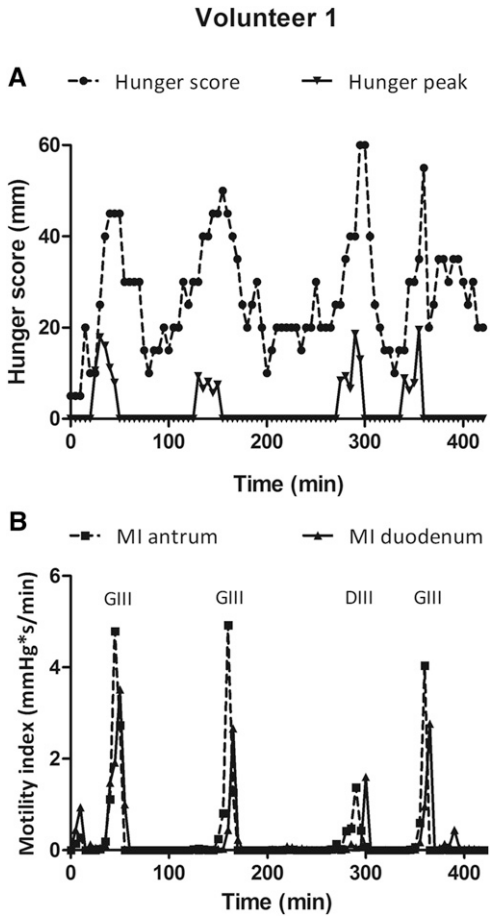

FIGURE 3 Representative tracing of a 7-h pressure measurement (physiologic protocol). Hunger was scored every 5 min on a 10-cm visual analog scale (A). Hunger peaks were identified if the difference between the original hunger score and the calculated baseline was >6 mm. MI of antral and duodenal region were calculated by averaging the MI of 6 antral and 6 duodenal channels (B). DIII, duodenal phase III; GIII, gastric phase III; MI, motility index.

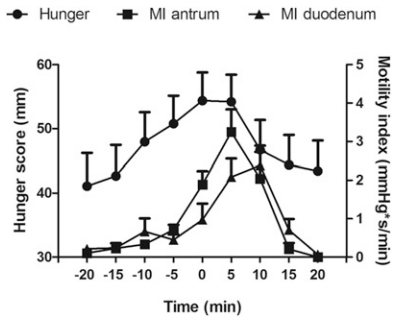

FIGURE 4 Hunger scores during phase III of the migrating motor complex (physiologic protocol). Mean hunger scores are shown together with the corresponding antral and duodenal MI during phase III of the migrating motor complex of 14 healthy volunteers. A significant association ($P < 0.0001$) between phase III and hunger peaks was found with generalized mixed-model analysis. Time point 0 indicates the start of phase III. Data are represented as means ± SEMs. MI, motility index.

hunger peaks detected by the algorithm and the peaks detected by the assessors.

Hunger ratings increase during phase III contractions with the formation of hunger peaks

Under physiologic conditions after a 12-h fast, 2.5 ± 0.3 phase III contractions were measured on average during the following 7-h fasting period with 80% of the phase III contractions starting in the antrum. **Figure 3** shows an individual tracing of a volunteer who had 4 phase III contractions during the measurement. All of these phase III contractions were associated with a hunger peak (Figure 3A), and 3 started in the antrum (Figure 3B). In the entire group of volunteers, the mean hunger scores increased significantly ($P < 0.0001$) with the occurrence of phase III contractions (**Figure 4**). The hunger score at the start of phase III contractions was on average 32% higher than 20 min before the start.

With use of the algorithm, individual hunger score tracings could be screened for hunger peaks. The mean duration of hunger peaks was 16 ± 2 min, and the mean number of hunger peaks during the 7-h measurement was 3.6 ± 0.6. In the generalized linear mixed-model analysis, a significant association was found between phase III contractions of the MMC and the presence of a hunger peak (OR: 6.125; 95% CI: 3.452, 10.866; $P < 0.0001$). The occurrence of hunger peaks was not time-dependent because there was not a significant association between time and the presence of a hunger peak (OR: 0.999; 95% CI: 0.996, 1.003; $P = 0.72$). The origin of phase III contractions did not affect the association between phase III contractions and the presence of hunger peaks because both gastric phase III contractions (OR: 5.888; 95% CI: 3.162, 10.962; $P < 0.0001$) and duodenal phase III contractions (OR: 4.782; 95% CI: 1.393, 16.418; $P = 0.01$) showed a significant association with hunger peaks.

Erythromycin induces hunger peaks through a cholinergic pathway

Pretreatment with placebo did not affect the ability of erythromycin to induce a gastric phase III contraction because 18 ± 2 min after administering erythromycin a gastric phase III contraction occurred in all volunteers. Administering atropine significantly increased the pulse rate (73 ± 5 beats/min compared with 95 ± 3 beats/min; paired t test; $P = 0.0008$), and all volunteers reported having a dry mouth after the administration of atropine. The significant association between erythromycin and gastric phase III contractions (OR: 74.011; 95% CI: 22.183, 246.931; $P < 0.0001$) was lost if atropine was given before the erythromycin administration (OR: 1.615; 95% CI: 0.346, 7.535; $P = 0.54$). A significant association was found between erythromycin administration pretreated with placebo and hunger peaks (OR: 2.832; 95% CI: 1.198, 6.695; $P = 0.018$). This was mediated through the induction of gastric phase III contractions because the significant association between erythromycin administration and hunger peaks turned nonsignificant (OR: 0.524; 95% CI: 0.178, 1.543; $P = 0.24$) when phase III (OR: 0.470; 95% CI: 0.129, 1.715; $P = 0.25$) was added as a mediator variable to the model. The association between erythromycin and hunger peaks was lost if atropine was given before the administration of erythromycin (OR: 1.017; 95% CI 0.412, 2.514; $P = 0.97$). **Figure 5**A illustrates the association between

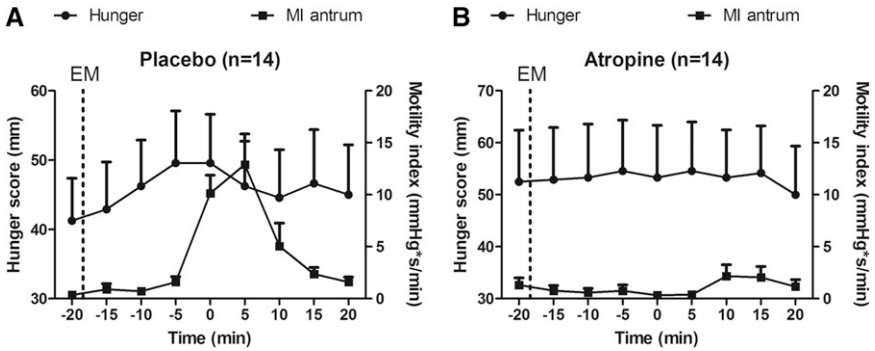

FIGURE 5 The role of a cholinergic pathway on the effect of EM to induce phase III contractions and hunger peaks (cholinergic protocol). In total, 14 healthy volunteers were included in this study. The administration of 40 mg erythromycin induced a gastric phase III together with a hunger peak (A). A significant association was found between EM administration and gastric phase III ($P < 0.0001$) and hunger peaks ($P = 0.018$). Data were analyzed with use of generalized linear mixed models. Pretreatment with 15 $\mu g \cdot kg^{-1} \cdot h^{-1}$ atropine abolished the induction of gastric phase III contractions and the increase in hunger at time points corresponding to the placebo pretreatment (B). No significant association was found between EM administration and gastric phase III ($P = 0.54$) or hunger peaks ($P = 0.97$) after atropine pretreatment. Data were analyzed with use of generalized linear mixed models. Time point 0 indicates start of phase III. The dotted vertical line indicates the start of EM administration. Pretreatment with placebo or atropine was given 10 min before EM administration. Data are represented as means ± SEMs. EM, erythromycin; MI, motility index.

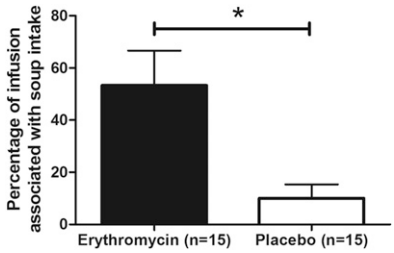

FIGURE 6 Soup intake can be stimulated via the administration of erythromycin (food intake protocol). The occurrence of soup intake during erythromycin and placebo infusion in 15 healthy volunteers is shown. Data were analyzed with use of Wilcoxon's signed rank test and are represented as mean ± SEM. $*P < 0.05$.

erythromycin-induced phase III contractions and hunger peaks. Figure 5B illustrates the effect of atropine pretreatment on hunger scores and antral motility at the corresponding time points of the placebo pretreatment.

Administration of erythromycin induces food intake

A phase III contraction with a gastric origin occurred in all volunteers 21 ± 3 min after the administration of erythromycin. On average, 50 ± 8 min after placebo administration a duodenal (47%) or gastric (53%) phase III contraction started (significantly different from the interval after erythromycin administration; paired *t* test; $P = 0.0057$). In comparison with placebo, administration of erythromycin significantly (Wilcoxon's signed rank test, $P = 0.015$) increased food intake, with 53% ± 13% of erythromycin infusions inducing food intake compared with 10% ± 5% for placebo administration (**Figure 6**). In all, 6 volunteers ingested only 1 soup meal during the measurement, 7 ingested 2 soups, and 2 had no meal intake. The interval until the first meal request was 207 ± 23 min; the interval until the second meal request was 326 ± 22 min. There was no significant ($P = 0.25$) change from baseline for smell ratings (**Figure 7**A). There was a significant (corrected P value = 0.031) increase from baseline for the second soup intake for visual appeal (Figure 7B). The desire to eat the meal was significantly increased for the first (corrected P value = 0.02) and second (corrected P value = 0.012) meal compared with baseline (Figure 7C). Palatability rating did not significantly ($P = 0.57$) differ between the 2 intakes (Figure 7D).

DISCUSSION

This study confirms the association between phase III contractions of the MMC, measured by HRM, and hunger peaks, identified through a newly developed and validated algorithm. Furthermore, infusion of the motilin receptor agonist erythromycin was associated with hunger peaks through a cholinergic pathway. Finally, for the first time to our knowledge we have shown that erythromycin can stimulate food intake in healthy human subjects.

The notion that fasting contractions of the GIT signal hunger was postulated in the last century by Cannon and Washburn (24) and Carlson (25). Bloom et al. (26), however, argued against the involvement of gastric and duodenal motility in determining hunger. The lack of association between interdigestive motility and hunger ratings is probably attributable to the fact that they measured hunger with use of a half-hourly interval between consecutive hunger scores (26). This interval is too long to perform association studies with the MMC given that phase III of the MMC has a mean duration of only <10 min. In this study, we measured MMC activity with use of antroduodenal HRM and linked this activity to hunger ratings obtained every 5 min. Both

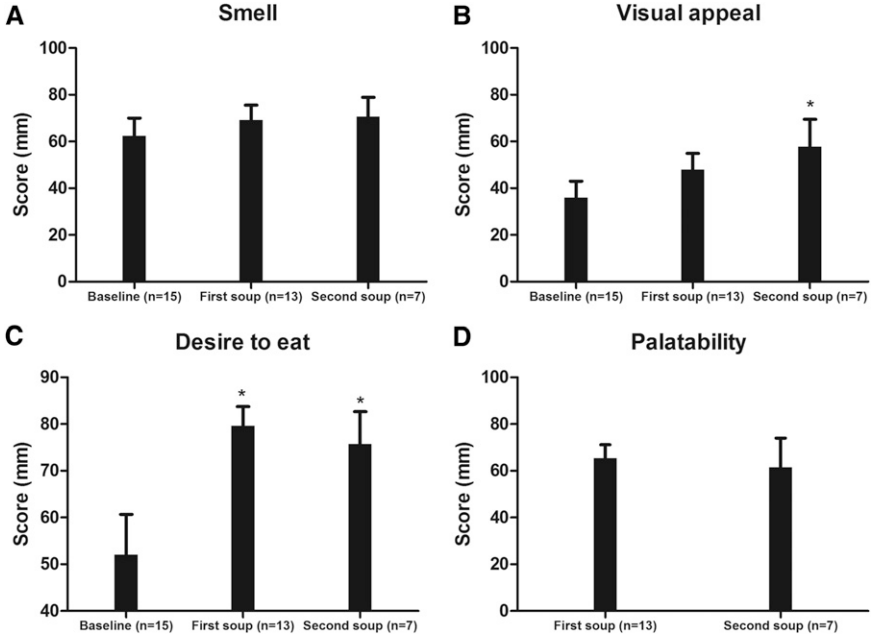

FIGURE 7 Assessment of the meal. The soup was scored for smell (A), visual appeal (B), desire to eat (C), and palatability (D) on a 10-cm visual analog scale. Data were analyzed with use of mixed-model analysis with either smell, visual appeal, desire to eat, or palatability as the dependent variable and intake (baseline, first soup, second soup) as the independent repeated variable. Data are represented as means ± SEMs. $*P < 0.05$ compared with baseline after Bonferroni correction.

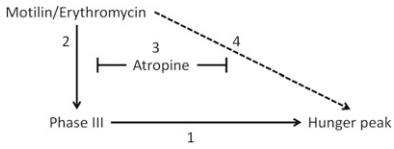

FIGURE 8 Schematic representation of the potential mechanism involved in the generation of hunger peaks (1). Physiologic measurement of the migrating motor complex showed a significant association between phase III and hunger peaks (2). Administration of erythromycin induced an antral phase III in all of the volunteers. This induced phase III was associated with a hunger peak (3). Pretreatment with atropine abolished the induction of phase III by erythromycin. Administration of erythromycin without the induction of phase III contractions did not induce hunger peaks (4). A possible direct cholinergic central effect of motilin on the induction of hunger peaks cannot be excluded from this study. Lines with arrow points indicate association between the 2 variables, and lines without arrow points indicate inhibition. The dotted line represents a secondary hypothesis.

antral and duodenal phase III contractions showed a significant association with hunger peaks in the this study. It has been reported previously that duodenal phase III contractions can be accompanied by a motilin plasma peak if they are preceded by intense gastric contractile activity (6). This is consistent with a recent study in which we identified motilin-induced phase III contractions as a hunger signal in humans (17). In our previous study, phase III contractions with a jejunal origin were not associated with hunger peaks, but the standard water-perfused manometry catheter we used assessed mainly antral and jejunal contractility, without detailed information on the duodenum (17). The current study shows that phase III contractions originating in the duodenum can also be associated with increased hunger sensations. The number of measured duodenal phase III contractions was too low, however, to differentiate between phase III contractions preceded by strong and weak gastric contractility.

We went on to demonstrate that administering the motilin receptor agonist erythromycin stimulates food intake and induces gastric phase III contractions (and consequently hunger peaks). Studies in rodents have shown that motilin administration can stimulate food intake (27, 28). However, these results need to be interpreted with caution because other studies reported that motilin and the motilin receptor exist only as pseudogenes in rodents and are therefore not functional (29, 30) Although the expression of motilin and the motilin receptor has been demonstrated in humans, studies on the effect of motilin on food intake in humans are lacking (13, 31, 32). In this study, we have shown that the effect of the motilin receptor agonist erythromycin on hunger is regulated via a cholinergic pathway because pretreatment with atropine abolished both the increase in hunger and the induction of phase III contractions. Previous studies already showed that erythromycin acts on gastric motility via 2 different pathways in humans: a low dose induces a premature phase III contraction through the activation of an intrinsic or vagal cholinergic pathway, whereas a high dose increases antral contractility via the activation of a muscular receptor (33). Whether this cholinergic pathway is directed via the vagus nerve, the myenteric plexus, or both has not yet been determined. Circumstantial evidence in both dogs and humans has shown that an intact vagus nerve seems necessary to stimulate phase III activity in the stomach, suggesting the presence of motilin receptors on the vagus nerve and the involvement of the vagus nerve in stimulating phase III activity (34–37). However, the only direct evidence to our knowledge for the presence of motilin receptors on the vagus nerve until now comes from in vitro *Suncus murinus* studies, in which motilin increased gastric vagal afferent fiber activity (38). *S. murinus* is a small animal model of interest for gastrointestinal research because it has an emetic reflex that is lacking in rodents. Furthermore, in contrast to rats and mice, it also expresses motilin (39). However, in the same species it has been shown that motilin induces phase III contractions independently of the vagus nerve via a myenteric cholinergic neural pathway (40, 41). It is unclear whether the administration of a low dose of erythromycin is still able to induce a phase III contraction in vagotomized patients. Studies in which erythromycin was administered as a prokinetic agent used a high dosage to improve gastroparesis after vagotomy (42, 43).

An association between plasma motilin levels and hunger through a direct hypothalamic effect cannot be excluded from this study, however. Another limitation of our study was the length of the catheter used to measure the antroduodenal motility. Phase III contractions that started in the jejunum could not be measured because of the limited length of the catheter. The full migration pattern of gastric and duodenal phase III contractions could also not be measured. In addition, the study design does not allow an exact analysis of causality or temporal sequence because hunger was not measured continuously like the gastrointestinal motility. The restriction of food intake to 2 low-caloric soup meals during the measurement is also a limitation because this may tempt the subjects to postpone food intake as long as possible to preserve the opportunity for eating at later time points. Future studies should address the impact of hunger peaks and phase III contractions on food intake that is unlimited in both time and size.

In conclusion, our results show that phase III contractions of the MMC in the stomach or proximal duodenum are associated with a hunger peak. The increase in hunger seems to be mediated via a cholinergic pathway because atropine pretreatment abolishes the induction of a hunger peak by erythromycin (**Figure 8**). Moreover, we have shown that food intake can be stimulated via erythromycin. It is therefore tempting to speculate that drugs that induce premature phase III contraction activity can be considered as therapeutic targets to stimulate food intake. Conversely, motilin receptor antagonists could be used to decrease food intake. Further research is needed to study whether eating disorders and obesity are associated with a distortion in the MMC or motilin plasma levels.

The authors' responsibilities were as follows—ED: conducted the research, analyzed the data, and wrote the manuscript; RV: provided essential material; PJ: provided essential material and wrote the manuscript; OVdB: designed the research and analyzed the data; LVO: designed the research, analyzed the data, and wrote the manuscript; ID: designed the research and wrote the manuscript; JT: designed the research, wrote the manuscript, and had primary responsibility for the final content; and all authors: read and approved the final version of the manuscript. ED was a Ph.D. fellow of the Fonds voor Wetenschappelijk Onderzoek at the time of the study. None of the other authors reported any conflicts of interest. The funders had no role in the design, implementation, analysis, or interpretation of the data.

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