Specific food structures suppress appetite through reduced gastric emptying rate

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Mackie AR, Rafiee H, Malcolm P, Salt L, van Aken G. Specific food structures suppress appetite through reduced gastric emptying rate. Am J Physiol Gastrointest Liver Physiol 304: G1038–G1043, 2013. First published April 11, 2013; doi:10.1152/ajpgi.00060.2013. —The aim of this study was to determine the extent to which gastric layering and retention of a meal could be used to reduce appetite using the same caloric load. Liquid (control) and semi-solid (active) meals were produced with the same protein, fat, carbohydrate, and mass. These were fed to 10 volunteers on separate days in a crossover study, and subjective appetite ratings, gastric contents, and plasma cholecystokinin (CCK) were assessed over a period of 3 h. The active meal showed food boluses in the stomach persisting for ~45 min, slower emptying rates, and lower plasma CCK levels over the first hour. After the first hour, both gastric emptying rates and plasma CCK levels were similar for both systems and slightly increased compared with the unfed situation. Despite the lower plasma CCK levels for the active meal over the first hour, this meal reduced appetite more than the control meal over the 3 h of the study. For a moderately increased caloric load, increased hunger and appetite. The use of emulsion systems with acid-stable emulsion also induced increased fullness and decreased hunger and appetite. The use of emulsion systems with tailored release properties is increasingly being considered to control hyperlipidemia (3, 24, 33).

These studies demonstrate the importance of food structure in the modulation of postprandial satiety-related physiology. Indeed, foods such as fresh whole fruits and vegetables, whole grain bread, and meat are digested more slowly and as a consequence are more satiating than foods that have a softer, more highly processed structure (25). However, a review of a cross section of these types of studies in which food structures have been developed to enhance satiety (35) shows that we are still a long way from fully understanding the complex processes of satiety, which involves physiological processes of the entire metabolism as well as psychological and social processes, although progress is being made in all these areas.

In this study, we have attempted to confirm that food structure alone can impact satiety and to determine the role of gastric retention and nutrient sensing as indicated by CCK secretion. The design of the meals in this study was based on the concept that gastric emptying is regulated by the caloric density of the chime delivered to the small intestine and the in
vitro observation (34) that liquid food emulsions can be designed to either form a creaming or sedimenting energy-rich phase in the stomach. This feature can be used to control the timing of energy release from the stomach, which in case of a sedimenting energy-rich layer would induce increased satiety compared with meals that remain homogeneous under gastric conditions. In the present study, we wanted to test this concept by comparing two isocaloric (same fat, protein, and carbohydrate content) meals to assess the impact of food structuring on gastric retention and short-term appetite regulation. Because we did not want to be dependent on the process of gastric acidification since the mechanism that would produce a sedimenting protein and fat-rich phase in the stomach, we constructed a sedimenting system in the form of a slurry of small, dense cheese particles. These cheese particles were suspended in yoghurt to form a thick semi-fluid. Detailed information about the dissolution of structures in the stomach and the volume of gastric contents were obtained by MRI, allowing the persistence of structure to be correlated with gastric flow rates, appetite, and CCK secretion.

**MATERIALS AND METHODS**

**The meals.** The two meals used in the study were prepared under food-grade conditions. The homogeneous meal referred to in this article as the control meal was made as follows. An emulsion was made comprising 27.5 g of sunflower oil and 242.5 g of 1.24% sodium caseinate solution in a blender (BL450 series, Kenwood). The shear cycle comprised 30 s at the low shear setting, 30 s of rest, 30 s at the high shear setting, 30 s of rest, and 30 s at high shear setting. The emulsion was then mixed with 199.5 g of a solution containing 1.24% sodium caseinate and 10% whey protein isolate (Bipro, Davisco). Sugar (6.1 g) was then added to the emulsion along with a few drops of vanilla flavoring. The emulsion was stored at 4°C until use (<24 h).

The structured meal referred to as the active meal was prepared by mixing 88 g of finely grated Gouda cheese (Waitrose Essential Dutch Gouda) and 73 g of low-fat yogurt (Waitrose Essential low-fat yogurt), both of which were purchased from the local supermarket. The meal was consumed with 339 ml of bottled water, which was stored with the cheese and yogurt mixture at 4°C until use (<24 h). The sodium content of the active meal was 64 mM based on a concentration in the cheese of 2.1%, whereas the sodium content in the control meal was 20 mM based on the protein content. Figures for the nutrient content of the meals are given in Table 1. As far as possible, the two meals were isocaloric, the only differences being the higher salt content and the large degree of proteolysis of the proteins introduced into the active meal with the cheese.

**Imaging of gastric contents.** The gastric contents of the volunteers were determined using a conventional 1.5-T magnetic resonance imaging (MRI) scanner (Siemens Avanto 1.5T). Imaging used a TRUFISP (fast imaging with steady-state precession) protocol developed to scan the stomach in a breath-hold of the order of 15–25 s, depending on the fullness of the stomach (repetition time (TR)/echo delay time (TE) 3.5/1.5 ms; field of view 24 × 32 cm; matrix 154 × 256; slice thickness 0.5 cm). This yields contiguous 5-mm axial slices through the stomach, enabling calculation of total stomach volume. Both transverse and coronal images were acquired to ensure that the gastric volume could be accurately defined. Total volumes of gastric contents (excluding gas) and the nature of layers formed as a result of sedimentation were determined at each time point using free-hand tracings of the region of interest around the stomach contents for each 5-mm-thick slice, and from this the total stomach volume was calculated using cardiac ventricular volume measurement software (Siemens Argus workstation). This involved assessment of the position of the pylorus. Each scan took ~5 min, and between scans the volunteers underwent minimal physical movement and remained seated upright close to the scanner. From the variation of the gastric volume with time, we deduced an apparent emptying rate, which gives and impression of, but is not precisely the same as, the rate at which the food emptied from the stomach, because of the inhomogeneous distribution of the food material inside the stomach and because of the simultaneous addition of gastric secretion.

**Visual analog scales.** We assessed volunteer satiety with a self-reported visual analog scale (VAS) technique (32). Before the meal and at specific time intervals post-meal, as given in Table 2, the volunteers completed a five-question satiety questionnaire with a VAS for each of the following questions: 1) “How hungry are you?”; 2) “How full do you feel?”; 3) “How satisfied do you feel?”; 4) “How big is your desire to eat?”; 5) “How thirsty are you?” The analog scores for each question were then converted to numeric scores based on the following: 1) 1 = not at all hungry, 10 = very hungry; 2) 1 = not full at all, 10 = very full; 3) 1 = not satisfied at all, 10 = very satisfied; 4) 1 = no desire to eat at all, 10 = very big desire to eat; 5) 1 = not thirsty at all, 10 = very thirsty.

**Determination of CCK.** At the start of each study session, volunteers were fitted with a cannula so that blood could be drawn periodically. At each required time point, 4 ml of blood was drawn and stored on ice for <2 h before being centrifuged. Blood was collected into tubes (Vacutainer K2 EDTA, Becton Dickenson) containing 170.9 μl of aprotinin (Sigma-Aldrich), and, after centrifugation for 10 min at 1,500 g and 4°C, the plasma was removed and stored in prelabelled tubes at −80°C. The plasma was subsequently analyzed for CCK content using a radio-immunooassay (RIA) (27, 28) performed by the TNO organization in The Netherlands.

**The study method.** The crossover study was designed to assess differences in gastric emptying, satiety indicators, and levels of the GI hormone CCK. The study included only male volunteers aged between 20 and 50 yr and with a body mass index (BMI) between 19 and 30. The mean age of the cohort was 35 yr, and the mean BMI was 24.7. All 10 volunteers recruited to the study were apparently healthy and provided written, informed consent before taking part in the study.

**Table 1. The nutritional composition of the two meals used in the study**

<table>
<thead>
<tr>
<th></th>
<th>Control Meal</th>
<th>Active Meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat, g</td>
<td>27.5</td>
<td>27.5</td>
</tr>
<tr>
<td>Carbohydrate, g</td>
<td>8.1</td>
<td>6.1</td>
</tr>
<tr>
<td>Protein, g</td>
<td>25.3</td>
<td>25.3</td>
</tr>
<tr>
<td>Total, g</td>
<td>58.9</td>
<td>58.9</td>
</tr>
<tr>
<td>Sodium, mM</td>
<td>20</td>
<td>64</td>
</tr>
<tr>
<td>Energy, kCal</td>
<td>373.1</td>
<td>373.4</td>
</tr>
<tr>
<td>Weight of meal, g</td>
<td>500</td>
<td>500</td>
</tr>
</tbody>
</table>

**Table 2. Timing of clinical activities relative to consumption of the meal (min)**

<table>
<thead>
<tr>
<th>Activity</th>
<th>MRI Scan</th>
<th>Blood Drawing</th>
<th>VAS Questionnaire</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>−15*</td>
<td>−10*</td>
<td>−5*</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>45</td>
<td>35</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>65</td>
<td>60</td>
<td>55</td>
</tr>
<tr>
<td>6</td>
<td>85</td>
<td>90</td>
<td>95</td>
</tr>
<tr>
<td>7</td>
<td>105</td>
<td>120</td>
<td>115</td>
</tr>
<tr>
<td>8</td>
<td>125</td>
<td>150</td>
<td>155</td>
</tr>
<tr>
<td>9</td>
<td>145</td>
<td>180</td>
<td>185</td>
</tr>
<tr>
<td>10</td>
<td>165</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Premeal was not considered to be critical, so these values are largely indicative.*
subsequent scans being undertaken as laid out in Table 2. The volunteers were asked to repeatedly complete a VAS satiety questionnaire and have a 4-ml sample of blood drawn, and the timing for these are given in Table 2.

Data analysis. SPSS for Windows software (SPSS for Windows, version 19.0) was used to analyze the data. The results are expressed as means and SE, with a P value of ≤0.05 (two-sided) as a criterion for the statistical significance. The statistical significance of the data was determined from differences in the areas under the curves using a paired two-sided t-test (1).

RESULTS

Satiety. The impact of the two meals on the scores for hunger and fullness are shown in Fig. 1, and the areas under the curves (AUC) summarized in Table 3. There is a significant difference in the hunger scores for the two meals, with the active meal reducing hunger more than the control meal at virtually every time point, although the slopes of the two curves are very similar. Statistical analysis of the AUC for the two data sets yielded a P value of 0.002 (Table 3). A similar pattern is seen for the fullness data, with the active meal eliciting a higher fullness score at every time point, although the difference was more pronounced over the first 45 min after ingestion. Interestingly, the liquid control meal gave the highest fullness score at the first point collected after the meal was consumed, whereas after the active meal the second time point was highest. Although this difference was not statistically significant, it may indicate a slightly slower onset of the sensation of fullness with the active meal. The VAS determination of satisfaction and desire to eat very much reflect the sensation of fullness with the active meal. The VAS thirst data would show a high value for the active meal. The VAS thirst data for the control meal fell into two different groups, as can be seen from the AUC data in Table 3. Given the composition of the active meal and the relatively high salt content, it might be expected that the VAS thirst data would show a high value for the active meal. However, the data in Fig. 1C shows consistently lower thirst scores for the active meal at every post-meal time point.

CCK modulation. The mean levels of CCK measured in the blood of the volunteers after consuming the two meals is shown in Fig. 2A. The data for both meals is an average for nine of the volunteers, since the data from the active meal for one was clearly an outlier (data not shown). It is clear that differences in the release of the hormone were only seen in the case of the control meal, there was a steep rise to a maximum of 1.6 pmol/l at 25 min post-ingestion. In fact, the liquid control meal gave the highest CCK concentration of 2.2 pmol/l and another with four

<table>
<thead>
<tr>
<th></th>
<th>Control Meal</th>
<th>Active Meal</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hunger</td>
<td>6,187 ± 697</td>
<td>4,477 ± 592</td>
<td>0.002053</td>
</tr>
<tr>
<td>Fullness</td>
<td>4,345 ± 627</td>
<td>6,538 ± 635</td>
<td>0.004911</td>
</tr>
<tr>
<td>Satisfaction</td>
<td>4,616 ± 711</td>
<td>6,759 ± 647</td>
<td>0.00881</td>
</tr>
<tr>
<td>Desire to eat</td>
<td>6,561 ± 685</td>
<td>4,787 ± 701</td>
<td>0.007301</td>
</tr>
<tr>
<td>Thirst</td>
<td>7,518 ± 358</td>
<td>5,598 ± 659</td>
<td>0.001162</td>
</tr>
</tbody>
</table>

P values shown are based on a paired, two tailed t-test.
volunteers where all the values were close to 1 pmol/l over the first 2 h. The data from both control groups was significantly different from that for the active meal over the first 25 min ($P = 0.029$, paired, two-sided $t$-test). The CCK levels generated by the active meal were significantly lower than the control meal for at least the first 40 min post-ingestion.

Gastric emptying and layering. The data shown above has been governed to a large extent by the behavior of the two meals in the gastric compartment. To assess this, we have used MRI as illustrated in Fig. 3. Figure 3A shows the active meal in the stomach 5 min after ingestion, and individual boluses of cheese are clearly visible as dark regions in a fairly homogeneous surrounding medium. The other image in the figure shows the control meal in the same volunteer some 25 min after consumption. In this image, layering of the gastric contents is clearly visible as the fat in the control meal starts to cream, leaving a darker, higher fat content region as an upper layer. We have measured the time over which these structures formed and persisted.

The structures in Fig. 3 reflect the heterogeneous and changing nature of the gastric contents and highlight the challenges in interpreting gastric retention times. The structures seen in Fig. 3A were persistent over the first 45 min after ingestion, and this time scale is typical of what was seen in the volunteers more generally. The layering seen in Fig. 3B tended to be persistent for rather longer and was typically still visible up to 105 min after ingestion. In addition to assessing intra-gastric layering, we measured mean gastric volume over the time course of the study, and the data are given in Fig. 4. After ingestion, there was little difference in the volume of gastric contents between the two meals for the first 25 min. The active meal showed a slightly higher volume at 5 min, but this was not statistically significant. The active meal also emptied slightly faster over the first 25 min, but again this was not statistically significant. Between 25 and 45 min, the two meals presented markedly different emptying rates, with the control meal emptying more than twice as fast as the active meal ($P = 0.0058$, paired, two-tailed $t$-test). From 60 min onward, the emptying rates of the two meals were very similar at $\sim$2 ml/min, showing that the cause of the 30-min difference in gastric half-times of 69 min and 100 min for the control meal and active meal, respectively, was behavior in the first 45 min after ingestion.

DISCUSSION

In the study described in this article, we have used two iso-caloric meals with different structures, semi-solid vs. liquid, to assess the impact of food structure on gastric emptying rates and short-term perception of appetite. We have used MRI to assess not just gastric volume but also the persistence of macroscopic structure in the gastric compartment, any layering formed, and its subsequent persistence. The active meal had a gastric retention half-time 30 min longer than the control meal. Although this seems to be in disagreement with a study showing that a mixed solid/liquid food empties faster and is less satiating than the same meal after homogenization to a “soup” (22), in that case the authors suggested that the mixed solid/liquid system initially emptied the liquid portion, which had a low energy density and in this way more quickly reduced the gastric volume and hence the sensation of fullness. Another study demonstrated faster gastric emptying when the viscosity of a meal was increased by adding pectin (30). However, in a study comparing liquid and semi-solid meals, the latter was more satiating, even though there was no difference in CCK-8 or GLP-1 secretion (36). It has also been shown that the detection of fat in the duodenum can significantly reduce hunger, increase fullness, and delay gastric emptying (20).

There are a number of feedback mechanisms that control gastric emptying, but one of the important ones involves the
peptide hormone CCK. Detection of nutrients in the proximal small intestine by I-cells leads to the release of CCK, which plays a key role in regulating a range of intestinal responses that integrate and optimize the digestion of fat and protein (15). CCK is released in response to nutrients in the duodenum, with fat and protein producing a greater postprandial release than carbohydrates (13). This regulation includes three physiological effects of threshold levels of plasma CCK, which are a stimulation of pancreatic secretion through relaxation of the sphincter of Oddi; gall bladder emptying through contraction of the gall bladder and a modulating effect on gastric emptying (7–9, 15, 17, 31). Normally, following ingestion of a meal, CCK levels rise rapidly from a resting value of ~1 pmol/l to a peak of 6–8 pmol/l during the first 15 min and then decline to a submaximal level, which is maintained for up to 2 h after eating (16, 17, 26). This peak in plasma CCK is responsible for gall bladder contraction (7). In the present study, the observed effect of the meals on CCK blood levels was smaller than typically observed for liquid emulsions (e.g., peaking to 8 pmol/l). This might be due to the relatively high viscosity of both meals in this study, which impeded the initial fast emptying found for thin liquids. The role of plasma CCK concentrations on inhibition of gastric motility and emptying seems more complex. CCK probably stimulates the mechanoreceptors in the gastric wall that signal gastric distension, which through the enteric nervous systems stimulates neurons that ultimately lead to a relaxation of the gastric fundus, a reduced motor activity of the antrum, and a reduced gastric emptying rate (14). The same type of effect is also induced by osmotic pressures in the duodenum (typically caused by sugars) and acidity (from the chime), independent of the CCK regulation (5–7).

Most significantly, in the present study, the main difference in emptying rates occurred up to ~50 min after ingestion, when the apparent emptying rate of the control meal was more than twice that for the active meal. This coincides with the time over which semi-solid parts of the active meal were seen to persist. This study paves the way for further work to assess the role of nutrient-controlled gastric emptying, extending over the whole time of gastric emptying. Although the greater feeling of fullness persisted for the duration of the study, the differences seen after 45 min were much reduced and only just statistically significant. As discussed above, CCK works through activation of vagal afferent mechanoreceptors in the stomach and in the duodenum. Consequently, the satiating effect of gastric distension increases the anorectic effects of CCK in humans (14).

In summary, in the study reported here, the more structured active meal suppressed the initial secretion of CCK compared with the liquid control meal, presumably because, for the active meal, initially a more viscous layer containing food boluses was present in the antrum, preventing significant early emptying. Thus a larger volume was retained longer in the stomach, leading to an increased sense of fullness. Altogether, this suggests that a nutrient-induced increase in serum CCK levels did not have a direct role in the control of appetite sensation in the active meal. In contrast, the liquid meal showed a peak in both emptying rate and plasma CCK at 30 min, related to the initial quick emptying of this meal. This is the first time that macroscopic structure persistence and formation have been linked to satiety via gastric retention and CCK secretion. The results suggest that, for the studied situation in which the plasma CCK level is moderately increased during nutrient-controlled gastric emptying, gastric retention was the key factor in decreasing appetite rather than the detection of nutrients in the duodenum and that plasma CCK was not directly linked to suppression of either gastric emptying or appetite. This study paves the way for further work to assess the role of other GI hormones and the impact of even more persistent structures.

**GRANTS**

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).
AUTHOR CONTRIBUTIONS

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