Specific food structures suppress appetite through reduced gastric emptying rate.

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Running title (<45 chars): Appetite suppressed by increased gastric retention.

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Abstract

Scope: 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.

Method and results: Liquid (Control) and semi-solid (Active) meals were produced with the same protein, fat, carbohydrate and mass. These were fed to ten volunteers on separate days in a crossover study and subjective appetite ratings, gastric contents and plasma CCK were assessed over a period of three hours. The Active meal showed food boluses in the stomach persisting for around 45 minutes, 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 three hours of the study.

Conclusions: For a moderately increased plasma CCK level in the fed state appetite was correlated with the volume of gastric contents rather than gastric emptying rates or plasma CCK. This suggests that enhanced gastric retention was the key factor in decreasing appetite and was probably mediated by a combination of intestinal nutrient sensing and increased viscosity in the stomach.
1.0 Introduction

Over the last 30 years there has been a steady rise in obesity and related health issues, such as diabetes. Type 2 diabetes and obesity are now major public health problems, with over 50% of the adult population being overweight and 1 in 20 having diabetes in the UK. Type 2 diabetes is associated with weight gain and both are associated with aging. A range of approaches have been used to address the increase in obesity based around dieting but the continued increase in the prevalence of obesity suggest that a greater understanding of the mechanisms involved in controlling appetite and satiety is needed. Nutrition studies have started to look in detail at how specific macroscopic food structures are broken down in the GI tract and how they release their nutrients. Such studies have shown that one of the major factors influencing nutrient release is the control of GI flow. In this regard nutrient sensing in the small intestine has also been shown to be important and acts through the release of GI hormones such as CCK, GLP-1 and PYY. Studies looking at the sensing of specific nutrients such as protein and lipids have shown that intra-duodenal protein or fat can contribute to the suppression of energy intake. Thus understanding the rules that link food structure to GI flow, nutrient sensing and satiety responses will be of primary importance in designing foods that are acceptable to the consumer but that provide the required physiological responses for maintaining health.

A number of studies have investigated the effect of food structure on gastric emptying and appetite regulation. At the most basic level, simple protein solutions have been assessed for their ability to affect gastric retention. In a study using dairy proteins, whey was compared with casein and cross-linked casein. Both casein and whey were more potent in lowering postprandial glucose than the cross-linked protein, which had a less pronounced peak in insulin and significantly lower cholecystokinin (CCK) release. Clemente et al. studied the effects of structure on nutrient release for a range of dairy based foods, showing that Mozzarella cheese was able to delay the peak in plasma triglycerides in comparison to milk. They also demonstrated a significantly faster reduction in antral volume as assessed by ultrasound, which may however not be directly related to a reduced gastric emptying time as suggested by the authors. Lipids form another group of macro nutrients that affect GI flow, and of particular interest for this are systems used to control the release of lipids. In a study comparing stable and unstable emulsions, gastric emptying was slower for an acid-stable emulsion, although the rate of energy delivery to the duodenum was not different up to 2 hours. The 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 in order to control hyperlipidaemia.

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. 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 process 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 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 to 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 in order 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 as 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.

2.0 Materials and Methods

2.1 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 was made as follows: An emulsion was made
comprising 27.5 g sunflower oil and 242.5 g of 1.24% sodium caseinate solution in a blender
(BL450 series, Kenwood UK). The shear cycle comprised 30 s at the low shear setting, 30 s rest,
30 s at the high shear setting, 30 s rest, 30 s 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 USA). 6.1g of sugar was then added to the emulsion along with a few drops of vanilla
flavouring. The emulsion was stored at 4 °C until use (< 24hours). The structured meal referred to
as the Active was prepared by mixing 88g of finely grated Gouda cheese (Waitrose Essential
Dutch Gouda) and 73g of low fat yoghurt (Waitrose Essential Low Fat Yoghurt), 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 yoghurt mixture at 4 °C until use (< 24 hours). 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 20mM 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 with the cheese.

2.2 Imaging of gastric contents
The gastric contents' of the volunteers was determined using a conventional 1.5T 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 15-25s depending on the fullness of the stomach (TR/TE 3.5/1.5ms, Field of view 24x32cm, matrix 154x256, slice thickness 0.5cm). This yields contiguous 5mm axial slices through the stomach enabling calculation of total stomach volume. Both transverse and coronal images were acquired in order 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 freehand tracings of the region of interest around the stomach contents for each 5mm 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 about 5 minutes 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.

2.3 Visual analogue scales
We assessed volunteer satiety with a self-reported visual analogue scale 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 visual-analogue scale (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 analogue 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".

2.4 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 4ml of blood was drawn and stored on ice for less than two hours before being centrifuged. Blood was collected into tubes (Vacutainer K2 EDTA, Becton Dickenson, USA) containing 170.9 µl of aprotinin (Sigma-Aldrich, UK) and after centrifugation for 10 minutes at 1500 x g and 4 °C the plasma was removed and stored in pre-
labelled tubes at -80 °C. The plasma was subsequently analysed for CCK content using a radio-immunoassay (RIA) (27, 28) performed by the TNO organisation in the Netherlands.

2.5 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 and with a BMI between 19 and 30. The mean age of the cohort was 35 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, which was approved by the local research ethics committee (Approval 11/EE/0192). Each volunteer attended the study centre on two occasions, at least 7 days apart consuming a different meal on each occasion. The order in which the meals were consumed was randomly allocated. All volunteers were able consume all of the test meals within 5 minutes.

On each study day volunteers were asked to eat their breakfast at home (before 09:00). They were allowed to drink as much water as they need but only until 10am. After this time no further consumption was allowed. The experimental protocol was started at between 13:30 and 14:00, which corresponds to the first time point in Table 2. After initial formalities each volunteer had a cannula inserted into an arm ready for blood drawing. They then underwent the first MRI scan, a 4 ml sample of blood was drawn and they were asked to complete a VAS questionnaire (baseline measurements). The volunteer consumed the meal, allocated at random. Immediately after the meal has been consumed the second MRI scan was performed with 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.

2.6 Data analysis

SPSS for Windows software (SPSS for WINDOWS, version 19.0, USA) were used to analyse the data. The results are expressed as mean and standard error of the mean (SEM) with a value $P \leq 0.05$ (2-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).

3 Results

3.1 Satiety

The impact of the two meals on the scores for hunger and fullness are shown in Figure 1 and the areas under the curves summarised 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 areas under the curves (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 minutes 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 data in Figure 1a and b 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 Figure 1c shows consistently lower thirst scores for the Active meal at every post meal time point.

3.2 Cholecystokinin modulation

The mean levels of CCK measured in the blood of the volunteers after consuming the two meals is shown in Figure 2a. The data for both meals is an average for 9 of the volunteers as 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 first hour after consumption of the meal. After the first hour serum CCK levels were increased from 0.6 pmol/L in the unfed state to about 1 pmol/L during gastric emptying for both meals. In the case of the control meal there was a steep rise to a maximum of 1.6 pmol/L at 25 minutes post ingestion. In fact the data for the Control meal fell into two different groups as shown in Figure 2b. One group of 5 volunteers with a maximum in CCK concentration of 2.2 pmol/L and another with 4 volunteers where all the values were close to 1 pmol/L over the first two hours. The data from both Control groups was significantly different from that for the Active meal over the first 25 minutes (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 minutes post ingestion.

3.3 Gastric emptying and layering

The data shown above has been governed to a large extent by the behaviour of the two meals in the gastric compartment. In order to assess this we have used MRI as illustrated in Figure 3. Figure 3a shows the Active meal in the stomach 5 minutes after ingestion and individual boluses of cheese are clearly visible as dark regions in a fairly homogenous surrounding medium. The other image in the figure shows the Control meal in the same volunteer some 25 minutes 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 Figure 3 reflect the heterogeneous and changing nature of the gastric contents and highlight the challenges in interpreting gastric retention times. The structures seen in Figure 3a were persistent over the first 45 minutes after ingestion and this timescale is typical of what was seen in the volunteers more generally. The layering seen in Figure 3b tended to be persistent for
rather longer and was typically still visible up to 105 minutes after ingestion. In addition to assessing intra-gastric layering, we measured mean gastric volume over the time course of the study and the data is given in Figure 4. After ingestion, there was little difference in the volume of gastric contents between the two meals for the first 25 minutes. The Active meal showed a slightly higher volume at 5 minutes but this was not statistically significant. The Active also emptied slightly faster over the first 25 minutes but again this was not statistically significant. Between 25 and 45 minutes the two meals presented markedly different emptying rates, with the Control meal emptying more than twice as fast as the Active (p=0.0058, paired two tailed T-test). From 60 minutes onward, the emptying rates of the two meals were very similar at circa 2 ml/minute, showing that the cause of the 30 minute difference in gastric half times of 69 minutes and 100 minutes for the Control and Active respectively, was behaviour in the first 45 minutes after ingestion.

4.0 Discussion

In the study described in this article we have used two iso-caloric meals with different structures, semi-solid versus liquid to assess the impact of food structure on gastric emptying rates and short term perception of appetite. We have used MRI in order 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 minutes longer than the Control meal. Whilst 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 homogenisation 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, 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 gallbladder and a modulating effect on gastric emptying (7-9, 15, 17, 31). Normally, following ingestion of a meal, cholecystokininin levels
rise rapidly from a resting value of about 1 pmol/L to a peak of 6 to 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 current 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 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 about
50 minutes after ingestion, when the apparent emptying rate of the control meal was more than
twice that for the Active. This coincides with the time over which semi-solid parts of the Active meal
were seen to persist. Over this same time period the rate of CCK secretion was significantly
suppressed relative to the Control meal, apparently related to a reduced release of energy in the
form of protein and fat during this time period. In fact, the observed steady state emptying rate of
about 2 ml/min for both meals after 60 min translates to an emptying rate of about 1.5 kcals/min,
which is typical for energy-controlled gastric emptying. Thus it seems that the body was better able
to control the initial emptying rate of the Active at an appropriate energy release rate than the
Control. This might be because the less viscous Control had already emptied by a substantial
amount before CCK-regulated control of the emptying rate became fully effective. This would also
explain why the Active meal also generated a significantly higher feeling of fullness over the same
period: since plasma CCK levels were similarly elevated in both cases, the remaining volume in the
stomach would have been sensed by the distension mechanoreceptors, signalling more fullness
for the Active meal compared to the Control, 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 minutes were much reduced and only just statistically significant. As discussed above,
CCK works through activation of vagal afferent mechanosensors 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 in comparison to the liquid Control, 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 minutes, 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.

Acknowledgements

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References


Figure Legends

Figure 1. VAS score data for (a) hunger (b) fullness and (c) thirst for the control (dashed line) and active
(solid line) meals. Mean and standard error values are shown.

Figure 2. (a) Blood plasma concentrations of CCK measured before and after consumption of the Control
(dashed line) and Active (solid line) meals. (b) Two groups of controls, those showing a significant increase in
CCK at 30 min (n=5, dashed line) and those that did not (n=4, solid line). Mean and standard error values
are shown in both graphs.

Figure 3. MRI images of (a) the active meal in the stomach (outlined) 5 minutes after consumption and (b)
the control meal in the stomach (outlined) 25 minutes after consumption.

Figure 4. Mean volumes of gastric contents (a) as a function of time before and after consumption of the
Control (dashed line) and Active (solid line) meals. The gastric emptying rates (derivatives) calculated from
the individual volume data are also shown (b). The data represent the Mean and standard error values.
Table 1. The nutritional composition of the two meals used in the study

<table>
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<td>Fat (g)</td>
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<td>Carbohydrate (g)</td>
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<td>Protein (g)</td>
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<td>Total (g)</td>
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<td>Sodium (mM)</td>
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<td>64</td>
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<td>Energy (kCals)</td>
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<td>373.4</td>
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<td>Weight of meal (g)</td>
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Table 2. Timing of clinical activities relative to consumption of the meal (minutes)

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<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>
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<td>3</td>
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</tr>
<tr>
<td>10</td>
<td>165</td>
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* pre-meal was not considered to be critical so these values are largely indicative.

Table 3. Mean areas under the curve +/- standard error of the VAS data collected for the control and active meals. The P-values shown are based on a paired, two tailed T-test.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Active</th>
<th>P value</th>
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<tbody>
<tr>
<td>Hunger</td>
<td>6187 ± 697</td>
<td>4477 ± 592</td>
<td>0.002053</td>
</tr>
<tr>
<td>Fullness</td>
<td>4345 ± 627</td>
<td>6538 ± 635</td>
<td>0.004911</td>
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<tr>
<td>Satisfaction</td>
<td>4616 ± 711</td>
<td>6759 ± 647</td>
<td>0.00881</td>
</tr>
<tr>
<td>Desire to eat</td>
<td>6561 ± 685</td>
<td>4787 ± 701</td>
<td>0.007301</td>
</tr>
<tr>
<td>Thirst</td>
<td>7518 ± 358</td>
<td>5598 ± 659</td>
<td>0.001126</td>
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