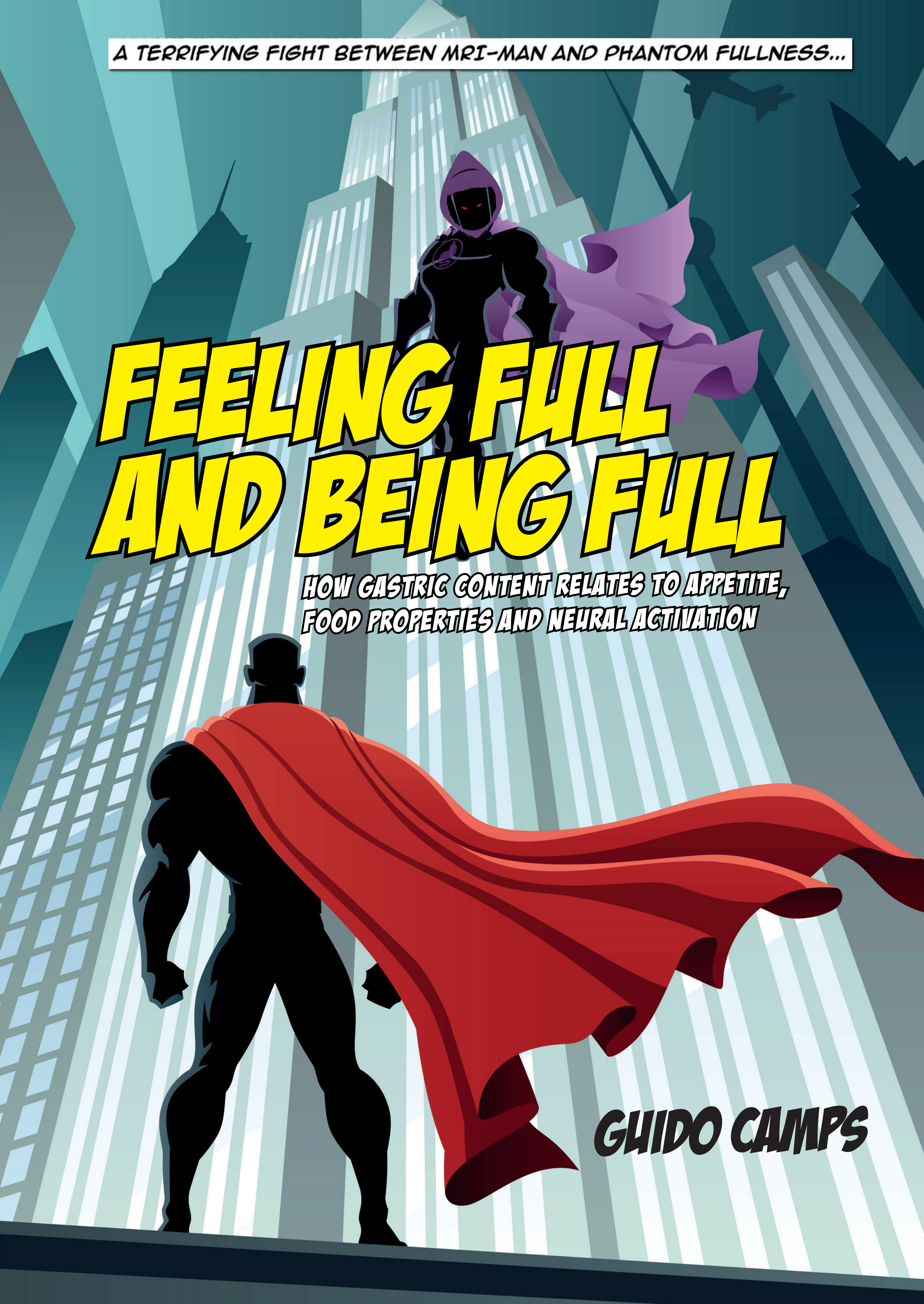
A TERRIFYING FIGHT BETWEEN MRI-MAN AND PHANTOM FULLNESS...



# FEELING FULL AND BEING FULL

## HOW GASTRIC CONTENT RELATES TO APPETITE, FOOD PROPERTIES AND NEURAL ACTIVATION

GUIDO CAMPS

# Feeling full and being full

how gastric content relates to appetite, food properties and neural activation

Guido Camps

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# Feeling full and being full

## how gastric content relates to appetite, food properties and neural activation

Guido Camps

**Thesis**

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# Chapter 1.

Introduction

*"When everyone had eaten as much as they could, the remains of the food faded from the plates, leaving them sparkling clean as before. A moment later the desserts appeared. Blocks of ice cream in every flavour you could think of, apple pies, treacle tarts, chocolate eclairs and jam doughnuts, trifle, strawberries, Jell-O, rice pudding -- As Harry helped himself to a treacle tart..."*

Chapter 7 – The Sorting Hat

Harry Potter and the Philosopher's Stone – J.K. Rowling

## Introduction

Not everyone merely eats to live. Some, including the author of this thesis, may sometimes feel as though they live to eat. Harry Potter is enjoying his first Hogwarts Feast, and after "*everyone had eaten as much as they could*" paradoxically the protagonists of the novel start to eat dessert. Food is consumed and enjoyed, and sometimes to such an extent that we may refer to it as overconsumption. Further developing our knowledge on ingestion and digestion may help us to understand why people eat what they eat, something which will only become more important considering the trends in consumption and obesity.

Harry Potter serving himself a treacle tart even though he is completely sated is but one recognizable example of the complex link between subjective and physiological fullness: our stomach may be filled by a large meal, but our satiation or desire to eat may not follow the state or one's stomach. Apparently our brain is not simply encoding the input from the stomach, there must be more to the story than this: appetite feelings apparently do not always accurately reflect the state of our stomach. Understanding the relation between feeling full and actually being full was studied in this thesis. In order to answer this question we must answer what is meant by *being full*?

We could measure when people stop eating, but as Read[1] so eloquently stated in 1992, we hardly listen to our physiology as there is "*a plethora of social and conditioning factors to influence what we eat.*" Instead of measuring intake, people can be asked how hungry or how full they are. To further explain the advantages and disadvantages of these appetite ratings, a short review of the literature on this subject will be given.

But ratings may only explain subjective experiences of fullness. In order to be able to compare the experience of fullness to an objective measure such as gastric content measurements, we will discuss insights about Magnetic Resonance Imaging (MRI) of the stomach.

Both appetite feelings and gastric sensations must somehow be encoded and associated in the brain. To relate these subjective and objective measures of fullness to one another functional MRI can be used. Therefore a short insight into how the brain can be measured with fMRI will be given. After these methodological parts, a selection of the current insights in literature in the area of food properties and food matrix effects on satiation and gastric emptying will be described. After this, insights in brain activity in combination with gastric distention and emptying will be reviewed, concluding with current gaps in the knowledge and the aim of this thesis. Lastly the individual chapters within this dissertation will be introduced, including their relationship to the overall aim of the thesis.

**Appetite**

Following the satiety cascade[2], the process of eating behavior can be separated into appetite sensations resulting in intake and the process of eating a meal, and the sated period following the meal[3,4]. In this cascade 'satiation' pertains to the process resulting in meal termination: when do we stop eating during a meal. 'Satiety' meaning the ensuing state in which new intake is not desired until the following meal.

Questionnaire scores are an often used measure to quantify appetite feelings. Research of appetite and eating behavior has led to standardization of the appetite questions asked[5]. Participants are asked to rate their hunger, fullness, desire to eat, the amount they could eat and thirst by marking their response on a 100-mm visual analogue sale

(**Figure 1**). The distance from the origin to the mark is measured, yielding a score ranging from 0 to a 100.

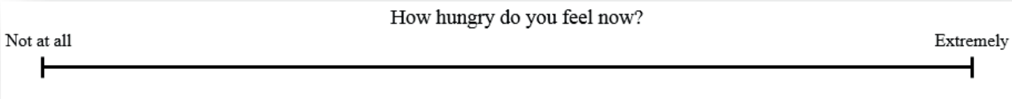


Figure 1. Example of a visual analogue scale (VAS) on which the participant is asked to mark the line where it intuitively corresponds to the feeling at that moment.

Alternatively, mostly in the medical field, participants are asked to score their feelings orally with a number going from zero to a hundred[6]. Subjective appetite ratings have shown to be reproducible and predictive of food intake[7-9]. However, there has also been work showing that rated appetite and actual intake may differ[10].
Therefore, it should be kept in mind that food intake may not reflect appetite feelings because food intake itself is influenced by many processes and cognitive cues. We may look for other biomarkers of appetite, including neural activation, to investigate appetite[11]. When it comes to the answer of '*how full do you currently feel?*', visualizing the stomach with MRI allows direct insight in how full the stomach actually is.

## Measuring the stomach

### *General*

After putting food into the mouth, it is chewed, swallowed and moved via the oesophagus to the stomach. Gastric emptying is the process of controlled release of chyme from the stomach into the duodenum.

Measuring gastric emptying can be done either by a direct measurement or by an indirect measurement. Direct measurements include echography, scintigraphy and MRI, indirect measurements methods include blood acetaminophen and stable isotope breath testing[12]. The advantage of direct measurement methods are that they allow real-time visualisation and determination of gastric content. An advantage of MRI over scintigraphy is that it does not require any radioactive tracer and that there are no known risks associated with its use.

*How does MRI work*

MRI uses the magnetic properties of protons and their distribution through tissue to produce detailed images. By placing a body in a strong magnetic field, such as an MRI scanner, the protons within the body align with the field. By using a radio frequency pulse at the correct frequency, certain protons are realigned absorbing energy from the radio frequency pulse. When the radio frequency pulse is stopped, these protons re-emit this energy. The signal based on this re-emission varies on the basis of tissue properties. This signal is captured with a coil and used to create images. The variation in the signal can be used to distinguish tissues[13].

*Gastric MRI measurement*

MRI has been validated as a method to measure gastric emptying[14-16]. Because food also contains protons, food can be visualized with a MRI scanner. This allows cross-sectional images of the body at the height of the stomach showing both tissue from the body as well intragastric food (**Figure 2**), making it feasible to discriminate the gastric content from the gastric lining and the surrounding tissue.

Figure 2. The author putting the body coil on a participant in the MRI scanner used for data acquisition in this dissertation.

These images can be used to manually delineate gastric content on every slice, which may include gastric secretions caused by ingestion[17]. For each time point, total gastric volume can be calculated by multiplying surface area of gastric content per slice with slice thickness, including gap distance, summed over the total slices showing gastric content (**Figure 3**).

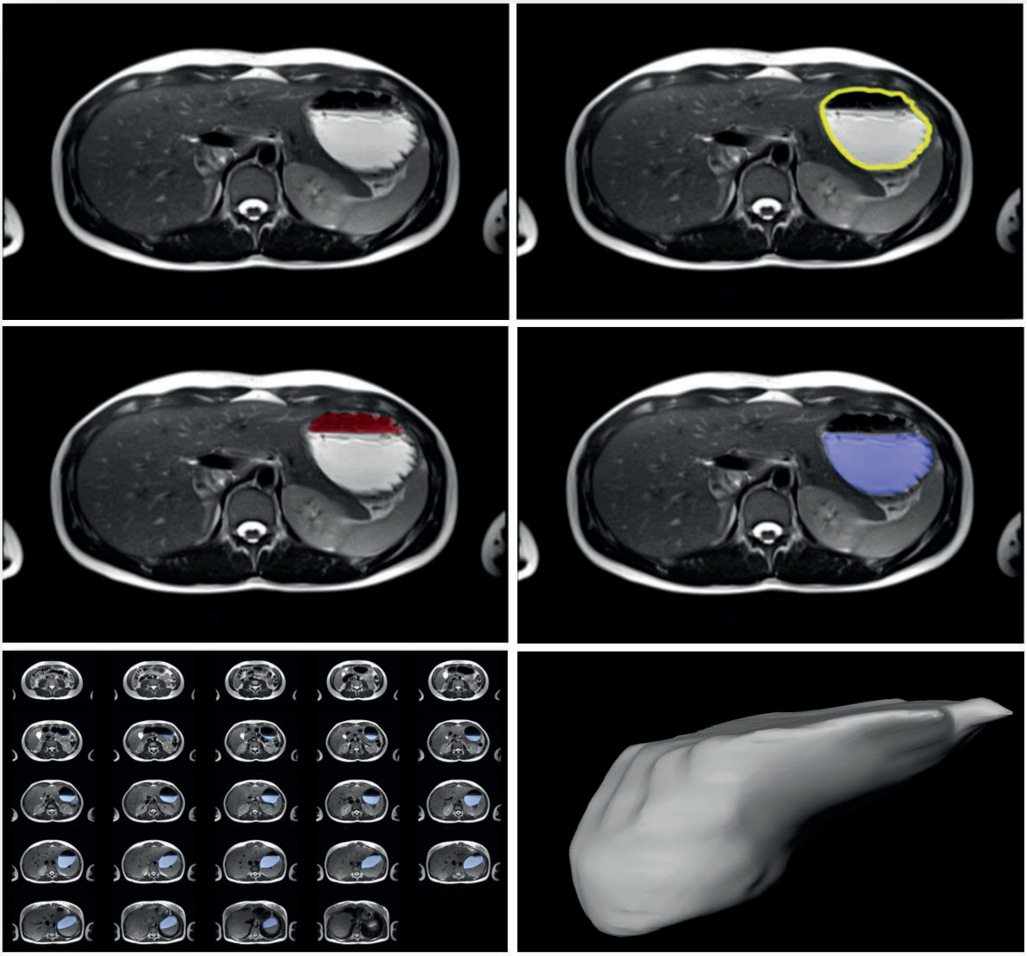

Figure 3. Top left: example of a cross sectional slice in which parts of the arms can be seen and the thorax in the middle. The liver can be seen on the left and the spine in the lower middle. On the bottom right the spleen is visible and above that the stomach. Top right: the gastric wall delineated in yellow. Middle left: air in the stomach marked in red. Middle right: fluid content in the stomach marked in blue. Bottom left: one full scan with all slices (one scan takes approximately 17 seconds) of a participant at a single time point. Gastric content is marked in blue. Combining all slices can be used to calculate content in mL. Bottom right: 3D rendering of the content based on the delineated slices[18].

Multiple measurements of gastric content at single time points can be plotted over time. On the basis of previous literature emptying curves have been determined. This allows a gastric emptying curve to be fitted to the measurements from individual participants[19,20](**Figure 4**). From this curve we can derive the emptying half time as an outcome measure for gastric emptying speed for any given treatment. Additionally, we may use the MRI determined mL gastric content at a specific time point as a covariate or reference point for other concurrent measurements.

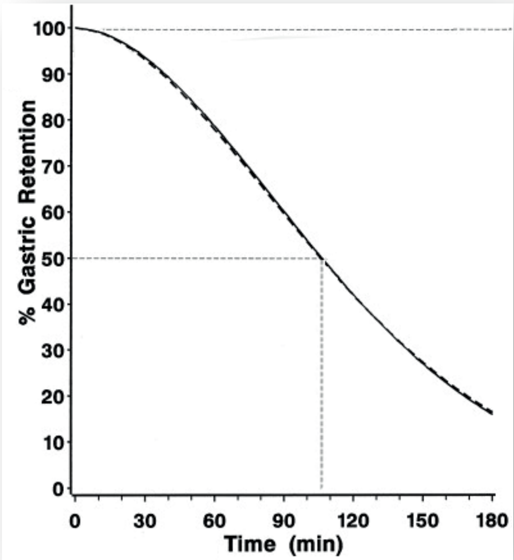


Figure 4. A theoretical curve which can be fitted to the data to be used to derive gastric emptying half time

## Measuring the brain

When it comes to unravelling how the different sensory inputs are translated into the appetite questionnaire responses and actions of research participants, somehow the answer must lie in the brain. Studying the brain is challenging, and many methods are used (e.g. positron emission tomography (PET), electro-encephalography (EEG)), all with different temporal and spatial resolution. Overall, functional Magnetic Resonance Imaging (fMRI) currently seems to be the best tool for understanding how the brain functions[21] (**Figure 5**). In the previous section, MRI was introduced to visualize anatomical structure (in that particular case the stomach).


Figure 5. A participant lying in the scanner just previous to undergoing a fMRI scan

Perfusion fMRI measurements provide a proxy of neural activity. The term 'fMRI' is often used in popular scientific articles with the means of the more specific blood oxygenation level dependent fMRI (BOLD fMRI). BOLD fMRI is a method of acquiring fMRI data which has many examples in nutrition research[22-25]. BOLD fMRI works on the principle that at the site of neuronal firing, increased local blood flow leads to a decreased concentration of deoxygenated haemoglobin, which creates localized distortions of the magnetic field, resulting a stronger fMRI signal. Though ubiquitous in the literature, BOLD is most suited to event-based experiments, with high repetition of a short stimulus such as repeated viewing of pictures[26]. When it comes to measuring longer term changes, there is a more suitable fMRI alternative to BOLD, namely arterial spin labelling (ASL) or perfusion fMRI. Perfusion fMRI uses the magnetic field to magnetically label tissue (water) by changing the molecular spin of the proton. By labelling specifically at the height of the neck, the main arteries which supply blood to the brain are included in the labelling slab. Tag and control images are made of the brain, in which a subtraction between the two allows for a calculation of the amount of labelled blood which has travelled from the neck to the brain[27](**Figure 6**). This allows absolute calculation of the perfusion per voxel within the brain. These values can be used to compare how food research interventions lead to perfusion changes within the brain over longer periods of time[28].

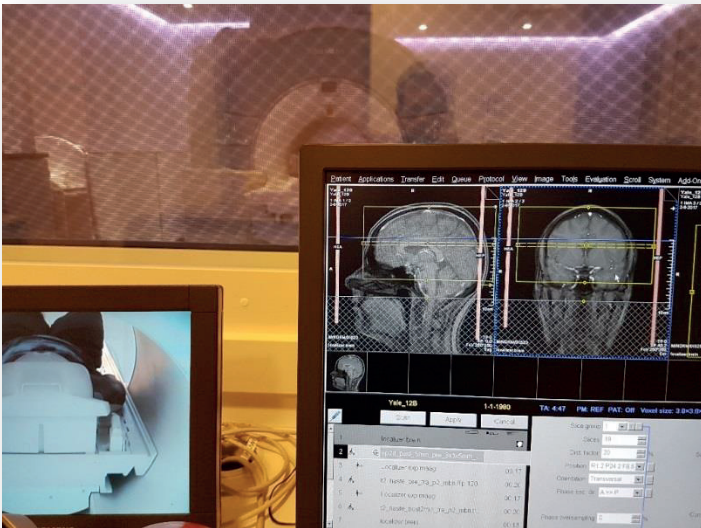


Figure 6. The terminal operating the scanner. The scanner can be seen through the glass, camera feed of the participant can be seen in the lower left corner.

## Satiation, food properties and the digestive tract

*Segmentation of the digestive tract*

The digestive tract can be divided on a physiological basis. In the image below a simple segmentation between the brain, the oral cavity, the stomach and the small and large intestine is shown (**Figure 7**).

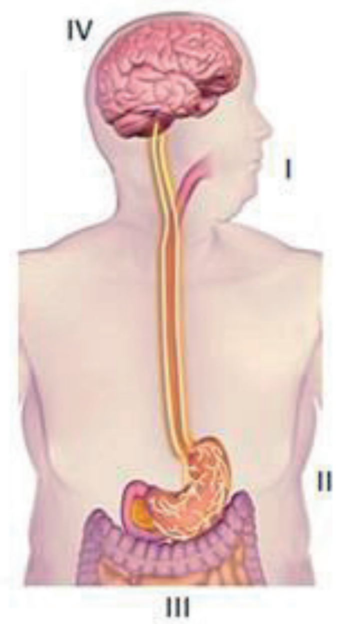


Figure 7. Simple segmentation of the structures relating to the digestive system: the oral cavity (I) and nose, the stomach (II), the small and large intestine (III) and the brain (IV)

Orosensory exposure to food (I) affects appetite sensations resulting in intake[29]. Food travels from the mouth to the stomach (II). Volume, energy density, viscosity, macronutrient content and other food matrix effects may infuence the time food spends in the stomach[30-32]. This gastric transit time, or gastric emptying time, is thought to be negatively associated with satiation (meal termination) and enhanced satiety[33]. Further digestion food happens in the small and large intestine (III). Hormonal effects within the digestive tract include ghrelin, implicated in mealtime hunger and meal initiation. Upper-intestinal satiation is associated with cholecystokinin and

lower-intestinal satiation with glucagon-like peptide-1, oxyntomodulin and the polypeptide–fold family including polypeptide YY[34].
The brain (IV) is the location where food intake is regulated and signals from the digestive tract are integrated.
In this dissertation, the focus lies on the relation between appetite, the stomach and the brain.

*Food properties and the food matrix*

Apart from water, the main components of food are carbohydrates, fats, protein and fiber, together these add to the energy content of food. Protein is considered the most satiating of the macronutrients[35], in contrast fats have been shown to be the least sating[36,37]. Nevertheless the individual contributions of the macronutrients to appetite have been elusive[38,39].

Fibers seem to be associated with lower energy intake[40,41]. But there are many types of fibers and they may not all have similar effects[42]. The satiating effect of fibers may in part be due to its physiochemical properties, as fiber increases viscosity[43].

Viscosity may also affect satiation and satiety by delaying transit through the digestive tract. Food components can influence flow and consistency of the content within the stomach[44]. Foods with a slower gastric emptying rate may be more sating[45], making the assessment of gastric emptying one aspect needed to understand appetite. We know that both energy load and viscosity affect gastric emptying[46], but additional work with other energy loads and viscosities is needed to complete the picture.

It has been known for some time that food consistency and energy density may influence the sating effects of different foods[47]. Increasing the distention of the stomach by incorporating water in food was shown to positively affect satiety[48]. In addition, manipulating the food matrix by creating a highly aerated version has been shown to increase satiation by suppressing hunger and reducing intake[49-51]. Similarly, carbonating drinks have been shown to induce an increase in gastric volume[52] and to increase satiety[53].

Food may not behave uniformly within the stomach[44,54,55]. Marciani et al. showed gastric sieving of the water fraction from a soup[56]. They were able to show that individual components may empty more rapidly than others, even though they are all part of the gastric content. Mackie et al.[57] manipulated gastric emptying rate using a liquid and a semi-solid meal. The semi-solid meal had a slower gastric emptying time which was attributed in part to emulsification leading to gastric layering.

In conclusion, more work is required with different energy loads and viscosities to determine their effect on appetite and the relationship with gastric emptying.

*Gastric emptying and gender*

Differences in gastric emptying between men and women have been documented[58-61]. Datz et al. showed slower gastric emptying for women, of both a liquid and a solid, when comparing the genders[58]. In their discussion they attribute these differences to hormonal contributions. For women, delayed gastric emptying was confirmed in subsequent research with solid meals[60,62]. Additonally, a slower transit time in the small bowel was shown for women compared to men[63].

In conclusion, gastric emptying seems to be slower in women compared to men, but this has yet to be shown in more detail using MRI, as well as showing whether women have increased appetite suppression and/or different neural activation compared to men post-ingestively.

*Neural activation and gastric distension*

Separating orosensory and gastric effects using modern methods of brain imaging can help to understand how gastric distention affects satiation. Gastric distention by a balloon has been shown to activate satiety related brain regions[64]. However, Spetter et al.[23] were able to show that the inclusion of orosensory stimulation yields different brain activation than distention alone, demonstrating the importance of orosensory exposure for reward sensations. Pure physical distension of the stomach does not equal normal ingestion: Oesch et al. showed that participants who underwent transient distension using an intragastric balloon prior to eating do not rate fullness higher and hunger lower, nor do they have a lower intake in a subsequent meal[65]. Orosensory stimulation plays an important role in appetite regulation[66,67]. An additional interesting conjecture is that the addition or orosensory exposure of food has been shown to affect gastric emptying[68]. The consequence of this is that to have real-world validity of the neural correlates of gastric emptying, research ought to use normal ingestion of food instead of infusion. The consequence of using ingested food to look at the distention effects on appetite and/or neural correlates is that real-time gastric content would have to be observed concurrently with brain activation.

There are currently no works in the literature where gastric measurements allow gastric distention of foods to be directly related to appetite ratings and brain activation. This would require concurrent MRI and fMRI measurements within the same session and participant, which has not been shown yet.

## Aim and outline

This thesis aims to further determine how gastric content relates to subjective experiences regarding appetite, how this relation is affected by food properties and whether this is visible in neural activation changes. This was studied using questionnaires, MRI of the stomach and fMRI of the brain. Because this relationship between the stomach and our appetite must result from processes in our brain.

In order to measure the gastric emptying process different methods may be used. **Chapter 2** of this thesis explores the correlation and discrepancies between a direct and indirect measurement method of gastric emptying. A comparison between MRI determined gastric emptying and an indirect method to determine gastric emptying applied to four food stimuli differing in viscosity and energy load.

**Chapter 3** of this thesis presents gastric emptying differences over a period of 90 minutes and how appetite changes over the same time. It aims to see how liquid foods differing in viscosity and energy load differ in gastric emptying rates, and to what extent subjective feelings correspond with the actual fullness of the stomach.

In **Chapter 4**, the gastric content is examined more closely, as we aim to see how the behaviour of food affects gastric emptying. We strive to see whether we can determine individual layering within the stomach and subsequently measure whether this layering affects emptying when compared to homogenous content.

**Chapter 5** of this thesis explores a new paradigm in gastric research. There is literature with MRI studies of the stomach and fMRI studies of the brain during food trials, but this chapter presents results of both MRI and fMRI measurements in the same subject during the same trial, striving to relate both appetite and gastric measurements directly to brain activation.

The work presented in the chapters up till now comes exclusively from male participants, but just before the finish line we are happy to introduce a female perspective on things:
**Chapter 6** shows gender differences when it comes to appetite, gastric emptying and brain responses after consumption of soda and an alcoholic beverage. We extend the examination of gastric content even further by looking at both the fluid as well as the air inside the stomach.

In the discussion of this dissertation, **Chapter 7**, a short summary of the most important results from the abovementioned chapters is presented. It follows with a discussion on the limitations and validity of the work. It concludes with an answer to the main research question and suggestions on where we should go from here.

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PHANTOM
FULLNESS

MRI-Man

# Chapter 2.

Indirect versus direct assessment of gastric emptying: a randomized crossover trial comparing C-isotope breath analysis and MRI

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Submitted for publication

**Abstract**

**Background:** Indirect methods to assess gastric emptying (GE), such as $^{13}C$ breath tests (BT), are commonly used. However, BT usually use a sampling time of 4+ hours.

**Objective**: This study aims to assess the validity of BT for four liquid meals differing in physicochemical properties. To this aim, we compared them to MRI GE-measurements.

**Methods**: 15 healthy males (age 22.6±2.4 y, BMI 22.6±1.8 kg/m$^2$) participated in a randomized 2x2 crossover experiment. Test foods were liquid meals, which were either thin/thick and 100/500 kcal, labelled with 100 mg of $^{13}C$-octanoate. GE was measured with MRI and assessed by $^{13}C$ recovery from breath. Participants were scanned every 10 min and at 6 time points breath samples were collected up to t=90 min. Two curves were fitted to the data to estimate emptying halftime ($t_{50\ Ghoos}$ and $t_{50\ Bluck}$). $T_{50}$ times were ranked per participant and compared between methods.

**Results**: On average, MRI and BT showed similar $t_{50}$ rankings for the four liquid meals. In comparison to MRI, $t_{50\ Ghoos}$ overestimated, while $t_{50\ Bluck}$ underestimated GE time. Moreover, more viscous foods were overestimated. In most participants individual $t_{50}$ time rankings differed significantly between methods.

**Conclusion**: BT can assess relative emptying differences on group level and collecting breath data for 90 minutes constitutes a lower burden for participants and the research facility. However, BT has severe shortcomings compared to MRI for individual GE assessment. Notably, food matrix effects should be considered when interpreting the results of BT.

## Introduction

Gastric emptying (GE) is an important element of gastro-intestinal physiology. For clinical applications, highly accurate measurements of GE on an individual level are required for diagnosis[1]. In a research setting GE measurements are often used to determine group differences in GE between treatments (for example[2,3]).

GE can be measured directly or indirectly. Direct methods include echography, scintigraphy and MRI[4]. MRI has been validated as an accurate method to measure gastric emptying[5]; it allows accurate real-time visualisation and determination of gastric content without the γ-radiation burden of scintigraphy. An exponential curve can be fitted to MRI gastric content measurements over time, from which the half-emptying time ($t_{50}$) can be estimated[6,7].

With indirect methods, the emptying process is estimated by labelling the meal with a tracer. Tracer recovery via breath - or blood for acetaminophen[8] - is used as a proxy to estimate gastric emptying[9]. Breath testing (BT) is performed with a carbon isotope ($^{13}C$) , which is absorbed and metabolised into $CO_2$. $^{13}C$ recovery in breath is used to estimate GE $t_{50}$ using an exponential β function or a Wagner-Nelson function[9]. The advantage of BT is that it requires less expensive equipment and is less invasive than direct methods. Disadvantages are that it provides a proxy measure which may be influenced by other factors and that sampling time is long.

It has been advocated by Bluemel et al. to only use $^{13}C$-acetate BT with prior validation by MRI[10]. They demonstrated an overestimation for the exponential β curve fit and an underestimation for the Wagner-Nelson curve fit, in a comparison of the $t_{50}$ of a 300-mL meal. This was measured by MRI and BT using 100 mg $^{13}C$-acetate.  It was suggested that these differences were caused by dilution by gastric secretions and the composition of the meal[10]. Accordingly, tracer interactions with fat components in a test meal have been shown to affect tracer absorption and subsequent excretion[11] and thus tracer-based estimates of GE.

Sanaka et al. propose a sampling period of at least 4 hours, and in specific pathological cases up to 6 hours, to allow accurate curve fitting[9]. However, this sample collection duration is both a practical limitation and a burden for the participant or patient. Longer sample collection duration may increase accuracy but this may not always be necessary. With liquid foods, recovery differences are apparent within two hours post ingestion[10,12]. Apparently, liquid meals empty relatively quickly, especially if their macronutrient content is low[13]. Therefore, for quick emptying foods a much shorter breath sampling time may be sufficient to detect differences.

The current study aims to compare gastric emptying over 90 minutes measured by $^{13}C$ breath analysis and MRI with four liquid meals, varying in energy load and viscosity. We hypothesized that food properties will affect GE as measured by both BT and MRI, and that $^{13}C$ breath analysis will show comparable group results to MRI.

## Materials and methods

### *Ethics*

The procedures followed were approved by the Medical Ethical Committee of Wageningen University (NL48059.081.14). This study was registered in the Dutch Trial Registry under number NTR4573. Written informed consent was obtained from all participants before participation. Participants received monetary incentives for participation.

MRI results from the same study have been published elsewhere[14].

### *Participants*

Participants were recruited via e-mail and social media in the Wageningen region. The participants were 15 healthy normal-weight males (age 22.6±2.3 y, BMI 22.6±1.7 kg/m$^2$). Inclusion criteria were: being male, aged between 18 and 35y, having a BMI between 18 and 25 kg/m$^2$, self-reported good general health, willing to comply with the study procedures, willing to be informed of incidental findings. Exclusion criteria were: unexplained weight loss or gain of >5 kg in the last two months, oversensitivity for any of the food products used in the experiment, any reported abnormalities of the gastrointestinal tract, use of medication which may influence gastrointestinal function, having contraindications for undergoing an MRI, and being employed or studying at the department of Human Nutrition at Wageningen University.

### *Design*

The study had a randomized 2x2 crossover design, with 4 different liquid meals that were different in viscosity (thin vs. thick) and energy load (100 kcal vs. 500 kcal). Participants visited the lab facilities 4 times for a test session with at least a 48 hour washout period in between. Each participant had his test session always on the same time of the day.

*Liquid meals*

The ingredients for the liquid meals were: cream (AH Basic, Albert Heijn B.V. Zaandam, the Netherlands), dextrin-maltose (Fantomalt Nutricia®, Cuijk, the Nederlands), vanilla sugar (Dr.Oetker®, Amersfoort, the Nederlands), whey powder (Whey Delicious Vanilla, XXL Nutrition, Helmond, the Netherlands), and tap water. The amount of ingredients was manipulated to create a 100 kcal and a 500 kcal variant. Locust bean gum was added to the meal shake to manipulate viscosity of the liquid meals, that is 20 g for the thick 100-kcal, 10 gram for the thick 500-kcal shake. A hundred milligram of $^{13}$C-octanoate (Campro Scientific GmbH, Veenendaal, The Netherlands) was added to all meal shakes to label them for the breath test. Ingredients and nutrient composition of the liquid meals can be found in **Table 1**.

Table 1. Energy content and nutrient composition of the four liquids per 100g, participants received 500g.

| | Thin 100 kcal | Thick 100 kcal | Thin 500 kcal | Thick 500 kcal |
|---|---|---|---|---|
| | | | | |
| **Protein powder*, g*** | 4.5 | 3.9 | 12.9 | 12.5 |
| **Cream*, g*** | 7.5 | 6.6 | 21.6 | 20.8 |
| **Dextrin-maltose*, g*** | 7.9 | 6.9 | 22.8 | 22 |
| **Vanilla sugar*, g*** | 6 | 5.2 | 3.4 | 3.3 |
| **Locust bean gum*, g*** | 0.7 | 13.1 | 0.5 | 4.1 |
| **$^{13}$C-octaonic acid, m*g*** | 100 | 100 | 100 | 100 |
| **Water, *g*** | 73.3 | 64.2 | 38.7 | 37.2 |
| **Total, *g*** | 100 | 100 | 100 | 100 |
| | | | | |
| **Energy[1], *kJ*** | 84 | 84 | 418 | 418 |
| **Energy, *kcal*** | 20.2 | 20.2 | 100.3 | 100.3 |
| **Carbohydrates, *g*** | 2.4 | 2.4 | 12 | 12 |
| **Of which mono- and dissacharides** | 0.4 | 0.4 | 2.1 | 2.1 |
| **Fat, *g*** | 0.6 | 0.6 | 3 | 3 |
| **Protein, *g*** | 1.2 | 1.2 | 6 | 6 |
| **Fiber, *g*** | 0.5 | 4 | 0.5 | 2.5 |

[1]Nutrient composition of the food stimuli resemble a mixed meal, with 50% of the energy load coming from carbohydrates, 30% from fats and 20% from protein.

*Test session procedures*

Participants were instructed to fast for at least three hours prior to the test session. They were only allowed to drink water during that time and nothing in the last hour before the session. After arrival participants provided two baseline breath samples and were scanned for baseline stomach content. After this, participants were taken out of the scanner and instructed to consume the shake through a 1-cm diameter straw within 2 min. After that, they were positioned in the scanner again in a supine position and remained there for 90 minutes, undergoing a gastric MRI scan every 10 minutes (see **Figure 1** for a session overview). in the same time schedule breath samples were obtained at t=10, 20, 30, 50, 70 and 90 minutes.

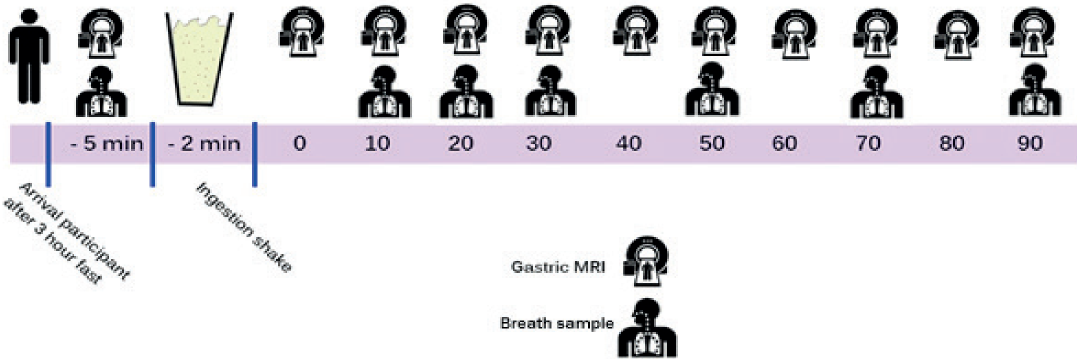



Figure 1. Overview of the procedure of one experimental session.

*Direct gastric emptying: gastric content (MRI)*

Participants were scanned with the use of a 3-Tesla Siemens Verio (Siemens AG, Munich, Germany) MRI scanner using a $T_2$-weighted spin echo sequence (HASTE, 24 6-mm slices, 2.4 mm gap, 1.19 x 1.19 mm in-plane resolution). The duration of one scan was approximately 19 sec, with a breath hold command on expiration to fixate the position of the diaphragm and the stomach. Syngo fastView MRI software (Siemens AG, Munich, Germany, http://www.healthcare.siemens.com/medical-imaging-it/syno-special-topics/syngo-fastview) was used to manually delineate gastric content on every slice (**Figure 2**). For each time point, total gastric volume (TGV) was calculated by multiplying surface area of gastric content per slice with slice thickness, including gap distance, summed over the total slices showing gastric content. Data was fitted using the NLME package in R (R3.2.2, R Foundation for Statistical Computing, Vienna, Austria). The gastric content per time point fitted using a linear exponential model[6,7], this was subsequently used to determine MRI gastric emptying $t_{50}$ (MRI $t_{50}$)[6].

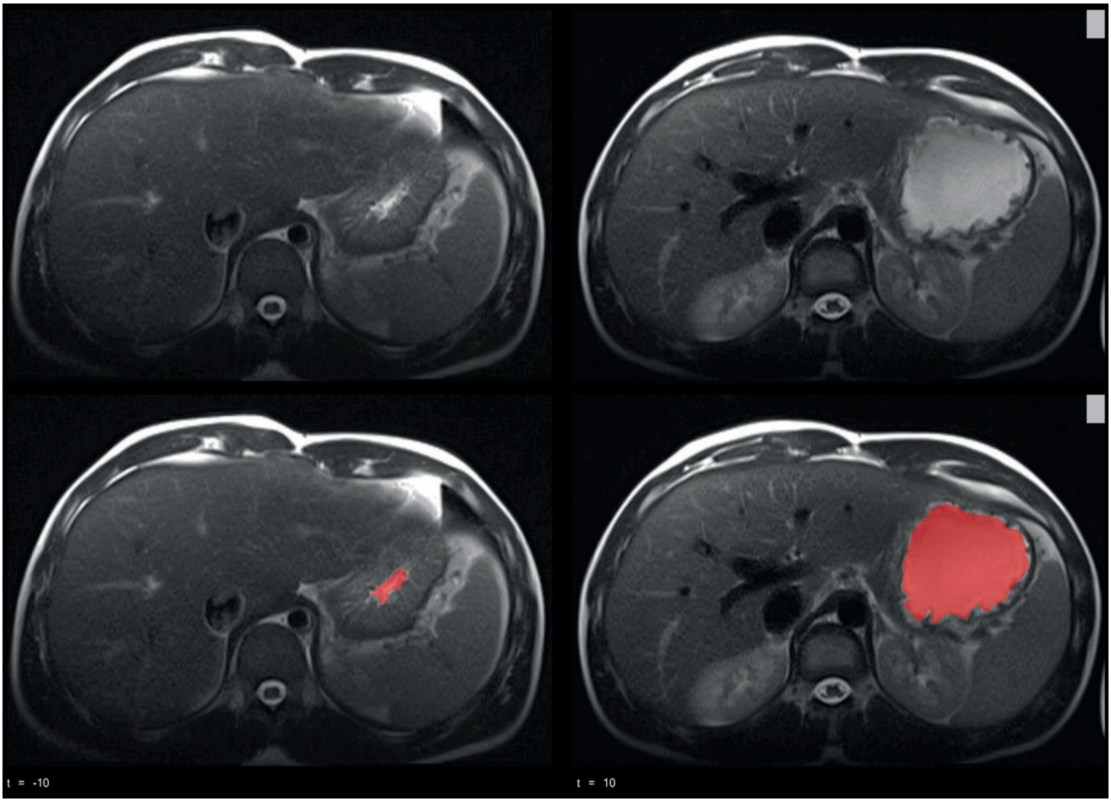



Figure 2. Transversal slice at the height of the liver, showing pre ingestion on the left and 10 minutes post ingestion on the right. The bottom has the surface area of the gastric content highlighted in red. Surface area was used to calculate gastric volume at the moment of a scan.

*Indirect gastric emptying: $^{13}C$ expiration (Breath test)*

Breath samples were collected with single-use one-way valves attached to collection bags (F201-VP-5a, FAN, Leipzig, Germany). Samples were obtained by requesting the participant to exhale through the valve filling the bag to capacity. Breath samples were analysed on the day of sampling on a spectrometer (IRIS 3, ID nr. 109849, Wagner Analysen Technik GmbH, Bremen, Germany). The spectrometer was calibrated before each measurement following the supplier's instruction. Two baseline samples were taken to provide solid baseline $^{13}C$ concentrations. Subsequently, delta over baseline (DOB) was calculated for the other time points. The $^{13}C$ was converted to a percentage of the dose $^{13}C$ recovered (PDR). The PDR was fitted using the original method following Ghoos et al.[15-17] as well as a more recent modified method[7,18], and these models were subsequently used to determine $^{13}C$ gastric emptying $t_{50}$ ($^{13}C$ $t_{50\ Bluck}$ and $^{13}C$ $t_{50\ Ghoos}$)[10,18-22].

*Calculations and Statistical analyses*

All data presented are means and SD unless mentioned otherwise. Significance level was set at a p-value of 0.05. A Sidak adjusted general linear mixed model using treatment and time as factors was used to test main effects on $t_{50}$. A Sidak corrected post-hoc test was performed to test differences between the treatments per time point (IBM SPSS 20 (IBM, Armonk, USA).

A Sidak adjusted general linear mixed model with viscosity and energy load was used to test for the main effects of food composition on $t_{50}$ between treatments (IBM SPSS 20 (IBM, Armonk, USA).

Additionally, outcomes were ranked per treatment for each participant. If the ranking between MRI $t_{50}$ and $^{13}C$ $t_{50}$ were dissimilar Kendall τ distance of the two rankings was computed. Kendall τ distance indicates how many pair-wise swaps are necessary to attain similar rankings. A distance of 0 indicates completely similar rankings, and the maximal distance of 6 indicates a completely reversed order. Correlation was tested by computing Kendall's τ coefficient.

**Results**

An overview of the mean $t_{50}$ per treatment, significance of the model factors and post hoc results for both MRI and $^{13}C$ PDR can be found in **Table 2**.

Table 2. Effect of viscosity and energy load on $t_{50}$

| | Thin 100 kcal (min) | Thick 100 kcal (min) | Thin 500 kcal (min) | Thick 500 kcal (min) | Viscosity p | Energy load P | Viscosity* Energy load p |
|---|---|---|---|---|---|---|---|
| **MRI $t_{50}$** | 27±11$^{A1}$ | 44±20.7$^{B}$ | 69±22$^{C}$ | 82±31$^{C}$ | 0.006 | <0.001 | 0.83 |
| **$^{13}C$ $t_{50}$** Bluck | 27±7$^{A}$ | 41±10$^{B}$ | 43±7$^{B}$ | 44±6$^{B}$ | 0.001 | <0.001 | 0.003 |
| **$^{13}C$ $t_{50}$** Ghoos | 80±25$^{A}$ | 120±26$^{B}$ | 149±37$^{C}$ | 158±47$^{D}$ | 0.003 | <0.001 | 0.06 |

[1]different letters indicate Sidak adjusted post-hoc pair-wise comparisons showed significant differences between the $t_{50}$.

*Direct Gastric emptying: MRI*

Total gastric volume over time can be seen in **Figure 3**. There was a significant effect of viscosity ($p = 0.006$) and energy load ($p < 0.001$), but their interaction was not significant ($p = 0.83$). The thin 100 kcal shake had the shortest GE $t_{50}$ of the treatments (95% CI 11.9 – 41.4 min). The thick 100 kcal had longer GE $t_{50}$ (95% CI 26.4 – 55.9 min), followed by the thin 500 kcal (95% CI 54.9 – 84.4 min). The thick 500 kcal had the highest $t_{50}$ (95% CI 67.3 – 96.8 min). Results show significant effects of energy content for both low ($p < 0.001$) and high viscosity ($p < 0.001$). The effect of viscosity was significant for the low energy load ($p = 0.033$), but not the high energy load condition ($p = 0.065$).

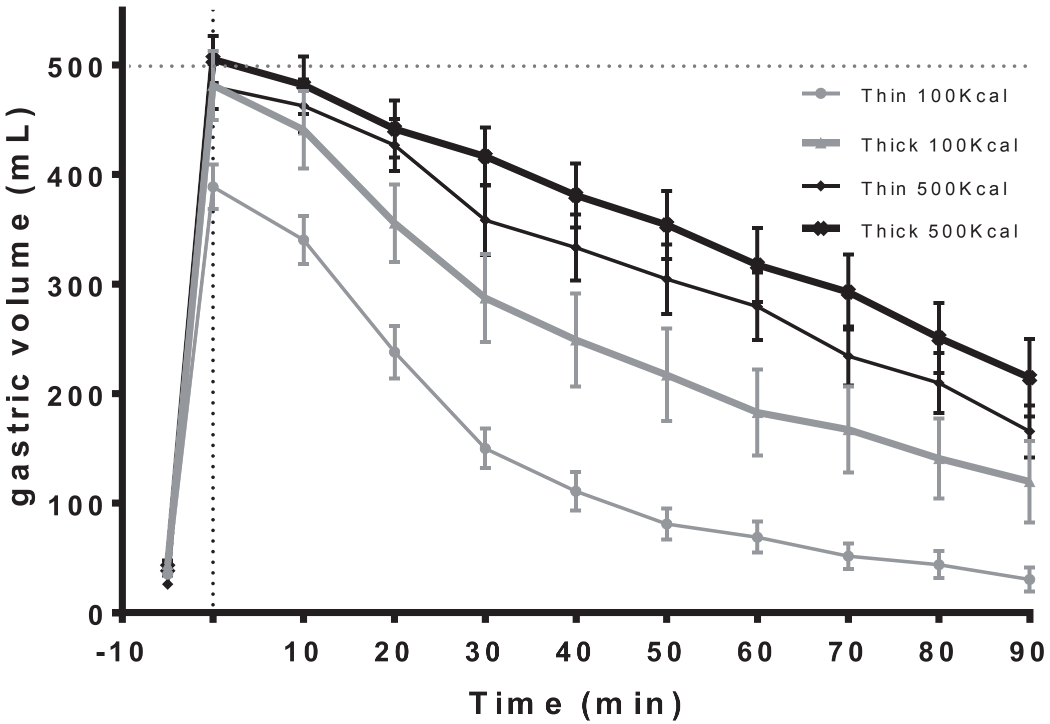



Figure 3. Overview of the MRI volume over time of the four treatments (mean±SEM). These volumes were used to fit emptying curves and extract $t_{50}$. At time points 0 and 10 the volume of the thin 100 kcal treatment was significantly different from the other treatments, at the following time points there was a significant difference between all but the two 500 kcal treatments.

*Indirect gastric emptying: $^{13}$C Breath test*

Percentage dosage recovered over time is shown in **Figure 4**.

$^{13}$C $t_{50\ Bluck}$: there was a significant effect of viscosity ($p = 0.001$), energy load ($p < 0.001$) and a significant interaction effect ($p = 0.003$) indicating that the effects of viscosity and energy load are not independent. Breath testing $t_{50}$ was shortest for the thin 100 kcal shake (95% CI 15.1 – 41.5 min). The thick 100 kcal had a longer $t_{50}$ (95% CI 28.6 – 55 min) followed by the thin 500 kcal (95% CI 31.3 – 57.8 min). There was a significant effects of energy load for the low viscosity (p < 0.001) but not the high viscosity condition ($p = 0.25$). The effect of viscosity was significant for the low energy load ($p < 0.001$), but not the high energy load condition ($p = 0.80$).

$^{13}C$ $t_{50\ Ghoos}$: there was a significant effect of viscosity ($p = 0.003$), energy load ($p < 0.001$) but no interaction effect ($p = 0.06$). Breath testing $t_{50}$ was shortest for the thin 100 kcal shake (95% CI 60.1 – 100.4 min). The thick 100 kcal had a longer $t_{50}$ (95% CI 100.9 – 140.7 min) followed by the thin 500 kcal (95% CI 128.9 – 168.7 min). The thick 500 kcal had the highest $t_{50}$ (95% CI 138.5 – 178.3 min). There was a significant effect of energy load for both the low ($p < 0.001$) and high viscosity condition ($p = 0.002$). The effect of viscosity was significant for the low energy load ($p = 0.001$), but not the high energy load condition ($p = 0.40$).

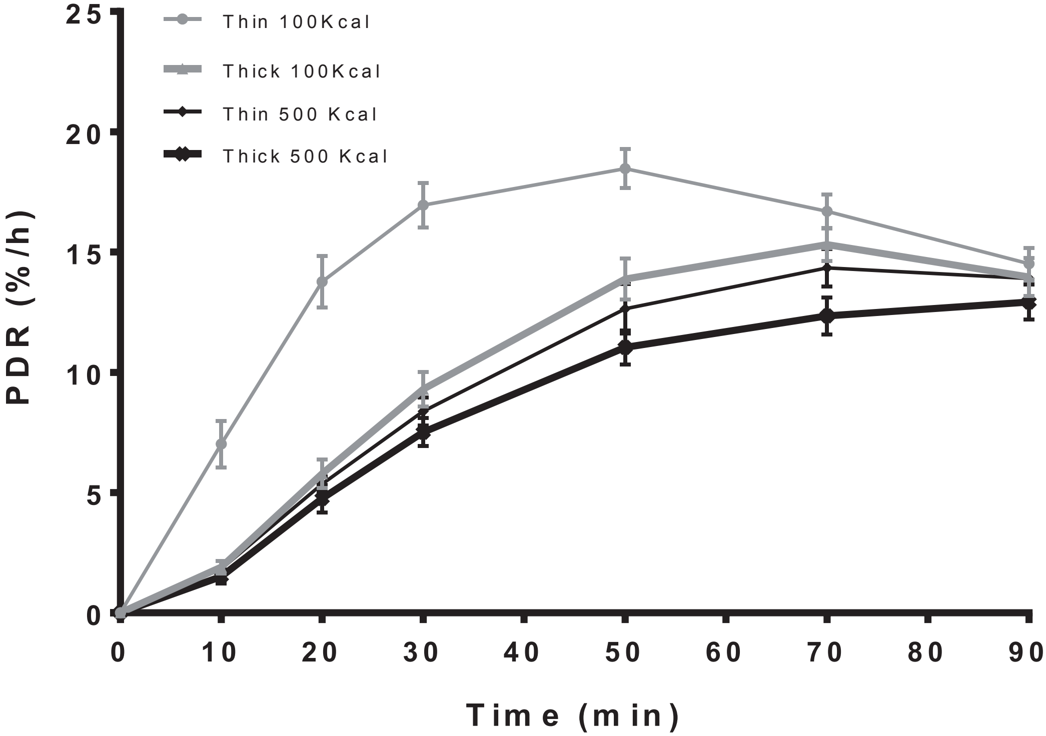
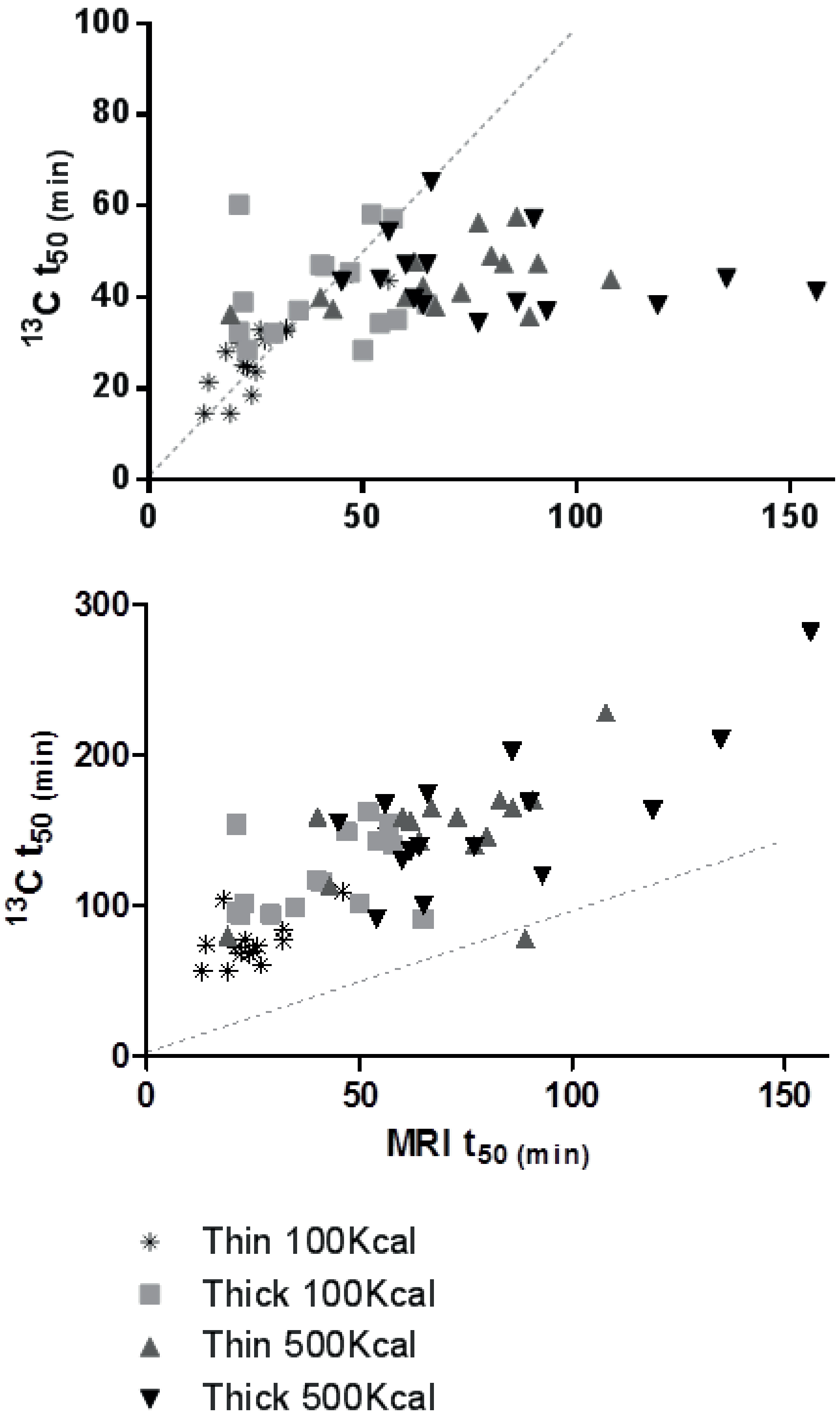



Figure 4. Percentage dosage recovered (PDR) over time for the four treatments (mean±SEM). These values were used to derive $t_{50}$. At time points 10 and 20 the thin 100 kcal was significantly different from all treatments. At 50 and 70 minutes there were significant differences between the 100 kcal and the 500 kcal treatments. At 90 minutes there was no difference.

*Comparison of BT with MRI*

Each session measured with MRI $t_{50}$ and $^{13}C$ $t_{50}$ is plotted in **Figure 5**. Six out of 15 participants showed complete similarity in rankings for $^{13}C$ $t_{50\ Bluck}$ and 6 participants had a Kendall distance >1. Four out of 15 participants showed complete similarity in rankings for $^{13}C$ $t_{50\ Ghoos}$ and 8 participants had a Kendall distance >1.

Estimated $t_{50}$ values were significantly correlated, with Kendall's $\tau = 0.412$ ($p < 0.001$) for MRI and $^{13}C$ $t_{50\ Bluck}$ and Kendall's $\tau = 0.555$ ($p < 0.001$) for MRI and $^{13}C$ $t_{50\ Ghoos}$.


100
80
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$^{13}$C $t_{50\ (min)}$
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$^{13}$C $t_{50\ (min)}$
0
50
100
150
MRI $t_{50\ (min)}$
Thin 100Kcal
Thick 100Kcal
Thin 500Kcal
Thick 500Kcal

Figure 5. MRI $t_{50}$ and $^{13}C$ $t_{50}$ plotted against each other for all sessions, One dot indicates one session. Scatterplot above shows $^{13}C$ $t_{50\ Bluck}$ and scatterplot below shows $^{13}C$ $t_{50\ Ghoos}$. In relation to a linear x = y relationship (grey dotted line) $^{13}C$ $t_{50\ Bluck}$ underestimated emptying times and $^{13}C$ $t_{50\ Ghoos}$ overestimated emptying times. Estimated $t_{50}$ values were significantly correlated, with Kendall's $\tau = 0.412$ ($p < 0.001$) for MRI and $^{13}C$ $t_{50\ Bluck}$ and Kendall's $\tau = 0.555$ ($p < 0.001$) for MRI and $^{13}C$ $t_{50\ Ghoos}$.

## Discussion

The current study aimed to compare gastric emptying times measured by $^{13}C$ breath analysis and MRI among four liquid foods varying in energy load and viscosity by measuring their gastric emptying concurrently with $^{13}C$ breath analysis and MRI over 90 min.

Gastric emptying times were increased for the liquid meals by both viscosity and energy load. Although $^{13}C$ $t_{50\ Bluck}$ underestimated emptying rate, $^{13}C$ $t_{50\ Ghoos}$ overestimated emptying rate when looking at individual results; only a minority of participants, 6 for $t_{50\ Bluck}$ and 4 for $t_{50\ Ghoos}$ had matching rankings. However, on the group level they were similar to MRI $t_{50}$. Viscosity and energy load showed a significant positive interaction effect for $^{13}C$ $t_{50\ Bluck}$ which was not apparent when looking at the MRI $t_{50}$ results.

Our ranking data suggest that MRI and $^{13}C$ $t_{50}$ estimates are congruent on agroup level, which is illustrated by the curves shown in Figure 3 and 4. However, individual rankings were different; six participants (40%) had a Kendall distance greater than 1, indicating that there were at least two treatments ranked different in relative GE between measurement methods. From our results we conclude that short-term BT may be a suitable method for relative GE emptying differences of fast emptying food stimuli on agroup level, but not for individual estimates.

The outcomes of BT are often used to diagnose dyspepsia. In clinical practice, the $^{13}C$ GE test is commonly performed using an egg sandwich. Knight et al. showed that something as seemingly insignificant as the cooking method of the egg may already influence tracer behaviour[23]. It should therefore be strongly advised to look further into the validity of current clinical practice using BT to diagnose abnormal GE.

*Limitations*

The current study assessed gastric emptying over 90 minutes with both BT and MRI, whereas other studies kept collecting breath samples after finishing the MRI measurements[10,11]. Our MRI measurements showed that at the end of the sampling time not all of the meal had emptied from the stomach. For research in which relative gastric emptying rates between groups are compared, gathering breath samples for 90 min can be sensitive enough to measure different emptying rates as these differences occur already quickly after ingestion.

For this study we chose to include only male participants in order to maintain a homogenous group. Moreover, isotope measurements for short periods such as 90 minutes are not standard procedure. We have a relatively large dataset of 60 gastric emptying events of both techniques simultaneously, which allows within subject rankings of both techniques. When we look at individual data large discrepancies can be seen between the ranking based on BT and that based on MRI. The rankings are very different in up to 40% of participants. In our opinion, this can not only be the result of our relatively short sample gathering time.

*The challenges of indirect measurement methods*

GE measurements with the indirect BT method have been used in many different studies[15,19,24-26]. Nevertheless, there has been discussion on the validity of indirect measurements and the GE times which are inferred from them[27-30]. BT results are based on the solubility, bio-availability, absorption, metabolization and excretion of the used tracer. To guarantee reliable study results it is important that the tracer is distributed evenly throughout the food stimulus, that it is bio-available, directly absorbed by the intestine, and metabolized by the liver upon availability. A likely explanation for discrepancies between direct and tracer-based methods is that the behaviour of the tracer and its interaction with the intestine and liver is influenced by the food matrix in which it is delivered.

In addition, different tracer compounds have different chemical properties. It has been suggested that $^{13}C$ acetate might lead to higher $^{13}C$ levels within the $CO_2$ in breath more quickly than octanoic acid due to faster metabolism of acetate[31]. This would make $^{13}C$ acetate a better tracer for short duration trials, however, acetate has a large affinity for water. $^{13}C$ octanoic acide is a lipophilic compound and has been used in experiments with both liquid and solid test foods[1,32]. We used octanoic acid as a tracer because of the fat component of the meals. Octanoic acid has affinity for fatty foods[33]. With fat containing foods intragastric lipid layering can occur[34,35]. Intragastric lipid layering is of concern as experimental foods may contain a fat component. This fat component can affect tracer distribution and may influence measurement results. When layer formation occurs within the stomach, labelling techniques are inherently unreliable; The label will move with either the water, fat or sediment phase (dependent on the chosen molecule) and thus either over- or underestimate the actual emptying rate.

Very recent evidence for this effect of gastric lipid content on tracers has been reported by Parker et al.[11]. This study used acid stable and unstable stimuli, and compared three differed tracing agents (including acetate and octanoic acid) with MRI measurements to assess whether the distribution of tracing agents was influenced by the food matrix. Their results show that octanoic acid measurements correlate better with fat movement through the stomach than acetate and $^{13}C$ trioctanoin measurements, indicating that the chemical properties of the tracer may have influenced GE.

Such food matrix effects may have resulted in over- or underestimation in previous tracer-based work. The food stimuli we used contained all macronutrients of a normal meal in relative percentages. The rationale for this was to find results valid for actual meals and also to exclude specific macronutrient effects[34,36-38]. However, using regular foods makes the tracer choice challenging, because intragastric tracer behaviour becomes less predictable as the food matrix becomes more complex. A possible solution would be to combine multiple tracers or use a combination of a radioactive marker and a tracer. However, also in that case prior validation is required to ascertain that the labelling works as intended.

*Conclusion*

MRI and $^{13}C$ breath testing show agreement on the group level; with a relatively short time of data collection (90 min), we were able to establish similar differences in GE between four foods for both methods. In contrast, on the individual level our results show over- or underestimations of half emptying times depending on the curve fitting method used and different ranked GE times between treatments.

In contrast to BT, MRI does not depend on tracer binding and recovery. Therefore, we advise researchers and clinicians to validate their BT-protocol for each type of food stimulus with MRI before using it as a measure of gastric emptying time.

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Author contributions

GC, MM, BW, CdG and PS designed the study. GC did the data acquisition, analyses and wrote the manuscript. MM, BW, CdG and PS revised the manuscript.

All authors have approved the manuscript.

Disclosure

The authors declare no conflicts of interest.

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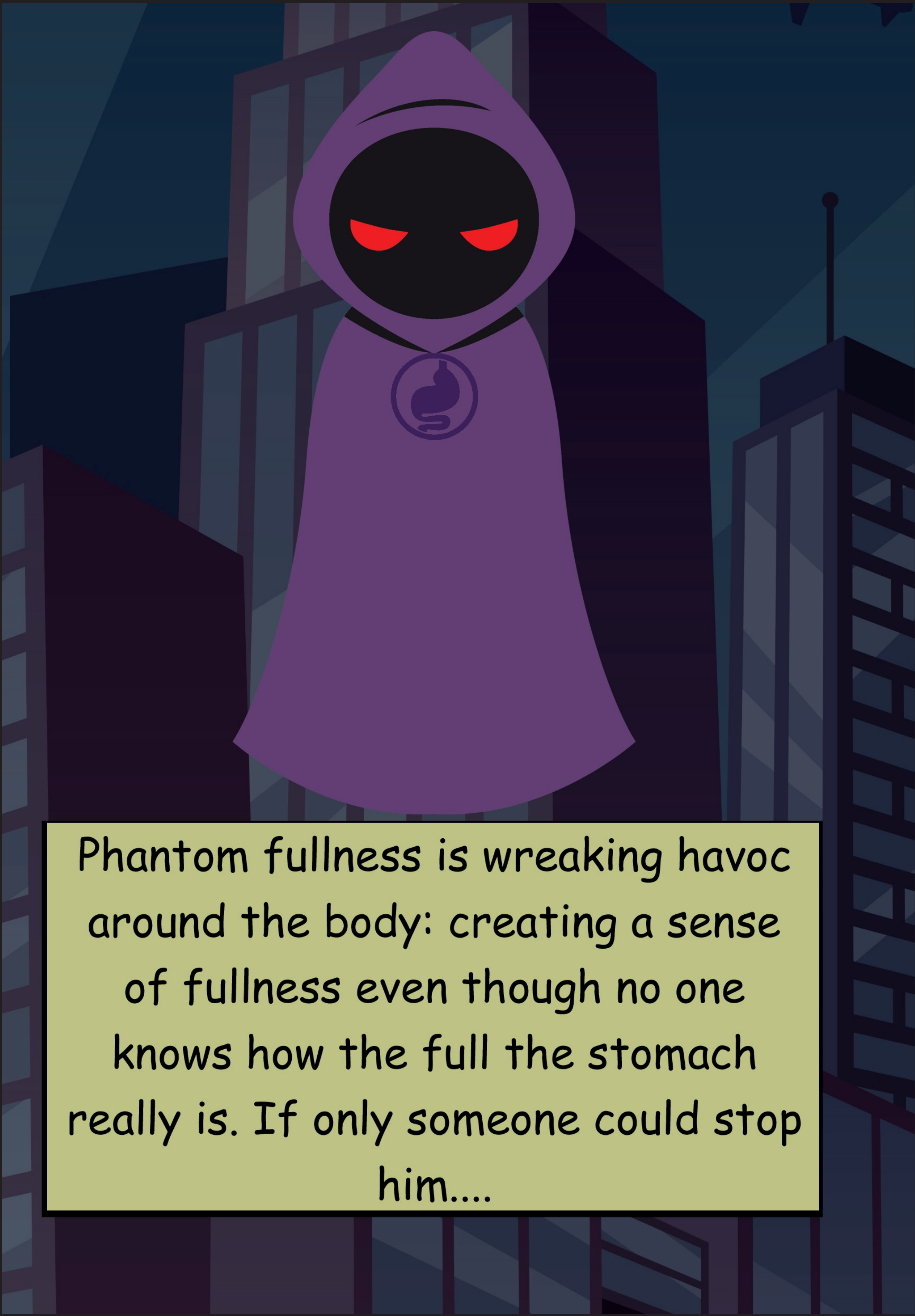
Phantom fullness is wreaking havoc around the body: creating a sense of fullness even though no one knows how the full the stomach really is. If only someone could stop him....

# Chapter 3.

Empty calories and phantom fullness: a randomised trial studying the relative effects of energy density and viscosity on gastric emptying determined by MRI and satiety

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**Abstract**

**Background:** Stomach fullness is a determinant of satiety. Though both viscosity and energy content have been shown to delay gastric emptying, their relative importance is not well understood.

**Objective:** To compare the relative effects of, and interactions between, viscosity and energy density on gastric emptying and perceived satiety.

**Design:** 15 healthy males (age 22.6±2.4y, BMI 22.6±1.8kg/m$^2$) participated in an experiment with a randomized 2x2 crossover design. Participants received a dairy based shake (500 mL, 50%CHO, 20%PRO, 30%FAT), differing in viscosity (thin/thick) and/or energy density (100 kcal corresponding with 0.2 kcal/mL vs 500 kcal corresponding with 1 kcal/mL). After ingestion participants entered an MRI scanner where abdominal scans and oral appetite ratings on a 100-point scale were obtained every 10 minutes till 90 minutes post-ingestion. From the scans gastric content volumes were determined.

**Results:** Overall gastric emptying half time (GE t50) was 54.7±3.8min. The thin 100 kcal shake had the lowest GE t50 (26.5±3.0min), followed by the thick 100 kcal(41±3.9min), followed by the thin 500 kcal (69.5±5.9min) and the thick 500 kcal had the highest GE t50 (81.9±8.3min).

With respect to appetite, the 100 kcal thick shake led to higher fullness (58 pts at 40 minutes) than the thin 500 kcal shake (48 pts at 40 minutes).

**Conclusions:** Our results show that increasing viscosity is less effective in slowing gastric emptying than increasing energy density. However, viscosity is more important to increase perceived fullness. This underscores the lack of satiating efficiency of ‘empty calories’ in quickly ingested drinks such as sodas. The increase in perceived fullness due solely to increased viscosity, a phenomenon we refer to as ‘phantom fullness’, may be useful to lower energy intake.

## Introduction

Understanding satiety may help decrease overconsumption. Many factors contribute to satiety, from portion size[1] to orosensory exposure[2,3]. Post-ingestive feedback from the stomach and intestinal tract on nutrient content[4,5] and volume[6] also affect satiety.

Gastric feedback affects satiety through stretch receptors via distension, rate of emptying and nutrient and energy content. The increase of feelings of satiety through gastric distension has been shown in earlier work[7,8]. Mackie et al. manipulated gastric emptying rate by using isovolumetric and isocaloric liquid and semi-solid meals. It was shown that the semi-solid condition slowed gastric emptying, resulting in larger distension, inducing enhanced satiety[9].

Viscosity has been shown to have an effect on satiation and satiety in multiple studies[10-13]. However, the literature is inconsistent when it comes to whether viscosity directly slows gastric emptying. In certain studies it did not slow gastric emptying[14,15], in others viscosity did delay gastric emptying rate[16,17]. Hoad et al. reported no difference in gastric emptying between a control, guar gum and intragastric gelling stimulus, but did find different fullness scores[18]. The demonstrated satiating effects of viscosity may be related to oral exposure and could be independent of gastric feedback[12]. Marciani et al. are, to our knowledge, the only group which studied viscosity and energy density on gastric emptying in one single study. They showed that gastric emptying is slowed by both viscosity and energy content[19] and concluded that energy content delays gastric emptying more effectively than increased viscosity. Concurrently, they found that perceived satiety is affected more by viscosity. However, the authors state that further work is necessary with other levels of energy and viscosity. More specifically, there are two areas where this work can be extended: the energy condition (320 kcal) is opposed to shakes with zero energy (excluding dextrose for osmolarity), and the stimuli contained only carbohydrates and fats. It may be important to include the effects of protein[20]. More importantly, it may be interesting to compare a smaller and larger energy load with one another, in order to see what the relative delaying effect of increased energy density is. On top of looking at gastric emptying by measuring changes in gastric volume as is commonly practised, it should prove interesting to also compare gastric emptying per time unit in order to further understand gastric emptying dynamics.

Therefore, in this study we aim to further elucidate the contributions and effects of viscosity and energy on gastric emptying dynamics and appetite, measured through ratings and intake. We hypothesize that viscosity and energy will slow gastric emptying. Based on the studies mentioned before[18,21] we expect that a higher energy content will decrease gastric emptying rate more than increased viscosity does.

## Subjects and Methods

*Subjects*

The participant group consisted of 15 healthy males (age 22.6±2.4y, BMI 22.6±1.8kg/$m^2$). Healthy adult males were recruited by website and flyers around the campus of Wageningen University. Inclusion criteria were: being male, aged between 18 and 35y, having a BMI between 18 and 25 kg/$m^2$, being of self-reported good general health, willing to comply with study procedures, willing to be informed of incidental findings. Exclusion criteria were: unexplained weight loss or gain of >5kg in the last two months, oversensitivity to any of the food items used in the experiment, any reported pathologies relating to the gastrointestinal tract which might influence results, use of any medications which may influence gastrointestinal function, having any contraindications for undergoing an MRI, not signing the informed consent form, not signing the MRI safety questionnaire before each session and being employed or studying at the department of Human Nutrition at Wageningen University.

Potential participants filled out an inclusion questionnaire to screen for eligibility. Subsequently they attended a screening meeting that included measurement of weight and height and explanation of the study procedures, including MRI procedures. The participants were unaware of the exact aim of the study; they were only informed about the fact that we were investigating the digestive system. We kept the participants naive to the fact that we varied the energy content of the shakes.

Before the study, a sample size calculation was performed. The calculation was based on an estimated effect size, with an expected difference in minutes of ~11 and a standard deviation of ~7 minutes. With statistical significance set at 0.05, significance level in a two sided cross-over study, with a power of 90% would be achieved with 15 subjects. The power calculation was included for review by the Medical Ethical Committee.

The procedures followed were approved by the Medical Ethical Committee of Wageningen University in accordance with the Helsinki Declaration of 1975 as revised in 2013 (NL48059.081.14). This study was registered with the Dutch Trial Registry under number NTR4573.
Written informed consent was obtained from all subjects.

*Design*

Subjects came to our facilities 4 times in a randomized 2x2 crossover design. Each participant was always scanned on the same time of day. participants were offered to drink one of the 4 shakes the order of which was based on a scheme generated by use of the website site http://www.randomization.com. The scheme was created using balanced permutations.
Five randomly selected willing participants came for one additional session after completing the 4 sessions to perform a control measurement with ingestion of 500 mL of water.

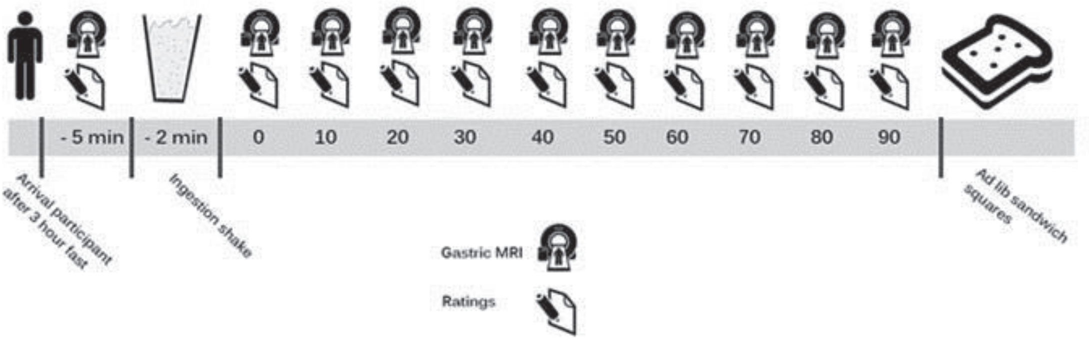


Figure 1. Overview of one experimental session for one participant

*Session procedures*

Subjects were instructed to fast for at least three hours, only drinking watery fluids in that time and not in the last hour before each session. After arrival participants provided baseline appetite ratings and were scanned for baseline stomach content. After this, participants exited the scanner and consumed the shake. The shakes were consumed from a blinded cup with a 1 cm diameter straw to allow consumption of both the thick and thin shakes through the straw. Participants were instructed to consume the shake within 2 minutes. All participants finished the shake within this time. The shakes were then rated on a 100-mm VAS on liking, sweetness, thickness and creaminess. After that, participants were positioned in the scanner and stayed there for 90 minutes, giving subjective ratings via the intercom and undergoing a gastric MRI scan every 10 minutes (**Figure 1** for session overview). At the end of the session the participants were offered a sandwich meal, of which food intake was recorded.

*Treatments*

The ingredients for the shake were cream (AH Basic, Albert Heijn B.V. Zaandam, the Netherlands), dextrin-maltose (Fantomalt Nutricia®, Cuijk, the Nederlands), vanilla sugar (Dr.Oetker®, Amersfoort, the Nederlands),whey powder (Whey Delicious Vanilla, XXL Nutrition, Helmond, the Netherlands), and water. Fibre in the form of locust bean gum was added to the shakes (1 gram for the thin shakes, 20 gram for the 100 kcal, 10 gram for the 500 kcal shake) to manipulate viscosity. Viscosity was visibly affected, rheology measurements were performed in line with previous work[10] and flow behaviour can be found in **Appendix Figure 1**. Energy was manipulated by differing grams of added ingredients. The macronutrients of the shake were combined to reflect a mixed meal in which 50% of the energy came from carbohydrates, 30% from fat and 20% from protein,  the composition can be found in **Table 1**. Shakes were mixed in a container with an internal whisking ball for approximately 30 seconds. All shakes were prepared about 15 minutes before intake, and offered at 20 ºC.

Table 1 also gives an overview of the liking, creaminess, sweetness and thickness VAS ratings. Liking was significantly different between shakes with different energy loads. Creaminess was significantly higher for both the thick as well as the 500 kcal condition. Sweetness was significantly higher for the 500 kcal shake. Viscosity and energy significantly increased reported thickness. Perceived thickness was greater for the high viscosity condition as opposed to the low viscosity condition. Additionally a greater energy load increased perceived thickness both in the low and the high viscosity condition.

Table 1. Energy content and nutrient composition of the shakes per 100g, viscosity and sensory characteristics.

| | Thin 100 kcal | Thick 100 kcal | Thin 500 kcal | Thick 500 kcal |
|---|---|---|---|---|
| | | | | |
| **Protein powder*, g*** | 4.5 | 3.9 | 12.9 | 12.5 |
| **Cream*, g*** | 7.5 | 6.6 | 21.6 | 20.8 |
| **Dextrin-maltose*, g*** | 7.9 | 6.9 | 22.8 | 22 |
| **Vanilla sugar*, g*** | 6 | 5.2 | 3.4 | 3.3 |
| **Locust bean gum, *g*** | 0.7 | 13.1 | 0.5 | 4.1 |
| **Water, *g*** | 73.4 | 64.3 | 38.8 | 37.3 |
| **Total gram** | 100 | 100 | 100 | 100 |
| | | | | |
| **Energy[1], *kJ*** | 84 | 84 | 418 | 418 |
| **Carbohydrates, *g*** | 2.4 | 2.4 | 12 | 12 |
| **Of which mono- and** | 0.4 | 0.4 | 2.1 | 2.1 |

| | | | | |
|---|---|---|---|---|
| **dissacharides** | | | | |
| **Fat, *g*** | 0.6 | 0.6 | 3 | 3 |
| **Protein, *g*** | 1.2 | 1.2 | 6 | 6 |
| **Fiber, *g*** | 0.5 | 4 | 0.5 | 2.5 |
| | | | | |
| **Pleasantless[2]** | 35.7±4.3 | 30.3±4.5 | 65.5±3.7 | 61.9±4.2 |
| **Creaminess[2]** | 27.9±5.2 | 60.8±5.6 | 48.6±5.1 | 71.1±3.1 |
| **Sweetness[2]** | 37.0±6.1 | 30.2±3.9 | 67.1±5.4 | 71.5±3.1 |
| **Thickness[2]** | 14.3±2.6[a] | 72.7±4.1[b] | 30.8±4.2[c] | 53.5±4.9[d] |

[1]Nutrient composition of the shake resembles a mixed meal, with 50% of the energy load coming from carbohydrates, 30% from fats and 20% from protein. [2] 100 mm VAS (mean ± *SEM)*

*Outcomes subjective appetite ratings*

Subjects rated hunger, fullness, prospective consumption, desire to eat and thirst on a 100-mm VAS at baseline, i.e., before intake of the shake. After ingestion these ratings were obtained orally every 10 minutes over the scanner intercom system and orally scored by the participant from 1 – 100 points[22].

*Gastric volume*

Subjects were scanned with the use of a 3-Tesla Siemens Verio (Siemens AG, Munich, Germany) MRI scanner using a $T_1$-weighted spin echo sequence (HASTE, 24 6-mm slices, 2.4 mm gap, 1.19 x 1.19 mm in-plane resolution), with breath hold command on expiration to fixate the position of the diaphragm and the stomach. The duration of one scan was approximately 19 seconds. Syngo fastView MRI software (Siemens AG, Munich, Germany, http://www.healthcare.siemens.com/medical-imaging-it/syngo-special-topics/syngo-fastview) was used to manually delineate gastric content on every slice (**Figure 2**). Gastric volume on each time point was calculated by multiplying surface area of gastric content per slice with slice thickness including gap distance, summed over the total slices showing gastric content. Gastric emptying was defined as the decrease in gastric content in mL over time.

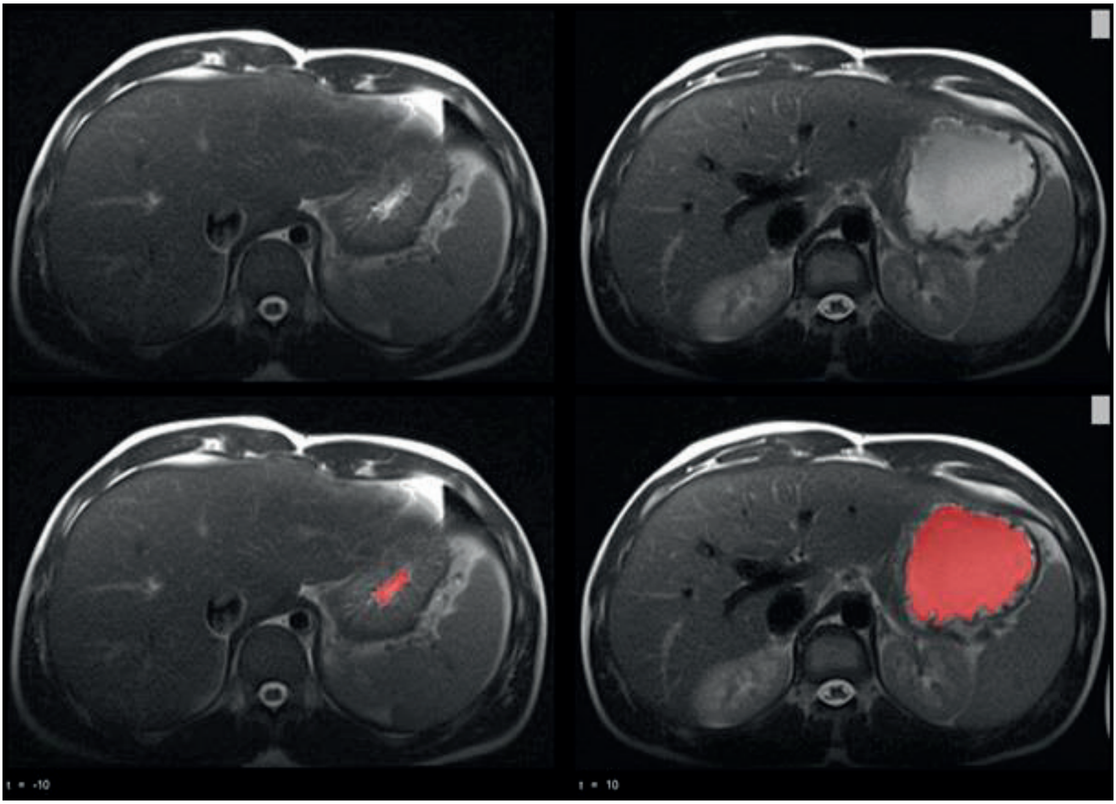


Figure 2. Transversal slice at the height of the liver, showing pre ingestion on the left and 10 minutes post ingestion on the right. The bottom has the surface area of the gastric content delineated in white. Surface area was used to calculate gastric volume at the moment of a scan.

*Food intake*

After 90 minutes, participants were offered a 500 mL bottle of water and a surplus of ham-cheese sandwich squares. The serving consisted of 20 squares with the message that more was available if required, one square was approximately 312 kJ/ 75 kcal. Participants were instructed to eat until they were pleasantly satisfied. The number of sandwich squares eaten was recorded and used to calculate intake.

*Control session with water*

A subset of five participants returned for a fifth session, in which they underwent the same procedure with 500 mL of water instead of a shake. Because of  the rapid emptying of the water, scanning time for those sessions was limited to 25 minutes after ingestion. Results including the water control are shown in **Appendix Figure 2** and **3**.

*Statistical analyses*

Gastric half emptying time (GE t50) was calculated following the linear exponential model for gastric emptying developed on the basis of earlier models in Zürich[23,24]. The data were fitted to the model and GE t50 extracted using R (R Foundation for Statistical Computing, Vienna, Austria). Gastric emptying rate (GER) was calculated by subtracting gastric volumes to what amount of mL was emptied in the 10 minutes between scans.
AUC over 90 minutes was calculated for subjective ratings. The AUC was calculated using Graphpad Prism 5 (Graphpad Software, La Jolla, USA), following the trapezoidal rule.
All data were quantative. Data were expressed as mean±SD unless otherwise stated. Testing for the differences between treatments for gastric emptying was done by a general linear mixed model on the GE t50 scores, for GER by a general linear mixed model on the emptied amounts, and for appetite scores this was done by means of a general linear mixed model of the AUC. Energy load and viscosity were included as fixed factors and participants were included as a random factor in the model. For the subjective appetite ratings analysis, baseline measurements were included as a covariate[25]. Analyses were performed assuming a first-order

autoregressive covariance structure. Post hoc Šídák adjusted tests were performed to further examine the main effects. Statistics were performed using IBM SPSS 20 (IBM, Armonk, USA). Significance level was set at a p-value of 0.05.

## Results

*Gastric emptying*

**Figure 3** shows the gastric volume over time of the different treatments.

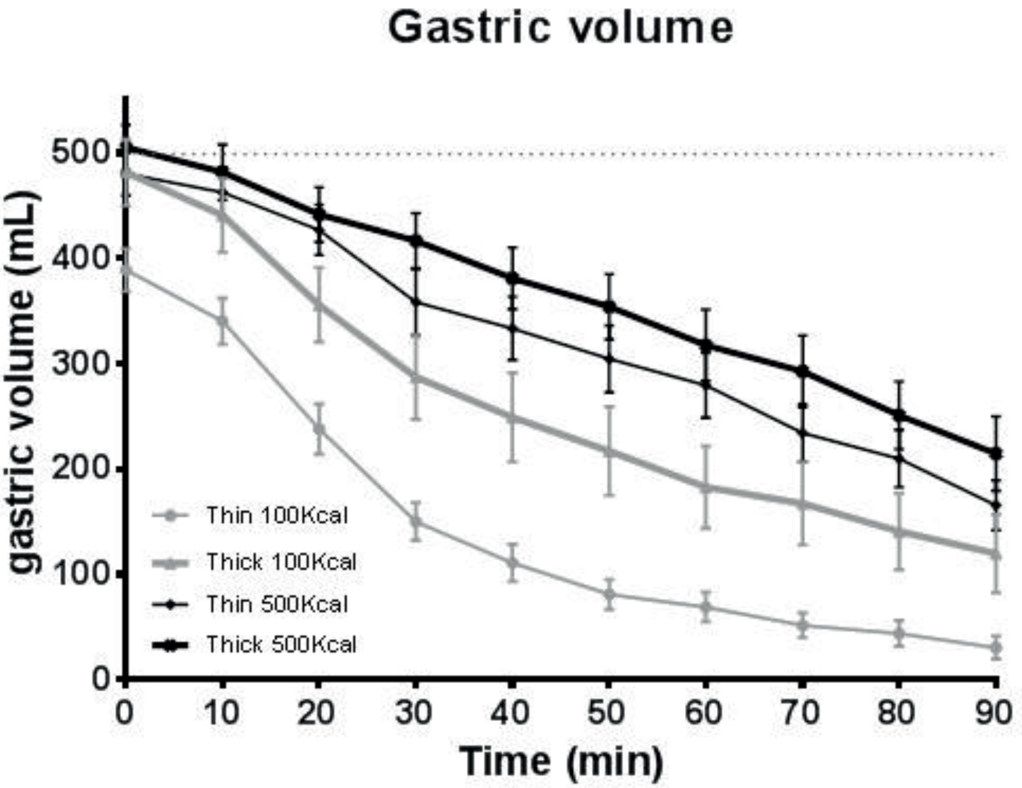


Figure 3. Gastric volume as measured by multiplying slice surface area with summed slice distance and thickness. Error bars show SEM. From the emptying curve of the individual participant the gastric emptying halftime (GE t50) was extracted and the mean GE t50 calculated per treatment: thin 100 kcal 26.5±3.0min, thick 100 kcal 41±3.9min, thin 500 kcal 69.5±5.9, thick 500 kcal 81.9±8.3. Mixed model analysis yielded that viscosity significantly increased t50 ($p=0.006$, $F=8.4$) as did energy content ($p<0.001$, $F=81.8$). Post hoc tests showed that all pairwise comparisons were significant, with the exception of the thin and thick 500 kcal shakes. $n = 15$.

The thin 100 kcal shake had the lowest t50 (26.5±3.0, 95% Confidence Interval (95% CI) 11.9 – 41.4), followed by the thick 100 kcal(41±3.9, 95% CI 26.4 – 55.9), followed by the thin 500 kcal (69.5±5.9, 95% CI 54.9 – 84.4) and the thick 500 kcal had the highest t50 (81.9±8.3, 95% CI 67.3 – 96.8).

For GE t50, there was a significant effect of viscosity ($p=0.006$, $F=8.4$) and energy content ($p<0.001$, $F=81.8$). Estimated means were closer together for viscosity (100 kcal: 48.2±5.4 500 kcal: 61.6±5.4) than for energy load (thin: 33.9±5.4 thick:75.8±5.4). Post hoc tests showed GE t50 was significantly higher under both the low viscosity condition ($p<0.001$, $F=43.0$) and the high viscosity condition ($p<0.001$, $F=38.9$). Gastric emptying time was significantly higher because of increased viscosity under the low energy load condition ($p=0.033$, $F=4.8$), but not under the high energy load condition ($p=0.065$, $F=3.6$). Significant effects of increasing energy load on GE t50 were an increase of 42.9 min under the thin condition, and of 40.9 min under the thick condition. Viscosity increased GE t50 by 14.5 min in the 100 kcal condition.

The curve for GER is shown in **Figure 4**.

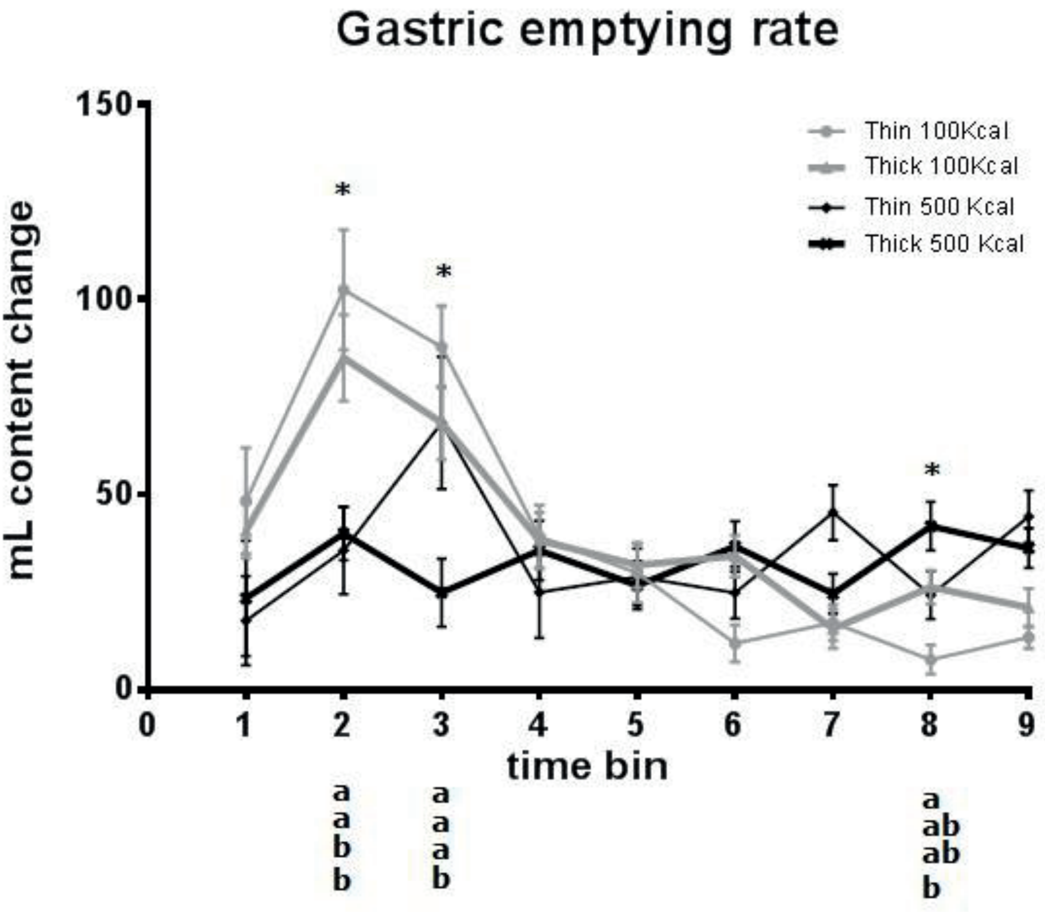


Figure 4. Mean volume difference between scans per shake. Error bars show SEM. Each time bin corresponds with 10 minutes and shows the difference between determined gastric content at the beginning and end of the 10 minutes in mL. Mixed model analysis showed significant differences in bin 2, 3 and 8 (marked with an * above the graph). Below the x-axis significant pairwise comparisons are shown, different letters means significant differences, the order of the letters follows the legend. n = 15

*Appetite ratings*

An overview of all AUC mean values and p values for the main effects can be found in **table 2**. Hunger was not significantly affected by viscosity or energy load. Fullness ratings over time can be seen in **Figure 5**. AUC for fullness was significantly higher after increasing viscosity ($p=0.007$, $F=8.1$, estimated means of the AUC under the thin condition were: 3976.3±264.6 and under the thick condition: 4718.6±264.6). The effect of increasing viscosity on fullness was an increase of 984.2 points on the AUC. For prospective consumption there was a significant interaction effect ($p=0.038$). Increases on prospective consumption ratings of energy load under the high viscosity condition ($p=0.043$, $F=4.4$) and increases on prospective consumption ratings of viscosity under the high energy load condition ($p=0.001$, $F=13.0$) were significant. The effect of increasing viscosity was an increase of 1129.9 points on the AUC. The effect of increasing energy load was an increase of 654 points on the AUC. For desire to eat there was a significant interaction effect as well ($p=0.026$). Post hoc analysis showed that desire to eat ratings were significantly decreased by increasing viscosity, but only under the high energy load condition ($p=0.018$, $F=6.1$). The effect of increasing viscosity was an increase of 1210.3 points on the AUC.

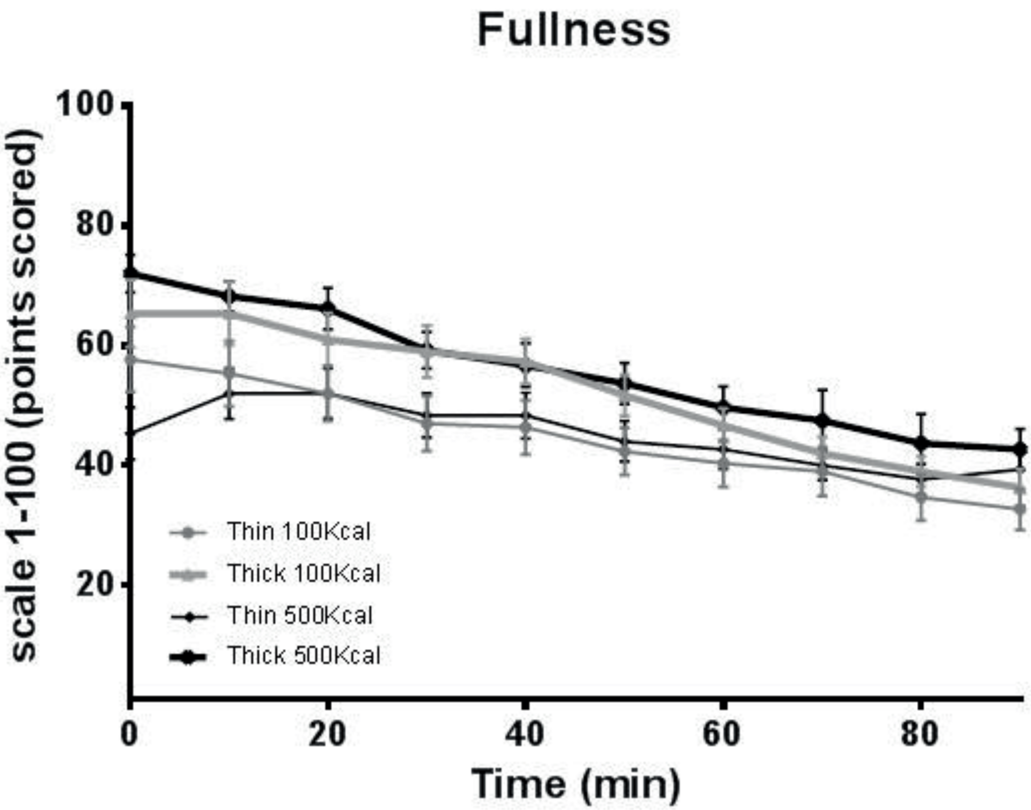


Figure 5. Fullness scores Mean±SEM fullness scores shown per time point. t=0 is the moment the stimulus has been consumed. n = 15.

Table 2. Appetite ratings area under curve over 90 minutes (mean ± *SD*)[1].

| Subjective response | Shake | | | | Viscosity | Energy load | Viscosity * energy load |
|---|---|---|---|---|---|---|---|
| | Thin 100 kcal | Thick 100 kcal | Thin 500 kcal | Thick 500 kcal | P[2] | p | p |
| **Hunger** | 4487±1645 | 4306±1624 | 4573±1253 | 3797±1723 | 0.071 | 0.544 | 0.065 |
| **Fullness** | 4063±1390 | 4774±1151 | 4108±1056 | 5071±1109 | 0.007 | 0.599 | 0.357 |
| **Prospective consumption** | 5258±1316[a] | 4851±1360[a] | 5346±1143[a] | 4299±1418[b] | 0.005 | 0.422 | 0.038 |
| **Desire to eat** | 5122±1585[ab] | 4691±1608[ab] | 5392±1554[a] | 4206±1725[b] | 0.248 | 0.633 | 0.026 |
| **Thirst** | 4375±1551 | 4776±1653 | 4373±1629 | 4531±1641 | 0.124 | 0.877 | 0.122 |

[1]Following the trapezoidal rule, AUC's were calculated per session over 90 minutes per participant and averaged.

[2] General linear mixed model analysis main effects. If interaction was significant post hoc Sidak tests were performed, significant difference is shown using different superscript lettering following the AUC score. n = 15.

*Ad libitum intake*

Participants consumed around 7 sandwich squares after the session, which corresponds with 1 and ¾ ham and cheese sandwich. There were no significant differences in intake of sandwiches between the treatments (effect of viscosity p=0.190, F=1.8; effect of energy load p=0.804, F=0.062) (**Table 3)**.

Table 3. Intake of sandwich squares 90 minutes after shake intake.

| | Thin 100 kcal | Thick 100 kcal | Thin 500 kcal | Thick 500 kcal |
|---|---|---|---|---|
| **Number sandwich squares[1] consumed, *mean±SD*** | 8.40±4.91 | 6.40±4.61 | 7.27±3.80 | 7.13±4.31 |
| **Energy of sandwich squares consumed, *kJ±SD*** | 2618±1529 | 1995±1438 | 2266±1186 | 2222±1345 |

[1]Mixed Model analysis yielded no significant differences between treatments. n = 15.

**Discussion**

In the current experiment we studied the effects of viscosity and energy load on gastric emptying and satiety. Increasing energy load leads to slower gastric emptying over time, increasing viscosity only significantly slowed emptying under the low energy load condition. Gastric emptying rate was very similar for the two 100 kcal conditions despite the substantial difference in viscosity. Viscosity did significantly change appetite ratings: fullness was scored higher and desire to eat was scored lower.

Confirming our hypothesis, we found that gastric emptying of a low energy load is quicker than that of a stimulus with higher energy load. This corresponds with a study of Marciani et al.[19]. Additionally, gastric emptying was delayed by increasing viscosity, albeit less effectively[21]. Our findings indicate that the difference in gastric emptying between a thin and thick low energy fluid can be completely explained by quicker drainage within the very first moments after consumption. If we correct for the draining in the beginning by looking at gastric emptying rate per 10 minutes, viscosity has little to no effect.

It is known that gastric sieving of water occurs, which can be mitigated by blending the water with the stimulus[26]. Blending a stimulus may influence both the energy load sensed in the duodenal of the emptied gastric content, as well as the viscosity of the content in the stomach. Because of this phenomenon it remains unclear whether this blending slows emptying by increasing energy load, increasing viscosity or both. Our research shows that increasing energy load by a factor 5, from 100 kcal to 500 kcal, slows gastric emptying significantly more than increasing viscosity by a much larger factor. Therefore, our results indicate that the gastric sieving effect is due to the sieved fluid, namely water, having a low caloric content. In the blended condition the fluid fraction will be much more caloric. These calories, regardless of viscosity can be the cause of delayed emptying.

The thin 100 kcal shake in our experiment showed an approximately 100 mL lower starting volume than the other three shakes. This lower starting volume was also apparent in the water control treatment. About 100 mL seeps through before gastric content is retained. The slope of the subsequent emptying curve seems very similar between the 100 kcal thin and thick shakes. This is also reflected in the gastric emptying rates, which appear to be very similar for the shakes of equal energy load. This suggests that in our experiment gastric emptying rate is not driven by viscosity, and that previously reported differences[27] might be due to 'gastric seeping' of the first millilitres of a stimulus which is both thin and contains a low energy load. This seeping through of the first 100 mL may also be an important effect to confound results in studies using indirect methods to assess differences in gastric emptying dynamics such as plasma acetaminophen concentration or $C^{13}$ labelling. Further research in this area is warranted to establish the effects of viscosity and energy density on this initial seeping through of gastric contents. Secondarily, it may be important in the future to measure gastric volume during ingestion as well, in order to 'catch' the content before it seeps through.

It has been suggested and it seems logical that slower gastric emptying increases feelings of satiety[4]. Clegg et al. found a larger AUC for fullness after consumption of a smooth soup as opposed to a chunky or solid meal[4]. They conclude on the basis of their gastric emptying data that this effect is due to longer gastric distension by the soup. Our results may suggest an alternative explanation for the satiating power of soup. We find that subjective ratings are more heavily influenced by viscosity than they are by energy load, which is most apparent in self-reported fullness. One would expect that perceived fullness follows gastric volume quite naturally. However, we find higher ratings for the thick 100 kcal shake as compared to the 500 kcal thin shake, even though the former is emptied much more quickly. For example, we find that both thick shakes and both thin shakes have comparable fullness ratings at 40 minutes after ingestion, however, on average the thick shakes are scored only about 8 points lower on a 100 point scale. When we look at the gastric content at t40, the 500 kcal shakes still have around 350 mL within the stomach, but the 100 kcal thick shake 250 mL, and the thin shake only 110 mL. Thus, the thin shakes yield similar fullness ratings, even though the stomach is filled with 350 mL in one condition, and 110 mL in the other.

Concurrently, we find low fullness ratings, without the post-prandial peak of the other stimuli for the thin 500 kcal shake. This well-known effect is often referred to in popular science as the *empty calories* in sodas. Not only do we find an *empty calories* effect of the thin 500 kcal shake, but we also find the other side of the coin: high fullness even though the shake is emptied quickly. Thus, increasing perceived thickness creates a kind of ‘phantom fullness’: a perceived feeling of fullness which is not congruent with actual gastric content. This hypothesis is supported by results from work with low calorie foams[28], which reduce appetite.

The link between ‘subjective fullness’ and ‘gastric fullness’ may thus be weaker than it sometimes appears in previous work[29]. The importance of effort and mouthfeel has been shown in multiple studies[30]. Hunger is suppressed more or for a longer period[31,32], intake is reduced[3,12] and through longer mastication satiety is increased[33,34]. We propose that taste and mouthfeel are perhaps contributing factors to subjective feelings of fullness. This may offer an alternative explanation of findings in earlier studies which concluded that greater satiety was caused by delayed gastric emptying[35]; both may have actually resulted from greater oral and sensory exposure of viscous stimuli. The importance of the perception in the mouth is underlined by a study which demonstrated that gastric emptying is only slowed down when stimuli are administered orally, and not when administered intragastrically[36]. A design utilizing sham-feeding showed that the oral exposure through sham-feeding in itself was not enough to delay gastric emptying[37]. In order to further understand this, similar paradigms with inclusion of hormone level measurements would be highly interesting. If taste and mouthfeel contribute as to fullness feelings as we propose, manipulating mouthfeel should change hormone levels such as CCK. Research with MRI requires a supine position. In our study participants remained completely stationary in their supine position for 90 minutes. This supine position means that gastric content flows slower through the pyloric sphincter, due to gravity not propagating flow as effectively as it would in a standing or sitting position. Our observed emptying may be slower than in a more natural position[38], which means that appetite scores and calculated emptying time may not transfer directly to the natural situation due to overestimation of the absolute effects. A second effect of the supine position is that observed emptying may represent more active emptying by gastric contractions.

We conclude that gastric emptying is slowed down by both increases in energy load and in viscosity. However, increasing energy load is the most important factor. Viscosity loses its retarding effect if energy load is increased to a meal-size 500 kcal. This indicates that viscosity may not affect satiety and satiation through delaying gastric emptying. We find significant changes in subjective ratings due to viscosity, indicating an increased satiation and satiety are affected by viscosity through mouthfeel and oral exposure. Moreover, participants show no tendency to compensate for the caloric intake during the meal afterwards. A thick shake containing 100 kcal will yield higher fullness ratings and lead to similar intake when compared to a thin shake containing 500 kcal. We may find use for this 'phantom fullness' effect, i.e., a sense of fullness and satiation caused by the taste and mouthfeel of a food which is irrespective of its caloric content. Thick low caloric liquid meals may leave people to feel fuller and satiated, preventing compensatory overconsumption afterwards.

**Acknowledgements**

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GC, MM, CdG and PAMS declare no potential conflicts of interest in conducting the research.

GC, MM, KG and PAMS designed the study, GC conducted the research, GC performed parameter extraction, GC and MM performed statistical analysis; GC wrote the paper MM, CdG and PAMS provided feedback, PAMS had primary responsibility for final content.

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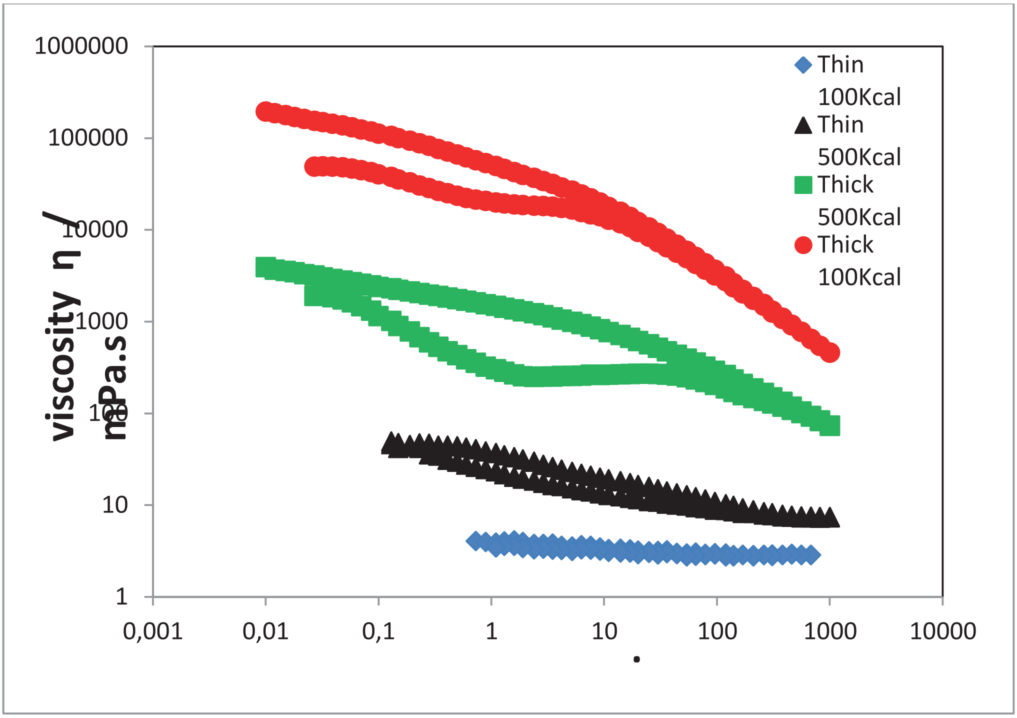
Figure 1. Shear rates of the shakes




The flow behaviour of the four shakes was determined using a rheometer (Anton Paar MCR 502 equipped with concentric cylinders). Viscosities were measured first at shear rates increasing from 0.01 $s^{-1}$ to 1000 $s^{-1}$ and then at shear rates decreasing from 1000 $s^{-1}$ to 0.01 $s^{-1}$ at 37$^0$C. Sixty measurements points were recorded for each shake with a measurement duration of 10 s per point. The supplement file shows the flow curves of the four shakes. The viscosity differed greatly between the four shakes. The 100Kcal/thin shake was the least viscous sample which displayed the lowest viscosity of all samples. At any shear rate, the 500Kcal/thin shake displayed a higher viscosity than the 100Kcal/thin shake and a lower viscosity than the 500Kcal/thick shake. At any shear rate, the 500Kcal/thick shake displayed a higher viscosity than the 500Kcal/thin shake and a lower viscosity than the 100Kcal/thick shake. The 100Kcal/thick shake was the most viscous sample which had the highest viscosity of all samples at any shear rate.

Figure 2. Gastric volume including the water condition (n=5)

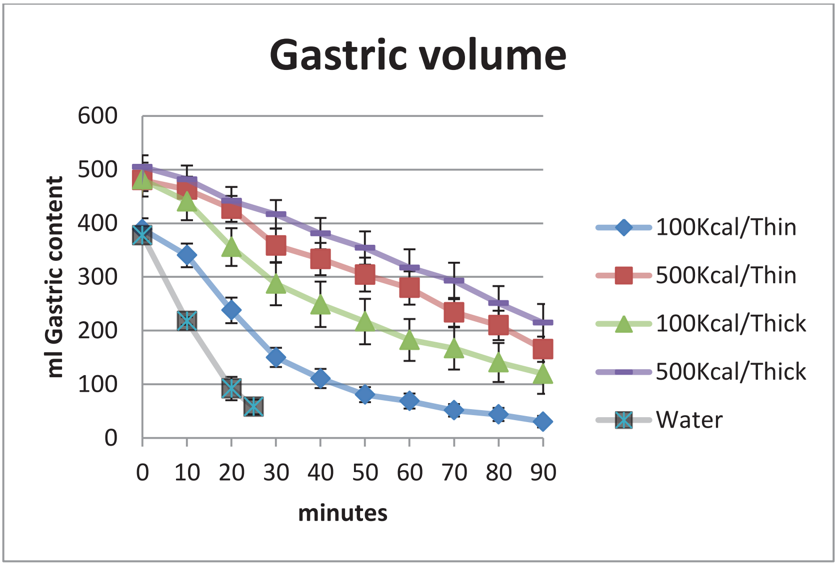
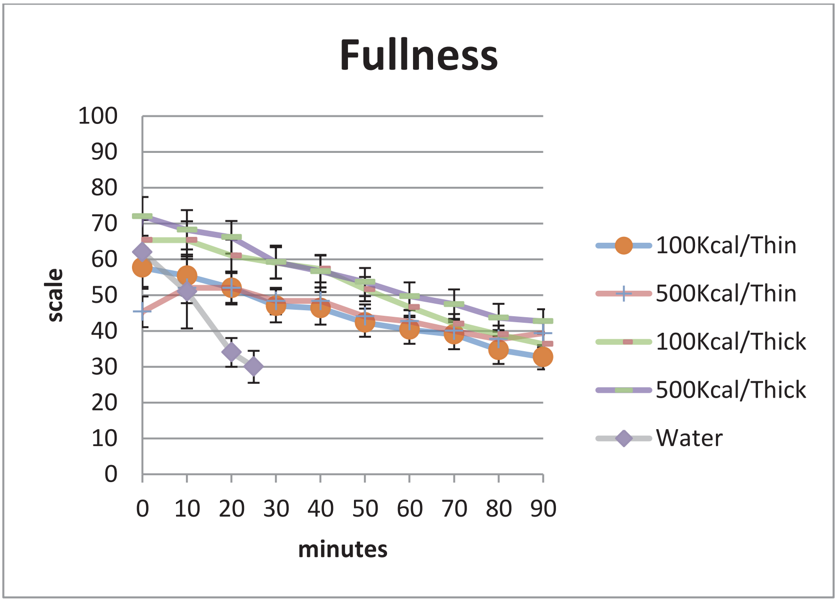



Figure 3. Fullness ratings including the water condition (n=5)



Known by day as a simple PhD student in Wageningen.

He reveals his secret identity...

**Beware Phantom Fullness, for here is MRI-Man!!!!**

# Chapter 4.

A tale of gastric layering and sieving: gastric emptying of a liquid meal with water blended in or consumed separately

Guido Camps
Monica Mars
Kees de Graaf
Paul AM Smeets

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**Abstract**

**Background:** The process of gastric emptying determines how fast gastric content is delivered to the small intestine. It has been shown that solids empty slower than liquids and that a blended soup empties slower than the same soup as broth and chunks, due to the liquid fraction emptying more quickly. This process of 'gastric sieving' has not been investigated for liquid foods.

**Objective**: To determine whether gastric sieving of water can also occur for liquid foods.

**Method**: Two groups of men participated in a parallel design (n = 15, age 22.6±2.4y, BMI 22.6±1.8kg/m$^2$, and n = 19, age 22.2±2.5 y, BMI 21.8±1.5 kg/m$^2$) and consumed an isocaloric shake ( 2093 kJ, CARBOHYDRATES: 71g, FAT: 18g PROTEIN:34g ), either in a 500-mL version (MIXED) or as a 150-mL shake followed by 350 mL water (SEPARATE). Participants provided appetite ratings and were scanned using MRI to determine gastric emptying rate and volume at three time-points within 35 min post ingestion.

**Results:** Gastric emptying the percentage emptied in 35 min was significantly smaller for MIXED (29±19%) than for SEPARATE (57±11%, p<0.001).

**Conclusion**: In the present study we show that gastric sieving can occur for liquid foods; water is able to drain from the stomach while a layer of nutrient rich liquid is retained. In indirect gastric emptying measurements, the behavior of labelling agents may be affected by the layering and confound emptying measurements.

## Introduction

Gastric emptying is an important part of digestion, as the stomach acts as a gatekeeper distributing nutrients to the small intestine, in order to optimize digestion. The dynamics of stomach content flow and passage are still not completely understood. Gastric emptying was studied in relation to food qualities in animals, namely canines, in the seventies. This work showed that solids empty slower than liquids[1,2].

Subsequent work from the nineties showed congruent results in humans; the liquid fraction of the gastric content was dispersed quickly and drained quickly as well, with solid pieces being retained for longer periods in the stomach[3]. Additionally, Collins et al. showed differences in gastric content in the proximal and distal parts of the stomach. A subsequent study compared a solid meal given together with water versus a homogenised soup-like stimulus made out of the same ingredients[4]. In this study, the homogenized version yielded significantly higher feelings of fullness and slower gastric emptying. The authors attributed this to greater distension of the antral region by the homogenized version in combination with the slower emptying. Rolls et al. extended this by showing that subsequent intake can be reduced by incorporating water into a food dish, instead of serving water concurrently with the dish[5].

In 2012 it was shown - using MRI - that a possible mechanism for this greater satiety resulting from incorporated water is caused by the fact that when the solid and water fractions are not homogenized, the water sieves from the gastric content and empties quickly[6]. This gastric sieving can be prevented by blending the two fractions together to create a homogeneous caloric food. Additionally, emulsifiers have been used to manipulate the dispersion of fats throughout the gastric content and thereby manipulate gastric emptying[7].

Many commercial meal replacements in liquid form are currently available. Liquid meals are also often used in studies because they offer practical benefits over solid meals, for example when feeding must take place at standardised rates or through a tube. Although gastric sieving of water has been shown with solid and semi-solid foods, it has to our knowledge never been investigated using a liquid meal. MRI is optimal measurement method understanding gastric passage of liquid meals and the interaction with water consumption. Understanding this passage will help create novel experimental designs in the future.

In this research we sought to determine whether gastric sieving of water can also occur with liquid meals. We hypothesized that the stomach empties more quickly when water is consumed separate from a liquid meal, as compared to when water is consumed as part of a liquid meal (mixed in). We also hypothesized that mixed consumption will suppress appetite more.

**Participants and Methods**

*Design*

The design for this analysis is a two sample comparison between two treatments. Participants participated in one of two larger trials (Dutch Trial Register (NTR4573 (published:[8]) and NTR5507)).

*Test products*

An overview of the nutrient content of the test products can be found in **Table 1**.

The shake consisted of 50 g cream (Albert Heijn B.V, Zaandam, The Netherlands), 53 g Fantomalt (Nutricia®, Cuijk, The Netherlands), 30 g whey powder (Whey Delicious Vanilla, XXL Nutrition, Helmond, The Netherlands) and 8 g vanilla-sugar (Dr.Oetker®, Bielefeld, Germany). These ingredients were used to create a liquid shake either with 100 g of water or with 450 g of water (SEPARATE or MIXED). The shakes were mixed by adding the ingredients into a closed 600-mL beaker and whisking with an internal spherical whisk (diameter 3.5 cm). In case of the smaller shake, 350 g of water was consumed directly after finishing the shake.

Table 1. Energy content and nutrient composition of the shakes.

| | MIXED | SEPARATE |
|---|---|---|
| **Ingredients, per 100g shake** | | |
| **Protein powder, *g*** | 5.1 | 12.4 |
| **Cream, *g*** | 8.5 | 20.8 |
| **Dextrin-maltose, *g*** | 8.9 | 21.9 |
| **Vanilla sugar, *g*** | 1.3 | 3.3 |
| **Water, *g*** | 76.2 | 41.6 |
| **Total, *g*** | 100 | 100 |

| | | |
|---|---|---|
| **Nutrients, 100g shake** | | |
| **Energy[1], *kJ*** | 418 | 1393 |
| **Carbohydrates, *g*** | 12 | 40 |
| **Of which mono- and dissacharides** | 2.1 | 7 |
| **Fat, *g*** | 3 | 10 |
| **Of saturated** | 2.1 | 7 |
| **Protein, *g*** | 6 | 20 |
| **Fiber, *g*** | 0.5 | 1.7 |
| | | |
| **Total ingested** | | |
| **Shake weight, *g*** | 591 | 241 |
| **Amount of water served after shake, *g*** | 0 | 350 |
| **Shake energy, *kJ (kcal)*** | 2093 *(500)* | 2093 *(500)* |
| **Total volume, *mL*** | 500 | 500 |

[1]Nutrient composition of the shake resembles a mixed meal, with 50% of the energy load coming from carbohydrates, 30% from fats and 20% from protein.

*Participants*

Two groups of healthy men, SEPARATE (n = 15, age 22.6±2.4 y, BMI 22.6±1.8 kg/m$^2$) and MIXED (n = 19, age 22.2±2.5 y, BMI 21.8±1.5 kg/m$^2$), participated. There were no participants partaking in both trials. Participants were recruited via email. To be eligible, participants had to be male, healthy and 18-35 years old. Potential participants were screened to be of normal weight (BMI 18.5-25.0 kg/m$^2$) and willing to comply with the study procedures. By use of a questionnaire, potential participants were excluded if they had a self-reported hypersensitivity for any components which were present in the test products, if they were on a diet or had unexplained weight loss or gain in the past months, or if they reported any MRI contraindications. The procedures were approved by the Medical Ethical Committee of Wageningen University in accordance with the Helsinki Declaration of 1975 as revised in 2013. Written informed consent was obtained from all participants.

*Study procedures*

Participants were instructed to have a light meal the night before the session and not do any rigorous exercise or strenuous activities that day. Participants were instructed to fast for at least three hours, only drinking water in that time and nothing during the last hour before each session. After arrival participants provided baseline appetite ratings, that is ratings of hunger, fullness, prospective consumption, desire to eat and thirst. These were orally scored from 1 – 100 points[9].

Subsequently participants were scanned for baseline stomach content (to confirm it was empty). After this, participants exited the scanner and consumed the shake (MIXED) or shake and water (SEPERATE).

Each participant consumed the shake within 2 minutes as instructed. After that, they were positioned in the scanner and provided appetite ratings via the intercom and underwent gastric MRI scans up to 40 minutes after ingestion. For MIXED scans and scores were obtained directly after ingestion, at 10, 20, 30 and 40 min. Data for for 30 and 40 min were interpolated. For SEPARATE scans and scores were obtained directly after ingestion and at 15 and 35 min.

*MRI*

Participants were scanned with the use of a 3-Tesla Siemens Verio (Siemens AG, Munich, Germany) MRI scanner using a $T_2$-weighted spin echo sequence (HASTE, 24 6-mm slices, 2.4 mm gap, 1.19 x 1.19 mm in-plane resolution, effective TE of 87ms with parallel imaging (grappa, factor 2), with breath hold command on expiration to fixate the position of the diaphragm and the stomach. The duration of one scan was approximately 19 seconds. Syngo fastView MRI software (Siemens AG, Munich, Germany, http://www.healthcare.siemens.com/medical-imaging-it/syngo-special-topics/syngo-fastview) was used to manually delineate gastric content on every slice. Gastric volume on each time point was calculated by multiplying surface area of gastric content per slice with slice thickness including gap distance, summed over the total slices showing gastric content.

*Statistical analyses*

AUC over 35 minutes was calculated for subjective ratings and gastric content using Graphpad Prism 5 (Graphpad Software, La Jolla, USA), following the trapezoidal rule. Change in appetite ratings was tested using an ANOVA with time, treatment and the interaction as fixed factors and subject as random factor and baseline measurement as a covariate. Post hoc LSD corrected test were performed in case of significant effects. Differences between the AUC values were tested using a t-test. Emptying percentage of the gastric content in 35 minutes was calculated by correcting for baseline and dividing the content at 35 minutes by the starting volume. The difference between percentage emptied in this period was tested using a t-test. Significance level was set at $p=0.05$. Data are expressed as mean±SD. All tests were performed using IBM SPSS 22.0 (IBM, Armonk, USA).

**Results**

*Gastric content*

Gastric emptying curves for both treatments of the meal can be found in **Figure 1**. **Figure 2** shows example MRI images from both treatments. Percentage emptied in 35 minutes was significantly smaller for MIXED (29±19% ) compared to SEPARATE (57±11%, $p<0.001$). **Table 2** shows the gastric content and percentage at each timepoint.

MIXED AUC ( 18,813±3,170mL.min$^{-1}$) was significantly greater than SEPARATE AUC (13,170±1,708 mL.min$^{-1}$., $p=0.035$).

Table 2. Gastric content per timepoint.

| **Time** | **MIXED, *mL±SD*** | **% emptied** | **SEPARATE, *mL±SD*** | **% emptied** |
|---|---|---|---|---|
| **Baseline** | 26±27 | | 18±15 | |
| **2** | 480±77 | 0 | 543±37 | 0 |
| **10** | 463±89 | 4% | | |
| **15** | | | 391±82 | 28% |
| **20** | 427±89 | 11% | | |
| **30** | 358±119 | 25% | | |
| **35** | | | 229±50 | 58% |
| **40** | 346±113 | 28% | | |

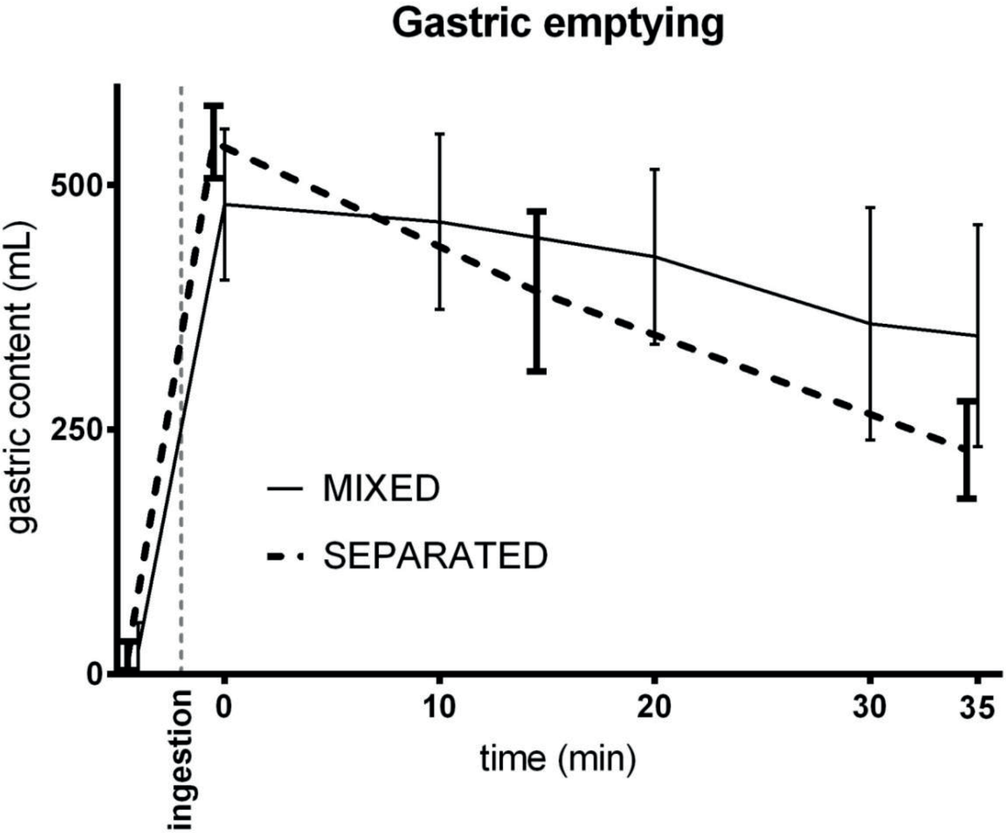


Figure 1. Gastric emptying curves for both treatments are shown over 35 minutes. SEPARATE empties significantly more quickly over 35 minutes.

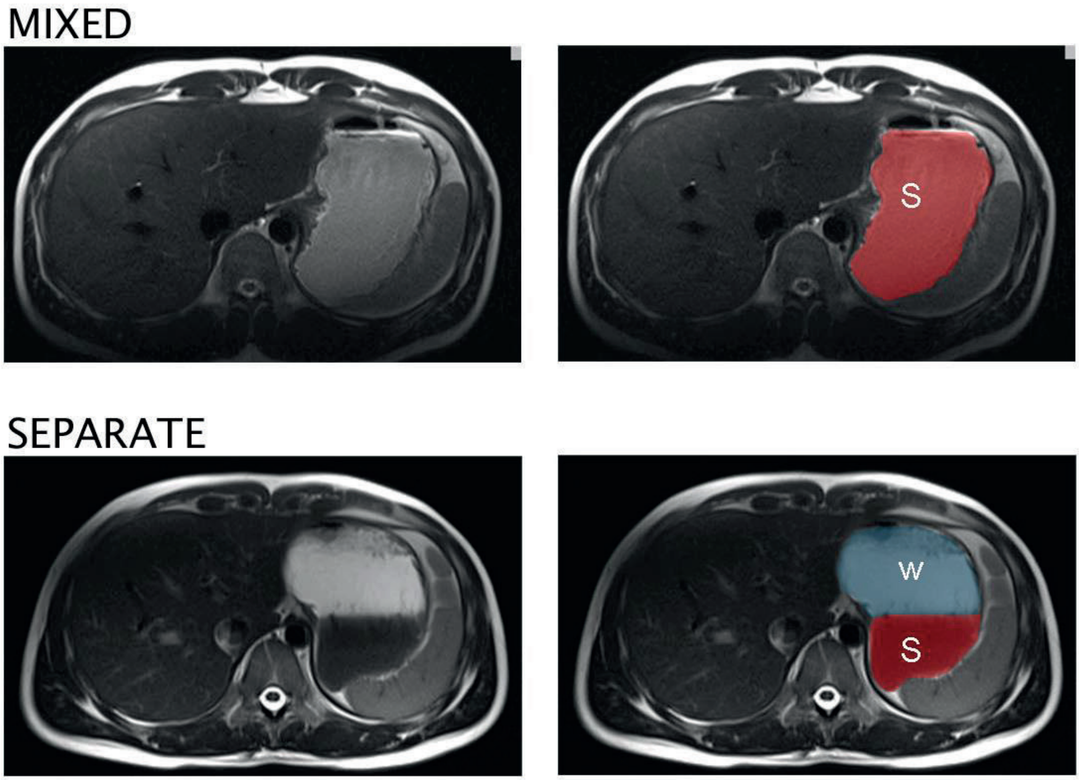


Figure 2. Example gastric MRI images. Shown are transverse slices at the height of the liver, directly after ingestion. Left: original images. Right: the same slices as shown on the left but with the gastric content highlighted manually based on signal strength as indicated by colorization. S: shake fraction. W: water fraction.
Water yields high signal in a $T_2$-weighted scan, and therefore appears bright. In the MIXED condition a uniform coloration of the gastric content can be observed. In the SEPARATE condition, owing to the watery content, a whiter layer can be observed on top of the shake layer.

*Subjective ratings*

None of the subjective ratings were significantly different between treatments when comparing AUCs.

Appetite curves for both treatments can be found in **Figure 3**.

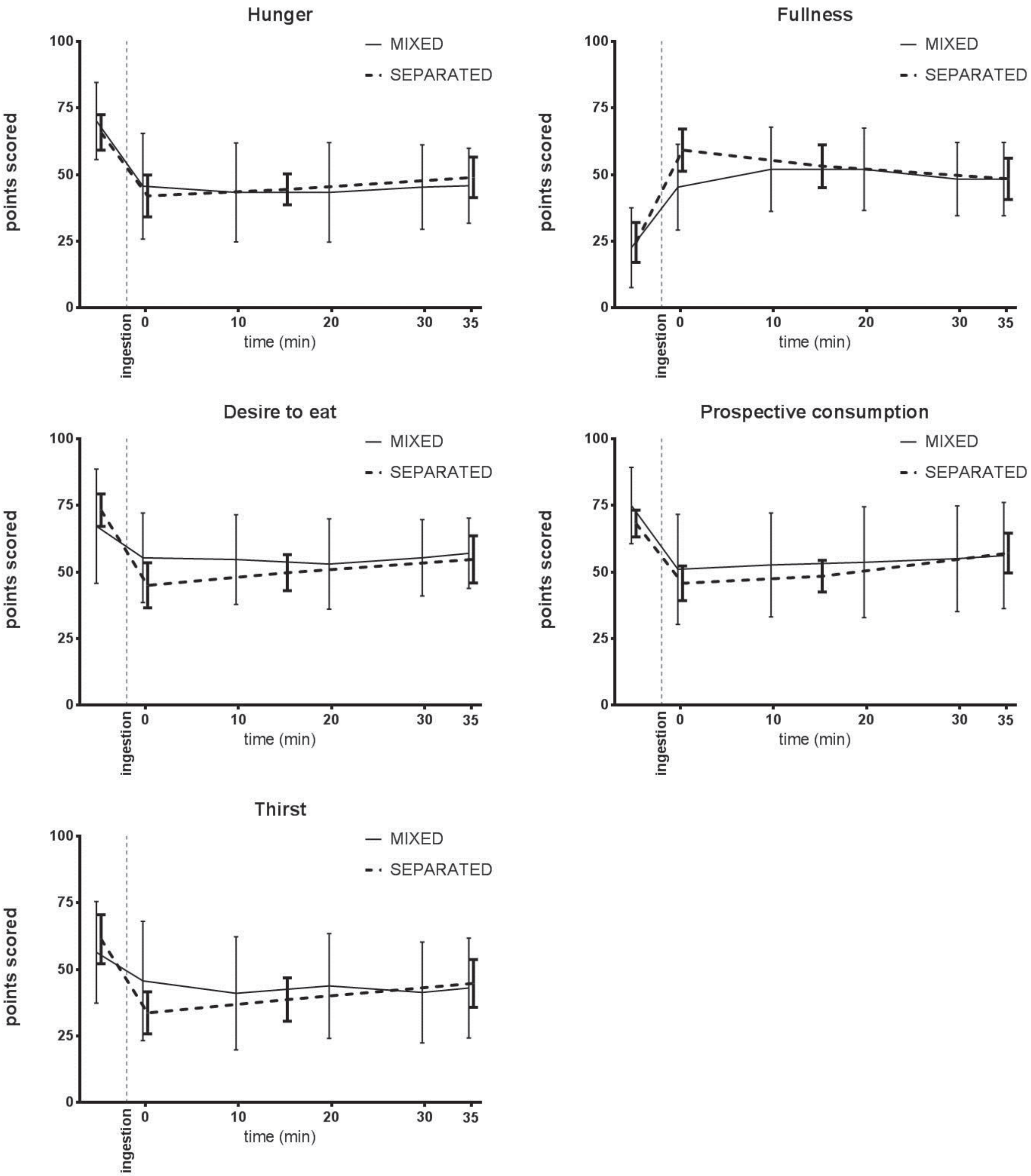


Figure 3. Appetite curves for both treatments are shown over 35 minutes. There were no significant differences between treatments.

## Discussion

In the current analyses we aimed to show gastric sieving of water with liquid gastric food content. We found that a liquid meal followed by a drink of water empties about twice as fast in the first 35 minutes compared to the same amount of water incorporated within the liquid meal. Despite dissimilar emptying rates we found similar changes in appetite feelings.

Our results show, in line with our hypothesis, that 'gastric sieving' can also occur with a liquid food. It has been shown recently that a nutrient rich filling of the stomach which absorbs water (bread), leads to a greater delay in gastric emptying than one which does not absorb water as readily (rice)[10]. We observed that if liquid food and water do not mix in the stomach, a layer rich in nutrients including fat, can remain separate. That watery, low nutrient layer can subsequently drain from the stomach. This prevents the nutrient content from entering the duodenum and delaying gastric emptying, thereby creating a relative increase in gastric emptying when compared to the mixed shake. Gastric MRI images taken after ingestion allow us to clearly discern the layer of water beneath the nutrient rich liquid in the stomach (**Figure 2**). This finding strongly resembles research from 2013 in which different gastric layers were observed for an isocaloric liquid and semi-solid meal[11].

SEPARATE had a higher post ingestive volume than MIXED. We attribute this to the fact that the smaller shake volume is thicker and thereby contains more air bubbles, which yields a slightly larger starting volume. The volume increase for SEPARATE was 24 ± 22 mL air, which corresponds to a ratio of 0 to 25%. This larger volume may have stimulated gastric emptying. However, this difference in starting volume is relatively small and further research is necessary to determine whether this could explain the observed difference in emptying.

For SEPARATE there was a larger variance of gastric content than for MIXED. This could be due  differences in stomach anatomy between participants. Stimulation of the antral region of the stomach by the nutrient rich layer may be more or less pronounced depending on the anatomical shape of the stomach. This would be an interesting avenue for further research.

We did not find any significant differences in appetite ratings between the two treatments, with the exception that if water is consumed separately it quenches thirst more than when incorporated within a shake. The lack of appetite suppression may be due to there not being an effect, or to the fact that significant differences will only occur at a later moment and that our measurement window was simply too short to observe these differences. The latter seems plausible as effects on satiety have been reported in other work with longer measurement windows[6]. Alternatively, the fact that we did not find any difference might be due to the fact that both stimuli were liquid, yielding smaller differences.

These results show significant differences in the emptying time between the treatments when comparing two groups in a parallel design. However, a limitation of this work is that we compare between different groups. Differences between the individual participants may have confounded the results.

Ideally, the work would have been carried to using one sample and a crossover design, making MRI scans at the exact same time. In future work, the data collection period should be lengthened, as this will give more insight in the complete emptying curve. Though the studied period was long enough to find very significant differences,  additional measurement points will allow better analysis of the emptying curve.

Gastric content at different time points has been used in other publications to fit an emptying curve and thereby estimate the gastric emptying t[5012]. In this study the number of data points was not sufficient to do curve fitting. However, we feel that using the AUC of the emptying curve for this specific comparison is justifiable since in similar studies the largest differences in emptying occur within the first 35 minutes[6,11].

Participants were scanned while remaining in a supine position. Therefore, gastric emptying rate may have been slower than in a standing or sitting position[13]. However, we conject that relative differences will be similar, although the overall emptying may be slower than that in an upright position.

Additionally, in other experiments positioning of the subject in a supine position was accompanied with adjustments with a pillow to manipulate the pyloric and antrum location. This prevents a delivery of the rich top fatty layer into the duodenum or stimulating the antrum leading to slowed gastric emptying[14-16]. In our experiment both treatments had the same completely supine position, and the position was not adjusted using a pillow, which may increase the effect of the layering.

In the design for SEPERATE we purposefully had the participants drink the calorie rich shake first, because we feared that if water was consumed first it may seep through the stomach as gastric emptying is mainly determined by nutrient load[17,18]. The sensing of calories in the duodenum will retard emptying and thereby make sure that the following water does not directly pass.

We conject that because the shake is different in density and viscosity as compared to water, it stays separated from the water fraction. This is potentially mitigated by the emulsification of the cream in the shake and its additives. It would be possible that if the two fractions are more similar in composition the layers would not stay separated.

The gastric layering with fluid we and others have observed may explain differences between gastric emptying measuring methods[19]. Indirect gastric emptying measurement methods such as isotope labelling and acetaminophen plasma levels can be confounded if the measured agent is in either layer: underestimation of emptying time if it is in the water fraction, and overestimation of emptying time if it is in the nutrient rich layer. This implies that if a research paradigm includes both nutrient ingestion and drinking water or there is a potential for post-ingestive gastric layering, MRI measures or MRI piloting is advisable. MRI allows us very detailed insights of the emptying process, which may lead to new avenues for strategies to influence appetite.

We showed using MRI that gastric sieving, which has been demonstrated for solid and semi-solid foods, can also occur in liquids. We attribute this to gastric sieving of water from the stomach, by water draining from above a layer of nutrient rich liquid. This can have large effects on gastric emptying rate, even when the total nutrient content of the gastric load is the same. Also, this suggests that indirect gastric emptying measurements may be affected by gastric layering.

**Acknowledgements**

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GC, MM, CdG and PAMS designed the study, GC conducted the research, GC performed statistical analysis; GC, MM, CdG and PAMS wrote the paper, PAMS had primary responsibility for final content.

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I'VE BEEN EXPECTING YOU, MRI-MAN...
SO WE FINALLY MEET, PHANTOM FULLNESS!

# Chapter 5.

Just add water: effects of added gastric distention by water on gastric emptying and brain activity

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Monica Mars

Kees de Graaf

Paul AM Smeets

Submitted for publication

**Abstract**

**Background:** Gastric distention contributes to meal termination. There is little research on the neural correlates of gastric distention by food. Moreover, to date, neural measures have not been done concurrently with measurements of gastric distention and emptying.

**Objective:** To study 1) how offering a small versus a large water load following a standardized meal affects gastric distention over time and 2) to assess associations between appetite experiences and brain activity and the degree of gastric distention.

**Method:** 19 healthy males (age 22.2±2.5 y, BMI 21.8±1.5 kg/$m^2$) participated in a randomized crossover study with two treatments: ingestion of a 500-kcal 150-mL liquid meal shake followed by either a low (LV, 50 mL) or a high volume (HV, 350 mL) water load. At baseline and at three times after ingestion appetite feelings were scored, MRI scans were made to determine total gastric content volume (TGV) and functional MRI scans were made to measure cerebral blood flow (CBF).

**Results:** TGV was significantly higher for HV compared to LV at all three time points ($p < 0.001$) with a relative increase of 292±37 mL directly after ingestion, and of 182±83 mL at t=15 min and 62±57 mL at t=35 min. Hunger AUC decreased ($p = 0.002$) and fullness AUC increased ($p = 0.045$) significantly for HV compared to LV. Overall, ingestion increased CBF in the inferior frontal gyrus and the insula . There were no differences in brain activation between treatments.

**Conclusion:** Performing concurrent gastric MRI and fMRI measurements is a promising method to investigate neural correlates of gastric distention. Increased distention by 300 mL water added to a 150-mL 500-kcal load did not induce significantly greater brain activation. Future research should further examine the role of the inferior frontal gyrus in ingestion.

## Introduction

In the effort to better prevent obesity, one strategy may be to limit caloric intake. To limit caloric intake we could strive to promote earlier meal termination (satiation)[1]. Satiation can be increased by increased stomach distention[2]. For example, when the stomach is distended with a water filled intragastric balloon meal intake is reduced[3].

Wang et al. aimed to find neural correlates of gastric distention using fMRI by inflating and deflating an intragastric balloon with water[4]. They observed that distention induced brain activation in satiety related brain areas, such as the amygdala and insula.

In work comparing naso-gastric infusion and ingestion of a liquid nutrient stimulus, Spetter et al. showed differences in brain activation and hormone responses between gastric infusion and ingestion (i.e., gastric plus oro-sensory stimulation) of the same 500-mL load using fMRI[5,6]. Normal ingestion increased activity in the thalamus, amygdala, putamen and precuneus compared to matched gastric infusion of the same load. However, gastric emptying was not assessed in this work. This would provide more detailed information on stomach distention and the rate of gastric emptying.

Gastric distention with a balloon and naso-gastric infusion are suitable tools to investigate orosensory and gastric contributions to satiation. However, they are not very naturalistic and the results obtained with such approaches may have limited ecological validity. Also, such approaches do not provide ways to enhance satiation and thereby limit energy intake. Therefore, researchers have used food manipulations to increase gastric distention such as the incorporation of air[7], water[8] and the addition of gelling fibers[9]. However, this introduces a possible confounder, as incorporated air or water inevitably affect the texture of the test food and the eating speed, both of which have been shown to affect satiation[10-16].

The presence of calories slows gastric emptying[17,18]. Therefore, a possible way to control both orosensory exposure as well as manipulate gastric distention would be to introduce a caloric load first, and then manipulate gastric distention with water. Using water loads is not new: increased water consumption during the day or with a meal has been shown to increase satiety[19-21]. What we propose is more specific: the caloric load is ingested first, allowing the subsequently added water to increase stomach distention. A benefit of this approach would be that the eating speed and associated orosensory experience is similar. Additionally, if the water remains separated from the nutrient load intragastrically it would not change caloric density of the nutrient liquid and only add pure distention. Water taken with a meal may sieve from the stomach[22], however, this would still create added distention for some time.

The primary aim of this study was to assess how offering a small versus a large water load following a standardized meal affects gastric distention over time. The secondary aim was to examine the associations between subjective appetite feelings, brain activity and gastric distention.

**Participants and Methods**

*Design*

Participants came to our facilities 2 times in a randomized crossover design. Each participant was always scanned on the same time in the morning after an overnight fast. Participants were offered a standardized liquid meal followed by either a small (50 mL, LV) or a large (350 mL, HV) water load in a random and balanced order.

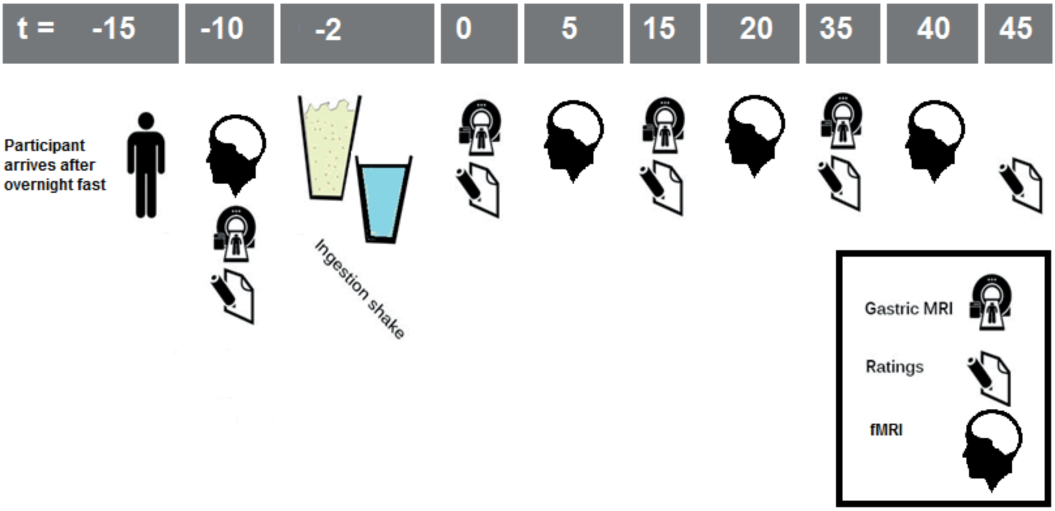



Figure 1. Overview of one experimental session for one participant.

*Participants*

19 healthy males (age 22.6±2.4 y, BMI 22.6±1.8 kg/m$^2$) participated. They were recruited by website and flyers around the campus of Wageningen University. A flowchart of the study can be found in **Supplemental Figure 1** Inclusion criteria were: being male, aged between 18 and 35 y, having a BMI between 18 and 25 kg/m$^2$, being of self-reported good general health, willing to comply with study procedures, willing to be informed of incidental findings. Exclusion criteria were: unexplained weight loss or gain of >5 kg in the last two months, oversensitivity to any of the food items used in the

experiment, any reported pathologies relating to the gastrointestinal tract which might influence results, use of any medications which may influence gastrointestinal function, having any contraindications for undergoing an MRI, not signing the informed consent form, and being employed or studying at the Division of Human Nutrition at Wageningen University. Potential participants filled out an inclusion questionnaire to screen for eligibility. Subsequently, they attended a screening meeting that included measurement of weight and height and explanation of the study procedures. During screening, potential participants participated in a mock scanner trial to familiarize them with the scan procedures[23]. Participants were unaware of the exact aim of the study; they were only informed about the fact that we were investigating the digestive system and brain activation.
The study procedures were approved by the Medical Ethical Committee of Wageningen University and in accordance with the Helsinki Declaration of 1975 as revised in 2013 (NL48059.081.14). The study was registered with the Dutch Trial Registry under number NTR4573. Results from the HV condition were used in[24].
Written informed consent was obtained from all participants.

*Stimulus*

The stimulus consisted of a meal shake and a water load, both offered in a paper cup. The ingredients of the shake were 50 g cream (AH Basic, Albert Heijn B.V. Zaandam, the Netherlands), 53 g dextrin-maltose (Fantomalt Nutricia®, Cuijk, the Nederlands), 8 g vanilla sugar (Dr.Oetker®, Amersfoort, the Nederlands), 30 g whey powder (Whey Delicious Vanilla, XXL Nutrition, Helmond, the Netherlands), and 100 mL water (**Table 1**). The macronutrients of the shake resembled a mixed meal in which 50% of the energy came from carbohydrates, 30% from fat and 20% from protein. Shakes were mixed in a container with an internal whisking ball for approximately 30 seconds. All shakes were prepared about 15 minutes before intake, and offered at ~18 ºC. The shake was consumed normally from a cup within 40 sec. The subsequent water load was 50 mL for LV and 350 mL for HV.

Table 1. Energy content and nutrient composition of the shake.

| Ingredients, per 100g shake | |
|---|---|
| **Protein powder, *g*** | 12.4 |
| **Cream, *g*** | 20.8 |
| **Dextrin-maltose, *g*** | 21.9 |
| **Vanilla sugar, *g*** | 3.3 |
| **Water, *g*** | 41.6 |
| **Total, *g*** | 100 |
| | |
| Nutrients, per 100g shake | |
| **Energy[1], *kJ*** | 1,393 |
| **Carbohydrates, *g*** | 40 |
| **Of which mono- and dissacharides** | 7 |
| **Fat, *g*** | 10 |
| **Of which saturated** | 7 |
| **Protein, *g*** | 20 |
| **Fiber, *g*** | 1.7 |
| | |
| Total ingested | |

| | |
|---|---|
| **Shake weight, *g*** | 241 |
| **Shake volume[2], *mL*** | 150 |
| **Shake energy, *kJ (kcal)*** | 2,093 *(500)* |

[1]Nutrient composition of the shake resembles a mixed meal, with 50% of the energy load coming from carbohydrates, 30% from fats and 20% from protein.
[2]The shakes for the two treatments were completely similar, but followed by either 50 mL of water (LV) or 350 mL of water (HV), making total consumed volume 200 mL or 500 mL.

*Scan session procedures*

See **Figure 1** for an overview of the session. After arrival participants provided baseline appetite ratings. Following this, a baseline scan for stomach content and a baseline perfusion fMRI scan of 5 minutes was performed. After this, participants exited the scanner and first consumed the shake, followed by the water load, within 2 minutes. After consumption, participants were positioned in the scanner and stayed there for approximately 50 minutes. During this time we performed gastric MRI scans and obtained appetite ratings at 2, 15 and 35 minutes post ingestion. At 5, 18 and 40 minutes post ingestion we performed perfusion fMRI scans with a duration of 5, 8 and 5 minutes, respectively. The MRI body coil and 32-channel head coil were exchanged during the session in order to scan both sequences.

*Appetite ratings*

Subjective appetite ratings were given via the intercom. Participants verbally scored hunger, fullness, prospective consumption, desire to eat and thirst between 1 and 100 points[25].

*Gastric volume*

Participants were scanned with the use of a 3-Tesla Siemens Verio (Siemens AG, Munich, Germany) MRI scanner using a $T_2$-weighted spin echo sequence (HASTE, 24 6-mm slices, 2.4 mm gap, 1.19 x 1.19 mm in-plane resolution), with breath hold command on expiration to fixate the position of the diaphragm and the stomach. The duration of one scan was approximately 18 seconds. A custom tool created in MeVisLab (MeVis Medical Solutions AG, Bremen, Germany) was used to manually delineate gastric content on every slice[26]. Gastric content volume on each time point was calculated by multiplying surface area of gastric content per slice with slice thickness including gap distance, summed over the total slices showing gastric content. The different layers within the stomach were segmented separately and then summed to determine total gastric volume (TGV) (**Figure 2**).

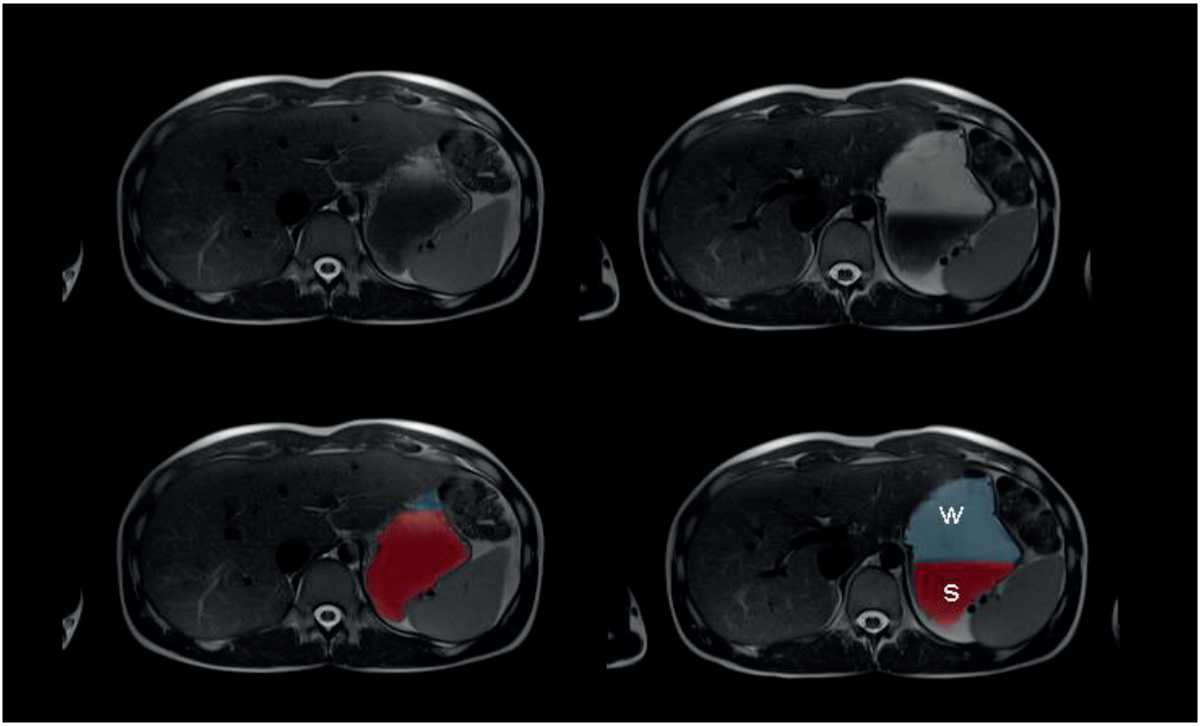


Figure 2. MRI images of transverse slices at the height of the liver, shortly after ingestion of the shake. Left: original images. Right: the same slices as shown on the left but with the gastric content delineated manually based on signal strength as indicated by colorization. Water yields high signal in a T2 weighted scan, and therefore appears bright. Owing to the watery content a higher signal yielding, and thus whiter, layer can be observed above the nutrient rich layer beneath.In the HV image the nutrient rich fraction has been marked with an S and the water fraction with a W.

*Brain activity- Data acquisition*

Cerebral blood flow (CBF) was measured with a 32-channel head coil using perfusion MRI[27] on the same scanner. Images were obtained with a PICORE Q2T ASL sequence, using a frequency offset corrected inversion pulse and echo planar imaging readout for acquisition. A total of 19 axial slices were acquired in ascending order. Each measurement consisted of 100 alternating tag and control images with the following imaging parameters: inversion time (TI), TI1 = 700 ms, TI2 = 1800 ms, repetition time = 2800 ms, echo time = 13 ms, in plane resolution = 3 × 3 mm, field of view = 192 × 192 mm, and flip angle = 90°, with a slice thickness of 5 mm. The first image of the series was used to estimate the equilibrium magnetization of the blood (M0B) to allow for absolute Cerebral Blood Flow (CBF) quantification. At 27 minutes post ingestion a high-resolution $T_1$-weighted anatomical image was acquired (magnetization-prepared rapid gradient echo (MPRAGE), matrix size = 256 × 256, 192 sagittal slices, 1 × 1 × 1 mm isotropic voxels, TR = 1900 ms, TE = 2.26 ms, TI = 900 ms).

*Image processing*

Image processing was performed using functions from the ASLtbx[28] in conjunction with SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK) similar to Kullmann et al[29]. The tag and control ASL images were separately motion corrected and a common mean image was created.

Subsequently, the ASL images were coregistered to the anatomical image and smoothed with a 3-dimensional isotropic Gaussian kernel of 6 mm full-width at half-maximum.
Relative CBF maps were generated by subtracting the control-tag differences. The one compartment model was used for absolute quantification of CBF[30,31] using the following parameters: inversion efficiency (α)=0.95, water partition coefficient (λ)=0.9 ml/g, T1 of arterial blood ($T_{1a}$)=1684 ms. $T1_2$ was incrementally adjusted per slice with 39.5 ms. The anatomical image was normalized using SPM8 unified segmentation , and the resulting parameter file was used to normalize the CBF maps to MNI space retaining 3 × 3 × 3 mm resolution.

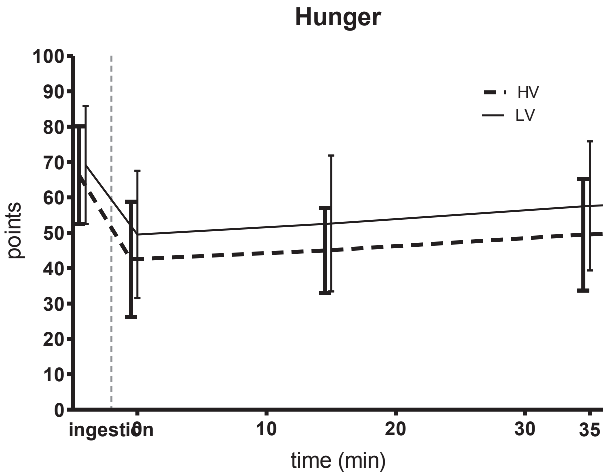
*Statistical analyses*
For all subjective ratings AUC over 45 minutes was calculated. For total gastric volume AUC over 35 minutes was calculated. AUCs were calculated using Graphpad Prism 5 (Graphpad Software, La Jolla, USA), following the trapezoidal rule.  Differences between treatments were tested with paired t-tests in SPSS (IBM, Armonk, USA). A linear mixed model with treatment*time as a random factor was performed to check for significant differences per time point. Significance level was set at a p-value of 0.05. Data are expressed as mean±SD unless otherwise stated.

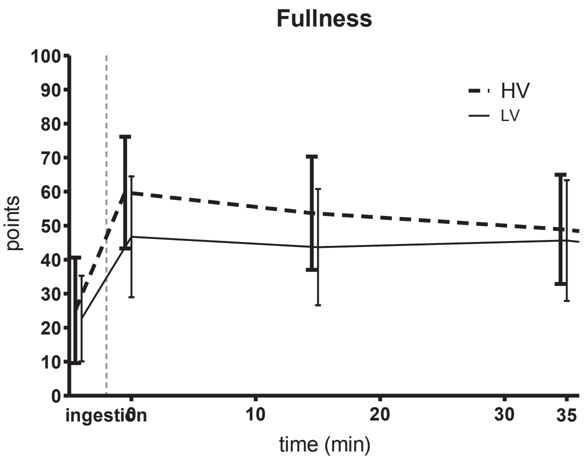
Whole-brain group level analyses were performed in SPM12 (Wellcome Trust Centre for Neuroimaging, London, UK). To investigate CBF changes a full factorial analysis was conducted including the factors treatment (LV, HV) and time (baseline, post ingestion, 15 and 35 min). The threshold for significance was set at a family wise error–corrected (FWE) peak P-value = 0.05. In case of significant clusters, CBF values of the different anatomical areas were extracted using the WFU PickAtlas[32,33] and MarsBaR toolbox[34] (marsbar.sourceforge.net).

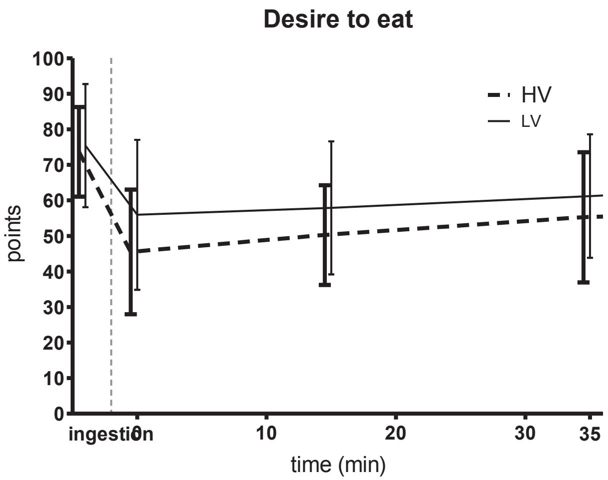
## Results

*Appetite ratings*

Appetite and thirst ratings over time can be seen in **Figure 3.** HV showed significantly decreased hunger AUC compared to LV (p = 0.015), HV showed significantly increased fullness AUC compared to LV (p = 0.045), and HV showed significantly decreased desire to eat AUC and prospective consumption compared to LV (p = 0.01, p = 0.01). Thirst AUC was significantly decreased for HV in comparison to LV (p < 0.001).




Desire to eat
points
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LV
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90
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70
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50
40
30
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10
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ingestion
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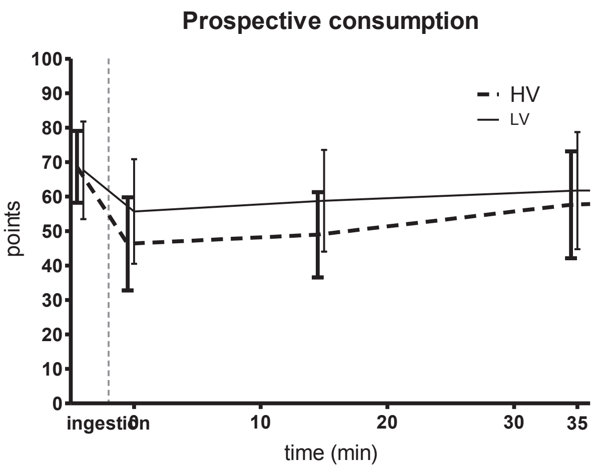
35
time (min)


Prospective consumption
points
HV
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ingestion
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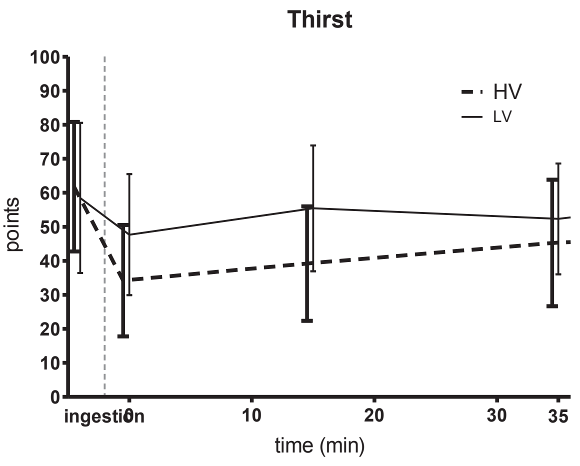
time (min)


Thirst
points
HV
LV
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50
40
30
20
10
0
ingestion
0
10
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30
35
time (min)

Figure 3. Hunger, fullness, prospective consumption, desire to eat and thirst plotted over time. All appetite ratings were scored significantly different when comparing AUC. n = 19.

### *Total gastric volume*

A graph of the TGV for both treatments can be seen in **Figure 4**. **Figure 5** shows TGV per treatment, as well as differences in volume of the water and shake layers.

LV TGV was 251±24 mL directly after ingestion, 209±28 mL at t = 15 min and 166±28 mL at t = 35 min. HV TGV was 543±37 mL directly after ingestion, 391±82 mL at t = 15 min and 229±50 mL at t = 35 min. TGV AUC was significantly greater for HV between treatments ($p < 0.001$). TGV was significantly greater for HV compared to LV at all three time points ($p < 0.001$) with a relative increase of 292±37 mL directly after ingestion, and of 182±83 mL at t=15 min and 62±57 mL at t=35 min.

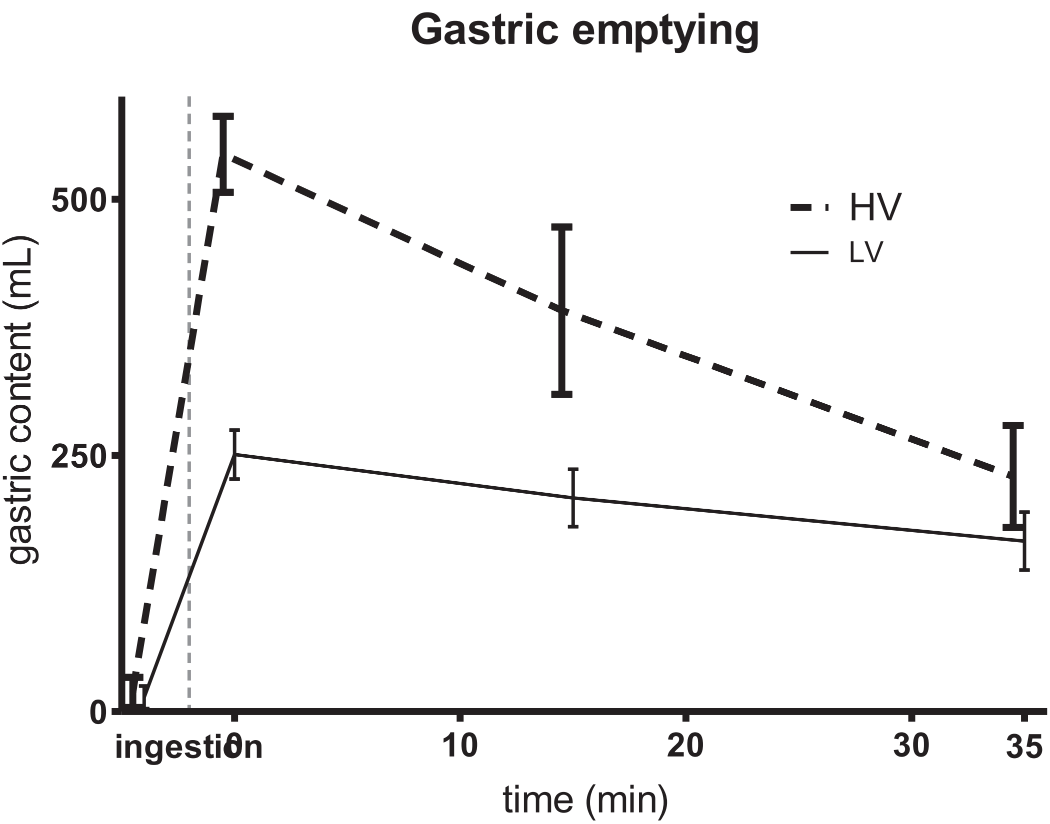
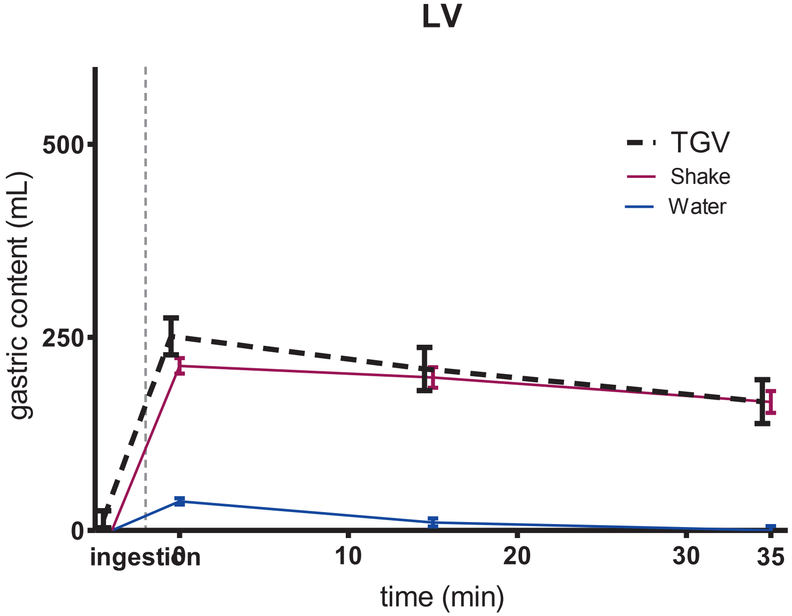



Figure 4. TGV plotted over time. AUC TGV was significantly greater for HV in comparison to LV. TGV at all three time points was significantly different between treatments.  n = 19.



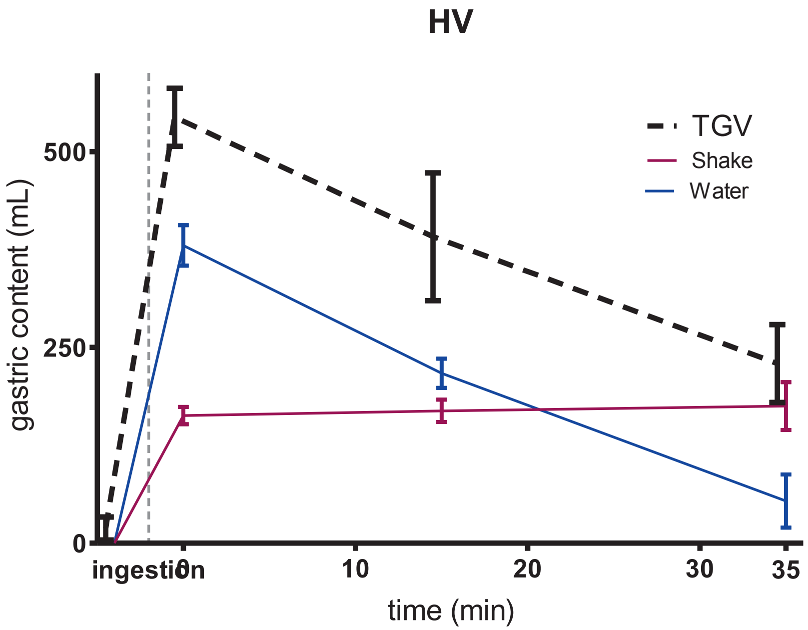




Figure 5. TGV plotted over time, as well as difference between the individual layers within the stomach. n = 19.

*Brain activation*

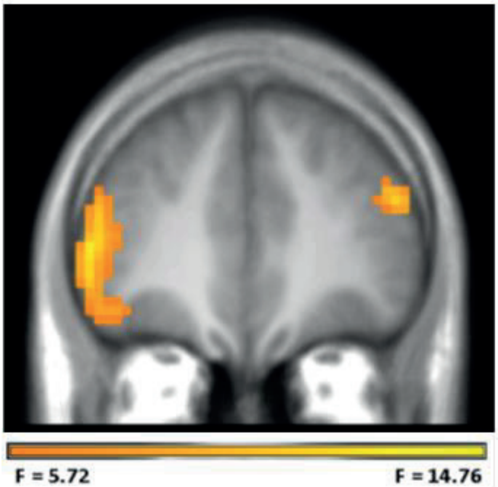
An overview of significant clusters can be found in **Table 2**. There was no significant main effect of treatment on brain activation. There was, however, a significant main effect of time in the opercular part of the left inferior frontal gyrus (MNI(-57, 17, 22), Z = 5.49, k = 938, $P_{fwe}$ = 0.001). This cluster extended into the triangular and orbital parts of the inferior frontal gyrus. There was a contra-lateral cluster in the triangular part of the right inferior frontal gyrus (MNI(54, 29, 22), Z = 4.81, k = 319, $P_{fwe}$ < 0.001). This cluster extended into the right middle frontal gyrus and the insula.

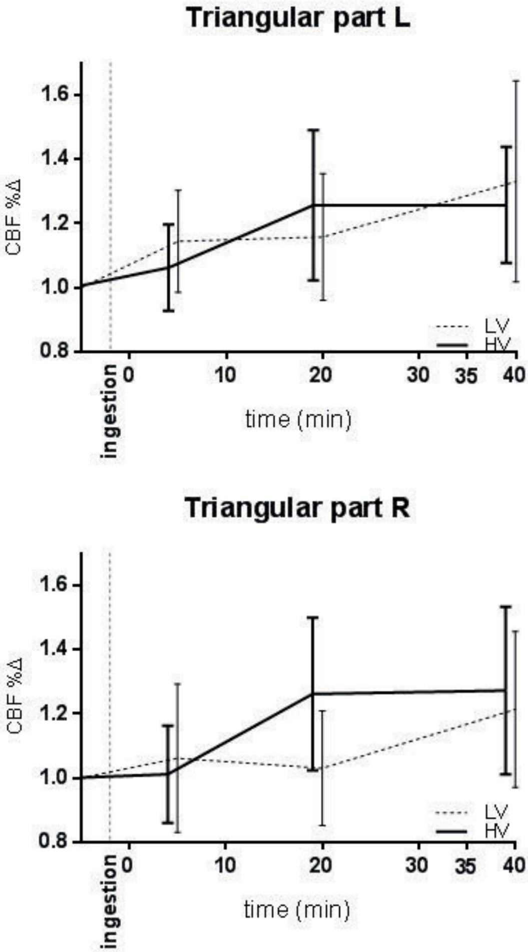
There was no significant interaction between time and treatment.

Table 2. Whole brain analysis of LV and HV in 19 healthy men[1]

| Area | k[2] | MNI[3] | | | Z | $P_{fwe}$ |
|---|---|---|---|---|---|---|
| | | **x** | **y** | **z** | | |
| **Opercular part of the inferior frontal gyrus L** | 938 | -57 | 17 | 22 | 5.49 | 0.001 |
| **Triangular part of the inferior frontal gyrus L** | | -51 | 38 | 7 | 5.16 | 0.006 |
| **Orbital part of the inferior frontal gyrus L** | | -51 | 35 | -5 | 5.02 | 0.012 |
| **Triangular part of the inferior frontal gyrus R** | 319 | 54 | 29 | 22 | 4.81 | 0.031 |
| **Middle frontal gyrus R** | | 48 | 38 | 22 | 4.76 | 0.039 |
| **Anterior insula R** | | 36 | 14 | -11 | 4.53 | 0.101 |

[1] Main effect of time in a 2 x 4 full factorial model with treatment and time as factors. [2]cluster size. [3]Voxel coordinates in Montreal Neurological Institute space.


F = 5.72
F = 14.76


Triangular part L
CBF %Δ
1.6
1.4
1.2
1.0
0.8
ingestion
0
10
20
30
35
40
time (min)
LV
HV
Triangular part R
CBF %Δ
1.6
1.4
1.2
1.0
0.8
ingestion
0
10
20
30
35
40
time (min)
LV
HV

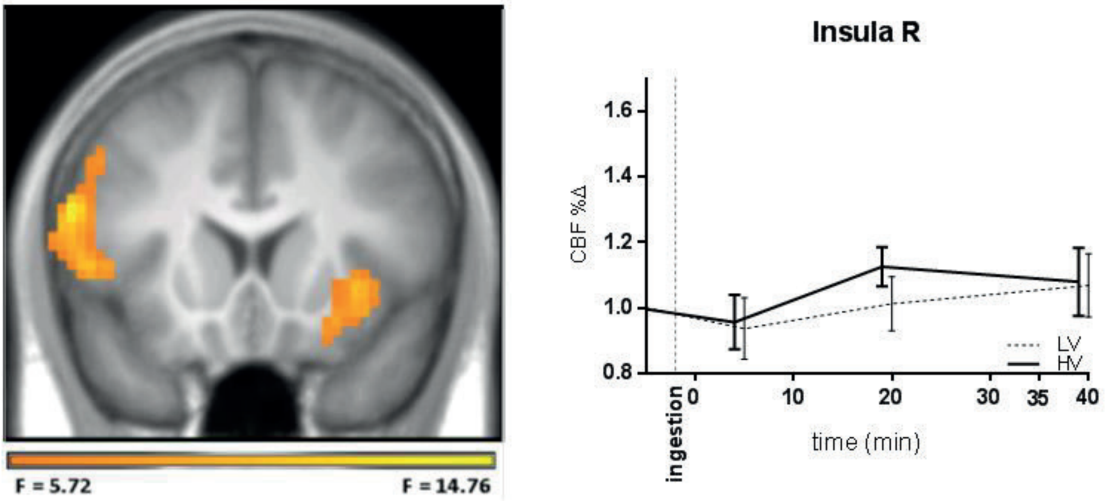



Figure 6. Color coded F-map overlaid onto study population mean anatomical image (F = 5.72 corresponds to p = 0.001). In the graph: percentual deviation from baseline CBF values per treatment over time for the bilateral inferior frontal gyrus – triangular part (y = 38) and the right anterior insula (y = 14). n = 19.

## Discussion

The primary aim of this study was to examine how offering a small versus a large water load following a standardized meal affects gastric distention over time. Gastric MRI data show that TGV was significantly larger for HV over the course of the measurement period. In addition, appetite was suppressed more for HV than for LV. CBF increased over time in parts of the bilateral inferior frontal cortex and adjacent insula. However, differences between treatments were not significant, although HV consistently tended to increase brain activity more than LV.

Our work shows that it is possible to concurrently measure both gastric volume and brain activity in the same subject. By changing the MRI coils (body coil and head coil) in quick succession we were able to obtain one baseline and three post-ingestive measurements of both gastric content and CBF. To our knowledge this is the first paradigm to allow direct comparison of MRI determined gastric content with neural activation. One effect of the introduction of water after ingestion of the shake was that it did not mix into it, as can be seen in Figure 2. The data indicates that the water ingested after the liquid food stimulus floats on top and empties relative quickly from the stomach, as studied in more detail in Chapter 5[24]. This is in line with other work showing gastric sieving of low caloric watery fluid while retaining more calorie-dense gastric content[22].

In line with our hypotheses activation in the HV condition consistently tended to be higher than that in the LV condition, although the difference was not significant. This may imply that our manipulation was not strong enough to invoke measurable treatment differences. A load of 500 mL is consistent with earlier paradigms that did find a brain response to gastric distention[4,5], however larger volumes have also been used[35]. Ly et al. report activation due to distention induced by a balloon in the right insula, but this activation decreased when a nutrient stimulus of the same volume was infused.

Our results show activation in the inferior frontal gyrus, which has been shown to be associated with gastric stimulation with a liquid meal[36]. Gut hormones such as cholecystokinin, GLP-1, Peptide YY (PYY) and ghrelin are known to affect brain activity[37]. PYY has been implicated in regulating gastric emptying[38]. Weise et al. report a positive correlation between plasma PYY and right inferior frontal gyrus resting state CBF in the same region[39]. Interestingly, stable plasma PYY levels have been shown after non-nutrient distention of the stomach[40], which is in line with work showing that PYY release is mediated by caloric chime[41]. This could imply that nutrients combined with distention of the stomach mediate the release of PYY inducing activation in the inferior frontal gyrus.

Our data show an increase and subsequent decrease in activity in the right anterior insula after ingestion. There was greater insula activation for HV than for LV although this difference was not significant. Insular activation is associated with visceral and sensory integration[42]. The anterior insula is activated by pure gastric distention with an intragastric balloon[4]. Gastric manipulations and viscerosensation of the stomach has been shown to be associated with anterior insular activation, and more so in the right hemisphere.[43-45]. Our results are consistent with this, as we show significant CBF changes within the anterior insula over the course of our measurements.

In our study participants were scanned in a supine position. The supine position required in our research is different from real life, and consequently fluid dispersion throughout the stomach is different as well. Stimulating the antral gastric wall has been shown to be important in increasing emptying contractions[46], and antral stimulation may be different due to the position of the gastric content relative to the body in a  supine position. However, in the literature a supine position is common for gastric MRI studies, and studies which compared positions have found similar emptying[47]. Additionally, in a within-subject design relative gastric emptying differences remain intact[48].

Gastric emptying is usually measured over a longer period[9,17,22]. Our data shows that for this combination of a liquid food stimulus and water gastric, the difference in volume between LV and HV declines from 250-300 mL to around 60 mL in 35 minutes. This shows that ourCBF measurement fell right into the period with the most divergent gastric volumes between the treatments, indicating that the paradigm was in principle well suited to measure differences in CBF changes between the treatments.

Our protocol allows concurrent measurement of gastric volume and brain activation in the same subject. There has been work showing different effects of gastric distention of a balloon versus the same volume infused as a nutrient rich liquid[49]. However, it was unknown for how long the infused nutrients were retained in the stomach, and what the volume of the gastric content was during the concomitant fMRI measurements. Our work opens up possibilities to use nutrient loads as a more specific tool for manipulating gastric distention.

*Conclusions*

This study is the first to employ concurrent interleaved gastric MRI and fMRI measurements. Offering a large versus a small water load after a standardized meal significantly increases gastric distention for over 35 minutes and suppresses appetite. A liquid meal with or without an increased intragastric volume of 300 mL water do not differ enough to induce CBF changes. However, this method is an easy and valid non-caloric method to increase gastric distention.

**Acknowledgements**

This research is supported by the European Union Seventh Framework Programme (FP7/2007–2013) under Grant Agreement 607310 (Nudge-it). Use of the MRI scanner was made possible by Wageningen University Shared Research Facilities.

GC, RV, MM, CdG and PAMS declare no potential conflicts of interest.

GC, MM, KG and PAMS designed the study, GC conducted the research, GC, PAMS and RV performed the statistical analysis; GC wrote the paper. RV, MM, CdG and PAMS provided feedback, PAMS had primary responsibility for final content.

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POW


MRI-MAN IS TOO MUCH FOR YOU, PHANTOM!

# Chapter 6.

Bloating, bellies and the brain: effects of soda and beer on appetite, gastric liquid and gas content and neural activity

Guido Camps
Kees de Graaf
Paul AM Smeets

Submitted for publication

**Abstract**

**Background:** The most commonly consumed carbonated beverages are soda and beer. $CO_2$ in beverages increases gastric volume, which can lead to gastric discomfort (bloating). Women are more susceptible to this, however, correlations with neural activity and gastric distention are unknown.

**Objective:** This study sought to determine the subjective, gastric and neural correlates of bloating in men and women.

**Methods:** 34 healthy normal-weight adults (17 women) participated in a randomized crossover study with 2 treatments: ingestion of 500 mL beer or soda over 20 min. Before and after consumption gastric content and brain activity were measured with MRI. Participants rated fullness, bloating, hunger and nausea at baseline and at t=0, 20 and 30 min after consumption in parallel with gastric MRI. Brain activity (CBF) was measured at baseline and at t=5 and 35 min. Liquid, gas and total gastric volume (TGV) were determined from manual segmentations of the gastric MRIs. For subjective ratings and gastric data AUCs were calculated.

**Results:** Changes in subjective ratings did not differ between drink types or genders, except for an increase in nausea for women in the beer condition. TGV AUC was significantly greater for women ($p = 0.019$) and caused by a difference in liquid content. There was no difference in gas content between the genders, and no difference between the drinks. Bloating correlated strongest with TGV ($r = 0.45$), nausea correlated strongest with the liquid fraction AUC ($r = 0.45$).

CBF did not differ between the drinks. Men showed greater activation than women in the left precentral and postcentral gyrus at t=5.

**Conclusions:** There are differences between the sexes when it comes to appetite ratings, gastric fluid retention and neural activation. Bloating in women may be related to fluid rather than gas in the stomach, as they retain more fluid than men.

**Introduction**

The most commonly consumed carbonated beverages are soda and beer (world consumption is 12.5% for carbonated soft drinks and 11.2% for beer)[1]. Carbon dioxide contained in beverages increases gastric volume, which stimulates stretch receptors in the gastric wall. This may subsequently induce a feeling of epigastric discomfort[2,3], commonly referred to as 'bloating'.

Gastric discomfort is comprised of unpleasant sensations induced by distention of the stomach[4]. Nausea, gastrict pain, discomfort and bloating have all been shown to increase when the stomach is distended with a balloon[5].The neural correlates of gastric discomfort have mainly been studied in the clinical context of painful gastric distention e.g. comparing irritable bowel syndrome patients with healthy controls[6,7]. There are to our knowledge no studies that have examined the neural correlates of gastric discomfort caused by food or drinks.

Balloon distention of the stomach activates pain regions, but interestingly, infusion of a matched volume of nutrients leads to deactivation in among others the insula and ventrolateral prefrontal cortex[8]. Apparently nutrients are a prerequisite for gastric tolerance of normal meal volumes without pain. Therefore, as carbonated drinks are commonly associated with bloating, but can also contain nutrients, it is interesting to further examine the effects of nutrient rich carbonated drinks like beer and soda on gastric discomfort and associated brain activity.

Different bodies of evidence show that there are clear differences in visceral perception and gastric processes between men and women. Women are more prone to dyspepsia, and gastric discomfort[9-11]. Also, neuroimaging work in healthy controls and patients with visceral medical conditions has shown stronger responses in women[7,12], although these are not always very pronounced[13]. Gender differences in gastric processes as well as within-subject fluctuations over time appear to be significantly modulated by the sex hormones involved in the menstrual cycle[14].

Alcohol has been shown to delay gastric emptying in men[15,16]. In comparing genders, Sekime et al. found no delay in gastric emptying between non-alcoholic drinks and their alcoholic counterparts for women, but they did find delayed emptying for men[17]. In follow up work they found that women did show delaying effects of alcohol in the follicular phase of the menstrual cycle, but not in the luteal phase[18]. Interestingly, older work has shown effects of menstrual cycle hormones on the emptying of solid, but not liquid foods[19]. Thus, the hormonal differences between men and women may explain differences in their gastric emptying rate and other gastric factors.

Here, we aimed to determine the subjective, gastric and neural correlates of bloating in men and women by means of carbonated soda and carbonated alcoholic drink ingestion. We hypothesized that women will experience greater bloating and fullness than men. For gastric emptying we expect slower emptying of beer due to the alcohol content, but not necessarily differences between the genders. Neurally, we expect greater activation in the insula and ventromedial prefrontal cortex in women due to greater experienced discomfort.

## Participants and Methods

### *Design*

The study had a randomized crossover design with two treatments: ingestion of 500 mL beer or soda. Each participant was always scanned on the same time in the afternoon after a 3 hour fast. Soda and beer were offered in a random order.

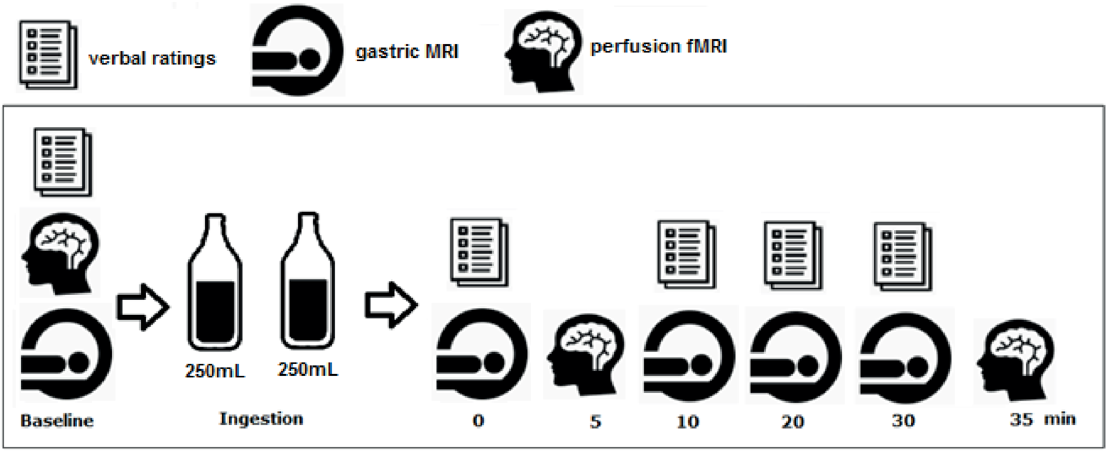


Figure 1. Overview of one experimental session for one participant.

### *Participants*

The subject group consisted of 34 healthy adults (17 female, age 21.8±1.9 y, height 171±8 cm, BMI 22.3±1.9 kg/m$^2$, 17 male, age 24.4±3.7 y, height 181±7 cm, BMI 22.8±1.8 kg/m$^2$). Potential participants were recruited by website and flyers around the campus of Wageningen University. Inclusion criteria were: being aged between 18 and 35 y, having a BMI between 18 and 25 kg/m$^2$, being of self-reported good general health, willing to comply with study procedures, willing to be informed of incidental findings. Exclusion criteria were: unexplained weight loss or gain of >5kg in the last two months, hypersensitivity to any of the food items used in the experiment, any reported pathologies relating to the gastrointestinal tract which might influence results, smoking on average more than one cigarette/cigar a day, having a history of or current alcohol consumption of on average more than 12 units per week, use of any medication which may influence gastrointestinal function, having a contraindication for undergoing an MRI, and being employed or studying at the Division of Human Nutrition of Wageningen University, for women: having the intention to become pregnant, pregnancy during the last 6 months or lactating.

The procedures followed were approved by the Medical Ethical Committee of Wageningen University in accordance with the Helsinki Declaration of 1975 as revised in 2013. This study was registered with the Dutch Trial Registry under number NTR5418. Written informed consent was obtained from all participants.

*Training / screening session*

Potential participants filled out an inclusion questionnaire to screen for eligibility. Subsequently, if potential participants still wished to partake and were eligible, they attended a screening session that included measurement of weight and height and further explanation of the study procedures, including MRI procedures and consumption of 500 mL of beer in 20 minutes to assess whether they could comply with study procedures. After beer consumption, participants engaged in a mock MRI scanner session to see if they were comfortable to do so after ingestion of 500 mL beer and to accustom them to the MRI procedures.

*Stimulus*

For the soda treatment subjects received a 500 mL bottle of soda (Sprite, The Coca-Cola Company, Atlanta, USA). For the beer treatment subjects received two 250 mL bottles of a draft lager beer (Heineken NV, Amsterdam, The Netherlands). The beer and soda were matched on nutrient composition (the soda had 39 kcal per 100 mL, beer 42 kcal per

100 mL, both had zero grams of fat and very low amounts of protein) and had similar acidity (pH ~3.5 for soda and ~4 for beer) and carbonation. The drinks were served chilled (~5 degrees celsius) in blinded bottles, to prevent any branding effects.

*Scan session procedures*

See **Figure 1** for an overview of a scan session. After arrival, participants provided baseline appetite ratings and baseline stomach content and baseline CBF were measured. After this, participants exited the scanner and were offered the drinks. Subjects had a clock and some leisure reading material available, and were instructed to pace their drinking such that they drank 250 mL spread evenly over 10 minutes and 250 mL over the second 10 minutes, consuming a total of 500 mL.

After consumption, gastric MRI scans were made and subjective ratings were obtained at t=0, 10, 20 and 30 minutes post ingestion. At t=5, and 35 minutes CBF was measured.

*Subjective ratings*

Participants verbally scored fullness, bloating, hunger and nausea over the scanner intercom system on a 100-point scale[20].

*Gastric MRI*

Participants were scanned with the use of a 3-Tesla Siemens Verio (Siemens AG, Munich, Germany) MRI scanner using a $T_2$-weighted spin echo sequence (HASTE, 24 6-mm slices, 2.4 mm gap, 1.19 x 1.19 mm in-plane resolution), with breath hold command on expiration to fixate the position of the diaphragm and the stomach. The duration of one scan was approximately 18 seconds. A custom software tool created in MeVisLab (MeVis Medical Solutions AG, Bremen, Germany) was used to manually delineate gastric contents on every slice[21]. Volumes on each time point were calculated by multiplying surface area of gastric content per slice with slice thickness including gap distance, summed over the total number of slices showing gastric content. Liquid and gas volumes were measured separately (**Figure 2**) and summed up to obtain total gastric volume (TGV).

*Brain activity (CBF)*

*Data acquisition*

Cerebral blood flow (CBF) was measured with a 32-channel head coil using perfusion MRI[22] on the same scanner. Images were obtained with a PICORE Q2T ASL sequence, using a frequency offset corrected inversion pulse and echo planar imaging readout for acquisition. A total of 19 axial slices were acquired in ascending order. Each measurement consisted of 100 alternating tag and control images with the following imaging parameters: inversion time (TI), TI1 = 700 ms, TI2 = 1800 ms, repetition time = 2800 ms, echo time = 13 ms, in plane resolution = 3 × 3 mm, field of view = 192 × 192 mm, and flip angle = 90°, with a slice thickness of 5 mm. The first image of the series was used to estimate the equilibrium magnetization of the blood (M0B) to allow for absolute CBF quantification. A $T_1$-weighted anatomical image was acquired (magnetization-prepared rapid gradient echo (MPRAGE), matrix size = 256 × 256, 192 sagittal slices, 1 × 1 × 1 mm isotropic voxels, TR = 1900 ms, TE = 2.26 ms, TI = 900 ms).

*Image processing*

Image processing was performed using functions from the ASLtbx[23] in conjunction with SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK) similar to Kullmann et al.[24]. The tag and control ASL images were separately motion corrected and a common mean image was created. Subsequently, the ASL images were coregistered to the anatomical image

and smoothed with a 3-dimensional isotropic Gaussian kernel of 6 mm full-width at half-maximum.
Relative CBF maps were generated by subtracting the control-tag differences. The one compartment model was used for absolute quantification of CBF[25,26] using the following parameters: inversion efficiency (α)=0.95, water partition coefficient (λ)=0.9 mL/g, T1 of arterial blood ($T_{1a}$)=1684 ms. $T1_2$ was incrementally adjusted per slice with 39.5 ms. The anatomical image was normalized using SPM8 unified segmentation and the resulting parameter file was used to normalize the CBF maps to MNI space retaining 3 × 3 × 3 mm resolution. CBF maps were baseline corrected. CBF maps of both drink types were averaged per participant to create an overall ingestion CBF maps for t= 5 and one for t =35.

*Statistical analyses*
AUCs for appetite ratings and gastric volumes were calculated using Graphpad Prism 5 (Graphpad Software, La Jolla, USA), following the trapezoidal rule. For all subjective ratings AUC over 35 minutes was calculated. For total gastric volume AUC over 35 minutes was calculated. Statistical analyses were performed in factors in SPSS (IBM, Armonk, USA). Differences in the subjective ratings and liquid, gas and total gastric volume were tested using a Sidak adjusted linear mixed model with gender and drink type as fixed factors. Pearson correlation coefficients were calculated between bloating, fullness, nausea and gastric content. Significance level was set at a p-value of 0.05. Data are expressed as mean±SD unless otherwise stated.

Whole-brain analyses were performed in SPM12 (Wellcome Trust Centre for Neuroimaging, London, UK). Average effect of consumption at t = 5 min was tested using a one sample t-test on baseline corrected ingestion CBF maps. Gender effects were tested by performing two sample t-tests for the t=5 averaged ingestion CBF maps and for the t=35 averaged ingestion CBF maps.

Effects of drink type were tested in a paired t-test for the t=5 and for the t=35 CBF maps.

Factorial models with gender and drink type as factors were used to test for an interaction between gender and drink type for both time points.

The threshold for significance was set at a family wise error–corrected (FWE) P-value = 0.05.

## Results

*Subjective ratings*

There were no significant differences in AUC for fullness, bloating and nausea between the drink types, nor between the genders. An overview of the ratings can be seen in **Figure 2**.

For nausea AUC, there was also no significant effect of drink type. However, nausea AUC was significantly larger for women compared to men in the beer condition (p = 0.045).

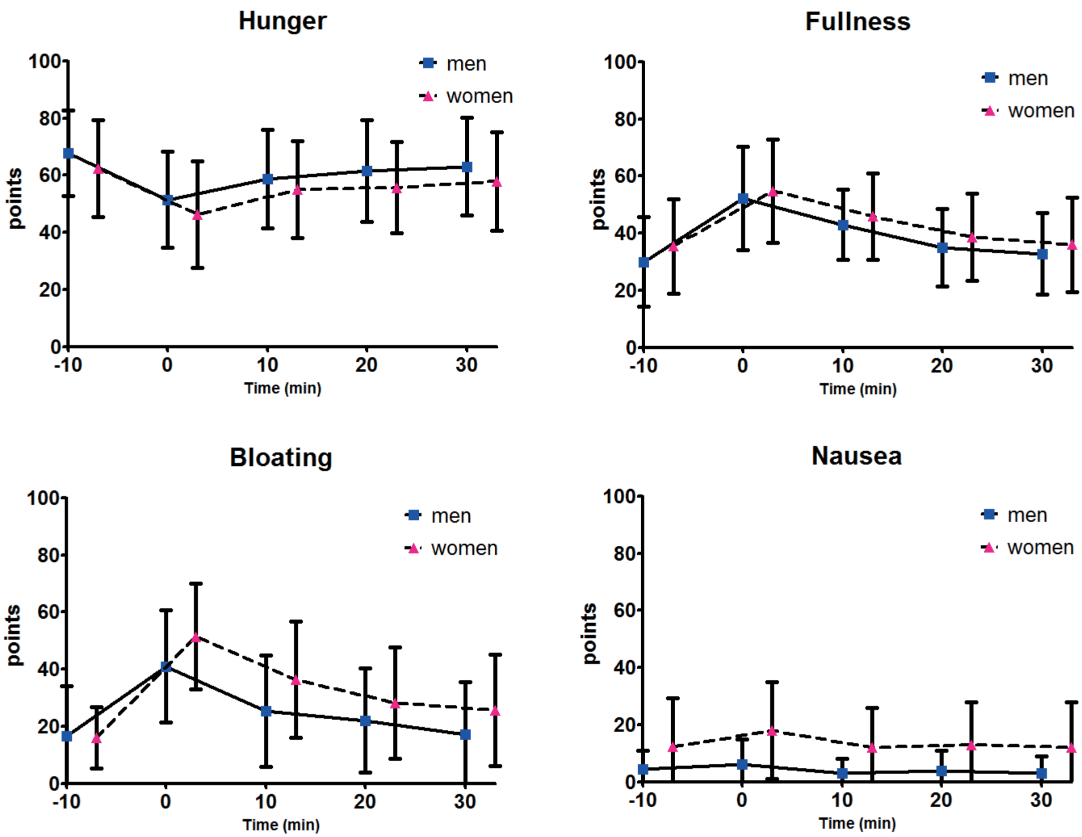


Figure 2. Average ratings of fullness, bloating and nausea for both drink types, per gender. Ingestion was finished at t= 0. There were no significant differences in AUC for hunger, fullness, bloating and nausea between  drink types and the genders. There was a significant increase in nausea for female participants in the beer condition (p = 0.045). n = 17 for both groups.

*Gastric volumes*

Overview of gastric volumes for both drink types, genders and both fluid and gas volumes can be seen in **Figure 3**.

For the liquid content fraction, there was a significant main effect of gender with greater volumes for women (women: 6667±2204 mL*min, men: 4596±2158 mL*min, $p < 0.001$). There was no effect of drink type.

There was no significant effect of gender or drink type on the gas fraction AUC.

For TGV, there was a significant main effect of gender with greater volumes for women (women: 12209±3991 mL*min, women: 9684±3751 mL*min, $p = 0.019$). There was no effect of drink type and no interaction effect.

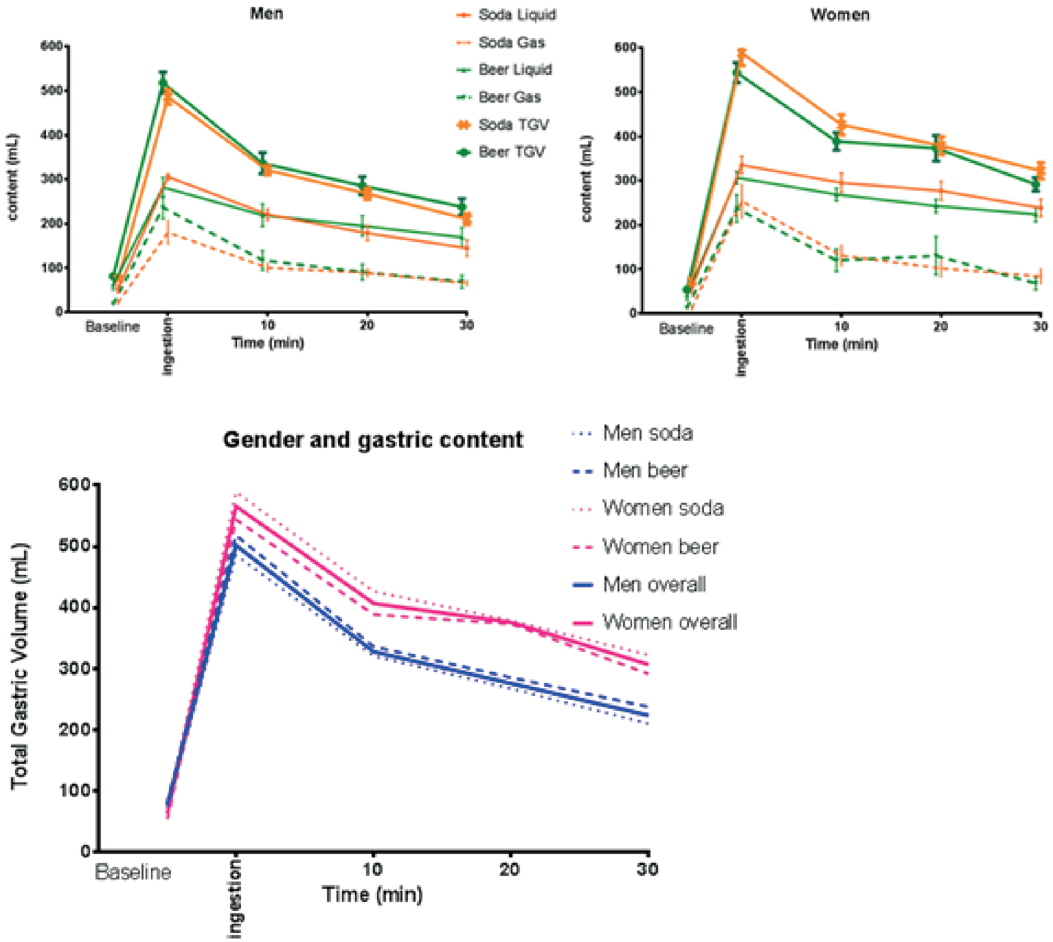


Figure 3. TGV, liquid and gas fraction within the stomach for men (n = 17) and women (n = 17). Graphs shows mean±SEM. For the liquid content fraction, there was a significant main effect of gender (female: 6667±2204 mL*min, male: 4596±2158 mL*min, $p < 0.001$). For TGV, there was a significant main effect of gender (female: 12209±3991 mL*min, male: 9684±3751 mL*min, $p = 0.019$). There was no significant effect of gender or drink type on the gas fraction in the stomach and there was no effect of drink type.

*Brain activation*

An overview of the fMRI results can be found in **Table 1**.

At t=5 there was a significant increase in CBF compared with baseline in the left inferior frontal gyrus - triangular part and adjacent anterior insula (peak voxel MNI( -45, 20, 7), Z = 5.07, k=2672, $P_{fwe}$ = 0.008), right inferior frontal gyrus - triangular part (peak voxel MNI(45, 38, 25), Z = 4.65, k = 504, $P_{fwe}$ = 0.046) and the right precentral gyrus (peak voxel MNI(63, -4, -32), Z = 4.63, k = 277, $P_{fwe}$ = 0.050).

At t=35 there was a significant increase in CBF compared with baseline in the bilateral occipital cortex and hippocampus (peak voxel MNI(-27, -43, 4), Z = 6.16, k = 6969, $P_{fwe}$ < 0.001), the left inferior frontal gyrus - triangular part (peak voxel MNI(-45, 29, 25), Z = 4.78, k = 569, $P_{fwe}$ < 0.001), left caudate nucleus (peak voxel MNI(-6, 11, -5), Z = 4.73, k = 75, $P_{fwe}$ = 0.027) and middle frontal gyrus - orbital part (peak voxel MNI( 21, 38, -17), Z = 4.67, k = 50, $P_{fwe}$ = 0.034).

At t=5 there was greater activation in men compared to women in the left precentral gyrus (peak voxel MNI( -45, 4, 52), Z = 5.03, k = 68, $P_{fwe}$ = 0.007) (**Figure 4**).

There were no significant differences between the genders at t=35 and no significant interaction effect.

Table 1. Significant activation differences due to consumption, gender and drink type[1]

| **Test** | **Area** | **k**[2] | **MNI**[3] | | | **Z** | **$P_{fwe}$** |
|---|---|---|---|---|---|---|---|
| | | | **x** | **y** | **z** | | |
| **T5 Main effect consumption** | L Inferior Frontal Gyrus Triangular Part, | 2672 | -45 | 20 | 7 | 5.07 | 0.008 |
| | L Anterior Insula | | -36 | 23 | -2 | 4.35 | <0.001 |
| | L Middle Temporal Gyrus | | -33 | 20 | -20 | 5.07 | 0.008 |
| | L Inferior Frontal Gyrus Orbital Part | | -55 | -13 | -23 | 4.98 | 0.012 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | R Inferior Frontal Gyrus Triangular Part | 504 | 45 | 38 | 25 | 4.65 | 0.046 |
| | R Inferior Temporal Gyrus | 277 | 62 | -4 | -32 | 4.63 | 0.050 |
| | | | | | | | |
| **T30 Main effect consumption** | L Hippocampus | 6929 | -27 | -43 | 4 | 6.16 | <0.001 |
| | Occipital Cortex | | -39 | -91 | 4 | 5.97 | <0.001 |
| | R Hippocampus | | 27 | -40 | 7 | 5.8 | <0.001 |
| | L Middle Frontal Gyrus | 569 | -45 | 29 | 34 | 4.78 | 0.022 |
| | L Caudate Nucleus | 75 | -6 | 11 | -5 | 4.73 | 0.027 |
| | R Middle Frontal Gyrus Orbital Part | 50 | 21 | 38 | -17 | 4.67 | 0.034 |
| | | | | | | | |
| **T5 Beer versus Soda** | | | | | | | n.s. |
| **T35 Beer versus Soda** | | | | | | | n.s. |
| | | | | | | | |
| **T5 M > F** | L Precentral gyrus | 71 | -45 | -4 | 52 | 5.03 | 0.007 |
| | L Postcentral gyrus | | -51 | -22 | 55 | 4.25 | 0.194 |
| | | | | | | | |
| **T35 M < F** | | | | | | | n.s. |
| **T5 Interaction drink type x gender** | | | | | | | n.s. |

| | |
|---|---|
| **T35 Interaction drink type x gender** | n.s. |

[1] Main effect of gender was tested by averaging the images of the drink types per participant and performing a two sample t-test of the images from t=5 and a paired t-test for the images of t=35. Main effect of consumption was tested by performing a paired t-test of the images from t=5 and a paired t-test for the images of t=35. A factorial model with gender and drink type as factors was performed to test for interaction between gender and drink type for both time points. The threshold for significance was set at a family wise error–corrected (FWE) P-value < 0.05, n = 34 (17 males, 17 females). [2]cluster size. [3]Voxel coordinates in Montreal Neurological Institute space

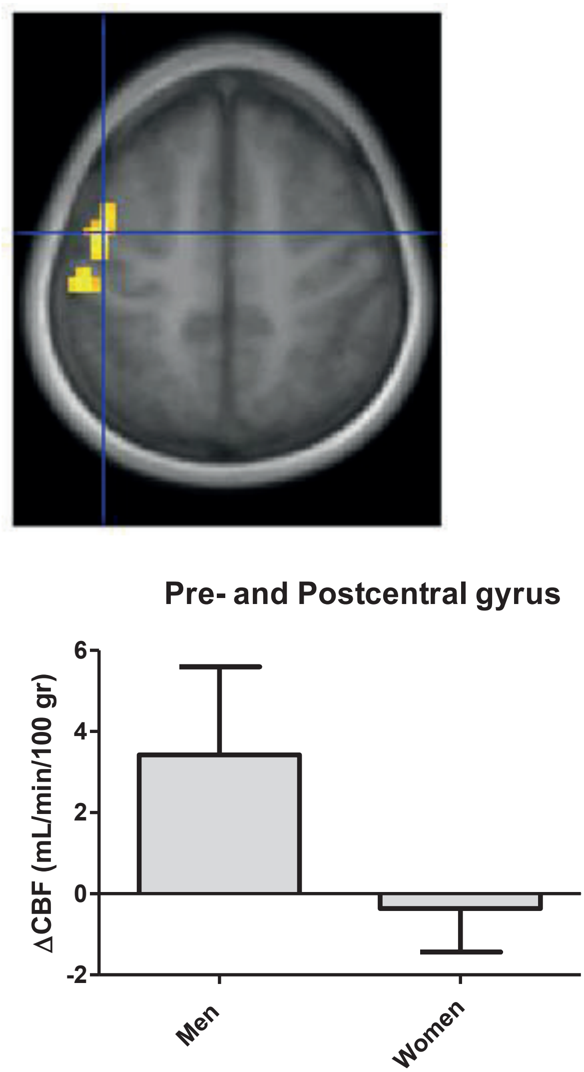


Figure 4. At t=5 there was greater activation in men compared to women in the left precentral gyrus (Peak at MNI( -45, 4, 52), Z = 5.03, k = 68, $P_{fwe}$ = 0.007, indicated by the crosshair). The cluster extends into the postcentral gyrus.

*Correlations*

An overview of all correlations can be found in **Table 2**.

Nausea and bloating were significantly positively correlated (r = 0.57, $p < 0.001$), as were nausea and fullness (r = 0.36, p = 0.003). Fullness and bloating correlated strongly (r = 0.72, $p < 0.001$).

TGV AUC was significantly positively correlated with bloating AUC (r = 0.45, $p < 0.001$), nausea (r = 0.31, p = 0.010) and fullness AUC (r = 0.29, p = 0.016). Interestingly, nausea  and fullness AUC correlated strongest with the liquid fraction AUC (nausea: r = 0.45, $p < 0.001$ and fullness: 0.29, p = 0.016).

Table 2. Correlations between gastric content and subjective rating AUCs[1]

| | Hunger | Nausea | Bloating | Fullness |
|---|---|---|---|---|
| **Liquid** | n.s. | r = 0.45** | r = 0.38** | r = 0.29* |
| **Gas** | n.s. | r = 0.31* | r = 0.38** | n.s. |
| **Total Gastric Volume** | n.s. | r = 0.31** | r = 0.45** | r = 0.27* |
| **Hunger** | | n.s. | r = -0.241* | r = -0.26* |
| **Nausea** | | | r = 0.57* | r = 0.36** |
| **Bloating** | | | | r = 0.72** |

[1]Shown are Pearson correlation coefficients. * Correlation significant at p = 0.05 level. ** Correlation significant at p = 0.01. n = 34.

## Discussion

In this study we found that 500 mL of beer consumed over 20 minutes by men and women yields increased feelings of nausea among female participants for beer. In contrast to our hypothesis all other subjective ratings did not differ significantly between the genders. When it comes to gastric emptying, women retained significantly more fluid in their stomach than men irrespective of drink type. There was only a gender difference at t=5, with men showing greater activation than women in the left precentral gyrus. There were no differences in brain activation between the drinks. When comparing correlations between subjective ratings and intragastric liquid and gas and TGV, nausea and fullness correlated strongest with the liquid fraction within the stomach, and bloating strongest with TGV. Subjective ratings correlated weakest with the gas fraction.

Differences between men and women in gastric transit have been well documented and have been linked with sex hormone levels[27-30]. Datz et al. were the first to describe slower gastric emptying for women compared to men, of both a liquid and a solid food[27].

Our results confirm these findings of greater retention through slower gastric emptying in women for caloric liquids. This is in contrast to Gill et al. who found no difference for liquids[19]. Thus, gender differences may be important in clinical assessment of gastrointestinal pathology. Mori et al. specifically suggest amending diagnostic criteria to accommodate different values for women[31], and our research supports this.

Our data show that women have a higher initial post-ingestive stomach content volume. Apparently, during the preceding 20 minutes of ingestion, for some reason women let less liquid flow through into the small intestine. Mearadji et al. showed that women are more sensitive when it comes to feeling gastric distention and that they rate fullness and abdominal pressure higher for the same degree of distention than men[32]. Additionally, they found that women rate nausea higher and have greater retention of a test meal from 30 to 90 minutes post ingestion. A combination of this greater sensitivity and the greater gastric retention in women may explain why they are more susceptible to bloating and discomfort[9]. However, our data shows few differences in appetite as well as in brain activity between the genders, with the exception of increased activation for men in the pre- and postcentral gyrus. Future work will have to show whether differences between the genders become more pronounced with greater stimuli volumes.

The pre- and postcentral gyrus are also known as primary motor and somatosensory cortex. These areas show greater resting state brain activity of functional dyspepsia patients versus controls[33] and increased activity during gastric balloon distention[34]. In apparent contrast, we found greater activation of these regions in men compared to women, even though men had a lower gastric volume. This greater activation could also be a nutrient effect, which has been shown to affect this region as well[8]. Men in our study had lower gastric volumes than women and thus emptied more nutrients into the small intestine, which in turn caused greater activation in the pre- and postcentral gyrus.
In summary, our results confirm the involvement of the pre- and postcentral gyrus in gastric processes. The gender difference we observed warrants further investigation. Apart from the precentral gyrus, our finding of nutrient induced brain activation in the hippocampus, inferior frontal gyrus and insula, is in line with earlier work using a liquid caloric load[35].

In contrast to earlier work[15,16], we did not find any effect of alcohol on gastric emptying. Additionally, the presence of alcohol in the drink did not lead to differences between the genders in emptying. This is partly in line with Sekime et al.[17]who also found no difference between alcoholic and non-alcoholic drinks for women, but did find delaying effect for men which we did not replicate. Kaibara et al. did find slower emptying for alcoholic drinks compared to non-alcoholic drinks in women[18]. However, they showed this delayed emptying only in the follicular phase of the menstrual cycle. We did not control for possible effects of menstrual status on gastric emptying, which is a limitation of our work.

Our study had a large sample size, considering other endeavours in the field[2,36]. The two drinks were relatively well matched on carbonation, calories and pH, and both were commercially available products offered in commonly consumed volumes, which makes our findings more ecologically valid than experiment specific stimuli. However, participants were in a supine position during the stomach and brain scans. Our observed emptying may therefore be slower than that in a normal upright position[37]. Though this drawback of MRI, we have shown in **Chapter 2** that MRI is superior to indirect methods such as $^{13}C$ breath measurements[38,39]. Moreover?, MRI allows intragastric visualisation and accurate quantification of the gas and liquid fractions in the stomach, which is impossible with $^{13}C$ labelling and scintigraphy.

The ingestion of 500 mL of beer or soda was spread over 20 minutes. Ingestion within a shorter timeframe was both difficult and undesirable because participants might have experienced discomfort. However, this does result in a measurement lag of 20 minutes after the onset of consumption. We might have been able to induce greater effects of bloating by more rapid ingestion.

*Conclusions*

In conclusion, there are differences between the sexes when it comes to appetite ratings, gastric fluid retention and neural activation. However, our results show that beer and soda do not differ in gastric retention or brain activation. Beer did increase nausea for women and nausea was strongly correlated to the size of the gastric liquid fraction. Our results confirm that women have different gastric emptying dynamics than men and suggest that bloating may in part be due to intragastric liquid rather than gas.

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GC, CdG and PAMS declare no potential conflicts of interest.

GC, KG and PAMS designed the study, GC conducted the research, GC and PAMS performed the statistical analysis; GC wrote the manuscript. CdG and PAMS provided revision remarks. All authors approved the final manuscript.

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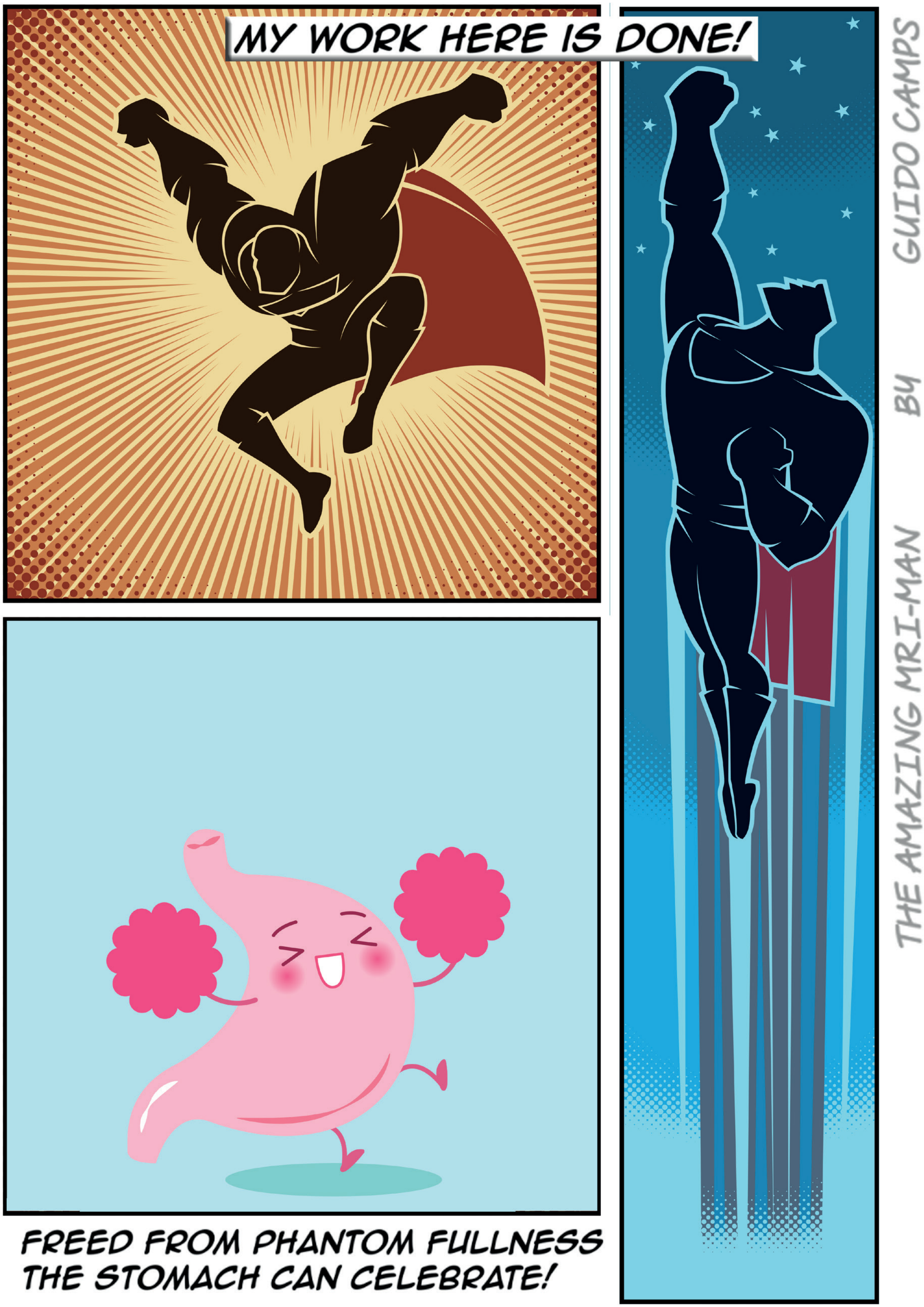
MY WORK HERE IS DONE!
FREED FROM PHANTOM FULLNESS THE STOMACH CAN CELEBRATE!
THE AMAZING MRI-MAN BY GUIDO CAMPS

# Chapter 7.

Discussion

## Summary of main results

This thesis aimed to further determine how gastric content relates to subjective experiences regarding appetite, how this relation is affected by food properties and whether this is visible in neural activation changes. This was studied using questionnaires, MRI of the stomach and fMRI of the brain. **Figure 1** gives an overview of the study designs that were used.

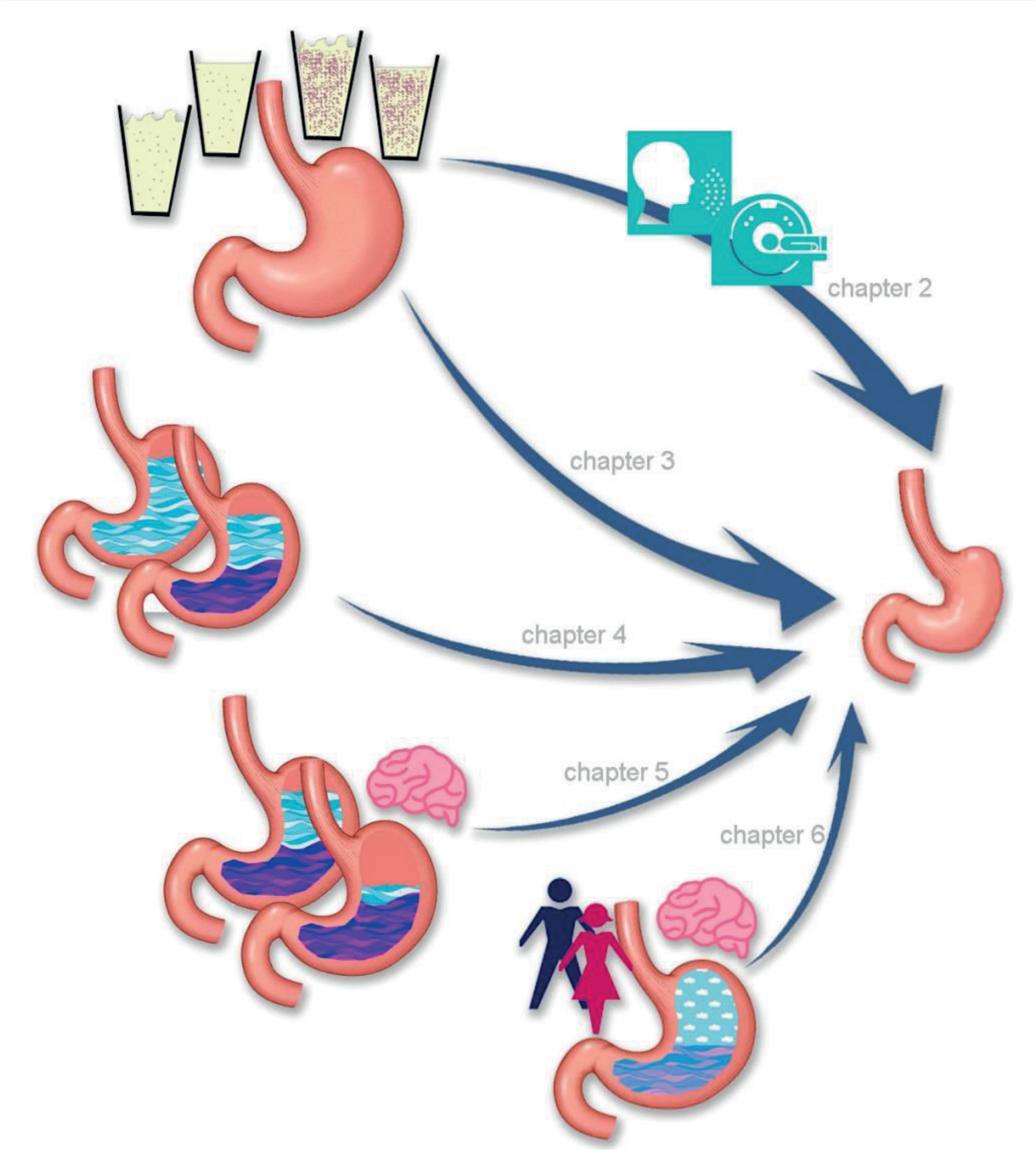


Figure 1. Visual study designs overview: Chapter 2 compares gastric emptying methodologies. Chapter 3 focuses on the effects of viscosity and nutrient load on gastric emptying. Chapter 4 compares a layered to a mixed food stimulus and the effect on gastric emptying. Chapter 5 shows the effect of adding water to a food stimulus on gastric distension and neural activation. Lastly, Chapter 6 compares men and women, with outcomes being appetite ratings, gastric liquid and air content and brain activation after consumption of a carbonated drink.

These are the main results of the chapters within this thesis.

*Measuring gastric emptying*

MRI measurements of the stomach as opposed to an indirect measurement by proxy, such as $^{13}C$ breath testing are to be preferred. Both MRI and $^{13}C$ breath analysis provide insight in the gastric emptying process and both these measures can be used to compute a gastric emptying $t_{50}$. However, on an individual level results diverged considerably between methods. MRI allows visualisation of intragastric food behaviour and unlike breath testing is not affected by food matrix effects (**Chapter 2**).

*Effects of viscosity and calories on gastric emptying and subjective fullness*

We show that gastric emptying is affected by energy load, and to a much smaller extent by viscosity. Additionally we show that a thick shake containing 100 kcal will yield higher fullness sensations than a thin shake containing 500 kcal. In the chapter we name this phenomenon 'phantom fullness', i.e., a sense of fullness and satiation caused by the taste and mouthfeel of a food which is irrespective of actual stomach fullness (**Chapter 3**).

*Gastric layering and sieving*

When water is in the stomach together with nutrient rich pieces, gastric sieving of the water can occur, allowing the water to drain from the stomach while retaining the solid masses. This can be mitigated by blending the water with the stimulus. Combining results from two studies, we show that this process can occur for liquids as well: a liquid meal followed by a drink of water empties about twice as fast in the first 35 minutes compared to the same amount of water incorporated within the liquid meal. Using MRI we were able to show layering within the stomach and increased emptying of this watery layer (**Chapter 4**).

The possibility to measure both gastric and functional MRI in conjunction, while also recording subjective ratings, allows new insight in the effect of gastric distention. We manipulated gastric distention by having participants drink a small or large volume of water after an energy dense liquid meal. With 300mL of increased gastric content inducing distention, appetite was lowered. Ingestion led to significant changes in activation in the right insula and parts of the left and right inferior frontal cortices over time, but there was no significant difference between the control and 300 mL of added distention (**Chapter 5**).

Women retain significantly more fluid after a carbonated drink in their stomach than men. When comparing correlations between subjective ratings and intragastric liquid and gas and total gastric volume, nausea and fullness correlated strongest with the liquid fraction within the stomach, bloating strongest with total gastric volume. Subjective ratings correlated weakest with the gas fraction. When it comes to brain activation, men had stronger activation in the primary motor and somatosensory cortex (**Chapter 6**).

## Methodological considerations and validity

*Internal validity: posture, anatomy and gastric emptying*

*"A good stance and posture reflect a proper state of mind"*
*Morihei Ueshiba*

Though the founder of Aikido undoubtedly speaks true words in the quote above, we may wonder whether the same goes for one of the main outcomes of this dissertation: does posture affect gastric emptying? In this thesis participants remained completely stationary in their supine position for the duration of the study. Work in this area has shown that a supine posture (i.e. laying down as when undergoing an MRI scan) may slow emptying[1]. Positional change, especially sitting up, increases intra-abdominal pressure[2]. By keeping participants in a supine position for the duration of the session we avoided positional changes, thereby minimizing changes in pressure allowing undisturbed measurement of emptying. However, today's world people tend to eat and digest whilst sitting, and consideration should be given to the fact that the participant is in a different position in the MRI scanner.

A supine position means that gastric content flows slower through the pyloric sphincter, due to gravity not propagating flow as effectively as it would in a standing or sitting position. However, it has shown no overall effect of posture on gastric emptying, and only shows effects on trans-pyloric flow using Doppler sonograms and on the distribution of food within the stomach[3]. Admirably, researchers from Zürich have gone so far as to obtain ethical permission to put people upside down within an MRI scanner in order to induce changes in gastric emptying[4]. They conclude: *´the stomach maintains the rate of gastric emptying despite radical changes in body position and intragastric distribution of gastric contents.*' Thus, the effects of a supine position versus sitting may be relatively small. Other more sideways body positions have been studied, but also in that case the differences were minimal[5]. Given the above literature, there is only a relatively small effect of posture on gastric emptying rate.

*Gender*

All but one study described in this thesis was performed in men. As described in the introduction it is known that men show quicker gastric emptying than women[6-8]. A slower transit time in the small bowel has also been shown for women compared to men[9]. Our studies had a within-participant design, so the interpretation of our results should not be affected by the gender difference. However, this gender difference, which is confirmed by our own work in chapter 6, should be used in future clinical assessment of gastrointestinal physiology. Mori et al. specifically suggest amending diagnostic criteria to accommodate different reference values for women[10].

The greater retention in women may be caused by proximal gastric motor differences, inducing different sensory experiences, as concluded by Mearadji et al[11]. Combining these studies and the work presented in this dissertation, the conclusion may be that results from work with single-sex participants are more fundamental findings. Analogous to the subsection 1 on body position in the scanner, the results from the second, third, fourth and fifth chapters can only be viewed in relative terms, and not in absolute terms because the work was exclusively performed in male participants. Though subsequent research may benefit from the use of a dual gender sample, our results show significant within-participant effects.

*External validity*

Research in academia is often performed with a participant group, largely consisting of undergraduate students. This does however lead to a problem with external validity, as undergraduate students are often younger and healthier than the general population.

More importantly, in the research presented in this thesis as well as in most nutrition research we actively exclude overweight and obese individuals. However, when we look to the general population, the norm is shifting towards being overweight[12]. This means that our sample or normal weight individuals may inadvertently contain a selection bias toward people who are less responsive to the obesogenic environment. We should be careful to extrapolate the results of young and healthy individuals, including the ones in this research, to the general population. The research in this dissertation mainly aims to show proof of concept. Work in the field of nutrition can be aided by replication of findings, using participants who are overweight, when research aims to confirm real-world application.

## Conclusions

*Food properties, the food matrix and the stomach*

Confirming the results of Marciani et al. we show that calorie density is most important in determining gastric emptying speed[13]. However, our results show that the satiating effect of fibers may not be exclusively dependent on delayed gastric emptying[14], as fullness scores were higher for a viscous shake though it emptied more quickly (**Figure 2**).

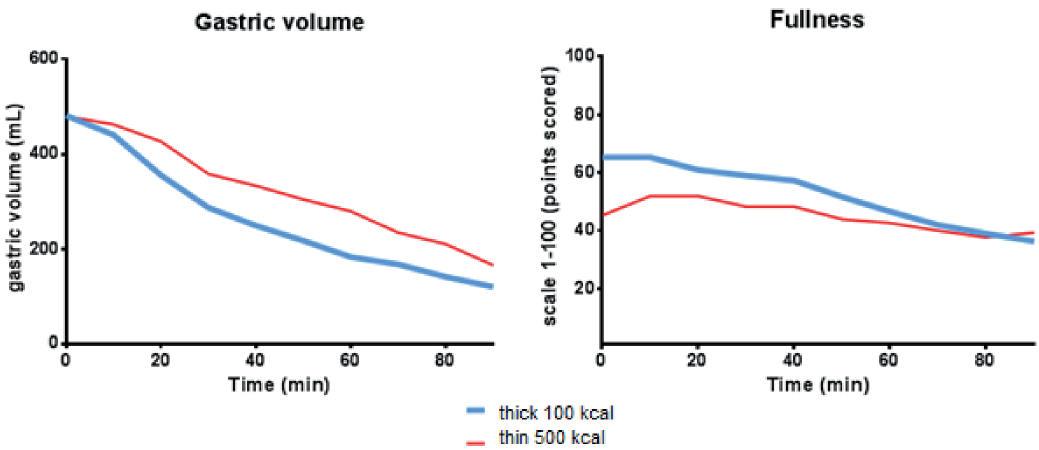


Figure 2. Gastric volume plotted on the left and fullness on the right showing greater fullness scores for a liquid food stimulus emptied more quickly and vice versa.

Apart from macronutrients, water, fiber, $CO_2$ and air can all be a part of the food matrix. The different physicochemical properties of these compounds may affect intragastric behavior.

MRI allows visualization of separation of the matrix as well: air can be separated from liquid content and different layers can be seen within a liquid. Within this dissertation we have exclusively used liquid stimuli. It is an important consideration that behavior of the food matrix, and its ensuing gastric emptying effects such as gastric sieving, become even more complex with liquid, semi-solid and solid gastric content. On top of this solid content may become liquid[15], and liquids may become solid[16].

A normal meal can consist of different food items and drinks which may go from non-caloric (water) to highly caloric (milk shake). Intragastrically, this meal may lead to mixture of solid chunks of different sizes, together with more or less semi-solid content and more or less caloric fluid. The relative changes within the stomach in viscosity, (macro)nutrients and other properties, of such a mixture are highly complex, and that complexity will affect gastric emptying.

This highly complex mixture will lead to non-uniform intragastric behavior, such as for example layering. As we have shown gastric layering increases gastric emptying rates, confirming earlier work[17]. On top of this it is impossible to label such a highly complex mixture, as we and others have shown with much simpler stimuli[18].

In conclusion, the work in this thesis uses relatively simple foods and we observe significant food matrix effects, and these will probably become more diverse and more significant if more components are added to this matrix such as would be the case in normal ingestion. MRI is the only technique which can give insight in the intragastric behavior and gastric emptying of highly complex food matrices.

*Gastric emptying and gender*

Our work confirms emptying differences between the genders, with women emptying significantly slower than men[6,7]. This confirmation is significant because it extends the current knowledge by using a superior technique in MRI, but also a liquid instead of a solid stimulus. Whether or not this can be attributed to hormonal differences cannot be derived from our work, but it is an interesting avenue to pursue[19-21].

*Neural activation and gastric distension*

In the work from **Chapters 5** and **6** we find activation in areas that could be expected from the literature to be relevant for ingestion and gastric stimulation, such as the insula, hippocampus and orbitofrontal cortex[22-24]. However, we also find activation in the orbital and triangular parts of the inferior frontal cortex in both data sets. Given that we found activation in this region in two distinct data sets, using different foods, they may be associated with ingestion. The inferior frontal gyrus could be an interesting candidate for region of interest analyses in future fMRI studies manipulating ingestion.

Lastly, we show that in **Chapter 5** and **6** that within-session body coil and head coil exchange within the MRI scanner allows interwoven gastric volume measurements and brain activation measurements allowing further insight in neural correlates of gastric distention.

*Main conclusion*

Concluding, in this thesis we have demonstrated that subjective fullness and gastric fullness are not the same. Additionally we have shown that the food matrix may lead to complex behaviour of meals within the stomach, an example of this is layering. The gastric dynamics caused by food properties affect gastric emptying. MRI is a good technique to visualize these dynamics of gastric emptying including different phases and food behaviors. Lastly we have confirmed the known association between ingestion and certain areas within the brain and also identified brain areas which are interesting for future fMRI research on ingestion and distention of the stomach.

## Implications and further research

*Designing food for satiation*

**Chapter 3** introduced the concept of *phantom fullness*, feeling full even though the stomach is relatively empty due to orosensory exposure. Our work made it possible to allow direct comparison of the gastric content and appetite ratings between liquid food stimuli varying in caloric load. This principle can be taken further to increase satiation and satiety by designing foods to make them more 'difficult' to eat. Reducing the eating rate, and thereby increasing the orosensory exposure time has been suggested as a viable way to increase satiation and satiety[25]. Beverages have been made more sating by adding fiber[26]. Melnikov et. al and Peters et al. show a reduction in appetite by giving participants low caloric foam instead of a liquid[27,28]. Future research into using food design to limit caloric intake should delve further into manipulating the food matrix so that it will increase satiation.

*Following through: measurement from food to gut*

Apart from gastric sensations, oral processing is also relevant for satiation[29]. The research in this dissertation shows we can accurately view gastric emptying using MRI, and others have shown MRI of the small bowel to be possible[9,30,31]. As can be seen in **Figure 3,** MRI of the oral cavity is possible as well. This means that it should be technically possible to visualize food using MRI from ingestion, chewing, including estimating particle size, through the stomach into the small bowel. This would allow further understanding of digestion and combine oral processing with gastric insights.

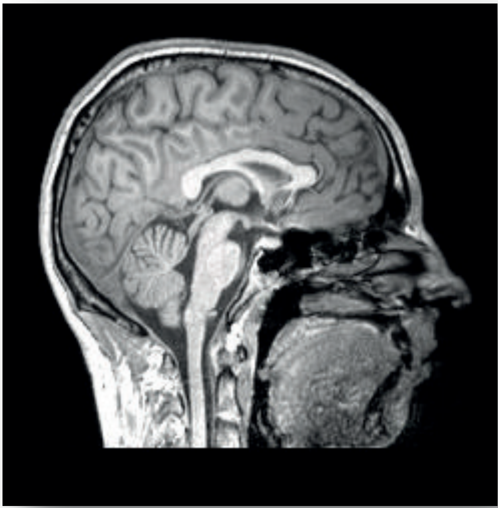

Figure 3. Anatomical MRI slice of the head of a participant. The tongue can be seen within the oral cavity bordered by the airway on the left (black) and palate on the top.

*Clinical applications of MRI*

In **Chapter 2** we show the advantages of MRI as compared to indirect measurements. The clinical assessment of gastric emptying is currently often still performed via these indirect methods. If indirect methods are used, scintigraphy is often used as the golden standard[32-36]. Further research should focus on furthering MRI as a preferable alternative to scintigraphy, not only because it allows accurate measurement, but also because it means there is no radiation burden on the patient and no requirement for a radioactive tracer to be incorporated into the test meal. This would require the creating of a standardized meal and reference system for MRI and validation of this within the clinical setting.

*From correlation with brain regions to causal models*

Further integration of research techniques may allow us to go beyond correlation. fMRI techniques allow insight in brain processes, but for insight into causal relationships we need other approaches. Roelofs et al. show how to combine fMRI with direct activation of brain areas in animal models using Designer Receptor Exclusively Activated by Designer Drug (DREADD)[37]. Translational research would be a possible avenue to pursue in order to obtain fundamental insight into cause and effect of regional brain activation. Amygdala, hippocampus, precuneus and insula are usual suspects in food fMRI research[38], but close cooperation in translational projects with animal model scientists will make it possible to elucidate the causal relations between these centres in the brain and perhaps discover the exact causal mechanism of satiation.

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# Chapter 8.

Summary

**Summary**

This thesis aimed to further determine how gastric content relates to subjective experiences regarding appetite, how this relation is affected by food properties and whether this is visible in neural activation changes. This was studied using appetite questionnaires, MRI of the stomach and fMRI of the brain.

**Chapter 2** shows the main advantages of MRI measurements of the stomach as opposed to an indirect measurement by proxy, such as $^{13}C$ breath testing. Both MRI and $^{13}C$ breath analysis provide insight in the gastric emptying process and both these measures can be used to compute a gastric emptying $t_{50}$. However, on an individual level results diverged considerably between methods. In contrast to tracer dependent methods, MRI is not affected by food matrix effects and the behaviour of the food can be investigated.

In **Chapter 3** we show that gastric emptying is affected by energy, and to a much lesser extent by viscosity. Additionally we show that a thick shake containing 100 kcal will yield higher fullness than a thin shake containing 500 kcal. In the chapter we name this phenomenon 'phantom fullness', i.e., a sense of fullness and satiation caused by the taste and mouthfeel of a food, irrespective of actual stomach fullness.

When water is in the stomach together with nutrient rich pieces, gastric sieving of the water can occur, allowing the water to drain from the stomach while retaining the solid masses. **Chapter 4** shows this process can occur for liquids as well: a liquid meal followed by a drink of water empties about twice as fast in the first 35 minutes compared to the same amount of water incorporated within the liquid meal. Using MRI we were able to show a watery and a nutrient rich layer within the stomach and the subsequent emptying of this watery layer.

In **Chapter 5** we show for the first time the possibility to measure subjective measurements in conjunction with both gastric and functional MRI. We manipulated gastric distention by having participants drink water after an energy dense liquid meal. With 300mL of increased gastric content inducing distention, appetite was lowered. Ingestion led to significant changes in activation in the right insula and parts of the left and right inferior frontal cortices over time, but there was no significant difference between the control and 300 mL of added distention.
We show in **Chapter 6** that women retain significantly more fluid in their stomach after a carbonated drink compared to men. When comparing correlations between subjective ratings and intragastric liquid, gas and total gastric volume, nausea and fullness correlated strongest with the liquid fraction within the stomach, bloating strongest with total gastric volume. Subjective ratings correlated weakest with the gas fraction. When it comes to brain activation, men had stronger activation in the primary motor and somatosensory cortex.

Concluding, in this thesis we have demonstrated that subjective fullness and gastric fullness are not the same. Additionally, we have shown that the food matrix may lead to complex behaviour of meals within the stomach, an example of this is layering. The gastric dynamics caused by food properties affect gastric emptying. MRI is a good technique to visualize these dynamics of gastric emptying, including phase separation and intragastric food behaviour. Lastly, we have confirmed the known association between ingestion and certain areas within the brain and identified brain areas which are interesting for future fMRI research on ingestion and distention of the stomach.

# Chapter 9.

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2017 Elected PhD representative Student Staff Council Wageningen University

2017 Chair Student Staff Council Wageningen University

2017 PhD representative working group Future of Wageningen University

2017 Invited column writer Resource – Wageningen University and Research magazine

## Conference abstracts

*Poster presentations:*

Changes in brain activity associated with stomach distention during gastric emptying, Camps, G., Spetter, M.S., de Graaf. K., Smeets, P.A.M., Organization for Human Brain Mapping, , Honolulu, US, 2015

Bloating and the brain: effects of beer and soda on gastric perceptions, gastric distension and brain responses in men and women, Camps, G., de Graaf, K., Smeets, P.A.M., Society for the Study of Ingestive Behavior 2017 Montreal, Canada

*Oral presentations:*

Relationship between reported fullness and actual gastric content, Camps, G., Smeets, P.A.M., Mars, M., de Graaf, K. British Feeding and Drinking Group, 2015, Wageningen, the Netherlands

Effects of viscosity and nutrient load on gastric emptying as determined by MRI, Camps, G., Mars, M., de Graaf, K., Smeets, P.A.M., Society for the Study of Ingestive Behavior, 2015, Denver, USA

Just add water: effects on gastric distension, appetite and brain activity, Camps, G., Veit, R., Mars, M., de Graaf, K., Smeets, P.A.M., Society for the Study of Ingestive Behavior 2016 Porto, Portugal

## Overview of training activities

**Discipline specific**

Organization for Human Brain Mapping 2014 Hamburg, Germany

Nudge-IT Project meeting 2014 Edinburgh, UK

Advanced course in Sensory evaluation and food preferences 2014 Kopenhagen, Denmark

Nudge-IT Project meeting 2014 Kopenhagen, Denmark

Organization for Human Brain Mapping 2015 Honolulu, USA

Society for the Study of Ingestive Behavior 2015 Denver, USA

British Feeding and Drinking Group 2015 Wagningen, the Netherlands

Nudge-IT Project meeting 2015 Tübingen, Germany

Society for the Study of Ingestive Behavior 2016 Porto, Portugal *(New Investigator Travel Award winner)*

Nudge-IT Project update meeting 2016 Utrecht, the Netherlands

Society for the Study of Ingestive Behavior 2017 Montreal, Canada

**General courses**

OHBM Educational Course on Review papers, 2014

Pitching and presenting scientific content, 2016

fMRI analysis, Tübingen, 2016

Course on Scientific writing, WGS, 2017

European Nutrition Leadership Program – essentials, 2017

**Optionals**

Preparing PhD research proposal, 2014

PhD study tour to the East Coast USA, 2015

MUFFIN research study group, UMCU, Utrecht, 2014 - 2017

Sensory Science research study group, Wageningen, 2014 – 2017

**Colophon**

The research described in chapters 2, 3, 4 and 5 in this thesis was conducted as part of the Nudge-it project. Nudge-it is a European Commission-funded FP7 project (under Grant Agreement 607310) bringing together dozens of scientists from 16 institutions across six European countries, the US and New Zealand. The project engages internationally leading experts in the neurobiology of motivational behaviour, reward and regulation of appetite, experimental psychology, functional brain imaging, behavioural economics and computational modelling. The project is developing innovative tools that link understanding across these interacting disciplines. The overall aim is to better understand decision-making in food choice and to build predictive models to contribute to improving public health policy.

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