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Novel strategies to address disrupted sensing and signalling of satiety

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Novel strategies to address disrupted sensing and signalling of satiety

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ter verkrijging van de graad van doctor aan de Universiteit Maastricht,
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volgens het besluit van het College van Decanen,
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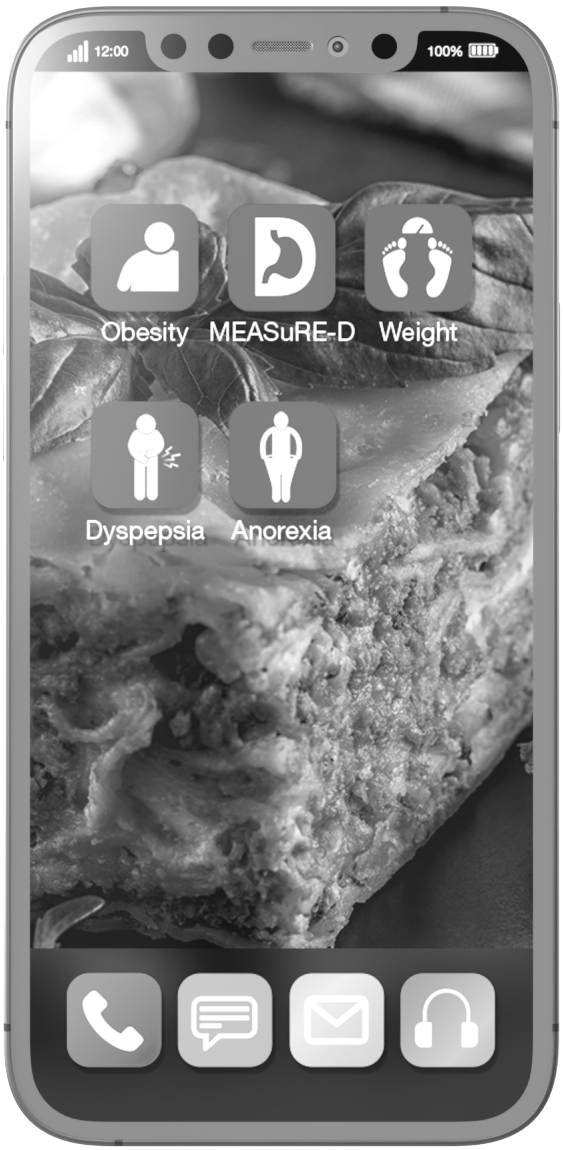
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100%
Obesity
MEASuRE-D
Weight
Dyspepsia
Anorexia

Chapter 1

General introduction

Disturbances in sensing and signalling of satiety and food intake

Food intake and sensing and signalling of satiety can be disturbed in various clinical situations. A scenario of increased food intake and diminished satiety signalling is seen in the case of overweight and obesity.[1,2] On the other hand, decreased food intake and increased satiety can be seen in the aging population, also known as anorexia of aging.[3,4] Moreover, satiety signalling and food intake can be influenced by abnormal sensations such as recurring pain, stress, anxiety, *et cetera*. This can result in food intake disorders such as avoidant/restrictive food intake disorder (ARFID).[5-7] In other words, disturbances in satiety signalling can have important clinical repercussions and the direction of these changes will have an important impact on health outcome. In this thesis, conditions and scenarios that influence satiety signalling will be explored in order to develop more efficient treatment and monitoring strategies.

Overweight and obesity

Obesity is defined as a body mass index (BMI) of 30 kg/m^2 and higher and overweight is defined as a BMI of 25.0 to 29.9 kg/m^2.[1] The prevalence of obesity in the general population has nearly tripled since 1975.[8] The burden of obesity and overweight is evident. A study has shown that every 5-unit increase in BMI above 25 kg/m^2 increased overall mortality (29%), vascular mortality (41%), and diabetes-related mortality (210%).[9] Moreover, medical costs of obese individuals are 30% higher compared with people with normal weight.[10] This highlights the severe economic burden on healthcare systems rising from overweight and obesity.

It is generally accepted that overweight and obesity result from small, but cumulative imbalances between energy intake and energy expenditure resulting in a positive energy balance and thus weight gain.[2] One of the most used treatment strategies to lose weight is to limit energy intake by means of a reduced-energy diet. However, compliance is notoriously low due to persistent hunger feelings. An energy-restricted diet often results in an initial weight loss plateau, which can be explained by a lack of diet adherence.[11] Moreover, maintaining the reduced weight has proven to be difficult for the majority of individuals, resulting in regaining weight. A regain of more than half of the weight lost after 2 years and more than three-quarters after 5 years can be seen according to a meta-analysis of

weight-loss studies.[12] Currently, the only effective strategy for long term weight loss is bariatric surgery, such as the Roux-en-Y gastric bypass surgery.[13] However, gastric bypass surgery is invasive and only indicated in a small group of morbidly obese subjects. This urges the need for non-invasive treatment strategies in battling the obesity epidemic that are also effective on the long term. The GI tract exerts its regulating effects on eating behaviour via its ability to produce and secrete GI peptides from food-derived triggers and neutrally, through receptors on vagal afferents.[14-17] Therefore, the gastrointestinal (GI) tract is believed to play an important role in regulating eating behaviour.

In order for the gut to affect eating behaviour, food constituents need to be sensed in the gut.

Satiety signalling by the intestines is hypothesised to be mainly exerted by nutritive triggers.[18] It has been shown that macronutrient infusion in the intestine reduces subsequent food intake and is accompanied by the release of GI peptides.[19-21] This is commonly referred to as "intestinal brake". Moreover, it has been shown that this intestinal brake is operational in a proximal to distal gradient, with generally more pronounced effects on no-absorbed nutrients in the more distal parts of the small intestine.[22] A recent study showed similar decreases in food intake after high concentrations of proteins, carbohydrates, and lipids.[21] This indicates nutrient sensing ability throughout the intestine for all macronutrients. However, macronutrients differ with respect to caloric contents. The use of a satiety inducing intervention without caloric content would be preferable. In this respect, much attention is given to tastants as non-caloric substances.

Currently, five prototypical tastes can be distinguished by humans: sweet, bitter, umami, sour, and salty. Stimulation of taste receptors on the taste buds on the tongue elicits the taste that we perceive. Sensing of sour and salty is mediated by ion channels, whereas sensing of sweet, bitter, and umami is mediated by two families of taste receptors. Taste receptor family 1 (TAS1) is responsible for sensing sweet and umami taste. Bitter taste is perceived by taste receptor family 2 (TAS2).[23] These taste receptors are not only present on the tongue, they are expressed throughout the entire human GI tract.[24-27] The taste receptors that are found in the GI tract are co-localised with entero-endocrine cells (EECs). Activation of these taste receptors *in vitro* results in GI peptide release.[28-30] In this perspective, gastrointestinal delivery of non-caloric tastants appears to be a potentially attractive treatment option in battling the obesity epidemic.

Underweight and loss of appetite

The gastrointestinal sensing and signalling processes can also become impaired in a way that it results in reduced food intake and weight loss. For instance, a progressing decrease in hunger and food intake can be seen during aging. This is called 'anorexia of aging'.[31-33] It must be noted that energy expenditure and resting metabolic rate also decline with aging. However, energy intake often decreases to a greater extent than energy expenditure, resulting in weight loss.[34,35] Elderly are predisposed to weight loss and loss of muscle mass leading to sarcopenia and frailty due to this 'anorexia of aging'.[36] There is still debate on the factors that contribute to 'anorexia of aging'. It is hypothesised that the GI tract is an important factor in this regard.

The initial satiating effect that occurs directly after ingesting a meal mainly results from distension of the stomach wall. The stomach is aligned with mechanoreceptors that sense an increase in stomach volume. This triggers neural circuits that inhibit food intake behaviour.[18,37] It has been shown that sensations of satiation and fullness are evoked after small increases in stomach volume, whereas sensations of pain and nausea are evoked after larger increases in stomach volume.[38] Moreover, several studies have shown that hunger feelings are negatively correlated with feelings of distension after inflation of a gastric balloon.[39,40] Most studies describe protocols using balloons placed in the proximal stomach. However, other studies showed that distal stomach filling is also a determinant of satiation.[3,41] Moreover, a recent study displayed comparable mechanosensitivity in the proximal and distal stomach.[42] In elderly, postprandial fullness and satiation are found to be increased compared to younger subjects. It is hypothesised that this results from delayed gastric emptying, a larger antral area and overactive sensing profile of GI mechanoreceptors.[3,4] These findings point to an impaired accommodation response to meal intake in elderly, possibly attributable to an altered sensing profile of mechanoreceptors.

Therefore, interventions aimed at reducing the sensing of mechanoreceptors in the stomach could prove to be a therapy for underweight and loss of appetite. This could be achieved by using a local topical anaesthetic to block the mechanoreceptor signalling. Application of a local anaesthetic in rats' stomachs has been shown to significantly increase food intake of a subsequent meal.[43] Currently, no human studies have been performed to investigate the effects of intragastric application of local anaesthetics on food intake.

Functional dyspepsia and patient reported outcome measures (PROMs)

Another clinical condition that can be associated with altered perception of food-induced stimuli is functional dyspepsia (FD). Functional dyspepsia symptoms are common with an estimated prevalence of 10-15% in the general population, making it one of the most common functional gastrointestinal disorders.[44] The Rome IV criteria for disorders of the brain-gut interaction are used to define FD. FD is defined by the presence of various symptoms in the absence of evidence for systemic, metabolic, or organic diseases that could explain the complaints.[45] Four core symptoms have been defined among the heterogeneous presentation of FD patients: epigastric pain, epigastric burning, early satiation, and postprandial fullness.[45,46] Based on the occurrence of these core symptoms, three subtypes of FD can be distinguished: postprandial distress syndrome (PDS), epigastric pain syndrome (EPS), and overlap syndrome (OS).[45] The pathophysiology of FD is currently incompletely understood and multifactorial.[45,47,48] It has been hypothesised that different pathophysiological mechanisms can contribute to the variety of predominant symptoms. Commonly accepted pathophysiological mechanisms are visceral (hyper)sensitivity, gastric emptying time, and gastric accommodation. Moreover, it has been shown that the extent to which these symptoms add to the existence of FD can vary between FD subtypes.[49-51] As mentioned before, impaired gastric accommodation could be a product of hypersensitivity of mechanoreceptors, resulting in a decrease in food intake. Moreover, a recent meta-analysis showed an association between delayed gastric emptying and early satiation/fullness.[52] Visceral hypersensitivity and pain resulting from food intake can lead to a clinical phenomenon called ARFID. ARFID is a fairly novel diagnostic category defined by the DSM V and represents a distinct eating disorder characterised by reduced food intake without body image distortion.[5-7] ARFID can lead to nutritional deficiency and subsequent weight loss. Visceral hypersensitivity could result in avoiding overall food intake or specific food products that trigger symptoms.

There is a need to develop effective treatment strategies, as economic and societal burdens of FD are high. Up to 40% of FD patients consults a physician, indicating financial implications.[53]

Moreover, quality of life and work productivity are impaired in FD patients.[54] Dividing patients into subtypes according to their predominant symptoms helped to develop 'symptom-based' treatment strategies as opposed to 'FD diagnosis-

based' treatment strategies.[55] However, new treatment strategies should be thoroughly evaluated.

The Food and Drug Administration (FDA) recommends the use of well-defined patient reported outcome measures (PROMs) in clinical trials to evaluate treatment outcome.[56] The definition of a PRO is *"a measurement of any aspect of a patient's health status that comes directly from the patient (i.e., without the interpretation of the patient's responses by a physician or anyone else).*[56] According to a recent review, 20 PROMs can be used to assess dyspeptic symptoms. However, not a single of those instruments underwent all the development steps recommended by the FDA. Therefore, there is currently no consensus on the most relevant outcome measure in FD patients.[57] Current assessment methods that evaluate dyspeptic symptoms are self-reported retrospective questionnaires characterised by daily or weekly monitoring. This generates limitations such as recall bias,[58] ecological bias,[59-61] and lack of compliance or fake compliance.[62] Monitoring symptoms in FD patients is cardinal, as this is the only available clinical outcome measure to evaluate treatment efficacy. However, abovementioned limitations indicate that currently available questionnaires are suboptimal. This underlines the need for a more reliable assessment method of symptom monitoring to evaluate treatment efficacy in functional dyspepsia.

The Experience Sampling Method (ESM), also referred to as Ecological Momentary Assessment (EMA), might be able to overcome aforementioned limitations. ESM is an electronic structured questioning method that is characterised by random, repeated assessments in a patient's current state and environment. A digital device is used to complete ESM assessments shortly after an auditory signal is emitted. This makes sure that the patient's response to questions adequately reflects their current symptoms. ESM takes into account contextual, social and psychological factors, which might affect symptom reporting.[63,64] Therefore, ESM might be able to capture symptom variability over time and reduce the risk of recall bias. ESM has been extensively used in psychologic and psychiatric disorders. However, the use of ESM for GI disorders is currently limited. Two studies evaluated the use of ESM in patients with irritable bowel syndrome (IBS). Those studies found significant correlations between symptom scores on ESM and on commonly used questionnaires. However, significantly higher scores for abdominal pain were reported on retrospective questionnaires compared with mean scores derived with ESM. Interestingly, these abdominal pain scores seemed to be represented by the peak scores recorded with ESM.[65,66]

According to the FDA criterium for IBS, research in IBS should mainly focus on abdominal pain and bowel habits.[67] As these are core symptoms in IBS, questionnaires for FD should thus focus on core symptoms in the various subtypes of FD. Moreover, ESM has the potential to assess individual triggers that can result in complaints of early satiation/postprandial fullness. This could provide more insight in the factors that play a role in the emergence of satiety related complaints in patients with FD. Additionally, triggers for other symptoms can also be investigated using ESM, which could lead to personalised therapeutic strategies.
Therefore, ESM has the potential to assist in disease self-management and to assess factors that play a role in the emergence of satiety related complaints. The use of ESM in FD patients has not been described previously. Recently, an FD ESM-questionnaire was created by item selection after a systematic literature search, patient focus groups, and an international expert meeting.[68]

Aim and outline of the thesis

This thesis aims to provide an in-depth understanding of food-gut interactions in order to develop and optimise therapeutic strategies aiming at correcting conditions with disturbed (*i.e.*, too high or too low) energy intake. Moreover, a novel tool to provide more insight into disturbed sensing and signalling of satiety as seen in FD was investigated.

Taste receptors can be found throughout the entire GI tract. A function of these taste receptors is to sense the presence of food in the gut. The systematic review and meta-analysis described in **Chapter 2** provides an overview of the effects of gastrointestinal taste receptor activation on eating behaviour.

Previous research showed a decrease in food intake after activation of duodenal taste receptors using a tastant mixture of bitter, sweet, and umami. The optimal location of taste receptor activation to affect eating behaviour is unknown. The study in **Chapter 3** aimed to assess the optimal location(s) of intestinal taste receptor activation to affect eating behaviour.

Chapter 2 showed that, among tastants, bitter agents seem to be the most potent tastant to affect eating behaviour. However, the optimal location to exert their effect is still unknown. Since bitter taste is linked to toxic substances, more

proximal activation of bitter tastants could exert a larger inhibitory effect on food intake. The study in **Chapter 4** aimed to assess the effect of gastric-, oral-, and synchronous gastric- and oral delivery of a bitter tastant on eating behaviour.

Conditions such as FD and anorexia of aging can negatively influence food intake behaviour. A possible explanation for this is an increase in sensitivity of mechanoreceptors. In **Chapter 5,** we aimed to explore the effect of lidocaine infusion directly into the stomach on eating behaviour.

Dyspeptic symptoms can be triggered by food intake. Current assessment methods to evaluate dyspeptic symptoms and treatment efficacy have several limitations. In the studies depicted in **Chapter 6 and 7**, we examined a novel tool to assess symptoms and treatment efficacy in FD patients.

Finally, in **Chapter 8**, the main findings of the studies presented in this thesis will be presented. Moreover, our findings will be discussed in relation to data from other studies and implications for future research will be mentioned.

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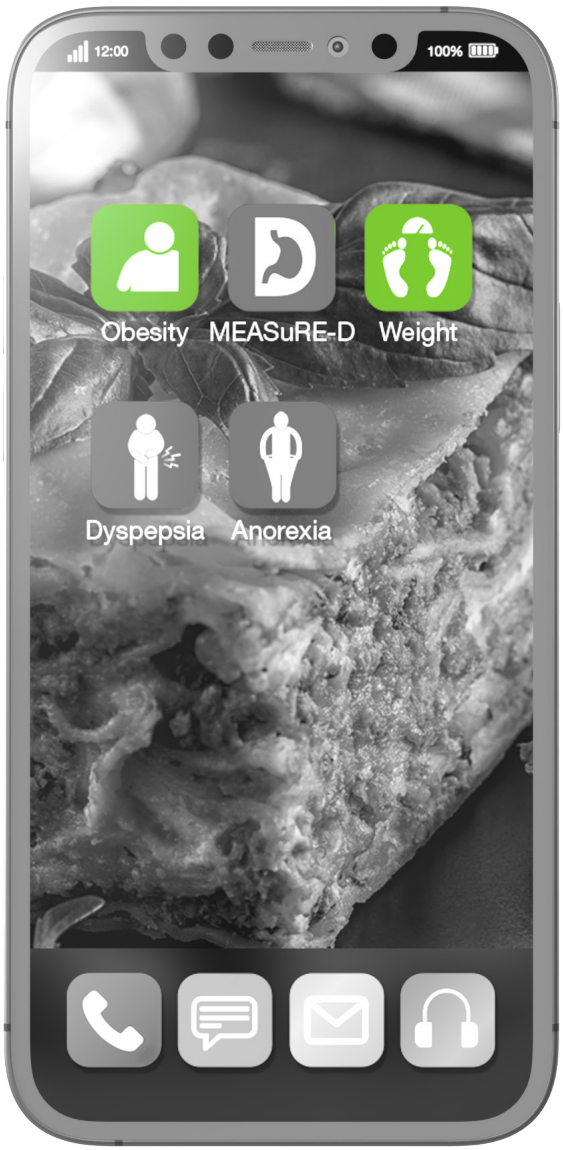
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Chapter 2

Effects of gastrointestinal delivery of non-caloric tastants on energy intake: A systematic review and meta-analysis

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Abstract

Purpose

Taste receptors are expressed throughout the gastrointestinal tract. Activation of post-oral taste receptors using tastants could provide a non-invasive treatment option in combating the obesity epidemic. The aim of this review was to examine the effect of post-oral delivery of non-caloric tastants on eating behaviour reflected by primary outcome energy intake and secondary outcomes GI symptoms and perceptions and potential underlying mechanisms. This review was conducted according to the PRISMA guidelines for systematic reviews.

Methods

A systematic literature search of the Cochrane, PubMed, Embase, and Medline databases was performed. This systematic review and meta-analysis was registered in the PROSPERO database on 26 February 2020 (ID: CRD42020171182). Two researchers independently screened 11912 articles and extracted information from 19 articles. If at least two studies investigated the effect of the same taste compound on primary outcome energy intake, a meta-analysis was performed to determine pooled effect sizes.

Results

Nineteen papers including healthy volunteers were included. In the 19 papers analysed, effects of various tastants were investigated in healthy volunteers. Most extensively investigated were bitter tastants. Meta-analysis of effects of bitter tastants showed a significant reduction in energy intake of 54.62 Kcal (95% CI: -78.54 – -30.69, p=0.0014).

Conclusions

Bitter stimuli are most potent to influence eating behaviour. Energy intake decreased after post-oral delivery of bitter tastants. This highlights the potential of a preventive role of bitter tastants in battling the obesity epidemic.

Introduction

There are at least five prototypical basic tastes that can be distinguished by humans: sweet, sour, bitter, salty, and umami More recent studies have pointed to the existence of other basic tastes (*i.e.*, fat and starch)[1,2] as well as taste disorders such as metallic taste in cancer patients treated with chemotherapy.[3] Moreover, there is a phenomenon known as chemesthesis, which refers to chemical sensations that are perceived as warmth, heat, irritation, cooling, or pungency.[4] A prototypical pungent stimulus is capsaicin, resulting in a sizable number of studies investigating the effects of capsaicin as a weight loss intervention.[5,6]

As far as the prototypical basic tastes are concerned, these can be sensed by taste buds present on the tongue. Ion channels mediate the sensing of salty and sour taste, whereas sensing sweet, bitter and umami taste is mediated by two families of taste receptors. Taste receptor family 1 (TAS1) generally senses sweet and umami taste and taste receptor family 2 (TAS2) primarily senses bitter taste.[7] It is hypothesised that these prototypical tastes exist in order to predict the type of food that is ingested (*i.e.*, sweet for saccharides, umami for glutamate, and bitter for potential toxic substances).[8] However, it should be noted that several studies show that the negative affective response to bitter can be decoupled by, for instance, the positive response to caffeine.[9,10] In addition, several studies have shown that the activation of oral taste receptors can result in the release of gastrointestinal (GI) peptides such as peptide YY (PYY), glucagon-like peptide 1 (GLP-1), and cholecystokinin (CCK).[11,12] These GI peptides have been shown to influence eating behaviour by reducing appetite sensations and food intake after intravenous administration.[13-18]

Taste receptors are not only present on the tongue but are expressed throughout the entire human gut.[19-22] In the GI-tract, entero-endocrine cells (EECs) are co-localised with these taste receptors. *In vitro* studies have shown that activation of these taste-receptors results in the release of GI peptides.[23-25]

Activation of taste receptors can be elicited using non-caloric tastants. Taste receptor activation using non-caloric tastants to influence eating behaviour is potentially considered as a non-invasive treatment option in combating the obesity epidemic.[26] This concept deserves further evaluation. To date, a significant number of papers[27-45] describing the effects of post-oral delivery of non-caloric tastants (*i.e.*, exposure to tastants anywhere distal to the oral cavity) on eating behaviour, and in particular energy intake, have been published. However, due to inconsistent results reported in these papers, the effect of post-oral delivery of non-caloric tastants on eating behaviour remains unclear and a detailed overview

of the literature on the effects of post-oral delivery of non-caloric tastants on eating behaviour is lacking. Therefore, we conducted a systematic review and meta-analysis using the PRISMA guidelines for systematic reviews. In order to keep this review and meta-analysis concise, we focused on the prototypical basic tastes; novel taste entities and chemesthesis were deemed out of scope.
Our aim was to systematically address randomised controlled trials investigating the effects of post-oral delivery of prototypical non-caloric tastants versus placebo on energy intake in healthy volunteers. Our secondary aims were to evaluate the effects of post-oral delivery of non-caloric tastants versus placebo on GI symptoms and perceptions and potential underlying mechanisms in healthy volunteers. We hypothesised that post-oral delivery of non-caloric tastants results in decreased energy intake compared with placebo in healthy volunteers. Moreover, we hypothesised that post-oral delivery of non-caloric tastants results in increased satiation and the release of GI peptides as the primary mechanism of action. A meta-analysis was performed in case at least two studies described the use of non-caloric tastants of the same taste on the primary outcome energy intake and clinical heterogeneity was acceptable.

Methods

Search strategy

This systematic review and meta-analysis was registered in the PROSPERO database on 26 February 2020 (ID: CRD42020171182). The present systematic review and meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[46] The description of the PICOS (participants, intervention, comparison, outcome, and setting) criteria used to define the research question are depicted in Table 2.1. A structured search in the Cochrane, PubMed, Embase and Medline databases was performed up to 26 February 2020 with the following search strategy: ((((((((((tastant) OR Taste) OR Taste receptor) OR bitter taste) OR quinine) OR denatonium benzoate) OR umami) OR sodium glutamate) OR monosodium glutamate) OR sweet) OR non-nutritive sweeteners)) AND ((((((((((((((((energy intake) OR intake) OR food intake) OR appetite sensation) OR satiation) OR satiety response) OR satiety) OR satiety hormones) OR glucagon-like peptide-1) OR peptide YY) OR Ghrelin) OR leptin) OR cholecystokinin) OR motilin) OR motility) OR gastric emptying).

Table 2.1 Description of the PICOS criteria used to define the research question.

Parameter	Description
Participants	Healthy individuals
Intervention	Prototypical non-caloric tastants at least once
Comparison	Prototypical non-caloric tastants vs. placebo
Outcomes	Energy intake, GI symptoms and perceptions, and mechanisms of effect
Setting	Randomised controlled trials with a parallel or crossover design
Research question	What is the effect of post-oral delivery of non-caloric tastants on energy intake in healthy volunteers? Secondary: what is the effect of post-oral delivery of non-caloric tastants on GI symptoms and perceptions and what is the effect of post-oral delivery of non-caloric tastants on mechanisms of action in healthy volunteers

Selection criteria

Eligibility of each paper was assessed independently by two researchers (T.K. and D.K.) according to predefined criteria. Papers reporting the effects of post-oral delivery of non-caloric tastants on eating behaviour (*e.g.*, energy intake, GI peptides, appetite sensations, GI motility, GI symptoms, brain signalling and other effects) were included. In order to investigate the function of gastrointestinal taste receptors, papers needed to properly bypass oral taste effects. Therefore, studies investigating delivery methods using catheters, capsules or other methods resulting in adequate masking of oral taste were included. Moreover, papers were excluded if they were reviews, comments, replies on an original paper, or abstracts without available full text. No limitations on publication date were set. Paper inclusion was agreed upon by both reviewers. A third reviewer (A.M.) was consulted with regard to inclusion in case of disagreement between the two reviewers.

Outcome measures

This systematic review looked at various aspects of eating behaviour after post-oral delivery of non-caloric tastants. Outcome measures of interest were (1) energy intake, (2) GI symptoms and perceptions, and (3) mechanisms of effect.

Data extraction

Two reviewers (T.K.) and (D.K.) carried out the data extraction. Two authors were contacted to elaborate their data and they replied. Name of author, year of publication, country, sample size, age of subjects, BMI of subjects, tastants used, method of administration, energy intake, appetite sensations, GI symptoms, GI peptides, GI motility, and brain signalling in homeostatic and hedonic regions

were abstracted and presented in tables. Principle summary measures are differences in means.

Quality assessment

Two independent reviewers (T.K. and D.K.) used the revised Cochrane risk of bias tool to assess risk of bias in randomised trials (RoB 2) to assess the quality of included papers.[47] The quality of the paper was assessed only once when a paper described multiple studies. A third reviewer (A.M.) was consulted in case of discordance between the two reviewers. The RoB 2 tool, assessing the quality of randomised controlled trials, consists of five domains covering bias arising from the randomisation process, bias due to deviations from intended interventions, bias due to missing outcome data, bias in the measurement of the outcome, and bias in selection of the reported result. The scoring system assesses the risk of bias on these domains (low risk of bias, some concerns, high risk of bias). When an individual domain received score of a particular level of bias, overall risk of bias was determined to be at least as severe.

Statistical analysis

A meta-analysis was performed if at least two studies described the use of non-caloric tastants of the same basic taste on energy intake and clinical heterogeneity between studies was acceptable. Clinical heterogeneity was discussed by two independent reviewers (T.K and D.K). In case of discordance between these reviewers, a third reviewer (A.M.) was consulted. Meta-analyses were performed using a random effect model by the metaphor package in R (version 3.6.3).[48] Energy intake in Kcal after tastants and control were pooled using the data provided by included studies. Sensitivity analyses were performed when same studies employed different doses of tastants in order for those subjects to not influence the results to a greater extent than subjects form other studies. The I^2 was used to quantitively measure statistical heterogeneity between studies (p-value <0.05).

Data reporting

Paper inclusion, exclusion, and reasons for exclusion are presented in a diagram (Figure 2.1) according to the PRISMA statement for reporting a systematic review and meta-analysis.

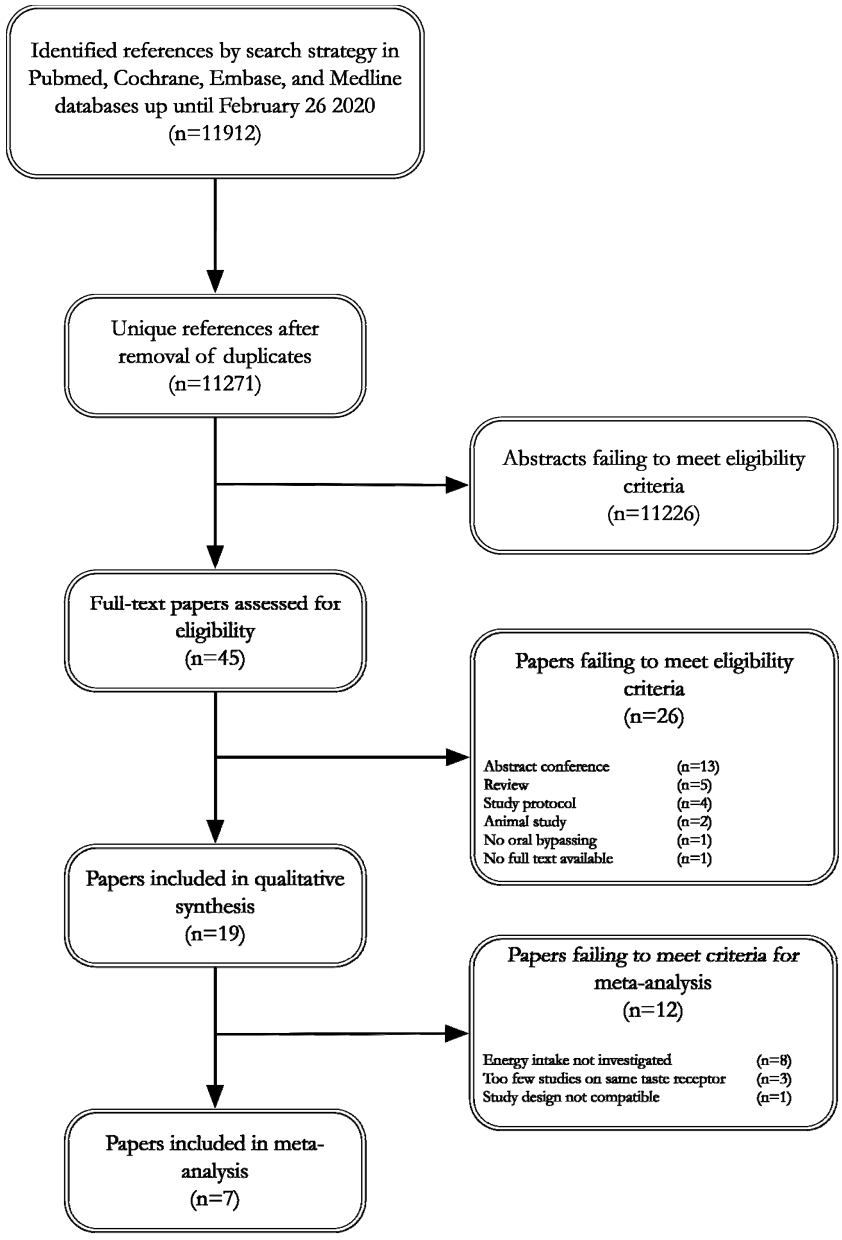

Figure 2.1 **Flow chart of the selection process; from identification of possible eligible papers to papers included in this review and meta-analysis.**

Results

Systematic approach to paper selection

After removal of duplicates, a total of 11271 abstracts were assessed, and 45 full texts were screened for eligibility (Figure 2.1). A total of 19 papers, describing 25 studies met the inclusion criteria. Twenty-six papers were excluded for various reasons: abstract for a conference (13), review (5), study protocol (4), animal study (2), no adequate bypassing of oral taste receptors (1), no full text available (1). The flow-chart for screening and inclusion of papers is depicted in Figure 2.1.
Supplementary Table S2.1 provides an overview of the results of the included papers.

Quality assessment

The results of the quality assessment are summarised in Figure 2.2. Overall, papers scored decently on the risk of bias assessment. Bias arising from the randomisation process was determined to raise some concerns by the majority of papers due to lacking information on the method of randomisation. Most papers described randomisation of subjects but provided no information on the method of randomisation.

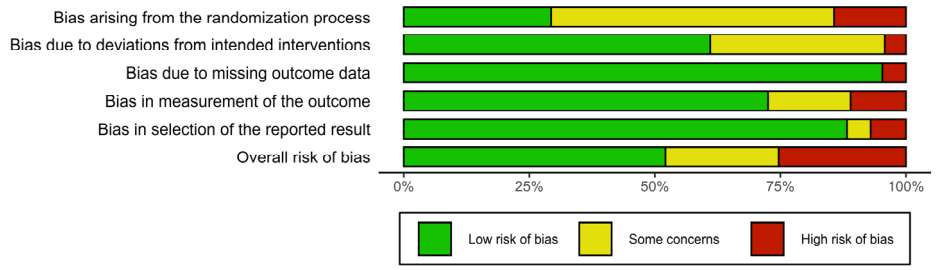

Figure 2.2 **Risk of bias summary.** Author's judgements broken down for the domains according to the revised tool to assess risk of bias in randomised trials (RoB 2)

Study characteristics

Study population

The characteristics of the 25 studies described in 19 papers are summarised in Supplementary Table S2.1. All studies included healthy volunteers.[27-45] Most studies included volunteers with a normal BMI between 18 and 25 kg/m^2.[27-38,40-42,44,45] One study described inclusion of both subjects with a BMI between 18 and 25 kg/m^2 and subjects with a BMI greater than 30 kg/m^2.[43] One study described inclusion of subjects with a BMI between 23 and 32 kg/m^2.[39] Eleven studies described inclusion of both women and men,[27,28,34-38,40-43] four studies described inclusion of men only,[29,30,44,45] and four studies described inclusion of women only.[31-33,39]

Tastants used

Table 2.2 provides an overview of the tastants used by included studies and the ligand receptors that are activated per tastant.

Sweet tastants

Nine studies (reported on in eight papers) investigated post-oral delivery of sweet tastants. Aspartame alone was used in three studies (two papers),[40,44] one study used aspartame and saccharin,[35] one study used aspartame, Acesulfame potassium (Ace-K), and sucralose,[41] one study used sucralose,[36] one study used Rebaudioside A (Reb-A),[42] one study used xylitol and erythritol,[43] and one study used only Ace-K.[38] It should be noted that Reb-A, Ace-K, Saccharin, and sucralose are known to activate bitter taste receptors aside from sweet taste receptors. TAS2R4 and 14 are activated by Reb-A, TAS2R43 and 44 are activated by Ace-K, TAS2R43 and 44 are activated by saccharin, and TAS2R1, 4, 5, 7, 8, 10, 39, 41, 46 are activated by sucralose.[49] However, given their predominant sweet taste[50-52] and their main role as non-nutritive sweeteners, for this paper, they were described as sweet tastants.

Bitter tastants

Sixteen studies (12 papers) investigated post-oral taste delivery of bitter tastants. Six of these studies used quinine alone,[27,29-31,33,29,42] five studies (two papers) used denatonium benzoate (DB) alone,[28,32] one study investigated quinine and naringin,[35] one study used a bitter mixture consisting of raisin flavour, sucrose octa acetate, and quassia extract,[39] one study used bitter secoiridoids,[37] and one study used bitter New Zealand hop extracts.[45] Different bitter compounds activate

different (combinations of) TAS2 receptor subtypes in humans.[49,53] However, most extensively investigated are quinine and denatonium benzoate, which both activate four of the same TAS2 receptor subtypes, among other subtypes. DB activates eight TAS2 receptor subtypes in humans (TAS2R 4, 8, 10, 13, 39, 43, 46, and 47), whereas quinine activates nine subtypes of TAS2 receptor in humans (TAS2R 4, 7, 10, 14, 39, 40, 43, 44, and 46).[49,53]

Table 2.2 **Ligand receptors of the tastants described in the included studies**. Ligand receptors for TAS2Rs adapted from BitterDB.[49]

Tastant	Ligand receptors
Aspartame	TAS1R2/TAS1R3 heterodimer
Saccharin	TAS1R2/TAS1R3 heterodimer, TAS2R43, TAS2R44
Sucralose	TAS1R2/TAS1R3 heterodimer, TAS2R1, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R10, TAS2R39, TAS2R41, TAS2R41, TAS2R46
Ace-K	TAS1R2/TAS1R3 heterodimer, TAS2R43, TAS2R44
Reb-A	TAS1R2/TAS1R3 heterodimer, TAS2R4, TAS2R14
Xylitol	TAS1R2/TAS1R3 heterodimer
Erythritol	TAS1R2/TAS1R3 heterodimer
Naringin	N/A
Quinine	TAS2R4, TAS2R7, TAS2R10, TAS2R14, TAS2R39, TAS2R40, TAS2R43, TAS2R44, TAS2R46
QHCl	TAS2R4, TAS2R7, TAS2R10, TAS2R14, TAS2R39, TAS2R40, TAS2R43, TAS2R44, TAS2R46
DB	TAS2R4, TAS2R8, TAS2R10, TAS2R13, TAS2R39, TAS2R43, TAS2R46, TAS2R47
Bitter secoiridoids (Gentiana lutea extract, contains amarogentin)	TAS2R1, TAS2R4, TAS2R39, TAS2R43, TAS2R46, TAS2R47, TAS2R50
Raisin flavour	N/A
Sucrose Octa Acetate	TAS2R46
Quassia extract	TAS2R4, TAS2R10, TAS2R14, TAS2R46, TAS2R47
Amarasate extract	N/A
MSG	TAS1R1/TAS1R3 heterodimer

TAS1R: taste receptor 1, TAS2R: taste receptor 2Ace-K: acesulfame potassium, Reb-A: rebaudioside A, N/A: no data available, QHCL: quinine hydrochloride, DB: denatonium benzoate, MSG: monosodium glutamate.

Umami tastants

Only one study investigated post-oral delivery of an umami tastant. Monosodium glutamate was used in this study.[42]

Combination of tastants

Two studies investigated post-oral delivery of a combination of tastants (sweet, bitter, and umami). Both of these studies used quinine, Reb-A, and monosodium glutamate.[34,42]

Comparators

Most studies describe the use of a placebo. For studies using nasogastric, nasoduodenal or naso-duodenal-ileal delivery of tastants either tap water,[32,34,35,38,41-43] saline,[28-30,36] or milli-Q water[31,33] was used as a comparator. Most studies that used capsules to deliver the tastants used placebo capsules as a comparator.[27,39,40,45] One study used a capsule to deliver tastants and used water without a capsule as comparator.[44] One study added microencapsulated bitter taste to a pudding and used only the coating as a comparator.[37]

Energy intake

An overview of the studies describing effects of post-oral delivery of non-caloric tastants on energy intake is provided in Table 2.3.

Sweet tastants

Four studies (three papers) reported the effect of sweet tastants on energy intake.[40,42] Two studies (one paper) showed a significant decrease of 138, 150, and 175 Kcal ($p<0.05$, $p<0.01$, and $p<0.02$, respectively) of an *ad libitum* buffet meal after intragastric delivery of aspartame in various concentrations compared with placebo.[40] However, another study showed no effect on energy intake or macronutrient preferences during an *ad libitum* buffet meal after intragastric delivery of aspartame.[44] In line with this, one study failed to demonstrate a difference between Reb-A and placebo on *ad libitum* food intake intraduodenal delivery.[42]

The data reported in two papers were not described in sufficient detail to use for pooling.[40,44] Authors of these papers were contacted. Both authors responded and declared that raw data were not available anymore, since the studies were performed over 30 years ago. Therefore, these studies could not be pooled.

Table 2.3 **Studies describing the effects of post-oral delivery of non-caloric tastants on energy intake.**

Taste	Reference	Subjects	Tastants and comparators used	Method of administration	Interval intervention to meal	Energy intake (Kcal)	Direction of effect
Sweet	Rogers *et al.* (1990)[40] UK	12 subjects (6 men, 6 women, 18-26y, BMI 20.8)	Aspartame capsule (234 mg) Comparator: Placebo capsule	Gastric capsule	60 mins	-175 Kcal	↓
		15 subjects (10 men, 5 women, 19-24y, normal BMI	Aspartame capsule (235 mg) Aspartame capsule (470 mg) Comparator: Placebo capsule	Gastric capsule	60 mins	-138 Kcal for 235mg aspartame -150 Kcal for 470 mg aspartame	↓
	Black *et al.* (1993)[44] Canada	18 subjects (18 men, 19-25y, BMI 21-25)	Aspartame capsule (340 mg) Comparator: Water	Gastric capsule	60 mins	Slight non-significant increase in energy intake (numbers not shown). No effect on macronutrient composition	No effect
	Van Avesaat *et al.* (2015)[42] The Netherlands	15 subjects (6 men, 9 women, 22.4 y, BMI 22.4)	Reb-A (540 mg) Comparator: Tap water	Nasoduodenal catheter	75 mins	-24 Kcal (n.s.)	No effect
Bitter	Andreozzi *et al.* (2015)[27] Italy	20 subjects (8 men, 12 women, 27 y, BMI 24)	QHCl capsule (18 mg) Comparator: Placebo capsule	Acid resistant capsules	60 mins	-82 Kcal	↓
	Van Avesaat *et al.* (2015)[42] The Netherlands	15 subjects (6 men, 9 women, 22.4 y, BMI 22.4)	QHCl (75 mg) Comparator: Tap water	Nasoduodenal catheter	75 mins	-44 Kcal (n.s.)	No effect

Table 2.3 (continued)

Taste	Reference	Subjects	Tastants and comparators used	Method of administration	Interval intervention to meal	Energy intake (Kcal)	Direction of effect
Bitter	Mennella *et al.* (2016)[37] Italy	20 subjects (11 men, 9 women, 25.3 y, BMI 22.1	Microencapsulated bitter secoiridoids (100 mg) Comparator: Coating only	Microencapsulation to mask oral tasting. Exact location of effect in GI tract unknown	180 mins (lunch) 24 h energy intake	Lunch: - 88 Kcal (n.s.) Post-lunch: -252 Kcal 24h energy intake: - 340 Kcal	↓
	Peters *et al.* (2016)[39] The Netherlands	57 subjects (all women, 40.5 y, BMI 26.5)	Bitter mixture containing: Raisin flavour (22.0 mg) Sucrose Octa Acetate 0.88 mg) Quassia extract (0.088 mg) Comparator: Placebo capsule	Intragastric capsule, 2 times daily for 14 days	60 mins (breakfast) 300 mins (lunch) 60 mins (dinner) All day energy intake	Day 0 vs. day 14: Meals only: -109 Kcal (n.s.) Meals + snack: -86 Kcal (n.s.) Breakfast: -30 Kcal (n.s.) Lunch: -61 Kcal (n.s.) Dinner: -1 Kcal (n.s.) Snacks: +41 Kcal (n.s.)	No effect
	Deloose *et al.* (2017)[32] Belgium	20 subjects (all women, 23 y, BMI 22)	DB (0.447 mg/kg body weight) Comparator: Tap water	Nasogastric catheter	40 mins	-76 Kcal (n.s.)	No effect
	Bitarafan *et al.* (2019)[30] Australia	14 subjects (14 men, 25 y, BMI 22.5)	QHCl (37.5 mg, Q37.5)) QHCl (75 mg, Q75)) QHCl (225 mg, Q225)) Comparator: Saline	Nasoduodenal catheter	60 mins	Q37.5: - 31Kcal (n.s.), Q75: -59 Kcal (n.s.), Q225: -11 Kcal vs. Control (n.s.)	No effect
	Iven *et al.* (2019)[33] Belgium	16 subjects (16 women, 24.5 y, BMI 21.9)	QHCl (3.6 mg/kg body weigh) Comparator: Milli-Q water	Nasogastric catheter	40 mins	-67.6 Kcal	↓
	Bitarafan *et al.* (2020)[29] Australia	12 subjects (12 men, 26 y, BMI 23.1)	QHCl (275 mg, Q275) QHCl (600 mg, Q600) Comparator: Saline	Nasogastric catheter	30 mins	Q275: +26 Kcal, Q600: -53 Kcal (n.s.)	No effect

Table 2.3 (continued)

Taste	Reference	Subjects	Tastants and comparators used	Method of administration	Interval intervention to meal	Energy intake (Kcal)	Direction of effect
Umami	Van Avesaat *et al.* (2015)[42] The Netherlands	15 subjects (6 men, 9 women, 22.4 y, BMI 22.4)	MSG (2 g) Comparator: Tap water	Intraduodenal catheter	75 mins	+ 5 Kcal (n.s.)	No effect
Combination	Van Avesaat *et al.* (2015)[42] The Netherlands	15 subjects (6 men, 9 women, 22.4 y, BMI 22.4)	Tastant mixture: Reb-A (540 mg) QHCl (75 mg) MSG (2 g) Comparator: Tap water	Nasoduodenal catheter	75 mins	-64 Kcal	↓
	Klaassen *et al.* (2019)[34] The Netherlands	14 subjects (3 men, 11 women, 25.6 y, BMI 22.3)	Tastant mixture: Reb-A (540 mg) QHCl (75 mg) MSG (2 g) Comparator: Tap water	Naso-duodenal-ileal catheter	75 mins	Duodenal +16.7 Kcal (n.s.), Ileal +28.1 Kcal (n.s.), Combined duodenal and ileal +31.5 Kcal (n.s.)	No effect

y: years, BMI: body mass index, Reb-A: rebaudioside A, n.s.: not significant, QHCl: quinine hydrochloride, GI: gastrointestinal, DB: denatonium benzoate, MSG: monosodium glutamate.

Bitter tastants

Eight studies showed the effect of post-oral delivery of bitter tastants on energy intake. Three of these described a decrease in energy intake after intragastric,[33] intraduodenal,[27] or post-oral delivery of bitter tastants.[37] On the other hand, five studies showed no effect on energy intake after gastrointestinal delivery of bitter tastants. However, most of these studies described a modest decrease in energy intake that did not reach statistical significance.[29,30,32,39,42]

Iven *et al.* showed a decrease of 67.7 Kcal of hedonic eating after intragastric infusion of quinine compared with control.[33] Andreozzi *et al.* showed a decrease of 82 Kcal after an acid resistant capsule containing quinine compared with a placebo capsule.[27] Mennella *et al.* showed no significant decrease in lunch intake (-88 Kcal) after microencapsulated bitter secoiridoids compared with control, but a significant decrease of post-lunch energy intake (-252 Kcal) and 24h energy intake (-340 Kcal).[37]
Van Avesaat *et al.* showed a non-significant decrease of 44 Kcal after intraduodenal infusion of quinine compared with control.[42] Peters *et al.* investigated energy intake after a two week, two times daily consumed capsule containing a bitter mixture compared with control.[39] They showed a non-significant decrease of daily meal intake (-109 Kcal), daily meal intake including snacks (-86 Kcal), breakfast (-30 Kcal), lunch (-61 Kcal), and dinner (-1 Kcal). A non-significant increase of 41 Kcal on snacks only was found.[39] Deloose *et al.* showed that intragastric infusion of DB resulted in a non-significant decrease of 76 Kcal compared with control.[32] In one study, Bitarafan *et al.* showed a non-significant decrease in food intake following various doses of intraduodenally administered quinine compared with control (-31 Kcal for 37.5 mg, -59 Kcal for 75 mg, and -11 Kcal for 225 mg).[30] In another study, Bitarafan *et al.* showed a non-significant increase in energy intake of 26 Kcal after intragastric administration 275 mg of quinine, whereas intragastric administration of 600 mg quinine showed a non-significant decrease of 53 Kcal.[29]
Seven studies investigating the effect of gastrointestinal delivery of bitter tastants on energy intake could be pooled[27,29,30,32,33,37,42] and are depicted in Figure 2.3. One study looked at total energy intake during the day as well as energy intake during the lunch.[37] Pooled effects were calculated with the outcome of lunch intake for this study in order to minimise clinical heterogeneity. One study investigating the effect of intragastric bitter tastant delivery on energy intake could not be pooled, because the design employed in that study differed too much from the other

designs.[39] Two studies employed various doses of QHCl in the same population.[29,30] In order for those subjects to not influence the results to a greater extent than subjects form other studies, the meta-analysis was performed for all the combinations of doses. The lowest dose for both studies is depicted in Figure 2.3. Pooled effects show a significant reduction in caloric intake of 54.62 Kcal (95% CI: -78.54 -30.69, p=0.0014). A sensitivity analysis was performed for all the combinations of doses employed by Bitarafan *et al.* and results are depicted in Supplementary Figure S2.1. Decrease in caloric intake varies between 53 and 58 Kcal for the different combinations, all statistically significant.[29,30]

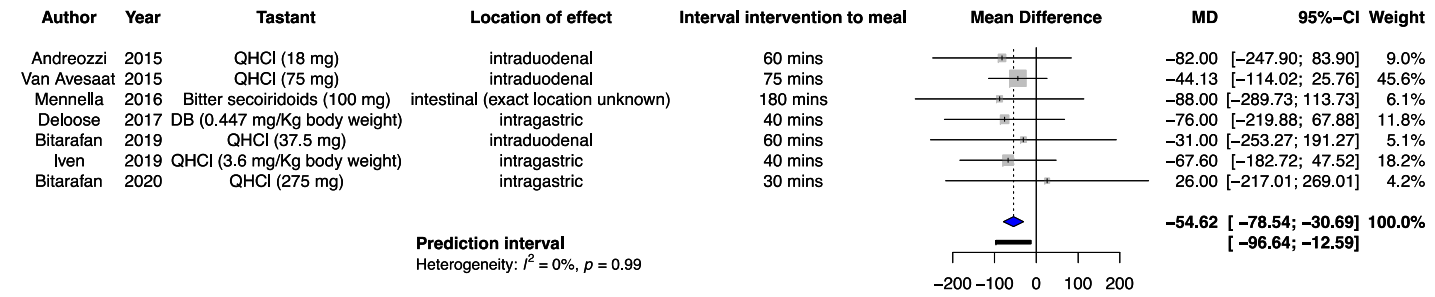

Figure 2.3 Forest plot for pooled mean difference in energy intake after bitter components versus placebo. For the papers Bitarafan *et al.* 2019[30] and Bitarafan *et al.* 2020[29] the lowest dose is depicted. QHCl: quinine hydrochloride, DB: denatonium benzoate.

Umami tastants

Van Avesaat *et al.* showed no effect on energy intake after intraduodenal delivery of monosodium glutamate compared with placebo.[42]

Combination of tastants

Van Avesaat *et al.* showed a significant decrease of energy intake of 64 Kcal after intraduodenal delivery of a combination of quinine, Reb-A and monosodium glutamate. A study conducted by the same research group showed no effects on energy intake after intraduodenal and/or intraileal delivery of the same tastant mixture.[42] These studies were not pooled, due to high clinical heterogeneity.[34]

GI symptoms and perceptions

An overview of the studies describing effects of post-oral delivery of non-caloric tastants on GI symptoms and perceptions is provided in Table 2.4.

Sweet tastants

Seven studies investigated the effects of gastrointestinal delivery of sweet tastants on appetite sensations. Five of these studies showed no effects on appetite sensations.[35,41-44] One study showed that intragastric delivery of aspartame reduced desire to eat without influencing fullness.[40] Another study demonstrated a strong initial decrease in hunger and increase in satiety, with faster returns to baseline after intragastric delivery of Ace-K compared with control.[38]
Three studies examined the effects of post-oral administration of sweet tastants on GI symptoms and other adverse events.[38,42,43] Wölnerhanssen *et al.* showed that intragastric administration of xylitol and erythritol leads to bloating and diarrhoea in 70% and 60% of subjects respectively.[43] Other studies reported no GI symptoms or adverse events.[38,42]

Bitter tastants

Five studies (four papers) showed that post-oral delivery of bitter tastants resulted in a decrease of hunger and prospective food consumption and an increase of satiation/satiety and fullness.[28,32,33,45] However, six studies showed no effects on appetite sensations after post-oral delivery of bitter tastants.[27,29,30,35,37,42]

Table 2.4 Studies describing the effects of post-oral delivery of non-caloric tastants on GI symptoms and perceptions.

Taste	Reference	Subjects	Tastants and comparators used	Method of administration	GI symptoms and perceptions	Direction of effect
Sweet	Rogers *et al.* (1990)[40] UK	15 subjects (10 men, 5 women, 19-24y, normal BMI	Aspartame capsule (235 mg) Aspartame capsule (470 mg) Comparator: Placebo capsule	Gastric capsule	Aspartame capsules reduced desire to eat and hunger scores Aspartame capsules tended to increase fullness compared with placebo (n.s.)	↓ desire to eat/hunger No effects fullness
	Black *et al.* (1993)[44] Canada	18 subjects (18 men, 19-25y, BMI 21-25)	Aspartame capsule (340 mg) Comparator: Water	Gastric capsule	No effects of aspartame on appetite sensations	No effects
	Little *et al.* (2009)[35] UK	10 subjects	Saccharin (50 mg) Aspartame (200 mg) Comparator: Tap water	Nasogastric catheter	No effects of aspartame or saccharin on hunger or fullness	No effects
	Steinert *et al.* (2011)[41] Switzerland	12 subjects (6 men, 6 women, 23.3 y, BMI 23.0)	Aspartame (160 mg) Ace-K (200 mg) Sucralose (62 mg) Comparator: Tap water	Nasogastric catheter	Artificial sweeteners reduced hunger, and increased satiety and fullness ratings to an intermediate amount between water and carbohydrate sugars (n.s.)	No effects
	Van Avesaat *et al.* (2015)[42] The Netherlands	15 subjects (6 men, 9 women, 22.4 y, BMI 22.4)	Reb-A (540 mg) Comparator: Tap water	Nasoduodenal catheter	Reb-A did not influence appetite sensations. Reb-A did not induce GI symptoms	No effects

Table 2.4 (continued)

Taste	Reference	Subjects	Tastants and comparators used	Method of administration	GI symptoms and perceptions	Direction of effect
Sweet	Wölnerhanssen *et al.* (2016)[43] Switzerland	20 subjects 10 lean subjects (5 men, 5 women, 26.6 y, BMI 21.7) 10 obese subjects (5 men, 5 women, 27.2 y, BMI 40.0)	Xylitol (50 g) Erythritol (75 g) Comparator: Tap water	Nasogastric catheter	Both sweeteners did not affect appetite sensations. Xylitol and erythritol led to bloating and diarrhoea in 70% and 60% of subjects respectively.	No effects appetite sensations ↑Side effects
	Meyer-Gerspach *et al.* (2018)[38] Belgium	12 subjects (6 men, 6 women, 23 y, BMI 23)	Ace-K (220 mg) Comparator: Tap water	Nasogastric catheter	Hunger: Strong initial decrease in hunger after Ace-K with a faster return of hunger after first time point and slower return of hunger in last part of curve after Ace-K vs. Control Satiation: Strong initial increase in satiation after Ace-K vs. control with faster decrease after first time point and slower decrease in last part of curve after Ace-K vs. control No adverse events	↓Hunger ↑Satiation No adverse events
Bitter	Little *et al.* (2009)[35] UK	12 subjects	Naringin (1 mM) Quinine (0.198 mM) Comparator: Tap water	Nasogastric catheter	No effects of naringin or quinine on appetite sensations	No effects
	Andreozzi *et al.* (2015)[27] Italy	20 subjects (8 men, 12 women, 27 y, BMI 24)	QHCl capsule (18 mg) Comparator: Placebo capsule	Acid resistant capsules	QHCl did not affect satiety or desire to eat scores vs. Control. No adverse events	No effects No adverse events
	Avau *et al.* (2015)[28] Belgium	12 subjects (5 men, 30.6 y, BMI 23.8)	DB (1 μmol/kg body weight) Comparator: Saline	Nasogastric catheter	DB made subjects feel satiated earlier and at lower volumes during constant nutrient infusion No adverse effects	↑satiation No adverse events

Table 2.4 (continued)

Taste	Reference	Subjects	Tastants and comparators used	Method of administration	GI symptoms and perceptions	Direction of effect
Bitter	Van Avesaat *et al.* (2015)[42] The Netherlands	15 subjects (6 men, 9 women, 22.4 y, BMI 22.4)	QHCl (75 mg) Comparator: Tap water	Nasoduodenal catheter	Quinine did not influence appetite sensations. Quinine did not induce GI symptoms	No effects No GI symptoms
	Mennella *et al.* (2016)[37] Italy	20 subjects (11 men, 9 women, 25.3 y, BMI 22.1	Bitter secoiridoids (100 mg) Comparator: Coating only	Microencapsulation to mask oral tasting. Exact location of effect in GI tract unknown	no effect of bitter encapsulate on fullness, satiety, hunger or desire to eat	No effects
	Deloose *et al.* (2017)[32] Belgium	20 subjects (10 men, 10 women, 27 y, BMI 24)	DB (1 µmol/Kg body weight) Comparator: Tap water	Nasogastric catheter	Women: Switch from gastric to duodenal phase 3 origin was accompanied by lower percentage change of hunger scores after DB vs. Control Men: Percentage change in hunger scores during phase 3 contraction did not differ after DB vs. Control (n.s.) No adverse events after DB administration	↓Hunger in women
		12 subjects (all women, 31 y, BMI 22)	DB (1 µmol/Kg body weight) Comparator: Tap water	Nasogastric catheter	No adverse events after DB administration	No adverse events
		13 subjects (all women, 28 y, BMI 23)	DB (1 µmol/Kg body weight) Comparator: Tap water	Nasogastric catheter	Hunger scores after a standardised meal were lower after DB vs. Control. Satiety scores were higher after a standardised meal after DB. No adverse events after DB administration	↓Hunger ↑Satiety No adverse events
		20 subjects (all women, 23 y, BMI 22)	DB (1 µmol/Kg body weight) Comparator: Tap water	Nasogastric catheter	No adverse events after DB administration	No adverse events

Table 2.4 (continued)

Taste	Reference	Subjects	Tastants and comparators used	Method of administration	GI symptoms and perceptions	Direction of effect
Bitter	Deloose *et al.* (2018)[31] Belgium	10 subjects (10 women, 33 y, BMI 22)	QHCl (10 µmol/kg body weight) Comparator: Milli-Q water	Nasogastric catheter	No adverse events	No adverse events
	Bitarafan *et al.* (2019)[30] Australia	14 subjects (14 men, 25 y, BMI 22.5)	QHCl (37.5 mg, Q37.5)) QHCl (75 mg, Q75)) QHCl (225 mg, Q225)) Comparator: Saline	Nasoduodenal catheter	No differences in VAS scores for hunger, desire to eat, prospective consumption, or fullness after Q37.5, Q75, or Q225 vs. Control. No adverse events, no effects of Q37.5, Q75, or Q225 on nausea or bloating.	No effects No GI symptoms No adverse events
	Iven *et al.* (2019)[33] Belgium	16 subjects (16 women, 24.5 y, BMI 21.9)	QHCl (10 µmol/Kg body weigh) Comparator: Milli-Q water	Nasogastric catheter	Hunger scores increased after control and decreased after QHCl (n.s.) Prospective food consumption scores decreased after QHCl vs. Control Satiety scores increased after QHCl vs. Control fullness scores increased after QHCl vs. control Minimal nausea scores reported (n.s.)	↓Prospective food consumption ↑Satiety ↑Fullness No GI symptoms
	Walker *et al.* (2019)[45] New Zealand	30 subjects (30 men, 24y, BMI 23.1)	Amarasate extract (500 mg, HD) Amarasate extract (200 mg, LD) Comparator: Placebo capsule	Acid resistant capsule	From T=90 onwards HD and LD show lower mean changes in hunger and fullness Lower mean changes in fullness for HD from t=120 onwards, only t=180 and t=330 for LD. No nausea. 3 participants in HD and 1 in LD had liquid loose bowel movements	↓Hunger ↑Fullness No GI symptoms

Table 2.4 (continued)

Taste	Reference	Subjects	Tastants and comparators used	Method of administration	GI symptoms and perceptions	Direction of effect
Bitter	Bitarafan *et al.* (2020)[29] Australia	15 subjects (15 men, 26 y, BMI 23.2)	QHCl (275 mg, Q275) Quinine-HCl (600 mg, Q600) Comparator: Saline	Nasogastric catheter	No effects of Q275 or Q600 on hunger, desire to eat, prospective consumption, or fullness scores. No effects of Q275 or Q600 on bloating or nausea vs. Control. No other adverse effects	No effects No GI symptoms No adverse events
		12 subjects (12 men, 26 y, BMI 23.1)	QHCl (275 mg, Q275) QHCl (600 mg, Q600) Comparator: Saline	Nasogastric catheter	No effects of Q275 or Q600 on hunger, desire to eat, prospective consumption, or fullness scores. No effects of Q275 or Q600 on bloating or nausea vs. Control. No other adverse effects	No effects No GI symptoms No adverse events
Umami	Van Avesaat *et al.* (2015)[42] The Netherlands	15 subjects (6 men, 9 women, 22.4 y, BMI 22.4)	MSG (2 g) Comparator: Tap water	Intraduodenal catheter	MSG decreased hunger and desire to eat but did not influence satiation or fullness. MSG did not induce GI symptoms	↓ Desire to eat/hunger No effects satiation/fullness No GI symptoms
Combination	Van Avesaat *et al.* (2015)[42] The Netherlands	15 subjects (6 men, 9 women, 22.4 y, BMI 22.4)	Tastant mixture: Reb-A (540 mg) QHCl (75 mg) MSG (2 g) Comparator: Tap water	Nasoduodenal catheter	The tastant mixture decreased hunger and desire to eat, but not satiation or fullness. The tastant mixture did not induce GI symptoms	↓ Desire to eat/hunger No effects satiation/fullness No GI symptoms
	Klaassen *et al.* (2019)[34] The Netherlands	14 subjects (3 men, 11 women, 25.6 y, BMI 22.3)	Tastant mixture: Reb-A (540 mg) QHCl (75 mg) MSG (2 g) Comparator: Tap water	Naso-duodenal-ileal catheter	No effects of duodenal-, ileal- or combined duodenal and ileal delivery of non-caloric tastants on appetite sensations. The tastant mixture did not induce GI symptoms	No effects No GI symptoms

y: years, BMI: body mass index, n.s.: not significant, Ace-K: acesulfame potassium, Reb-A: rebaudioside A, QHCl: quinine hydrochloride, DB: denatonium benzoate, GI: gastrointestinal, MSG: monosodium glutamate.

Seven studies examined the effects of post-oral delivery of bitter tastants on GI symptoms and/or adverse events. None of these reported side effects or adverse events.[27,29-33,42]

Umami tastants

One study described that post-oral delivery of umami decreased desire to eat and hunger, without influencing satiation, fullness or GI symptoms.[42]

Combination of tastants

Two studies described the effects of post-oral delivery of a combination of sweet, bitter and umami tastants on appetite sensations. One study described a decrease of desire to eat and hunger, whereas satiation and fullness were not attenuated.[42] The other study showed no effects on appetite sensations.[34]
No GI symptoms or adverse events after post-oral delivery of this combination of sweet, bitter, and umami tastants were reported.[34,42]

Mechanisms of effect

An overview of the studies describing effects of post-oral delivery of non-caloric tastants on the mechanisms of effect involved in regulating eating behaviour is provided in Table 2.5. Mechanisms of interest were GI peptide release, GI motility, and brain signalling.

Sweet tastants

One study described a drop in intragastric pressure after intragastric administration of Ace-K.[38] Intragastric administration of xylitol and erythritol also resulted in slower gastric emptying.[43] However, another study showed no changes in gastric emptying after intragastric administration of saccharin or aspartame.[35]
Five studies investigated the effects of post-oral delivery of sweet tastants on GI peptides.[36,38,41-43] Four of these showed no effects on GI peptide plasma levels.[36,38,41,42] One study described that intragastric administration of xylitol and erythritol increases plasma CCK, GLP-1, and glucose.[43]

Table 2.5 **Studies describing the effects of post-oral delivery of non-caloric tastants on the mechanisms of effects that influence eating behaviour such as GI peptides, GI motility and brain signalling.**

Taste	Reference	Subjects	Tastants and comparators used	Method of administration	Mechanisms of effect	Direction of effect
Sweet	Little *et al.* (2009)[35] UK	10 subjects	Saccharin (50 mg) Aspartame (200 mg) Comparator: Tap water	Nasogastric catheter	No effects of aspartame or saccharin on GE	No effects GE
	Ma *et al.* (2009)[36] Australia	7 subjects (24 y, BMI 21.6)	Sucralose (80 mg) Sucralose (800 mg) Comparator: Saline	Nasogastric catheter	No effects of sucralose on GE, plasma glucose, plasma insulin, plasma GLP-1, or plasma GIP.	No effects GE No effects GI peptides
	Steinert *et al.* (2011)[41] Switzerland	12 subjects (6 men, 6 women, 23.3 y, BMI 23.0)	aspartame (160 mg) Ace-K (200 mg) sucralose (62 mg) Comparator: Tap water	Nasogastric catheter	Sweeteners did not affect plasma GLP-1, PYY, ghrelin, glucose, insulin, or glucagon	No effects GI peptides
	Van Avesaat *et al.* (2015)[42] The Netherlands	15 subjects (6 men, 9 women, 22.4 y, BMI 22.4)	Reb-A (540 mg) Comparator: Tap water	Nasoduodenal catheter	Reb-A did not affect plasma CCK, GLP-1, or PYY	No effects GI peptides
	Wölnerhanssen *et al.* (2016)[43] Switzerland	20 subjects 10 lean subjects (5 men, 5 women, 26.6 y, BMI 21.7) 10 obese subjects (5 men, 5 women, 27.2 y, BMI 40.0)	Xylitol (50 g) Erythritol (75 g) Comparator: Tap water	Nasogastric catheter	Plasma CCK, plasma GLP-1, Plasma glucose increased after xylitol and erythritol vs. control Plasma insulin increased after xylitol, but not after erythritol vs. control Gastric emptying was slowed during the first 60 mins after xylitol and erythritol vs. Control	↓GE ↑plasma CCK, GLP-1, Glucose, insulin

Table 2.5 (continued)

Taste	Reference	Subjects	Tastants and comparators used	Method of administration	Mechanisms of effect	Direction of effect
Sweet	Meyer-Gerspach *et al.* (2018)[38] Belgium	12 subjects (6 men, 6 women, 23 y, BMI 23)	Ace-K (220 mg) Comparator: Tap water	Nasogastric catheter	No effect of Ace-K on plasma motilin, octanoylated ghrelin, active GLP-1, CCK, gastrin, and glucose. GI motility did not differ between Ace-K and control. A faster linear decrease in IGP from first post infusion time point, quicker return of IGP and quicker flattening of the curve during IGP recovery after Ace-K vs. Control with faster return to baseline in last part of the IGP curve after Ace-K vs. control	No effects GI motility ↓ IGP No effects GI peptides
Bitter	Little *et al.* (2009)[35] UK	12 subjects	Naringin (1 mM) Quinine (0.198 mM) Comparator: Tap water	Nasogastric catheter	No effects of naringin or quinine on gastric emptying compared with water	No effects on GE
	Andreozzi *et al.* (2015)[27] Italy	20 subjects (8 men, 12 women, 27 y, BMI 24)	QHCl capsule (18 mg) Comparator: Placebo capsule	Acid resistant capsules	CCK: Higher ΔT90 vs T0 and ΔT90 vs T60 after QHCl vs. Control GE (evaluated in 8 subjects): no differences in GE between QHCl (87 mins) vs. Control (88 mins)	No effect on GE ↑ CCK
	Avau *et al.* (2015)[28] Belgium	12 subjects (5 men, 30.6 y, BMI 23.8)	DB (1 μmol/kg body weight) Comparator: Saline	Nasogastric catheter	Less drop in IGP after DB	↑ IGP
	Van Avesaat *et al.* (2015)[42] The Netherlands	15 subjects (6 men, 9 women, 22.4 y, BMI 22.4)	QHCl (75 mg) Comparator: Tap water	Nasoduodenal catheter	Quinine did not affect plasma CCK, GLP-1, or PYY levels.	No effects on GI peptides

Table 2.5 (continued)

Taste	Reference	Subjects	Tastants and comparators used	Method of administration	Mechanisms of effect	Direction of effect
Bitter	Mennella *et al.* (2016)[37] Italy	20 subjects (11 men, 9 women, 25.3 y, BMI 22.1	Bitter secoiridoids (100 mg) Comparator: Coating only	Microencapsulation to mask oral tasting. Exact location of effect in GI tract unknown	Bitter encapsulate decreased plasma GLP-1 at 30 mins, but had no effect on blood glucose, plasma amylin, plasma ghrelin, plasma glucagon, plasma GIP, plasma insulin, plasma leptin, plasma PP, or plasma PYY levels vs. Control	↑ GLP-1 at 30 mins No effects on other GI peptides
	Deloose *et al.* (2017)[32] Belgium	20 subjects (10 men, 10 women, 27 y, BMI 24)	DB (1 µmol/Kg body weight) Comparator: Tap water	Nasogastric catheter	Women: DB reduced number of gastric phase 3 contractions from 67% (control) to 33% (DB) in women. Interval between IG administration and occurrence of phase 3 did not differ between control (76 min) and DB (93 min) in women (n.s.). Men: No difference in origin of phase 3 contractions between control (57% gastric) and DB (40% gastric) in men (n.s.). Interval between IG administration and occurrence of phase 3 did not differ between control (76 min) and DB (111 min) in men (n.s.).	↓ Gastric phase 3 contractions in women
		12 subjects (all women, 31 y, BMI 22)	DB (1 µmol/Kg body weight) Comparator: Tap water	Nasogastric catheter	Plasma motilin was lower after DB vs. Control. No differences between plasma total ghrelin or octanoylated ghrelin after DB vs. Control	↓Plasma motilin No effect ghrelin
		13 subjects (all women, 28 y, BMI 23)	DB (1 µmol/Kg body weight) Comparator: Tap water	Nasogastric catheter	GE (measured in 6 subjects) did not differ between control and DB (both 109 mins.	No effects on GE

Table 2.5 (continued)

Taste	Reference	Subjects	Tastants and comparators used	Method of administration	Mechanisms of effect	Direction of effect
Bitter	Deloose *et al.* (2018)[31] Belgium	10 subjects (10 women, 33 y, BMI 22)	QHCl (10 μmol/kg body weight) Comparator: Milli-Q water	Nasogastric catheter	Plasma motilin and plasma ghrelin levels decreased after QHCl. No difference in plasma octanoylated ghrelin levels Time* treatment effect for antral motility. No main effect of treatment. No effects of QHCl on duodenal motility.	↓Antral motility ↓ plasma motilin and ghrelin
	Bitarafan *et al.* (2019)[30] Australia	14 subjects (14 men, 25 y, BMI 22.5)	QHCl (37.5 mg, Q37.5)) QHCl (75 mg, Q75)) QHCl (225 mg, Q225)) Comparator: Saline	Nasoduodenal catheter	No effects of Q37.5, Q75, and Q225 on plasma CCK or blood glucose vs. Control. No effect of Q37.5, Q75, or Q225 on antral pressure waves, basal pyloric pressure, isolated pyloric pressure waves, and duodenal pressure waves vs. Control.	No effects on GI motility No effects on GI peptides
	Iven *et al.* (2019)[33] Belgium	16 subjects (16 women, 24.5 y, BMI 21.9)	QHCl (10 μmol/Kg body weigh) Comparator: Milli-Q water	Nasogastric catheter	Decreases in total ghrelin, octanoylated ghrelin, and motilin after QHCl vs. control Brain activity in homeostatic and hedonic regions: Increased activity after QHCl vs. Control in anterior insula, ACC, amygdala, putamen, nucleus accumbens, pallidum, caudate head and caudate body, medial and lateral OFC, hypothalamus and midbrain. Decreased activity in brainstem/medulla	↓ plasma ghrelin and motilin ↑Activity in homeostatic and hedonic brain regions ↓ activity in brainstem/medulla

Table 2.5 (continued)

Taste	Reference	Subjects	Tastants and comparators used	Method of administration	Mechanisms of effect	Direction of effect
Bitter	Bitarafan *et al.* (2020)[29] Australia	15 subjects (15 men, 26 y, BMI 23.2)	QHCl (275 mg, Q275) QHCl (600 mg, Q600) Comparator: Saline	Nasogastric catheter	Plasma insulin was increased 30 mins after Q275 and Q600 vs. Control. No effects of Q275 or Q600 on plasma glucose, plasma glucagon, or plasma GLP-1. After mixed nutrient drink: Q275 and Q600 lowered glucose Q275 and Q600 increased plasma insulin No difference in glucagon response after nutrient drink. Q275 increased plasma GLP-1, Q600 did not. No effects of Q275 or Q600 on gastric emptying.	No effects on GE ↑ plasma insulin after intervention alone ↓ glucose after nutrient drink ↑ plasma insulin after nutrient drink ↑ plasma GLP-1 after nutrient drink
Umami	Van Avesaat *et al.* (2015)[42] The Netherlands	15 subjects (6 men, 9 women, 22.4 y, BMI 22.4)	MSG (2 g) Comparator: Tap water	Intraduodenal catheter	MSG did not affect plasma CCK, GLP-1, or PYY levels.	No effects on GI peptides
Combination	Van Avesaat *et al.* (2015)[42] The Netherlands	15 subjects (6 men, 9 women, 22.4 y, BMI 22.4)	Tastant mixture: Reb-A (540 mg) QHCl (75 mg) MSG (2 g) Comparator: Tap water	Nasoduodenal catheter	The tastant mixture did not affect plasma CCK, GLP-1, or PYY levels.	No effects on GI peptides

y: years, BMI: body mass index, GE: gastric emptying, GLP-1: glucagon-like peptide 1, GIP: glucose-dependent insulinotropic polypeptide, GI: gastrointestinal, Ace-K: acesulfame potassium, PYY: peptide yy, Reb-A: rebaudioside A, CCK: cholecystokinin, IGP: intragastric pressure, QHCL: quinine hydrochloride, DB: denatonium benzoate, PP: pancreatic polypeptide, IG: intragastric, n.s.: not significant, ACC: anterior cingulate cortex, OFC: orbitofrontal cortex, MSG: monosodium glutamate.

Bitter tastants

Eight studies (seven papers) investigated the effects of post-oral delivery of bitter tastants on GI motility.[27-32,35] Two of these showed a decrease in gastric phase 3 contractions after intragastric delivery of bitter tastants.[31,32] One study described a relative increase in intragastric pressure after DB compared with placebo.[28] Four studies showed no effect of post-oral delivery of bitter tastants on gastric emptying.[27,29,32,35] Another study showed no effect of intraduodenal delivery of quinine on antral, pyloric or duodenal pressure waves.[30]
One study showed that intragastric delivery of quinine resulted in increased brain activity in homeostatic and hedonic brain regions.[33]
Eight studies examined the effects of post-oral delivery of bitter tastants on GI peptides.[27,29-33,37,42] Two studies showed that post-oral delivery of bitter tastants did not result in changes in GI peptides.[30,42] One study showed an initial effect of quinine on plasma insulin, but not on plasma glucose, glucagon or GLP-1. However, a decrease of glucose and an increase of insulin was found after a standardised nutrient drink following quinine administration.[29] Another study showed a decrease in GLP-1 30 mins after intervention, but no effects on other GI peptides, nor an overall intervention effect on plasma GLP-1 levels.[37] Three studies showed a decrease in motilin and/or ghrelin after intragastric delivery of bitter tastants.[31-33] Only one study showed an increase of CCK after intraduodenal delivery of quinine.[27]

Umami tastants

One study investigated the mechanisms of effect of post-oral delivery of monosodium glutamate, showing no changes in GI peptides (CCK, GLP-1, and PYY) after intraduodenal administration of MSG.[42]

Combination of tastants

Only one study investigated the effect of post-oral delivery of a combination of tastants. No effect of intraduodenal administration of a combination of sweet, bitter and umami tastant mixture on plasma CCK, GLP-1, or PYY was found.[42]

Discussion

In this systematic review and meta-analysis, the currently available data on the effects post-oral delivery of non-caloric tastants has been evaluated. This review

shows that the effects of post-oral delivery of non-caloric tastants on eating behaviour are inconclusive and inconsistent thus far.

Tastants: sweet, biter and umami

Most studies described the effects of post-oral delivery of sweet[35,36,38,40-44] or bitter[27-33,35,37,39,42,45] stimuli. Only one study used an umami stimulus[42] and two studies described the effects of a tastant mixture.[34,42] It has been hypothesised that taste can predict the type of food that is being ingested (*i.e.*, bitter for potential toxic substances, umami for glutamate and sweet for saccharides),[8] although this theory is probably an oversimplification and does not sufficiently reflect the complexity of the underlying biological responses. According to traditional beliefs, carbohydrates are considered as fuel for the human body,[54] whereas one wants to avoid toxic substances.[55] Therefore, it is conceivable that most researchers hitherto have generally focused on sweet and bitter substances. However, umami taste is a predictor of amino acids. Up to now, little is known about solely post-oral effects of umami taste, although it has been widely accepted as a basic taste since the discovery of umami taste receptors in 2002.[56,57] However, more data are available on oral delivery of umami stimuli. Oral delivery of umami stimuli has been shown to elicit a GLP-1 response.[58,59] Moreover, adding MSG to a novel flavour is able to condition liking for that flavour.[60] Furthermore, Dermiki *et al.* showed that adding MSG to novel flavoured soups resulted in increased food intake in elderly subjects without eliciting increased liking.[61] Given the effects of oral MSG on food liking, food intake, and GI peptides, the effect of post-oral delivery of umami tastants should be further elucidated.

Energy intake

This review and meta-analysis clearly shows that the most potent stimuli to influence eating behaviour and reduce food intake are the bitter substances. The obvious explanation for this is the innate aversion for bitter taste.[62] That is, delivery of solely bitter tastants, in the absence of other flavours, would result in a warning signal with the intention to stop the intake of that particular substance. However, it should be noted that several studies show that this negative affective response to bitter can be decoupled by, for instance, the positive response to caffeine.[9,10] This process is called flavour-consequence learning.[63] Moreover, Higgins *et al.* showed an increase in pale ale intake in individuals with increased bitter perception.[64] This indicates that, over the years, humans have learned to

appreciate bitter tastes, mainly when combined with other flavours (*i.e.*, liking for black coffee and beers).
The question, however, arises whether any such mechanism would also hold true for post-oral taste receptor stimulation.
Data on post-oral delivery of sweet tastants are limited to three studies showing contradictory results.[40,42,44] Interestingly, more data are available on the effect of oral consumption of non-caloric sweeteners on energy intake. A review and meta-analysis by Rogers *et al.* that also included studies using oral stimulation showed a reduction of energy intake after consumption of low caloric sweeteners when compared with sugar consumption, but not when compared to consumption of water in a short-term setting.[65] Since there is a preference for sweet foods and beverages in humans, further research to investigate the effects of post-oral delivery of sweet tastants on energy intake is warranted.
Eight studies described effects on energy intake after post-oral delivery of bitter tastants. These studies show a modest decrease in energy intake following post-oral delivery of bitter tastants.[27,29,30,32,33,37,39,42] These decreases in energy intake varied from 11–88 Kcal in the acute setting to 340 Kcal on a daily basis. Pooled effects of post-oral delivery of bitter tastants show a significant decrease of 54 Kcal compared with placebo in the acute setting. In itself, a reduction of 54 Kcal in a single meal is rather small. However, in case this reduction in energy intake can be replicated several times daily over a longer period of time, this may indeed lead to a daily caloric deficit and subsequent weight loss. It should be noted, however, that these modest effects on caloric intake point towards a role in weight control rather than weight loss.

GI symptoms and perceptions

Based on the included papers, no clear effects of post-oral delivery of non-caloric tastants on appetite sensations was found. However, it must be noted that appetite sensations are rarely measured as a primary outcome. Consequently, most studies might not have been adequately powered to detect differences in appetite sensations. Therefore, interpretation of these results should be done with care.
Interesting to point out is the finding of Deloose *et al.* showing a longer sustained satiation in response to bitter after a standardised test meal.[32] This observation indicates that adding a bitter tastant to a caloric carrier could result in a delay until the next meal. Such a combination could result in a decrease in snacking in between meals. In line with this, Mennella *et al.* found in their study a reduction in

caloric consumption during the day, after intake of a breakfast containing an encapsulated bitter mixture leading to prolonged satiation.[37]
Only one study investigated the effect of post-oral delivery of an umami stimulus. A decrease in desire to eat and hunger was found.[42]

Safety

According to the studies described in this review, noncaloric tastant administration is considered to be safe. One study reported side effects after administration of high doses of xylitol and erythritol, which was not surprising given the doses employed.[43] Other studies showed no GI-symptoms or other side effects after post-oral delivery of non-caloric tastants.[27-34,38,42,45] It should be taken into account that almost all studies focused on acute effects of post-oral delivery of non-caloric tastants and not on prolonged, daily administration.
The United States Food and Drug Administration (U.S. FDA) issues an acceptable daily intake (ADI) for various food components, including tastants. For example, the U.S. FDA issued a code of regulations stating soft drinks are allowed to contain 83 parts per million quinine.[66] Moreover, a systematic review describing the use of quinine to treat muscle cramps showed an increase of gastrointestinal complaints, headache and tinnitus after daily intake of 200 mg to 500 mg of quinine for three days up to several weeks.[67] Even more, the Medicines and Healthcare products Regulatory Agency (MHRA) issued a reminder on the dose-dependent effects of on the QT interval.[68] Therefore, prolonged intake of high doses or combination with other drugs that prolong the QT interval should be avoided. Interestingly, in studies included in this review, the dose of the bitter tastant quinine ranged from 18 mg to 600 mg.[27,29-31,33-35,42] This did not result in side effects in the studies investigating the acute effects of these compounds. However, when applying quinine daily to prevent obesity, the maximum dose should be carefully considered. This should be considered in future study protocols.

Mechanisms of effect

Gastric emptying was delayed by high doses of xylitol and erythritol.[43] However, other studies showed no effect of post-oral delivery of non-caloric tastants on gastric emptying.[27,29,32,35] Therefore, gastric emptying does not seem to be attenuated by post-oral delivery of non-caloric tastants.
GI motility appears to be attenuated as one research group has consistently found gastric motility and the GI peptides motilin and ghrelin to be affected by post-oral

delivery of non-caloric tastants.[28,31-33] Moreover, this research group recently published a review that elaborated the role of motilin as a regulator of hunger and food intake in humans,[69] which indicates motilin as a possible target in combating the obesity epidemic. These interesting data are awaiting replication by other research groups.

Four studies showed increased CCK and/or GLP-1 levels after post-oral delivery of non-caloric tastants.[27,29,37,43] Other studies that investigated these traditional satiety peptides did not find any effect of post-oral delivery of non-caloric tastants.[30,36,38,42] This raises the question whether the focus should shift from more traditional satiety peptides towards motilin and/or ghrelin. It must be noted that all studies reported on *plasma* levels of GI peptides, pointing to systemic effects. Up to now, it is still unclear what the effect of taste receptor activation is on local secretion of GI peptides. It is conceivable that GI peptides are elevated at a splanchnic level or that they exert a more local or paracrine effect.

Only one study investigated brain signalling after post-oral delivery of non-caloric tastants. These authors found an increase in activity in homeostatic and hedonic brain regions and a decrease of activity in the brain stem and medulla after intragastric delivery of quinine.[33] These are interesting findings, since these data suggest additional mechanisms of effect of post-oral delivery of non-caloric tastants on eating behaviour. It remains to be further elucidated whether these changes are mediated by orexigenic GI peptides.

Implications of present data

In this review and meta-analysis, we have summarised the current knowledge on the effects of non-caloric tastants on energy intake. Using non-caloric tastants to reduce energy intake could provide a useful tool to combat the obesity epidemic.

Specifically, the use of bitter tastants appears to be promising. It is important to note that most studies described in this review have been performed in healthy adults with a normal BMI or in slightly overweight healthy adults. In several studies blunted postprandial levels of PYY[70,71] and GLP-1[72-74] were observed in obese subjects, and reductions in plasma CCK after weight loss.[75,76] This points to alterations in the sensitivity of various receptors to GI peptides and in the magnitude of peptide secretion in obesity. Non-caloric tastants, because of their modest reduction in caloric intake, appear to be more suitable as a weight control intervention in a population that is worried about, but has not yet gained excess weight.

This review and meta-analysis clearly illustrates the potential of bitter tastants in reducing caloric intake. However, several weaknesses of the currently available data should be elucidated. First, data are lacking uniformity and most studies were underpowered: only small numbers of subjects have been included. Second, different study designs have been employed, making it difficult to compare results between various publications. An important variable with respect to energy intake reduction is the varying time interval between post-oral delivery of non-caloric tastants and meal intake. These intervals varied among studies between 30 mins - five hours but usually an interval around 60 min was chosen. The optimal interval between intervention and *ad libitum* meal is currently unknown, but standardisation is necessary.

Third, most of the studies included in this review described the acute effects of post-oral delivery of non-caloric tastants on eating behaviour. Only two studies described energy intake during the day[37,39] and only one of those described an intervention period of two weeks.[39] Therefore, data on the long-term effects of post-oral delivery of non-caloric tastants are lacking. Consequently, it is unknown whether adaptation to the effects occurs. A fourth limitation is the lack of knowledge on the effects of post-oral delivery of umami tastants on eating behaviour, as this was described in only one paper.[42] Fifth, only one study investigated several target locations in the GI tract.[34] Sixth, based on the present data it is unknown which bitter stimulus elicits the greatest effect on energy intake. Most studies described the use of either QHCl or DB.

Future perspectives

In order to evaluate the effects of gastrointestinal delivery of non-caloric tastants on eating behaviour, future studies should preferably standardise study design and doses of tastants. First, standardisation could be achieved by creating consensus from the lead experts in the field. Second, mechanisms of effect should be more thoroughly investigated. For this, more research on the effects of post-oral delivery of non-caloric tastants on GI motility and systemic and local GI peptides secretion is needed. Based on the current data, we propose to focus on motilin and gastric motility. Third, compared with other tastants, knowledge on the effects of umami tastants is lacking. Therefore, the effects of post-oral delivery of umami tastants should be further elucidated. Fourth, taste receptors are expressed throughout the entire GI-tract.[19-22] The most appropriate location(s) for tastant delivery to modulate eating behaviour is unknown. More research investigating delivery of tastants in different locations in the GI-tract is needed. Fifth, the

optimal dose of tastants to exert an effect on eating behaviour is unknown. Studies should focus on finding the balance between the maximum possible effect without occurrence of side effects. Sixth, it should be investigated which bitter stimulus elicits the largest effect on energy intake. For this, studies should compare different bitter stimuli. We propose to focus on QHCl and DB, as most data are available on these bitter stimuli and both stimuli activate a wide range of bitter receptors. Lastly, when all the foundations are laid out, the field should move towards implementation of the successful interventions in battling the obesity epidemic. For this, the longer-term effects of post-oral delivery of non-caloric tastants on energy intake and ultimately body weight control should be investigated.

Conclusion

The current data show that, among tastants, bitter compounds are most effective in influencing eating behaviour. Energy intake, in the acute setting, decreased modestly after post-oral delivery of bitter tastants. This highlights the potential preventive role of bitter tastants in battling the obesity epidemic. However, there are still several gaps in knowledge, for which recommendations have been provided. Systematically addressing these issues is warranted and worldwide collaboration could provide a welcome solution.

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Supplemental material

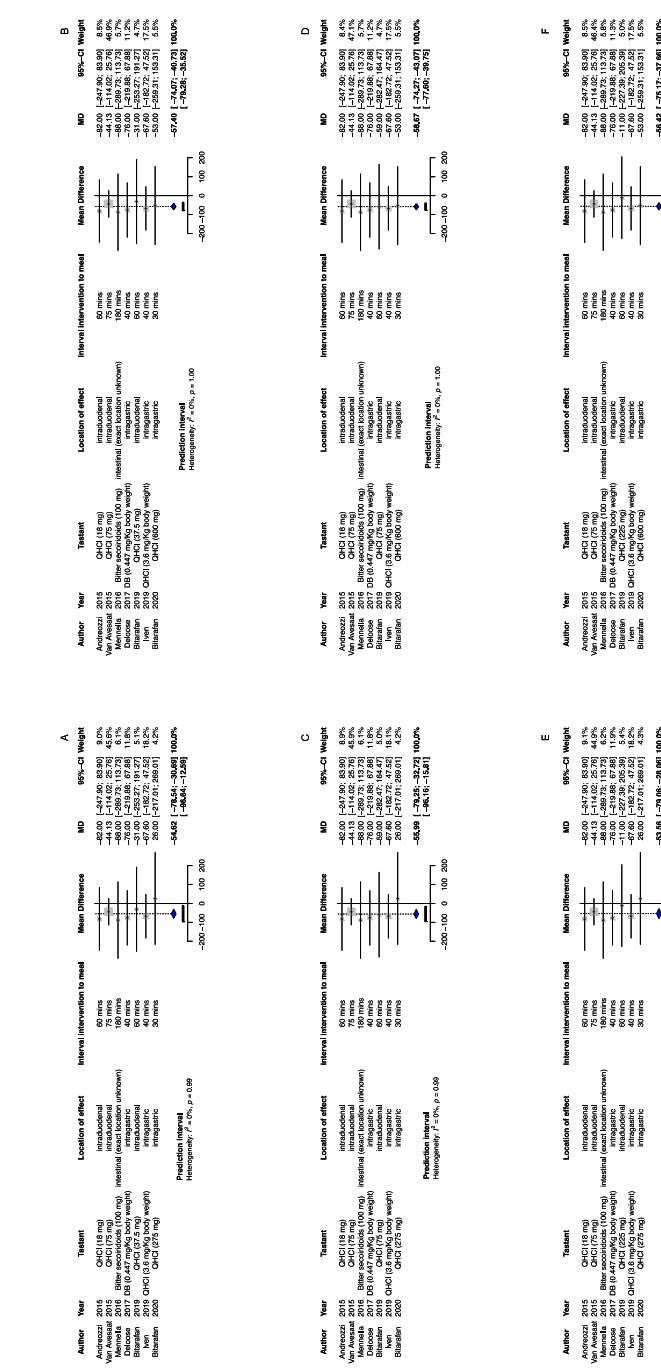

Figure S2.1 Forest plot for pooled mean difference in energy intake after bitter components versus placebo. Pooled mean differences were calculated for the various combinations of dosages of studies Bitarafan *et al.* 2019[15] and Bitarafan *et al.* 2020[18] respectively in order to eliminate a certain set of subjects having a large influence on the result: 37.5 mg QHCL and 275 mg QHCl (A), 37.5 mg QHCL and 600 mg QHCl (B), 75 mg QHCL and 275 mg QHCl (C), 75 mg QHCL and 600 mg QHCl (D), 225 mg QHCL and 275 mg QHCl (E), 225 mg QHCL and 600 mg QHCl (F). QHCl: quinine hydrochloride, DB: denatonium benzoate.

Table S2.1 Overview of included studies.

Taste	Reference	Subjects	Tastants and comparators used	Method of administration	Energy intake (Kcal)	GI symptoms and perceptions	Mechanisms of effect
Sweet	Rogers *et al.* (1990)[1] UK	12 subjects (6 men, 6 women, 18-26y, BMI 20.8)	Aspartame capsule (234 mg) Comparator: Placebo capsule	Gastric capsule	-175 Kcal	N/A	N/A
		15 subjects (10 men, 5 women, 19-24y, normal BMI	Aspartame capsule (235 mg) Aspartame capsule (470 mg) Comparator: Placebo capsule	Gastric capsule	-138 Kcal for 235mg aspartame -150 Kcal for 470 mg aspartame	Aspartame capsules reduced desire to eat and hunger scores Aspartame capsules tended to increase fullness compared with placebo (n.s.)	N/A
	Black *et al.* (1993)[2] Canada	18 subjects (18 men, 19-25y, BMI 21-25)	Aspartame capsule (340 mg) Comparator: Water	Gastric capsule	No effect on energy intake No effect on macronutrient composition	Aspartame capsules had no effect on appetite sensations	N/A
	Little *et al.* (2009)[3] UK	10 subjects	Saccharin (50 mg) Aspartame (200 mg) Comparator: Tap water	Nasogastric catheter	N/A	No effects of aspartame or saccharin on hunger or fullness	No effects of aspartame or saccharin GE
	Ma *et al.* (2009)[4] Australia	7 subjects (24 y, BMI 21.6)	Sucralose (80 mg) Sucralose (800 mg) Comparator: Saline	Nasogastric catheter	N/A	N/A	No effects of sucralose on gastric emptying, plasma glucose, plasma insulin, plasma GLP-1, or plasma GIP.

Table S2.1 (continued)

Taste	Reference	Subjects	Tastants and comparators used	Method of administration	Energy intake (Kcal)	GI symptoms and perceptions	Mechanisms of effect
Sweet	Steinert *et al.* (2011)[5] Switzerland	12 subjects (6 men, 6 women, 23.3 y, BMI 23.0)	Aspartame (160 mg) Ace-K (200 mg) sucralose (62 mg) Comparator: Tap water	Nasogastric catheter	N/A	Artificial sweeteners reduced hunger, and increased satiety and fullness ratings to an intermediate amount between water and carbohydrate sugars (n.s.)	Sweeteners did not affect plasma GLP-1, PYY, ghrelin, glucose, insulin, or glucagon levels
	Van Avesaat *et al.* (2015)[6] The Netherlands	15 subjects (6 men, 9 women, 22.4 y, BMI 22.4)	Reb-A (540 mg) Comparator: Tap water	Nasoduodenal catheter	-24 Kcal (n.s.)	Reb-A did not influence appetite sensations. Reb-A did not induce GI symptoms	Reb-A did not affect plasma CCK, GLP-1, or PYY
	Wölnerhanssen *et al.* (2016)[7] Switzerland	20 subjects 10 lean subjects (5 men, 5 women, 26.6 y, BMI 21.7) 10 obese subjects (5 men, 5 women, 27.2 y, BMI 40.0)	Xylitol (50 g) Erythritol (75 g) Comparator: Tap water	Nasogastric catheter	N/A	Both sweeteners did not affect appetite sensations. Xylitol and erythritol led to bloating and diarrhoea in 70% and 60% of subjects respectively (n.s.).	Gastric emptying was slowed during the first 60 mins after xylitol and erythritol vs. Control Plasma CCK, plasma GLP-1, Plasma glucose increased after xylitol and erythritol vs. control Plasma insulin increased after xylitol, but not after erythritol vs. control

Table S2.1 (continued)

Taste	Reference	Subjects	Tastants and comparators used	Method of administration	Energy intake (Kcal)	GI symptoms and perceptions	Mechanisms of effect
Sweet	Meyer-Gerspach *et al.* (2018)[8] Belgium	12 subjects (6 men, 6 women, 23 y, BMI 23)	Ace-K (220 mg) Comparator: Tap water	Nasogastric catheter	N/A	Hunger: Strong initial decrease in hunger after Ace-K with a faster return of hunger after first time point and slower return of hunger in last part of curve after Ace-K vs. Control Satiation: Strong initial increase in satiation after Ace-K vs. control with faster decrease after first time point and slower decrease in last part of curve after Ace-K vs. control No adverse events	GI motility did not differ between Ace-K and control. A faster linear decrease in IGP from first post infusion time point, quicker return of IGP and quicker flattening of the curve during IGP recovery with faster return to baseline in last part of the IGP curve after Ace-K vs. control No effect of Ace-K on plasma motilin, octanoylated ghrelin, active GLP-1, CCK, gastrin, and glucose.
Bitter	Little *et al.* (2009)[3] UK	12 subjects	Naringin (1 mM) Quinine (0.198 mM) Comparator: Tap water	Nasogastric catheter	N/A	No effects of naringin or quinine on hunger or fullness scores compared with water	No effects of naringin or quinine on gastric emptying compared with water

Table S2.1 (continued)

Taste	Reference	Subjects	Tastants and comparators used	Method of administration	Energy intake (Kcal)	GI symptoms and perceptions	Mechanisms of effect
Bitter	Andreozzi *et al.* (2015)[9] Italy	20 subjects (8 men, 12 women, 27 y, BMI 24)	QHCl capsule (18 mg) Comparator: Placebo capsule	Acid resistant capsules	-82 Kcal	QHCl did not affect satiety or desire to eat scores vs. Control. No adverse events	GE (evaluated in 8 subjects): no differences in GE between QHCl (87 mins) vs. Control (88 mins) CCK: Higher ΔT90 vs T0 and ΔT90 vs T60 after QHCl vs. Control
	Avau *et al.* (2015)[10] Belgium	12 subjects (5 men, 30.6 y, BMI 23.8)	DB (1 μmol/kg body weight) Comparator: Saline	Nasogastric catheter	N/A	DB made subjects feel satiated earlier and at lower volumes during constant nutrient infusion No adverse effects	Less drop in IGP after DB
	Van Avesaat *et al.* (2015)[6] The Netherlands	15 subjects (6 men, 9 women, 22.4 y, BMI 22.4)	QHCl (75 mg) Comparator: Tap water	Nasoduodenal catheter	-44 Kcal (n.s.)	Quinine did not influence appetite sensations. Quinine did not induce GI symptoms	Quinine did not affect plasma CCK, GLP-1, or PYY levels.
	Mennella *et al.* (2016)[11] Italy	20 subjects (11 men, 9 women, 25.3 y, BMI 22.1	Microencapsulated bitter secoiridoids (100 mg) Comparator: Coating only	Microencapsulation to mask oral tasting. Exact location of effect in GI tract unknown	Lunch: - 88 Kcal (n.s.) Post-lunch: -252 Kcal 24h energy intake: - 340 Kcal	no effect of bitter encapsulate on fullness, satiety, hunger or desire to eat	Bitter encapsulate increased plasma GLP-1 30 mins after intervention, but had no effect on blood glucose, plasma amylin, plasma ghrelin, plasma glucagon, plasma GIP, plasma insulin, plasma leptin, plasma PP, or plasma PYY vs. Control

Table S2.1 (continued)

Taste	Reference	Subjects	Tastants and comparators used	Method of administration	Energy intake (Kcal)	GI symptoms and perceptions	Mechanisms of effect
Bitter	Peters *et al.* (2016)[12] The Netherlands	57 subjects (all women, 40.5 y, BMI 26.5)	Bitter mixture containing: Raisin flower (22.0 mg) Sucrose Octa Acetate (0.88 mg) Quassia extract (0.088 mg) Comparator: Placebo capsule	Intragastric capsule, 2 times daily for 14 days	Day 0 vs. day 14: Meals only: - 109 Kcal (n.s.) Meals+snack: - 86 Kcal (n.s.) Breakfast: -30 Kcal (n.s.) Lunch: -61 Kcal (n.s.) Dinner: -1 Kcal (n.s.) Snacks: +41 Kcal (n.s.)	N/A	N/A
Bitter	Deloose *et al.* (2017)[13] Belgium	20 subjects (10 men, 10 women, 27 y, BMI 24)	DB (1 μmol/kg body weight) Comparator: Tap water	Nasogastric catheter	N/A	Women: Switch from gastric to duodenal phase 3 origin was accompanied by lower percentage change of hunger scores after DB vs. Control Men: Percentage change in hunger scores during phase 3 contraction did not differ after DB vs. Control (n.s.) No adverse events after DB administration	Women: DB reduced number of gastric phase 3 contractions from 67% (control) to 33% (DB) in women. Interval between IG administration and occurrence of phase 3 did not differ between control (76 min) and DB (93 min) in women (n.s.). Men: No difference in origin of phase 3 contractions between control (57% gastric) and DB (40% gastric) in men (n.s.). Interval between IG administration and occurrence of phase 3 did not differ between control (76 min) and DB (111 min) in men (n.s.).

Table S2.1 (continued)

Taste	Reference	Subjects	Tastants and comparators used	Method of administration	Energy intake (Kcal)	GI symptoms and perceptions	Mechanisms of effect
Bitter	Deloose *et al.* (2017)[13] Belgium	12 subjects (all women, 31 y, BMI 22)	DB (1 μmol/kg body weight) Comparator: Tap water	Nasogastric catheter	N/A	No adverse events after DB administration	Plasma motilin was lower after DB vs. Control. No differences between plasma total ghrelin or octanoylated ghrelin after DB vs. Control
		13 subjects (all women, 28 y, BMI 23)	DB (1 μmol/kg body weight) Comparator: Tap water	Nasogastric catheter	N/A	Hunger scores after a standardised meal were lower after DB vs. Control. Satiety scores were higher after a standardised meal after DB. No adverse events after DB administration	GE (measured in 6 subjects) did not differ between control and DB (both 109 mins.
		20 subjects (all women, 23 y, BMI 22)	DB (1 μmol/Kg body weight) Comparator: Tap water	Nasogastric catheter	-76 Kcal (n.s.)	No adverse events after DB administration	N/A
	Deloose *et al.* (2018)[14] Belgium	10 subjects (10 women, 33 y, BMI 22)	QHCl (10 μmol/kg body weight) Comparator: Milli-Q water	Nasogastric catheter	N/A	No adverse events	Plasma motilin and plasma ghrelin levels decreased after QHCl. No difference in plasma octanoylated ghrelin levels Time* treatment effect for antral motility. No main effect of treatment. No effects of QHCl on duodenal motility.

Table S2.1 (continued)

Taste	Reference	Subjects	Tastants and comparators used	Method of administration	Energy intake (Kcal)	GI symptoms and perceptions	Mechanisms of effect
Bitter	Bitarafan *et al.* (2019)[15] Australia	14 subjects (14 men, 25 y, BMI 22.5)	QHCl (37.5 mg, Q37.5)) QHCl (75 mg, Q75)) QHCl (225 mg, Q225)) Comparator: Saline	Nasoduodenal catheter	Q37.5: - 31Kcal (n.s.), Q75: -59 Kcal (n.s.), Q225: -11 Kcal (n.s.)	No differences in VAS scores for hunger, desire to eat, prospective consumption, or fullness after Q37.5, Q75, or Q225 vs. Control. No adverse events, no effects of Q37.5, Q75, or Q225 on nausea or bloating.	No effect of Q37.5, Q75, or Q225 on antral pressure waves, basal pyloric pressure, isolated pyloric pressure waves, and duodenal pressure waves vs. Control. No effects of Q37.5, Q75, and Q225 on plasma CCK or blood glucose vs. Control.
	Iven *et al.* (2019)[16] Belgium	16 subjects (16 women, 24.5 y, BMI 21.9)	QHCl (10 µmol/Kg body weigh) Comparator: Milli-Q water	Nasogastric catheter	-67.6 Kcal	Hunger scores increased after control and decreased after QHCl (n.s.) Prospective food consumption scores decreased after QHCl vs. Control Satiety scores increased after QHCl vs. Control fullness scores increased after QHCl vs. control Minimal nausea scores reported	Decreases in total ghrelin, octanoylated ghrelin, and motilin after QHCl vs. control Brain activity in homeostatic and hedonic regions: Increased activity after QHCl vs. Control in anterior insula, ACC, amygdala, putamen, nucleus accumbens, pallidum, caudate head and caudate body, medial and lateral OFC, hypothalamus and midbrain. Decreased activity in brainstem/medulla

Table S2.1 (continued)

Taste	Reference	Subjects	Tastants and comparators used	Method of administration	Energy intake (Kcal)	GI symptoms and perceptions	Mechanisms of effect
Bitter	Walker *et al.* (2019)[17] New Zealand	30 subjects (30 men, 24 y, BMI 23.1)	Amarasate extract capsule (500 mg, HD) Amarasate extract capsule (200 mg, LD) Comparator: Placebo capsule	Acid resistant capsule	N/A	From T=90 onwards HD and LD show lower mean changes in hunger and fullness Lower mean changes in fullness for HD from t=120 onwards, only t=180 and t=330 for LD. No nausea. 3 participants in HD and 1 in LD had liquid loose bowel movements	N/A
	Bitarafan *et al.* (2020)[18] Australia	15 subjects (15 men, 26 y, BMI 23.2)	QHCl (275 mg, Q275) QHCl (600 mg, Q600) Comparator: Saline	Nasogastric catheter	N/A	No effects of Q275 or Q600 on hunger, desire to eat, prospective consumption, or fullness scores. No effects of Q275 or Q600 on bloating or nausea vs. Control. No other adverse effects	No effects of Q275 or Q600 on gastric emptying. Plasma insulin was increased 30 mins after Q275 and Q600 vs. Control. No effects of Q275 or Q600 on plasma glucose, plasma glucagon, or plasma GLP-1. After mixed nutrient drink: Q275 and Q600 lowered glucose Q275 and Q600 increased plasma insulin No difference in glucagon response after nutrient drink. Q275 increased plasma GLP-1, Q600 did not.

Table S2.1 (continued)

Taste	Reference	Subjects	Tastants and comparators used	Method of administration	Energy intake (Kcal)	GI symptoms and perceptions	Mechanisms of effect
Bitter		12 subjects (12 men, 26 y, BMI 23.1)	QHCl (275 mg, Q275) QHCl (600 mg, Q600) Comparator: Saline	Nasogastric catheter	No effect of treatment on energy intake Q275: +26 Kcal, Q600: -53 Kcal	No effects of Q275 or Q600 on hunger, desire to eat, prospective consumption, or fullness scores. No effects of Q275 or Q600 on bloating or nausea vs. Control. No other adverse effects	N/A
Umami	Van Avesaat *et al.* (2015)[6] The Netherlands	15 subjects (6 men, 9 women, 22.4 y, BMI 22.4)	MSG (2 g) Comparator: Tap water	Intraduodenal catheter	+ 5 Kcal (n.s.)	MSG decreased hunger and desire to eat but did not influence satiation or fullness. MSG did not induce GI symptoms	Monosodium glutamate did not affect plasma CCK, GLP-1, or PYY levels.
Combination	Van Avesaat *et al.* (2015)[6] The Netherlands	15 subjects (6 men, 9 women, 22.4 y, BMI 22.4)	Tastant mixture: Reb-A (540 mg) QHCl (75 mg) MSG (2 g) Comparator: Tap water	Nasoduodenal catheter	-64 Kcal	The tastant mixture decreased hunger and desire to eat, but not satiation or fullness. The tastant mixture did not induce GI symptoms	The tastant mixture did not affect plasma CCK, GLP-1, or PYY levels.

Table S2.1 (continued)

Taste	Reference	Subjects	Tastants and comparators used	Method of administration	Energy intake (Kcal)	GI symptoms and perceptions	Mechanisms of effect
Combination	Klaassen *et al.* (2019)[19] The Netherlands	14 subjects (3 men, 11 women, 25.6 y, BMI 22.3)	Tastant mixture: Reb-A (540 mg) QHCl (75 mg) MSG (2 g) Comparator: Tap water	Naso-duodenal-ileal catheter	Duodenal +16.7 Kcal (n.s.), ileal +28.1 Kcal (n.s.), combined duodenal and ileal +31.5 Kcal (n.s.)	No effects of duodenal-, ileal- or combined duodenal and ileal taste receptor activation on appetite sensations. The tastant mixture did not induce GI symptoms	N/A

y: years, BMI: body mass index, N/A: not applicable, n.s.: not significant, GE: gastric emptying, GLP-1: glucagon like peptide 1, GIP: glucose-dependent insulinotropic polypeptide, Ace-K: acesulfame potassium, PYY: peptide yy, Reb-A: rebaudioside A, GI: gastrointestinal, CCK: cholecystokinin, IGP: intragastric pressure, QHCL: quinine hydrochloride, DB: denatonium benzoate, PP: pancreatic polypeptide, IG: intragastric, ACC: anterior cingulate cortex, OFC: orbitofrontal cortex, MSG: monosodium glutamate.

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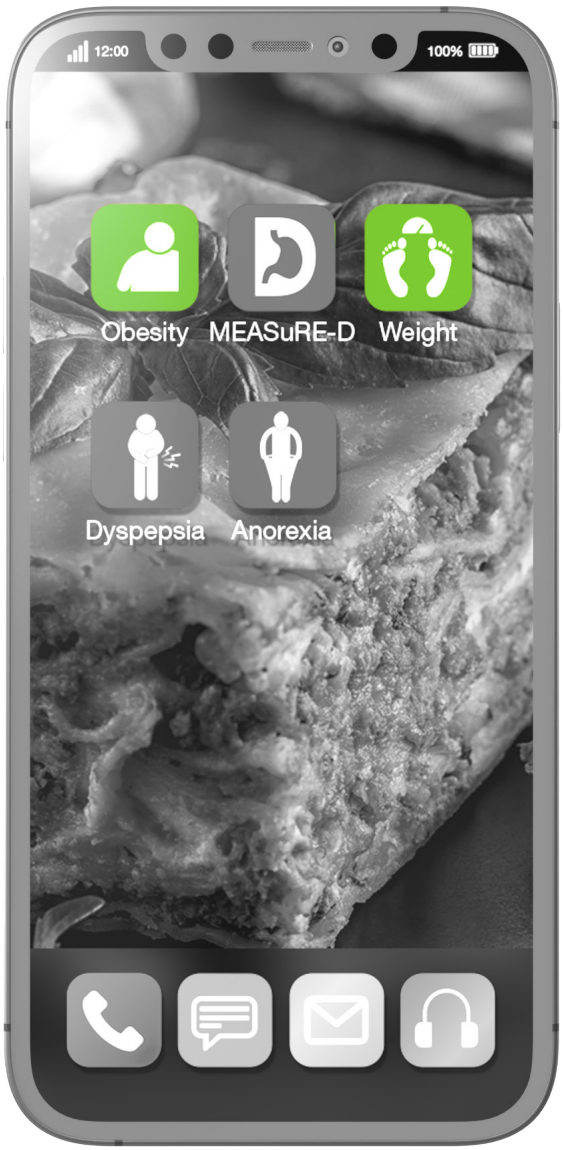
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Weight
Dyspepsia
Anorexia

Chapter 3

Intraintestinal delivery of tastants using a naso-duodenal-ileal catheter does not influence food intake or satiation

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Abstract

Intraduodenal activity of taste receptors reduces food intake. Taste receptors are expressed throughout the entire gastrointestinal tract. Currently, there are no data available on the effects of distal taste receptor activation. In this study, we investigate the effect of intraduodenal and/or intraileal activation of taste receptors on food intake and satiation. In a single-blind randomised crossover trial, fourteen participants were intubated with a naso-duodenal-ileal catheter and received four infusion regimens: duodenal placebo and ileal placebo (DPIP), duodenal tastants and ileal placebo (DTIP), duodenal placebo and ileal tastants (DPIT), duodenal tastants and ileal tastants (DTIT). Fifteen minutes after cessation of infusion, subjects received an *ad libitum* meal to measure food intake. Visual analogue scale scores for appetite sensations were collected at regular intervals. No differences in food intake were observed between the various interventions (DPIP: 786.6 ± 79.2 Kcal, DTIP: 803.3 ± 69.0 Kcal, DPIT: 814.7 ± 77.3 Kcal, DTIT: 834.8 ± 59.2 Kcal, $p=0.59$). No differences in appetite sensations were observed. Intestinal infusion of tastants using a naso-duodenal-ileal catheter did not influence food intake or appetite sensations. Possibly, the burden of the four-day naso-duodenal-ileal intubation masked a small effect that tastants might have on food intake and satiation.

Introduction

Obesity is considered a major healthcare problem with worldwide obesity almost being tripled since 1975.[1] Therefore, there is an increasing need for non-invasive therapies for weight management. Gastrointestinal (GI) hormones, such as cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1), have been shown to reduce food intake and hunger after intravenous administration.[2-4] Therefore, the GI-tract is an interesting target for non-invasive therapies to reduce food intake and induce satiety/satiation.

Intestinal macronutrient infusion decreases food intake and induces the release of CCK, GLP-1, and peptide YY (PYY).[5] This mechanism is commonly referred to as intestinal- or ileal brake.[6,7] A recent review proposed a proximal to the distal gradient in the small intestine, where a more profound effect on food intake can be found after distal compared to proximal macronutrient infusion.[8] Previous studies have demonstrated that besides macronutrients, substances referred to as tastants are able to activate certain taste receptors in the GI-tract which are coupled to enteroendocrine cells (EEC) and can trigger the release of satiety hormones (*i.e.*, CCK, GLP-1, and PYY).[9-13] These taste receptors can be found throughout the entire GI-tract. Expression levels for the various taste receptor differ throughout the gut. Table 3.1 gives a simplified visual representation of the relative expression of taste receptors throughout the human gut based on current literature.[14-17]

In a recent study, van Avesaat *et al.* have shown that duodenal infusion of a combination of sweet, bitter, and umami tastants significantly decreased *ad libitum* meal intake, whilst increasing satiation and decreasing hunger. These effects were not accompanied by changes in systemic levels of GLP-1, PYY, and CCK.[18] Up to now, no data are available on the effect of activation of taste receptors in the more distal small intestine. Since one of the functions of taste receptors in the gut is to sense food being present in the lumen, it should be investigated whether the beforementioned proximal to distal gradient found for the intestinal brake is operative for taste receptor activation.

Therefore, in the present study, we compared the effects of intraduodenal infusion versus intraileal infusion of a combination of tastants (sweet, bitter, and umami) on *ad libitum* food intake, satiation, and GI-complaints in healthy subjects. Since sweet and umami taste are sensed by various subtypes of the taste receptor family 1 (TAS1R) taste receptor family and bitter taste is sensed by the taste receptor family 2 (TAS2R), the combination will activate a wide range of taste receptors. We hypothesised that infusing tastants at both infusion sites

(duodenum and ileum) will decrease food intake and increase satiation to the greatest extent when compared with infusion of placebo or single port infusion. Infusing in solely the duodenum or the ileum will also decrease food intake and increase satiation when compared to placebo, albeit to a lesser degree than infusing at both infusion sites simultaneously. Furthermore, we expect intraileal delivery of tastants will decrease food intake and increase satiation to a greater extent when compared with intraduodenal delivery of tastants.

Table 3.1 **A simplified visual representation of the relative expression of taste receptors and gustducin throughout the human GI-tract.**

	Stomach	Duodenum	Jejunum	Ileum	Colon
TAS1R1 (Bezencon *et al.*[14])	++	+	++	+	+/−
TAS1R2 (Bezencon *et al.*[14])	−	++	+	+/−	+
TAS1R2 (Young *et al.*[15])	−−$	+	++#	N/A	N/A
TAS1R3 (Bezencon *et al.*[14])	+	++	++	+	+
TAS1R3 (van der Wielen *et al.*[16])	N/A	+	+	+	+
TAS1R3 (Young *et al.*[15])	+$	++	++#	N/A	N/A
TAS2R102–TAS2R144 (Gu *et al.*[17])	N/A	+	+	+	N/A
Gustducin (Bezencon *et al.*[14])	−−	++	++	+	−
Gustducin (Young *et al.*[15])	−$	+	++#	N/A	N/A

Expression levels are relative to each other and a simplified visual representation with ++ indicating very high expression, + indicating high expression, +/− indicating medium expression, − indicating low expression, and −− indicating very low expression. $ Young *et al.* displayed the stomach as fundus, body, and antrum. For details, please refer to Young *et al.*[15] # Young *et al.* displayed jejunum as proximal jejunum and distal jejunum. For details, please refer to Young *et al.*[15] N/A: not available. * T2R family is expressed throughout the entire small intestine in a comparable fashion with some subtypes more abundant proximally and some distally. For details, please refer to Gu *et al.*[17]

Materials and methods

This study was approved by the Medical Ethics Committee of the Maastricht University Medical Centre+ (MUMC+), Maastricht, the Netherlands, and performed in full accordance with the Declaration of Helsinki (latest amendment by the World Medic Association in 2013) and Dutch Regulations on Medical Research Involving Human Subjects (WMO, 1998). This study was registered in

the US National Library of Medicine (http://www.clinicaltrials.gov, ID NCT03140930). All subjects gave written informed consent before screening.

Subjects

Healthy men and women were recruited by local advertisements. Inclusion criteria were age between 18 and 65 years, a body mass index (BMI) between 18 and 25 kg/m^2, with a stable weight over the past six months (<5% body weight change). Exclusion criteria were gastrointestinal complaints, history of chronic or severe disease, use of medication influencing endpoints within 14 days prior to testing, administration of investigational drugs which interfere with this study, major abdominal surgery, dieting, pregnancy or lactation, excessive alcohol consumption (>20 alcoholic consumptions per week), smoking, weight <60 kg, non-tasters of sweet, bitter or umami stimuli, evidence of monosodium glutamate (MSG)-hypersensitivity.

Prior to testing, screening was performed where abovementioned inclusion and exclusion criteria were checked, and a taste perception test was performed. Subjects tasted quinine (0.5 mmol/L), Reb A (50 mmol/L), MSG (50 mmol/L), and tap water blindly and had to indicate their sense of taste. Subjects had to identify each taste correctly in order to be eligible for the study. Furthermore, their length and weight were measured to calculate their BMI.

A sample size calculation was based on the difference in meal intake between duodenal infusion of a combination of tastants and duodenal infusion of placebo as reported by van Avesaat *et al.*[18] Using a difference in means of 64 Kcal, a standard deviation of difference of 63, a power of 80%, and an alpha of 1.67%, a total number of 13 subjects were needed. An alpha of 1.67% was used to correct for multiple testing.

Study design

In this single-blind randomised, placebo-controlled crossover study, subjects received the combination of tastants (sweet, bitter, and umami) and/or placebo (tap water) in the duodenum and/or the ileum for four consecutive test days. This results in four combinations which were infused on the various test days: duodenal placebo and ileal placebo (DPIP), duodenal tastants and ileal placebo (DTIP), duodenal placebo and ileal tastants (DPIT), duodenal tastants and ileal tastants (DTIT).

Catheter positioning

A 305 cm long silicon 9-lumen (8-lumen, 1 balloon inflation channel, the outer diameter of 3.5 mm) custom-made naso-ileal reusable catheter (Dentsleeve International, Mui Scientific, Mississauga, Canada) was used for intubation.
One day prior to the first test day, subjects arrived at 7:40 AM at the Maastricht University Medical Centre+ (MUMC+) after an overnight fast. If preferred by the subject, local anaesthesia of nasal mucosa using xylocaine (10% spray, AstraZeneca, Zoetermeer, the Netherlands) was applied. After placement of the catheter in the stomach, the catheter was guided through the pylorus and into the duodenum under intermittent fluoroscopic control. Progression of the catheter from duodenum to ileum was performed as described earlier.[19] Fluoroscopy was used to check the positioning of the catheter on the first and the last test day. Radio-opaque markers were added to the infusion ports on the catheter, which accounted for the determination of the catheter position. On all test days, intestinal fluid was sampled from various infusion ports, and pH was measured using pH strips (MColorpHast™, Merck, Darmstadt, Germany) in order to estimate the catheter positioning.

Preparation and infusion of tastants

The combination of three tastants was infused in the duodenum, the ileum, or both the duodenum and the ileum. In order to prevent side effects from occurring, 75% of acceptable daily intake (ADI) of these tastants was infused. 540 mg Rebaudioside A (Reb A, Stevija Natuurlijk, Drachten, the Netherlands), 75 mg Quinine (Arnold Suhr, Hilversum, the Netherlands), and 2 g Monosodium Glutamate (MSG, Ajinomoto, Hamburg, Germany) were dissolved in 120 mL tap water and was used as tastant mixture for infusion, as was done by van Avesaat *et al.*[18] All tastants used were non-caloric and yielded no nutritional value. The placebo infusion consisted of 120 mL of tap water. A magnetic stirrer was used to dissolve the tastants. The mixture was infused over a 60-min period with an infusion rate of 2 mL/min. This was consistent with the infusion rate of van Avesaat *et al.* mimicking the slow influx from the stomach to duodenum and slow transit through the gut in the ileum.

Protocol

On each test day, after an 8 h overnight fast, subjects arrived at 8:00 AM at the MUMC+. Subjects were instructed to consume the same habitual meal on the

evening prior to testing. Hereafter, at t=0 min, a standardised liquid breakfast meal (250 mL Goedemorgen drinkontbijt (Vifit); energy 145 Kcal per portion, 20.25 g carbohydrates, 8.5 g protein, and 2 g fat) was consumed. One hundred and fifty min (at t=150 min) after breakfast consumption, a syringe containing the mixture for infusion was connected to the duodenal and ileal infusion port. The infusion was performed in 60 min at an infusion rate of 2 ml/min. Subjects received a standardised *ad libitum* lunch meal (Lasagne Bolognese (Plus supermarket); energy density per 100 g: 152 Kcal, 11 g carbohydrates, 7.1 g protein, and 8.6 g fat) fifteen min (at t = 225 min) after cessation of the infusion. The test meal was offered in excess and subjects were instructed to eat until they felt satiated.

VAS for appetite sensations and GI-complaints

Appetite sensations and GI-complaints (*e.g.*, satiation, hunger, stomach pain, and nausea) were measured using visual analogue scales (VAS, 0-100 mm) scores at various time points (t=−30, 30, 90, 150, 165, 180, 195, 210, and 240 min) during the day. Subjects were asked to indicate on a line, anchored at the low end with the lowest intensity feelings, with opposing terms at the high end, which place on the scale best reflected their feeling at that moment.[20]

Statistical analyses

Data were analysed using IBM SPSS statistics 24 (IBM Corporation, Armonk, NY, USA). A visual check of the normality of the data was performed. The primary outcome of this study was the amount of food intake in Kcal during an *ad libitum* lunch meal. Secondary outcomes were VAS scores for appetite sensations and GI-symptoms.

Age, BMI, and gender were calculated by descriptive statistics. Food intake in Kcal and area under the curve (AUC) for VAS scores were compared using a linear mixed model with intervention (DTIP, DPIT, and DTIT, and DPIP), test day and the interaction of intervention × test day as fixed factors. When no significant interaction was found, the interaction was removed from the model to get the best model fit.

For VAS scores, a linear mixed model that included abovementioned fixed factors with the addition of fixed factors time and time × treatment interaction was also performed.

Data are presented as mean ± standard error of the mean (SEM) (unless specified otherwise), and a $p<0.05$ was considered statistically significant.

Results

Subjects

In total, 19 subjects met the inclusion and exclusion criteria. Two subjects dropped out due to discomfort induced by the naso-ileal catheter, two subjects dropped out due to incorrect position of the catheter on the first test day, and one subject was excluded after not properly following the instructions for the *ad libitum* meal on the first test day. Therefore, 14 healthy volunteers (11 female, age 25.6 ± 10.5 years, BMI 22.3 ± 1.7 kg/m^2) completed the study protocol and were included in the analyses.

Food intake

No intervention × test day interaction was found. No differences in *ad libitum* food intake in Kcal were observed after intraduodenal, intraileal or combined infusion of tastants versus placebo infusion (DPIP: 786.6 ± 79.2 Kcal, DTIP: 803.3 ± 69.0 Kcal, DPIT: 814.7 ± 77.3 Kcal, DTIT: 834.8 ± 59.2 Kcal; p=0.59) (Figure 3.1). Furthermore, as depicted in Figure 3.2, no trends in individual responses were found.

Satiation/Satiety scores

The mean VAS scores for the desire to eat, hunger, satiation, and fullness are depicted in Figure 3.3. No differences in area under the curve ($AUC_{150–210}$) for these VAS scores were observed between the various interventions. Furthermore, no intervention × timepoint interactions were found for these VAS scores.

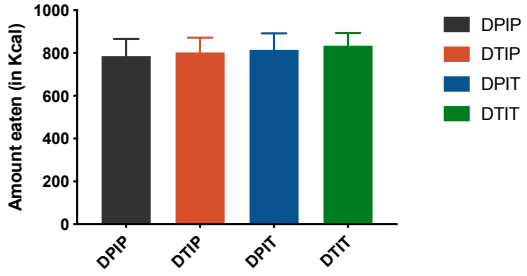

Figure 3.1 **Food intake.** The amount eaten in Kcal (mean + SEM) 15 min after cessation of the infusion of placebo both intraduodenal and intraileal (DPIP), tastants intraduodenal and placebo intraileal (DTIP), placebo intraduodenal and tastants intraileal (DPIT), and tastants both intraduodenal and intraileal (DTIT). Based on a linear mixed model, no difference in food intake was observed between the conditions (p=0.59).

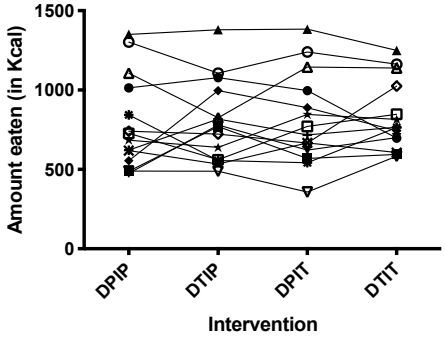

Figure 3.2 **Individual food intake.** An individual representation per subject of amount eaten in Kcal 15 min after cessation of the infusion of placebo both intraduodenal and intraileal (DPIP), tastants intraduodenal and placebo intraileal (DTIP), placebo intraduodenal and tastants intraileal (DPIT), and tastants both intraduodenal and intraileal (DTIT). Treatment order was randomised for each subject. Each line with a unique symbol represents an individual subject. Based on a linear mixed model, no difference in food intake was observed between the conditions (p=0.59).

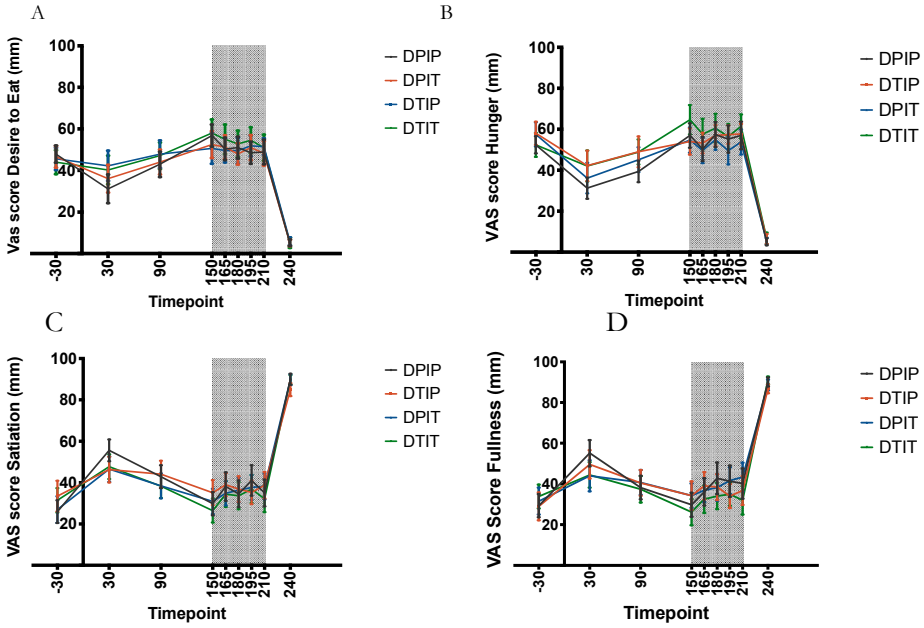

Figure 3.3 **Appetite sensations.** VAS scores for desire to eat (**A**), hunger (**B**), satiation (**C**), and fullness (**D**) (mean + SEM) before, during, and after the infusion of placebo both intraduodenal and intraileal (DPIP), tastants intraduodenal and placebo intraileal (DTIP), placebo intraduodenal and tastants intraileal (DPIT), and tastants both intraduodenal and intraileal (DTIT). VAS scores were measured at t=−30, 30, 90, 150, 165, 180, 195, 210, and 240 min. No VAS scores were taken at t=225 min. At t=0 min, subjects received a standardised breakfast, infusion of mixtures was performed from t=150 until t=210 min, and *ad libitum* test meal was presented at t=225. Based on a linear mixed model of mean scores and area under the curve ($AUC_{150–210}$), no differences in desire to eat, hunger, satiation, and fullness were observed between the various conditions.

GI-complaints

The mean VAS scores for stomach pain, bloating, and nausea are depicted in Figure 3.4. No differences in area under the curve ($AUC_{150–210}$) for these VAS scores were observed between the various interventions. Furthermore, no intervention × timepoint interactions were found for these VAS scores.

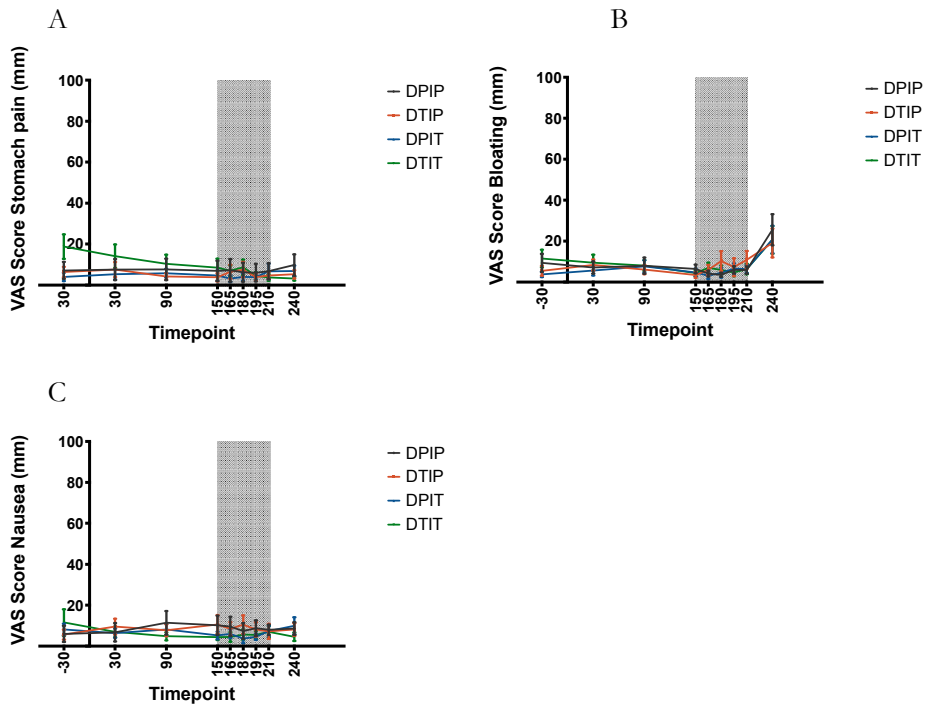

Figure 3.4 **GI complaints.** VAS scores for stomach pain (**A**), bloating (**B**), and nausea (**C**) (mean + SEM) before, during, and after the infusion of placebo both intraduodenal and intraileal (DPIP), tastants intraduodenal and placebo intraileal (DTIP), placebo intraduodenal and tastants intraileal (DPIT), and tastants both intraduodenal and intraileal (DTIT). t=−30, 30, 90, 150, 165, 180, 195, 210, and 240 min. No VAS scores were taken at t=225 min. At t=0 min, subjects received a standardised breakfast, infusion of mixtures was performed from t=150 until t=210 min, and *ad libitum* test meal was presented at t=225 min. Based on a linear mixed model of mean scores and area under the curve ($AUC_{150–210}$), no differences in stomach pain, bloating, and nausea were observed between the various conditions.

Discussion

Our results do not reveal any difference in appetite sensations or food intake between combined duodenal- and ileal administration of placebo, duodenal

administration of a tastant mixture, ileal administration of a tastant mixture, or combined duodenal and ileal administration of a tastant mixture. Moreover, no GI-complaints were caused by infusing a tastant mixture or placebo into the duodenum and/or the ileum.

Van Avesaat *et al.* have investigated the effect of intraduodenal infusion of the same tastant mixture on food intake.[18] In that study, intraduodenal infusion of this combination of tastants, in similar study design, using the same amount of tastants significantly reduced food intake by 64 Kcal and was accompanied by increased satiation. However, it must be noted that this is a small difference, which on its own might not be clinically significant. Repeating this effect multiple times per day with each meal might result in a clinically significant decrease of caloric intake. This difference in results of food intake between the two studies may be related to differences in study design. In the study of van Avesaat *et al.*, the subjects were intubated with a naso-duodenal catheter on every test day for the administration of tastants. The catheter was removed immediately thereafter before the subjects were presented with the *ad libitum* test meal. In the present study, subjects were intubated for several days with a naso-ileal catheter, and therefore this catheter was present while meals were offered and ingested. We hypothesise that having a naso-ileal catheter in situ for multiple days negatively influences meal ingestion to such a degree that this masks the smaller magnitude of effect that infusion of non-caloric tastants into the intestine has. On the other hand, mean caloric intake showed no major differences between the two studies.

Previous studies from our group investigating the 'intestinal brake' by infusing macronutrients in the ileum have repeatedly shown that infusion of even low doses of macronutrients results in a significant reduction of food intake, ranging between 64–188 Kcal, corresponding to a percentual decrease of 11.7%–32% of caloric intake during a single meal.[5,21] This indicates a negative feedback mechanism on food intake that arises from nutrient sensing. These data demonstrate that magnitude of the effect of macronutrient infusion on food intake is greater than the effects of infusing tastants.

Conclusively, studies investigating differences in food intake should be aware that naso-ileal intubation might mask a small effect. Therefore, other delivery options, such as encapsulation, should be considered in the future.

Results of studies investigating the effects of single tastants on food intake, satiation/satiety, and GI peptides are not consistent. An initial strong decrease of hunger with a steep increase thereafter has been observed after administration of a non-caloric sweetener.[22] Ingestion of low caloric sweeteners did not influence energy intake compared with a control condition (intake of water).[23] Adding an

umami tastant to a meal did not affect appetite sensations but has been shown to result in an increase of subsequent food intake.[24] Recently, increased attention has been given to the effects of bitter substances on satiation and food intake. Intake or infusion of bitter substances (quinine, denatonium benzoate) not only reduced antral motility[25,26] but also increased satiation/satiety scores and resulted in a significant decrease in food intake.[27] A possible mechanism explaining the strong aversive effects of bitter tastants is that bitter taste is evolutionarily linked to toxic substances, as has been showed by presenting new-born infants with bitter substances.[28]

Alleleyn *et al.* have shown that the inhibition of food intake shows a proximal to the distal gradient, with higher effects observed after distal versus proximal administration of nutrients.[8] Based on our data, such a gradient was not observed for intestinally administered tastants. Intestinal taste receptor expression varies for various taste receptors, where some taste receptors are more profound proximally in the GI-tract, while expression of other taste receptors is higher in the more distal intestine.[14-17]

We thought the proximal to distal gradient found for macronutrient infusion might be operable for taste receptor activation, which was clearly not the case. It is possible that taste receptors inhibit food intake in a different fashion than macronutrients. For instance, it has been speculated that taste receptors function by sensing the type of food (*i.e.*, sweet for carbohydrates, umami for amino acids, and bitter for toxic substances).[29] Since bitter tastants are linked to toxic substances, another working mechanism for bitter tastants could be through an aversive reaction of subsequent food intake.

From an evolutionary perspective, a more pronounced inhibitory or aversive effect for toxic substances could be expected to occur in the most proximal parts of the GI tract. However, there are no data available with respect to activation of oral (bitter) taste receptors on subsequent food intake. It is therefore unclear, whether activation of more proximal taste receptors will reveal more pronounced effects on food intake and satiation/satiety. Consequently, further studies are needed to investigate whether more proximal activation of taste receptors results in a stronger decrease in food intake.

Published data on the role of GI peptides in the regulation of food intake after administration of tastants are not in line. Van Avesaat *et al.* found a clear effect of intraduodenal administration of tastants on food intake that was not accompanied by changes in GLP-1- or PYY level.[18] Other studies, however, did show a decrease in systemic ghrelin- and motilin levels[25,26] and an increase in systemic CCK levels[27] after administration of a bitter tastant.

A limitation of our study is that the wash-out period consisted of only one day. Prolonging the wash-out period over one day would have resulted in a longer period of naso-ileal catheter intubation increasing the discomfort to our volunteers. No interaction effect between intervention and test day was found on food intake, satiation scores or GI-complaints, indicating that no carry-over effect was present.

Another limitation of the present study was the absence of systemic GI hormone measurements. This would have provided a complete analysis of the effects of intestinal tastant administration on eating behaviour. However, van Avesaat *et al.* showed a decrease in food intake and an increase in satiation scores, which was not accompanied by changes in systemic GI hormone levels.[18] Therefore, no systemic GI hormone measurement was conducted in the present study.

It has to be noted that the ideal duration of administration of the intervention and of the timing between intervention and serving the *ad libitum* meal is unknown. We employed a design similar to that of van Avesaat *et al.* based on their positive results.[18] Future research protocols should consider these factors.

Studies investigating the effects of tastants on food intake up to now focus on only acute effects in a single *ad libitum* meal. It is not known whether repetitive or chronic administration of tastants will lead to other results. More data are needed on the long-term effects of tastants, especially on daily energy intake.

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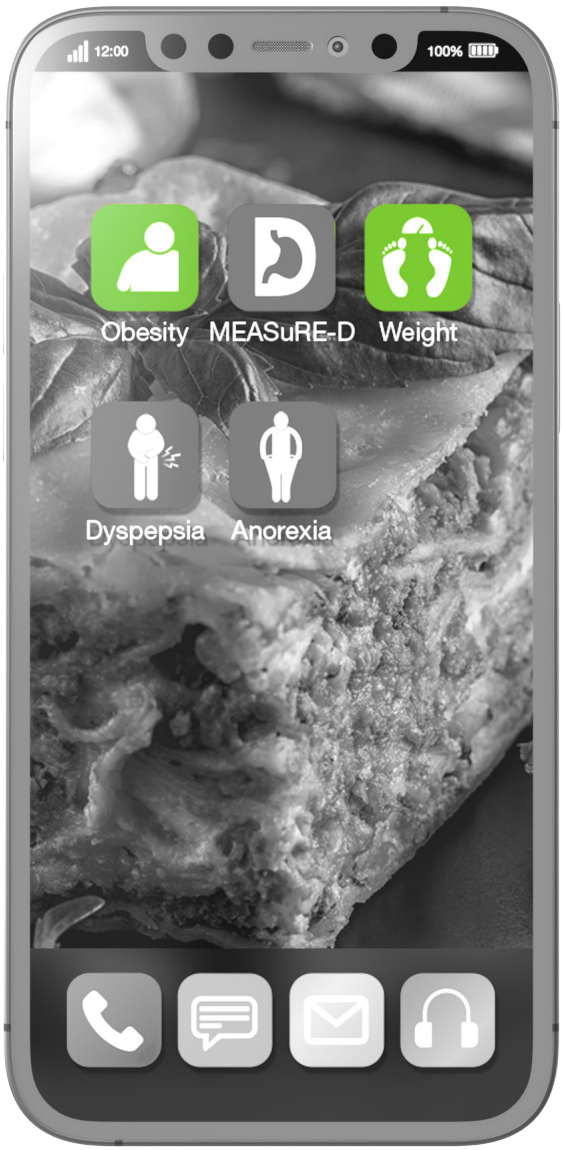
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Chapter 4

Effect of oral or intragastric delivery of the bitter tastant quinine on food intake and appetite sensations: a randomised crossover trial

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Abstract

Stimulation of gastrointestinal taste receptors affects eating behaviour. Intraduodenal infusion of tastants leads to increased satiation and reduced food intake, whereas intraileal infusion of tastants does not affect eating behaviour. Currently, it is unknown whether oral- or intragastric administration of tastants induces a larger effect on eating behaviour. This study investigated the effects of oral- and/or intragastric administration of quinine on food intake, appetite sensations, and heart rate variability (HRV). In a blinded randomised crossover trial, 32 healthy volunteers participated in four interventions with a one-week washout: oral placebo/intragastric placebo (OPGP), oral quinine/intragastric placebo (OQGP), oral placebo/intragastric quinine (OPGQ), and oral quinine/intragastric quinine (OQGQ). On test days, 150 minutes after a standardised breakfast, subjects ingested a capsule containing quinine or placebo and were sham-fed a mixture of quinine or placebo orally. Fifty min after intervention, subjects received an *ad libitum* meal to measure food intake. Visual analogue scales for appetite sensations were collected and HRV measurements were performed at regular intervals. Oral- and/or intragastric delivery of the bitter tastant quinine did not affect food intake OPGP: 3273.6 (SEM 131.8) KJ, OQGP: 3072.7 (SEM 132.2) KJ, OPGQ: 3289.0 (SEM 132.6) KJ, OQGQ: 3204.1 (SEM 133.1) KJ, $p=0.069$). Desire to eat and hunger decreased after OQGP and OPGQ compared with OPGP ($p<0.001$ and $p<0.05$, respectively), whereas satiation, fullness and HRV did not differ between interventions. In conclusion, sole oral sham feeding with- and sole intragastric delivery of quinine decreased desire to eat and hunger, without affecting food intake, satiation, fullness, or HRV.

Introduction

Since 1975, the prevalence of obesity has nearly tripled, being a major healthcare problem worldwide[1] urging the need for non-invasive treatment strategies. The gastrointestinal (GI) tract plays a crucial role in the regulation of eating behaviour via its ability to produce and secrete GI peptides upon food-derived triggers.[2-4]
In order for the GI tract to modulate eating behaviour, food constituents need to be sensed in the gut. It has been shown that intestinal infusion of macronutrients reduces food intake that is accompanied by the release of GI peptides, generally called the intestinal brake,[5-7] which is operable in a proximal to distal intestinal gradient.[8]
Another way to perceive food constituents in the gut is via taste receptors, which are expressed throughout the entire GI tract.[9-11] and have been shown to trigger the release of GI peptides *in vitro*.[12-15] Duodenal delivery of a tastant mixture containing non-caloric sweet, bitter and umami tastants, resulted in significant decrease in food intake, without affecting systemic GI peptide levels.[16] However, the proximal to distal gradient operable for macronutrients was not applicable to tastants.[17]
Inhibition of food intake via taste receptors may occur via pathways different from those via nutrient receptors. It has been hypothesised that taste predicts the type of food that is ingested (*i.e.*, bitter for toxic substances, umami for amino acids, and sweet for carbohydrates).[18] Since bitter taste is linked to toxic substances, subsequent food intake could be limited via an aversive reaction to bitter tastants. Currently, data on sole oral effects of (bitter) tastant delivery (*i.e.*, without ingesting) on eating behaviour is lacking.
Besides endocrine signalling, the autonomic nervous system (ANS) plays a role in appetite signalling. Changes in the balance of ANS can occur prior to changes in GI peptide levels in presence of a shift from hunger to satiety.[19,20] Increased sympathetic tone is suggested to indicate satiation in humans.[21] In addition to aversive responses in reaction to bitter substances, bitter taste also exerts a strong sympathetic activation.[22] A simple, non-invasive method to measure ANS is heart rate variability (HRV), a physiological variation in time interval between consecutive heartbeats.[23]
This study investigated the effects of oral- versus intragastric versus synchronous oral- and intragastric delivery of the bitter tastant quinine on eating behaviour in healthy subjects, with *ad libitum* food intake as primary outcome and HRV, appetite sensations, and GI-complaints as secondary outcomes. We hypothesised that synchronous oral and intragastric delivery of quinine decreases food intake

compared with placebo or delivery of quinine solely oral- or intragastric. Moreover, we expected that solely oral- or intragastric delivery of quinine would decrease food intake compared with placebo, albeit to a lesser extent than synchronous oral- and intragastric delivery of quinine. Finally, we expect oral delivery of quinine to decrease food intake to a greater extent than intragastric delivery of quinine.

Methods

This study was approved by the Medical Ethics Committee of the Maastricht University Medical Centre+ (MUMC+), Maastricht, the Netherlands (ID METC163047), and performed in full accordance with the Declaration of Helsinki (latest amendment by the World Medic Association in 2013) and Dutch Regulations on Medical Research involving Human Subjects (WMO, 1998). The study was performed at the Maastricht University Campus Venlo from August 17th 2018 until August 15th 2019. This study was registered in the US National Library of Medicine (http://www.clinicaltrials.gov, ID NCT 03565133). All subjects gave written informed consent before screening.

Subjects

Healthy men and women were recruited by local advertisements. Inclusion criteria were age between 18 and 65 years, a body mass index (BMI) between 18 and 25 kg/m^2, with a stable weight over the past six months (<5% bodyweight change). Exclusion criteria were GI complaints, history of chronic or severe disease, use of medication influencing study endpoints within 14 days prior to testing, administration of investigational drugs which interfere with this study, major abdominal surgery, dieting, pregnancy or lactation, excessive alcohol consumption (>20 alcoholic consumptions per week), smoking, and non-tasters of the bitter stimulus quinine.

Prior to testing, a screening was performed where abovementioned in- and exclusion criteria were checked and a taste perception test with quinine was performed. Subjects tasted quinine (0.5 mmol/L) and tap water blindly and had to indicate their sense of taste. Subjects had to correctly identify the bitter taste of the quinine sample in order to be eligible for the study. Furthermore, length and weight were measured to calculate BMI.

Study design

In this blinded randomised, placebo-controlled crossover study, subjects received a capsule containing quinine or placebo (cellulose, which is tasteless and odourless in itself[22,24] and subjects performed oral sham-feeding with quinine dissolved in tap water or placebo (tap water) on four test days separated by at least one-week wash-out. An independent researcher not involved in execution of the study generated the randomisation list using www.sealedenvelope.com. On each test day, participants received one of the following interventions in random order: oral placebo and intragastric placebo (OPGP), oral quinine and intragastric placebo (OQGP), oral placebo and intragastric quinine (OPGQ), and oral quinine and intragastric quinine (OQGQ). Subjects and researchers were blinded for the containments of the capsule, but not for the substance used for oral sham feeding as subjects cannot be blinded for the taste of oral sham feeding intervention. Moreover, due to an evolutionary aversive reaction to tasting a bitter substance,[25] subjects would involuntarily show whether oral sham feeding with tap water or with quinine dissolved in tap water is being performed. Therefore, the researcher could not be blinded for the oral sham condition.

Oral sham feeding

For oral sham feeding 75 mg of quinine-hydrochloride (QHCl) (Arnold Suhr, Hilversum, the Netherlands) was dissolved in 120 ml tap water. A magnetic stirrer was used to dissolve the quinine. As placebo condition, 120 ml tap water was used. Sham feeding was performed by letting subjects take several sips (habitual size as if they would take a sip of a drink) out of one cup and letting them swish the contents around with closed mouth for 20-30 seconds before expectorating it in a different cup according to previously described protocols.[26,27] This induced two to three minutes of sensory stimulation.

Intragastric capsule

Capsules were manufactured by *'Verenigde Apotheken Limburg (VAL) Bereidingsapotheek'*, Maastricht, the Netherlands. Gelatine capsules were filled with 75 mg QHCl and cellulose. As placebo, capsules were filled with cellulose only. The capsules were indistinguishable in terms of appearance and weight. According to the VAL capsule breakup time was < nine minutes (unpublished results). A dosage of 75 mg QHCl was used, as this aligns with previous research from our group.[16,17]

Protocol

On each test day, after an 8 h overnight fast, subjects arrived at 8:30 AM at the Maastricht University Campus Venlo. Subjects were instructed to consume the same habitual meal on the evening prior to testing. At t=-150 min subjects consumed a standardised liquid breakfast meal (250 ml Goedemorgen drinkontbijt (Vifit, Friesland Campina, Amersfoort, the Netherlands); energy 648.5 KJ (155 Kcal) per portion, 22 g carbohydrates, 8.5 g protein, and 2 g fat). One hundred and fifty min (at t=0 min) after breakfast consumption, subjects ingested the capsule with 120 ml tap water and directly thereafter performed the oral sham feeding. Participants were not allowed to rinse their mouths after the sham feeding. Participants reported a persistent bitter taste in their mouth, although this was not quantified. Fifty min after the intervention (at t=50), subjects received a standardised *ad libitum* lunch meal. During the test day, heart rate variability (HRV) measurements (at t=-150 min, t=-15 min, t=5 min, and t=35 min) were performed and VAS scores for appetite sensations (t=-20 min, t=-10 min, t=0min, t=10 min, t=20 min, t=30 min, t=40 min, t=50 min, and t=END) were collected. An overview of the timeline of the test days can be found in Figure 4.1.

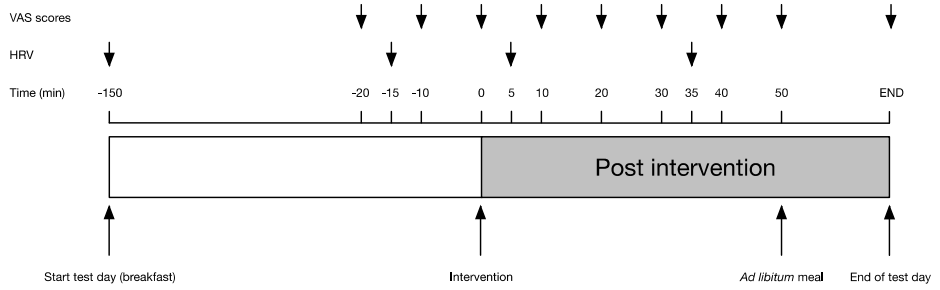

Figure 4.1 **Timeline of test day**. A standardised breakfast was consumed at t=-150 min. At t=0 min participants ingested a capsule containing quinine or placebo and performed oral sham feeding with quinine dissolved in tap water or solely tap water. Fifty mins after the intervention an *ad libitum* test meal was offered. Heart rate variability (HRV) measurements were performed and VAS scores for appetite sensations and GI complaints were collected over the course of the test day. Endpoint of the study and last VAS scores varied per subject, depending on duration of test meal intake.

Ad libitum test meal

Subjects received a standardised *ad libitum* lunch meal (Lasagne Bolognese (Plus supermarket, Utrecht, the Netherlands); energy density per 100 g: 581.6 KJ

(139 Kcal); food constituents: 10.6 g carbohydrates, 8.1 g protein, and 6.7 g fat) fifty min (at t=50 min) after the intervention. The test meal was offered in excess (a portion of 1 kg (5815.7 KJ (1390 Kcal)), which is more than half of the recommended daily intake. No participant finished the entire portion) and subjects were instructed to eat until they felt satiated.

VAS for appetite sensations and gastrointestinal complaints

Appetite sensations and GI-complaints (*e.g.*, satiety, hunger, stomach pain, and nausea) were measured using visual analogue scales (VAS, 0-100 mm) over the course of the test day. Subjects were asked to indicate on a line, anchored at the low end with the lowest intensity feelings, with opposing terms at the high end, which place on the scale best reflected their feeling at that moment.[28]

Heart rate variability

Utilising only standard and uni-polar leads, short time electrocardiogram (ECG) records were obtained using computer electrocardiograph "Poly-Spectrum-8/E" (Neurosoft®, version 5.3.1.0, Ivanovo, Russia). Five minutes recordings were performed after a resting period of at least 5 minutes. Measurements were performed in a semi-recumbent position in order to minimise ectopic activity. With a sampling frequency of 2 kHz, successive heartbeats or inter-beat intervals were determined and analysed according to HRV standards using "Poly-Spectrum-8" (Neurosoft®, version 5.3.1.0, Ivanovo, Russia) software.[23] The power spectral analysis was performed with a fast Fourier transformation. Total power (TP, 0.01 to 0.40 Hz), high frequency power (HF, .15 to 0.40 Hz), low frequency power (LF, 0.04 to 0.15 Hz), and very low frequency power (VLF, 0.01 to 0.04 Hz) were calculated. For this study heart rate and LF/HF ratio were investigated.

Statistical analyses

A sample size calculation was based on the difference in food intake after duodenal infusion of the bitter tastant quinine in comparison to food intake after duodenal infusion of placebo as reported by van Avesaat *et al.*[16] We anticipated the same magnitude for change comparing the combined oral and gastric administration vs. placebo. Using a difference in means of 184.1 KJ (44 Kcal), a standard deviation of difference of 355.6 KJ (85 Kcal), a power of 80%, and an alpha of 5%, a total number of 32 subjects was needed.

Data was analysed using IBM SPSS statistics 25 (IBM Corporation, Armonk, NY, USA). Kolmogorov-Smirnov tests as well as a visual check of normality of the data were performed. The primary outcome of this study was food intake during an *ad libitum* lunch meal. Secondary outcomes were VAS scores for appetite sensations and GI-complaints, and HRV measurements. Data are presented as mean ± standard error of mean (SEM) (unless specified otherwise).
Age, BMI and sex were calculated by descriptive statistics.
Food intake measurements were compared using an autoregressive (1) linear mixed model with intervention, test day and the interaction of intervention × test day as fixed factors. In case no intervention × test day interaction was found, this factor was removed from the model to get the best model fit.
VAS scores for appetite sensations and GI complaints were compared using an autoregressive (1) linear mixed model with intervention, test day, time and the interactions of intervention× test day and time × intervention as fixed factors. In case no intervention × test day interaction was found, this factor was removed from the model to get the best model fit.
HRV measurements were compared using an autoregressive (1) linear mixed model with intervention, test day, time and the interactions of intervention × test day and time × intervention as fixed factors. In case no intervention × test day interaction was found, this factor was removed from the model to get the best model fit.
For all analyses a $p<0.05$ was considered statistically significant. Whenever a main effect was found, Bonferroni correction for multiple testing was performed per outcome parameter.

Results

Subjects

In total 34 subjects met the in- and exclusion criteria. Two subjects dropped out after the first test day due to personal circumstances. Therefore, 32 healthy volunteers (22 female, age 25.3 ± SD 3.8 years, BMI 22.5 ± SD 1.9 kg/m^2) completed the study protocol and were included in the analyses. No other adverse events were reported and all subjects tolerated the interventions well.

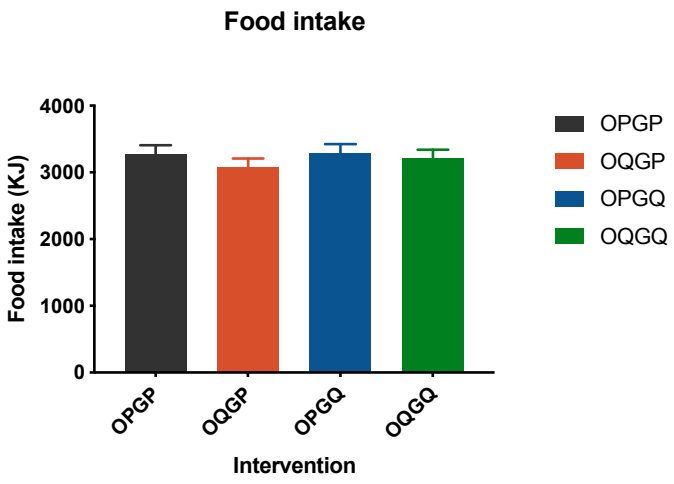

Figure 4.2 **Food intake.** Amount eaten in KJ (mean + SEM) 50 minutes after oral placebo and intragastric placebo (OPGP), oral quinine and intragastric placebo (OQGP), oral placebo and intragastric quinine (OPGQ), and quinine both oral and intragastric (OQGQ). Based on an autoregressive (1) linear mixed model, no difference in food intake was observed between the conditions (p=0.069).

Food intake

Based on estimated means, no differences in *ad libitum* food intake were observed after oral sham feeding with quinine, intragastric delivery of quinine or combined oral sham feeding with- and intragastric delivery of quinine versus placebo (Placebo: 3273.6 (SEM 131.8) KJ, oral sham feeding with quinine: 3072.7 (SEM 132.2) KJ, intragastric delivery of quinine: 3289.0 (SEM 132.6) KJ, oral sham feeding with- and intragastric delivery of quinine: 3204.1 (SEM 133.1) KJ, p=0.069) (Figure 4.2). Furthermore, no trends in individual responses were found (Figure 4.3).

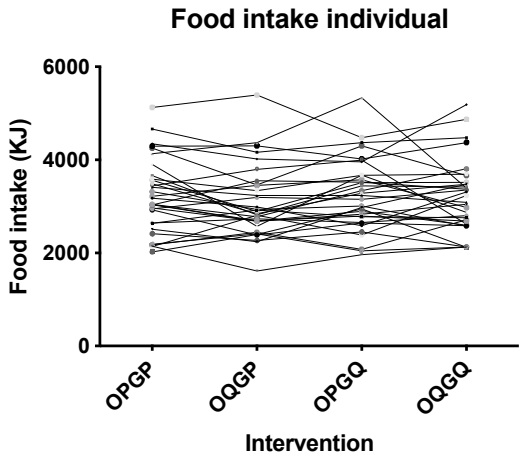

Figure 4.3 **Individual food intake.** Individual representation per subject of amount eaten in KJ (mean + SEM) 50 minutes after oral placebo and intragastric placebo (OPGP), oral quinine and intragastric placebo (OQGP), oral placebo and intragastric quinine (OPGQ), and quinine both oral and intragastric (OQGQ). Based on an autoregressive (1) linear mixed model, no difference in food intake was observed between the conditions ($p=0.069$).

Appetite sensations

The mean VAS scores for desire to eat, hunger, satiation, and fullness are depicted in Figure 4.4. Based on a linear mixed model, significant main effects of intervention were found for desire to eat and hunger scores, indicating an overall intervention effect on these scores. Post hoc pairwise comparisons showed a significant reduction in desire to eat and hunger scores for oral sham feeding with quinine vs. placebo (both $p<0.001$), intragastric delivery of quinine vs. placebo (both $p<0.05$), and oral sham feeding with quinine vs. oral sham feeding with- and intragastric delivery of quinine (both $p<0.01$). No main effects of intervention were found for satiation and fullness scores. No intervention × time interactions were found for any of the VAS scores, thus intervention effects did not differ on specific timepoints.

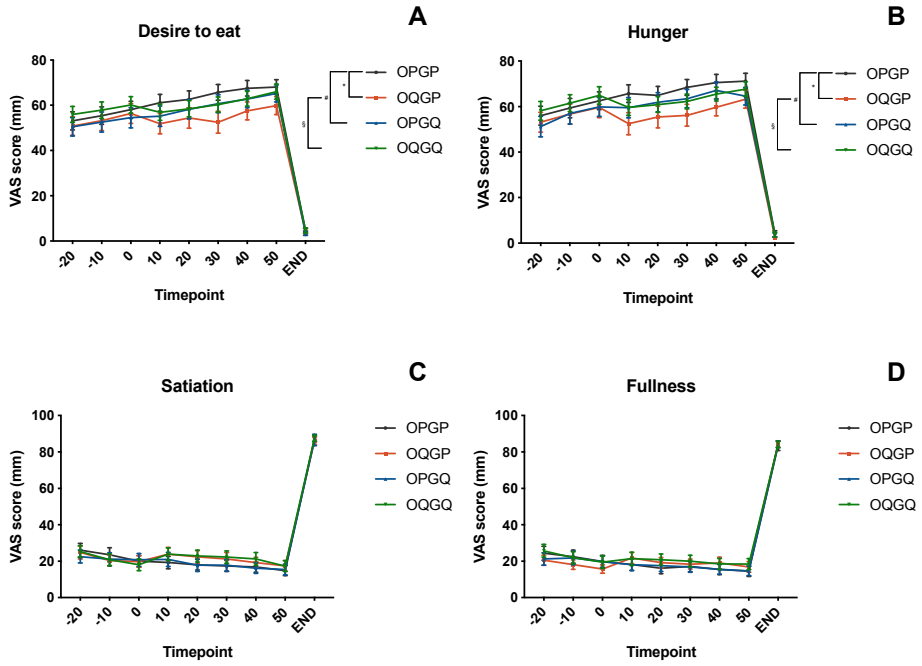

Figure 4.4 **Appetite sensations.** VAS scores for desire to eat (**A**), hunger (**B**), satiation (**C**), and fullness (**D**) (mean + SEM) before and after oral placebo and intragastric placebo (OPGP), oral quinine and intragastric placebo (OQGP), oral placebo and intragastric quinine (OPGQ), and quinine both oral and intragastric (OQGQ). Based on an autoregressive (1) linear mixed model, intervention effects were found for desire to eat and hunger scores ($p<0.001$). Bonferroni correction was applied for post-hoc pairwise comparisons. *<0.001, #<0.05, §<0.01.

Gastrointestinal complaints

The mean VAS scores for stomach pain, bloating, and nausea are depicted in Figure 4.5. No intervention effects for these VAS scores were found. Moreover, no intervention × timepoint interactions were found for these VAS scores.

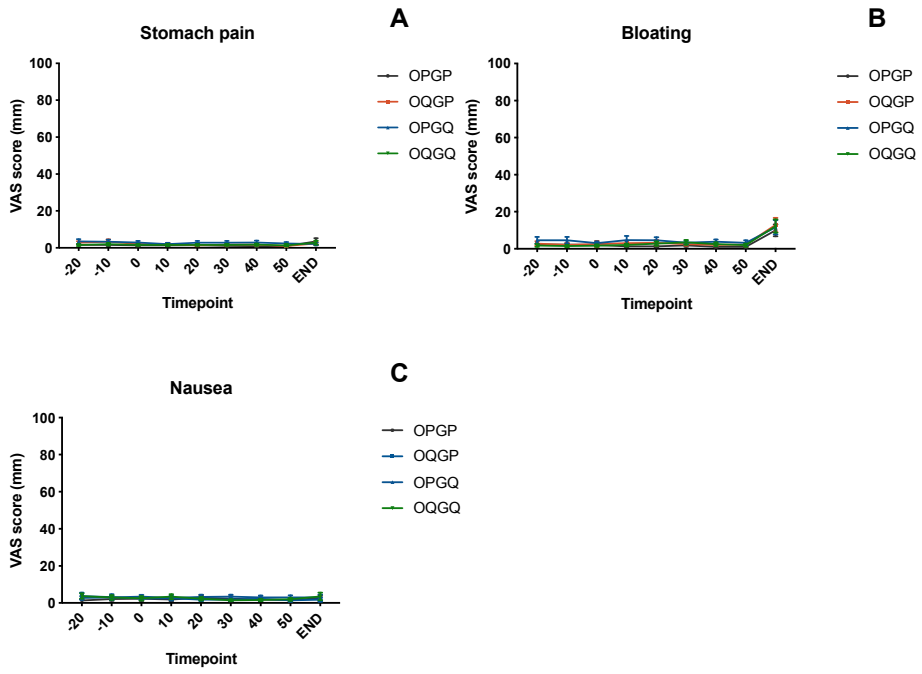

Figure 4.5 **GI complaints.** VAS scores for stomach pain (**A**), bloating (**B**), and nausea (**C**) (mean + SEM) before and after oral placebo and intragastric placebo (OPGP), oral quinine and intragastric placebo (OQGP), oral placebo and intragastric quinine (OPGQ), and quinine both oral and intragastric (OQGQ). Based on an autoregressive (1) linear mixed model, no differences were observed between conditions.

Heart rate variability

Heart rate and LF/HF ratio are depicted in Figure 4.6. No intervention effects for these HRV measurements were found. Moreover, no intervention × timepoint interactions were found for these HRV measurements.

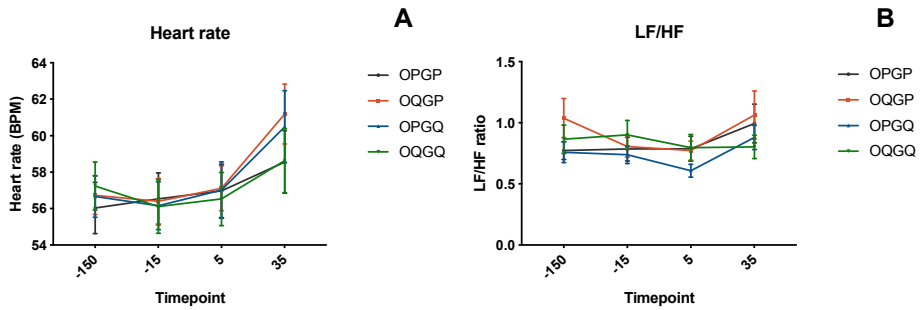

Figure 4.6 **Heart rate variability (HRV).** HRV depicted by heartrate (**A**) and low frequency (LF)/high frequency (HF) ratio (**B**) before and after oral placebo and intragastric placebo (OPGP), oral quinine and intragastric placebo (OQGP), oral placebo and intragastric quinine (OPGQ), and quinine both oral and intragastric (OQGQ). Based on an autoregressive (1) linear mixed model, no differences were observed between conditions.

Discussion

Our results show that food intake is affected neither by oral sham feeding of quinine, nor intragastric delivery of quinine, nor synchronous oral sham feeding and intragastric delivery of quinine compared with placebo. Moreover, no differences have been found in VAS scores for satiation or fullness and HRV measurements. However, VAS scores for desire to eat and hunger decreased significantly after oral sham feeding and intragastric delivery of quinine.

To the best of our knowledge, the effects of oral sham feeding with (bitter) tastants have not been described in literature previously. Moreover, the effects of bitter tastants on eating behaviour have been conducted only in small-sized studies. This is the first study to investigate the acute effects of a bitter tastant on eating behaviour in an adequately sized study.

The results of studies on the effects of bitter tastants on food intake, appetite sensations, and GI peptides are not conclusive. Most studies reported only a trend towards a decrease in food intake after intragastric or intraduodenal delivery of bitter tastants.[16,29-32] Some studies, however, reported a statistically significant reduction in food intake.[33,34] In one study, no initial effect of encapsulated bitter compounds on food intake was observed, while over the day a significant decrease in food intake was found.[35] Deloose *et al.* showed a longer sustained satiety effect after a standardised meal when the meal was preceded by intragastric administration of denatonium benzoate (DB).[29] Taken together, these results

indicate that delivery of bitter tastants in the presence of- or followed by a caloric content, exerts a more pronounced effect on eating behaviour. With respect to our study, it is possible that a more marked decrease in food intake could have been found if food intake was measured over the course of the day. However, our aim was to explore in detail the acute effects of oral sham feeding and/or intragastric delivery of the bitter tastants quinine on caloric intake during the next meal and not over a longer period.

Intragastric administration of bitter compounds (quinine and DB) has been shown to reduce antral motility[29,36] and to decrease systemic motilin and ghrelin levels,[29,34,36] whereas effects of bitter compounds on overall gastric emptying rate, have not been demonstrated in most studies.[29,32,33,37] A recent study did show slowed gastric emptying after both intragastric and intraduodenal administration of quinine.[38] These recent findings are in line with pre-clinical trials in rodents.[39,40] Therefore, the effects of gastrointestinal delivery of tastants on circulating GI peptide levels have not been fully elucidated. Several studies did not observe any changes in glucagon like peptide-1 (GLP-1), polypeptide YY (PYY), and/or cholecystokinin (CCK).[16,30,32] In one study an increase in plasma CCK was observed after intraduodenal delivery of the bitter tastant quinine, but systemic ghrelin levels were not affected.[33]

Where systemic GI peptide levels are considered to be an objective parameter to predict prospective food intake, VAS scores for appetite sensations can give insight in subjective feelings relating to prospective food intake. In the present study, oral sham feeding or intragastric delivery of quinine decreased the desire to eat and hunger sensations compared with placebo. Interestingly, there seems to be an overall trend of an upward slope for appetite throughout the test day. In this study, subjects were provided with the test meal around noon. Therefore, this might reflect the natural tendency to get an increased appetite towards habitual time of meal intake, due to conditioned hunger.[41] Important to note is that synchronous oral sham feeding with- and intragastric delivery of quinine did not alter desire to eat and hunger scores compared with placebo. This rather unexpected finding could be explained by the higher starting point for this intervention, influencing a possible overall intervention effect.

Contrary to desire to eat and hunger sensations, satiation and fullness sensations were not affected. Since there was no intervention effect on actual food intake, this raises the question whether actual food intake might be more dependent on satiation and fullness rather than desire to eat and hunger. In addition, caloric intake might be influenced by a large array of different sensations and factors that are not necessarily captured by a single VAS score. Various studies that did find a

significant decrease in food intake after gastrointestinal delivery of tastants showed mixed results on VAS scores for appetite sensations. Iven *et al.* showed a decrease in hunger and an increase in satiety and fullness.[34] Van Avesaat *et al.* found a decrease in desire to eat and hunger and an increase in satiety, but no effects on fullness.[16] Andreozzi *et al.* found no differences in satiation or desire to eat scores.[33] It must be noted that in the present study no intervention × time interaction was found and therefore conclusions on timing of the effect of oral sham feeding with- and/or intragastric delivery of quinine on appetite sensations cannot be drawn. Moreover, interpretation of VAS scores for appetite sensations should always be done with care, since this usually is a secondary outcome parameter in studies on food intake and most studies might not have been adequately powered for such outcomes – as is the case for our study.

A strength of the design of the present study is the large sample size when compared to other research in the field. Moreover, possible inter-subject confounding factors (*i.e.*, habitual meal size, daily energy requirements) were countered by the cross-over design. However, this study also has several limitations. A limitation of this study is generalisability to daily life. In this study, subjects consumed their test meal without external stimuli (*i.e.*, phone use, watching television) and were asked to stop eating when comfortably full. In daily life people might not be focussed on their appetite sensations and be distracted by said external stimuli, which are known to have an increasing effect on eating behaviour.[42,43] However, before implementing interventions in daily life, their effects should first be investigated under controlled conditions, as was done in the present study. Another important limitation of this study is that systemic GI peptide levels were not assessed. Measurement of GI peptides could have provided a more complete overview of the effect of oral sham feeding with- and/or intragastric delivery of quinine on eating behaviour. However, a previous study from our group showed a decrease in food intake and increase in satiation without marked changes in systemic GI peptide levels.[16] Although *in vitro* stimulation of intestinal cell lines with tastants results in markedly increased levels of GI peptides, these results have not always been reproduced *in vivo*, as stated above. It should be noted that all studies measured plasma levels of GI peptides, in search for systemic effects. It remains to be determined what the effect of gastrointestinal delivery of tastants is on local splanchnic excretion of GI peptides or on possible paracrine effects. Measurement of local levels of GI peptides requires complex invasive techniques. We focussed on other, non-invasive, markers that can indicate a shift in hunger/satiation, such as the balance in ANS,

which can occur before systemic GI peptide changes can be detected[19,20] and used HRV measurements.[23]

In the present study, no differences in HRV measurements were observed between the various interventions, which is in line with the fact that there was no shift in hunger/satiation, as indicated by the VAS scores for fullness and satiation. Moreover, food intake was not influenced by the various interventions. A recent meta-analysis showed that HRV is sensitive to changes in the autonomic nervous system as a response to stress.[44] Although two studies showed meal intake to influence HRV,[45,46] it is uncertain whether HRV measurements are sensitive enough to detect satiation responses. Therefore, future studies should ascertain whether the application of HRV measurements in detecting satiation responses has an added value.

Furthermore, it has to be noted that the ideal interval between tastant delivery and the timing of the *ad libitum* test meal is unknown. In the present study, we employed a timing identical to that of studies from other groups investigating the effects of bitter compounds on food intake.[29,30,33] However, these studies investigated only intragastric or intraduodenal effects of bitter tastants. It is possible that effects of oral sham feeding with quinine could have been found if the test meal was offered sooner after the intervention, since tasting a substance orally might have more acute effects. Moreover, our results suggest a fast response on desire to eat and hunger scores after intervention which could have driven the overall effect that was found. It is possible that earlier presentation of the test meal would have resulted in a reduction in energy intake after administration of quinine. On the other hand, the possibility of an increase in energy intake, due to the need to quell their symptoms (*i.e.*, get rid of the bad taste of quinine), cannot be excluded. Future research protocols should consider timing as a factor to be investigated.

Another important aspect to mention is the dosage of quinine. According to the code of federal regulations stated by the United States Food and Drug Administration (U.S. FDA), soft drinks such as tonic water are allowed to contain 83 parts per million quinine.[47] In this protocol we chose to administer 75 mg of QHCl which is lower than this amount and aligns with previous studies conducted by our group.[16,17] Due to this relatively low dosage of quinine, should we have found significant effects on eating behaviour, this dosage of quinine could have been easily implemented for daily intake. Other studies employ various dosages of quinine administration ranging from 18 mg to 600 mg.[29,30,32-34,36]. The higher dosages may give rise to medical problems when implemented for daily intake. A systematic review on the usage of quinine as treatment for muscle cramps showed

an increase in minor adverse events such as GI complaints, headache, and tinnitus after daily intake of 200 mg to 500 mg for three days up to several weeks.[48] This aspect should also be considered when investigating the effects on eating behaviour of higher dosages of quinine.

Up to now, most studies investigating the effects of tastants on food intake focussed on acute effects in single test meals. Repetitive or chronic administration of tastants has not been investigated, warranting studies to be conducted on long-term effects of tastants on energy intake.

In conclusion, we have shown that oral sham feeding with- or intragastric delivery of the bitter tastant quinine decreased desire to eat and hunger feelings, without affecting food intake, feelings of satiation and fullness, and HRV when compared with placebo.

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Obesity
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Dyspepsia
Anorexia

Chapter 5

Effect of intragastric lidocaine on ad libitum food intake and satiation in healthy male volunteers: a double-blind randomized crossover study

EMBARGOED

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Chapter 6

Psychometric evaluation of an experience sampling method-based patient-reported outcome measure in functional dyspepsia

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Abstract

Background

Due to important biases, conventional end-of-day and end-of-week assessment methods of gastrointestinal symptoms in functional dyspepsia (FD) are considered suboptimal. Real-time symptom assessment based on the experience sampling method (ESM) could be a more accurate measurement method. The present study aimed to evaluate validity and reliability of an ESM-based patient-reported outcome measure (PROM) for symptom assessment in FD.

Methods

Thirty-five patients with FD (25 female, mean age 44.7 years) completed the ESM-based PROM (a maximum of 10 random moments per day) and an end-of-day symptom diary for 7 consecutive days. On day 7, end-of-week questionnaires were completed including the Nepean Dyspepsia Index (NDI) and Patient Assessment of Gastrointestinal Symptom Severity Index (PAGI-SYM).

Key results

ESM- and corresponding end-of-day scores for gastrointestinal symptoms were significantly associated (ICCs ranging from 0.770-0.917). However, end-of-day scores were significantly higher (Δ0.329-1.031) than mean ESM scores ($p<0.05$). Comparing ESM with NDI and PAGI-SYM scores, correlations were weaker (Pearson's r ranging from 0.467-0.846). Cronbach's α coefficient was good for upper gastrointestinal symptoms ($\alpha=0.842$). First half-week and second half-week scores showed very good consistency (ICCs ranging from 0.913-0.975).

Conclusion & inferences

The present results demonstrate good validity and reliability of a novel ESM-based PROM for assessing gastrointestinal symptoms in patients with FD. Moreover, this novel PROM allows to evaluate individual symptom patterns and can evaluate interactions between symptoms and environmental/contextual factors. ESM has the potential to increase patients' disease insight, provide tools for self-management, and improve shared decision-making. Hence, this novel tool may aid in the transition towards personalised healthcare for patients with FD.

Introduction

Functional dyspepsia (FD) is one of the most common functional gastrointestinal disorders, recently renamed in disorders of gut-brain interaction, with an estimated prevalence of 10-15% in the general population.[1] According to the Rome IV criteria for functional gastroduodenal disorders, it is defined by the presence of various symptoms in the absence of organic, systemic, or metabolic diseases that could explain complaints.[2] Among the heterogeneous presentation of patients with FD, four core symptoms have been defined: early satiation, postprandial fullness, epigastric burning, and epigastric pain.[2,3] Quality of life and work productivity are impaired in patients with FD.[4] Moreover, up to 40% of patients will consult a physician, having substantial financial implications.[5] The diagnosis of FD largely relies on symptoms, since underlying pathophysiologic mechanisms remain unclear and specific biological markers are currently lacking. Hence, symptom assessment is warranted to evaluate treatment efficacy. The Food and Drug Administration (FDA) recommends the use of well-defined patient-reported outcome measures (PROMs) for evaluation of treatment outcomes in clinical trials.[6] A recent systematic review identified 20 PROMs for assessment of dyspeptic symptoms. However, no single instrument has undergone all the development steps recommended by the FDA. Therefore, no consensus has yet been reached with regard to the most relevant outcome measure in patients with FD.[7]

The currently used assessment methods to evaluate dyspeptic symptoms and response to treatment are mainly retrospective, self-reported questionnaires, based on daily or weekly monitoring. This has important limitations. Firstly, retrospective questionnaires are prone to recall bias.[8] Secondly, symptom variability in functional disorders can occur due to external triggers, such as intake of food or psychological factors,[9-11] which cannot be accurately captured by retrospective assessments, thereby resulting in ecological bias. Thirdly, lack of patient adherence or fake adherence are common problems that arise with the use of paper questionnaires.[12] These limitations underline the need for a reliable method for symptom assessment to evaluate treatment efficacy in patients with FD.

The experience sampling method (ESM) might overcome these limitations. ESM is characterised by random, repeated assessments in a patient's current state and environment. Assessment has to be completed within a short time after an auditory signal and questions always relate to current symptoms, contextual factors, and psychological factors. Therefore, ESM might be able to reduce the risk of recall and ecological bias and capture symptom variability over time.[13,14] Current use of ESM in gastrointestinal disorders is limited. Several studies evaluated the use of ESM in patients with irritable

bowel syndrome (IBS) and found good correlation between symptom scores on ESM and retrospective questionnaires. However, abdominal pain scores were significantly higher in retrospective questionnaires compared with mean scores derived from ESM. Interestingly, the scores for abdominal pain on retrospective questionnaires seemed to represent the peak scores measured by ESM.[15-17] Currently, there are no data available on the use of ESM in patients with FD. Recently an ESM-based PROM was developed for patients with FD.[18] Therefore, the present study aimed to assess the validity and reliability of this novel FD-specific ESM-based PROM.

Methods

The study protocol was approved by the Medical Ethics Committee of the Maastricht University Medical Centre+ (MUMC+), Maastricht, the Netherlands (ID METC19-077), and performed in full accordance with the Declaration of Helsinki (latest amendment by the World Medic Association in 2013) and Dutch Regulations of Medical Research involving Human Subjects (WMO, 1998). This prospective observational study was performed at the MUMC+ from 29th May 2020 until 1st October 2020. This study was registered in the US National Library of Medicine (http://www.clinicaltrials.gov, ID NCT04204421).

Subjects

Recruitment of patients with FD, aged between 18 and 75 years, took place at the outpatient clinic of Gastroenterology and Hepatology of the MUMC+, a secondary/tertiary academic hospital. Additionally, patients with FD that participated in other studies of the MUMC+ were contacted to participate in the current study (NCT02522000, NCT03652571). Functional dyspepsia, including subtype assessment, was diagnosed according to the Rome IV criteria, which were evaluated by a trained clinical researcher in a face-to-face interview. For all subgroups of FD, subjects needed to have symptoms for at least 6 months. As per Rome IV definitions, in order to fulfil criteria for postprandial distress syndrome (PDS), subjects needed to experience either 1) an uncomfortably full feeling after meal of a normal portion size for at least 3 days or more per week in the past 3 months that restricted their normal activities, or 2) have an uncomfortable feeling of early satiation that resulted in inability to finish a normal sized meal for at least 3 days or more per week in the past 3 months. In order to fulfil criteria for epigastric pain syndrome (EPS), subjects needed to experience either 1) epigastric pain or 2) burning for at least 1-2 days per week that restricted their normal activities. In order

to fulfil criteria for overlap syndrome (OS), subjects needed to fulfil both PDS and EPS criteria. Apart from the Rome IV criteria, no specific minimum symptom frequency/intensity on a weekly basis was used as an entry criterion. Exclusion criteria were the initiation of regularly used medication from 1 month before inclusion until the end of the study period, a history of upper gastrointestinal surgery, history of radiation therapy to the abdomen, and pregnancy. Subjects could only participate if they understood the Dutch language and were able to use the smartphone application. Moreover, as studies in the general population show patients fulfilling criteria for both FD and IBS are common,[19-23] fulfilling criteria for IBS was not deemed an exclusion criterion, as to adequately reflect FD patients in the general population.

Data collection

ESM and an end-of-day symptom diary were collected during seven consecutive days. On day 7, subjects completed validated symptom questionnaires using an electronic case report form (eCRF) system (CastorEDC).

ESM

The MEASuRE-D application was developed for the use of ESM in patients with FD.[18] Figure 6.1 displays the home screen of the application. The subjects downloaded this app on their smartphones. During their regular daily life, subjects completed ESM for seven consecutive days. In order to complete the real-time questionnaires as often as possible, subjects were instructed to carry their smartphone with them during the week. The MEASuRE-D app sent out a haptic, auditory, and written signal 10 times per day between 07:00 and 22:00 at randomly chosen moments, with a time-interval of at least 15 minutes between consecutive signals. Following a signal, the ESM-questionnaire (called "Beep vragenlijst", Figure 6.1) was available for 10 minutes. On all measurement moments, the questions were repeated in the same order, and scored on an 11-point numeric rating scale (NRS) (0=not at all to 10=very severely). The development of this ESM-based questionnaire has been described previously.[18]

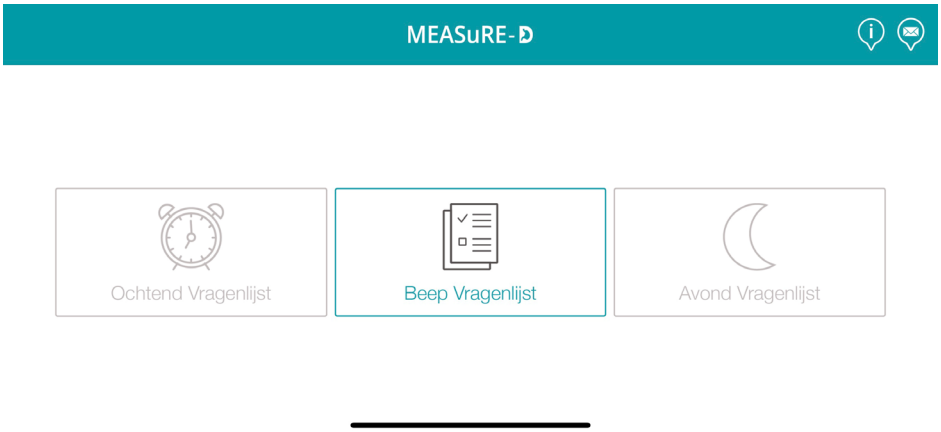

Figure 6.1 Home screen of the MEASuRE-D smartphone application.

End-of-day diary

A 7-day end-of-day symptom diary was used to evaluate symptom severity on a daily basis. Gastrointestinal symptoms (*i.e.,* upper abdominal fullness, upper abdominal heaviness, bloating, upper abdominal pain, upper abdominal burning sensation, lower abdominal pain, nausea, belching, heartburn, regurgitation, ability to eat normal portion sizes, vomiting, as well as urge to defecate) were scored using an 11-point NRS (0=not at all to 10=very severely) at the end of each test day. This symptom diary was built into the MEASuRE-D app and made available between 19:00 and 0:00. Subjects were instructed to manually open the application to fulfil this diary, as no signal was sent to the smartphone to indicate availability of this questionnaire. In the app this list was called "Avondvragenlijst" (Figure 6.1).

End-of-week questionnaires

At the end of the study period, validated questionnaires were completed using an eCRF, assessing upper gastrointestinal symptoms and mental health status. Regarding upper gastrointestinal symptom severity, the Nepean Dyspepsia Index (NDI; 0-4 scale for occurrence of core complaints, 0-5 scale for severity of core complaints, 0-4 scale for hinderance due to core complaints, recall period 14 days)[24-26] and Patient Assessment of Gastrointestinal Symptom Severity Index (PAGI-SYM; 1-6 scale; composes sub scores for postprandial fullness, nausea/vomiting, bloating, upper abdominal pain, lower abdominal pain, heartburn/regurgitation, recall period 14 days)[27] were completed.

The Generalised Anxiety Disorder-7 (GAD-7; 0-3 scale; total composite score for severity of anxiety symptoms; recall period of 14 days),[28] the Hospital Anxiety and Depression Scale (HADS; 0-3 scale, total composite scores for severity of anxiety and depression, recall period one week),[29] and the Patient Health Questionnaire-9 (PHQ-9; 0-3 scale; total composite score for severity of depressive symptoms, recall period 14 days)[30] were collected regarding anxiety and depression.

Statistical analyses

Sample size was based on previous studies using ESM-data that have shown sample sizes between 20 and 30 subjects to be sufficient for analyses.[31,32] Moreover, in IBS patients sample sizes of 26-37 were used to evaluate a novel ESM-based PROM.[16,33,34] The present study was an exploratory study on the use of an ESM-based PROM in patients with FD. Therefore, we aimed to include at least 30 valid cases with a maximum of 36. Subjects were included in the analyses only when at least 1/3 of the total number of ESM-assessments (*i.e.*, 23 out of 70) were completed.[35,36]

All analyses were performed using R version 3.6.3. Continuous outcomes are presented as mean ± standard deviation (SD), and tested using paired or independent samples t-test. Proportions for categorical variables were tested using the χ^2-test. For all analyses, $p<0.05$ was considered statistically significant.

ESM scores were compared with end-of-day diary scores and with end-of-week questionnaire scores to assess concurrent validity. In order to compare ESM scores with end-of-day diary scores, mean and maximum scores for ESM were calculated for each of the 7 days. Associations between ESM scores and end-of-day scores were tested using a linear mixed-effects model with end-of-day score as the dependent and ESM score as the independent variable, a random intercept, and correcting for repeated measures by using an autoregression (AR1) correlation structure. The level of agreement between these scores was assessed by calculating intra-class correlation coefficients (ICC), based on a single-rating, consistency, two-way model. Additionally, intercept-only linear mixed-effects models with the delta scores (*i.e.,* difference between ESM and end-of-day diary) as the dependent variable were used to assess differences between assessment methods.

In order to compare ESM scores with end-of-week questionnaire scores, average ESM scores were calculated per subject. Paired samples t-test and Pearson correlations were calculated to assess the differences between measurement methods. A Pearson r above 0.7 reflects a strong correlation, a Pearson r of 0.50-0.70 reflects a good correlation, a Pearson

r between 0.3 and 0.5 reflects a moderate correlation, and a Pearson r below 0.30 reflects a poor correlation.[37] For the PAGI-SYM questionnaire the sub scores for postprandial fullness, nausea/vomiting, bloating, upper abdominal pain, lower abdominal pain, and heartburn/regurgitation were used. Corresponding ESM scores that were used were fullness, nausea, bloating, upper abdominal pain, lower abdominal pain, and heartburn, respectively. For comparison between ESM scores and NDI or PAGI-SYM, end-of-week scores were rescaled to an 11-point scale by multiplying the NDI and PAGI-SYM scores by $\frac{11}{6}$.

Reliability was assessed with the internal consistency and test-retest reliability. For assessment of internal consistency with the Cronbach's alpha coefficient, the ESM-PROM items were divided into five domains (*i.e.*, upper gastrointestinal symptoms, lower gastrointestinal symptoms, physical non-GI symptoms, mental positive affect, and mental negative affect). Good internal consistency is reflected by a Cronbach's alpha of 0.7-0.9.[38]

For assessment of test-retest reliability, we assumed that ESM scores during the first half of the study period (*i.e.,* days 1, 2, and 3) would show moderate to good consistency with ESM scores during the second half of the study period (*i.e.,* day 5, 6, and 7). For each symptom, mean scores were calculated per subject for these two time periods. A paired samples t-test was performed to test the differences between these study periods in order to exclude a time effect. Agreement was assessed by calculating an ICC between the time periods. For this, a two-way model based on average-measures and absolute agreement was used. ICC values above 0.75 are considered good, whereas ICC values between 0.5 and 0.75 are considered moderate.[39]

Results

Subjects

In total, 36 patients with FD met the inclusion criteria. One patient did not complete at least 1/3 of the total number of ESM-assessments. Therefore, 35 patients (25 female (71.4%), age 44.7 (SD 15.7) years, GAD-7 4.8 (SD 4.5), HADS-Anxiety 5.3 (SD 4.5), HADS-Depression 4.9 (SD 4.3)) were included in the analyses. Ten patients fulfilled the criteria for EPS (28.6%), seven for PDS (20%), and 18 for OS (51.4%). Of the 35 included patients, 13 had comorbid IBS (37%, one in the EPS group, three in the PDS group, and nine in the overlap group). During the study, no adverse events were reported by the subjects.

Compliance

The completion rate of ESM assessments was 62.2%. Over the 7-day period, a mean number of 43.5 measurements was completed per individual (range: 23-68). The majority of subjects completed between 31 and 60 assessments during the study period (Figure 6.2).

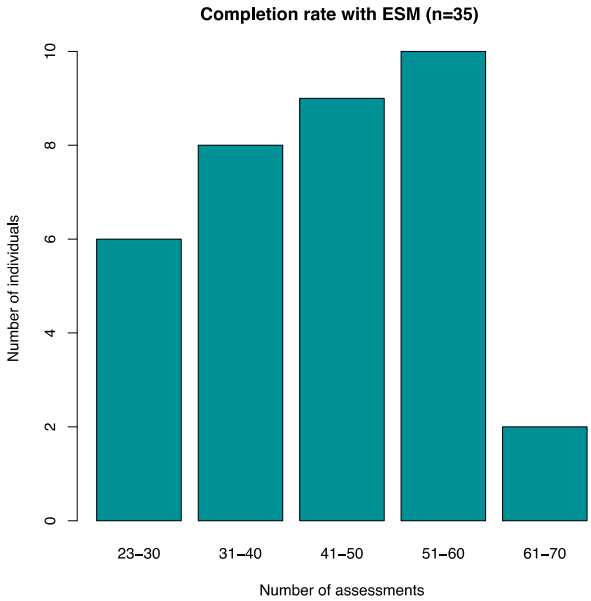

Figure 6.2 Number of individuals (y-axis) per category of completed number of assessments (x-axis).

Concurrent validity

ESM scores compared with end-of-day diary scores

Both ESM and the end-of-day diary scored the following gastrointestinal symptoms: upper abdominal fullness, upper abdominal heaviness, bloating, upper abdominal pain, upper abdominal burning sensation, lower abdominal pain, nausea, belching, heartburn, and regurgitation.

Mean scores on ESM and end-of-day scores were all significantly associated, which indicates that both assessment methods measure the same construct (Table 6.1). Furthermore, ICCs between ESM scores and end-of-day diary scores were all above 0.75, indicating good agreement between these assessment methods (Table 6.1). However, symptom scores were all significantly higher in end-of-day diaries compared with mean ESM-scores (Table 6.2). Furthermore, symptom scores were all significantly lower in end-

of-day diaries compared with maximum ESM-scores. Therefore, end-of-day diary scores were placed in between the mean and maximum ESM scores for all gastrointestinal symptoms. This concept is illustrated in Figure 6.3 for fullness scores.

These differences between end-of-day scores and both mean and maximum ESM scores can be made insightful for individual patients. Figure 6.4 depicts the fullness scores on the end-of-day diary and the ESM for one individual patient. A highly fluctuating pattern for fullness is observed during the 7-day study period with ESM: the patient reported multiple time-points without fullness (*i.e.*, score 0) or with low feelings of fullness (*i.e.*, scores below 5) and only a few time-points with higher symptom scores (*i.e.*, above 5). Instead of real-time symptom assessment, end-of-day diary scores reflect scores of the entire day. For this individual, this resulted in end-of-day fullness scores higher than 5 for 6 out of 7 days. Figure 6.4 highlights the discrepancy between assessment methods.

Table 6.1 Associations and intraclass correlation coefficients between mean ESM scores and end-of-day diary scores.

Symptom	Associations		Intraclass correlations	
	Estimate	SE	ICC	95%-CI
Fullness	0.952 ***	0.084	0.790	0.729 – 0.839
Heaviness	1.025 ***	0.060	0.803	0.745 – 0.849
Bloating	1.092 ***	0.054	0.865	0.824 – 0.898
Upper abdominal pain	0.953 ***	0.062	0.833	0.783 – 0.873
Upper abdominal burning	0.735 ***	0.069	0.885	0.849 – 0.913
Lower abdominal pain	1.103 ***	0.078	0.770	0.703 – 0.823
Nausea	1.147 ***	0.052	0.872	0.832 – 0.903
Belching	1.045 ***	0.061	0.861	0.818 – 0.894
Heartburn	1.107 ***	0.043	0.917	0.891 – 0.938
Regurgitation	1.154 ***	0.078	0.771	0.704 – 0.824

Mixed linear models with end-of-day diary score as dependent variable and ESM mean scores as independent variable, corrected for repeated measures (AR1 covariate structure) were used to test significance. Strength and direction of the association is depicted by estimate. SE: Standard Error; ICC Intraclass Correlation Coefficient. ***p<0.001.

Table 6.2 Difference between ESM mean and maximum scores and end-of-day diary scores.

Symptom	ESM mean vs. end-of-day		ESM maximum vs. end-of day	
	Difference	SE	Difference	SE
Fullness	1.031 ***	0.191	-1.001 ***	0.233
Heaviness	0.875 ***	0.127	-1.133 ***	0.164
Bloating	0.793 ***	0.133	-0.991 ***	0.101
Upper abdominal pain	0.563 ***	0.148	-0.712 ***	0.132
Upper abdominal burning	0.426 **	0.144	-0.484 ***	0.124
Lower abdominal pain	0.562 ***	0.151	-0.459 ***	0.120
Nausea	0.524 ***	0.128	-0.597 ***	0.113
Belching	0.677 ***	0.150	-0.387 ***	0.115
Heartburn	0.459 ***	0.125	-0.398 ***	0.103
Regurgitation	0.329 *	0.131	-0.359 ***	0.080

A positive difference indicates a higher score, and a negative difference indicates a lower score in end-of-day diary compared with ESM. Mixed linear models with the delta score (*i.e.,* difference between ESM mean/max score and end-of-day diary score) as the dependent variable, corrected for repeated measures (AR1 covariate structure) were used to test significance. Strength and direction of the association is depicted by estimate. SE: Standard Error; ICC Intraclass Correlation Coefficient. *p<0.05; **p<0.01; ***p<0.001.

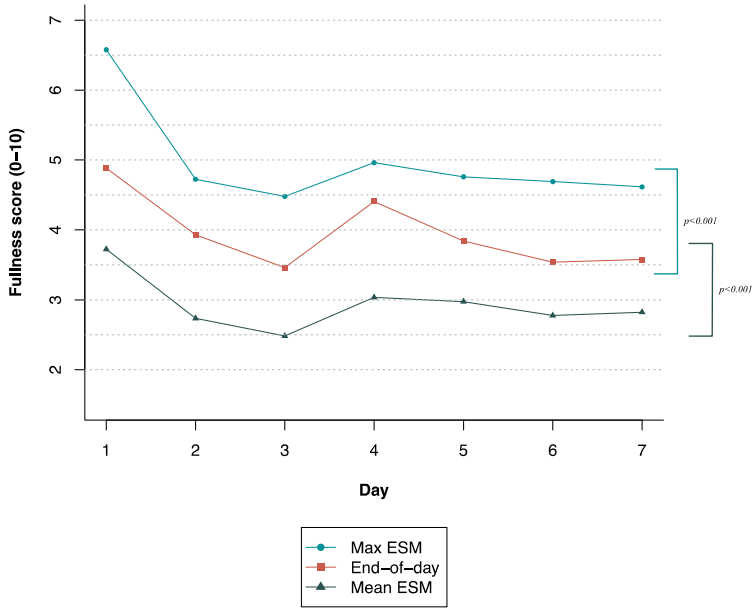

Figure 6.3 Fullness scores. Daily fullness scores based on end-of-day diary scores and mean and maximum ESM scores (11-points NRS). Mixed linear models with the delta score (*i.e.,* difference between ESM mean/max score and end-of-day diary score) as the dependent variable, corrected for repeated measures (AR1 covariate structure) were used to test significance.

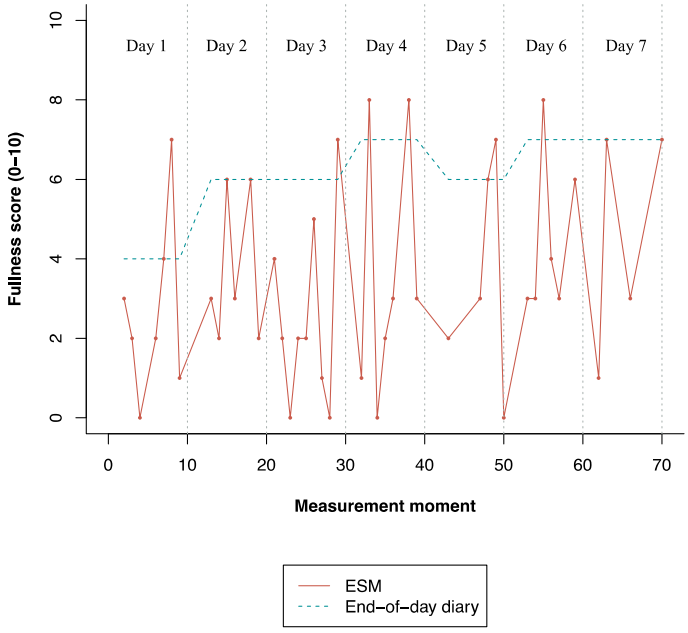

Figure 6.4 **Individual fullness scores.** Fullness scores (on an 11-point NRS) for one FD patient on ESM and end-of-day diary over the seven-day study period. Each day, at maximum 10 assessments were available for ESM. One assessment was completed electronically at the end of each day.

ESM scores compared with end-of-week scores

The comparison and correlation between ESM scores and end-of-week scores are depicted in Table 6.3. ESM scores for postprandial fullness, nausea, bloating, upper abdominal pain, lower abdominal pain, heartburn, upper abdominal burning, and belching were all lower compared with end-of-week scores on the NDI and PAGI-SYM. Scores for upper abdominal burning, heartburn, bloating, and belching on the NDI were strongly correlated with ESM scores. Scores for upper abdominal pain, fullness, and nausea on the NDI showed good correlation with ESM scores. Reflux scores on the NDI showed a moderate correlation with ESM scores. Bloating, upper abdominal pain, and heartburn/regurgitation sub scores on the PAGI-SYM were strongly correlated with ESM scores. All other PAGI-SYM sub scores showed good correlation with ESM scores.

In addition to gastrointestinal symptoms, psychological factors were assessed with ESM. Several psychological factors corresponded with the PHQ-9 or GAD-7, namely "feeling down", "feeling anxious", "feeling worried", "feeling irritated", and "feeling relaxed". Answering scales were substantially different and, therefore, did not allow for

harmonisation of the scores. Hence, no mean scores could be compared between assessment methods. However, correlations between the two assessment methods could be calculated. Strong correlations between ESM and end-of week scores were found for "feeling down", "feeling worried", "feeling irritated", and "feeling relaxed". Good correlations were found for "feeling anxious".

Table 6.3 ESM scores compared with end-of-week scores. Mean scores based on subject-level.

NDI vs. ESM	**ESM score** mean ± SD	**NDI score** mean ± SD	**Pearson correlation**
Upper abdominal pain	1.88 ± 2.05	3.72 ± 2.29 ***	0.617
Upper abdominal burning	1.37 ± 2.01	2.10 ± 2.56 **	0.846
Heartburn	1.35 ± 1.96	2.51 ± 2.71 ***	0.737
Reflux	0.58 ± 1.05	1.94 ± 2.39 ***	0.465
Fullness	2.88 ± 1.98	4.66 ± 2.24 ***	0.574
Bloating	2.86 ± 1.95	4.03 ± 2.84 ***	0.838
Nausea	1.35 ± 1.91	3.25 ± 2.85 ***	0.556
Belching	1.23 ± 1.63	2.67 ± 2.79 ***	0.779
PAGI-SYM vs. ESM		**PAGI-SYM subscale score** mean ± SD	
Postprandial fullness	2.88 ± 1.98	3.89 ± 2.06 **	0.636
Nausea/vomiting	1.35 ± 1.91	1.64 ± 2.05	0.556
Bloating	2.86 ± 1.95	4.64 ± 2.89 ***	0.728
Upper abdominal pain	1.81 ± 2.05	3.75 ± 2.44 ***	0.804
Lower abdominal pain	1.16 ± 1.56	2.20 ± 2.29 ***	0.680
Heartburn/regurgitation	1.35 ± 1.96	1.92 ± 1.87 **	0.802
PHQ-9/GAD-7 vs. ESM			
Down			0.828
Anxious			0.586
Worried			0.865
Irritated			0.724
Relaxed			0.836

NDI vs. ESM: NDI scores transformed from 6-point scale to 11-point scale by multiplying with $\frac{11}{6}$. PAGI-SYM sub scores vs. ESM: PAGI-SYM scores transformed from 6-point scale to 11-point scale by multiplying with $\frac{11}{6}$. Corresponding ESM scores tested with PAGI-SYM scores: Early satiety and Fullness, Nausea/vomiting and Nausea, Bloating and Bloating, Upper abdominal pain and Upper abdominal pain, Lower abdominal pain and Lower abdominal pain, Heartburn/regurgitation and Heartburn. PHQ-9/GAD-7 vs. ESM: answering scales do not allow comparison between mean scores. Paired samples t-test was used to test for differences. **p<0.01; ***p<0.001.

Internal consistency

Internal consistency of the ESM-PROM for FD was determined by categorising the items in five constructs, namely upper gastrointestinal symptoms, lower gastrointestinal symptoms, physical non-gastrointestinal symptoms, positive affect, and negative affect. Table 6.4 lists the Cronbach's α coefficients for these constructs. Very good internal consistency was found for upper gastrointestinal symptoms and negative affect. Good

internal consistency was found for physical non-gastrointestinal symptoms. An acceptable internal consistency was found for positive affect. Consistency was relatively low for lower gastrointestinal symptoms, indicating that these two symptoms might not perfectly reflect the same construct for lower gastrointestinal symptoms.

Table 6.4 Internal consistency of five ESM-PROM symptom domains, reflected by Cronbach's α coefficient.

Symptoms	Cronbach's α
Upper gastrointestinal symptoms	0.842
Fullness	
Upper abdominal heaviness	
Upper abdominal pain	
Upper abdominal burning	
Nausea	
Vomiting	
Belching	
Heartburn	
Regurgitation	
Lower gastrointestinal symptoms	0.483
Bloating	
Lower abdominal pain	
Physical – non-gastrointestinal	0.786
Palpitations	
Sweating	
Dyspnoea	
Dizziness	
Mental – Positive affect	0.584
Good	
Relaxed	
Mental – Negative affect	0.917
Down	
Anxious	
Irritated	
Stressed	
Worried	

Test-retest reliability

Mean ESM scores for the first (*i.e.*, day 1, 2, and 3) and second (*i.e.*, day 5, 6, and 7) half week of the study period are depicted in Table 6.5. Only the scores for "feeling down", "feeling anxious", "feeling irritated", "feeling stressed", and "feeling worried" differed, with all scores lower in the second half-week. All other scores did not differ between the first half-week and the second half-week. All symptoms showed good consistency between the measurements, as reflected by the ICCs. These findings suggest sufficient test-retest reliability.

Table 6.5 **Mean scores for ESM-reported gastrointestinal symptoms, non-gastrointestinal physical symptoms, and mental status in the first half-week and second-half week of the study period. Agreement between first and second half-week is reflected by the ICC.**

	First half-week Mean score ± SD	Second half-week Mean score ± SD	ICC [95%-CI]
Gastrointestinal symptoms			
Fullness	2.94 ± 1.93	2.79 ± 2.08	0.913 [0.828 – 0.956]
Upper abdominal heaviness	2.58 ± 1.72	2.48 ± 1.91	0.935 [0.871 – 0.967]
Bloating	2.99 ± 1.86	2.74 ± 2.13	0.934 [0.869 – 0.966]
Upper abdominal pain	1.74 ± 1.89	1.86 ± 2.22	0.956 [0.913 – 0.978]
Upper abdominal burning	1.40 ± 1.90	1.36 ± 2.11	0.957 [0.915 – 0.978]
Lower abdominal pain	1.24 ± 1.57	1.13 ± 1.65	0.947 [0.896 – 0.973]
Nausea	1.43 ± 2.00	1.28 ± 1.82	0.970 [0.940 – 0.985]
Belching	1.27 ± 1.67	1.24 ± 1.77	0.942 [0.884 – 0.971]
Heartburn	1.30 ± 1.82	1.41 ± 2.16	0.915 [0.832 – 0.957]
Non-gastrointestinal physical symptoms			
Palpitations	0.68 ± 1.33	0.67 ± 1.52	0.953 [0.907 – 0.976]
Sweating	1.50 ± 2.19	1.37 ± 2.27	0.958 [0.918 – 0.979]
Dyspnoea	1.20 ± 1.96	1.35 ± 2.12	0.975 [0.950 – 0.987]
Dizziness	0.65 ± 1.34	0.67 ± 1.47	0.955 [0.910 – 0.977]
Pressure on chest	0.89 ± 1.62	1.06 ± 1.95	0.955[0.910 – 0.977]
Tired	4.77 ± 2.36	5.03 ± 2.35	0.966 [0.930 – 0.983]
Mental status			
Good	6.11 ± 1.62	6.32 ± 1.50	0.931 [0.862 – 0.965]
Down	1.41 ± 1.85	0.98 ± 1.67 **	0.929 [0.822 – 0.968]
Anxious	0.85 ± 1.58	0.48 ± 1.44 **	0.914 [0.804 – 0.959]
Irritated	1.32 ± 1.90	1.02 ± 1.80 *	0.963 [0.914 – 0.982]
Stressed	2.26 ± 2.27	1.79 ± 2.33 *	0.938 [0.858 – 0.971]
Relaxed	6.15 ± 1.80	6.22 ± 1.83	0.929 [0.859 – 0.964]
Worried	2.07 ± 2.61	1.66 ± 2.54 *	0.941 [0.878 – 0.971]

First half-week reflects day 1, 2, and 3. Second half-week reflects day 5, 6, and 7. Paired samples t-test was used to test for significance. SD: standard deviation, ICC: intraclass correlation coefficient. *p<0.05, **p<0.01.

Discussion

The present study evaluated the validity and reliability of an ESM-based PROM for symptom assessment in patients with FD. The development of this tool has been previously described.[18] This study demonstrated significant associations between ESM scores for gastrointestinal symptoms and end-of-day scores, as well as moderate to strong correlations between ESM scores and end-of-week scores, confirming concurrent validity. Besides validity, reliability was considered adequate based on moderate-to-good internal consistency and excellent test-retest reliability.

Prior to the implementation of a novel PROM in patient care or clinical trials, adequate validity and reliability have to be demonstrated. Throughout the years, a plethora of

statistical methods to test psychometric properties of novel PROMs have been described.[6,38,40] In this study, measures of validity and reliability that are most applicable to the novel ESM-based PROM are used. Regarding concurrent validity, significant associations for all mean ESM and end-of-day scores for gastrointestinal symptoms were found. Given the good agreement between these assessment methods, it can be stated that the ESM-PROM and end-of-day diary measure similar constructs regarding gastrointestinal symptoms. Interestingly, in this study, no peak symptom score in end-of-day reporting was shown, contrary to studies performed in IBS patients.[15,16] End-of-day diary symptom scores tended to be in between the mean and maximum ESM scores. This points towards over-reporting of gastrointestinal complaints when subjects need to provide one score over the entire day. The difference between mean ESM scores and end-of-week scores was even more pronounced. This indicates that subjects tend to remember the moments that they were aware of complaints and neglect the moments without complaints when providing scores over a longer period of time, emphasising the usefulness of ESM in generating accurate individual symptom patterns.

This study demonstrated very good internal consistency for upper gastrointestinal symptoms, whereas a poor consistency was found for lower gastrointestinal symptoms. This suggests that the two items chosen to represent lower gastrointestinal symptoms (*i.e.*, bloating and lower abdominal pain) should possibly be considered to reflect different constructs. It should be noted that the items were selected based on focus group interviews with FD patients. A previous study developed an ESM-based PROM for IBS patients that included more items reflecting lower gastrointestinal symptoms.[33] It could have been possible to combine both PROMs. However, this would have increased the number of total symptom items substantially and, therefore, the patient's burden. Instead, we decided to focus on the symptoms deemed essential by patients with FD, as reflected by the item selection in the focus groups. However, internal consistency might have been improved by expanding this domain with lower gastrointestinal symptoms that are deemed more appropriate.

In the present study, continuous repeated measurements were performed for 1 week. The strength and purpose of ESM is real-time assessment. Test-retest reliability of ESM was investigated by comparing and correlating mean scores of the first half-week with those of the second half-week. We hypothesised that test-retest reliability of the ESM would not be perfect due to the fluctuating nature of FD symptoms and the influence of subjects' daily life on (gastrointestinal) symptoms. The probability that within-subject differences are smaller than between-subject differences is plausible. Therefore, it is possible that a subjects' own symptom pattern can be identified by using ESM. For all upper- and lower

gastrointestinal symptoms, as well as non-gastrointestinal symptoms and positive affect, no significant differences in mean scores were found, when the first half of the week was compared with the second half. Symptoms reflecting negative affect tended to be significantly lower during the second half-week. For all scores, high correlations between the first half-week and second half-week were demonstrated. Most subjects started the test period in the beginning of the week. Consequently, many of the second half-week assessments were scheduled on one or more weekend days. It is possible that feelings of negative affect were lower in the second half-week, due to less daily life hassles (*i.e.*, no work pressure).

The regulatory authorities recommend the use of end-of-day reporting in FD.[41] ESM represents a higher burden for patients than end-of-day reporting, as multiple assessments during the day are likely to be more time-consuming than most conventional assessment methods. The accuracy of the assessment method would be at stake if this had resulted in low adherence. A completion rate of 33% for ESM-based PROMS is conventionally accepted.[35,36] In the present study, 62.2% of the total assessments was completed. Reported completion rates are reliable and accurate, since ESM assessments were only available for a set period of time and electronic date- and time-stamps were registered for each assessment.

A particular strength of the present study is the validation of this novel tool according to the recommendations of de FDA.[6] An important addition is that conventionally static measures of health status at specific time-points are used for comparison. In contrast to end-of-day or end-of-week health questionnaires, ESM, is able to detect short-term fluctuations in symptoms by its dynamic assessment of symptomatology. Therefore, ESM has the ability to provide a more detailed and individual assessment of symptom patterns. Moreover, this study demonstrated the suitability of ESM to provide an overview of patients' symptoms over the 7-day period as well as a detailed insight into within-day fluctuations of symptoms. Furthermore, by assessing other symptoms at the exact same moments, ESM offers the opportunity to investigate associations between concurrent symptoms, environmental factors, and psychological symptoms.[13,42] This means that ESM is capable of investigating symptom formation, in other words, how symptoms impact on symptoms. More insight in symptom dynamics may reveal a better understanding of the underlying pathophysiology in FD and a more customised treatment trajectory. A recent study in psychiatry even demonstrated the capability of ESM-based self-monitoring combined with positive emotion enhancement to enhance treatment effects in patients treated for depression.[43] This emphasises the capability of ESM to aid in disease insight, self-management, and improved shared decision making.

Moreover, FD is characterised as a heterogeneous disorder often accompanied by general somatic complaints and/or psychological disturbances. This is reflected by a considerable number of studies describing lower levels of physical and mental quality of life.[44-48] Therefore, items reflecting frequently reported physical complaints and mental state were included in addition to gastrointestinal symptoms. Previous studies have described the assessment of patients' mental state by using ESM-based PROMs.[32,49,50] However, the main focus of the current study was the gastrointestinal complaints as reflected in this PROM.

A potential shortcoming of the present study is the relatively small sample size. For the evaluation of validity and reliability of outcome measures, it has been recommended to include at least 50 subjects.[51] However, the large number of repeated measures per subject provides a significant increase in power, which is a substantial strength of ESM concerning required sample sizes in clinical trials. Moreover, in this small sample size, adherence was reasonable. However, adherence should be evaluated in a larger pragmatic trial in order to adequately evaluate compliance to the present smartphone app. Furthermore, potential user bias should be considered, as this study required subjects to own a smartphone and to be able to adequately operate the smartphone app requiring sufficient digital skills. Additionally, intensive recording using this smartphone app was mandatory. Another important aspect in quality testing of PROMs is assessment of responsiveness (*i.e.*, the sensitivity to detect change over time).[38,40,52] In the present study, responsiveness was not evaluated, and this has to be performed before the use of this novel ESM-based PROM as a tool to evaluate treatment efficacy in patients with FD. In addition, cross-cultural validation remains to be performed.

In conclusion, adequate concurrent validity, moderate-to-good internal consistency, and very good test-retest reliability were demonstrated for the novel ESM-based PROM for patients with FD. Moreover, the ESM-based PROM has the advantage of evaluating individual symptom patterns, providing the opportunity to evaluate interactions between symptoms and environmental factors. It must be noted that this was not evaluated in the present study as the goal of the present study was merely the validation of the used smartphone app. Future studies should evaluate the potential of this tool to evaluate these interactions, as this may lead to increased insight into their illness and tools for self-management, as well as improved shared decision-making. Thus, this novel tool has the ability to aid in the transition towards more personalised healthcare for patients with FD. Future research for assessment of responsiveness of this novel ESM-based PROM is warranted, in order to determine its place in the evaluation of treatment efficacy.

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100%
Obesity
MEASuRE-D
Weight
Dyspepsia
Anorexia

Chapter 7

The interplay between stress and fullness in functional dyspepsia and healthy controls: an exploratory experience sampling method study

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Submitted

Abstract

Background and aims

Fullness is a cardinal symptom in functional dyspepsia (FD). The use of real-time symptom assessment might provide more insight in factors, such as stress, that can influence fullness. Furthermore, it is unknown whether real-time assessment of fullness is able to capture the construct of early satiation. Therefore, this study aimed to use a real-time, repeated measurement method (Experience Sampling Method, ESM) to primarily assess the association between stress and fullness in FD patients and healthy controls (HC). Additionally, this study aimed to evaluate whether real-time assessment of fullness can capture the construct of early satiation.

Methods

Thirty-five FD patients (25 female, mean age 44.7 years) and 34 HC (24 females, mean age 44.1 years) completed ESM (a maximum of 10 random moments per day) for seven consecutive days. Stress, fullness, and ability to finish meal scores were rated on an 11-point Numeric Rating Scale.

Results

FD patients scored 2.24 points higher on fullness ($p<0.001$), 1.37 points higher on stress ($p<0.01$), and 1.94 points lower on ability to finish meals ($p<0.001$) compared with HC. In FD, fullness scores increased with 0.14 for every 1-point increase in concurrent stress scores ($p=0.01$) and increased with 0.20 for every 1-point decrease in ability to finish meal ($p<0.001$). Fullness scores at $t=0$ increased with 0.09 for every 1-point increase in stress scores at $t=-1$ ($p=0.02$). No associations between stress scores or ability to finish meals and fullness scores were found for HC.

Conclusion

Concurrent and preceding stress scores are positively associated with fullness scores in FD patients, but not in HC, indicating a difference in response to stress. Moreover, real-time assessment of the symptom fullness is able to capture the construct of early satiation.

Introduction

Functional dyspepsia (FD) is one of the most common functional gastrointestinal (GI) disorders, with an estimated prevalence of 10-15% in the general population.[1] According to the Rome IV criteria, FD is defined by the presence of various symptoms in the absence of evident organic, systemic or metabolic diseases that could explain the complaints.[2] Among the heterogeneous presentation of FD patients, four core symptoms have been defined: early satiation, postprandial fullness, epigastric burning, and epigastric pain.[2,3] Based on the occurrence of these core-symptoms, three subtypes of FD can be distinguished: postprandial distress syndrome (PDS), epigastric pain syndrome (EPS), and overlap syndrome (OS).[2] Among FD patients, 60% can be diagnosed with the PDS subtype and herewith, PDS appears to be the predominant subtype.[4] In that regard, symptom generation is part of a complex interaction between the gastroduodenal region and the brain, and is triggered by factors including food, stress, and psychosocial events.[5] Given that PDS is the predominant subtype among FD patients, specific triggers that can generate fullness complaints should be thoroughly investigated, including its core symptom of early satiation.

FD is known to be associated with anxiety and depression, daily stress and life hassles.[6,7] Ly *et al.* demonstrated that acute anxiety, induced by a stressor, results in a disturbance of gastric sensory and motor function, manifested by an impairment of the gastric accommodation reflex.[8] Moreover, it has been shown that even the anticipation of a stressor already elicits alterations in brain activity in FD patients.[9] These findings point towards the capability of acute stress to interfere with gastrointestinal sensory-motor responses resulting in abnormal sensations of early satiation/fullness, which are both defining characteristics of the PDS subtype of FD. However, it is still unclear whether GI symptoms are more a cause or a consequence of psychological characteristics.[10-12]

Currently, most studies assessing psychological symptoms and GI symptoms are cross-sectional or assess these symptoms in retrospect over a longer period of time and are therefore prone to recall bias.[7,12-14] Unfortunately, these methods of evaluation are not able to take symptom fluctuation over time into account. Symptoms of functional GI disorders are known to vary over time[15,16] and stress is also characterised by daily fluctuations.[17,18] Therefore, daily life symptom variability should be taken into account when evaluating triggers for early satiation/fullness complaints in FD patients.

For this purpose, an electronic patient-reported outcome measure (PROM) based on the experience sampling methodology (ESM) for patients with FD was developed.[19] ESM is characterised by collection of repeated measurements on random moments during the day. Due to the repeated and momentary nature, ESM might be able to capture fullness complaints and possible influencing factors, such as stress, over the course of the day.

Therefore, ESM offers the opportunity to provide insight in the interplay between daily life stress and fullness complaints. Moreover, using ESM, it could be investigated whether real-time assessment of fullness can capture the construct of early satiation.
Primary aim of this study was to evaluate the association between stress and fullness complaints, using concurrent and time-lagged assessments, by using ESM in FD patients and healthy controls (HC). Our secondary aim was to evaluate whether real-time assessment of the symptom fullness is able to capture the construct of early satiation. We hypothesised that stress levels and fullness levels will be associated in FD patients, but not in HC. Moreover, we hypothesised that fullness scores, provided a meal had been consumed, is able to capture the construct of early satiation.

Methods

The study protocol was approved by the Medical Ethics Committee of the Maastricht University Medical Centre+ (MUMC +), Maastricht, the Netherlands (ID METC19-077), and performed in full accordance with the Declaration of Helsinki (latest amendment by the World Medic Association in 2013) and Dutch Regulations of Medical Research involving Human Subjects (WMO, 1998). This prospective observational study was performed at the MUMC+ from 29 May 2020 until 1 October 2020. This study was registered in the US National Library of Medicine (http:///www.clinicaltrials.gov, ID NCT04204421).

Subjects

Recruitment of FD patients, aged between 18 and 75 years, took place at the outpatient clinic of Gastroenterology and Hepatology of the MUMC+, a secondary/tertiary hospital. Additionally, FD patients that participated in other studies of the MUMC+ were contacted to participate in the current study (NCT02522000, NCT03652571). FD and FD subtypes were diagnosed according to the Rome IV criteria, which were evaluated by a trained clinical researcher in a face-to-face interview.
Healthy controls, aged between 18 and 75 years, were recruited through local advertisements. Healthy controls were eligible if they did not fulfil Rome IV criteria for FD and did not have a past or present diagnosis of any organic or functional GI disorder.
Exclusion criteria for all subjects were initiation of regularly used medication from one month before inclusion until the end of the study period, a history of upper digestive surgery influencing end points, history of radiation therapy of the abdomen, and

pregnancy. Subjects could only participate if they understood the Dutch language and were able to use the smartphone application.

Data collection

ESM was collected during seven consecutive days. On day seven, subjects completed validated symptom questionnaires using an electronic CRF (eCRF) system (CastorEDC).

ESM

The MEASuRE-D application was developed for the use of ESM in FD patients,[19] and was downloaded on the subjects' smartphones for the purpose of the current study. During their regular daily life, subjects completed ESM for seven consecutive days. In order to complete the real-time questionnaires as often as possible, subjects were instructed to carry their smartphone with them during the week. The MEASuRE-D app sent out a haptic, auditory and written signal ten times per day between 07:00 and 22:00 at randomly chosen moments, with a time-interval of at least 15 minutes between consecutive signals. Following a signal, the ESM-questionnaire was available for 10 minutes. On all measurement moments, the questions were repeated in the same order and questions were scored on an 11-point Numeric Rating Scale (0: not at all to 10: very severely). Questions were asked in Dutch and translate as follows. Fullness was defined as "I am having full feeling in my upper abdomen" (0: not at all, 10: very severely). Early satiation was defined as "I am able to finish a normal sized meal" (0: not at all, 10: very much). Question for early satiation was only prompted when a preceding question "I have eaten … since the last beep" (breakfast, lunch, dinner, a snack, none of these) was not answered "none of these". This question was not analysed when participants answered with "a snack". Stress was defined as "I am feeling stressed" (0: not at all, 10: very severely). Contrary to fullness and stress constructs, early satiation was not momentary, as this feeling is always in relation to eating a meal. The development of this ESM-based questionnaire was previously described.[19]

Questionnaires

At the end of the seven-day study period the Generalised Anxiety Disorder (GAD-7; 0-3 scale, total composite score for severity of anxiety, recall period of two weeks)[20] and the Hospital Anxiety and Depression Scale (HADS; 0-3, total composite scores for severity of anxiety and depression, recall period of one week)[21,22] were completed.

Statistical analyses

Sample size was based on several previous studies using ESM-data that have shown sample sizes between 20-30 subjects to be sufficient for analyses.[23,24] Moreover, in IBS patients sample sizes of 26-37 were used to evaluate a novel ESM-based PROM.[25-27] The present study was an exploratory study on the usage of an ESM-based PROM in FD patients. To date, no data on the use of ESM in FD are available. FD is a functional GI disorder, as is IBS, in which abovementioned trials were performed. Therefore, we aimed to include at least 30 valid cases with a maximum of 36. Subjects were included in the analyses only when at least 1/3 of the total number of assessments (*i.e.*, 23 out of 70) were completed.[28,29]

All analyses were performed using R version 3.6.3. Continuous outcomes are presented as mean ± standard deviation (SD), and tested using paired or independent samples t-test. Proportions for categorical variables were tested using the χ^2-test. For all analyses, $p<0.05$ was considered statistically significant.

A linear mixed-effects model was used with repeated measures (level 1) nested within subjects (level 2) to compare levels of fullness, levels of stress, and early satiation between FD patients and HC. ESM scores were indicated as dependent variable and group (*i.e.*, FD, HC) was used as predictor variable. An autoregressive covariate (AR1) was used to correct for repeated measures and autocorrelation.

Similar analyses were performed to evaluate associations between fullness and stress and between fullness and early satiation.

First, fullness scores were used as dependent and stress or early satiation scores were used as independent variables to investigate concurrent fullness and stress and concurrent fullness and early satiation.

Second, assessing whether fullness scores could be predicted by stress scores preceding the measurement of fullness, lagged scores (*i.e.*, t=-1) were used as the predictor variable. Similarly, association between stress scores and preceding fullness scores were investigated by using lagged scores for fullness as predictor variables with stress scores as dependent variables. The association between lagged scores for early satiation and fullness was not investigated, as the measurement of early satiation was dependent on meal intake prior to the assessment and was, therefore, in itself not momentary. Random slopes for predictor variables were tested in all models. Best model fit was reported based on Akaike Information Criterion.

Linear mixed model analyses were not performed to evaluate associations between early satiation and stress, since the perception of early satiation is unsuitable for momentary assessment, as it is dependent on consumption of a meal, which does not occur at random.

In patients with FD, individual regression coefficients were calculated for the associations between fullness and stress and the associations between fullness and early satiation. In both cases, FD patients were selected that showed a positive association, a negative association, and a very weak association in order to visually represent inter-individual differences.

Results

Subjects

In total, 36 FD patients and 36 HC met the inclusion criteria and not the exclusion criteria. One FD patient and two HC completed less than 1/3 of the total number of ESM assessments. Therefore, 35 FD patients and 34 HC were included in the analyses. Baseline characteristics are summarised in Table 7.1.
In the FD patients, ten patients fulfilled the criteria for EPS (28.6%), seven for PDS (20%) and 18 for OS (51.4%). Of the 35 included patients, 13 had comorbid IBS (37%, one in the EPS group, three in the PDS group, and nine in the OS group). During the study, no adverse events were reported by the subjects.

Table 7.1 Demographics of included subjects.

	FD patients (n=35)	**Healthy controls (n=34)**
Age	44.7 (± 15.7)	44.1 (± 13.9)
Gender female (%)	25 (71.4%)	24 (70.6%)
GAD-7	4.8 (± 4.5)**	1.5 (± 1.9)**
HADS-Anxiety	5.3 (± 4.5)*	2.9 (± 2.2)*
HADS-Depression	4.9 (± 4.3)**	0.9 (± 1.0)**

Demographics of FD patients and healthy controls. Means and standard deviations (SD) are depicted. *p<0.01, **p<0.001

Compliance ESM

Figure 7.1 depicts the compliance rate for FD patients and HC. For FD patients, completion rate of ESM assessments was 62.2%. Over the seven-day period, a mean number of 43.5 measurements were completed per individual (range:23-68). The majority of subjects completed between 31 and 60 assessments during the study period.
For HC, completion rate of ESM assessments was 69,7%. Over the seven-day period, a mean number of 48.8 measurements were completed per individual (range: 24-69). The majority of subjects completed between 41 and 60 assessments during the study period.

It should be noted that feedback in general revealed that the question "I have eaten … since the last beep" was difficul to answer, as subjects could not always remember whether they already reported the meal with the last beep. Therefore, some meals might be overreported by subjects.

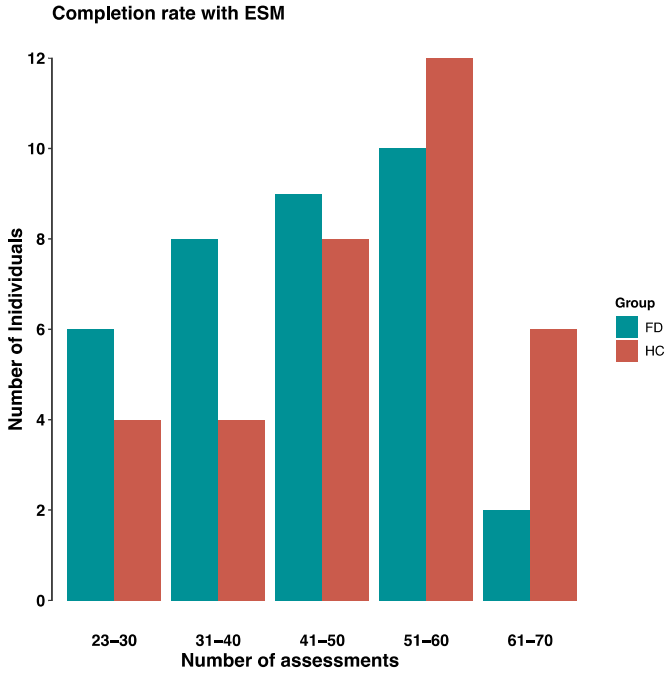

Figure 7.1 **Number of individuals (y-axis) per category of completed number of assessments (x-axis) for FD patients (n=35) and HC (n=34).**

Fullness, stress and ability to finish meal

Mean scores for fullness, stress, and ability to finish meals for FD patients and HC are depicted in Figure 7.2. Fullness scores were, on average, 2.23 (SE 0.37, $p<0.001$) points higher in FD patients compared with HC (2.88 (SE 0.33) vs. 0.66 (SE 0.16)). FD patients also reported higher mean stress scores. Stress scores were, on average, 1.37 (SE 0.30, $p<0.01$) points higher in FD patients compared with HC (1.99 (SE 0.29) vs. 0.62 (SE 0.30)).

Ability to finish meal scores were, on average, 1.94 (SE 0.46, $p<0.001$) points lower in FD patients compared with HC (6.27 (SE 0.32) vs. 8.21 (SE 0.33)).

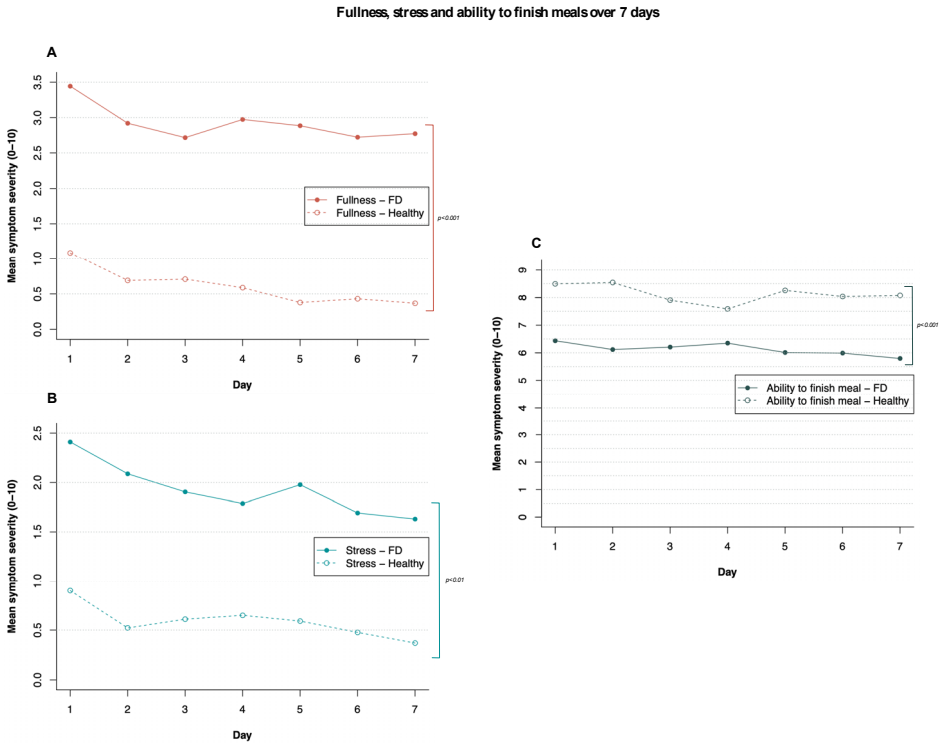

Figure 7.2 **Symptom severity.** Symptom severity for fullness (**A**) and stress (**B**) and ability to finish meals (**C**) over the 7-day study period, separately for functional dyspepsia patients and healthy controls. Mean scores per day as recorded using experience sampling method over 7 days.

Associations between concurrent fullness and stress scores

In FD patients a 1-point increase in stress scores was associated with an increase in fullness of 0.138 (SE 0.05, p=0.01). On the other hand, stress and fullness scores were not associated in HC (p=0.97). In Figure 7.3, repeated measures for stress and fullness for three FD patients are depicted. Between-subject differences in the association between fullness and stress are clearly seen here, as the plots show varying relationships between fullness and stress for these three patients. These inter-individual differences are confirmed when performing the linear mixed-effects model for each FD patient separately. The coefficients for the association between fullness and stress range from -0.62 to 1.59. This indicates that, in a particular patient, a 1-point increase in stress corresponds with a *decrease* in fullness of 0.62 points, while another patient has an *increase* of 1.59 points on fullness for every 1-point increase in stress. Figure 7.3 depicts the distribution of the individual coefficients for fullness and stress among FD patients. Three

patients, selected based on varying individual regression coefficients (-0.17, 0.03, and 0.32), were depicted to demonstrate inter-individual differences.

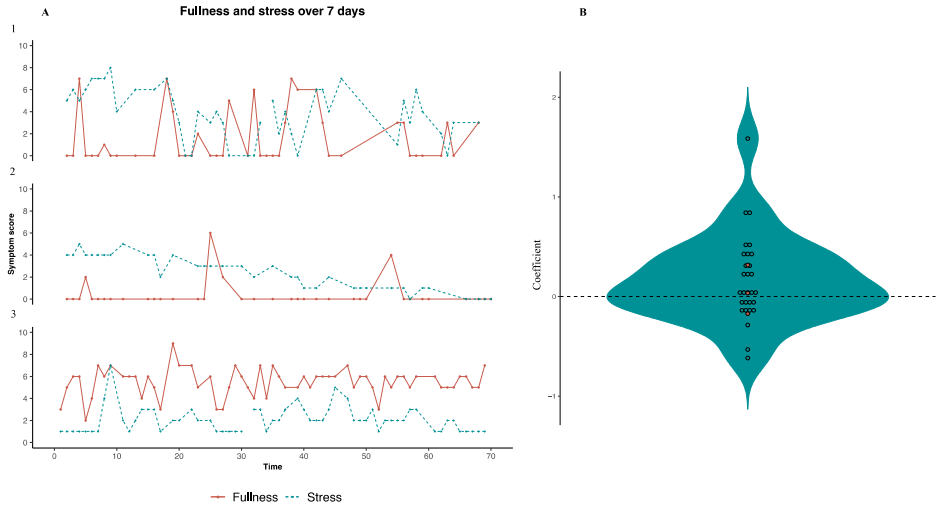

Figure 7.3 **Individual representation of fullness and stress scores. A:** Individual representation of symptom severity scores for fullness and stress for 3 functional dyspepsia patients on 70 random time points over 7 consecutive days. On the x axis: 70 consecutive assessments, each within a 90-minute timeframe between 07:00 AM and 22:00 (day 1: 1-10; day 2:11-20; day 3: 21-30; day 4: 31-40; day 5: 41-50; day 6:51-60; day 7: 61-70. Different patterns of stress and fullness indicate the heterogeneity between FD patients. This is also reflected by differences in the corresponding regression coefficients for (1) -0.17, (2) 0.03, and (3) 0.32. **B**: Distribution of individual coefficients for fullness and stress, based on linear mixed effects model per individual, among FD patients. Selected patients in panel A are highlighted in orange.

Associations between fullness and lagged stress scores

For FD patients a 1-point increase in lagged-stress scores was associated with an increase in fullness scores of 0.09 (SE 0.04, p=0.02). On the other hand, fullness and lagged stress scores were not associated for HC (p=0.65).

Associations between stress and lagged fullness scores

Fullness scores at t=-1 were not a significant predictor for stress at t=0 in FD patients (p=0.42) nor in HC (p=0.73).

Associations between fullness and early satiation

In FD patients, a 1-point decrease in ability to finish meal was associated with an increase in fullness scores of 0.20 (SE 0.08, p<0.001). Whereas ability to finish meal and fullness

scores were not associated in HC (p=0.08). In Figure 7.4, repeated measures for fullness and ability to finish meals (if a meal was consumed since the last beep) are depicted for three FD patients. Between-subject differences in the association between fullness and early satiation are clearly shown in these three patients. The plots show varying relationships between fullness and early satiation between the three patients. These inter-individual differences are confirmed when performing the linear mixed-effects model for each FD patient separately. The coefficients for the association between fullness and ability to finish meal range from -1.34 to 1.31. This indicates that, in a particular patient, a 1-point increase in ability to finish the meal corresponds with a *decrease* in fullness of 1.34 points, while another patient has an *increase* of 1.31 points on fullness for every 1-point increase in ability to finish meal. Figure 7.4 depicts the distribution of the individual coefficients for fullness and early satiation among FD patients. Three patients, selected based on varying individual regression coefficients (-0.34, 0.01, and 0.42), were depicted to demonstrate inter-individual differences.

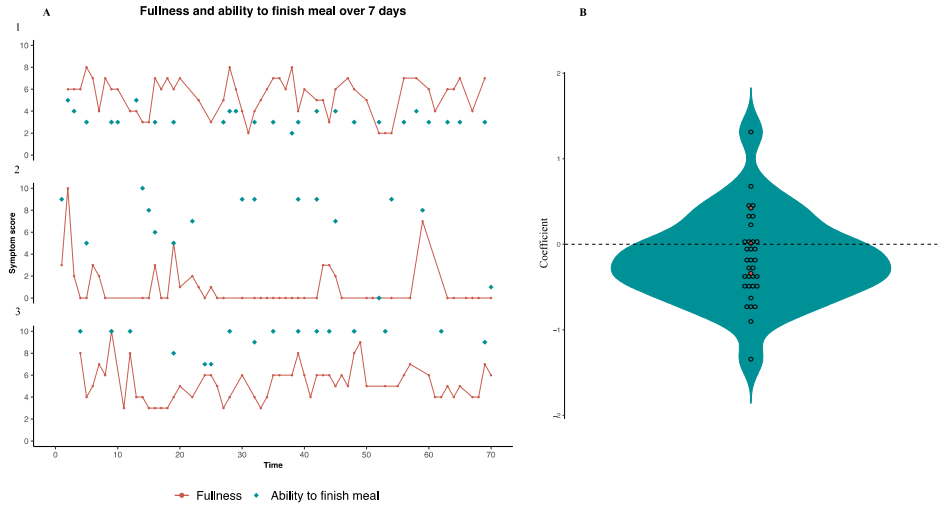

Figure 7.4 **Individual representation of fullness and ability to finish meal scores. A:** Individual representation of fullness and ability to finish meal scores for 3 functional dyspepsia patients on 70 random time points over 7 consecutive days. Ability to finish meal is based on self-reported meal intake (breakfast, lunch or dinner) preceding the assessment. A high score on ability to finish meal indicates good ability to finish said meal. On the x axis: 70 consecutive assessments, each within a 90-minute timeframe between 07:00 AM and 22:00 (day 1: 1-10; day 2:11-20; day 3: 21-30; day 4: 31-40; day 5: 41-50; day 6:51-60; day 7: 61-70. Different patterns of ability to finish meals and fullness indicate the heterogeneity between FD patients. This is also reflected by differences in the corresponding regression coefficients for (1) -0.34, (2) 0.01, and (3) 0.42. **B**: Distribution of individual coefficients for fullness and early satiation, based on linear mixed effects model per individual, among FD patients. Selected patients in panel A are highlighted in orange.

Discussion

To our knowledge, the current study is the first to examine a real-time association between stress and fullness in FD patients compared with HC using momentary assessment. Our results provide more detailed information on the associations between daily life stress and sensations such as fullness and early satiation that are both defining characteristics in FD. Indeed, concurrent fullness and daily stress levels were significantly associated in FD patients, but not in HC. In HC, stress levels were substantially lower compared with FD patients. Furthermore, the ability to finish meals was associated with fullness scores in FD patients, but not in HC.

Both average fullness levels and average stress levels were significantly higher in FD patients compared with HC. This was also true for the scores on GAD-7, and HADS questionnaires completed at the end of the week. In contrast to our findings in FD patients, previous ESM studies in IBS patients showed daily stress levels in IBS patients to be comparable to HC, whereas abdominal pain scores were significantly higher in IBS patients compared to HC.[27,30] We compared the results of the present study with data from the ESM study in IBS patients (data not shown).[27] Stress scores were higher in FD patients compared with IBS patients (1.94 vs. 1.26, $p<0.001$). It should be noted, that in the present study, only subjects from a secondary/tertiary referral centre were included, whereas in the study with IBS patients subjects were also included from primary practice. Interestingly, mean stress scores of HC in our study appeared to be lower when compared with mean stress scores of HC in the previously performed ESM study by our group (0.59 vs. 1.06, $p<0.001$). . It should be taken into account that the present study was performed for a large part during the summer holiday season in the Netherlands. This could have resulted in lesser daily-life hassles and stress, as previous studies demonstrated lower daily life stress during vacation periods.[31-33] This may point towards other factors (*i.e.*, not work-related) resulting in daily life stress or a more chronic state of daily life stress in FD patients compared with HC.

The association found between stress and functional GI complaints does not reveal whether stress and FD symptoms are cause or consequence. The association between fullness and stress in FD patients, which could not be confirmed in healthy subjects, at least points towards a susceptibility in FD patients for the effects of daily life stress. We have described similar findings using ESM in IBS patients.[27] Other studies have also demonstrated that patients with functional GI disorders are also susceptible to a different form of stress, namely early life stress events.[34,35] On the other hand, the presence of invalidating FD symptoms may also lead to stress.[6] The higher stress levels found in FD

patients compared with HC in this study may reflect, thus, a cause-effect relationship. However, this study showed an association between lagged daily stress scores and fullness, which indicates that stress scores were able to predict fullness scores for the subsequent questionnaire. These findings lead us to assume that stress triggers the onset or worsening of fullness complaints in FD patients, rather than the other way around. Possibly, this could be related to the effects of stress on gastric accommodation or neurohormonal processing which affects gastrointestinal sensing and signalling. This is in line with findings by Ly *et al.* who demonstrated disturbed gastric sensory and motor function after inducing acute anxiety in FD patients.[8] Moreover, stress induces an inhibition of propulsive gastric motor activity in FD patients.[36] Other studies showed modified gastroduodenal motility after either a physical stressor or mental stress in healthy volunteers.[37,38] On the other hand, Lunding *et al.* previously showed that gastric accommodation was not influenced by induction of acute emotional stress in FD patients or healthy volunteers.[39] Furthermore, in healthy volunteers, corticotropin-releasing hormone was not able to modulate gastric accommodation.[40] Based on our results, no definite conclusions with respect to the direction of the interplay between stress and fullness can be made, as our ESM analyses indicate associations rather than causality. However, the association between fullness and lagged stress scores demonstrated by the present study are in favour of stress as a predictor of fullness, in terms of symptom formation, in FD patients.

Overall, this study demonstrates a high heterogeneity between FD patients in the magnitude of the stress-fullness association. The inter-individual heterogeneity in the interplay between stress and fullness points towards variations in stress-sensitivity in FD patients. It is known that each individual has different coping mechanisms for stress.[41] This study demonstrates the usefulness of ESM in characterising individualised symptom patterns, including influencing factors.

The association between fullness scores and ability to finish meals in FD patients points towards the ability of fullness to capture the construct of early satiation, as this sensation only occurs in conjunction with meal consumption and is therefore unsuitable for momentary assessment. In that regard, a major advantage of ESM in comparison to conventional questionnaires is the repeated measure moments over the day. This allows for fullness scores on ESM to reflect both satiation and satiety. Although commonly used as synonyms, satiation and satiety are known to be separate entities. Satiation refers to feelings of fullness during ingestion of a meal, acting as terminating factor of meal intake, whereas satiety reflects feelings of fullness in between meals, determining the length of the inter-meal interval.[42] Subjects informed us about their difficulties in answering the question "I have eaten … since the last beep". It was difficult for them to recall whether a

meal has previously been reported during the preceding beep. This may have resulted in over-reporting of the same meals and it underlines the impact of recall bias in questionnaires that are not based on momentary reporting. Our momentary assessment method does not systematically capture meal intakes over the day. This should be improved in future versions, perhaps by asking subjects to manually indicate the onset, end, and content of a meal.

Strengths and limitations

Here, we provided an in-depth analysis of the relation between stress and fullness complaints in FD patients and HC. These in depth-analyses can be performed for a variety of factors that can influence eating behaviour. Since sample size was small, this study only focused on the interplay between stress and fullness. In the present study, we demonstrated the potential of ESM to investigate the role of specific triggers that can influence eating behaviour and satiety signalling in healthy and diseased. A strength of this study was the inclusion of healthy controls. Therefore, conclusions on this interplay in FD patients could accurately be drawn. Moreover, including all tree subtypes of FD patients led to generalisable results reflecting the entire FD patient group. Collection of data was performed with an FD-specific ESM tool developed according to FDA guidelines for the development of PROMs.[19] Furthermore, correction for autocorrelation was performed in all statistical models.[43]

Using ESM also poses several limitations. The occurrence of missing data is inevitable. This study accounted for missing data to the greatest possible extent by correcting for repeated measures and within-subject autocorrelation using advanced statistical modelling. Moreover, subjects who failed to complete at least one-third of the total numbers of assessments were excluded from the analyses. Furthermore, ESM might be experienced as burdensome, which can result in selection bias. Despite of multiple measurements, results should be interpreted with care, as this study's sample size still is relatively small. Moreover, the present study focused on merely two aspects, namely stress and fullness/early satiation, while probably more factors playing a role in the interplay are involved. Furthermore, due to the small sample size, groups for each subtype were rather small (*i.e.*, 10 EPS and 7 PDS). Therefore, analyses on subtypes were not performed. Future studies should aim to expand sample size, allowing a) analyses of and correction for additional factors that might influence fullness complaints in FD patients and b) comparison between FD subgroups.

In conclusion, using ESM for real-time symptom assessment, a positive association between stress levels and fullness scores was observed in FD patients. Moreover, the momentary assessment of fullness scores was able to capture the concept of early satiation.

Additionally, fullness scores were predicted by preceding stress levels, but not the other way around. This suggests the presence of a longitudinal relation between stress and fullness, existing next to the in-the-moment association. Moreover, ESM can depict within-day changes in stress-perception, abdominal symptoms, psychosocial aspects, and food intake, providing healthcare professionals with the tools to evaluate patient-specific symptom patterns. By knowing these symptom patterns, healthcare professionals can be enabled to offer tailor-made treatment options to the individual patients. This study, therefore, emphasises the importance of assessing individual symptom patterns in FD patients and supports the use of real-time measurement methodologies.

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Chapter 8

General discussion

Main findings

The gastrointestinal tract plays a pivotal role in the regulation of satiety and food intake. Several clinical scenarios can disrupt the sensing and signalling of satiety resulting in increased or decreased food intake behaviour. Disordered food intake can result in disease conditions at both ends of the spectrum: obesity can be the result of an increase in food intake,[1,2] whereas the aging population shows decreased food intake, mainly due to decreased appetite, resulting in weight loss.[3,4] Proof of concept studies investigating treatment and/or preventive treatment strategies are described in **chapter 2, chapter 3, chapter 4,** and **chapter 5**.

Satiety signalling can also be disrupted by abnormal sensations such recurring abdominal pain, resulting in abdominal complaints such as functional dyspepsia and food intake disorders such as avoidant/restrictive food intake disorder (ARFID), which can also result in weight loss, albeit through a different mechanism.[5-7] In **chapter 6** a new monitoring tool for functional dyspepsia was described and was validated. **Chapter 7** elucidated the interplay between stress and fullness complaints in FD patients and healthy controls.

Previous studies have shown that gastrointestinal delivery of non-caloric tastants can result in a reduction of food intake.[8-10] In **chapter 2** of this thesis, we showed that, among non-caloric tastants, bitter tastants are the most potent to reduce food intake. The optimal location in the GI tract for tastants to exert their effect on food intake is unknown. Therefore, **chapter 3** and **chapter 4** aimed to assess the optimal location of respectively a tastants mixture and the bitter tastant quinine. No effect was found on food intake or appetite sensations after intraduodenal, intraileal or synchronous intraduodenal and intraileal delivery of a tastant mixture (**chapter 3**). Moreover, oral, intragastric, or synchronous oral and intragastric delivery of the bitter tastant quinine did not result in a reduction of food intake. However, desire to eat and hunger scores did decrease in the quinine interventions, whereas satiation and fullness scores were not affected (**chapter 4**).

Although the optimal location for tastants to sufficiently influence eating behaviour was not found in the set of studies we performed, the results from various studies analysed in more detail in the systematic review and meta-analysis described in **chapter 2** are promising with respect to bitter agents, but standardisation of protocols and of interventions, as well as collaboration in this research field is a pre-requisite.

Mechanoreceptors in the stomach are the first step in the satiation cascade. An increase in sensitivity of these mechanoreceptors could result in a decrease in food intake. In **chapter**

5, we showed that acute intragastric administration of lidocaine in order to blunt the mechanoreceptor signalling did not result in an increase in food intake or appetite sensations in healthy volunteers. However, results were promising and no safety issues occurred. As next step a chronic intervention study in subjects suffering from early satiation, reduced food intake or cachexia is warranted.

Negative signals such as pain can result in avoidant food intake behaviour as a conditioned adaptive mechanism aimed at decreasing symptoms (*i.e.*, avoiding food intake altogether or avoiding specific food products that trigger complaints). **Chapter 6** showed an adequate concurrent validity, moderate-to-good internal consistency, and very good test-retest reliability for the novel experience sampling method (ESM) based questionnaire on symptoms of functional dyspepsia (FD). **Chapter 7** additionally showed that fullness complaints were associated with stress levels in FD patients but not in healthy controls.

Effects of non-caloric tastants on food intake behaviour

Nutritive triggers in the intestine are known to elicit satiety signalling.[11] One way for the gut to perceive food intraluminally is via taste receptors found throughout the entire GI tract.[12-15] In the GI tract, these taste receptors are localised on enteroendocrine cells (EECs). Activation of these taste receptors *in vitro* results in GI peptide release, indicating their possible role in the satiation cascade.[16-18] Interestingly, in **chapter 3** and **chapter 4** we found no effect of duodenal, ileal or intragastric delivery of tastants on energy intake. This was in line with some previous findings.[19-22] However, several other studies did show reduced energy intake after gastrointestinal delivery of non-caloric tastants.[8-10,23,24] It should be taken into account that among most studies that showed no significant decrease in energy intake, a non-significant decrease was found. Therefore, we conducted a systematic review and meta-analysis to investigate the effect of gastrointestinal delivery of tastants on eating behaviour (**chapter 2)**. This review and meta-analysis clearly showed that among the various tastants, bitter agents are the most potent to influence eating behaviour. Overall, a significant reduction in food intake was found after gastrointestinal delivery of bitter tastants. The effects of gastrointestinal delivery of tastants on GI peptide release were inconclusive. Most recently, the role of motilin as opposed to the more conventional GI peptides was elaborated to be a regulator of hunger and food intake in humans.[25] It must be noted, however, that generally *plasma* levels of GI peptides are measured, indicating systemic instead of local effects. The effects of taste receptor activation on local secretion of GI peptides *in vivo* are entirely unknown. Since elevated GI peptides are consistently found after taste receptor activation *in vitro,*[16-18] it is conceivable

that, at local and splanchnic level, GI peptides levels are elevated. However, both splanchnic blood collection and using catheters to investigate local intestinal tissue levels of GI peptides are invasive and complex procedures. Interestingly, recent studies consistently show attenuated gastric motility and serum motilin as possible mediators of the effect of bitter tastants on eating behaviour.[21,23,26] Therefore, future research projects in this field should focus on motility and motilin as outcome measures to investigate underlying mechanisms of action.

Although GI delivery of bitter tastants seems promising with respect to influencing eating behaviour, it must be noted that the reduction in energy intake that was found is small. Several studies showed blunted postprandial levels of GI peptides PYY[27,28] and GLP-1 in obese subjects.[29-31] Moreover, a reduction in plasma CCK levels has been shown after weight loss.[32,33] These observations point to alterations in the sensitivity of various receptors to GI peptides in obesity. Due to the modest caloric reduction in energy intake, bitter tastants appear to be more suitable to prevent obesity rather than treat (morbid) obesity. Further research is needed to elucidate the long-term efficacy and safety of gastrointestinal bitter tastant delivery before it can be made available for consumers. At first, repetitive stimulation should be investigated to examine whether desensitisation of taste receptors occurs or not. Thereafter, a long-term intervention study should be conducted to evaluate the effects of bitter tastant administration on eating behaviour, actual weight loss. Moreover, safety issues should be addressed. We propose such a first long-term intervention study to last at least 3-6 months, as most pilot studies investigating initial weight loss employ an intervention period of 3-6 months.[34-36]

It must be noted that the currently available data is highly heterogenic with different modes and sites of delivery of tastants, different dosages of tastants. The field should move towards uniformity in study designs, preferably by meeting each other and discussing further steps.

One specific aspect should also be taken into account: differences in response between subjects classified as 'tasters' or 'non-tasters'. Up to now only one study addressed this issue and found a difference in response between 'tasters' and 'non-tasters', with a higher reduction in energy intake in 'tasters'.[8] Classifying 'tasters' and 'non-tasters' in that study was performed based on the ability to recognise phenylthiocarbamide (PTC) and 6-N propylthiouracil (PROP).[37] Bitter taste is sensed by the taste receptor family 2 (TAS2R). Currently, there are 25 known subtypes of TAS2Rs in humans, with different compounds activating various (combinations of) subtypes. Both PTC and PROP are recognised only by TAS2R38.[38] **Chapter 2** showed that denatonium benzoate (DB) and quinine are most

commonly used in studies investigating the effects of bitter compounds on eating behaviour. DB is known to activate 8 TAS2R subtypes (TAS2R 4, 8, 10, 13, 39, 43, 46, and 47), whereas quinine is known to activate 9 subtypes of TAS2R (TAS2R 4, 7, 10, 14, 39, 40, 43, 44, and 46).[38] Therefore, it essential to characterise 'tasters' and 'non-tasters' based on the tastant that is being used in the study. This challenge can be met by using a gustometer, a taste delivery system able to deliver a precise amount of taste.[39-41] The gustometer could also be used to predict interventional success based on tastant responders when applied in the screening setting.

Furthermore, studies should focus on feasible, and in daily life implementable methodology such as capsules rather than using naso-gastric or naso-intestinal catheters, as effect sizes of gastrointestinal tastant delivery are small. The potentially negative and aversive effects of catheters may mask or override the subtle effects that tastants exert on eating behaviour. Regarding bitter agents, studies in new-born infants showed bitter taste to be evolutionarily linked to toxic substances.[42] Therefore, one should realise that oral ingestion of bitter tastants is not pleasant and may even be aversive. Such effects could result in loss of compliance over time. Taken together, we consider the stomach as best target location for bitter tastants to exert their effect and we advise to focus on the effects of intragastrically delivered bitter tastants on eating behaviour.

Effects of lidocaine on food intake behaviour

Directly after ingestion of a meal, the initial satiating effect occurs due to distension of the stomach wall. Along the stomach wall are mechanoreceptors that sense an increase in stomach volume, which in turn triggers neural circuits that inhibit food intake behaviour.[11,43] A recent study applicated a local topical anaesthetic to rats' stomachs and found a significant increase in energy intake during a subsequent meal.[44]

In **chapter 5**, we described the first in-human study to investigate safety and acute effects on eating behaviour of lidocaine infusion into the stomach. Lidocaine infusion resulted in a modest, non-significant, increase in energy intake. Interestingly, this increase in energy intake was found on treatment day 2 compared with treatment day 1. It is known that stressors can influence energy intake in both directions, dependent on strength, type, and impact of the stressor.[45-47] As a nasogastric catheter was used to administer the lidocaine, we believe this treatment day effect to arise from the subjects not being familiar with the procedures of catheter placement and removal. Based on this knowledge, the option of

using a mock test day to familiarise subjects to study protocols should be considered for future studies involving invasive procedures.

Moreover, in this study we employed a rather low dosage of lidocaine when compared with the dosage of topical anaesthetics used in the beforementioned animal study. It is likely that a higher dosage of lidocaine could have resulted in a more pronounced, statistically significant increase in energy intake. However, it should be taken into account that applying higher dosages of lidocaine may increase the risk of hazardous side-effects, such as cardiac arrythmias.[48,49] These aspects should be taken into account when designing future studies.

In this study we did not exclude subjects based on their capability to eat large meals. One could speculate that subjects with the ability to consume large volume meals are able to eat until maximum distension of the stomach. If this holds true, desensitising the stomach mechanoreceptors using local topical anaesthetics would not result in an additional increase in energy intake in these individuals. Therefore, we expect lidocaine to have a certain window of efficacy. We anticipate no effects to occur in response to lidocaine on an empty stomach, as there is no stretch or distension of the stomach wall. On the other hand, in a fully distended stomach it is physiologically impossible to further increase the volume.

Another aspect that should be noted is the fact that this study was performed in healthy, young individuals. The aging population, on the other hand, is known to have alterations in the signalling of their sensory pathways in the gastrointestinal tract.[50-52] Moreover, impaired gastric accommodation is found in patients suffering from functional dyspepsia (FD) and is associated with hypersensitivity of mechano-receptors. As these populations of elderly and dyspeptics show signs of oversensitivity of mechanoreceptors, interventions directed towards desensitisation of mechano-receptors may represent new and viable treatment options to improve nutrient intake and reverse the observed weight loss. Therefore, effects of gastric anaesthetics on eating behaviour should be investigated in individuals suffering from anorexia of aging and FD patients with predominant postprandial distress syndrome. As gastric anaesthetics have been shown to be safe in healthy individuals, future research should address the balance between treatment efficacy and potentially hazardous side-effects in the abovementioned patient groups.

Besides the selection of the patient groups, one should consider applying gastric anaesthetics using a capsule rather than via a naso-gastric catheter, as we have indicated possible negative effects of catheter usage and intubation on eating behaviour. Moreover, in patients with FD nutrient drink tests are often used to estimate gastric sensation and accommodation.[53-55] It could be interesting to use nutrient drink test as an outcome rather than an *ad libitum* meal, as this possibly reflects gastric stretch more accurately. Therefore, we would propose to investigate the effect of gastric anaesthetics without using naso-

gastric catheters and the nutrient drink test as primary outcome measure in patients with postprandial distress syndrome.

Real time assessment to indicate triggers for postprandial distress

According to the Rome IV criteria, functional dyspepsia is a symptom-based diagnosis.[56] Use of patient reported outcome measures (PROMs) is recommended in both clinical and research settings, as objective outcome measures such as biological markers are lacking.
Experience Sampling Method (ESM) is an attractive method to overcome limitations posed by retrospective questionnaires. This novel technique has proven to be reliable and valid in psychiatric patients.[57] ESM is not only able to assess symptoms and evaluate treatment efficacy. ESM has the ability to provide detailed feedback to patients in order to enhance insight in their complaints. Therefore, ESM has the potential to increase patient compliance, facilitate self-management, and improve shared decision making.[58,59]
The use of ESM in patients with gastrointestinal disorders is limited compared with the use of ESM in patients with psychiatric disorders. Several studies from our group that evaluated ESM in patients with irritable bowel syndrome (IBS) found correlations between commonly used questionnaires and symptom scores on ESM. However, peak abdominal pain scores found using ESM were reported as mean abdominal pain scores on retrospective questionnaires.[60,61] Moreover, ESM was also able to demonstrate interactions between stress and abdominal complaints.[62,63]

In **chapter 6** and **Chapter 7**, we described the first use of an ESM-based PROM used for patients with FD. We found good concurrent validity, internal consistency and test-retest reliability. One important aspect of quality testing of this new PROM that is yet to be investigated, is the responsiveness of this PROM. The responsiveness of a PROM refers to its ability to detect change.[64,65] This is an important aspect to evaluate prior to evaluating treatment efficacy in FD patients by using this novel ESM-based PROM.
In **Chapter 7**, we showed the ability of ESM to evaluate the construct early satiation in FD patients. Moreover, both concurrent and preceding stress levels were associated with fullness levels in FD patients, but not in healthy controls. These findings lead us to assume that stress triggers the onset or worsening of fullness complaints in FD patients, rather than the other way around. Other studies have also shown the relationship between functional dyspepsia and psychological factors.[66,67] Another study demonstrated disturbed gastric sensory-motor function, that was manifested in an impaired gastric accommodation

reflex after acute anxiety, using a stressor.[68] Moreover, another study has shown altered brain activity in FD patients that were in anticipation of a stressor.[69]
We demonstrated that the novel ESM-based PROM can distinguish individual symptom patterns as well as interplays between concurrent and preceding symptoms.
Based on these results, we postulate that this novel ESM-based PROM has the potential to aid us in obtaining more insight into the disorder FD, in underlying mechanisms and symptom triggers, to improve shared-decision making, and self-management of patients with functional dyspepsia.

Conclusions and future directions

In this section of the discussion, we present suggestions for further research focusing on gastrointestinal delivery of tastants and blunting mechanoreceptors on food intake. Moreover, recommendations for further studies investigating PROMs in diagnosing and the management of functional dyspepsia are presented.

As demonstrated in **chapter 2**, the use of non-caloric tastants to reduce food intake could provide a useful tool to combat the obesity epidemic. However, data are in consistent and study procedures lack uniformity. Studies generally employ small sample sizes, different study designs, and different doses of tastants. Moreover, data on the long-term effects of gastrointestinal delivery of non-caloric tastants on eating behaviour are lacking. However, we believe this to be a field that should be further explored using more standardised and uniform protocols.

In order to standardise the study designs, we suggest several steps to be taken. First, it is unclear whether potential effects of tastant administration on eating behaviour diminish over time. Therefore, effects of repetitive administration of tastants on eating behaviour should be investigated. Second, underlying mechanisms on effect of gastrointestinal tastant administration should be more thoroughly investigated. As recent results show attenuated gastric motility and serum motilin, we propose to focus on these aspects.[21,23,26] Third, taste receptors are expressed throughout the entire GI tract.[12-15] **Chapter 3** and **Chapter 4** are the first studies to assess the effects of tastant administration on food intake in different locations in the GI tract. The optimal location of administration is yet to be elucidated. More research investigating delivery of tastants in different locations in the GI-tract is therefore needed. Fourth, the optimal dose of tastants to influence eating behaviour is unknown. Future studies should focus on finding the balance between the maximal possible effect without inducing side effects. A last and crucial step relates to the

implementation of successful interventions. Crucial is that longer-term effects of gastrointestinal delivery of tastants on eating behaviour are shown that ultimately result in body weight reduction.

In **chapter 5,** we showed that intragastric lidocaine infusion did not result in a significant increase in energy intake in this first in-human explorative study. However, we observed a significant increase in energy intake in treatment day 2 compared with treatment day 1. Future protocols should consider other delivery methods such as capsules. Moreover, the dose of lidocaine used was only 2.6 mg/kg body weight. As this dose proved to be safe and did not result in (cardiac) side effects, studies exploring the optimal dose of gastric lidocaine administration on energy intake should be conducted.
Furthermore, as local administration of anaesthetics in the stomach proved to be safe in healthy volunteers, the effects of gastric lidocaine administration on eating behaviour should be investigated in a population that show impaired energy intake. Moreover, outcome measures such as the nutrient drink test should be considered in these populations.

In **chapter 6** and **chapter 7**, we showed a novel tool to address symptoms of FD patients. Moreover, we indicated that concurrent stress and fullness levels are associated in FD patients. Future studies should evaluate the responsiveness (*i.e.*, the ability to detect change) of this novel ESM tool. Thereafter, researchers should make use of the ESM method to investigate patient-specific intervention methods in managing functional dyspepsia. Subsequently, this novel ESM tool should be optimised before implementation in patient care.

Concluding remarks

The studies presented in this thesis help to expand our understanding of potential novel treatment-, preventive-, and monitoring options in disrupted sensing and signalling of satiety. We focused on the potential of (bitter) tastants to reduce food intake and assessed the possibility of blunting the effects of mechanoreceptors in order to increase food intake. Moreover, we investigated the potential of real time assessment of PROMs in order to monitor FD and to search for specific triggers that can lead to fullness complaints.

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Summary

Various clinical situations can influence energy intake and sensing and signaling of satiety. Increased energy intake and diminished satiety signaling can be seen in the case of overweight and obesity. On the other hand, decreased energy intake and increased satiety can be seen in the aging population, also known as anorexia of aging. Furthermore, abnormal sensations such as recurring pain, stress, and anxiety can influence energy intake and satiety signaling. This in turn can result in food intake disorders such as avoidant/restrictive food intake disorder (ARFID). This thesis contains studies that provide an in-depth understanding of food-gut interactions in order to develop and optimize therapeutic strategies aiming at correcting conditions with disturbed (being too high or too low) energy intake. Furthermore, a novel tool to provide more insight into disturbed sensing and signaling of satiety as seen in functional dyspepsia was investigated.

Effects of non-caloric tastants on food intake behavior

Humans are able to detect five basic tastes: sweet, bitter, umami and salty. Interestingly, taste receptors are not only found on the tongue. Taste receptors can be found throughout the entire GI tract. A function of these taste receptors is to sense the presence of food in the gut. The systematic review and meta-analysis described in **Chapter 2** provides an overview of the effects of gastrointestinal delivery of non-caloric tastants on eating behavior. This review and meta-analysis showed that among non-caloric tastants, bitter tastants are the most potent to reduce food intake. However, further research and international collaboration is needed to move forward in this field.

One aspect that was not thoroughly investigated was the optimal location of effect of tastants on eating behavior. **Chapter 3** of this thesis describes a study investigating the effects of a tastant mixture (consisting of a sweet, bitter, and umami tastant) on *ad libitum* food intake and appetite sensations. Fourteen healthy volunteers were intubated with a naso-duodenal-ileal catheter with infusion ports in the duodenum and the ileum for four days. Hereafter, four test days with the following infusion combinations followed: duodenal placebo and ileal placebo, duodenal tastant mixture with ileal placebo, duodenal placebo with ileal tastant mixture, duodenal tastant mixture with ileal tastant mixture. We found no differences in food intake or appetite sensations between these four interventions. Herewith, we showed that a more distal infusion of tastants does not result in a stronger decrease in food intake compared with proximal infusion of tastants.

The findings of **chapter 2** indicated that among tastants, the bitter agents seem most potent in affecting eating behavior. As bitter is taste is evolutionarily linked to toxic substances, a more proximal delivery of bitter tastants might exert a larger inhibitory effect on food intake. In **chapter 4** of this thesis, we therefore investigated the effect of oral and/or intragastric delivery of the bitter tastant quinine on *ad libitum* food intake and appetite sensations. Thirty-two healthy volunteers underwent four regimens: both mouth wash and intragastric capsule with placebo, mouth wash with quinine and intragastric capsule with placebo, mouth wash with placebo and intragastric capsule with quinine, both mouth wash and intragastric capsule with quinine. We found no differences in food intake between the interventions. However, significant reductions in hunger and desire to eat scores were found for the quinine conditions compared with placebo. Herewith, we demonstrate the ability of a bitter tastant to influence appetite sensations that did not result in a reduction of food intake in this study.

Effects of lidocaine on food intake behavior

After ingestion of a meal, distension of the stomach wall is responsible for the initial satiating effect of a meal. Mechanoreceptors along the stomach wall sense the increase in stomach volume, in turn triggering neural circuits to inhibit food intake. In **chapter 5**, we described the first in-human study that investigated the effects of lidocaine infusion into the stomach on *ad libitum* food intake and appetite sensations. Moreover, safety of intragastric lidocaine infusion was assessed. Twenty-six healthy volunteers underwent two regimens: lidocaine and placebo infusion into the stomach. We found a modest, non-significant, increase in food intake after lidocaine infusion. Moreover, lidocaine infusion in the stomach was safe and no (cardiac) side effects were reported. Herewith, we demonstrated, in healthy volunteers, the potential of intragastric delivery of lidocaine to influence food intake behavior. Further studies need to clarify the role of intragastric lidocaine administration in patients suffering from postprandial distress syndrome.

Real time assessment to indicate triggers for postprandial distress

Functional dyspepsia (FD) is a symptom-based diagnosis. Objective outcome measures, such as biological markers are lacking. Therefore, the use of patient reported outcome measures (PROMs) is recommended in both research and clinical settings. Retrospective questionnaires pose several limitations that might be overcome by using the experience sampling method (ESM). ESM is an electronic structured questioning method that is characterized by random, repeated assessments in a patient's current state and environment. ESM has the ability to provide detailed feedback to patients in order to enhance insight in their complaints. Therefore, ESM has the potential to increase patient

compliance, facilitate self-management, and improve shared decision making. In **chapter 6**, we described the validation of an ESM-based PROM used for patients with FD. In this study, 35 patients with FD completed the previously developed ESM-based PROM for seven consecutive days. Moreover, the subjects completed end-of-day and end-of-week questionnaires that are currently being used in both clinical and research settings. We found good concurrent validity, internal consistency, and test-retest reliability of our novel PROM. Moreover, individual symptom patterns could be investigated using this PROM.

The ESM-based PROM provides us with the opportunity to investigate associations between concurring symptoms. Daily life stress is known to be able to influence a disturbance in gastric sensory and motor function. In **chapter 7**, we investigated the associations between fullness complaints and stress in patients with FD and healthy volunteers. Moreover, we evaluated whether real-time assessment of fullness is able to capture the construct early satiation, a cardinal symptom seen in patients with FD. Thirty-five patients with FD and 34 healthy volunteers completed the previously developed ESM-based PROM for seven consecutive days. We found that in patients with FD, but not in healthy volunteers, fullness showed significant associations with both concurrent and preceding stress scores. This indicates that stress induces fullness complaints in patients with FD. Moreover, we found that the construct of early satiation could be captured by the real-time measurement of fullness. Furthermore, we found high heterogeneity in symptom patterns between patients with FD. This once more emphasizes the need for a more individualistic approach in healthcare for patients with FD. We are convinced that this novel ESM-based PROM has the potential to aid in the transition towards personalized healthcare for patients with FD.

In **chapter 8**, we summarized and integrated the main findings of this thesis and discussed new insights and future directions.

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Samenvatting

Er zijn diverse klinische situaties die voedselinname en het waarnemen en signaleren van verzadiging kunnen beïnvloeden. Een verhoogde voedselinname en verzwakt verzadigingssignaal wordt gezien in het geval van overgewicht en obesitas. Aan de andere kant kan men verminderde voedselinname en een verhoogde verzadiging zien in bijvoorbeeld de oudere populatie. Dit wordt anorexia van het ouder worden genoemd. Hiernaast kunnen abnormale gevoelens in de buik, zoals terugkerende buikpijn, maar ook stress en angst voedselinname en verzadiging beïnvloeden. Dit kan leiden tot eetstoornissen zoals vermijdende/restrictieve voedselinname stoornis. Dit proefschrift bevat studies die zorgen voor een betere kennis over voedsel-darm interacties om zo therapeutische en preventieve strategieën te ontwikkelen en optimaliseren voor condities met verstoorde (zijnde te hoge of te lage) voedselinname. Ook is er een nieuw instrument ontwikkeld dat meer inzicht kan geven in de verstoorde waarneming en signalering van verzadiging gezien bij de ziekte functionele dyspepsie.

Effecten van niet-calorische smaakstoffen op voedselinname

De mens is in staat om vijf basissmaken te onderscheiden: zoet, bitter, umami, zuur en zout.
Smaakreceptoren worden niet alleen op de tong gevonden, maar komen voor in het gehele maagdarmstelsel. Een van de functies van deze smaakreceptoren is het waarnemen dat er voedsel aanwezig is in de darm. Het overzichtsartikel met meta-analyse beschreven in **Hoofstuk 2** geeft een actueel overzicht over de effecten van het toedienen van niet-calorische smaakstoffen in het maag-darmkanaal op eetgedrag. Uit dit overzichtsartikel komt naar voren dat van de niet-calorische smaakstoffen, bittere smaakstoffen het meest potent zijn in het reduceren van voedselinname. Echter, verder onderzoek en internationale samenwerking is nodig om de volgende stappen in dit onderzoeksveld te maken.

Een aspect dat onvoldoende onderzocht was is de optimale locatie van toediening van smaakstoffen voor het grootste effect op eetgedrag. **Hoofdstuk 3** van dit proefschrift beschrijft een studie waarin de effecten van een smaakstofmengsel (bestaande uit een zoete, een bittere en een umami smaakstof) op voedselinname en verzadigingsgevoelens zijn onderzocht. Voor dit onderzoek hebben we 14 gezonde vrijwilligers geïntubeerd met een neus-ileum sonde met infusieplaatsen in het duodenum (het eerste deel van de dunne darm, ook wel de twaalfvingerige darm genoemd) en het ileum (het laatste deel van de dunne darm, ook wel de kronkeldarm genoemd). Hierna kregen zij op vier verschillende

dagen de volgende infusiecombinaties: duodenaal en ileaal placebo, duodenaal het smaakstofmengsel en ileaal placebo, duodenaal placebo en ileaal het smaakstofmengsel, duodenaal en ilaal het smaakstofmengsel. In deze studie vonden we geen verschillen in voedselinname of verzadigingsgevoelens. Hiermee hebben we aangetoond dat het meer distaal, dus verderop in de darm, toedienden van smaakstoffen niet zorgt voor een sterkere rem op voedselinname vergeleken met meer proximale, dus meer aan het begin van de darm, toediening van smaakstoffen.

Een belangrijke bevinding uit **Hoofdstuk 2** was dat van de niet-calorische smaakstoffen, bittere smaakstoffen het meest potent bleken in het beïnvloeden van eetgedrag. Gezien bittere smaak evolutionair gekoppeld is aan giftige stoffen, zou het kunnen zijn dat het meer proximaal, dus hogerop in het maagdarmkanaal, toedienen van bittere smaakstoffen een sterker remmend effect op voedselinname heeft. In **Hoofdstuk 4** van dit proefschrift hebben we daarom de effecten van orale en/of intragastrische toediening van de bittere smaakstof kinine op voedselinname en verzadigingsgevoelens onderzocht. Voor dit onderzoek hebben 32 gezonde vrijwilligers vier testdagen ondergaan, waarbij zij de volgende interventiecombinaties kregen: mondspoeling en maagcapsule met placebo, mondspoeling met een kinine en maagcapsule met placebo, mondspoeling met placebo en maagcapsule met kinine, mondspoeling en maagcapsule met kinine. Uit dit onderzoek kwam naar voren dat er geen verschil was in voedselinname tussen de interventies. Echter, een significante afname in honger en de wens om te eten werd gevonden voor de kinine interventies vergeleken met placebo. Hierbij lieten wij zien dat kinine in staat is om verzadigingsgevoelens te beïnvloeden zonder dat dit leidde tot een verminderde voedselinname.

Effecten van lidocaïne op voedselinname

Het uitzetten van de maagwand zorgt voor het initiële verzadigingsgevoel na het innemen van een maaltijd. Er zijn zogeheten mechanoreceptoren te vinden op de maagwand die de rek van de maagwand en daarmee toename van het maagvolume waarnemen. Deze receptoren zorgen er vervolgens via neurale circuits voor dat voedselinname gestaakt wordt. Lidocaïne is een lokaal verdovingsmiddel, bekend van het gebruik door de tandarts bij het trekken van een kies. Mogelijk kan lidocaïne ook de bovengenoemde mechanoreceptoren verdoven. In **Hoofstuk 5** beschreven we de eerste humane studie die de effecten van toediening van lidocaïne in de maag op voedselinname en verzadigingsgevoelens onderzocht. Daarnaast hebben we in deze studie gekeken naar de veiligheid van het toedienen van lidocaïne in de maag. Voor dit onderzoek kregen 26 gezonde vrijwilligers op 2 dagen een neus-maagsonde waarmee op de ene dag lidocaïne en

de andere dag placebo werd ingespoten in de maag. We toonden een gematigde, niet-significante, toename in voedselinname. Daarnaast lieten we zien dat het toedienen van lidocaïne in de maag veilig was en niet leidde tot (cardiale) bijwerkingen. Hierbij hebben we het potentiële effect van lidocaïne toediening in de maag op voedselinname laten zien in gezonde vrijwilligers. Verder onderzoek is nodig om de rol van deze interventie bij patiënten die lijden aan een syndroom van vroege verzadiging, ook wel postprandiale nood genoemd, vast te stellen.

Real-time beoordeling van symptomen om triggers voor postprandiale nood te onderzoeken

Functionele dyspepsie (FD) is een symptoom-gebaseerde diagnose. Er zijn geen objectieve uitkomstmaten zoals biologische markers. Daarom wordt geadviseerd om patiënt gerapporteerde uitkomstmaten (PROMs) te gebruiken in zowel de onderzoeks- als de klinische setting. Retrospectieve vragenlijsten zorgen echter voor belangrijke tekortkomingen die mogelijk ondervangen worden door gebruik te maken van de experience sampling methode (ESM). ESM is een elektronisch gestructureerde manier van vragen stellen die gekarakteriseerd wordt door willekeurige, herhaalde metingen in de huidige status en omgeving van een patiënt. ESM heeft de mogelijkheid om gedetailleerde feedback te geven aan patiënten om zo inzicht in hun klachten te verschaffen. Daarom biedt ESM de mogelijkheid om therapietrouw van patiënten te verbeteren, zelfmanagement van symptomen te faciliteren en gedeelde besluitvorming te verbeteren. In **Hoofdstuk 6** hebben we de validatie van een op ESM gebaseerde PROM te gebruiken voor patiënten met FD beschreven. Voor deze studie hebben 35 patiënten met FD een eerder ontwikkelde op ESM gebaseerde PROM gedurende 7 dagen ingevuld. Ook vulden zijn einde-van-de-dag en einde-van-de-week vragenlijsten in die conventioneel worden gebruikt in zowel onderzoeks- als klinische setting. In deze studie vonden we goede validiteit ten opzichte van de conventionele vragenlijsten, goede interne consistentie en goede test-hertest betrouwbaarheid van dit nieuwe instrument. Daarnaast biedt deze nieuwe PROM de mogelijkheid om individuele symptoompatronen te onderzoeken.

Deze nieuwe op ESM gebaseerde PROM biedt ons de mogelijkheid om associaties tussen symptomen op hetzelfde moment te onderzoeken. Dagelijkse stress is een factor waarvan we weten dat deze in staat is om een verstoring in zowel de sensorische als motorische functie van de maag te bewerkstelligen. In **Hoofdstuk 7** hebben we de associatie tussen 'een vol gevoel' en stress onderzocht in patiënten met FD en gezonde vrijwilligers. Daarnaast hebben we bekeken of het real-time meten van 'een vol gevoel' in staat is om het construct vroege verzadiging, een belangrijk symptoom dat gezien wordt in patiënten

met FD, te vangen. Voor deze studie hebben 35 patiënten met FD en 34 vrijwilligers gedurende 7 dagen een op ESM gebaseerde PROM ingevuld. Hieruit kwam naar voren dat 'een vol gevoel' significant geassocieerd was met zowel gelijktijdige als voorafgaande stress scores in patiënten met FD, maar niet in gezonde vrijwilligers. Daarnaast hebben we aangetoond dat het construct vroege verzadiging gevangen kan worden door het real-time meten van 'een vol gevoel'. Ook zagen wij dat er een hoge heterogeniteit was in symptoompatronen tussen patiënten met FD. Dit onderstreept eens te meer dat er een meer individualistische aanpak nodig is in de zorg voor patiënten met FD. Wij zijn ervan overtuigd dat deze nieuwe op ESM gebaseerde PROM de mogelijkheid biedt om een rol te spelen in de transitie richting gepersonaliseerde gezondheidszorg voor patiënten met FD.

In **Hoofdstuk 8** hebben we de resultaten van dit proefschrift samengevat en geïntegreerd. Daarnaast hebben we nieuwe inzichten en vervolgstappen bediscussieerd.

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Addendum

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Since 1975, the prevalence of obesity in the population has nearly tripled. Obesity and overweight pose an evident burden and are linked to the occurrence of several diseases such as diabetes mellitus and cardiovascular diseases. Currently, only surgical options are an effective strategy for long-term weight loss. However, surgical treatment is invasive and only available for a selected group of morbidly obese subjects. Therefore, novel non-invasive treatment and preventive strategies are needed to battle the obesity epidemic. On the other hand, decreased food intake and increased satiety feelings are frequently seen in the aging population. A possible explanation could be altered sensitivity of pressure and nutrient and taste sensors in the stomach and small bowel. The gastrointestinal tract plays an important role in regulating food intake behaviour via release of gut peptides. Therefore, food-gut interactions could provide us with a strategy to influence and manipulate food intake behaviour.
The signalling of appetite can also be disrupted by abnormal sensations such as stress, anxiety, and recurrent pain. This can result in avoidance of food intake. Functional dyspepsia is a disorder in which patients experience symptoms such as bothersome early satiation, postprandial fullness, epigastric pain, and/or epigastric burning. However, no evidence for systemic, metabolic, or organic diseases can be found that is able to explain these symptoms. The presence of these symptoms is already sufficient to result in avoiding further food intake.

The aim of this thesis was to provide an in-depth understanding of interactions between food and the gastrointestinal tract related to satiety and eating behaviour. Hereby, we aimed to provide leads and thereafter develop and optimise therapeutic and preventive strategies that can correct disturbed (*i.e.*, too high or too low) energy intake. Moreover, this thesis described the validation and application of a novel tool that will provide more insight into the disturbed sensing and signalling of appetite as observed in patients with functional dyspepsia.

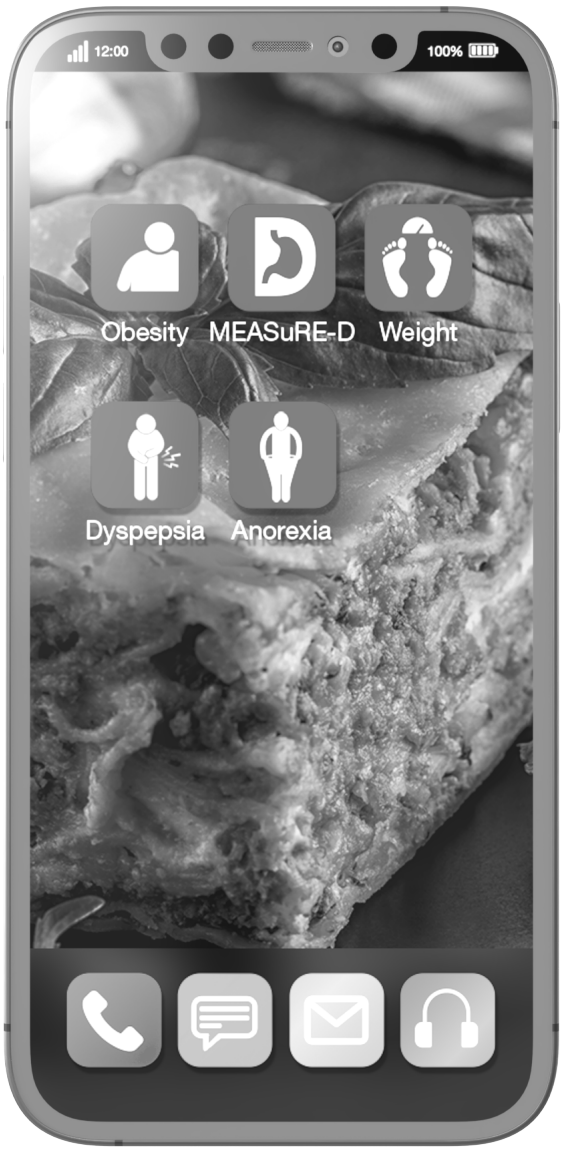
This thesis highlighted the potential role of non-caloric bitter substances in decreasing food intake. However, this decrease in food intake is rather modest. Appetite signalling in obese individuals is substantially dysregulated. Therefore, the place of bitter substances in battling the obesity epidemic should be more as a preventive strategy, rather than a treatment strategy. This is also in line with the current concept of shifting the focus in healthcare from therapeutic care and cure towards prevention. In this thesis the scientific steps that need to be undertaken before implementing this preventive strategy were

provided. The most important step is harmonising the designs of studies conducted by different research groups in order to generate more consistent data.

An intriguing observation in this thesis was that that local application of lidocaine, a local anaesthetic, in the stomach in humans was able to modify food intake although the overall effect was not statistically significant. However, in order to apply lidocaine a rather invasive method was used. We cannot exclude that the intubation negatively influenced the study results. Therefore, we advise future studies to consider other methods of administration of lidocaine in the stomach in order to further substantiate this concept.

Additional studies in this thesis investigated the usefulness of a novel assessment method for patients with functional dyspepsia. In these studies, we demonstrated the ability of this assessment method to capture the same constructs as conventional questionnaires. Moreover, we demonstrated the ability of the new assessment method to characterise individual symptom patterns. This provides us with a tool that can help move towards a more personalised approach in treating this functional symptom-based disorder. Using this novel tool, patients will be able to develop disease insight, shared-decision making can be improved, and patients can be provided with tools for self-management of their disease.

In summary, the first part of this thesis focused on generating knowledge on a strategy that has the potential to prevent the expansion of the obesity epidemic. Second, the first in-human experiment investigating a novel treatment tool for individuals with reduced food intake has been described. This paves the way for research to further elucidate the concept of modifying gastrointestinal responses to specific luminal triggers to influence satiety signalling and eating behaviour. Third, a novel tool that can aid functional dyspepsia patients in the transition towards personalised health care has been evaluated and was validated. Overall, this thesis, therefore, provided novel strategies to address disrupted sensing and signalling of satiety and eating behaviour.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List of publications

Wilbrink J, Masclee GMC, **Klaassen T**, van Avesaat M, Keszthelyi D, Masclee AAM. Review on the regional effects of gastrointestinal luminal stimulation on appetite and energy intake: (pre)clinical observations. Nutrients. 2021;13 (1601).

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Beckers AB, Snijkers JTW, Weerts ZZRM, Vork L, **Klaassen T**, Smeets FGM, Masclee AAM, Keszthelyi D. Digital instruments for reporting of gastrointestinal symptoms in clinical trials: comparison of end-of-day diaries versus experience sampling method.

Oral presentations

Digestive Disease Days, spring 2021, Veldhoven, the Netherlands – The interplay between stress and fullness in functional dyspepsia and healthy controls: an exploratory experience sampling method study.

Digestive Disease Days, autumn 2020, Veldhoven, the Netherlands – Oral or intragastric delivery of the bitter tastant quinine does not influence food intake.

NUTRIM symposium 2019, Maastricht the Netherlands – Oral or intragastric delivery of the bitter tastant quinine does not influence food intake.

Poster presentations

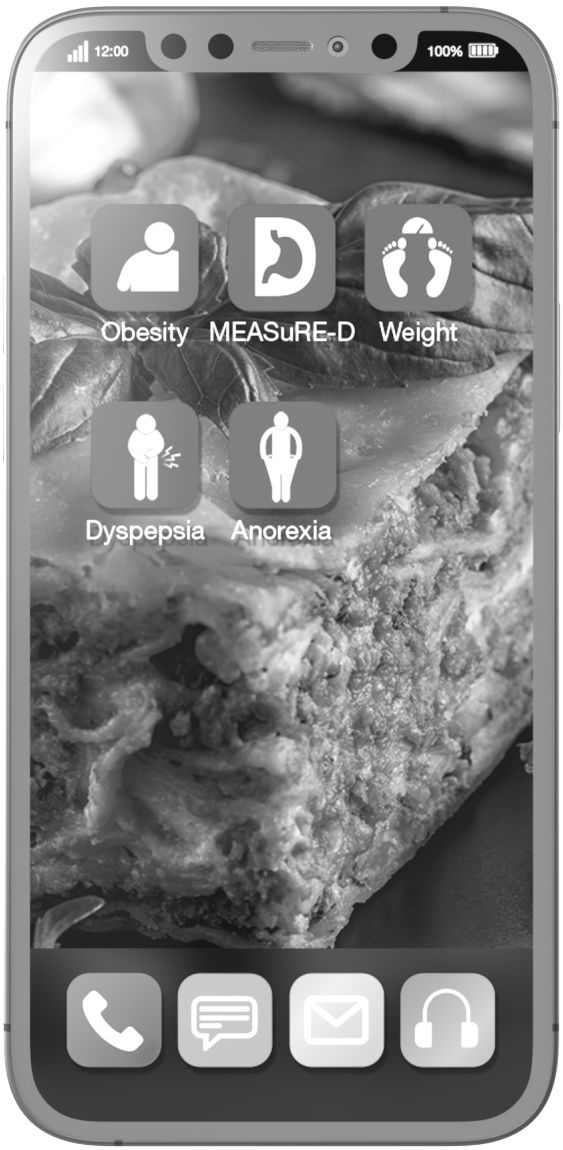
Digestive Disease Days, spring 2021, Veldhoven, the Netherlands – Psychometric evaluation of an experience sampling method-based Patient-Reported Outcome Measure in Functional Dyspepsia.

NUTRIM symposium 2019, Maastricht the Netherlands – Oral or intragastric delivery of the bitter tastant quinine does not influence food intake.

Neurogastro 2019, Lisboa, Portugal – Intraintestinal delivery of tastants using a naso-duodenal-ileal catheter does not influence food intake or satiety.

Wetenschapsdag MUMC+ 2019, Maastricht, the Netherlands – Intraintestinal delivery of tastants using a naso-duodenal-ileal catheter does not influence food intake or satiety.

NUTRIM symposium 2018, Maastricht, the Netherlands – Intraintestinal delivery of tastants using a naso-duodenal-ileal catheter does not influence food intake or satiety.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Dankwoord

Wat een fijn gevoel om mijn woorden van dank neer te mogen schrijven. Er zijn veel mensen direct of indirect betrokken geweest bij het tot stand komen van dit proefschrift. Daarvoor mijn onuitputtelijke dank.

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ben je een mooi mens met het hart op de goede plek. En ja, jij bent het beste in tafelvoetbal. Ik ben blij dat je naast me wilt staan tijdens mijn verdediging en kijk uit naar de gezamenlijke onderwijsmomenten tijdens de opleiding!

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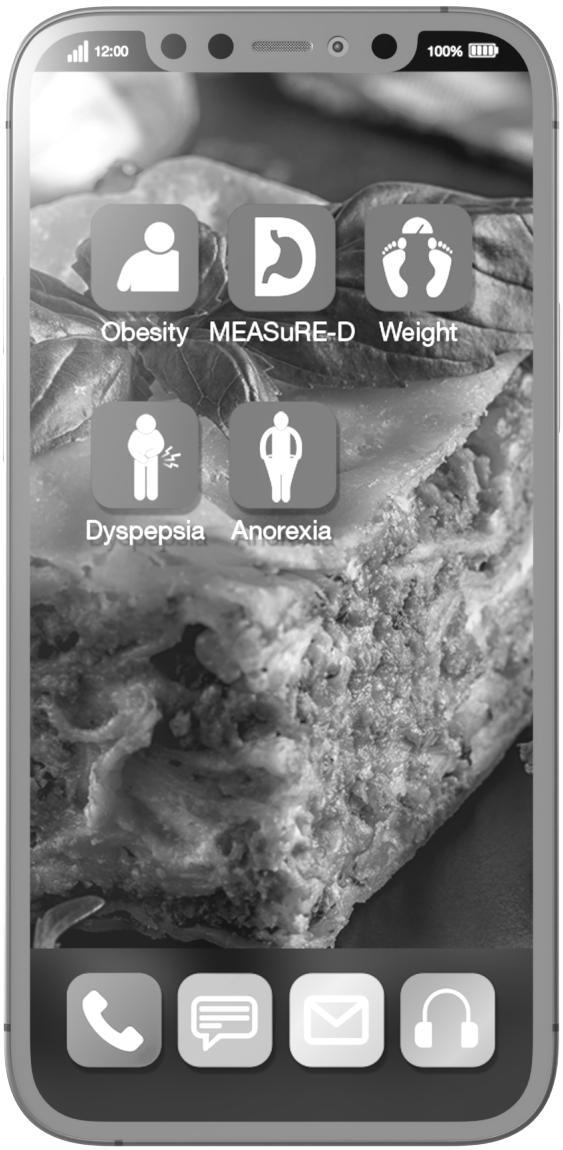
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Curriculum Vitae

Tim Klaassen was born on the 5th of September 1990 in Helmond, the Netherlands. After finishing secondary school at the Udens College in 2008, he studied Biomedical sciences at the University of Amsterdam and obtained his BSc degree in 2011. Hereafter, he studied Psychology at the University of Amsterdam and obtained his propaedeutic diploma with merit in 2012. Hereafter, he studied the master Physician – Clinical investigator at Maastricht University and obtained his MSc in 2016. During this master, he did his internship 'mother and child' at Academical Hospital Paramaribo in Paramaribo, Surinam. After graduating in 2016, Tim started with the research project described in this thesis: "Novel strategies to address disrupted sensing and signalling of satiety", supervised by Prof. dr. A.A.M. Masclee, Dr. D. Keszthelyi, and Dr. F.J. Troost at Maastricht University. During this project, Tim had the opportunity to publish manuscripts in international peer reviewed journals and to present his work at several conferences. In January 2021 he started his residency Gastroenterology-Hepatology under the supervision of Dr. J.W. Kruimel.