A Feeling of Full-Filment

Sensory and physiological processes involved in satiation



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A Feeling of Full-Filment

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“I began to realize how important it was to be an enthusiast in life.

If you are interested in something, no matter what it is,

go at it at full speed ahead. “

— Roald Dahl

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Chapter 1

General Introduction

General introduction

You are eating your favorite meal but after eating for some time, you feel fulfilled and decide to stop eating. In short; you feel satiated. Even though some foods are similar in caloric and nutrient content, they are not necessarily equally satiating. How much we need to eat to feel satiated is dependent on the taste and texture of the food that is eaten. For example, soft foods with little taste are less satiating, i.e. we need to eat more of these foods to feel full, compared to harder foods with higher taste intensity. Food products such as the latter may help control our food intake and prevent overconsumption (1-3). Overconsumption is when energy intake is exceeding energy expenditure, and chronic overconsumption leads to body weight gain (4-11). To control food intake such that it does not lead to overconsumption is difficult, especially for highly palatable energy dense foods that can be consumed quickly (12-15). Eating foods that have a high satiating value relative to their energy content may help us to control the amount we eat (16-18). This mechanism of controlling food intake was first defined by W.B. Cannon in 1912. He stated that the sensation of 'having had enough' or the 'feeling of satiation' is little understood, but has the function of protecting an organism against being overstocked with food or water (19, 20). To date, there is still much to be discovered about the mechanisms controlling food intake. Considering the high numbers of overweight and obese people world wide, understanding satiation seems more important than ever (21).

Satiation and the control of food intake

Plato described satiation in the dialogue *Timaeus*. In this dialogue he described that food and drinks are consumed in meals, that the stomach serves as a storage organ and that there are mechanisms that terminate meals (22, 23). Nowadays, satiation is defined as a proccess that occurs at the time of eating and involves inhibitory signals at a sensory, hormonal, digestive and cognitive level (24). Satiation attenuates hedonic reactions to the food eaten by down regulation of the reward system in the brain (25). Satiation signals eventually lead to meal cessation after which a period of satiety, the time between two eating occasions, starts (26).

Food texture and taste influence the proces of satiation. For example, liquids have been shown to be less satiating than solids (27, 28). One of the proposed mechanisms behind this is that solid products induce more oro-sensory exposure (in-mouth sensory stimulation of food), which induces stronger endocrine responses (27, 29). In addition, solids are ingested at a slower eating rate and are emptied from the stomach slower, compared to liquid food products (30-33).

The process of satiation thus involves sensory, endocrine and gastric signals that do not operate independently. The association between these systems and how sensory and gastric signals are processed in the brain will be described below in the following paragraphs (Figure 1).

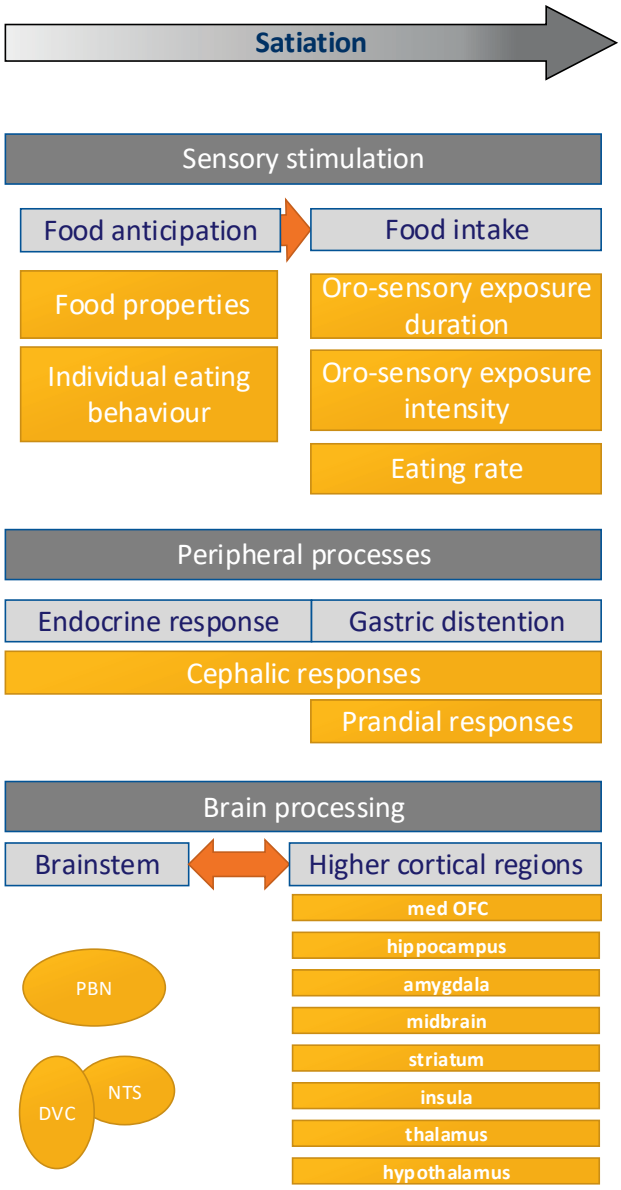

Figure 1. Diagram showing satiation as a function of the the three levels: sensory stimulation, peripheral processes and brain processing. Figure is partly based on Hopkins and Blundell *et al.* (17, 34). *PBN= parabrachial nuclei, NTS=nuclei of the solitary tract, DVC=dorsal vagal complex, med OFC= medial orbifrontal cortex*.

Sensory stimulation

The sensory properties of a food combined with individual eating behaviour determine the in-mouth exposure to the food or, in other words, the oro-sensory exposure (OSE). The degree of OSE has two determinants: the duration of exposure and the intensity of exposure. Together, these two aspects influence satiation.

OSE intensity and duration are affected by the texture and taste of food. Texture is the perception of the foods' structure, and from now on food structure is refered to as texture. Food texture can be classified into four categories: liquid, semi-solid, soft solid and hard solid (35). Different textures require different oral processes and both change over the course of a bite and meal (35). Foods that have a hard texture require more oral processing effort per bite to form a ready to swallow bolus (36). A food bolus is ready to swallow when the particle-size threshold is met (around 2 mm) and bolus lubrication is sufficient to safely swallow the food (37, 38). Because of the longer processing time of harder foods, hard foods are eaten at a slower rate compared to softer foods (39).

Besides the texture of food that influences OSE duration, taste influences the intensity of the exposure (40, 41). Oro-sensory exposure intensity depends on the taste that is perceived when tastants bind to the taste receptors on the tongue. Whether the tastant binds to the receptor depends on the solubility in saliva, which is determined by the food's texture (42). Food texture influences taste intensity due to the surface availability and the ability for tastants to dissolve. For example, two foods with the same amount of sucrose but differing in texture, (liquid vs solid), have different sweetness intensities (43, 44). For a solid food product to be equally sweet to a liquid, twice the amount of sucrose is needed (45, 46).

Eating behaviour also influences OSE. Two important eating behaviour factors are eating rate and oral processing, which are closely related. For example, when a person chews more, OSE duration is increased and often this leads to a decreased eating rate (47). Oral processing such as chewing behaviour and eating rate differs between individuals and is dependent on gender, age, ethnicity and weight status (64-67).

Orosensory duration and intensity both have been shown to affect food intake. Increased oral processing and reduced eating rate both have been shown to reduce food intake within a meal (27, 48-60). For example, liquids do not require oral processing and are therefore consumed fast and induce little OSE compared to semi-solid or solid foods that do require oral processing before a safe to swallow bolus is formed. This fast ingestion of foods that require little oral processing leads to a higher energy intake (27, 48-60). OSE therefore has an important role in satiation (53, 60-62).

The intensity of oro-sensory exposure or taste intensity has also been shown to affect the amount we eat. For example, foods that have low sweet taste intensities are consumed in larger amounts compared to the same food with a high sweetness intensity (41, 63-65). One of the proposed mechanisms is that low taste intensity signals low energy density and, therefore, these types of food are perceived as being less satiating (66). Another mechanism through wich OSE intensity may affect satiation is through sensory specific satiety, which is the decrease in liking for the food eaten relative to an uneaten food (67). Strong taste intensities may induce sensory specific satiation faster compared to low taste intensities.This decrease in liking may lead to meal termination.

OSE duration, intensity and eating behaviour thus interact, and together determine sensory perception and satiation (54, 68-72). However, the underlying physiological mechanisms through which sensory signals affect satiation are largely unknown.

Peripheral processes

Pre-absorptive endocrine responses

A proposed mechanism through which OSE affects food intake is through cephalic phase responses (CPRs) (18, 67, 73-75). CPRs are neurally mediated anticipatory and conditioned responses to food cues and are considered the first phase of digestion (73, 74, 76). Pavlov discoverd cephalic, meaning 'head related', responses in 1902. These, at the time called 'psychic secretions' were observed in the well-known bell study in which a dog learned to associate the ring of a bel with receiving food. Conditioned learning led the dog to increase saliva production when hearing the bell even without the presence of food (77). Besides the increase in saliva production, numerous other cephalic responses have been observed. These include production of gastric juice, secretion of bile by the gallbladder, increased gut motility, and gastric and pancreatic endocrine secretions (76, 78-81). The supposed function of CPRs is to prepare the body for incoming food and subsequent digestion (18, 82, 83). In addition, there seems to be a distinct function of early and late cephalic phase responses. Early CPRs prevent disruption of homeostasis when food is ingested, therefore CPRS may allow for larger meals. (18, 74). However, late CPRs are thought to aid in meal termination and hence help control food intake (74, 84). Reduced CPRs are associated with decreased appetite responses and weight gain as suggested by both animal (85-87) and human (74, 88) studies.

CPRs may not be triggered optimally when food is consumed quickly with little oro-sensory exposure (31, 89). Cephalic responses that may be affected by this are endocrine responses involved in satiation. Insulin, pancreatic polypeptide and ghrelin are among others hormones

secreted during the cephalic phase (74, 90-100). The most studied cephalic hormones are insulin and pancreatic polypeptide. Early meal (5-15 min after meal onset) increases in insulin and pancreatic polyppetide (PP) have been observed (93-96, 99). These early endocrine responses are thought to play an important role in glucose homeostasis and food intake control (90, 91). However, when OSE is of low intensity and short duration, the sensory stimulation may be too little to trigger a cephalic insulin or pancreatic polypeptide response and consequently the feeling of feeling full or satiated will be postponed, allowing for larger meal sizes.

Gastric distention

Next to cephalic phase responses, gastric distention and gastric emptying stimulate satiation (26, 101). The stomach is thought to be the primary gastro-intestinal organ that controls food intake (19). Factors that determine meal cessation through gastric signalling are meal volume, meal weight and indirectly gastric and intestine endocrine responses that slow down gastric emptying (102, 103). For example, stomach distention activates stretch and mechanoreceptors that send satiety signals to the brain through vagal afferent nerves (19). Besides this, gastric emptying of nutrients into the duodenum sends satiety hormone signals such as CCK, PYY and GLP-1 to the brain (104, 105).

Foods with a harder texture may induce earlier satiation indirectly through OSE by slowing down stomach empyting. For example, a study of Cecil *et al.* shows that gastric empyting is slowed down and satiety increased when the food is administered orally compared to intragastrically (106). Besides OSE, food texture also directly affects stomach kinetics. For example, more textured foods stay longer in the stomach, as larger solid particles require a longer grinding period to reduce particle size to a level appropriate for emptying (107). In addition, more viscous foods increase gastric distention by reducing the gastric emptying rate (108).

To summarize, foods with a hard texture require more oral processing which leads to increased OSE. OSE is important to elicit cephalic phase responses and it may also effect gastric emptying. Therefore, texture has an indirect effect (through OSE) on cephalic phase responses and gastric emptying rate. Furthermore, food texture may directly affect gastric emptying rate as longer griding is required to reduce particle size. These process induces satiation but how these signals interact and how they are processed in the brain is not fully understood.

Brain processing

Sensory, and gastric signals travel through the facial, glossopharyngeal and vagus nerve to the brain (51, 58). Besides this direct communication between the gastro- intenstinal (GI) tract and the brain, a slower endocrine system communicates the presence of food within the GI to the brain (109-111). All these food related signals arrive in the brainstem (109, 112-115). Specific areas involved in detection of these signals are the dorsal vagal complex (DVC) in the medulla and the the parabrachial nucleus (PBN) in the pons. The DVC includes the nucleus of the solitary tract (NTS), the dorsal motor vagal nucleus (DMV) and area postrema. Circulating metabolites and hormones such as glucose and CCK are detected in the DMV whereas tast signals are detected in the NTS and PBN. Gastric distention signals are specifically detected in the PBN. Integration of energy related signals and mid- and forebrain input takes place in the area postrema (109, 112-115).

The brainstem then projects these signals to hepatic and gastric efferent nerves and to other regions of the brain, such as the hypothalamus, amygdala, insula, parahippocampal gyrus, orbitofrontal cortex, striatum, and midbrain to regulate endocrine release, appetite and food intake (109, 110). The hypothalamus is the main part of the brain involved in food intake control (116-118). This part of the brain is continuously informed about the metabolic state of the body via the brainstem and controls homeostatic food intake by up-regulation of satiety peptides to decrease appetite (101).

However, these homeostatic appetite signals can be overruled by 'hedonic eating' when the food that is eaten is rewarding. The reward value of the food expressed in 'wanting to eat' the food item is processed in the reward system of the brain which includes the midbrain, striatum (including the putamen and caudate), from which it projects to the orbifrontal cortex, amygdala, anterior cingulate cortex and insula (117).

The current obesogenic environment includes many highly palatable foods that appear to cause a disbalance between these homeostatic and reward signals. This disbalance may lead to a drive to eat in the absence of hunger, when feeling satiated, which promotes overconsumption (17). Overconsumption seems to be less for foods with a hard texture and high taste intensity, that induce high oro-sensory exposure and slow down eating rate. However, little is known about how these sensory signals are processed in the brain and affect satiation.

To conclude, sensory and endocrine signals travel to the DVC and PBN in the brainstem to higher homeostatic and reward brain regions.Together these regions are involved in the control of food intake and satiation. However, how sensory and gastric signals are processed in the brain to affect satiation is not yet known.

Thesis aim and rationale

The overall aim of the research described in this thesis is to better understand the role of sensory signals in the physiological processes underlying satiation.

To study this, we investigated the effect of oro-sensory exposure (duration and intensity) and eating rate on food intake, associated (cephalic) endocrine responses and brain reactivity.

By understanding how these sensory and eating behaviour factors affect physiology and consequently satiation, we may bettter understand how the current obesogenic environment drives overeating. Insight in this process could guide dietary and behavioural strategies to maintain a healthy body weight.

Thesis outline

The research in this thesis is depicted in Table 1. The aim of **chapter 2** was to determine the independent contributions of oral processing time and taste intensity and their combined effect on satiation. Taste and texture of model foods was manipulated such that the intensity of sweet taste was high or low and texture hard or soft. Participants were instructed to eat until fullness of each type of model food in separate sessions.

To understand the physiological mechanism behind taste and texture effects on intake we used the same model foods to study endocrine cephalic phase responses when participants modified sham fed (chew and spit) the model foods (**chapter 3**). To determine whether oro-sensory exposure duration and/or eating rate had an effect on endocrine responses we set up a study where participants ate untill fullness of chocolate custard with and without fudge pieces (low or high oro-sensory exposure) at two different eating rates (slow or fast eating rate) (**chapter 4**). Furthermore, we weighed the existing data on cephalic phase insulin and pancreatic polypeptide responses in a systematic review (**chapter 5**).

Last, we studied vagal afferent taste signals and their role in satiation. To do this we performed an fMRI study where we measured neural reactivity in the brain(stem) to chocolate milk over the course of satiation (**chapter 6**). In **chapter 7**, the overall results and their implications are discussed and suggestions for future research are given.

Table 1. Factors studied within this thesis (Figure 1), and the associated outcome measures and chapters.

Main factor	**Sub factors**		**Outcome**	**Chapters**
Sensory stimulation	Food anticipation	Food properties	Hardness; stress and strain	**2**
			Sensory characteristics	**2,4,6**
	Food ingestion	Oro-sensory exposure intensity	Food intake Subjective appetite Eating behaviour	**2,6**
		Oro-sensory exposure duration Oral processing effort Eating rate	Food intake Subjective appetite Eating behaviour	**2,4**
Pre-absorptive Processes	Endorine	Cephalic responses	Insulin Pancreatic polypeptide Ghrelin Saliva production	**3,4,5**
		Prandial responses	Insulin Pancreatic polypeptide Ghrelin	**4**
	Gastric	Prandial responses	Stomach distention	**6**
Brain processing	Brainstem	Brain regions	Brain reactivity	**6**

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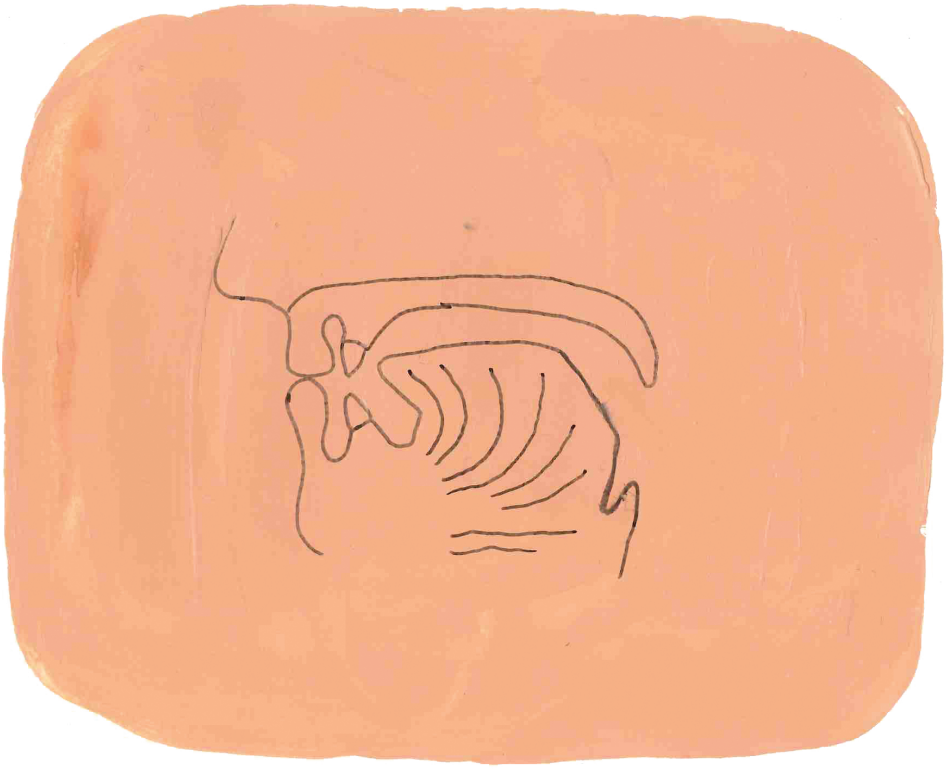

Chapter 2

Comparison of oro-sensory exposure duration and intensity manipulations on satiation

Previously published (with minor adaptations): Lasschuijt MP, Mars M, Stieger M, Miquel-Kergoat S, de Graaf C, Smeets PAM. Comparison of oro-sensory exposure duration and intensity manipulations on satiation. Physiology & Behaviour 2017;176:76-83. doi: 10.1016/j.physbeh.2017.02.003.

Abstract

Oro-sensory exposure (OSE) is an important factor in the regulation of food intake with increasing OSE leading to lower food intake. Oral processing time and taste intensity both play an important role in OSE but their individual contribution to satiation is unknown. We aimed to determine the independent and combined effects of oral processing time and taste intensity on satiation.

Fifty eight participants (23 ± 9 y, BMI 22 ± 2 kg/m^2) participated in a 2x2 factorial randomized crossover study in which they consumed one of four gel-based model foods until satiation during four sessions. Model foods were offered *ad libitum* and differed in texture (soft or hard texture, yielding shorter and longer oral processing time) and sweetness (low or high intensity). Model foods were isocaloric and were matched for flavour and palatability. Outcome measures were intake of the model food and the microstructure of eating behaviour, such as number of chews and eating rate.

There was an overall significant effect of texture ($p < 0.001$) but not sweetness ($p = 0.33$) on intake with a 29.2 % higher intake of the soft model foods compared to the hard model foods. After correction for palatability the difference in intake between the soft and hard model foods was 21.5 % ($p < 0.001$).

The number of chews was significantly lower for the soft (10.1 ± 6.2) than for the hard (26.9 ± 6.2) model foods ($p < 0.001$), which resulted in a significantly lower eating rate (soft, 26.3 ± 10.2 and hard, 15.3 ± 7.1 g/min, $p < 0.001$).

These results show that increasing texture hardness of gel model foods decreases food intake independent of sweet taste intensity. The higher number of chews and faster eating rate may cause this effect. In conclusion, oro-sensory exposure duration rather than taste intensity appears to be the main determinant of food intake.

Introduction

The abundance of palatable, high-energy dense foods in modern society is a major contributor to the obesity problem (1). This obesogenic environment offers many foods that can be eaten quickly (2-7). The fast rate by which consumers can ingest calories, results in overconsumption and weight gain, and has been associated with obesity (8-11). This rapid eating of food is associated with a short oral transit time and reduced oro-sensory exposure (OSE) to food. OSE is the collective stimulation of receptors on the tongue and in the oral cavity and is important for meal termination (12-17).

Oro-sensory stimulation affects intake and depends on food properties such as shape, odor, flavour, texture and taste (18-22). Enhanced sensory stimulation through taste and texture increases OSE and leads to early meal termination and limits food intake (12-15). Food texture does not only provide sensory stimulation *per se* but it also determines the duration of OSE by affecting the oral processing time that is needed to produce a food bolus that is safe to swallow (23). This may be the key reason why people easily consume more of foods that can be ingested quickly ('fast foods'). For example, Zijlstra *et al.* showed that participants consumed 30% more of a liquid compared to a semi-solid product that was similar in palatability, energy density and macronutrient composition (24). Moreover, the same authors showed that when oral processing time was increased, food intake decreased (25). This difference in intake has also been found comparing intake of solid foods with different hardness levels. Participants lowered their lunch meal intake with 13% when given hard lunch meal foods compared to soft lunch meal foods (26). However, the difference between soft and hard textures should be of a certain size in order to affect intake (27).

In addition to oral processing time, which determines the duration of oro-sensory stimulation, the degree of OSE is also determined by taste intensity (28). Foods containing low salt or sugar levels will provide less oro-sensory stimulation as they generally have low taste intensity (29-31). This effect of taste intensity on satiation has been shown for both sweet and salty foods (32, 33). One of the mechanisms behind this might be that low taste intensity signals low energy density and, therefore, these types of food are perceived as being less satiating (28).

Oral processing time and taste intensity are not independent; De Wijk *et al.* (2003) found that increased sweetness results in increased oral processing time and oral movements (34). Thus, the duration of OSE is not only determined by the food's texture but also by its taste intensity (35, 36). Likewise, texture determines the duration of the taste sensation in the mouth and influences the perception of taste intensity (37, 38). For example, solid foods require up to 200% more sugar to be perceived as being equally sweet as liquids (37, 38). It is therefore not

surprising that texture and taste also interact in their effect on satiation or food intake. This has been demonstrated by Forde *et al.* who found an interactive effect of high savoury taste and texture hardness on food intake (39).

Accordingly, foods that have a semi-liquid texture are less satiating and eaten in larger quantities compared to hard textured foods. In addition, low taste intensity foods seem to be less satiating compared to high taste intensity foods. However, the evidence for an effect of taste is not as strong as evidence for an effect of texture on food intake (13, 14, 40-43). The separate and interactive effects of oral processing time and taste intensity on satiation have yet to be studied.

To manipulate foods such that their properties promote lower food intake it is important to understand how food properties interact and which property contributes most to satiation; oral processing time or taste intensity. Therefore, the objective of this study was to determine the independent contributions of oral processing time and taste intensity and their combined effect on satiation. Our secondary aim was to explore how taste intensity and oral processing time affect microstructure eating behaviour and whether this may explain the differences in satiation. To study this we performed a 2x2 factorial randomized crossover study in which the degree of OSE stimulation was varied through different levels of texture hardness and sweetness. A higher degree of OSE (high taste intensity, hard texture) was hypothesized to lower food intake. In addition we expected that increased taste intensity and texture hardness would lead to a higher number of chews, longer oral processing time and slower eating rate.

Participants and methods

Participants

The study was performed at Wageningen University, The Netherlands. Participants were recruited from the surroundings of Wageningen using flyers and posters. In addition, emails were sent to persons in a database of volunteers who had previously expressed an interest in participating in nutrition studies. Participants had to be healthy, between 18-50 years old, and have a BMI between 18.5-27 kg/m^2. Participants were excluded if they indicated that they had a lack of appetite or had dental, chewing or swallowing problems. Participants were also excluded if they followed an energy-restricted diet or if they had gained or lost >5 kg of body weight during the past two months. In addition, they were not allowed to participate if they were high restrained eaters according to the Dutch Eating Behaviour Questionnaire (DEBQ): score >2.89 for men and >3.39 for women (44).

Personnel of the Division of Human Nutrition were omitted from participation together with students writing their Master thesis at the same division.

Potential participants were invited to a screening visit at Wageningen University. Height and body weight were measured. In addition, pre-defined hedonic and sensory attributes of the model foods were rated. Participants were excluded from participation if they disliked one of the model foods (score <4 on a nine point hedonic Likert scale) or had a stronger preference for one of the model foods (>2 point difference on a nine point hedonic Likert scale).

The study was approved by the Medical Ethical Committee of Wageningen University (ABR54634) and registered under NTR5523 (http://www.trialregister.nl). All participants signed informed consent prior to the screening. Participants were not informed on the exact aim until after the study, instead they were told that the study aimed to investigate eating behaviour in general. Participants received a financial compensation.

174 participants participated in the screening. After the screening, 66 participants were found eligible. However, three of them were not able to participate in the test sessions because of lack of time, three did not want to participate anymore and two were lost to follow up.

In total, 58 participants (14 men) completed the study. Participants were 23 ± 9 years old, had a mean (±SD) body mass index of 22 ± 2 kg/m^2 , men had an average DEBQ restraint score of 1.6 ± 0.4 and women had an average score of 2.8 ± 0.6 (44).

Experimental design

The study had a 2x2 factorial, randomized crossover-design where the models foods (MF) were formulated to have two levels of oral processing time (soft or hard texture), and two levels of sweetness (low or high). The MFs were similar in caloric content and palatability. During lunch visits, participants ate a portion of one of the four MFs, that is hard sweet- hard non-sweet, soft sweet or soft non-sweet. Treatment order was randomized, following a Latin square Williams design. Based on previous studies [11, 22, 31] we estimated an effect size of 15 gram with a within participant standard deviation of 30 gram. Including an estimated dropout rate of 10% we aimed to enroll 65 participants.

Model foods

The foods used in the study were strawberry flavoured model food gels, and were comparable with MFs used in previous studies (34, 45). To manipulate the oral processing time, the hardness of the gels was manipulated by altering the concentration of the thickening agents carrageenan (type CHP-2) and locust-bean gum (LBG). Thickening agents were added in the following amounts: 0.22/0.22 wt.% carrageenan/LBG for the soft and 0.66/0.88 wt.% carrageenan/LBG for the hard MF.

In order to manipulate the sweetness of the MF without changing the caloric content a non-caloric liquid sweetener (Natrena ® Classic: cyclamate, saccharine) was used. Other ingredients were cream (30% fat, AH Basic), strawberry flavoured pudding powder ('Jelly', dr. Oetker) and water. Per 100 gram the hard models foods contained 627 kJ (150 kcal), 16.5 g carbohydrates, 9.0 g fat and 0.8 g protein the soft model foods contained 615 kJ (147 kcal), 15.6 g carbohydrates, 9.0 g fat and 0.8 g protein. Model foods were offered to participants in small round slices (26 mm diameter, 10 mm height) of approximately 7 gram.

Selection of hardness and sweetness levels of model foods

We aimed to manipulate the hardness of the model foods in such a way that the eating duration of the hard model food was twice as long compared to the eating duration of the soft model food. In a first pilot study, 22 students of Wageningen University ate one piece of basic model food with a range of carrageenan/LBG concentrations and were instructed to measure their eating duration with a stop watch. Based on eating duration and feasibility of making the gel (lump forming) 0.22 wt.% carrageenan/LBG and 0.88 wt.% carrageenan with 0.66% LBG gels were chosen. In a second pilot study (n=20) participants were video recorded to determine number of chews and chewing duration of these model foods. Mean chewing duration in seconds for the soft model food was (11.33±5.0) and for the hard model food (20.23±6.55), number of chews for the soft model food was (9.25±4.96) and for the the hard model food (24.35±11.23). However, the model foods were generally disliked and liking significantly differed between the gels. Therefore strawberry flavoured jelly pudding powder was added for the next pilot study.

In a third pilot study, 40 Wageningen University students tasted the hard and soft model foods (pieces of ~7 gram) with a range of added sweetener concentrations. They rated pleasantness and relative-to-ideal sweetness intensity in duplicate. In addition, participants were asked to rate pleasantness on a 100-mm VAS anchored with "not pleasant at all" and "extremely pleasant" at respectively 10 and 90 mm. Means of the duplicate ratings were calculated and

plotted against the sweetness concentrations. The low sweet and high sweet concentrations were chosen, at each side of ideal based on equal pleasantness (10 mm difference for the hard gel and 40 mm difference for the soft gel on pleasantness ratings) based on group level palatability ratings. This method has been used and described in more detail in a previous study with salt from our laboratory (32, 46) and is in accordance with the method specified by Booth *et al.* (47).

Sensory and mechanical properties of the model foods

The 174 participants included in the screening, rated perceived intensity for a series of pre-defined sensory attributes of the four types of model foods in duplicate. Ratings were done on a 100 mm visual analogue scale with anchors 'not at all' and 'extremely' at 10% and 90% of the line scale. The sensory characteristics of the model foods can be found in table 1.

Mechanical properties of the MFs were determined using uniaxial compression tests with a texture analyzer (Model TA-XT plus, Stable Micro Systems-SMS) with a 75 mm steel probe. Cylindrical specimen of MFs (20 mm height, 26 mm diameter) were compressed up to 80% strain at a constant compression speed of 1 mm/s. Per MF-type 9 replicate measurements were performed and the average values obtained for fracture stress, fracture strain, fracture work and modulus can be found in table 2. The fracture stress of the hard gels is seven times larger than the fracture stress of the soft gels, which is reflected by the significant differences in perceived hardness by the participants (48).

Table 1. Sensory characteristics of the model foods expressed as 100 mm VAS ratings (N=178, mean ± SD).

	Soft		Hard		ANOVA Fixed Effects (p-value)		
	Low sweet	High sweet	Low sweet	High sweet	Texture	Taste	Taste *Texture
Pink color intensity	29 ± 14	29 ± 14	27 ± 14	28 ± 14	0.14	0.47	0.51
Odor intensity	42 ± 19	44 ± 19	38 ± 20	42 ± 20	0.01	0.001	0.50
Sweetness	55 ± 14^{a}	71 ± 13^{b}	36 ± 16^{c}	63 ± 16^{d}	<0.001	<0.001	<0.001
Flavour intensity	51 ± 15^{a}	66 ± 14^{b}	35 ± 16^{c}	64 ± 15^{d}	<0.001	<0.001	<0.001
Bitterness	15 ± 8.0	16 ± 11	19 ± 13	22 ± 16	<0.001	0.003	0.20
Hardness	24 ± 14	20 ± 10	67 ± 17	66 ± 16	<0.001	0.001	0.10
Brittleness	27 ± 19	28 ± 19	51 ± 23	50 ± 22	<0.001	0.19	0.82
Elasticity	25 ± 16	22 ± 14	58 ± 21	56 ± 20	<0.001	0.005	0.86
Creaminess	56 ± 17	58 ± 17	32 ± 18	36 ± 19	<0.001	0.003	0.20
Dryness	19 ± 12	17 ± 10	44 ± 23	40 ± 23	<0.001	0.002	0.15
Stickiness	30 ± 18	33 ± 20	24 ± 13	25 ± 14	<0.001	0.02	0.27
After taste intensity	45 ± 18^{a}	29 ± 14^{b}	34 ± 17^{c}	28 ± 14^{b}	<0.001	<0.001	<0.001

Different letters indicate a significant difference between model foods on that attribute (*Bonferroni post hoc, p* < 0.05).

Table 2. Mechanical properties of the model foods (mean ± SD*).

	Soft		Hard	
	Low sweet	High sweet	Low sweet	High sweet
Fracture stress (kPa)	49 ± 2	43 ± 1	360 ± 36	350 ± 20
Fracture strain (%)	58.5 ± 0.7	56.4 ± 0.6	69.4 ± 1.9	68.8 ± 1.2
Fracture work (kPa)	100 ± 4.0	88 ± 1.9	757 ± 86	758 ± 46
Modulus (kPa)	22.3 ± 3.1	25.3 ± 3.5	138.5 ± 9.1	139.6 ± 9.9

* based on 9 replicate measures per model food.

Study Procedures

Test sessions were conducted in the sensory booths at Wageningen University and were scheduled around lunchtime (between 11:00 – 14:00 h). Participants came in a hungry state and were assigned the same arrival time for all test sessions with at least one non-testing day in between. Participants were instructed to refrain from eating or drinking anything, except for water for four hours prior to the test sessions. As a compliance measure, participants were asked about the last time they ate and drank something before the start of each test session. The participants were then offered a standardized meal to equalize the level of satiety between sessions. This meal consisted of sandwiches made from two slices of white bread with butter (30% fat) and full-fat cheese (slices of 20 gram) cut in four, and a glass of water (100 ml). For each participant, the number of quarter sandwich pieces was calculated based on their estimated daily energy needs (Schofield; WHO, 1985) with an average estimated physical activity level (PAL) of 1.6. The amount of bread participants received was calculated based on 5% of their daily energy need which is which is equivalent to half of a standard Dutch lunch meal. Participants were instructed to consume the meal within 15 minutes.

- Food intake

Fifteen minutes after the standardized meal, participants were provided with a bowl containing 300 gram of a model food (approximately 40 pieces of 7 g). This amount is double the portion of a standard dessert portion in The Netherlands (150 g). MFs were served at room temperature. The bowl was opaque and participants were instructed to eat with a dessert fork and to eat naturally until they were comfortably full. Participants were told that they had 30 minutes to eat the MF and were offered an additional 150 g portion when they finished the first 300 g portion. In total, participants asked for an additional portion thirteen times (11 different participants); 10 times this concerned a soft MF and three times a hard MF. In four cases people ate both the 300 g and 150 g portion. When participants indicated that they were finished eating, the left over gels were removed and covertly weighed. *ad libitum* intake of the model food (main outcome measure) was calculated by subtracting the weight of the bowls before and after consumption. Products were weighed to the nearest of 0.1 g on a digital scale (model XP-3000, Denver Instruments, Gottingen Germany).

– Eating behaviour characteristics

To measure eating behaviour characteristics, participants were video recorded during each session. A digital camera (Logitech webcam, resolution 640 x 280 pixels) was positioned in front of the participant (face-on) where the lower frame was in line with the shoulders, and the upper frame above the top of the cranium and the sides at shoulder width. Participants were instructed to look straight into the webcam and try not to make excessive head movements. To ensure participants looked straight forward a PowerPoint slide show of neutral pictures (forest, flowers etc.) was presented on a computer screen just below the webcam. Eight different pictures were included that changed every 7 seconds.

Video recordings were analyzed with the use of Observer Noldus XT 11. Behaviours of interest were: chewing duration, number of masticatory cycles (chews) and total meal duration. Chewing duration was defined as the time between the first chew and swallowing, identified by the immediate swallow after the end of rhythmic rotary movements. A chew was defined as number of 'closing' masticatory actions of the jaw, and total meal duration was defined as the time between first bite and last swallow of the entire meal (49-51). From these variables chewing frequency was calculated by dividing the number of chews by the total chewing duration. The average number of chews per gram of food was calculated by dividing the total amount eaten by the number of chews. Eating rate was calculated by dividing the amount eaten in gram by the total meal duration.

– Satiety ratings and questionnaires

Participants rated their satiety feelings 10 minutes after consumption of the preload, immediately after the test meal, and 30 minutes after the start of the test meal. Participants rated hunger, fullness, desire to eat, appetite for something sweet, appetite for something savoury, prospective consumption in general, nausea and thirst on a 100-mm VAS anchored with 'not at all' at 10 mm and 'extremely' at 90 mm.

Following the satiety ratings, participants were asked to taste one piece of the model food (~7 gram) and rate palatability, sweetness, desire to eat, and prospective consumption of the model food on a VAS. Immediately after the test meal, participants were asked to indicate why they stopped eating. Participants could only choose one of the following options: "I was full", "I had enough of the strawberry taste of the MF", "I had enough of the sweet taste of the MF", "Eating the MF became boring", "I was nauseous", "I had enough of the texture of the MF".

Statistical analysis

Statistical analyses were performed using SAS (version 9.3; SAS Institute Inc., Cary, NC, USA). Results are presented as mean ± SD unless otherwise stated. P-values of < 0.05 were considered statistically significant.

Difference in *ad libitum* intake between textures and sweetness levels, and their interactions were tested with mixed model ANOVA (SAS proc mixed). Texture and sweetness were added as fixed effects and participant as random effect. The intake model was corrected for palatability by adding palatability as a covariate (fixed effect). Estimated marginal means and SE were calculated based on the average palatability of 55.9 (0-100 mm VAS). In case of an interaction effect, Tukey's correction for multiple comparisons was used in both models. The same ANOVA procedure was used to analyze differences between texture, sweetness and their interaction in: sensory ratings, satiety ratings, palatability and eating behaviour characteristics. Pearsons' chi-square analysis was used to analyze frequencies of the different reasons to stop eating after the *ad libitum* intake. In addition, factors that could have influenced *ad libitum* intake (such as palatability) were explored using Pearsons' partial correlations controlling for participant.

Spearman's correlation coefficients were calculated between model food qualities and eating behaviour characteristics vs. food intake per outcome measure; texture (hard, soft) and sweetness (low, high).

Results

Test food intake

There was a significant main effect of texture ($F = 28.2$, $p < 0.001$) on *ad libitum* intake, while there was no main effect for sweetness ($F = 1.0$, $p = 0.33$); Figure 1. Participants consumed 29.2% less of the hard compared to the soft MF (MF_{hard}= 147.7 ± 8.1 g, MF_{soft}= 208.5 ± 8.1 g, $p < 0.001$). There was no interaction between texture and sweetness on total intake ($F_{(1,3)} = 0.15$, $p = 0.70$).

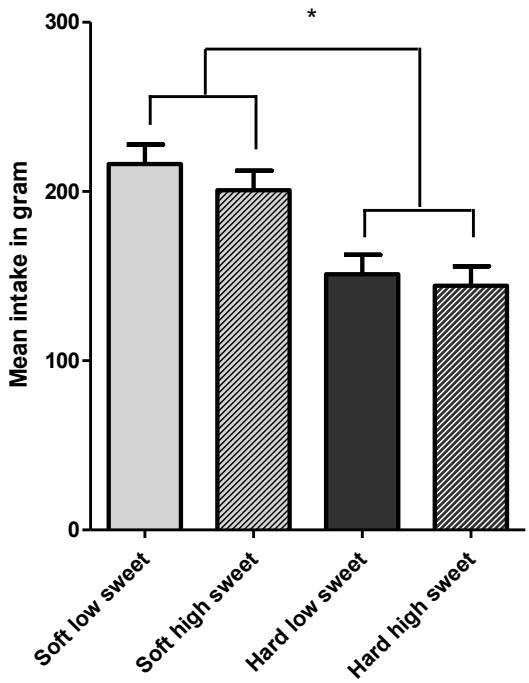

Figure 1. Mean (± SE) intake (gram) of the four model foods. * indicate significant difference. There was a significant main effect of texture ($p < 0.001$) but not of sweetness ($p = 0.33$).

Palatability

Despite trying to match MFs on palatability, palatability scores were significantly different between MFs ($F_{(1,3)}$ = 18.2, p < 0.001). The hard model foods were liked less than the soft model foods (mean± SE difference = 9.78±1.4 on a 100 mm VAS) (Supplement I). Intake was significantly correlated to palatability (r = 0.36, p < 0.001). Because of that, *ad libitum* intake of the MFs was corrected for palatability by adding palatability as a covariate to the model. Palatability had a significant effect ($F_{(1,3)}$ =20.7, p = <0.001) on intake. When the texture and sweetness effect on *ad libitum* intake was corrected for palatability, we found an overall texture effect but no effect for sweetness, similar to the uncorrected model (texture F = 13.5, p < 0.001, sweet F = 2.9 p = 0.10). Corrected for palatability the difference in intake between the hard and soft MFs was 21.5 % (p < 0.001) (Figure 2).

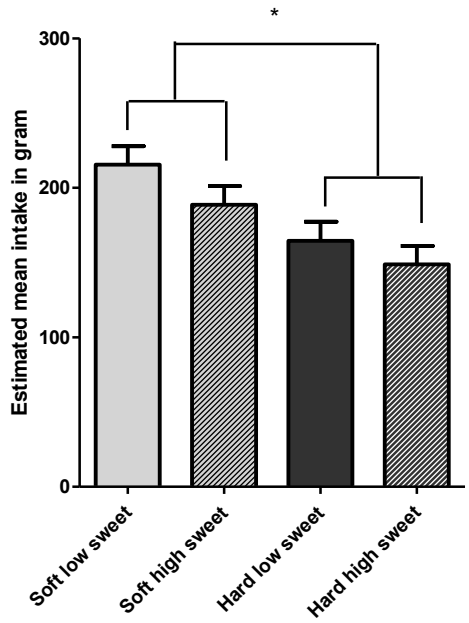

Figure 2. Estimated marginal means (± SE) *ad libitum* intake (gram) of the four model foods corrected for palatability. * indicate significant differences. There was a significant main effect of texture (p < 0.001) but not of sweetness (p = 0.10).

Reason to stop eating

Most participants (65.5%) reported that they stopped eating because they felt full. However no differences between the MFs were found. Participants reported in 9.1% of the cases (over all sessions) that they stopped eating because they got bored with the sweet taste, in 8.6% because they got bored with the texture, in 5.1% that they stopped eating because they got bored with the strawberry taste, in 5.6% that they felt nauseas and in 3.9 % that they stopped because they were bored with eating the gels.

Eating behaviour characteristics

All the measured elements of eating behaviour were significantly affected by texture, but not sweetness (Table 3). Number of chews was significantly higher for the hard texture MFs compared to the soft texture MFs ($F = 213.5$, $p < 0.001$). Participants chewed 2.5 times more per bite on the hard-, compared to the soft MFs ($MF_{hard} = 26.9 \pm 6.2$ $MF_{soft} = 10.1 \pm 6.2$). In addition, participants chewed on average 10 seconds longer per bite on the hard MFs compared to the soft MFs ($p < 0.001$). The chewing rate was slower for the hard MFs compared to the soft MFs, participants chewed 1.2 times per second on the hard MFs versus 0.9 times per second on the soft MFs ($p < 0.001$). Participants ate on average 10 gram less per minute from the hard MFs compared to the soft MFs ($MF_{hard} = 15.3 \pm 7.1$ and $MF_{soft} = 26.3 \pm 10.2$ g/min, $p < 0.001$). Furthermore, the duration of the meal was significantly longer when eating the hard MFs compared to eating the soft MFs ($p < 0.01$).

Table 3. Mean ± SD chewing behaviour characteristics.

	Soft		Hard		ANOVA Fixed Effect p-value		
	Low sweet	High sweet	Low sweet	High Sweet	Texture	Taste	Texture* taste
Number of chews (per MF piece)	10.4 ± 8.6	9.8 ± 8.9	27.2 ± 8.7	26.6 ± 8.7	<0.001	0.54	0.95
Chewing duration (per MF piece)	12.1 ± 7.9	11.5 ± 8.0	23.2 ± 8.0	22.5 ± 8.0	<0.001	0.85	0.65
Chewing rate (chews p/s)	0.9 ± 0.3	0.9 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	<0.001	0.73	0.91
Eating rate (g/min)	25.7 ± 8.6	25.5 ± 8.8	14.8 ± 8.6	14.6 ± 8.6	<0.001	0.97	0.85
Total meal duration (min)	8.75 ± 3.7	8.20 ± 3.7	10.2 ± 3.7	9.6 ± 3.7	0.006	0.19	0.96

Satiety feelings

All appetite ratings were similar across conditions at baseline ($p > 0.5$). Subjective hunger, prospective consumption, thirst, desire to eat and desire to eat sweet or savoury all significantly decreased comparing pre-, to directly after *ad libitum* intake ($p < 0.05$), Supplement I. Fullness ratings significantly increased directly after *ad libitum* intake ($p < 0.001$). Only thirst ratings were affected by texture ($p < 0.05$) and sweetness ($p < 0.05$) directly after the meal, no interaction effect was found.

Model food related properties significantly differed at baseline (pre-meal); texture affected desire to eat the gel ($p < 0.05$), palatability was affected by both texture ($p < 0.001$) and taste ($p < 0.05$) in addition sweetness significantly differed between texture ($p < 0.001$) and taste levels ($p < 0.001$). No interaction effects or differences over time (pre-, post-meal) were detected. The model food related properties and appetite ratings per model food type that were rated pre, direct, and 30 min. post intake can be found in Supplement I.

Correlations of model food qualities and eating behaviour characteristics with intake

Qualities of the model food that were most strongly correlated with intake of the model foods were expected satiation (how much participants thought they could eat of the MF before satiation) and wanting to eat the model foods (see table 4). The correlation between sweetness and intake was low ($r \leq 0.30$). Almost all eating behaviour characteristics were correlated with intake of the model foods. Chewing rate was least strongly correlated to intake and only the eating rate of the sweet version of both textures was related to intake. Chewing duration, eating rate and total meal duration most correlated with intake ($r \geq 0.50$).

Table 4. Spearman's correlation coefficients (r) between model food qualities and eating behaviour characteristics with intake.

	Soft		Hard	
	Low sweet	High sweet	Low sweet	High Sweet
Model food related qualities				
Sweetness	0.30*	0.23	0.08	0.28*
Palatability	0.26*	0.44**	0.23	0.28*
Wanting	0.40*	0.54**	0.37*	0.49**
Expected satiation	0.46**	0.53**	0.36*	0.50**
Eating behaviour characteristics				
Number of chews (per MF piece)	-0.35**	-0.16	-0.45**	-0.48**
Chewing duration (per MF piece)	0.57**	0.62**	0.58**	0.65**
Chewing rate (chews p/s)	0.13	0.29*	0.16	0.34*
Eating rate (g/min)	0.56**	0.50**	0.68**	0.76**
Total meal duration (min)	0.52**	0.57**	0.57**	0.56**

*Significant correlations between model food intake and variable *p=<0.05, **=< 0.001*

Discussion

We investigated the independent contribution of oral processing time and taste intensity and their combined effect on satiation. In addition, we explored how oral processing time and taste intensity affected eating behaviour characteristics.

A longer oral processing duration led to a lower intake of the model food; participants ate 21% less of the hard compared to the soft model foods. However, there was no effect of sweetness on intake. All eating behaviour characteristics differed between the soft and hard MF eating sessions. Participants chewed significantly more on the hard compared to the soft MFs. The observed number of chews differed by a factor of 2.5 between the soft and the hard model foods. The higher number of chews for the hard MFs also led to a longer chewing duration per MF, a faster chewing rate, a slower eating rate, and longer meal duration compared to the soft MFs. Overall, chewing duration, eating rate, total meal duration and expected satiation most strongly correlated with intake of the model foods.

Our findings are in accordance with earlier reports showing that increased oral processing time decreases food intake (13, 26, 40, 51-53). More specifically, our results support a recent review by McCrickerd *et al.* (54) which argues that texture exerts a larger effect on intake (30 %) than does taste (10%) since we found no effect of taste intensity on intake.

We matched our samples on palatability, as it is well known from literature that this is a strong driver of intake (54, 55). Although our model foods differed in sweetness intensity (range 39-67 mm, 100 mm VAS), it might be that – due to this matching in palatability - our perceived difference in taste intensity was presumably smaller than the perceived difference in texture. A more intense taste may only result in adjusted intake if texture differences are equal in perceived magnitude or when a person associates this sweet sensory experience with the presence of nutrients (54). In our study, participants may have been aware of and familiar with non-caloric sweeteners or the sweetness range in our study may have been within their habitual range causing them not to adjust their intake. This could explain why we did not detect an effect of taste intensity on intake. However, other studies have also reported no effect of sweetness on intake (56-58).

Differences in eating behaviour could explain the effects of texture hardness on intake. Several studies found that the number of chews and chewing duration strongly correlate with *ad libitum* intake (42, 59, 60). In addition, eating rate could explain part of the effect of texture on intake in this study. Which is inline with previous studies that have found that eating rate of a wide variety of foods is associated with (expected) satiation and meal size (9, 51, 61, 62).

We did not detect any differences in eating behaviour characteristics between the sweet and

low-sweet MFs. This finding is contrary to a study of Bolhuis *et al.* in which differences in bite size were found between salty taste levels of soup (32). The reason why we did not find any differences in eating behaviour characteristics could be due to the sweet- instead of salty-taste, or because of the same reasons why we did not find any differences in intake between sweetness levels.

Participants ate 21% less of the hard model foods; however, they did not feel less satiated after the meal compared to after eating the soft model foods. This is in accordance with previous studies reporting differences in intake but not in appetite ratings (24, 26).

Although texture can play an important role in determining intake (63, 64), other sensory characteristics such as palatability (54), and cognitive processes such as expectations, attention and memory could also play a role (65-67). The texture effect may be due to the increased OSE time, due to increased number of chews and lower eating rate, but it could also be a learned cognitive effect because foods that are perceived to be chewier and thicker are believed to be more filling (68, 69).

Despite matching the palatability of the MFs before the experiment, we still found significant differences in palatability between treatments. Because texture and taste are both drivers of palatability and food acceptance (54, 55), our difference in intake may have been partly due to differences in each individuals liking of these specific attributes. However, when we accounted for differences in palatability there was still a 21% difference in intake between the hard and soft MFs. A difference of 21% in intake is considered to be very relevant in decreasing food intake (70).

To be able to study the separate effects of texture and sweetness manipulations while keeping all other food properties equal we used gel-based model foods in this study. Other studies that compared intake of solid foods with different hardness levels found a 10% reduction in intake when comparing whole with mashed foods (39) and a 13% reduction comparing hard lunch meal items (hamburgers, rice salad) with soft lunch meal items (26). This effect is smaller than in the current study (21% reduction) presumably due to smaller contrast in texture hardness. The difference in texture in the present study is comparable to the range of texture differences among commercially available foods (4).

However, although the differences in texture, number of chews, chewing duration and eating rate between our hard and soft model foods seem to be ecologically valid compared to eating behaviour arrived from commercially available foods (4), the results of this study should be extrapolated with some caution (51). This study has been performed within a laboratory and observed reduced food intake only within one eating episode within a healthy and relatively

young study population. Future studies could extend the findings of this study outside the laboratory in a real-life setting using commercially available foods to investigate whether people will not compensate for the reduced intake and to see if such effects are sustained over the course of a day (26, 71). In a non-experimental setting the difference in hardness should be balanced such that intake is lowered, but the hedonic value and consumer acceptance of a food remains unchanged.

In addition, future studies should focus on whether the effect of texture on intake is, due to the longer processing time, slower eating rate or due to expected satiation. Future studies should focus on manipulating sweetness intensity based on individual preference level to investigate whether larger differences in taste intensity do lead to reduced food intake.

Conclusion

This study shows that increasing texture hardness decreases food intake independent of sweet taste intensity. Eating behaviour characteristics such as oral processing time may explain this effect. Therefore, orosensory exposure duration rather than intensity appears to be the main oro-sensory determinant of food intake. This suggests that texture is an important driver of intake that may be used to reduce food intake within a meal. Texture manipulations could therefore aid in naturally lowering food intake without dietary energy restrictions.

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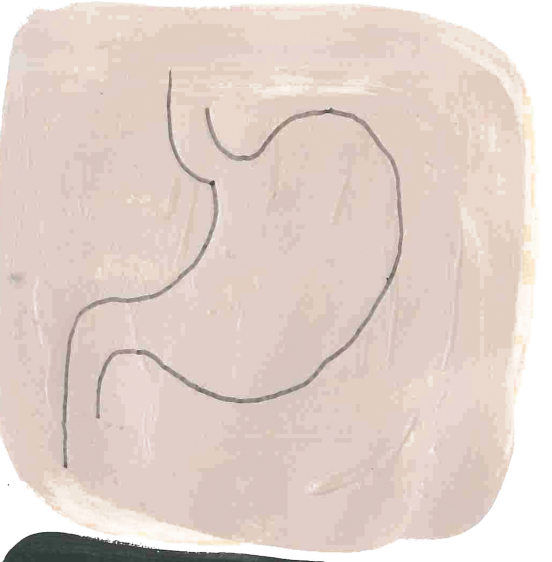
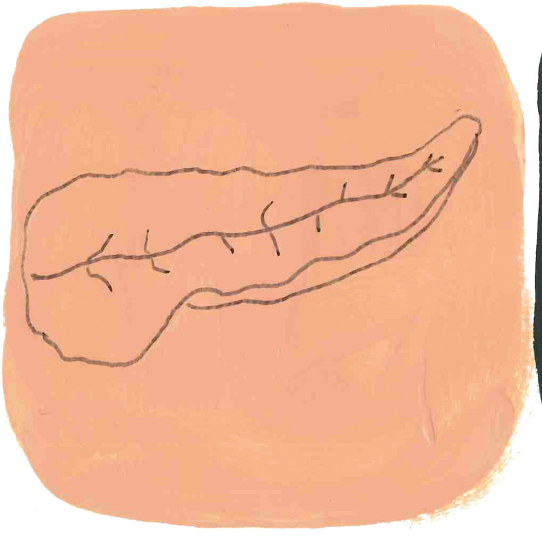
Supplement I. Table 1. Mean±SD appetite and MF property ratings per model food type: pre, direct, and 30 min. post intake.

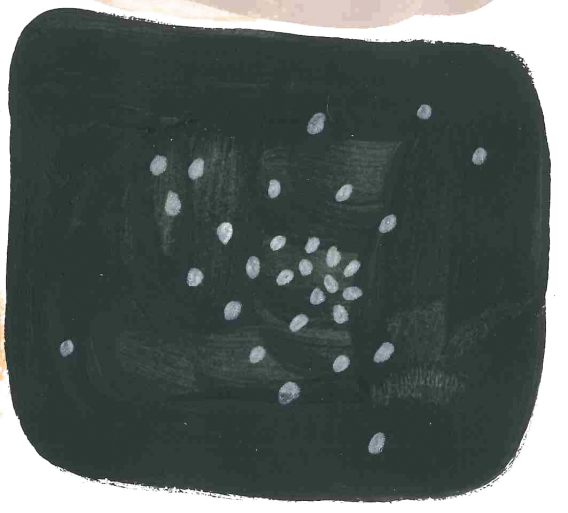
		Soft		Hard		ANOVA Fixed effect p-value		
		Low sweet	High sweet	Low sweet	High sweet	Texture	Taste	Texture* Taste
Appetite ratings								
Hunger								
	Pre	53 ± 15*	51 ± 17*	50 ± 15*	51 ± 17*	0.46	0.79	0.52
	Direct	19 ± 12*	19 ± 12*	20 ± 12*	21 ± 12*	0.33	0.85	0.80
	30 min.	27 ± 14*	27 ± 13*	27 ± 14*	28 ± 13*	0.99	0.79	0.91
Time (p-value)		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$			
Fullness								
	Pre	37 ± 14*	37 ± 14*	38 ± 14*	39 ± 14*	0.37	0.59	0.87
	Direct	67 ± 15*	68 ± 13*	68 ± 13*	68 ± 10*	0.68	0.76	0.88
	30 min.	61 ± 15*	59 ± 13	59 ± 19*	61 ± 14*	0.85	0.96	0.45
Time (p-value)		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$			
Prospective consumption								
	Pre	57 ± 14*	56 ± 15*	58 ± 14*	55 ± 16*	0.97	0.39	0.82
	Direct	26 ± 14*	27 ± 13*	27 ± 14*	25 ± 11*	0.80	0.77	0.34
	30 min.	33 ± 15*	36 ± 16*	33 ± 14*	35 ± 14	0.88	0.16	0.99
Time (p-value)		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$			
Desire to eat								
	Pre	58 ± 15*	56 ± 16*	57 ± 13*	57 ± 16*	0.93	0.47	0.67
	Direct	24 ± 15*	21 ± 14*	19 ± 15*	16 ± 15*	0.98	0.50	0.75
	30 min.	33 ± 18	32 ± 17*	29 ± 17	32 ± 15	0.46	0.74	0.51
Time (p-value)		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$			
Desire to eat sweet								
	Pre	54 ± 16*	53 ± 16*	53 ± 14*	53 ± 14*	1.00	0.83	0.72
	Direct	20 ± 14*	20 ± 14*	23 ± 15*	16 ± 11*	0.83	0.04	0.07
	30 min.	24 ± 14*	23 ± 13*	25 ± 15*	22 ± 16*	0.88	0.19	0.63
Time (p-value)		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$			

		Soft		Hard		ANOVA Fixed effect p-value		
		Low sweet	High sweet	Low sweet	High sweet	Texture	Taste	Texture* Taste
Desire to eat savoury								
	Pre	55 ± 16*	53 ± 17*	56 ± 16*	53 ± 16*	0.56	0.23	0.64
	Direct	36 ± 21*	41 ± 23*	40 ± 21*	38 ± 22*	0.77	0.62	0.25
	30 min.	41 ± 20	41 ± 21	40 ± 19	41 ± 21	0.91	0.83	0.85
Time (p-value)		p = 0.02	$p < 0.001$	$p < 0.001$	$p < 0.001$			
Thirst								
	Pre	52 ± 17*	56 ± 17*	52 ± 18*	53 ± 17*	0.64	0.32	0.62
	Direct	43 ± 18*	50 ± 21*	50 ± 16*	54 ± 19*	0.03	0.03	0.49
	30 min.	47 ± 18*	52 ± 19*	53 ± 16*	55 ± 17*	0.05	0.15	0.40
Time (p-value)		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$			
Model food characteristics								
Desire to eat the MFs								
	Pre	50 ± 17*	53 ± 16*	44 ± 16*	48 ± 18*	0.02	0.16	0.92
	Direct	24 ± 15*	21 ± 14*	19 ± 12*	16 ± 10*	0.01	0.09	0.89
	30 min.	26 ± 13*	29 ± 15	23 ± 11*	27 ± 15	0.20	0.10	0.75
Time (p-value)		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$			
Palatability								
	Pre	59 ± 13	62 ± 12	49 ± 14	53 ± 14	<0.001	0.024	0.72
	Direct	57 ± 14	57 ± 15	45 ± 16	48 ± 14	<0.001	0.49	0.60
	30 min.	57 ± 13	56 ± 16	49 ± 14	53 ± 14	0.004	0.34	0.30
Time (p-value)		p = 0.091	p = 0.714	p = 0.057	p = 0.292			
Sweetness								
	Pre	53 ± 14^{b}	67 ± 12^{a}	39 ± 13^{c}	63 ± 12^{a}	<0.001	<0.001	0.004
	Direct	51 ± 16^{b}	67 ± 14^{a}	35 ± 14^{b}	63 ± 16^{a}	<0.001	<0.001	0.003
	30 min.	53 ± 14^{b}	65 ± 13^{a}	41 ± 15^{b}	61 ± 15^{a}	<0.001	<0.001	0.02
Time (p-value)		p = 0.757	p = 0.808	p = 0.787	p = 0.139			









Chapter 3

Exacting responses: lack of endocrine cephalic phase responses upon oro-sensory exposure

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Abstract

Oro-sensory exposure to food plays an important role in the regulation of food intake. One proposed underlying mechanism is the occurrence of cephalic phase responses (CPRs). CPRs include the pre-digestive endocrine responses induced by food related sensory input. Yet, whether oro-sensory exposure duration or sweetness intensity affects CPRs is unknown. The objective of this study was to determine the independent and interactive effects of oro-sensory duration (chewing) and stimulation intensity (sweetness) on endocrine CPRs and satiation.

Eighteen males (22±2 y, BMI 22±2 kg/m^2) participated in a 2x2 randomized study with a control condition. Each session participants performed modified sham feeding (MSF) with one of four gel-based model foods. During the control session no MSF was performed. Model foods differed in chewing duration (hard or soft texture) and sweetness (low or high intensity). During each session eight blood samples were collected up till 25 min after MSF onset. Subsequently, food intake from an *ad libitum* lunch was measured.

No typical cephalic phase response was found for insulin, PP and ghrelin.

However, the overall PP response was 1.1 times greater for the hard sweet MSF condition compared to control (p=0.02). Overall ghrelin responses were 1.1 times greater for the hard model food compared to the soft model food conditions (p=0.003). These differences in endocrine response were not associated with differences in food intake at the subsequent meal. Exploratory sub analysis of the responsive insulin curves showed that after 2.5 minutes of MSF the hard texture model foods insulin concentrations were 1.2 greater compared to the soft texture. These findings indicate that texture hardness and sweetness increase the overall PP response and that MSF on hard texture increases the overall ghrelin response compared to soft texture model foods. However, MSF on model foods does not lead to a typical cephalic phase response.

This study, among others, shows that there are major dissimilarities in the endocrine responses to food stimulation between individuals. This emphasizes the importance of considering cephalic responders and non-responders. More research is needed to understand CPRs in relation to food texture and taste properties.

Introduction

The current food environment drives a positive energy balance leading to a growing number of obese and overweight people worldwide. (1, 2). Knowledge about the mechanisms by which food intake is controlled is key in finding solutions to this problem (3).

Food intake starts with the breakdown of food in the mouth; this process depends on the texture, the flavour, and the palatability of the food (4-7). Besides food qualities, oral processing is determined by the individuals' anatomy, such as mouth size, strength of the jaw muscle (masseter), and automated eating behaviour characteristics such as bite size and chewing rate (8-10). Intake of a food is inhibited when the afore mentioned food and individuals' characteristics lead to a slow ingestion rate (11). Several physiological mechanisms have been suggested to cause this effect, among which the oro-sensory exposure (OSE) to food (12, 13).

OSE can be defined as the release of nutrients, odor and taste molecules from the food matrix in the mouth during oral processing (14). Foods that do not require chewing before the bolus is ready to swallow induce only limited OSE, whereas foods that do require chewing enhance OSE (7). Beverages, for example, can be ingested in a relatively short time and therefore the oral-residence time and thus OSE is limited. This may be a reason why energy containing beverages suppress appetite and energy intake less compared to equicaloric solid foods (15, 16).

OSE is necessary for the initiation of cephalic phase responses (16). The cephalic phase is the first phase of digestion, including all physiological, endocrine and autonomic responses stimulated by cephalic phase sensory input such as taste, smell (OSE) and the sight of food (17, 18). The putative function of these anticipatory responses to food is to optimize digestion and to minimize the impact of meals on homeostasis (19-22). Initial cephalic responses signal stimulation of food intake, while continued sensory stimulation may induce satiation (18). Cephalic phase signals include the production of saliva and endocrine responses in insulin, pancreatic polypeptide (PP) and ghrelin.

A lack of, or diminished cephalic phase responsivity as a result of decreased OSE through rapid food consumption, has been suggested to affect physiological and psychological processes, such as glucose homeostasis, metabolism, and food reward and satiety systems in the brain (12). In both animal (23, 24) and human studies (25) it has been shown that these changes are related to decreased appetite responses and weight gain (26). OSE may therefore play a key role in the regulation of food intake through the induction of cephalic phase responses (10, 27, 28).

However, the importance of the duration and intensity of the OSE in order to induce cephalic phase responses is unknown. OSE duration affects the CPR as shown by studies of Teff. *et al.*

where cephalic responses of insulin and PP were found to mixed nutrient foods and solid foods but not to liquids (29, 30). Cephalic phase response could also be enhanced through taste (31).This is shown by a study of Just. *et al.* where an increase in insulin plasma concentration was found upon oral cavity stimulation with a sweet sucrose and saccharine solution but not for any of the other taste qualities (32). In addition, Teff *et al.* found a PP response to mixed nutrient sweet and salty foods with a higher magnitude of the response found for the sweet foods (29). This indicates that sweet taste may have a specific role for nutrient signaling that aids in controlling food intake and food digestion through cephalic phase responses (33).

This indicates that sweet taste may have a specific role for nutrient signaling that aids in controlling food intake and food digestion through cephalic phase responses (33).

Taken together; OSE can be varied in duration and intensity which may affect cephalic phase responses and consequently food intake. However, it is not known if, and to what extent OSE duration and taste intensity induce cephalic responses. Therefore, the main objective of this study was to determine the independent en interactive effects of oro-sensory duration (chewing) and stimulation intensity (sweetness) on the endocrine cephalic phase responses and subsequent food intake. To investigate this we performed a 2x2 factorial randomized crossover study with control condition. Endocrine responses were measured under conditions in which oro-sensory exposure duration (hard and soft texture) and taste intensity (low sweetness and high sweetness) were varied. We expected a higher peak of the endocrine responses (insulin, pancreatic polypeptide and ghrelin) for increased OSE magnitudes (i.e. hard texture; long chewing duration and high sweet intensity) and a consequently lower food intake.

Materials and methods

Participants

The study was performed at Wageningen University, The Netherlands. Participants were recruited from the surroundings of Wageningen using flyers and posters. In addition, emails were sent to persons in a database of volunteers who previously had expressed an interest in participating in nutrition studies. Healthy male participants between 18-35 years old with a BMI between 18.5-25 kg/m^2 were recruited. Participants had to eat three meals a day around the same time, and were excluded if they followed an energy-restricted diet or if they had gained or lost >5 kg of body weight during the past two months. Participants were also excluded if they had dental pathologies, chewing, swallowing or eating difficulties, self-reported taste or smell problems, braces or dentures, and when they used medication. In addition, they were not allowed to participate if they were high restrained eaters according to the Dutch Eating Behaviour Questionnaire (DEBQ): score >2.9 (34). Personnel and thesis students of the Division of Human Nutrition were omitted from participation. During the information meeting participants rated the model foods that were used in this study on liking. Participants were excluded from participation if they disliked one of the model foods (defined as score <4 on a nine point hedonic Likert scale) or had a stronger preference for one of the model foods compared to the others (defined as >2 point difference on a nine point hedonic Likert scale).

Potential participants were invited to a training visit at Wageningen University. During the training participants practiced with modified sham feeding (MSF) by repeatedly chewing and spitting out the entire model food bolus upon the moment they would normally swallow. In addition, height and body weight were measured and a research nurse measured the participants' Hb value (finger prick) and judged the forearm veins for suitability to place an intravenous cannula. Participants were excluded after the training session if their Hb value was not within 8.1-11 mmol/L or when the research nurse decided that the veins of the forearm were not suitable for placement of the intravenous cannula. In addition, participants were excluded if they had a recovery rate <85% of the dry weight of the model food. This 85% cut-off point has been used in previous studies (35).

The study was approved by the Medical Ethical Committee of Wageningen University, The Netherlands (ABR: NL5682408116) and registered in the Dutch trial register under NTR5870 (http://www.trialregister.nl). All participants signed informed consent prior to the training. Participants received a financial compensation for their time and effort.

Prior to the study, sample size calculations showed that a minimum of 18 participants was needed to show an effect of 10% between treatments. For this calculation we assumed a variation coefficient of 15% iAUC for the control condition and 20% iAUC for the experimental conditions. In addition, a correlation of within person measures of r=0.6 was assumed (22, 36). Power was set at 1- β=0.80 at a significant concentration of α= 0.05. In total, 74 participants joined the information meeting of which 42 participants were found eligible; however seven were lost to follow up. Finally, 35 participants joined the training session of which 22 participants were eligible to be included in the test sessions (Figure 1). Finally, 18 were invited for the study.

The participants that participated in the study were on average 22 ± 2 years old, had a mean (±SD) body mass index of 22 ± 2 kg/m^2 and an average DEBQ restraint score of 1.9 ± 0.5 (34).

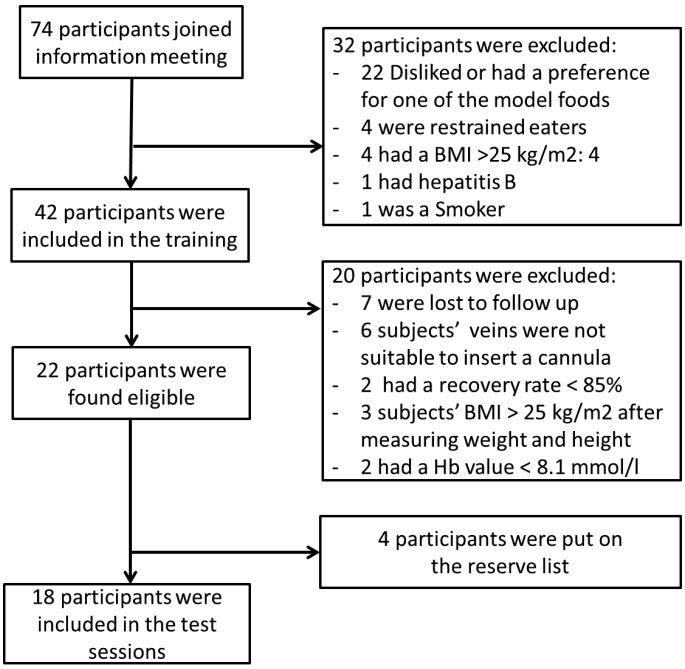

Figure 1. Flow chart of the in- and exclusion of participants

Experimental design

The study had a 2x2 factorial randomized crossover-design with a control condition. During the four treatment visits participants sham fed one of the four types of gel-like models foods. Model foods were formulated to have two levels of oral processing time (soft or hard texture), and two concentrations of sweetness (low or high) (see 2.3.1). During the control visit participants did not sham feed or eat anything. During each visit blood samples were taken at fixed time points and after the last blood sample collection participants received an *ad libitum* lunch meal. Conditions were randomized, following a Latin square Williams design with one-week washout in-between visits.

Experimental procedures

The evening preceding the test session participants were instructed to eat the same meal of their choosing to avoid confounding effects of previous food consumption. Participants were instructed to eat the meal between 18:00-20:00 hr after which the participant was not allowed to eat or drink anything except water until the next morning. Participants received two 500 ml cartons of a milk based breakfast drink (banana, kiwi, strawberry flavoured 'goedemorgen fruitontbijt' Vifit Wageningen, The Netherlands, Friesland Campina) to drink until fullness 4 hours prior to their arrival time. Participants were instructed not to eat or drink anything after their breakfast drink and to bring back the cartons. The breakfast drink cartons were covertly weighted to determine intake (average intake of the breakfast drink was 662±258ml). Participants were also instructed to avoid high-intensity exercise (everything besides regular speed walking and biking) and alcoholic drinks 24 hours preceding the test and to use the same mode of transport every time they visited. To control for compliance participants were asked to keep a diary of their food and beverage consumptions and physical activity the day before the test session.

Half an hour prior to the start of the test sessions an intravenous cannula was placed in the antecubital vein by a trained research nurse.

Blood drawings were done in a quiet room by a trained research nurse. Participants were provided with 15 pieces (of 7 gram each) of the model foods at t-2.5 (baseline sample) which they had to smell. After that participants started chewing on the model foods, starting to chew was defined as t=0. Blood samples were taken at time points t (min), t-2.5 (smell) t0 (start MSF) t2.5, t5, t10, t15 (stop MSF), t20 and t25.

Participants regularly filled in an appetite questionnaire. During the sham feeding session participants were recorded with use of a webcam to determine the number of chews and

chewing duration. After the sham feeding part of the test session participants were provided with an *ad libitum* lunch meal to measure intake as a measure of satiation. For an overview of the entire test session procedure (Figure 2).

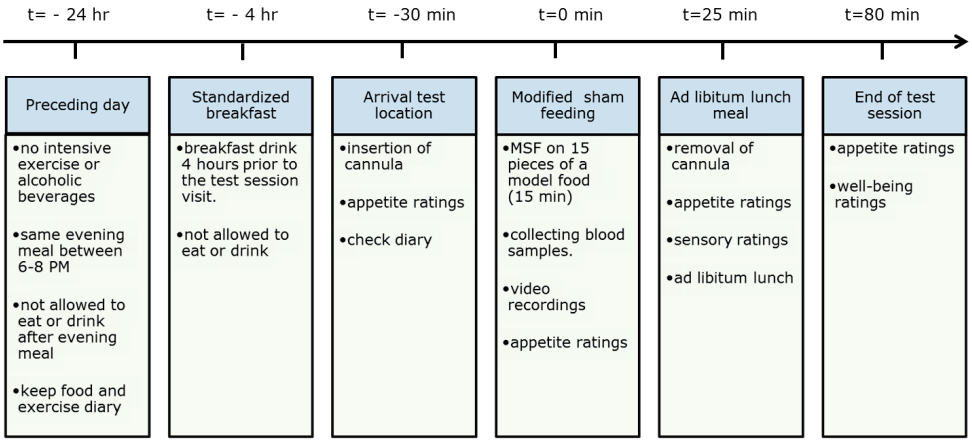

Figure. 2. Overview of experimental procedures

Model foods

Strawberry flavoured gel-based model foods similar to our previous study (37) were used. Ingredients used were cream (30% fat, AH Basic), sunflower oil, strawberry flavoured pudding powder ('Jelly', dr. Oetker) and water. To manipulate oral processing time, the hardness of the gels was altered by added thickening agents, that is carrageenan (type CHP-2) and locust-bean gum (LBG). Thickening agents were added in the following amounts: 0.22/0.22 wt% carrageenan/LBG for the soft and 0.66/0.88 wt% carrageenan/LBG for the hard model food.

In our previous study we were unable to increase the difference in perceived sweetness between the low and sweet version of the model foods without changing the liking of the model foods by using a non-caloric sweetener. For the present study multiple pilot studies were performed to enhance the sweetness difference between the low and high sweet model foods by adding table sugar. We were able to establish a (Mean±SEM) 16±2 mm difference (rated on a 100 mm VAS, $p<0.001$) in sweetness between the low and high sweet model foods, with no difference ($p=0.90$) in sweetness between textures (soft and hard) (Mean±SEM) 0.3±2.3 . By adding table sugar next to the non-caloric sweetener we were able to keep palatability of the model foods equal (Mean difference ±SE) 1.2±2.6, $p=0.80$).. . The added calories (table sugar) in the high

sweet conditions were thought to be negligible as participants sham fed the model foods (chew and spit) and did not ingest, in contrast to our previous study (37).

In the final study model foods significantly differed in sweetness concentration ($F_{(3, 51)}$=13.6, p<0.001) but the different types of model foods were equally liked (Mean±SE, 100-mm VAS: 56±3). Participants did not have a preference for a texture type (p=0.38) or sweetness concentration (p=0.23). The hard and soft texture low sweet variants were significantly less sweet 49±2 compared to the high sweet variants 66±2 (p<0.001). Perceived hardness significantly differed between texture concentrations ($F_{(3, 51)}$=56, p<0.001), mean±SEM perceived hardness of the hard model food was (58±2) and of the soft model foods (21±2). The average number of chews per model food piece significantly differed between texture variants ($F_{(1, 17)}$=138.8, p<0.001) but not between taste concentrations (p=0.64). Participants chewed on average 31±2 times on the hard model food and 20±2 times on the soft model foods. Chewing duration also differed between texture variants ($F_{(1, 17)}$=95.5, p<0.001) and not between taste concentrations (p=0.76). Average chewing duration on the hard model foods was 22±1 sec. and on the soft model foods 16±3 sec. These model food characteristics are similar to those found in our previous study (37). The ingredients, energy content and macronutrient composition of each of the model foods can be found in Table 1.

Table 1. Energy and macronutrient content of the model foods per 100 gram

Model food:	**Soft low sweet**	**Soft sweet**	**Hard low sweet**	**Hard sweet**
Energy (kJ/ kcal)	786 / 188	869 / 208	803 / 192	882 / 211
Protein (g)	0.8	0.8	0.8	0.8
Fat (g)	13.6	13.6	13.6	13.6
Carbohydrate (g)	15.6	20.5	16.5	21.4

Modified sham feeding

Participants were instructed to chew on the model food and to expectorate the entire bolus upon the moment they would normally swallow. This chew and spit method is called modified sham feeding (MSF) and has been used in previous studies (35, 38, 39). With this technique participants are orally exposed to the model foods without ingestion. Participants were trained to MSF during the training session.

Participants received 15 identical pieces of 7 gram of the model foods which they had to 'chew and spit'. To avoid the confounding effects of 'eating' rate rather than number of chews and chew effort on the metabolic and hormone response, participants received instructions on when to start chewing a new model food piece. After the maximum amount of time had passed (40 s)

they were however instructed to expectorate the model food if not previously done. The maximum amount of time of 40 s was determined based on a previous study performed with the same model foods (37). Compliance to the instructions for MSF not swallowing the (pieces of) model food was determined by analyzing the dry mass of model food that was spat out. Recovery percentage was calculated by dividing the dry mass of the expectorated boli by the dry mass of the model foods, in line with the method used by Wijlens *et al.*(35).

In this study the mean recovery percentage was (Mean±SE) 92.7 ± 0.8%. In grams this means that on average 2.4 ± 0.3 g of the model foods was swallowed. Per model food type this was: 92.0±0.7 % (2.2±0.2 g swallowed) for soft low sweet; 89.3±0.8 % (3.9±0.3 g swallowed) for soft high sweet; 94.1±0.4% (1.7±0.1 g swallowed) for hard low sweet; and 95.6±0.4 % (1.6±0.1 g swallowed) for hard high sweet. This is in line with other studies that also report recovery percentages between 89-97% (35, 40-42).

Blood collection and plasma analysis

Glucose concentrations were measured by collecting a blood sample via the cannula with a syringe and was directly measured using a blood glucose meter (FreeStyle Freedom Lite).

Insulin samples were collected in 3ml lithium heparin-coated vacutainer tubes and placed on ice immediately after acquiring. Insulin samples were centrifuged at 1300G for 10 min at 4 C°. Plasma samples for insulin measurements were stored at – 25°C until analysis at hospital 'De Gelderse vallei' in Ede, The Netherlands. The detection limit of the analysis ranged from 2-300 mU/l with an intra assay CV of 2.2% and inter assay CV of 5.7%.

PP blood samples were collected in 2ml EDTA tubes and stored on ice after acquiring and centrifuged at 2500G for 15 minutes at 4°C. Plasma samples were stored at -80 C° until analysis. PP was analysed by using Human pancreatic polypeptide Elisa kit (Millipore) with a detection range of 12.3-3000 pg/ml and a intra assay CV of 3.3% and a inter assay CV of 4.9%.

Ghrelin samples were collected in 2ml EDTA tubes with added AEBSF blocker (Pefabloc® SC) to a final concentration of 1 mg/ml. Samples were stored on ice after acquiring and centrifuged at 2500G for 15 minutes at 4°C. Ghrelin plasma samples were acidified to a final concentration of 0.05N before storage.

Human ghrelin total ELISA (Millipore) was used to analyse total ghrelin concentrations with a detection range of 50-5000 pg/ml and a intra assay CV of 1.11% and a inter assay CV of 5.18 %. In case of measured values below the detection limit the lowest detectable concentration of the analysis was used for data analysis.

Oral processing characteristics

To measure oral processing characteristics, participants were video recorded during each session. A webcam (resolution 640 x 280 pixels) was positioned in front of the participant (face-on) where the lower frame was in line with the shoulders, and the upper frame above the top of the cranium and the sides at shoulder width. Participants were instructed to limit their head movements. Video recordings were analyzed with the use of Observer Noldus XT 11. Behaviours of interest were chewing duration (sec) and number of masticatory cycles (number of chews). From these variables chewing frequency was calculated by dividing the number of chews by the total chewing duration.

Saliva excretion

Fluid content of the model foods was calculated by subtracting the dry weight of the model food from the total weight of the eaten model foods. In addition, the fluid content of what was spat out was determined by subtracting the dry weight of the spat out bolus from the total amount that was spat out. Saliva content was then calculated by subtracting the water content of the model foods from the fluid content of the expectorated boli (43).

Appetite ratings and wellbeing

Participants rated satiety feelings, wellbeing and model food characteristics on a 100-mm VAS anchored with 'not at all' at 10 mm and 'extremely' at 90 mm (Figure 3). Participants rated hunger, fullness and desire to eat at every time point. Due to time limitations; thirst, desire to eat (DTE), DTE sweet, DTE savoury, prospective consumption and nausea were rated at t=20, 25, 60 min (Figure 3). Participants rated their wellbeing after insertion of the cannula, at the same time of each blood sample collection and directly after the *ad libitum* lunch meal. Liking of the model food was rated at t=5 (liking of the taste) and after the sham feeding session at t=30. After the indwelling cannula was removed participants ate a model food piece and rated additional model food specific parameters; liking, desire to eat the gel, sweetness and hardness.

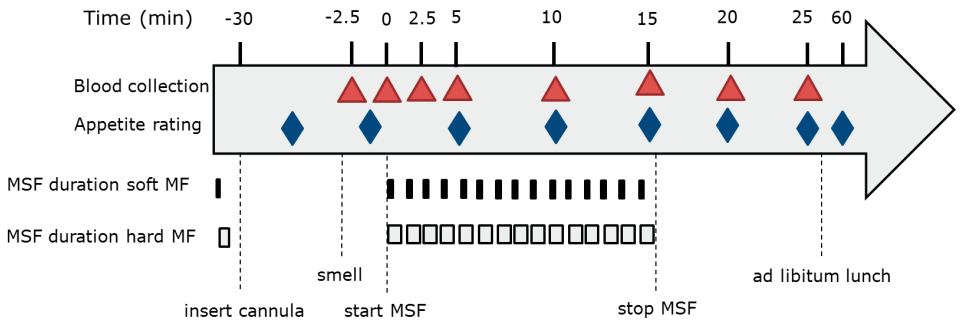

Figure 3. Modified sham feeding procedure overview including blood sample collection and appetite ratings

Food intake

Directly after the MSF experiment the cannula was removed and participants received an *ad libitum* lunch meal. The lunch meal consisted of sandwiches made from two slices of whole bread cut in four small pieces of bread with different types of topping; full fat cheese, ham, apricot jam or hazelnut spread. The amount of bread toppings was determined in such way that breads with different toppings were iso-caloric (50-52 kcal per quarter piece). To make sure there was a surplus of sandwiches (*ad libitum*), participants receive 200% of a normal portion size of bread for each of the different toppings. Based on an average lunch energy intake of 20% of the daily energy need for men (2500 kcal), participants should be offered 5 servings (2 slices of bread on top of each other with topping in between) equal to 20 sandwich quarters of each topping.

During these meals participants were seated in separate booths and were given 100 ml water with their lunch meal, that they were instructed to drink completely in sips between bites over the course of their lunch meal. They were instructed to eat until pleasantly full, and not to talk to each other. The weight and number of bread rolls was covertly weighed and counted to determine intake.

Statistics

Statistical analyses were performed using SAS (version 9.3; SAS Institute Inc., Cary, NC, USA). Results are presented as mean ± SEM unless otherwise stated. P-values <0.05 were considered statically significant. To test endocrine concentrations and appetite scores, mixed model ANOVA (PROC MIXED) was used. In this model, texture, taste, time and their interactions were added as fixed factors, the repeated statement was used to indicate the repeated measures over time per participant. Compound symmetry was used as a co-variate structure. Normality of the data was checked by visual inspection. Outcome variables that were not normally distributed (insulin, PP, ghrelin) were log10 transformed before analyses. For these variables geometric means and ratios with 95% CI are reported.

Outcomes were tested for an order and baseline effect and were found to be significant covariates. Therefore, outcomes (insulin, pp, ghrelin) were corrected for order and baseline concentrations by adding these two variables as a covariate. Tukey correction was used to compare means between treatments and Dunnett correction to compare means of treatments with control.

In addition, Incremental Area Under the Curves (IAUC) were calculated for all endocrine outcomes by means of the trapeziual rule and analyzed by means of a mixed model with conditions (texture, taste) as fixed factor, participant as random variable and baseline AUC as covariate. This model without covariate was also used to calculated differences in intake during the *ad libitum* lunch meal and differences in secreted saliva during MSF between treatments.

Results

Cephalic phase responses

– Glucose

Glucose concentrations remained constant over time ($F_{(7,199)}$=0.78, p=0.61) with no differences between treatments and the control condition at any of the time points($F_{(28,476)}$=0.59, p=0.96), (Figure 4).

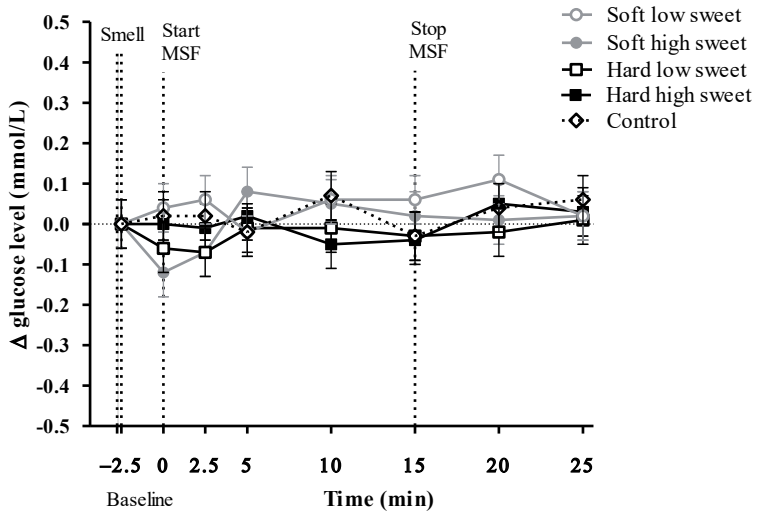

Figure 4. Mean±SEM Δ glucose concentration (mmol/l) from baseline over time for all treatments and the control condition. No significant differences were found between treatments or control condition at any time point (p=0.96).

– Insulin

We did not observe an insulin peak during any of the study treatments and we found no significant differences between treatments or control condition at any of the time points ($F_{(28,310)}$=0.53, p=0. 97) (Figure 5). Insulin concentrations changed over time ($F_{(7,199)}$=2.6, p=0.02), insulin levels at 5 min were 1.1 (95% CI: 1.0-1.2) times higher compared to insulin levels at 20 and 25 min. However, these differences in insulin concentrations between the time points could not be attributed to the texture ($F_{(7,119)}$=0.22, p=0.98) or taste ($F_{(7,119)}$=1.12, p=0.35) manipulations (Figure 5). IAUC did not differ between treatments ($F_{(1,50)}$=0.16, p=0.69) or between treatments and control condition ($F_{(4, 67)}$=0.72, p=0.58).

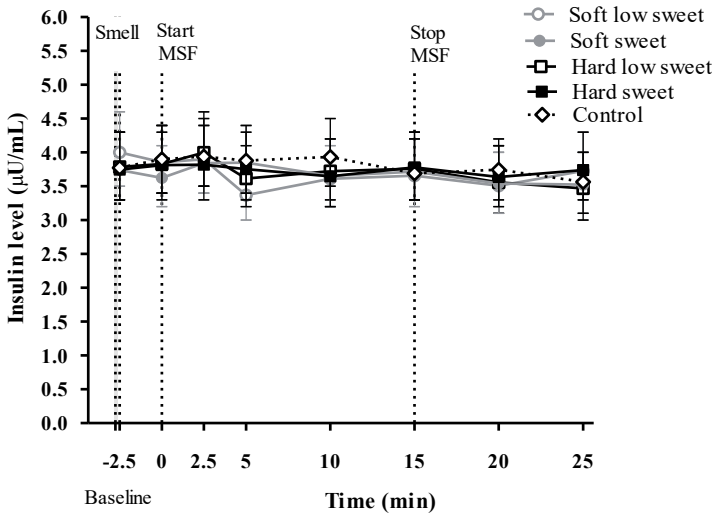

Figure. 5. Geometric mean±95%CI Insulin (µU/ml) concentrations over time per treatment and control condition. No significant differences were found between texture ($F_{(7,119)}$=0.22, p=0.98) and taste ($F_{(7,119)}$=1.12, p=0.35) manipulations.

– Pancreatic polypeptide

PP concentrations did not change over time ($F_{(7,119)}$=1.03, p=0.42), neither did the PP concentrations differ between treatments or control condition at any of the individual time points ($F_{(28,476)}$=6.18, p=0.72) (Figure 6). However, the total PP response to the hard sweet MSF condition was 1.1 times greater compared to control, which was significant (95%CI=1.0-1.2, p=0.02). In addition, we found a significant texture/taste interaction effect on PP concentrations ($F_{(1,17)}$=7.53, p=0.01), the response for the low sweet hard texture model food was 0.9 times lower compared to the sweet hard texture model food curve (95%CI=0.8-1.0, p=0.019). These effects were, however, too small to show significant differences in IAUC between treatments ($F_{(1,50)}$=2.6, p=0.11) or between treatments and control condition ($F_{(4, 67)}$=1.0, p=0.40).

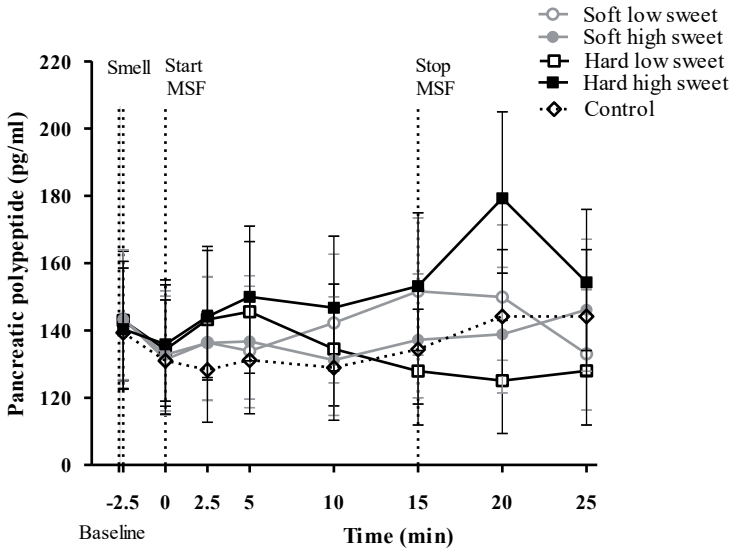

Figure 6. Geometric mean±95%CI pancreatic polypeptide concentrations (pg/ml) corrected for baseline concentration over time per treatment and control condition. No significant differences were found between treatments and control condition at any of the separate time points (p=0. 72).

– Ghrelin

No significant changes over time were found for any of the treatments ($F_{(28,476)}$=0.90, p=0.63) (Figure 7). In addition, we did not find differences between treatments or control condition at any of the individual time points. However, we did find a significant texture effect combining all time points ($F_{(1, 17)}$=12.3, p=0.003). Ghrelin curves of the hard model food conditions were 1.1 times higher compared to the soft model food conditions (95%CI=1.0-1.2, p=0.003). However, these differences were too small to result in a difference in IAUC between the treatments($F_{(1,50)}$=0.27, p=0.60) and control condition ($F_{(4,67)}$=0.48, p=0.75).

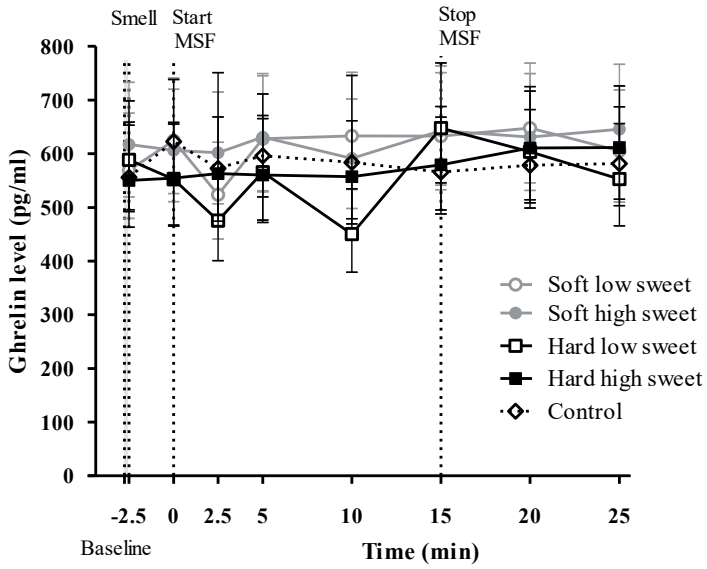

Figure 7. Geometric mean±95%CI ghrelin (pg/ml) concentrations corrected for baseline concentration per treatment and control condition. No significant differences were found between treatments and control condition at any time point (p=0.63).

– Saliva excretion

We found a significant effect of texture ($F_{(1,17)}$ =74.6, p<0.001) and taste ($F_{(1,17)}$=11.3, p=0.004) but no interaction effect ($F_{(1,17)}$=3.6, p=0.08) on expectorated saliva (Figure 8). When MSF on hard model foods participants expectorated 12±1.4 gram more saliva compared to MSF on the soft model foods (p<0.001). In addition, MSF on a sweet model food led to a 5±1.4 gram increased expectoration of saliva compared to MSF on a low-sweet model food (p=0.004).

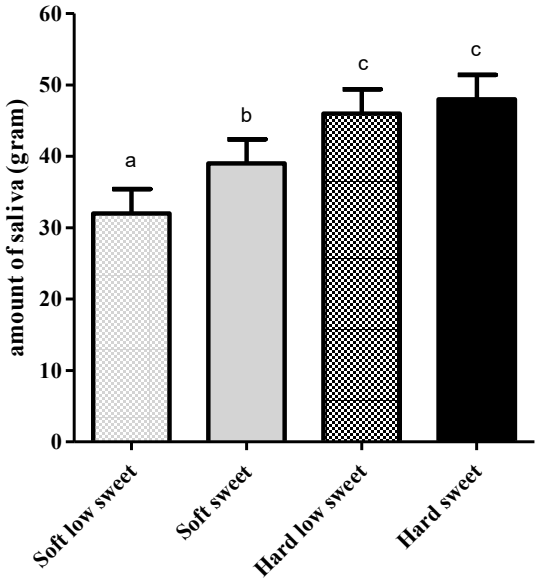

Figure 8. Estimated Mean±SEM saliva content (in gram) of expectorated boli per model food type. Bars with different letters are significantly different.

Exploratory findings: cephalic phase insulin responders and non-responders.

In line with the approach of Dhillon *et al.*(44) for each condition (including control)curves were considered responsive when insulin concentration increased at t=2.5 from baseline (t= -2.5). Based on this 54% of the curves were classified as responsive and 46% of the curves as non-responsive (Table 2). At a participant level, 33% could be considered responders, defined as having 3 or 4 responsive curves out of the four treatments.

For the responsive model we found a significant texture ($F_{(1,13)}$=4.84, p=0.047), taste ($F_{(1,17)}$=9.67, p=0.006), time ($F_{(7,119)}$=2.16, p= 0.042) and texture/taste interaction effect ($F_{(1,5)}$=9.72 , p=0.026) on insulin concentrations over all time points. Analyzing insulin concentrations per time point we found a texture effect ($F_{(1,47)}$=4.09, p=0.049), 2.5 minutes after starting sham feeding. Insulin concentrations at 2.5 min were 1.2 times (CI% 1.0-1.4) higher for hard compared to the soft texture conditions (p=0.049). In addition, we found a significant taste effect ($F_{(1,47)}$=8.89, p=0.005) after 5 min of MSF. Insulin concentrations were1.2 times (CI% 1.1-1.4) higher for low sweet compared to the sweet model foods (p=0.005) (Figure 9). In addition, a sub-analysis was performed of PP and Ghrelin concentrations based on the insulin

classification of responder, non-responder curves. For the PP non-responder and responder curves we did not find any effects (all, p≥0.3) or differences between treatments and control (p=0.06). For the ghrelin non-responder and responder curves we found a significant effect between the hard sweet curve and control curve (p=0.009). For the ghrelin responder curves we found a texture effect over all time points (p=0.007), hard texture was generally lower compared to the soft texture but not at any specific time point.

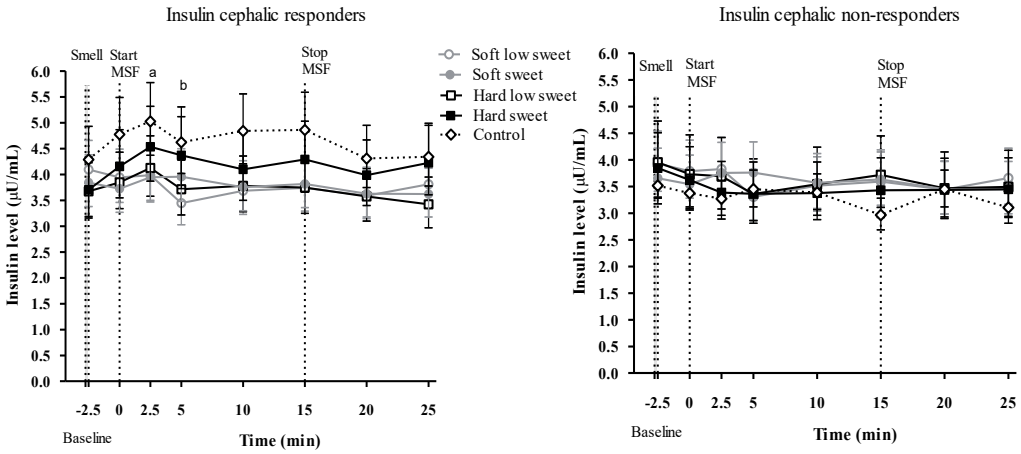

Figure 9. Cephalic phase mean±95%CI insulin responder and non-responder curves. Letter "a" indicates a significant texture effect (p=0.049), "b" indicates a significant sweetness effect (p=0.005).

Table 2. Percentage of responsive and non-responsive curves per treatment and control condition.

	Soft low sweet	Soft sweet	Hard low sweet	Hard sweet	control	total percentage
% responder	15	15	29	20	22	54
% non-responder	25	25	12	20	18	46

Appetite and well being

Hunger feelings over time were significantly lower for the hard high sweet, soft high sweet and low sweet model foods compared to control (Figure 10). After 10 min of MSF participants felt less hungry for the sweet compared to the low sweet model foods (Mean±SEM 9±3 difference, p=0.003).

Fullness ratings showed a similar trend; fullness over time was scored higher after MSF on the hard sweet and low sweet model foods and the soft sweet model food compared to control (Figure 11), significant time points are indicated by the bracket, ($p<0.05$). A small but significant taste effect was found 10 minutes after the MSF period; participants felt more full after the high sweet compared to the low sweet MSF treatments (mean difference= 6±4, $p=0.04$). Prospective consumption ratings post MSF on the sweet soft ($p=0.03$) and hard ($p<0.007$) model food differed from control. Prospective consumption ratings were lower (Mean±SEM 8±2) 10 minutes after MSF comparing the low ($p=0.03$) and high sweet ($p=0.012$) soft model food with the control condition. No significant changes (pre to post) were observed between treatments or control condition for desire to eat sweet or savoury. There were no differences in participants' wellbeing, dizziness, feeling to faint, nausea and thirst (before, or after MSF) between treatments and the control session (all, $p>0.32$).

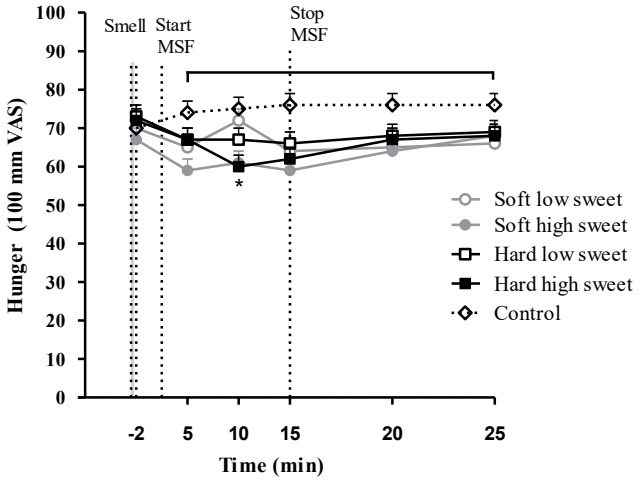

Figure 10. Mean±SEM hunger changes over time per treatment and control condition. Significant differences were found between treatments and control indicated by the bracket ($p<0.05$). * Significant taste effect ($p=0.003$).

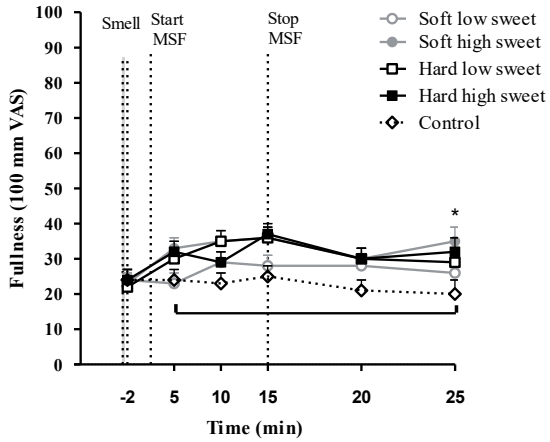

Figure 11. Mean±SEM fullness changes over time per treatment and control condition. Significant differences were found between treatments and control indicated by the bracket ($p<0.05$). * Significant taste effect ($p=0.04$).

Effect of MSF on food intake

There was no effect of texture ($F_{(1,17)}=0.03$, $p=0.87$) or taste ($F(1,17)=0.06$, $p=0.81$) or an interaction effect of texture and taste on intake($F_{(3,17)}=0.06$, $p=0.81$) (Figure 12). In addition, we did not find a significant difference between intake after the MSF sessions and control condition ($F_{(4,68)}=0.35$, $p=0.84$). In addition, no texture, sweetness or interaction effect on intake of the sweet toppings (jam and hazel nut spread) or savoury toppings (ham and cheese) was found.

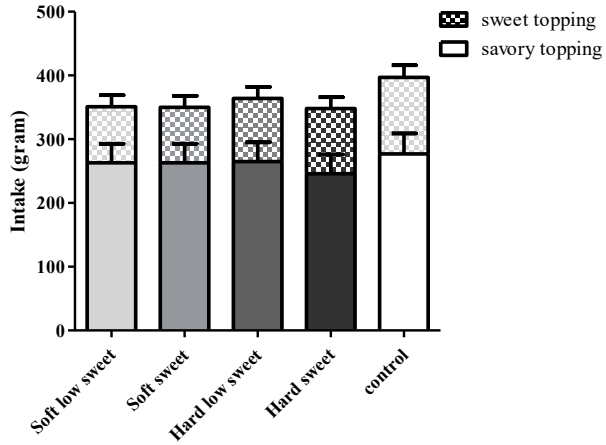

Figure 12. Estimated Mean±SEM post MSF lunch intake (in gram) per model food type. No significant differences were found.

Discussion

We investigated the independent and interactive effects of OSE duration (chewing) and stimulation intensity (sweetness) on endocrine cephalic phase responses, and subsequent food intake. In order to study this we successfully developed a model food system that aid in studying realistic texture and taste manipulations on endocrine responses. The model foods used in this study were equally liked but differed in sweetness level and chewing duration.

The findings of this study show that, insulin levels at 5 min after starting to MSF were 1.1 times higher compared to insulin levels at 20 and 25 min, but these differences between time points could not be attributed to the texture or taste manipulations. In addition, the overall PP response of the hard sweet MSF condition was 1.1 times greater compared to control and a significant texture/taste interaction effect was found. The PP curve of the low sweet hard texture model food was 0.9 times lower compared to the sweet hard texture model food curve. Comparing the total ghrelin curves, we found that the overall ghrelin response of the hard model food conditions was 1.1 times greater compared to the soft model food conditions. No typical cephalic phase peak response was found for any of the endocrine outcome measures. In addition, the found differences are small relative to the variation and would therefore be unlikely to have a physiological effect.

During MSF participants expectorated 12% more saliva during MSF on the hard compared to the soft model foods. In addition to this texture effect participants expectorated 5% more saliva when MSF on the sweet compared to the low-sweet model foods. Subjective appetite ratings showed that, in general, participants felt fuller and less hungry after MSF compared to no MSF. These differences in appetite ratings were, however, not reflected in subsequent food intake during the *ad libitum* lunch meal.

These outcomes are not in line with our hypothesis as we expected to find a typical cephalic peak/spike in the endocrine responses (insulin, pancreatic polypeptide) and an increase in ghrelin concentrations for increased OSE magnitudes (i.e. hard texture; long chewing duration and high sweet intensity) and a consequent decrease in food intake.

Our observed lack of a cephalic phase insulin response (CPIR) is in contrast with other studies that did find a CPIR upon sensory stimulation (36, 44-48). Based on conclusions of Teff *et al.* we expected a typical insulin response of approximately 25% above baseline or 1 % of a normal postprandial insulin release within 2-4 minutes after sensory stimulation, returning back to baseline concentrations by 8-10 minutes (36, 49). However, we observed no changes from baseline in this study for which there could be various explanations. Characteristics of the stimuli or food used to evoke the cephalic response are of great importance. The foremost

important aspect is palatability; it is assumed that the amplitude of the CPIR depends on the palatability of the stimuli used (50). Studies that have found a cephalic phase insulin response used highly palatable food stimuli such as apple pie (30) while the model foods used in this study were rated as neutral (average liking score was 56 mm on 100-mm VAS).

In addition to palatability, the stimuli should consist of multiple sensory modalities to induce insulin secretion as argued by Zafra *et al.* (12). This explains the lack of findings in studies where simple nutrient solutions (12, 51, 52) and sweet tablets (53) were used as stimuli. Only one study has shown an increase in insulin plasma concentration upon sucrose and saccharine oral cavity stimulation with a liquid (45 sec swirl and spit method) (just *et al.*) (32). However, this insulin response was smaller (1.5 µIU/mL rise from baseline for sucrose and 0.9 µIU/mL rise from baseline for saccharine) compared to the 25% above baseline; corresponding to a 2 µIU/mL increase, described by Teff *et al.* (36, 49). This emphasizes the importance of texture and taste qualities for the induction of detectable cephalic insulin response based upon which we expected to find a cephalic response in this study. Power *et al.* argues that the association between insulin response and palatability is more important than nutrients, similarly this may also hold for the sensory modalities being more important than nutrients (22).

Besides the stimuli properties, participant characteristics determine the magnitude of the CPIR. The participants included in this study were healthy and had a low, although healthy, BMI which could possibly explain the lack of CPIR; Teff at al. found that obese participant exhibit a larger CPIR compared to lean participants (54). Besides BMI there are several studies that report that there are cephalic phase responders and non-responders (44, 45, 55). In a recent study of Dhillon *et al.* insulin concentrations were measured during swirling of sweet drinks and MSF of sweet gelatin cubes. In this study sixty-four overweight and obese participants were included and a clear distinction could be made between responsive and non-responsive participants. Over all treatments, 45% of the measured insulin curves were considered to be responsive (rise in insulin 2 min after stimulation). Insulin responses were mostly observed after exposure to sucrose in solid form (44). In addition, Bellisle *et al.* also failed to observe a CPIR in 12% of the participants and Teff *et al.* found 50-75% of the participants to be responders (45, 55).

Based on the classification of Dhillon *et al.*(44) in the present study 54% of the insulin curves were considered responsive and 46% were considered non-responsive. Sub analysis of the responsive curves showed an increased insulin concentration at 2.5 min. after starting to MSF on the hard model foods, compared to soft. In addition, at 5 min an increased concentration for the sweet compared to the low sweet model foods was found. This indicates the importance of

considering insulin responsive and non-responsive participants when studying cephalic phase responses. However, the insulin responder, non-responder classifications were not confirmed by PP and Ghrelin curves. This stresses the need of clear responder non-responder criteria for the different cephalic hormones.

Although CPIR has been most extensively studied, pancreatic polypeptide is considered to show a more robust endocrine cephalic response that is not influenced by nutrients such as glucose but considered a vagal stimulation marker (36). Pancreatic polypeptide increases 100% above baseline starting 10 min after the onset of a meal or after MSF, and concentrations remain elevated for another 30 min (56). Because PP responses are of a larger magnitude compared to insulin responses they are likely a better measure of graded cephalic phase responses to OSE (such as duration and intensity as investigated in this study). However, we also did not find a cephalic PP response. This is not in line with our hypothesis as several other studies were able to detect a PP response in the absence of a CPIR (57, 58).

However, the lack of a cephalic PP response when MSF model foods is in line with the study findings of Mennella *et al.* that found no PP response when participants MSF pudding, which is comparable in texture to the soft model foods used in this study (59). Our findings are also in line with findings of Teff *et al.* that found no difference in cephalic PP response between sweetness concentrations when participants sham fed high sweet (unpalatable) and sweet (palatable) cream cheese crackers (29). In light of this finding, it is not surprising that we did not find a difference between sweetness concentrations that were within the palatability range and therefore did not differ largely in sweetness. This suggest that cephalic endocrine responses are not sensitive to small differences in sweetness levels.

Besides the lack of a CPIR and cephalic PP response we also did not find an increase in ghrelin concentrations comparing treatments to control. Based on other studies we expected an increase in ghrelin concentrations suggesting an initial appetizer effect of MSF (60). In addition, we expected a decrease in ghrelin towards the end of MSF as we hypothesized that continued sensory exposure would lower ghrelin levels (25, 40, 60). The fact that we did not observe either of these ghrelin responses could be due to the mixed macronutrient content of the model foods as it is hypothesized that carbohydrate meals decrease ghrelin concentrations whereas fat and protein stimulate ghrelin secretion (25).

Saliva release is one of the earliest described cephalic phase responses, by Pavlov in 1910 (61) and has been documented since by various other studies (62-65). We observed a significant difference in the amount of saliva produced upon MSF the hard and sweet model foods compared to the soft and low sweet model foods. This is in line with previous reports that

describe an up to five-fold increase of basal saliva release after 30 min of MSF a steak and French fries meal (66). The sensory receptors that are involved in saliva release are both chemical and mechanical (the movement of chewing stimulates saliva flow) (12). This can explain the differences found in saliva produced between texture and sweetness concentrations in our study. The act of chewing serves as a mechanical stimulant of the sensory receptors stimulating saliva flow. The fact that we found a sweetness effect on saliva production is in contrast with findings of Mattes and Pedersen et. al who showed that the macronutrient composition of the food determines the quality rather than the quantity of saliva. For example, sucrose and fructose stimulate amylase-rich saliva (67).

Compared to control, MSF decreased feelings of hunger and prospective consumption and increased fullness over the sham feed period up to 15 min. Differences between treatments and control were approximately 10 mm (100-mm VAS), which is modest but considered meaningful (68, 69). These findings are in line with previous studies that showed that prolonged oral stimulation alone suppresses hunger and increases fullness (35, 70). The differences in appetite ratings were not reflected in the amount eaten during the consequent meal, although, intake was 11% lower for the MSF conditions compared to the control condition but this was not significant. This in contrast with a study of Wijlens *et al.* that found a 15-19% decrease in intake after MSF cake for 8 minutes (35). Possibly, in our case, the total chewing duration was not long enough to affect intake significantly. A study of Mennella *et al.* also did not report an effect of MSF pudding on intake at a consequent meal (59). Few MSF studies have investigated the effect on subsequent food intake; most studies had a preload design with real food intake where participants were instructed to chew each bite for a fixed number of time after which they measured intake of a meal (71-73). For example, a study by Lavin *et al.* showed that chewing a sweet pastille ten times before swallowing compared to drinking a sweet liquid or water reduces intake (28). Another reason why we did not find a difference in intake after MSF might be because artificial sweeteners were used to manipulate sweetness of the model foods. It is contentious whether tasting (but not ingesting) artificial sweeteners has the same satiating capacity as glucose and sucrose (74). In addition, the fact that we did not see an effect of MSF on intake could be attributed to the time between MSF and the lunch; lunch was provided approximately 15 min after MSF because of the blood samples that were collected up to 10 min after MSF. This may have been too long to affect intake, which is confirmed by the appetite ratings that returned back to baseline 10 min after MSF.

To be able to manipulate texture and sweetness concentration while keeping all other food properties equal, model foods were used in this study. Although our model food system

facilitates a very controlled way of studying the effect of small changes in food properties on physiological responses, the disadvantage of using model foods is that participants are relatively unfamiliar with them, besides, our model foods were rated neutral for liking . Liking and expectations of food (ingestion) are both important to elicit a cephalic response, this could therefore be the reason why we did not find a cephalic insulin, PP or ghrelin respons, in contrast with other studies (12, 30, 50, 75).

Another explanation why we did not find a response could be because larger responses are seen in overweight participants while this study included healthy, normal weight participants. (55). In addition, 54% of the insulin curves in this study were responsive and 46% were non-responsive and because of that, on average, no effect of treatments could be found and no cephalic insulin response was seen

Taken together the before mentioned food and participant prerequisites to evoke measurable cephalic phase responses, it can be concluded that these are highly exacting responses that do not occur in every person at every eating occasion.

Conclusion

Our findings indicate that MSF on model foods does not lead to typical cephalic phase responses. Nevertheless, texture hardness and sweetness increases the total PP response, and MSF on hard texture increases the total ghrelin response compared to soft texture model foods. However, these effects are rather small and this study, among others, shows that there are major dissimilarities in cephalic phase endocrine responses to food stimulation between persons. This emphasizes that inter-individual factors need to be taken into account and stresses the importance of taking into consideration that there are cephalic responders and non-responders. These variable responses to food stimuli make it difficult to study the effect of realistic changes in food properties on cephalic phase responses. Therefore, more research is needed to further elucidate the effects of food texture and taste properties on cephalic phase responses.

Author Contributions

ML, PAM, MM, CG designed the research (project conception, development of overall research plan, and study oversight)

ML PAM MM wrote the manuscript

ML conducted the research

CG PAM MM read and approved the final version of the manuscript

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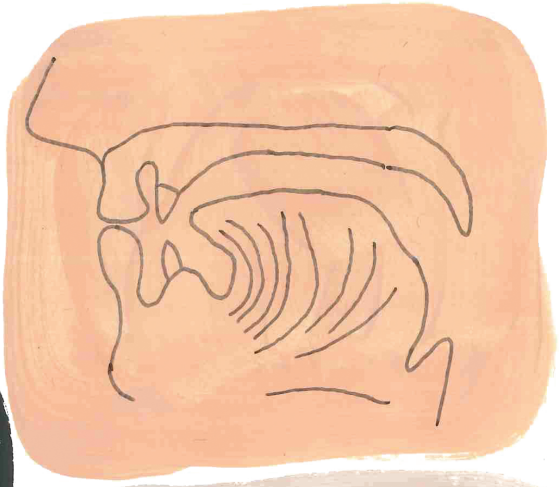

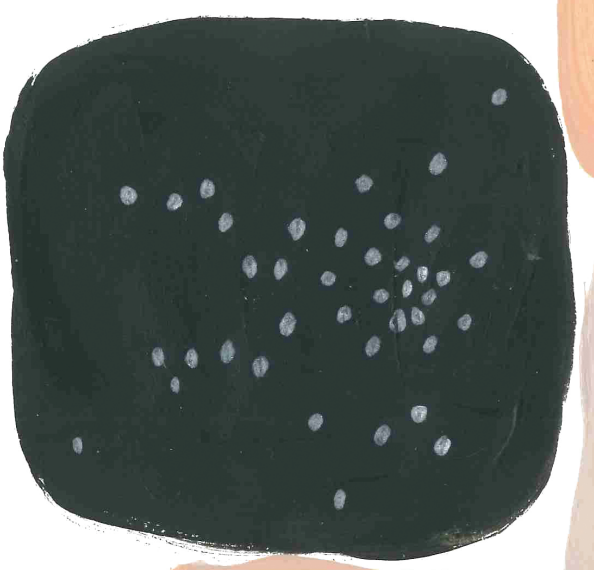

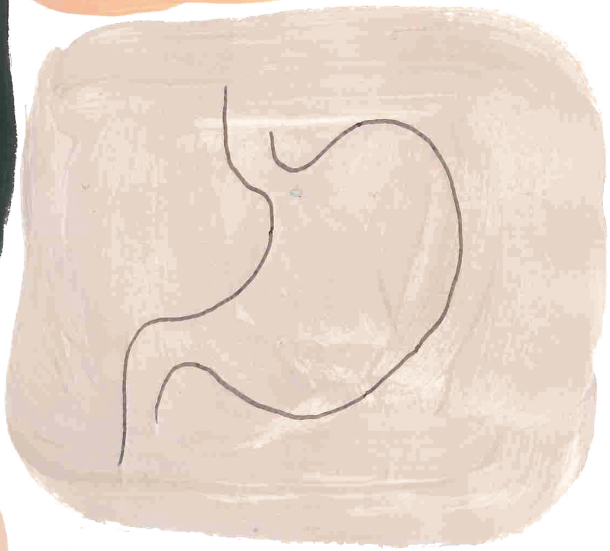

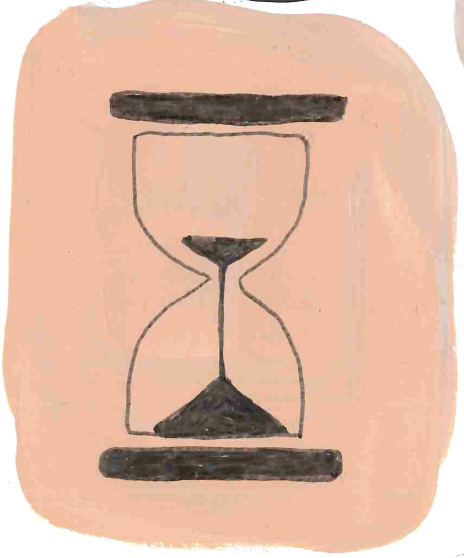

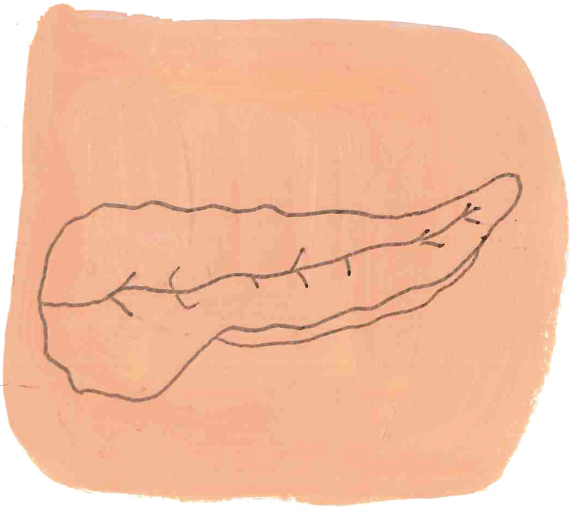

Chapter 4

How oro-sensory exposure and eating rate affect satiation and associated endocrine responses - a randomized trial

Submitted: Lasschuijt MP, Mars M, de Graaf C, Smeets PAM. How oro-sensory exposure and eating rate affect satiation and associated endocrine responses- a randomized trial

Abstract

Background: Longer oral processing decreases food intake. This can be attributed to greater oro-sensory exposure (OSE) and a lower eating rate (ER). How these factors contribute to food intake, and the underlying physiological mechanisms remain unclear.

Objective: To determine the independent and simultaneous effects of OSE and ER rate on satiation and associated endocrine responses.

Design: Forty participants in study 1 (24±4 y, BMI 22±2 kg/m2) and twenty in study 2 (23±3 y, BMI 23±2 kg/m2) participated in a 2x2 randomized trial. Participants ate chocolate custard with added fudge sauce (low OSE) or fudge pieces (high OSE) and with short (fast ER) or long breaks (slow ER) in between bites until they were full. In study 2 endocrine responses were measured during the meal.

Results: In study 1, participants ate 42±15 g less in the slow compared to the fast ER condition, only within the high OSE condition ($p=0.04$). In study 2, participants ate 66±21 g less in the high compared to the low OSE condition and there were no intake differences between ER ($p=0.35$). Eight min after starting to eat, insulin concentrations increased with 42-65% in all treatments compared to control. At the end of the meal insulin concentrations were 81% higher in the high OSE, slow ER compared to the low OSE, fast ER condition ($p=0.049$). PP increased with 62% at 5 min after meal onset in the low OSE, fast ER condition ($p=0.005$). There were no changes in ghrelin concentration.

Conclusions: Greater OSE increases insulin responsiveness. In contrast, PP responses are stronger when OSE is reduced and ER is fast. Insulin and PP responses may thus mediate the independent effects of OSE and ER on food intake. These may be beneficial eating strategies, particularly for type II diabetic patients, to control food intake and maintain glucose homeostasis.

Introduction

Eating behaviours that slow down food intake prevent overconsumption within a meal (1). Examples of such eating behaviours are the rate of eating and oral processing (1). Both these behaviours affect oro-sensory exposure. Oro-sensory exposure (OSE) can be defined as the in-mouth sensory perception. The duration of the exposure is determined by the time needed to form a ready-to-swallow bolus (Figure 1) (2). The oral processing needed to form a ready-to-swallow bolus takes longer for hard compared to soft texture foods. Because of this, hard foods induce higher OSE and are eaten at a slower rate compared to soft texture foods, which leads to smaller meal sizes of hard compared to soft foods (3, 4). This is exemplified by a study of Bolhuis *et al.* where both lower eating rate (ER) and greater OSE reduced intake of tomato soup, with 22% and 8 % respectively (5). However, when a food requires mastication the contribution of OSE to the satiation process may exceed that of ER, as mastication may decrease meal size not only through increased OSE but also through increased effort (6, 7).

The physiological mechanisms that mediate the effect of OSE and ER on food intake (4, 8-12) are not known. Based on literature we hypothesize that OSE may stimulated endocrine responses through vagal afferent signaling (13, 14). In addition, longer OSE duration (slower ER) may result in more time for hormones to respond within a meal (Figure 1) (3, 15). Varying OSE may thus affect endocrine responses within a meal or early pre-digestive responses (16, 17).

Pre-digestive responses or cephalic phase responses have been described for hormones like insulin, pancreatic polypeptide (PP) and ghrelin. Such endocrine responses can be stimulated by sensory food cues such as smell and taste rather than through nutrient uptake (18-24). For example, insulin responses early after meal onset (5 min) have been described in literature, without concomitant rise in blood glucose concentration (25). The assumed function of this early rise in insulin is to maintain glucose homeostasis although there is limited data underpinning this idea (20, 21). PP is an anorectic peptide inhibiting food intake and early PP response may therefore be important for food intake control (22, 23, 26). Ghrelin concentrations increase prior to an expected meal time, signaling hunger, and drop after food consumption (27). Continued sensory stimulation through modified sham feeding decreases ghrelin concentrations within a meal, independent of actual food intake (24).

It has been hypothesized that these endocrine cephalic phase responses may underlie the oro-sensory exposure control of food intake (28-30). This is supported by studies of Teff *et al.* and Dhillion *et al.* in which cephalic endocrine responses to solid foods that required oral processing

(greater OSE duration) were found but not to liquids or soft structured foods (limited OSE) (13, 14, 31).

However, to our knowledge, cephalic phase responses and the oro-sensory control of food intake have never been investigated simultaneously. Therefore, the objective of this study was to determine the independent and simultaneous effects of OSE and ER on satiation and associated endocrine responses. We expected that increased OSE and decreased ER would synergistically increase satiation (lower intake). In addition, we expected that increased OSE would lead to a higher initial (cephalic phase) insulin and PP response and a steeper decrease of ghrelin concentrations, independent of eating rate.

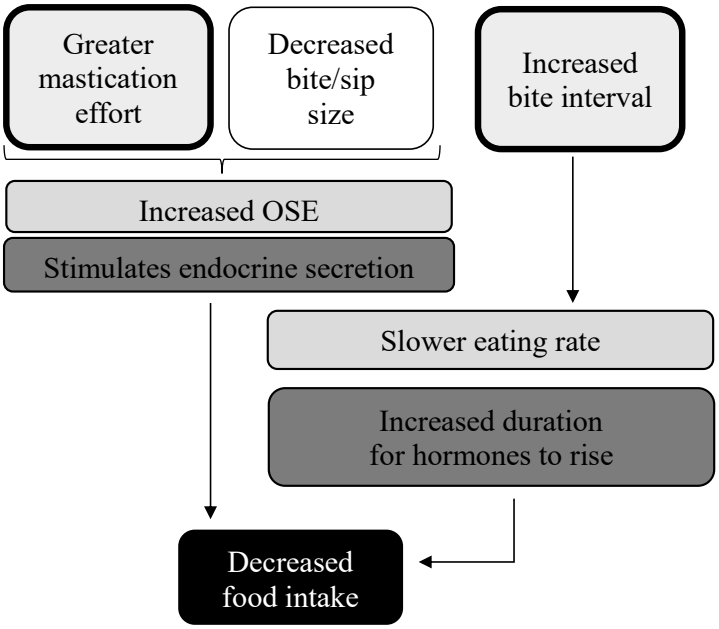

Figure 1. Proposed physiological mechanism behind the oro-sensory exposure and eating rate effect on food intake.

Materials and methods

Study Design

Two studies with a 2x2 randomized crossover design with 4 test sessions were performed. In each study condition participants were asked to eat chocolate custard until the felt full. Study conditions were low or high oro-sensory exposure and a slow or fast eating rate (Figure 2). In study 2, blood samples were collected to determine insulin, PP and ghrelin concentrations, and an additional control condition was included during which participants did not eat or drink anything. The order in which participants received the conditions was randomized following a Latin square Williams design with 1-week washout in-between visits.

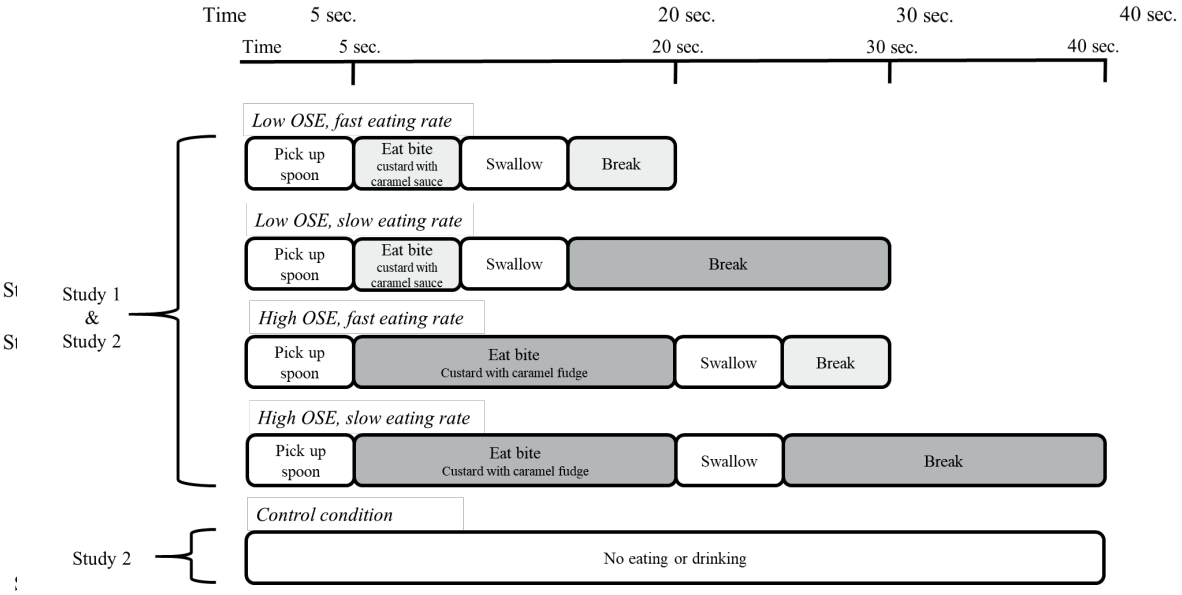

Figure 2. Overview of study conditions. During the treatment sessions, the blocks were repeated for each bite until the participant decided to stop eating.

Participants

The study was performed at Wageningen University and Research, The Netherlands. Healthy male participants between 18-35 years old with a BMI between 18.5-27 kg/m^2 were recruited from Wageningen and surrounding area using flyers and posters. In addition, emails were sent to volunteers in our database who previously had expressed an interest in participating in nutrition studies.

To be eligible participants had to; understand and speak English, commonly eat three meals a day around the same time, weekends not included. Participants were excluded from

participation if they reported to have a lack of appetite or dental, chewing or swallowing problems, or if they followed an energy-restricted diet or if they had gained or lost >5 kg of body weight during the past two months, if they drank more than 21 glasses of alcohol per week, used medication that influenced appetite or taste or smell, did intensive exercise for more than 8 h a week, or were high restrained eaters according to the Dutch Eating Behaviour Questionnaire (DEBQ): men score > 2.9 (32). Personnel and thesis students of the Division of Human Nutrition were not allowed to from participate.

During initial screening participants were screened based on the in- and exclusion criteria and participants rated their liking of the test foods (chocolate custard with and without pieces) in duplicate. Participants were excluded from participation if they disliked one of the custards (defined as score <4 on a nine- point hedonic Likert scale) or if they preferred one custard over the other (defined as >2 point difference in liking). During the meeting participants were also asked if they wanted to participate in study 1 or study 2. Participants that showed interest in participating in study 2 could only participate when they did not have a history of low blood pressure, did not recently donated blood (<1 month prior to the first study day) and did not have plans to donate blood during the study period.

Potential eligible participants were invited to a training session during which they practiced with the computerized eating rate instructions. In addition, participants were trained in scooping similar amounts of custard on their spoon for each bite. Subsequently, height and body weight were measured. Participants were excluded if their measured BMI was outside the 18.5-27 kg/m^2 range. Additionally, participants for study 2 were screened by a nurse. Their antecubital veins were checked for placing an intravenous cannula and some measurements were taken. Participants were included if Hb (8-11 mm/L) (finger prick), fasting blood glucose >3.5 mmol/L (finger prick) and blood pressure >90/60 mm/hg. Participant eligible to participate in both studies were allocated to study 1 or 2 based on their preference.

The study was approved by the Medical Ethical Committee of Wageningen University, The Netherlands (ABR: NL6215708117) and pre-registered in the Dutch trial register under NTR6732 (http://www.trialregister.nl). All participants signed informed consent during the information meeting and received financial compensation.

Sample size calculations showed that a minimum of 34 participants was needed to show an effect of 20% between treatments on custard intake (study 1), and 20 participants were needed for the hormone outcomes (study 2). The calculations of study 1 were based on a (mean±SD) 20±41% expected difference in intake between treatments based on our previous study (10), a correlation of within person measures of r=0.5 and a 15% dropout rate. For the sample size

calculation of study 2 we assumed a variation coefficient of 15% iAUC for the control condition and 20% iAUC for the experimental conditions for insulin (main outcome). A correlation of within person measures of r=0.6 was assumed and a dropout of 15% (25, 33). For both studies the Power was set at 1- β=0.80 at a significant concentration of α= 0.05.

In total, 122 participants joined the information meeting of which 97 potential participants were found eligible; 9 participants were lost to follow up (Supplement I). Finally, 88 participants joined the training session of which 60 participants were eligible for the test sessions. In study 1 (n=40) participants were on average 24±4 years old, had a BMI of 22±2 kg/m^2 and an average DEBQ restraint score of 2.0±0.5 (32). Participants in study 2 (n=20) were on average 23±3 years old and had a BMI of 23 ± 2 kg/m^2 and the DEBQ restrained eating score was 1.9 ± 0.4.

Experimental manipulations

Oro-sensory exposure duration was manipulated by having participants eat chocolate custard with caramel sauce (melted fudge, no mastication, low OSE condition) or caramel fudge pieces (to induce mastication, high OSE condition). Eating rate was manipulated by having long or short breaks between bites or spoonful's (bite interval). Study conditions were single- blinded such that each sample/condition was given a random 3 number code by the researcher. Participants were instructed on a computer when to take a bite. A visual representation of the study manipulations is shown in Figure 2.

Oral processing time and times between bites in the slow and fast condition were determined based on the data obtained during the training sessions. During the training participants were asked to record their eating duration (using a stop watch) for one bite eaten at a slow pace, one bite eaten at a fast pace and for one bite eaten at their normal eating rate for both chocolate custards. Eating chocolate custard with sauce in a slow manner it took participants on average 12±5 s to swallow one bite, when eating fast 4±2 s and when eating at their normal eating pace 7±3 s. For the chocolate custard with pieces participants took 20±10 s when instructed to eat in a slow manner 10±5 s when instructed to eat fast and 15±9s when eating at their normal eating pace. Based on the average normal eating speed, we decided on a 10s eating duration of the chocolate custard with sauce. Taking into account the large SD of the normal eating rate duration we decided on a 20s eating duration for the chocolate custard with pieces to give participants sufficient time to chew.

To manipulate eating rate to the same extent as the OSE duration, the intervals between bites were either short (10 s break) or long (20 s break), including 5 s to pick up the spoon for the next bite.

The amount of the first 5 bites of the custard meal that were standardized. Participants were given pre-weighted appetizer spoons with 10 g of custard that they had to consume in one bite. Standardization of the first 5 bites was important for the endocrine cephalic phase triggered by early OSE and therefore the exposure had to be the same (i.e. no difference in bite size) for all participants. After these 5 standardized spoons, participants were given a bowl with 450 g chocolate custard and were instructed to eat until they were satiated. Participants were encouraged to ask for another portion if desired. In total they could receive two additional standard sized bowls with 150 g after the first large 450 g bowl, i.e. 750 g in total (6239kJ/1492kcal). As it appeared that in study 1 participants stopped eating when they finished their first bowl, we increased the portion size to 1kg (8318kJ/1990kcal) in study 2.

Experimental procedures

Participants were instructed to eat a similar amount of the same meal (of their own choice) the evening preceding the test session. They were instructed to eat their meal between 18:00-19:30 h and not to eat or drink anything except water starting at 22:00 h until their standardized breakfast. Participants received a breakfast yoghurt (200 g, strawberry flavoured 'Breaker', Melkunie, The Netherlands) that they had to consume at home 4 h prior to the start of their test meal and to bring the empty package with them to the lab. Participants were instructed not to eat or drink anything but water after their breakfast yoghurt. The yoghurt package was covertly weighted to determine intake (mean±SD, 191±18 g). Participants were also instructed to avoid high-intensity exercise (everything besides regular walking and biking) and to use the same mode of transport every time they visited and to abstain from alcoholic drinks 24 h preceding the test. To assess compliance participants were asked to keep a 24-hour food and exercise diary.

- Study 1

Participants arrived at the test location 30 min before the test meal to receive instructions and to drink 100 mL water. Before starting the researcher checked the participants' food and exercise diary for compliance to the study rules (Table 1). After that, participants were seated in sensory booths where they filled in the appetite questionnaires on a computer and ate the test meal according to one of the study conditions (Figure 2).

We alternated and randomized the hand of eating (left/right) over sessions and participants. As in study 2, participants were forced to eat with their non-dominant hand when the intravenous cannula was placed in their dominant arm. To make sure participants would not use their dominant hand it was lightly taped to the table.

– Study 2

The participants of study 2 arrived 90 min prior to their test meal (Table 1). Upon their arrival their food and exercise diary and compliance to the study instructions was checked, after which a research nurse placed an intravenous cannula in an antecubital vein. After this participants waited for one hour before the first baseline blood sample was collected to eliminate any adrenalin-related stress effects due to the insertion of the cannula (34). Thirty min before the start of the test session, participants drank 100 mL tap water. Participants were seated in a quiet room and filled out the appetite questionnaire, after which they consumed the chocolate custard (except for in the control condition) until comfortably full, while following the computerized instructions (Figure 2). We wanted to ensure that participants ate until they were full, therefore participants were instructed before, and reminded after the test session, not to eat anything up to one hour after the end of the session.

Test product

Chocolate custard ('Chocolade vla', Friesland Campina, The Netherlands) with small 5x4x2 mm cubic fudge pieces (fudge sprinkles, 'Frusco Frisiana' caramel cubes, NiC Nederland BV, the Netherlands) or with caramel sauce (same fudge sprinkles but melted) was used.

The composition chocolate custard/fudge (sauce or sprinkles) was: 80 wt.% custard, 10 wt.% diary butter (full fat dairy butter 'Albert Heijn' The Netherlands), 10 wt. % caramel fudge. The dairy butter was used to make a caramel sauce of the fudge pieces, the same amount of dairy butter was melted and scooped through the chocolate custard with fudge pieces to have iso-caloric test foods.

Both custards contained 199 kcal/832 kJ, 2.7 g protein, 12.1 g fat, 19.4 g carbohydrates per 100 g. The chocolate custard was stored overnight in a 4 °C fridge and stored in a 6 °C fridge close to the test room the morning of the test sessions. Test meals were taken out of the fridge right before the start of the meal and the temperature was measured right before and after the meal. The increase in temperature pre- to post meal was 4±2 °C.

An overview of all sensory ratings of the test foods can be found in Supplement II, Table 1. In both studies, the two study products were equally liked (both, $p > 0.10$). Prior to the meal the average liking was 71±4 (100-mm VAS), which decreased to 50±5 mm directly after the meal. There were no differences for desire to eat (DTE) the product prior or directly after the meal between study products or conditions (all p-values >0.05), Supplement II.

Study measures

– Food intake and Eating behaviour

In both studies the spoons and bowls were covertly weighted after the session to determine food intake. During eating participants in both studies were video recorded to determine their eating behaviour. A webcam was positioned in front of the participant (face-on) where the lower frame was in line with the shoulders, and the upper frame above the top of the cranium and the sides at shoulder width. Video recordings were coded and analyzed with the use of Noldus Observer XT 11 (Noldus Information Technology, the Netherlands). Behaviours of interest were oral processing duration (s), duration between bites (s), number of chews and number of bites. From these variables, bite size was calculated by dividing the total amount eaten in gram by the total number of bites. Eating rate was calculated by dividing the meal duration by the number of bites (bites/min) and was also expressed in g/min by dividing the amount eaten by meal duration.

– Appetite ratings and wellbeing

At fixed time points participants rated their appetite and the sensory characteristics of the test foods on a 100-mm VAS anchored with 'not at all' to 'extremely'. Appetite parameters questioned were: hunger, fullness, thirst, DTE, DTE sweet, DTE savoury and prospective consumption. Participants rated their appetite after insertion of the cannula, right before the start of the meal t_{-8}, directly after food anticipation t_{-3}, at the end of the meal ($t_{variable}$) and 45 min after the start of the meal (t_{45}).

In addition, participants rated liking of the custard after the first and last bite of the meal and desire to eat the custard after food anticipation t_{-3}, the first bite t_0, the last bite of the meal ($t_{variable}$) and 45 min after the start of the meal (t_{45}). After the meal participants received a small (±20 g) sample cup to rate the sensory characteristics of the custard. Participants were asked to rate the following sensory properties: liking, creaminess, chocolate flavour, caramel flavour, odor intensity, sweetness, chewiness, and smoothness.

After rating the sensory properties, participant were asked to indicate the main reason(s) why they stopped eating from the following options; 'I was full', 'I got bored with the –texture, -flavour, -sweet taste, 'I got tired of chewing', 'I ate the portion I would normally eat', 'I got tired with the slow eating speed', 'I got tired with the fast eating speed' (Supplement III).

- Blood sampling and biochemical analysis

In study 2, blood samples were collected at 8 time points $t_{(min)}$: t_{-8} and t_{-6} (baselines), at t_{-4} participants anticipated on eating the custard and this effect was measured in t_{-2}, at t_0 participants started to eat and consequent blood samples were taken at t_2, t_5, t_8, t_{10} min. The last sample was collected directly after the last bite of the meal.

Blood glucose concentrations were measured directly after sampling with a blood glucose meter (FreeStyle Freedom Lite) using a blood sample collected via the cannula with a syringe.

Insulin, Ghrelin and PP blood samples were collected in 2-mL EDTA tubes. AEBSF blocker (Pefabloc, Sigma-Aldrich) was added to a final concentration of 1 mg/mL (4 mM) to the tubes intended for ghrelin concentration analysis. Additionally, ghrelin plasma samples were acidified to a final concentration of 0.05 N. All samples were placed on ice after which they were centrifuged immediately for 15 min at 2,500 G at 4°C. Plasma samples were stored on dry-ice until all samples were collected after which they were stored in a -80°C freezer until analysis. Plasma insulin concentrations were measured using the Mercodia ultrasensitive insulin ELISA kit with a detection limit of ≤ 0.15 μIU/mL, an intra assay CV of 5.4 % and an inter assay CV of 10.3 %.Plasma PP was analyzed by using a Human pancreatic polypeptide Elisa kit (Millipore) with a detection range of 12.3-3000 pg/mL and an intra assay CV of 2.8 % and an inter assay CV of 4.9 %.Human ghrelin total ELISA kit (Millipore) was used to analyze total ghrelin concentrations with a detection range of 50-5000 pg/mL and an intra assay CV of 2.9 % and an inter assay CV of 7.1 %.

Statistics

Statistical analyses were performed using SAS (version 9.3; SAS Institute Inc., Cary, NC, USA). Participant characteristics are presented as mean±SD. Results are presented as mean±SEM unless otherwise stated. P-values <0.05 were considered statically significant.

Mixed model ANOVA (PROC MIXED) was used to test treatment effects (oro-sensory exposure, eating rate) on food intake, endocrine concentrations, and appetite and wellbeing ratings. In this model oro-sensory exposure and eating rate and time and their interactions were added as a fixed factor. The repeated statement was used to indicate the repeated measures over time per participant. Compound symmetry (CS) was used as a co-variate structure as the variance was homogeneous and correlated consistently.

Normality of the data was checked by visual inspection of the distribution. Insulin, pancreatic polypeptide and ghrelin concentrations were not normally distributed and therefore log10 transformed before analysis. For these variables geometric means and back transformed 95% CI are reported. All outcomes were tested for an order effect. Significant order effects were found for the variables intake and eating behaviour characteristics in study 1 and insulin concentrations in study 2. These outcomes were corrected for order by adding order as a covariate in the model. Post-hoc Tukey corrected T-tests were used to compare means between treatments and Dunnett corrected t-tests were used to compare means of treatments with control. Correlations between eating behaviour characteristics and intake were calculated using Pearson Partial Correlations (PROC CORR), corrected for participant dependency.

Results

Intake and eating behaviour- study 1

As intended, oro-sensory exposure duration was significantly different between the low (6±0.4 s) and high (12±0.4 s) OSE conditions ($F_{(1,37)}$= 690, p<0.001). Moreover, eating rate was successfully manipulated; participants ate on average 46±4 g/min during the fast ER conditions and 34±4 g/min in the slow ER conditions (p=0.002). See Supplement IV for all eating behaviour characteristics per study condition.

We found a significant interaction between ER and OSE for food intake (F(1,39)=5.0, p=0.03) (Figure 3A). Participants ate significantly less (42±15 g) in the slow ER, high OSE compared to the fast ER, high OSE condition (p=0.04). No significant differences were found between ER within low OSE conditions (p=0.30).

Total meal duration was significantly longer for the high (12±1 min) compared to low OSE conditions (9±1 min), and for the fast (10±1 min) compared to the slow ER conditions (12±1min) (p<0.001) (Supplement IV-Table 1). A longer meal duration was associated with higher intake (r=0.68, p<0.001). Overall, larger bite sizes were associated with higher intake (r=0.28, p=0.001). However, bite size did not differ between conditions (p=0.96).

When correcting for both meal duration as well as bite size, we observed a main effect of both OSE and ER on intake (F(1,39)= 62.3, p<0.001, F(1,39)=80.1, p<0.001). Participants ate 86±11 g (19%) less in the low compared to high OSE conditions (p<0.001). In addition, participants ate 90±10 g (20 %) less in the slow compared to the fast ER conditions (p<0.001) (Figure 3B).

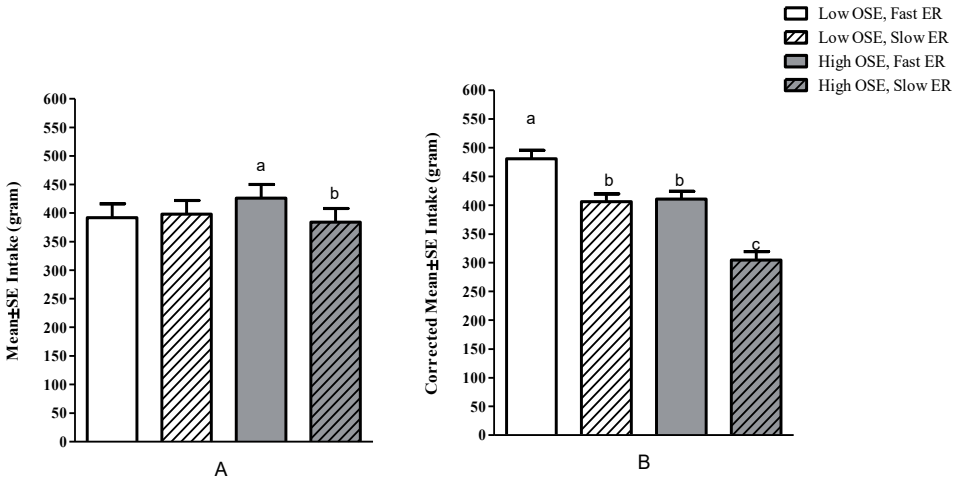

Figure 3. Mean±SEM food intake per study condition- study 1. A= uncorrected, right B=corrected for meal duration and bite size by adding as covariate. Bars with different letter differ significantly (Tukey corrections) p<0.05.

Appetite ratings- study 1

An overview of all appetite ratings can be found in Supplement II, Table 1. Hunger ratings were 7±3 mm lower in the high OSE exposure compared to the low OSE conditions ($p=0.008$). Fifteen min post meal intake DTE was 7±3 mm lower ($p=0.014$), DTE sweet was 4±2 mm lower ($p=0.01$) and prospective consumption was 6±2 mm lower ($p=0.005$), for the high compared to the low OSE conditions. There were no differences in fullness ratings directly after the meal or 15 min post meal. Reasons to stop eating and their frequencies are shown in Supplement III. The main reason to stop eating was feeling full; this was mentioned by 50% of the participants.

Intake and eating behaviour -study 2

Eating behaviour characteristics were similar to those described in study 1; OSE duration was 4±0.7 s per bite for the low and 12±0.7 s per bite for the high OSE conditions. Eating rate was 51±2 g/min for the fast and 35±2 g/min for the slow ER condition within the low OSE conditions. We found a significant interaction, ($p=0.03$) participants consumed 35±2 g/min in the high and 50±2 g/min in the low OSE conditions. See Supplement IV-Table 2 for eating behaviour characteristics per study condition.

Participants ate 66±21 g (14%) less in the high compared to the low OSE conditions ($p=0.04$). No differences in intake between fast and slow eating rates were found ($p=0.6$) (Figure 4A). Within the first 10 min of the meal, participants ate the most in the low OSE fast ER compared to the other study conditions (Figure 5). Larger bite sizes ($r=0.51$, $p<0.001$) and longer meal duration ($r=0.63$, $p<0.001$) positively correlated with intake. Bite size and meal duration were not significantly different across sessions ($p=0.31$ and $p=0.08$, respectively). However, similar to study 1, when adjusting food intake for meal duration and bite size, there was a significant effect of both factors on food intake (both $p<0.001$). Correcting for these factors participants consumed 120±18 (22%) g less in the high compared to the low OSE condition and 143±19 g (26%) less in the slow compared to fast ER condition (Figure 4B).

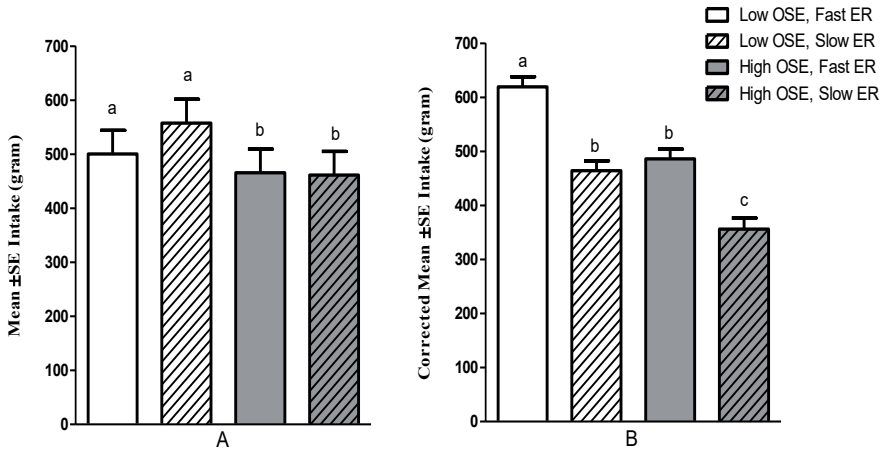

Figure 4. Mean±SEM food intake- study 2 per study condition. A= uncorrected, B= corrected for meal duration and bite size by adding as covariate. Bars with different letters differ significantly at $p<0.05$ (Tukey-corrected).

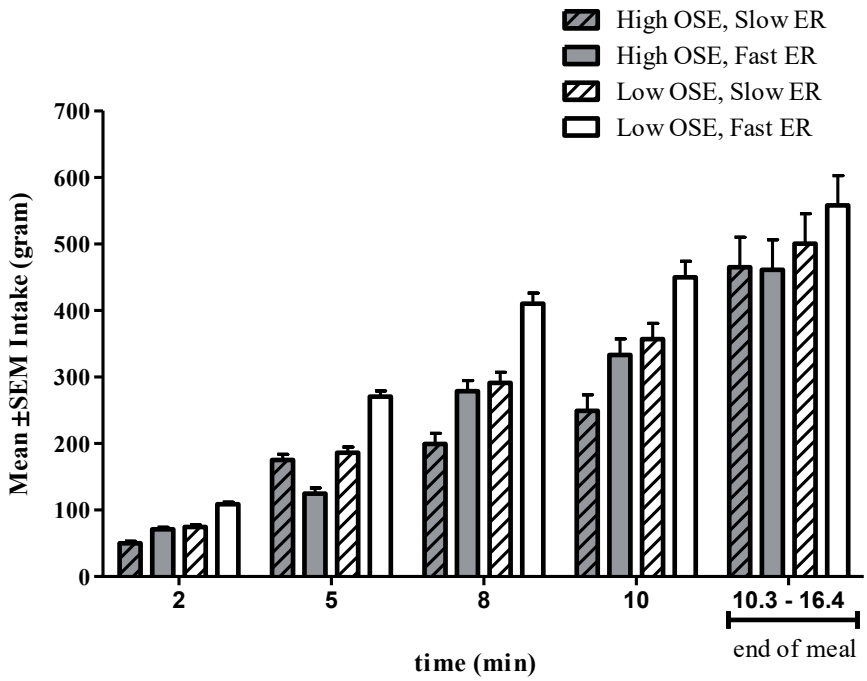

Figure 5. Intake per study condition per time point of study 2.

Appetite - study 2

An overview of all appetite ratings can be found in Supplement II, Table 2. Directly after the meal we found significant 5±3mm higher hunger ratings in the low compared to the high OSE conditions (p=0.03). Likewise, prospective consumption ratings were 5±3 mm lower for the high compared to low OSE conditions (p=0.014). The main reason to stop eating was feeling full (57%). Reasons to stop eating and their frequencies are shown in Supplement III.

Endocrine and glucose responses

- Blood glucose concentrations

In all treatment conditions glucose was significantly increased at the end of the meal (Figure 6); the 'end of the meal' time point significantly differed from all other time points (Δ range 0.84-0.92 mmol/L, all p<0.009) and from control (Δ 1.1-1.2 mmol/L, all p<0.04). No differences were observed between treatment conditions.

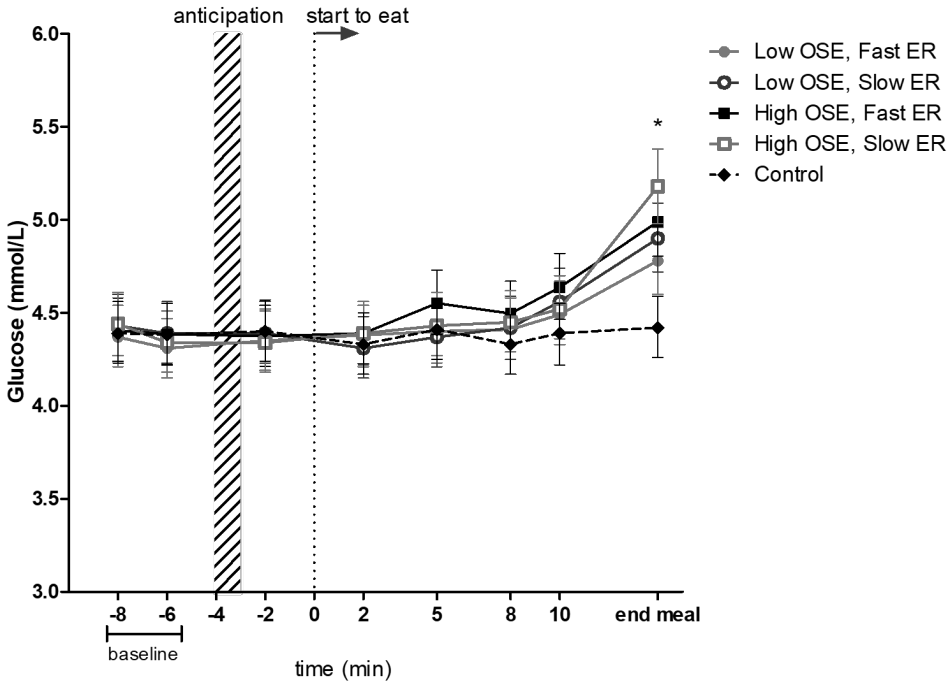

Figure 6. Mean±95%CI glucose (mmol/L) per time point for each condition. * 'End meal' of each study treatment was significantly different to all other time points and compared to the control condition.

– Plasma insulin concentrations

Eight min after starting to eat, insulin concentrations were increased by 1.3-2.0 µIU/mL in all treatment conditions compared to control (all p<0.001) (Figure 7). At 10 min this difference further increased to 4.1-6.3 µIU/mL above baseline (all p< 0.001) and to 12-25 µIU/mL at the end of the meal (all p< 0.001). At the end of the meal, insulin concentration of the low OSE, fast ER condition was 12.2 µIU/mL (95% CI= 9.7-14.5) lower compared to the high OSE, slow ER condition (p=0.049).

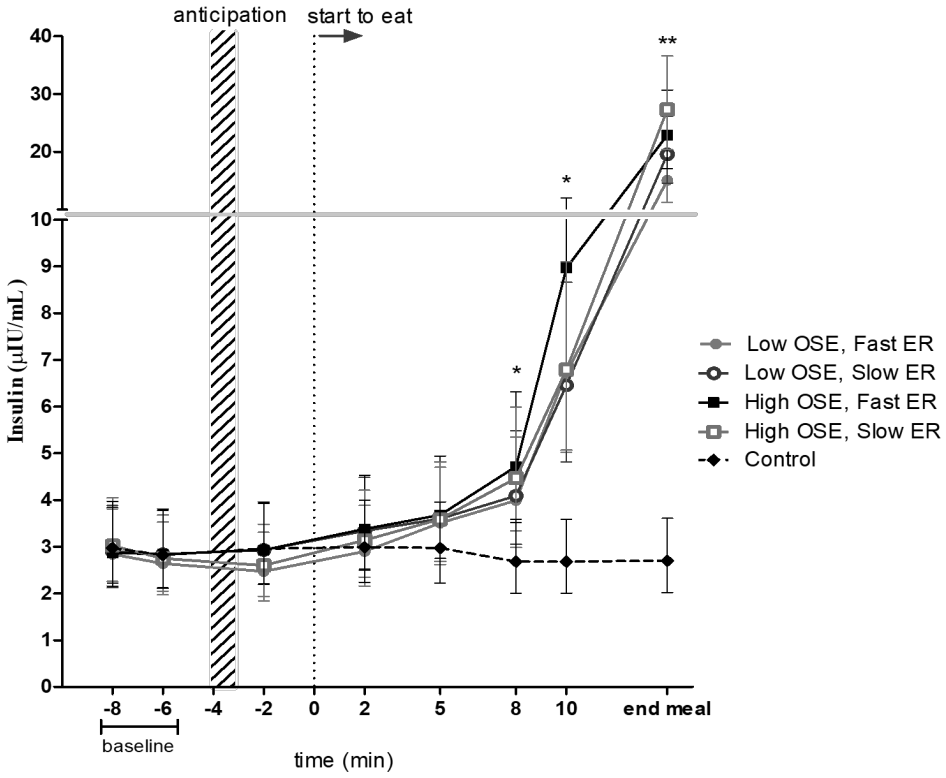

Figure 7. Geometric mean±95%CI Insulin (µIU/mL) concentrations per time point for each study condition. *Time points 8, 10 and end of meal significantly differed with control ** significant difference between low oro-sensory exposure (OSE), Fast eating rate (ER) and the High OSE, Slow ER condition.

– Plasma Pancreatic polypeptide concentrations

Five min after starting to eat, PP concentrations were 49.6 pg/mL higher in the low OSE, fast ER condition compared to control (p=0.005) (Figure 8). Eight min after the first bite, PP concentrations in all treatment conditions were between 78-123 pg/mL higher compared to control ($F(4, 67)=11.7$, all $p< 0.001$). After 10 min PP concentrations in the treatment conditions further increased to 121-235 pg/mL and to 192-209 pg/mL at the end of the meal (all, significantly different from control $p<0.001$). Ten min after starting to eat, PP concentrations in the Low OSE, Fast ER condition were 114 pg/mL higher compared to the High OSE, Slow ER condition (p=0.03).

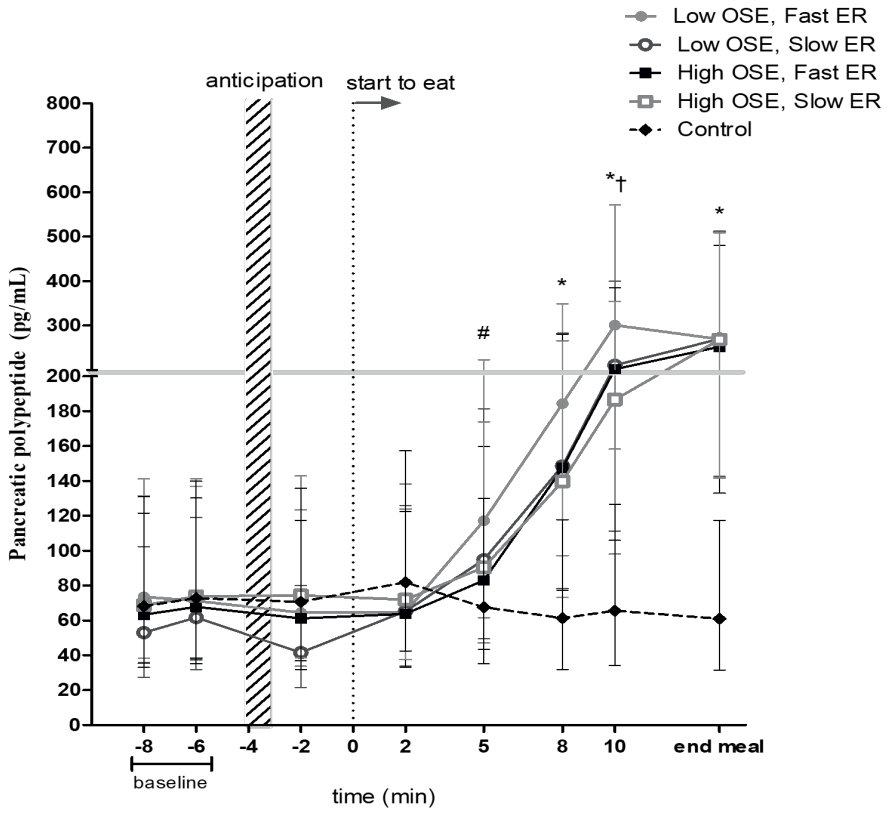

Figure 8. Geometric mean±95%CI Pancreatic polypeptide concentrations (pg/mL) over time, per condition. # significant difference between low OSE, fast ER condition compared to control. * PP concentrations in all treatment conditions were significantly higher compared to control. † significant difference between low oro-sensory exposure (OSE), fast eating rate (ER) condition and High OSE, Slow ER condition (p=0.03).

– Plasma ghrelin concentrations

No significant differences in ghrelin concentrations were found over time between any of the conditions (F(28,532)=0.75, P=0.82) (Figure 9).

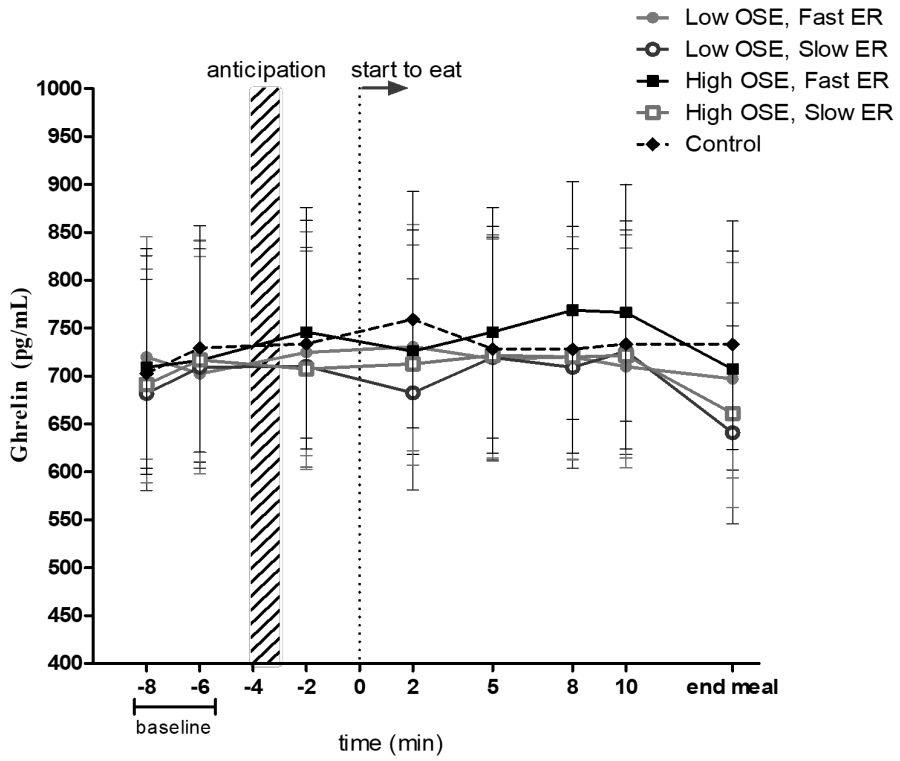

Figure 9. Geometric mean±95%CI Ghrelin concentrations (pg/mL) over time per study conditions. No significant differences over time between study conditions were found.

Discussion

The results of this study show clear effects of OSE and ER on satiation and endocrine responses. In study 1 we found that a reduced ER in the high OSE condition led to a 42g (10%) decrease in food intake. In study 2 greater OSE led to a 66g (14%) decrease in intake but with no effect of ER on intake.

In both studies, meal duration and bite size significantly affected food intake. When correcting intake for these factors we found that a reduced ER led to a 20% (study 1) and 26% (study 2) decrease in food intake. Moreover, greater OSE independently led to a 19% and 22% decrease in food intake, in study 1 and 2 respectively. Differences in intake could not be attributed to differences in liking, as the chocolate custard was equally liked and wanted in all 4 conditions, in both studies.

Both studies together suggest that a slower eating rate and higher oro-sensory exposure decrease food intake, but this may depend on the portion size that is given. When we gave 450g portions (study 1) participants ate less when ER was reduced, in the high OSE condition. This is in line with our hypothesis and with findings of previous studies of our lab (15, 35, 36). However, when we gave participants a larger portion of 1 kg, they ate less in the high compared to the low OSE condition, but without an effect of ER on intake. This is similar to findings of Mosca *et al.* that participants ate significantly less when granola with larger particles was added to yoghurt to induce longer OSE (37). In both studies appetite was rated significantly higher with longer OSE duration, supporting the finding that greater OSE reduces intake. Although differences in appetite ratings were small, they were consistent across the different appetite questions. Based on which we conclude that OSE increases subjective satiation.

We were successful in creating the four experimental conditions (high and low OSE and slow and fast IR); our data showed that in both studies participants ate 15 g/min less in the slow ER condition compared to the fast ER condition, and participants consumed a spoonful of chocolate custard in half the time in the low compared to the high OSE condition. In both studies we observed that bite size and meal duration significantly correlated with food intake. This has also been described by Spiegel *et al.* where eating rate was notably manipulated through smaller bite sizes and participants increased their meal duration with 4 min. (38). As participants compensated for eating rate by increasing meal duration, no differences in intake between the slow and fast eating rate were observed in this study. When we corrected the intake data in this study for differences in meal duration and bite size, greater OSE and slower ER independently decreased food intake by about 20% in both studies. This decrease is substantial but is similar to what previous studies that manipulated eating behaviour to slow down the eating process and

reduce food intake have observed (4, 7, 39). It may be that unconsciously manipulating oral processing and eating rate through, for example, food texture may not lead to compensatory eating strategies. This would then lead to a larger decrease in food intake than found in this study where participants were aware of the eating manipulation (i.e. instructed when to take a bite).

Next to the changes in eating behaviour, we were interested in the underlying endocrine responses. To study these, we used a highly controlled study setting with a relative large sample size (n=20) compared to other cephalic phase response studies (generally around 15 participants) (25). Our pre-testing conditions were strict and participants were highly compliant to enable us to detect small effects as underscored by Teff *et al.* (25). Early insulin increases of 42-65% (1.3-1.9 µIU/mL) were found at 8 min after meal onset in all treatment conditions compared to control. Glucose concentrations did not start to increase until 10 min after the first bite. This suggests that exogeneous glucose did not reach the blood stream before we measured the increase in insulin. However, whether or not the 8-min increase can be classified as a cephalic phase response is debatable. Previously, Teff *et al.* define a cephalic insulin response in normal weight participants as a 23% increase above baseline, corresponding to a 2 µIU/mL insulin increase within 5 min after food exposure (25, 40, 41). However, Morey *et al.* define it as a 5 µIU/mL increase in insulin from baseline (42) while Lucas *et al.* define it as a positive increase greater than twice the standard deviation of spontaneous insulin fluctuations, ranging between 1-17 µIU/mL (43, 44). In the current study we found an insulin increase of 42-65% (1.2-1.9 µIU/mL) above baseline, 8 min after meal onset. This would only qualify as a (late) cephalic phase insulin response according to the definition of Teff et. al (25, 40, 41). Therefore, we consider the insulin increase observed in this study as a response to early glucose absorption rather than as a conditioned insulin response.

At the end of the meal insulin concentrations were 12.2 µIU/mL (81%) higher in the high OSE, slow ER compared the low OSE, Fast ER condition. To determine the response independent of the amount eaten, responsivity was determined. This was done by dividing the increase in insulin concentration (% difference from baseline) by the amount eaten. Based on this, we indeed found that insulin was more responsive in the high OSE compared to the low OSE conditions (Supplement V). This confirms the findings of previous studies (16, 45). For example, a study of Zu *et al.* found higher insulin concentrations directly after the meal when participants chewed 40 compared to 15 times per bite (16). Another study found higher insulin concentrations after soup with chunks (chewing, higher OSE) compared to pureed soup (lower OSE) 10 min after meal onset (45). This indicates that for insulin to rise (sufficiently i.e. induce

satiation) within a meal, oro-sensory exposure combined with a slow ER are important. However, the higher insulin concentrations at the end of the meal in the High OSE, slow ER condition may in part be due to the 6 min longer meal duration compared to the low OSE, fast ER condition. This resulted in more time for insulin to rise before the end of the meal, which may promote meal termination (46). This is also supported by our data as participants ate a smaller meal (average 465±44 g) within a longer time frame in the High OSE, slow ER compared to the low OSE, fast ER condition (average 558±44 g).

PP concentrations were significantly higher in the low OSE, fast ER, compared to the high OSE conditions. This is not in line with our hypotheses, and also not in line with our insulin results discussed above. A statistically significant PP increase of 62% was found at 5 minutes after meal onset in the low OSE, fast ER condition. Albeit smaller than the 100% above baseline previously described as a cephalic PP response (33). In addition, this early steep rise in PP concentration continued and led to a 114 pg/mL (61%) significant higher PP concentration compared to the high OSE, slow ER condition, 10 min after meal onset. This finding is contrary to findings of Yeomans *et al.* where ingestion of a thick and creamy drink (greater OSE) led to a stronger increase in PP concentrations than ingestion of a thin drink (47). An explanation for the early increase in PP in the low OSE fast ER observed in this study might be the endocrine 'break' function. This 'break' function has been described for insulin when food is infused intragastrically and is thought to prevent rapid food intake before the body is able to process the incoming food (48). This hypothesis is also in line with the function of PP to slow down gastric emptying (49).

Ghrelin concentrations tended to be lower at the end of the meal, however, no significant increases with baseline or control were observed. This contradicts the literature about ghrelin which often describes ghrelin as a cephalic phase responder (24, 27, 50-52). Based on this and our previous study (53) we suggest that ghrelin responds slowly to food intake and may therefore not be involved in satiation. Summarizing findings of previous studies that do find a cephalic ghrelin response it seems that ghrelin follows a circadian rhythm and is involved in meal initiation (can be independent of a food cue) and thus plays a role in satiety and hunger (24, 27, 50-52).

Conclusion

In conclusion, greater oro-sensory exposure and reduced eating rate can independently decrease food intake. In addition, this study is the first to study early meal insulin and PP responses simultaneously with eating behaviour and food intake. The results show that greater OSE increases insulin but not PP responsiveness. In contrast, PP responses are greater when OSE is lower and ER is higher. Early PP responses may therefore have a 'break' function to prevent rapid consumption of food. Together, insulin and PP may thus mediate the effects of OSE and ER on satiation. Increasing oral processing duration and slowing down eating rate may especially be beneficial eating strategies for type II diabetic patients to control food intake and maintain glucose-homeostasis.

Acknowledgments

The authors declare no conflict of interest.

ML, PAM, MM, CG designed the research (project conception, development of overall research plan, and study oversight)

ML PAM MM wrote paper

ML conducted the research (hands-on conduct of the experiments and data collection)

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Supplement I- flow chart of in- and excluded participants

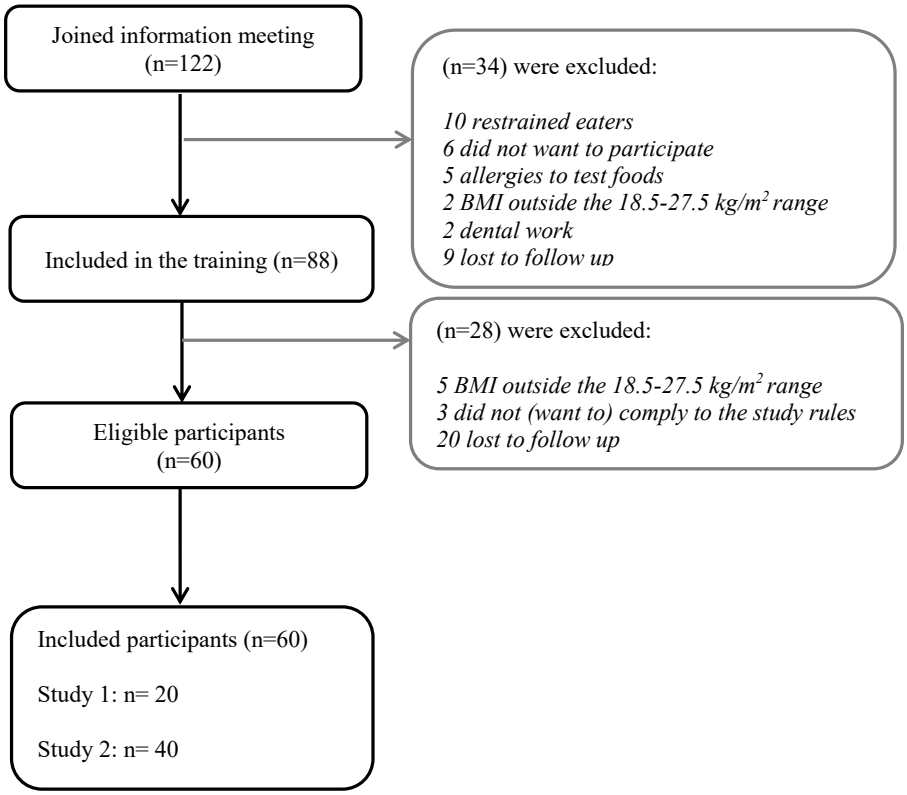


Figure 1. Flow chart of in- and excluded participants of the study

Supplement II- Appetite and sensory ratings

Table 1. Subjective appetite and sensory properties- study 1. All outcomes are order corrected

Characteristic	Low OSE		High OSE		ANOVA p-value		
	Fast	Slow	Fast	Slow	OSE	Rate	OSE*Rate
Hunger- *directly after meal*	25±3	21±3	25±3	23±3	0.65	0.11	0.48
Hunger- *15 min post meal*	38±3	33±3	28±3	30±3	0.008	0.41	0.46
Fullness- *directly after meal*	70±3	74±3	73±3	71±3	0.93	0.52	0.16
Fullness- *15 min post meal*	74±2	74±2	72±2	72±2	0.15	0.96	0.94
Desire to eat- *directly after meal*	27±3	25±3	25±3	25±3	0.64	0.68	0.41
Desire to eat- *15 min post meal*	39±3	36±3	31±3	31±4	0.014	0.63	0.40
Desire to eat sweet- *directly after meal*	13±2	10±2	10±2	10±2	0.49	0.42	0.46
Desire to eat sweet- *15 min post meal*	21±2	16±2	14±2	13±2	0.01	0.13	0.30
Desire to eat savoury- *directly after meal*	33±4	32±4	31±4	36±4	0.76	0.47	0.37
Desire to eat savoury- *15 min post meal*	46±4	39±4	36±4	38±4	0.06	0.40	0.07
Prospective consumption- *directly after meal*	28±2	24±2	27±2	26±2	0.6	0.23	0.15
Prospective consumption- *15 min post meal*	39±2	36±2	32±2	32±4	0.005	0.45	0.43
Thirst- *Baseline*	34±3	38±3	40±3	34±3	0.69	0.59	0.007
Thirst- *directly after meal*	55±4	53±4	52±4	51±4	0.29	0.34	0.77
Sensory properties and liking of study products							
Liking- *Baseline*	71±4	67±4	75±4	70±4	0.29	0.10	0.96
Liking- *directly after meal*	59±3	57±3	57±4	54±4	0.40	0.37	0.94
Desire to eat sample- *Baseline*	73±3	73±3	75±3	74±3	0.31	0.82	0.89
Desire to eat sample-*after meal*	31±4	28±4	28±4	29±4	0.70	0.51	0.85
Sweetness	64±3	65±3	60±3	66±3	0.43	0.14	0.30
Chocolate flavour intensity	54±3	55±3	48±3	52±3	0.16	0.35	0.58
Caramel flavour intensity	37±4	33±4	69±4	67±4	<0.001	0.83	0.41
Creamy	66±3	64±3	55±3	59±3	0.004	0.68	0.31

Table 2. Subjective appetite and sensory properties- study 2.

Characteristic	Low OSE		High OSE		ANOVA p-value		
	Fast	Slow	Fast	Slow	OSE	Rate	OSE*Rate
Hunger- *directly after meal*	18±3	17±3	13±3	13±3	0.03	0.95	0.72
Hunger- *15 min post meal*	23±3	23±3	20±3	24±3	0.71	0.53	0.47
Fullness- *directly after meal*	76±4	69±4	72±4	79±4	0.36	0.89	0.03
Fullness- *15 min post meal*	71±4	67±4	60±4	67±4	0.09	0.63	0.06
Desire to eat- *directly after meal*	16±3	19±3	20±3	14±3	0.72	0.54	0.13
Desire to eat- *15 min post meal*	24±4	25±4	28±4	18±4	0.77	0.23	0.11
Desire to eat sweet- *directly after meal*	8±2	11±2	12±3	6±3	0.74	0.51	0.03
Desire to eat sweet- *15 min post meal*	13±3	18±3	17±3	15±3	0.76	0.71	0.23
Desire to eat savoury- *directly after meal*	26±4	31±4	31±5	18±5	0.37	0.56	0.03
Desire to eat savoury- *15 min post meal*	37±4	35±4	40±5	35±5	0.85	0.37	0.74
Prospective consumption- *directly after meal*	17±3	23±3	14±3	16±3	0.03	0.06	0.29
Prospective consumption- *15 min post meal*	23±3	28±3	24±3	21±3	0.22	0.69	0.09
Thirst- *Baseline*	56±4	52±4	46±5	57±5	0.50	0.28	0.05
Thirst- *directly after meal*	42±4	38±4	43±4	34±4	0.60	0.02	0.27
Sensory and liking of study products							
Liking- *Baseline*	71±4	67±4	75±4	70±4	0.24	0.11	0.98
Liking- *directly after meal*	48±5	50±5	47±5	56±5	0.48	0.18	0.44
Desire to eat sample- *Baseline*	70±4	70±4	73±4	76±4	0.08	0.50	0.43
Desire to eat sample-*after meal*	20±4	25±4	17±4	17±4	0.11	0.52	0.49
Sweetness	64±5	59±5	64±5	63±5	0.62	0.57	0.45
Chocolate flavour intensity	53±5	45±5	43±5	48±5	0.37	0.66	0.07
Caramel flavour intensity	29±5	30±5	63±5	68±5	<0.001	0.56	0.70
Creamy	64±4	59±4	52±4	55±4	0.03	0.68	0.23

Supplement III- Reasons to end meal

Table 1. Reason to end the meal, frequency per study condition- study1(n=40)

Reason to end the meal	Low OSE		High OSE	
	Fast	Slow	Fast	Slow
I was full	22	26	26	28
I got bored with the texture	3	9	5	1
I got bored with the flavour	11	10	9	8
I got bored with the sweet taste	15	16	16	16
I got tired of chewing	2	1	9	7
I ate the portion I would normally eat	9	6	3	3
I got tired with the eating speed (too fast)	16	10	9	3
I got tired with the eating speed (too slow)	0	4	0	1

Table 2. Reason to end the meal, frequency

Reason to end the meal	Low OSE		High OSE	
	Fast	Slow	Fast	Slow
I was full	15	13	14	17
I got bored with the texture	1	1	1	0
I got bored with the flavour	1	2	1	1
I got bored with the sweet taste	4	5	6	0
I got tired chewing	0	0	2	3
I ate the portion I would normally eat	0	1	2	3
I got tired with the eating speed (too fast)	4	2	1	1
I got tired with the eating speed (too slow)	0	2	1	0

Supplement IV- Intake and eating behaviour

Table 1. Intake and eating behaviour characteristics- study 1 (n=40)

Study 1- all order corrected							
Characteristic	Low OSE		High OSE		ANOVA p-value		
	Fast	Slow	Fast	Slow	OSE	Rate	OSE*Rate
Intake (g)	392±24	398±24	426±24	384±24	0.30	0.12	0.03
Intake (g) Corrected: bite size & meal duration	481±15	406±14	411±14	305±15	<0.001	<0.001	0.08
OSE duration (s)	6.0±0.35	6.1±0.35	12.5±0.35	13.1±0.35	<0.001	0.14	0.36
Time between bites (s)	13±0.4	23±0.4	17±0.4	26±0.4	<0.001	<0.001	0.94
Number of chews per bite	2±0.4	2±0.4	9±0.4	10±0.4	<0.001	0.38	0.43
Eating speed (bites/min)	2.9±0.03	2.1±0.03	2.0±0.03	1.5±0.03	<0.001	<0.001	<0.001
Meal duration (min)	7±1	11±1	11±1	13±1	<0.001	<0.001	0.27
Eating rate (g/min)	51±4	36±4	40±4	31±4	0.03	0.002	0.40
Number of bites	24±1.5	24±1.5	24±1.5	23±1.5	0.76	0.31	0.33
Bite size (g/bite)	17±2	17±2	20±2	19±2	0.19	0.84	0.96
Temperature difference (Celsius)	3.7±0.4	4.7±0.4	5.0±0.4	5.4±0.5	0.002	0.03	0.43

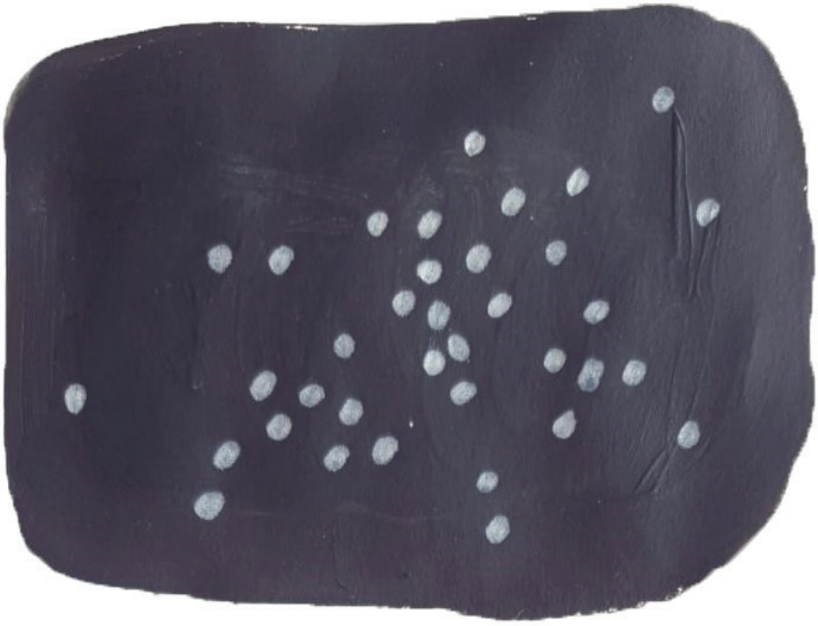
Supplement V- Endocrine responsiveness

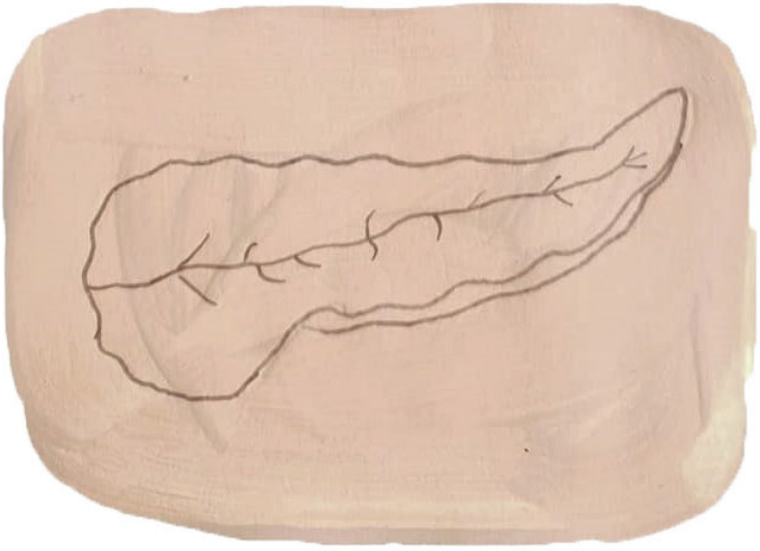
Table 1. Insulin responsiveness. Percentage insulin increase from baseline, per 10 g of food intake, per time moment after the first bite of the meal.

	Low OSE		High OSE	
	Fast	Slow	Fast	Slow
End of the meal	8.1	11.6	15.2	19.5

Table 2. PP responsiveness. Percentage pancreatic polypeptide increase from baseline, per 10 g of food intake, per time moment after the first bite of the meal.

	Low OSE		High OSE	
	Fast	Slow	Fast	Slow
End of the meal	5.7	6.3	6.4	6.3





Chapter 5

Endocrine cephalic phase responses to food cues – weighing the evidence

Submitted: Lasschuijt MP, Mars M, de Graaf C, Smeets PAM. Endocrine cephalic phase responses to food cues- Weighing the evidence

Abstract

Cephalic phase responses (CPRs) are conditioned anticipatory physiological responses to food cues. They occur before nutrient absorption and are hypothesized to be important for satiation and glucose homeostasis. Cephalic phase insulin (CPIR) and pancreatic polypeptide responses (CPPPR) are found consistently in animals, but human literature is inconclusive. We performed a systematic review of human studies to determine magnitude and onset time of these CPRs. Initially, 582 original research papers were found, 50 were included for analysis.

A CPIR (>1μIU/mL) was observed in 41% of the treatments (total n=119). In 22% of the treatments the increase was significant from baseline. Median(IQR) insulin increase was 2.5 (1.6-4.5) μIU/mL, 30% above baseline at 5±3 min after food cue onset. A CPPPR (>10 pg/mL) was found in 48% of the treatments (total n=42). In 21% of the treatments the increase was significant from baseline. Median(IQR) PP increase was 99(26-156) pg/mL, 68% above baseline at 9±4 min after food cue onset.

In conclusion, CPIRs are small compared to spontaneous fluctuations. Although CPPPRs are of a larger magnitude, both show substantial variation in magnitude and onset time. Based on the current evidence, we refute the hypotheses that CPRs improve satiation and glucose homeostasis in daily life.

Introduction

Mechanisms that help to control food intake are important for maintaining a healthy weight (1-3). The regulation of food intake already starts before the first bite with the thought of food and visual and olfactory stimulation(4). During this anticipatory process cephalic phase responses (CPRs) are elicited. CPRs were first discovered by Pavlov (1902), who originally named them 'psychic secretions'. The name later changed to CPRs since they are neurally mediated anticipatory and conditioned responses to food cues rather than responses to nutrients entering the digestive system (5-7).

CPRs are considered as the first phase of digestion and include physiological responses to food-related cues such as the thought, smell, sight and taste of food (8-10). CPRs described in literature include increased salivation, bile secretion by the gallbladder, production of gastric juice, increased gut motility, and gastric and pancreatic endocrine secretions (5, 10-13). The latter include leptin, glucagon, insulin and pancreatic polypeptide (PP) and ghrelin secretion(9). Of these endocrine CPRs, insulin and PP release have been studied most often. Insulin is produced in the pancreatic β-cells and is involved in glucose homeostasis and food intake regulation(14, 15). The cephalic phase insulin response (CPIR) is thought to occur within 2-4 minutes after sensory stimulation and to last for 8-10 min provided that no food is ingested (16-19). However, the magnitude of the CPIR is not well established and there are different definitions of what constitutes a CPIR (17, 18)(20).

PP is an anorectic hormone synthesized by the F-cells in the pancreas. It is mainly released upon fat and protein ingestion. The CPPPR is triggered through vagal activation and PP concentrations can increase up to 100% above baseline concentrations (16, 21). It is thought that cephalic PP concentrations remain elevated for about 30 min – if not followed by actual ingestion (16, 21).

The exact function of the CPIR and CPPPR is not fully understood. However, based on literature reviews that we, and others, did around 10 years ago we hypothesized that CPIR and CPPPR (among other CPP) are important for glucose homeostasis and for the control of food intake(22, 23)(9). CPRs may activate short term satiety-signals which may help to reduce meal size. However, CPRs may also allow for larger meals as the responses prepare the body for incoming nutrients by starting digestive processes in anticipation of incoming nutrients (24)(10, 25). In line with that, CPR magnitude has been shown to correlate positively with motivation to eat, through which they may indirectly affect meal size and total daily energy intake(22). From an evolutionary perspective the ability to accommodate larger meals is an advantage but in the modern food environment it may promote overconsumption(26).

Besides the control of food intake, CPIR may play an important role in glucose homeostasis. Work by Teff *et al.* showed that CPIRs can lead to a reduction in postprandial plasma glucose 16 min after food intake (27). Similarly, Ahren *et al.* found that blocking the neural pathways for CPRs through use of trimethaphan, results in higher postprandial plasma glucose levels (28). CPIR and CPPPR have been found consistently in rodents (29-33). Simple sucrose solutions have shown to be sufficient to trigger CPIR in rodents(30, 33). However, in humans, studies have failed to observe a CPIR or CPPPR (6, 34-41). A wide range of food cues has been used to study CPIR and CPPPR in humans. Examples are; anticipating consumption of favorite breakfast foods, modified sham feeding (MSF) of pizza, and ingestion of a mixed nutrient meal (42-44). The lack of a CPIR or CPPPR could in part be due to food cue specificity. It has been argued that multiple sensory modalities such as texture and flavour are needed to elicit a CPIR or CPPPR response in humans (7, 45). For example, larger cephalic insulin increases have been observed when participants MSF apple pie compared to when only they swirl a sweet solution in their mouth (46).

Additionally, the lack of CPIRs and CPPPRs in some human studies might be because the response is dependent of individual characteristics (47). For example, the response may be dependent of weight status, basal insulin levels and eating behaviour such as restrained eating or disordered eating (48-52). Studies also report cephalic phase insulin responders and non-responders, but have not found a common divider among responders yet(19, 34, 47, 53).

To summarize, endocrine CPRs are found consistently in animal (29-33), but not in human(6, 34-41) studies which could be due to individual characteristics and specificity to certain food cues (47). In previous review papers the literature on human CPIR and CPPPR has been summarized and hypotheses have been posited on their roles in satiation and glucose homeostasis. However, the strength of evidence for these hypotheses has not been assessed quantitatively. Therefore, the main aim of the current review was to determine the magnitude and the onset time of cephalic insulin and pancreatic polypeptide responses. In addition, their specificity for certain food cues and occurrences in specific population groups was explored. The secondary aim was to determine associations between CPIRs and CPPPRs and satiation and glucose homeostasis.

Methods

The study was pre-registered with the international prospective register of systematic reviews (PROSPERO, http://www.prisma-statement.org/) before the start of the literature search (CRD42018100675). The Cochrane Collaborations tool for assessing risk of bias was used to assess the quality of the studies included (54). Studies with a score below 5 were considered as having a 'low risk' of bias and studies with a score above 5 were considered to have a 'high risk' of bias. For descriptive purposes, additional quality parameters were included: whether or not the trial was (pre-) registered, whether a power calculation was done, whether dropouts or exclusion of participants was mentioned, whether there were compliance checks and the presence and quality of a control group or control condition.

Search strategy

The search strategy was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (55). To obtain the final set of research papers the following five databases were searched; Scopus (Elsevier), Science Direct, PubMed, Google Scholar, and The Cochrane Library. The terms included in the search strategy were (cephalic*) or (hormone response) or (endocrine response) combined with insulin and pancreatic polypeptide. See Supplement I for the detailed search strategy used in each database. An additional author and review search was performed for the most common authors occurring in the database. In the initial database we included only original research papers of human studies published in English between January 1945 and the date of search (August 2018). The search was later updated in august 2019 but none of the new found papers fulfilled the inclusion criteria. Review papers, commentaries and case-reports were not included.

All citations that came up in the different search databases were exported to the reference software EndNote™ X8.2. The titles and abstracts of the retrieved papers were screened by the first author (ML) to identify papers that potentially met the criteria as outlined below. To determine reliability of the screening Cohen's Kappa was calculated by having a second author (MM) screen 76 papers in duplicate. The number of papers screened in duplicate is in line with the Cohen's Kappa method. The interrater reliability Cohen's Kappa score was 0.64 (substantial) (56, 57). The full texts of the potentially eligible studies were independently assessed for eligibility by two reviewers (authors ML and MM). Full-texts that were rated differently were discussed by these reviewers until consensus was reached.

In- and exclusion criteria

Studies were included if blood levels of insulin or pancreatic polypeptide were measured and when the intervention was food related i.e. involved thought of food, anticipation of food consumption or other sensory food cues such as sight, smell and taste or actual food intake. Studies that included healthy participants (all weight classes) were included. To have a broader search range and because endocrine cephalic responses possibly play an important role in the (patho)physiology of diabetes and eating disorders, studies that included diabetic or eating disorder patient population groups were also included. Studies were excluded if they were related to other (chronic) diseases or surgery.

Cephalic phase endocrine responses are often described in literature as a peak response occurring within the first 2-10 min after exposure to a food cue (46, 58-60). We used a two-step approach to exclude papers that did not measure insulin and pancreatic polypeptide within this 'cephalic' time frame. First, a quick screening was done by two reviewers (authors ML and MM) to include studies that measured insulin or pancreatic polypeptide within 30 minutes after the food cue. The remaining papers were narrowed down to only the ones that reported insulin or PP concentrations or (I)AUC measures twice within 30 min after a food related cue with one time point measured within the first 15 min by one reviewer (author ML).

Paper selection

An overview of the entire selection procedure (PRISMA flow diagram (61) is shown in Figure 1. Using the search strategy as described previously, we identified 774 research papers. After removing duplicates this number was reduced to 582 unique research papers. These papers were screened based on their abstract and title to determine their eligibility. Main reasons for exclusion based on title/abstract were: related to surgery or chronic diseases (n=140), and non-human studies (n=121). The full text of the remaining 130 papers was screened and the numbers of papers removed due to the following exclusion criteria were: PP and/or insulin was not measured twice within 30 min after a food related intervention with one time point measured before the first 15 min (n=24), (n=8) papers did not measure PP or insulin within the first 30 min, or measured PP and insulin at all (n=12), (n=10) were non original research papers, (n=10) Insulin or PP response was drug induced, (n=6) non-human studies, (n=6) papers were not about cephalic phase, (n=3) papers were about surgery, chronic diseases or related to cancer. Finally, 50 papers were included for reviewing and data extraction. Out of these, 3 papers described 2 experimental studies. This review thus includes 53 studies.

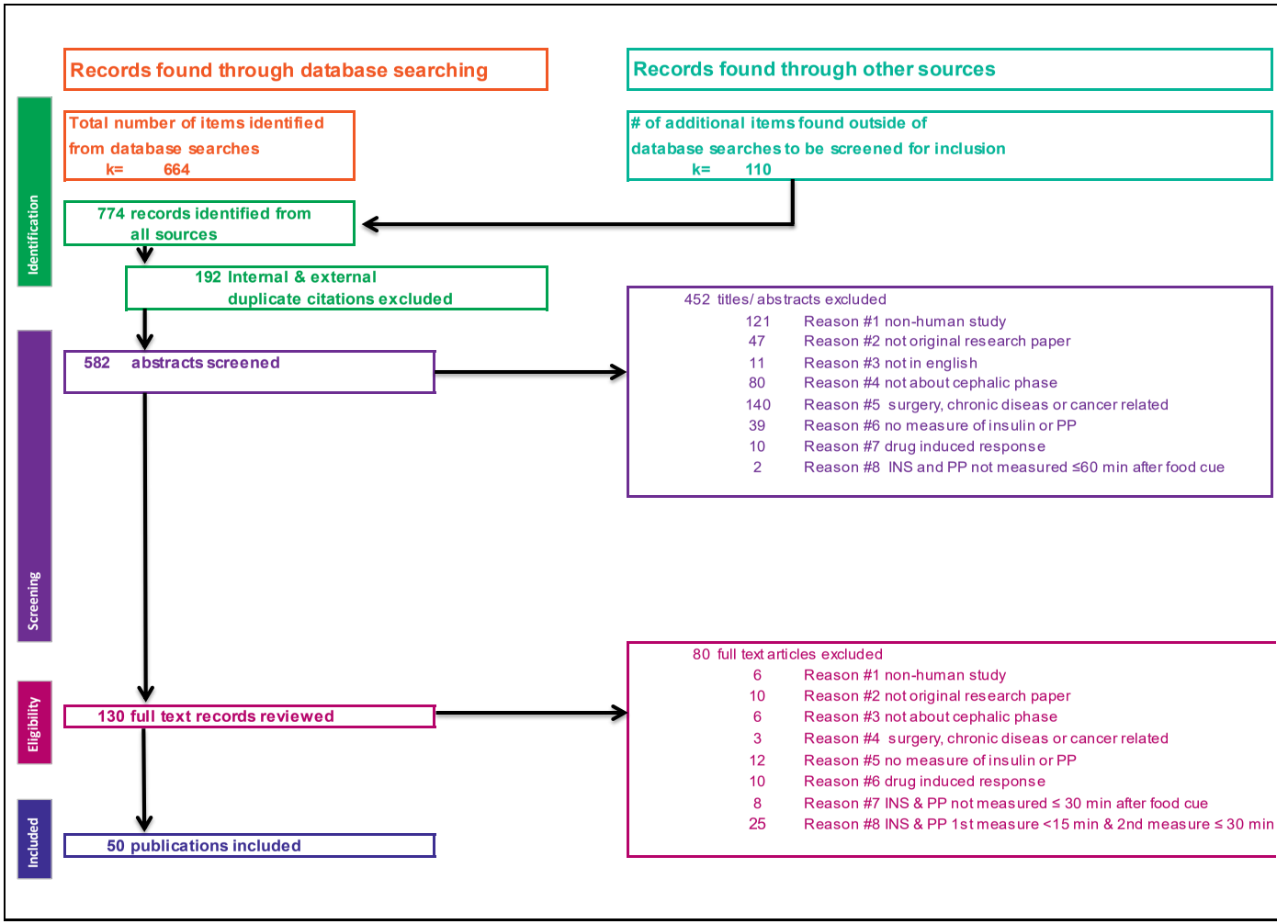


Figure 1. PRISMA flow chart showing paper selection.

Study characteristics and data extraction

Study characteristics such as the study design, (pre-) experimental conditions, participant characteristics, blood sample collection and blood sample analysis were retrieved from the included papers. In addition, for each study, we extracted the insulin, glucose and PP concentration for 5 different time points of a typical cephalic-(postprandial) curve, when applicable. These time points were baseline (where the time point closest to food cue onset was taken in case of multiple baselines), the first time point after food cue a blood sample was collected, the first significant increase, the first peak or increase (which would depict a cephalic response), and the concentration of the second peak (postprandial increase). These time points were denoted per study and study condition. Besides the timing the reported variability (SD, SE, 95%CI) of the peak was measured or derived from the text of the paper. The data extraction from the figures was done with the use of a measurement tool included in Adobe Acrobat Reader DC (version 19). With this tool the distance between two points can be measured with 0.1 mm accuracy.

Because many studies only report the changes relative to baseline and not actual baseline concentrations, we calculated the absolute increase from baseline for each of the 5 time points per study condition. All concentrations were converted to the same unit (μIU/mL for insulin and pg/mL for PP) as follows: For insulin, values in pmol/L were divided by 6.0 to convert them to μIU/mL. Based on a molecular weight of 5807.57 Da, 1 IU insulin equals 0.0347 mg (1-3). For PP, values in pmol/liter were divided by 0.239 to convert them to pg/ml based on a molecular weight of 4181.77 Da (2).

Summary of included studies, sub-populations and treatments

See Supplement II for an overview of all included studies and their findings. Of the 53 studies included 9 studies measured both insulin and pancreatic polypeptide blood concentrations (plasma or serum), 37 measured only insulin and 7 measured only pancreatic polypeptide. Combined, we found 46 studies that measured cephalic insulin responses and 16 that measured cephalic PP responses.

To determine if CPIR and CPPPR are specific to certain population groups or food cue type sub-groups were made (Supplement III). Study populations were classified base on the following sub-groups; healthy normal weight (BMI 18-25 kg/m^2), healthy overweight/obese (BMI >25 kg/m^2), diabetic (type I and II) and eating disorders (anorexia nervosa, bulimia nervosa, binge eating). Study conditions were classified as control (including fasting state and

water) and treatments were grouped in, food anticipation, rinsing of solutions/drinks, modified sham feeding (MSF), or actual food or drink intake.

Summary of methodologies used to study CPIR and CPPPR

- Participant characteristics

See Supplement III Table 1 for an overview of all studies included. The average sample size per treatment was n=14.5. Sample size ranged between n=4 and n=64. Out of the 53 included studies, 20 (38%) included both female and male participants, 20 (38%) included only males and 12 (22%) only females. Over all studies included, the average age (mean±SD) of the participants was 33.9±11.8 y with a range of 20.8-38.5 y.

- (Pre-) experimental conditions

The majority of the studies (37 out of 53, 70 %) was performed in the morning (7AM-12PM) and the number of fasting hours ranged from 3-15h. The majority of the studies had a 10-12h or overnight fast (n= 20, 38%). Other common pre-test conditions or instructions given to participants were: to eat a pre-load or standardized breakfast ca. 4 hours before the experiment, to refrain from exercise and alcohol and smoking tobacco products 24h before the study.

- Blood sample collection and analysis

The average acclimatization time between insertion of the cannula and the first blood sample was 39±49 min. N=18 studies (34%) chose a 30 min acclimatization time. However, 16 studies (30%) did not report acclimatization time. Studies included between 1 and 3 baseline samples and samples were drawn at a 1-5 min interval within the first 10-20 min after the food cue.

The majority of the studies collected blood plasma samples (63%) and most studies (61%) used a radioimmunoassay (RIA) to determine the insulin concentration. Other common analysis methods used were electrochemiluminescence immunoassay and enzyme linked immune absorptive assay. The inter- and intra- Assay coefficient of variability (CV) was reported in 28% of the studies, 3 (7%) reported only the intra- assay CV and 2 (4%) only the inter assay CV. To determine the PP concentrations most studies (75%) collected plasma samples and 81% of the studies used a RIA to determine PP concentration. Nine of the 16 studies (56%) reported both the inter and intra assay CV, and 1 reported only the inter assay CV.

– Overall quality of included studies

See Supplement IV for the Cochrane risk of bias assessment graph. Out of the 53 included studies, 4 studies (8%) registered their trial and 4 (8%) did a power calculation, 13 (25%) mentioned dropouts and 17 (32%) performed compliance checks. From these 53 studies, 35 studies (66%) had a within participant design, 7 (13%) had a within participant between groups design and 11 (21%) had a between participant design. Out of the 53 included studies, 43 studies (81%) had a proper control group or control condition.

To determine the quality of the studies Cochrane Collaborations tool for assessing risk of bias was used. In total, 46 studies (87%) had a score below 5 and were considered at 'low risk' of bias, 5 studies (9%) received a score between 4-5 and were considered at 'medium risk' of bias, 2 studies (4%) had scores above 5 and were considered to be at 'high risk' of bias (Supplement IV).

– Descriptive analysis

Statistical analyses were performed using SPSS (BM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.). Results are presented as median ± inter quartile range (IQR) unless otherwise stated. P-values <0.05 are considered statistically significant. To quantify an average response, time bins were made, time intervals were based on the average time intervals at which insulin and PP concentrations were measured in the original studies. Figure 2 and 5 include only the study treatments that showed an increase in insulin ≥ 1 µIU/mL within the first 10 min and those that showed an increase in PP ≥ 10 pg/mL within the first 15 min after food cue onset. Besides this, study treatments were included if they induced a significant increase from baseline according to the original study (even though the increase reported was <1 µIU/mL for insulin or <10 pg/mL for PP).

Results

CPIR

An increase in insulin ≥ 1 µIU/mL within 10 min after the food cue was observed in 41% (n=49) of the treatments. The median (IQR) insulin increase was 2.5 (1.6-4.5) µIU/mL at 5±3 min after food cue onset. In 22% of all treatments (n=119) the rise was reported as statistically significant from baseline (Figure 2 and Supplement V).

A median insulin increase of 33% compared to baseline was observed within the first 10 min after the food cue (based on the studies that included a baseline concentration). A median increase of 60% was found when only including the treatments that induced a statistically significant increase from baseline. Excluding the treatments that involved actual food intake, baseline insulin increased with 9% within the first 10 min after the food cue. The blood glucose concentration associated with these early insulin increases (≤10 min) did not change from baseline level with a median(IQR) concentration of 4.8 (4.5-5) mmol/L (Figure 3 and Supplement V).

Later than 10 min after the food cue, the median insulin concentration increased to 72% above baseline. Within this time frame glucose concentrations increased with 15%. When the intake treatments were excluded there was no rise in insulin >10 min after food cue onset and glucose remained at baseline level (Figure 3 and Supplement V).

Of the 49 treatments that increased insulin ≥1 µIU/mL, 18% were food anticipation treatments, 16% were induced by rinsing a solution, 31% by MSF and 35% by actual food intake (Figure 2). The relative contribution of each type of treatment is shown in Figure 4 and Supplement V.

The insulin response to food cues was measured in 57 sub-groups (Supplement III). In 61% of the 38 healthy normal-weight populations a CPIR was found, 37% of which were significantly different from baseline. Twelve studies measured insulin concentrations in overweight and obese participants. Within this population, 5 studies found a CPIR of which 3 were significant. Three studies investigated CPIR in participants with (familial) diabetes, 2 found a CPIR of which 1 was significant. Five studies examined CPIR in participants with an eating disorder of which 2 found a significant response (Supplement II).

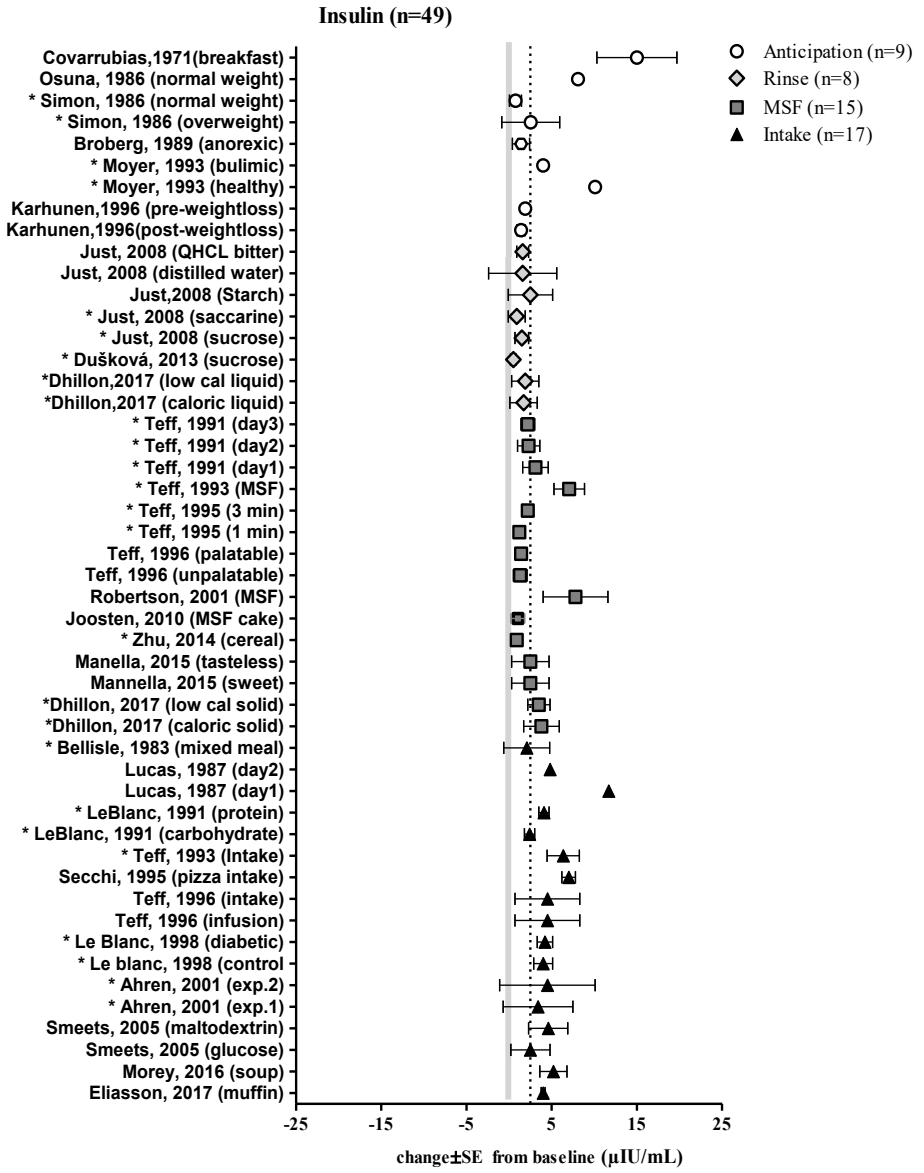


Figure 2. Mean±SEM insulin change from baseline per study (µIU/mL). Treatments of studies were included when the increase was significant according to the original paper or when an increase ≥1 µIU/mL was observed within 10 min (total n=49, i.e. 41% of all included treatment conditions). * indicates significance according to original study (n=26, 22%). The grey line indicates no change from baseline. The dotted line indicates the median change from baseline (median(IQR) = 2.5 (1.6-4.5) µIU/mL) over all studies. The different symbols indicate different treatment types. MSF=modified sham feeding.

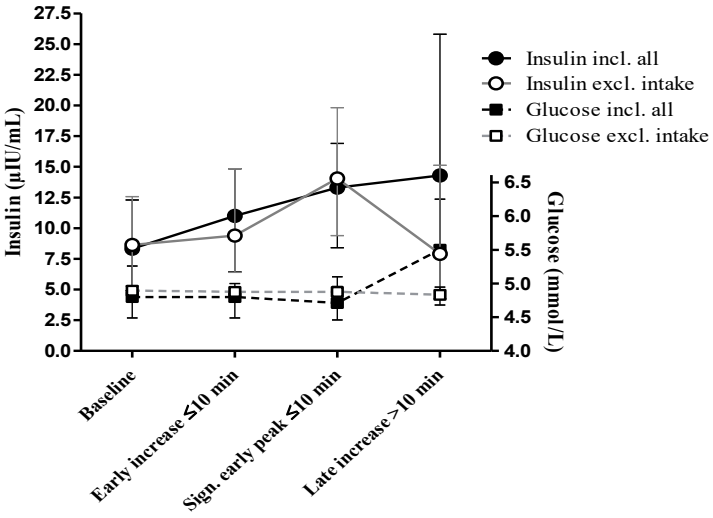


Figure 3. Insulin (µIU/mL) median±75th percentile change from baseline per time bin. Differently colored bar segments indicate the percentage contribution of each treatment type. MSF= modified sham feeding.

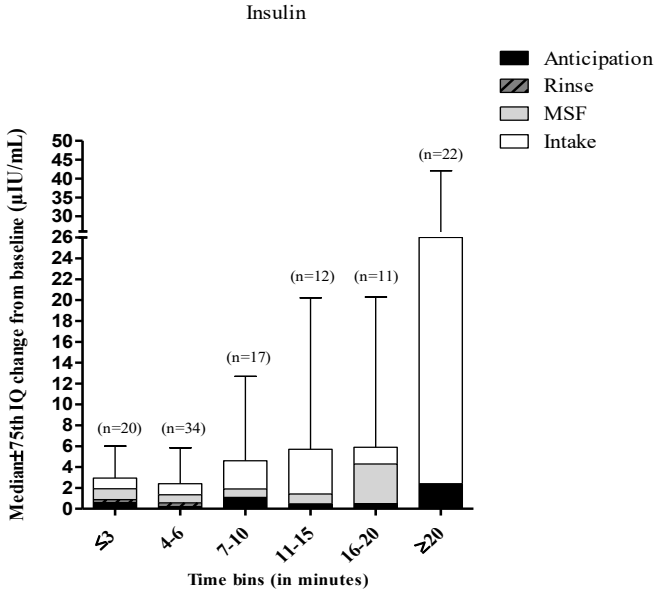


Figure 4. Median±IQR insulin (µIU/mL) and glucose (mmol/L) based on all included studies and treatments for baseline, early increase, significant early peak and late increase (when increases were observed). The number of observations per graph point can be found in Table 1 (Supplement II).

CPPPR

In 48% (n=20) of all treatments (n=42) a PP increase >10 pg/ml within the first 15 min after the food cue was found. The median (IQR) PP increase was 99 (26-156) pg/mL from baseline, at 9±4 min after food cue onset (Figure 5 and Supplement VI). In 21% (n=9) of all treatments (n=42), a significant increase from baseline was found, according to the original study.

We found a median PP increase of 68% from baseline within the first 15 min after food cue onset. Excluding the treatments that involved actual food intake the median PP increase was 17% from baseline within 15 min after food cue onset (Figure 6 and Supplement VI).

After the initial 15 min after the food cue we found a PP increase of 98% above baseline. This late increase in PP concentrations was not solely due to food intake treatments (Figure 6).

Of the 20 treatments that induced a PP response > 10 pg/mL, 15% were food anticipation treatments, 60% were induced by MSF, and 24% by actual food intake (Figure 5). The relative contribution of each treatment type per time bin is shown in Figure 7 and Supplement VI.

In 56% (n=7) of the studies that included healthy participants with a normal BMI (18.5-25 kg/m2) a >10 pg/ml increase in PP was found, 19% were significant increases from baseline.

Three studies measured PP responses to a food cue in overweight and obese participants and one (n=5) found a significant increase (200%) from baseline after exposure to the sight and smell of food (4). Only one study investigated cephalic PP responses in diabetic participants without autonomic neuropathy and found an increase similar to the increase found in healthy controls (5). No such increase was observed in diabetic patients with autonomic neuropathy (5).

We did not find studies examining cephalic PP responses in an eating disorder patient population (Supplement III).

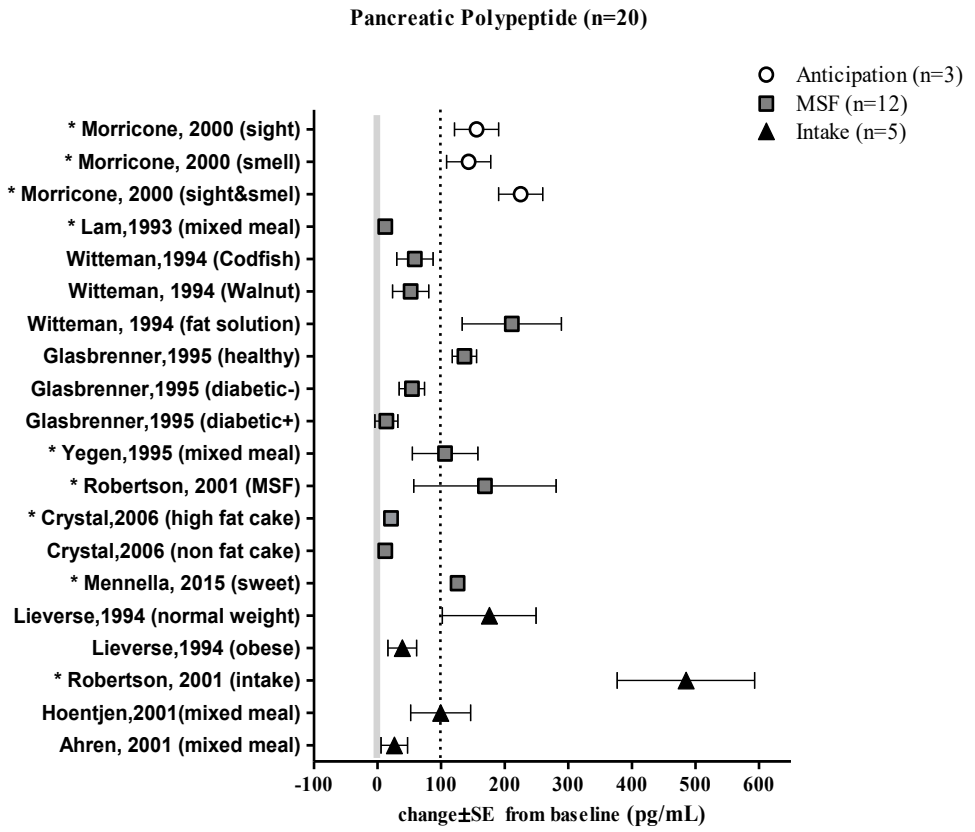


Figure 5. Mean±SEM pancreatic polypeptide change from baseline per treatment (pg/mL). Treatments were included when an increase >10 pg/ml was observed within 15 min or when the increase was significant according to the original paper (n=20, 48%). * indicates significance according to the original study (n=9, 21%). The grey line indicates no change from baseline. The dotted line indicates the median change from baseline (median(IQR), (99 (26-156) pg/mL) over all studies. The different symbols indicate different treatment types. MSF=modified sham feeding.

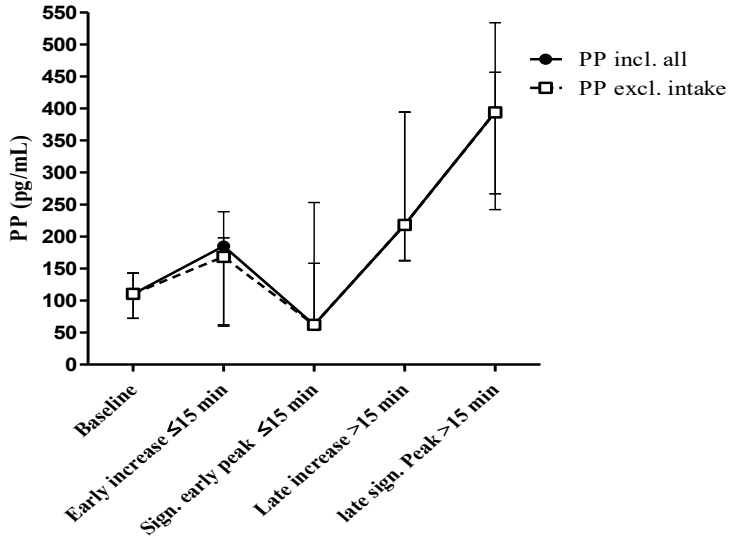


Figure 6. Median±IQR change in pancreatic polypeptide (pg/mL) per time bin based on all included studies and for studies not using food intake as treatment. The number of observations per time point can be found in Table 2.

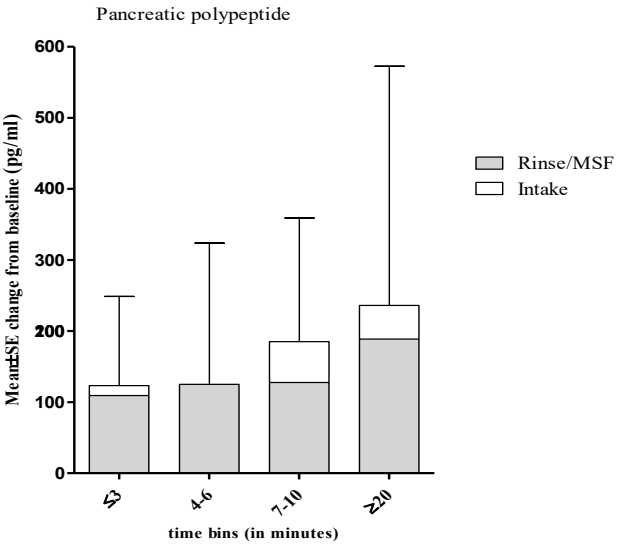


Figure 7. Median pancreatic polypeptide change from baseline (pg/mL) ±75th percentile, per time bin. Differently colored bar segments indicate different treatment types. Modified sham feeding and rinse were combined due to the small number of studies.

Relation between CPRs and food intake, and glucose homeostasis

Four studies investigated the relation of cephalic insulin responses on appetite or satiation (6-8)·(9). Teff *et al.* did not find any differences in ratings on hunger and motivation to eat between the hungry state, and when participants modified sham fed. Furthermore, there was no correlation between appetite ratings and the magnitude of the CPIR (6). This is similar to the study of Simon *et al.* the significant CPIR after presentation of a meal did not correlate with hunger or habitual food intake(9). This is also in line with the finding of Morey *et al.* that satiety ratings do not differ between oral (with cephalic stimulation) and intubated feeding (no cephalic stimulation)(8). However, higher prospective consumption ratings for cephalic phase insulin responders compared to non-responders were found, however these ratings did not correlate with iAUC of insulin (7). Out of the four studies investigating the relation between cephalic insulin responses and appetite or satiation only one study found indirect evidence for this (7).

To the best of our knowledge, only one study investigated the effect of a cephalic PP response on satiation. In this study, participants MSF a bitter (reduced CPPPR) or sweet pudding (greater CPPPR). PP responses after MSF the sweet pudding were 23% greater compared to the bitter pudding, however, no differences in subsequent energy intake were observed (10).

Five studies investigated the relation between CPIR and postprandial glucose homeostasis (6) (11) (9) (12) (13) . From these five, one study found a CPIR along with a significant decrease in glucose (6), while four studies did not observe a concomitant decrease in glucose. These studies observed a CPIR after MSF a peanut butter sandwich (11), after MSF a fat meal (13), after presentation of a meal (9) and after eating a muffin (12).

Discussion

In this systematic review we found that 41% of the 199 included treatments triggered a cephalic insulin response within 10 min after a food cue. Only in 22% of all treatments this rise was reported as statistically significant. The median increase in insulin 5 min after the food cue was 2.5 µIU/mL; this corresponded to an 30% increase compared to baseline - based on the median baseline concentration of 8.5 µIU/mL found in this review.

Whether this 30% increase at 5 min can be considered as a cephalic insulin response depends on the definition used. Three different definitions have been postulated. Teff *et al.* posit that a cephalic insulin increase is 25% above baseline corresponding to a minimum of 2 µIU/mL insulin increase (14, 15). However, more than twice the magnitude in insulin increase (5 µIU/mL) from baseline is defined as a cephalic response by Morey *et al.*(8). Using their definition, the median increase of 2.5 µIU/mL would not be considered a cephalic response. Lucas *et al.* poses a definition which is not dependent on baseline concentrations (16). They define a CPIR as a positive increase greater than twice the standard deviation of spontaneous insulin fluctuations (16). Two types of non-food related insulin fluctuations have been described by previous studies; ultradian- and pulsatile insulin fluctuations (17)·(18)·(19)·(20, 21)·(22, 23). Ultradian insulin fluctuations can easily been distinguished from cephalic increases as they occur within relatively slow time interval of 48-96 min(24). However, pulsatile insulin fluctuations cannot be distinguished from a CPIR as these occur within a 5-17 min time interval, and thus overlap with the 10 min after a food cue during which cephalic responses are ought to take place(16). Because CPIR cannot be distinguished from pulsatile insulin secretions based on time, the magnitude of the responses becomes most important to define a CPIR. The fluctuation amplitudes that have been reported range between 1.1 and 17 µIU/mL (16) (25). The median 2.5 µIU/mL increase we found falls well within this range and can therefore not be distinguished from naturally occurring fluctuations. According to these three definitions, which are all quite arbitrary, only the criterion of Teff *et al.* would define a median increase of 2.5 µIU/mL insulin from baseline as a CPIR (14, 15). A cephalic insulin response of this size would correspond to only 1% of the total postprandial insulin response (area under the curve) after a mixed nutrient meal[29]. Besides this less than half of the study treatments induced a rise in insulin to begin with, and in only a fifth this increase was significantly different from baseline. For these reasons the evidence for the existence of a physiological relevant cephalic phase insulin response seems minimal.

Fewer studies investigated the CPPPR; about half (48 %) of the 42 included treatments induced a PP increase within the first 15 min after a food cue. In 21% of these, this increase was reported

as being statistically significant from baseline. The median PP increase was 99 pg/mL (68%), 9 minutes after the food cue. The found CPPPRs are much larger compared to CPIRs. Across studies the median PP increase was 68% compared baseline. This is substantially smaller than the 100% above baseline that is described as a cephalic PP response (26). However, it does correspond to 50% of the post-nutrient uptake peak and can therefore be considered as a large response (27, 28). Although the magnitude of this PP response can be considered as large, it exhibits high variability and it was only observed in half of the treatments included in this review, and only significantly different from baseline in 23% of them. Thus, CPPPR cannot be considered as a very robust phenomenon. This conclusion is supported by a study that concluded that PP cannot be used as marker of vagal stimulation to diagnose neuropathy in diabetic patients due to the high variability in PP responses (5).

The secondary aim of this review paper was to determine whether responses occurred more frequently, or with a larger magnitude, for some type of food (cues) compared to others. Based on our classification of anticipation, rinsing, MSF and intake treatments we found that the majority of the treatments that induced a CPIR and CPPPR were MSF and food intake treatments. This in line with studies suggesting that multiple sensory modalities are needed to trigger a CPIR or CPPPR in humans (14, 29). This is in contrasts with observations from animal studies, where simple taste solutions consistently induce CPIR and CPPR. One of many explanations may be that humans have the cognitive ability to evaluate craving or wanting a food item. For other cephalic responses, such as salivation, it is known that they are most evident when a person is hungry and strongly craving the food item that they think of or is presented to them (30, 31). However, as yet no research has been done on the direct relation between food craving and CPIR and CPPPR. Some studies have investigated the effect of craving indirectly; they show that the magnitude of the CPIR and CPPPR is larger for palatable than for non-palatable food items (32)·(16). That individuals have to like the food cue in order to elicit a CPIR or CPPPR this explains why the response is not consistently found for the same food products in the same participants as shown by Just *et al.* (25).

Taken together, the data show that CPIR and CPPPR can occur but not at all consistently. Rather, if not due to non-food related fluctuations, they are highly specific and only occur in specific conditions in some individuals. One of our additional research questions was the occurrence of CPIR and CPPPR in specific subgroups. However, studies done in population groups such as overweight, obese, diabetic or eating disorder groups are limited and therefore no answers could be given to this question.

Besides the weak evidence for human CPIR and CPPRs we found no direct evidence for a relation between these responses and satiation or glucose homeostasis. Only four studies (6-9) investigated he effect of a CPIR on appetite or satiation, and only one of these found a relation between higher prospective consumption and a CPIR (7). One study investigated the effect of CPPPR on satiation (10). Tasting (without swallow) a sweet pudding elicited a 23% greater CPPPR than tasting bitter pudding, but no differences in energy intake were found in the subsequent meal (10).

The relationship between the cephalic responses and postprandial glucose homeostasis is especially of interest for people with diabetes. However, the hypothesized role of CPIR in glucose homeostasis, is mostly derived from indirect evidence. For example, it has been suggested that oral sensory stimulation elicits a CPIR which influences glucose metabolism (33). By bypassing oral sensory stimulation, through naso-gastric infusion of food, glucose and insulin levels increase more compared to when food is ingested normally (33, 34). Suggesting a role of the CPIR in glucose-homeostasis. Moreover, two studies, not included in this review as CPIR was studied simultaneously with infusion of dextrose (35) and trimetaphan (36), found direct evidence for a CPIR decrease in glucose. However, five studies (9, 11-13) (6) in our review investigated the direct relation between CPIR and glucose, and only one study found a CPIR 4 min after meal onset, along with a significant decrease in glucose (6). To summarize, there are only three studies that have shown a direct relationship between a CPIR and glucose-homeostasis, therefore evidence for an added value of CPIR in glucose-homeostasis is limited. This may be due to compensatory behaviour of glucagon or gastric emptying rate to prevent nutrients from entering the blood stream too fast (6, 34).

Besides the differences in food cue type, and population studied, other methodological differences may explain the inconsistency between studies' findings. For example, the duration between placement of the catheter or cannula and blood sample collection. For example a study from Alvarez *et al.* showed that serum insulin levels increased with 0.9 uIU/L up to 14 min after placement due to a stress response (37). Additionally, a wide range in the number of fasting hours before start of the intervention or food cue exposure was seen across the studies included, this may have caused differences in food craving and thus cephalic phase responses. Another methodological remark is that the measurements highly depend on the baseline fasting sample, therefore multiple baseline samples are needed to conclude whether the increase is due to the presented food cue or natural fluctuations (38). The vast majority of the studies only reported changes from baseline to correct for individual baseline differences. This is not in line with the advice of the Appetite Task Force of ILSI to correct for baseline differences by means of

ANCOVA rather than subtracting baseline (39, 40). Considering natural fluctuations over time in the baseline, variations in baseline are of interest and needed to draw conclusions. Especially in case of repeated measures, the chances of a type I error increase and statistical significance is therefore not of the primary interest. Instead, the size of the response relative to its (baseline) variation should be taken into consideration when drawing conclusions (41). The changes of a type I or II error depend on the sample size, only 8% of the studies did a power calculation prior to the study and the sample size in these studies ranged from 14-22 participants (42-45). Future studies should take the above-mentioned methodological issues into account and focus on individual (phenotype) differences in food perception and appreciation in relation to cephalic insulin and PP responses.

Conclusions

About half of the treatments observed a CPIRs and CPPPRs and of these only a fifth found a statistically significant increase compared to baseline concentrations. The size of the CPIRs increase (relative to spontaneous fluctuations) is small and there is substantial variation in magnitude and onset time of CPIRs and CPPPRs between food cues and individuals. Taken this into consideration, we conclude that there is only little evidence for a physiological relevant cephalic insulin or pancreatic polypeptide response. A large population level study where insulin and PP concentrations can be measured continuously throughout everyday life is needed to confirm these conclusions. More importantly, the controlled laboratory setting in which cephalic phase responses have been studied to date make a translation to a natural and realistic food environment inherently difficult. Therefore, based on the current evidence, we refute the long-standing hypotheses that cephalic phase responses improve satiation and glucose homeostasis in daily life.

Author contribution

ML, PAM, MM, CG designed the research (project conception)

ML PAM MM wrote the manuscript and reviewed papers for inclusion

ML conducted the systematic review and collected, organized and analyzed the data

All authors read, edited and approved the final manuscript.

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Supplement I – Search terms used per database

Scopus:
TITLE-ABS-KEY ("cephalic phase") OR TITLE-ABS-KEY (cephalic*) TITLE-ABS-KEY ("pancreatic polypeptide*") OR TITLE-ABS-KEY (pancreatic*) OR TITLE-ABS-KEY ("pancreatic polypeptide response") OR TITLE-ABS-KEY (insulin*) OR TITLE-ABS-KEY ("insulin response") AND TITLE-ABS-KEY ("cephalic phase") OR TITLE-ABS-KEY (cephalic*) OR TITLE-ABS-KEY ("hormone response") OR TITLE-ABS-KEY ("endocrine response") OR TITLE-ABS-KEY (endocrine*) OR TITLE-ABS-KEY (hormone*) OR TITLE-ABS-KEY (digest*) OR TITLE-ABS-KEY (physiology*) OR TITLE-ABS-KEY ("within meal") OR TITLE-ABS-KEY (satiation) OR TITLE-ABS-KEY (appetite) OR TITLE-ABS-KEY (hunger) OR TITLE-ABS-KEY (fullness) OR TITLE-ABS-KEY (vas) OR TITLE-ABS-KEY ("visual analogue scale") OR TITLE-ABS-KEY ("energy intake") OR TITLE-ABS-KEY ("energy consumption") OR TITLE-ABS-KEY ("energy ingestion") OR TITLE-ABS-KEY ("calor* intake") OR TITLE-ABS-KEY ("calor* consumption") OR TITLE-ABS-KEY ("calor* ingestion") OR TITLE-ABS-KEY ("food intake") OR TITLE-ABS-KEY ("food consumption") OR TITLE-ABS-KEY ("food ingestion") OR TITLE-ABS-KEY ("ad libitum") OR TITLE-ABS-KEY ("body weight") OR TITLE-ABS-KEY ("body mass INDEX") OR TITLE-ABS-KEY ("anthropometric") AND NOT TITLE (“rat”) AND NOT TITLE (“rats”) AND NOT TITLE (“dog”) AND NOT TITLE (“mouse”) AND NOT TITLE (“mice”) AND NOT TITLE (“rodents”) AND (LIMIT-TO (DOCTYPE , "ar ")) AND (LIMIT-TO (LANGUAGE , "English")

Pubmed:
((((((cephalic insulin [MeSH Terms]) OR cephalic pancreatic polypeptide[MeSH Terms]) OR (((cephalic [Title/Abstract]) AND pancreatic polypeptide[Title/Abstract]))) OR (((cephalic [Title/Abstract]) AND insulin[Title/Abstract])))) AND ((((((((((((((("digestion"[Title/Abstract]) OR "physiology"[Title/Abstract]) OR "within meal"[Title/Abstract]) OR "satiation"[Title/Abstract]) OR "appetite"[Title/Abstract]) OR "hunger"[Title/Abstract]) OR "fullness"[Title/Abstract]) OR "VAS"[Title/Abstract]) OR "visual analogue scale"[Title/Abstract]) OR ((("energy intake"[Title/Abstract]) OR "energy consumption"[Title/Abstract]) OR "energy balance"[Title/Abstract])) OR ((("calor* intake"[Title/Abstract]) OR "calor* consumption"[Title/Abstract]) OR "calor* balance")) OR (("food intake"[Title/Abstract]) OR "food consumption"[Title/Abstract])) OR (("ad libitum"[Title/Abstract]) OR "ad libitum intake")) OR "body weight"[Title/Abstract]) OR "body mass index"[Title/Abstract]) OR "BMI"[Title/Abstract]) OR "Anthropometric"[Title/Abstract])) NOT (((((((((patients[Title]) NOT tumor[Title]) NOT ((cancer[Title]) OR carcinoma[Title])) NOT pancreatitis[Title]) NOT Pancreatectomy[Title]) NOT "cells"[Title]) NOT "diabetic patients"[Title]) NOT "diabetes"[Title]) NOT "therapy"[Title])

Science direct:
(Cephalic phase AND insulin OR "pancreatic polypeptide") NOT "mice" NOT "rat" NOT "Rodent" NOT "cell" NOT "tumor"

Cochrane & google scholar:
“cephalic insulin”
OR
“Cephalic pancreatic polypeptide”

Supplement II. Overview of studies and findings

Table 1. Methods and results of studies investigating CPR to food cues of insulin and pancreatic polypeptide per participant group.

Study	N	Age, y Mean±SD or range	BMI, kg/m^2 Mean±SD or range	Population and food cue specifics	Exposure to Food cue	Exposure duration	Conditions	Effect on		
Healthy normal weight participants								Glu	Ins	PP
Lasschuijt, 2018[34]	18M/0F	22 ± 2	22 ± 2	strawberry gel model foods	MSF	15 min	Hard vs. soft and low vs. high sweet model foods	=	=	=
Kashima, 2017[85]	3M/5F	21±2	20.4 ± 2.1	15% glucose solution load	Intake	30 seconds max.	With and without sweet taste perception	⇨*	⇨*	n.a.
Morey, 2016[20]	10M/0F	37.8±3.4	18.5-30	Oral vs gastric infusion of 400 mL tomato cream soup	Intake	Freely	Oral	⇨	⇧⇧	n.a.
							Gastric	⇨	⇨	n.a.
Cedernaes, 2016[35]	16M/0F	22.9±0.7	22.9±0.5	With and without normal sleep duration evening before. Sucrose solution	Rinse	45 Sec	Little sleep (4.25 hr.)	=	=	n.a.
							Good sleep (8 hr.)	=	=	n.a.
Mennella, 2015[67]	10M/0F	28±1	22.7±0.6	milk pudding no taste (control), sweet, (liked), bitter (unliked)	MSF	3 min	Bitter pudding	n.s.	=	=
							Sweet pudding	n.s	⇧	⇧*
							Control (tasteless control pudding)	n.s.	⇧	=
Veedfald, 2015[94]	25M/0F	67.1±1	25±1	Glucose load	Intake	15 min	Intake glucose load	⇨	n.a.	⇨
Zhu, 2014[95]	10M/0F	27±n.s.	23.4±0.9	Normal to overweight participants. MSF on different macronutrient food items	MSF	3 min	Macadamia nuts	⇨*	=	n.a.
							Mozzarella cheese	⇨*	=	n.a.
							Kellogg frosted cereal	⇨*	⇧*	n.a.
							Water	⇨*	=	n.a.

Study	N	Age, y Mean±SD or range	BMI, kg/m^2 Mean±SD or range	Population and food cue specifics	Exposure to Food cue	Exposure duration	Conditions	Effect on		
Healthy normal weight participants								Glu	Ins	PP
Spetter, 2014[93]	14M/0F	24.6±3.8	22.3±1.6	gastric load (water), Gastric load(chocolate milk) oral load (chocolate milk)	Gastric vs oral intake	n.s.	oral caloric	⇨	⇨	n.a.
							gastric caloric	⇨	⇨	n.a.
							gastric non-caloric	⇨	=	n.a.
Dušková, 2013[96]	15M/0F	28.8±6.3	23.4±1.7	sucrose, sweetener and water rinse	Rinse	n.s.	sucrose	=	⇧*	n.a.
							aspartame	=	⇧	n.a.
							Water	=	=	n.a.
Ford, 2011[36]	1M/7F	22-27	18.8-23.9	load followed by rinse same solution. Control: 50 ml water preload with rinse sucralose	Intake+ Rinse	MSF 1 min per swallow	Water	=	=	n.a.
							cephalic sucralose	=	=	n.a.
							sucralose	=	=	n.a.
							Maltodextrin +sucralose	⇨	⇨	n.a.
Lindgren, 2011[37]	12M/0F	23.2±2.2	21.9±2	Oral ingestion of 3 ml/kg lipid emulsion	Intake	n.s.	Oral exposure to fat	⇨	⇨	n.a.
Bello, 2010[50]	0M/22F	24.8±6.5	23.1±2.7	yoghurt different fat% with added fat free cocoa	MSF	3 min	non-fat placebo, fat placebo, fat Naltrexone	=	=	n.a.
Massolt, 2010[38]	0M/12F	26.6±n.s.	18-25	Eating and smelling 30 gram od dark chocolate	Smell, Intake	5 min max	Control (fasted)	=	=	n.a.
							Smell	=	=	n.a.
							Eat	=	⇨	n.a.
Just, 2008 - final study[58]	11M/8F	26±5	23.3±2.3	Normal to overweight participants. 10 ml sweet taste solution	Rinse	45 sec	Sucrose	=	⇧*	n.a.
							Saccarine	=	⇧*	n.a.

Study	N	Age, y Mean±SD or range	BMI, kg/m² Mean±SD or range	Population and food cue specifics	Exposure to Food cue	Exposure duration	Conditions	Effect on		
Healthy normal weight participants								Glu	Ins	PP
Just, 2008 pilot study[58]	2M/3F	29±7.6	n.s.	Normal to overweight participants, 10 ml of different taste solutions	Rinse	45 seconds	Starch	=	⇧	n.a.
							QHCL (bitter)	=	⇧	n.a.
							citric acid (sour)	=	=	n.a.
							MSG (umami)	=	=	n.a.
							NACL (salty)	=	=	n.a.
							distilled water	=	⇧	n.a.
Crystal, 2006[49]	0M/22F	18-29	22.4±0.9	MSF high fat and non-fat cake and fasted control	MSF	3 min	high fat cake	=	=	⇧*
							non fat cake	=	=	⇧
							control(fasted)	=	=	=
Smeets, 2005 [97]	5M/0F	20.4±2.5	21.7±1.1	(sweet) water solutions	Intake	n.s.	Water	=	=	n.a.
							water+75 g glucose	⇨	⇧⇧	n.a.
							water+ aspartame	=	=	n.a.
							water+ maltodextrin	⇨	⇧⇧	n.a.
Hoentjen, 2001 [16]	3M/5F	19-24	n.s.	Bread, cheese, hamburger 20 g margarine 1 boiled egg and 150 ml tea	Saline infusion and MSF	30 min	mixed nutrient meal	n.a.	n.a.	⇧⇧
Robertson, 2001 [70]	4M/6F	35.5±n.s.	24.1±n.s.	cheese pizza served full fat milk and cream	MSF and Intake	10-15 min	water	=	=	=
							MSF	⇨	⇧	⇧
							meal intake	⇨	⇨	⇧⇧
Ahren, 2001 [28]	0M/12F	63±n.s.	27.7±n.s.	breakfast bread, margarine, marmalade, cheese (15% fat) cup of coffee.	Saline infusion and intake	5 min max	Experiment 1	⇨*	⇧*	⇧⇧
							Experiment 2	⇨*	⇧*	n.a.

Study	N	Age, y Mean±SD or range	BMI, kg/m^2 Mean±SD or range	Population and food cue specifics	Exposure to Food cue	Exposure duration	Conditions	Effect on		
Healthy normal weight participants								Glu	Ins	PP
Morricone, 2000[39] study 1	4M/8F	39.9±4.4	22.5±3.9	20 mg/10 ml saccharin/water solution and 5 ml lemon juice in 10 ml water. Control; water	Rinse	2 min	Saccharine, lemon, water	=	=	n.a.
LeBlanc, 1998 [98]	5M/3F	51±6	24.9±1.7	250 gram steak	Intake	Freely	Intake of steak	=	⇧⇧*	n.a.
Abdallah, 1997 [88]	12M/0F	18-27	20-24	sweet tablets with sucrose, aspartame or polydextrose	Suck on tablet	5 min	Sucrose	⇨	⇨	n.a.
							Aspartame	=	=	n.a.
							polydextrose	=	=	n.a.
Teff, 1996 [99]	0M/13F	19-27	18-24	palatable and unpalatable food items (based on preference participant)	MSF	2 min	palatable	=	⇧	n.a.
							unpalatable	=	⇧	n.a.
Teff, 1996 [27]	8M/0F	25.5±9.5	23.0±2.9	Intra-gastric infusion of glucose with or without MSF peanut butter sandwich	MSF	5 min	MSF	⇨	⇧⇧	n.a.
							No MSF	⇨	⇧⇧	n.a.
Yegen, 1995 [100]	15M/0F	19-31	n.s.	Saline infusion MSF mixed meal; steak, french fried potatoes, salad, bread	MSF	40 min	MSF mixed nutrient meal	n.a.	n.a.	⇧*
Secchi, 1995 [42]	N=5	25-35	n.s.	Intake of a pizza	Intake	5-10 min	Intake of pizza	=	⇧	n.a.

Study	N	Age, y Mean±SD or range	BMI, kg/m² Mean±SD or range	Population and food cue specifics	Exposure to Food cue	Exposure duration	Conditions	Effect on		
Healthy normal weight participants								Glu	Ins	PP
Glasbrenner, 1995 [65]	14M/10F	43±n.s.	n.s.	preparation of a sandwich and MSF a sandwich with butter bacon and two scrambled eggs	Visual & smell, intake	10 min visual 20 min MSF	Visual	n.s.	n.a.	=
							MSF	n.s.	n.a.	⇧
Teff, 1995 [46] study1	15M/0F	25±6	22.8±1.5	exposure to sweet solutions and apple pie	Rinse or MSF	1 min	water	=	=	n.a.
							aspartame	=	=	n.a.
							Saccharin	=	=	n.a.
							Sucrose	=	=	n.a.
							apple pie	=	⇧*	n.a.
Teff, 1995[46] study2	16M/0F	26±5	23.3±1.6	exposure to sweet solutions and apple pie	Rinse or MSF	3 min	water	=	=	n.a.
							aspartame	=	=	n.a.
							Saccharin	=	=	n.a.
							Sucrose	=	=	n.a.
							apple pie	=	⇧*	n.a.
Lieverse, 1994 [51]	3M/12F	35±2.5	21.2±0.4	mixed nutrient meal; hamburger, bread, mayonnaise	Intake	30 min	Intake Mixed nutrient meal	⇨	n.a.	⇧⇧
Johnson, 1994 [40]	0M/8F	26.6±n.s.	19.5±n.s.	mental imagery and viewing cookies and milk	Thinking of and viewing	2/2 min	Thought and view of Cookies and milk	=	=	n.a.
Witteman, 1994 [101]	4M/3F	48±n.s.	n.s.	Codfish (protein), walnut (fat), banana (CHO), fat solution	MSF	30 min	Codfish	n.a.	n.a.	⇧
							Walnut	n.a.	n.a.	⇧
							Banana	n.a.	n.a.	=
							Fat solution	n.a.	n.a.	⇧

Study	N	Age, y Mean±SD or range	BMI, kg/m^2 Mean±SD or range	Population and food cue specifics	Exposure to Food cue	Exposure duration	Conditions	Effect on		
Healthy normal weight participants								Glu	Ins	PP
Moyer, 1993 [102]	0M/11F	n.s.	19-25	Before and after lunch meal. Visual exposure and intake of chocolate chip cookies	Viewing and intake (1 cookie)	4 min visual 8 min intake	Before lunch	n.s.	=	n.a.
							After lunch	n.s.	⇧⇧*	n.a.
Teff, 1993 [68]	15M/0F	24±3	22.9±0.9	Peanut butter sandwich	Fasting, MSF, intake	2 min	Fasting	=	=	n.a.
							MSF	=	⇧*	n.a.
							Intake	⇨	⇧⇧*	n.a.
Lam, 1993[103]	7M/0F	18-25	n.s.	Mixed nutrient meal; hamburger, bread, margarine, tea	MSF	30 min	MSF mixed nutrient meal	=	n.a.	⇧⇧*
Teff, 1991 [19]	20M/0F	28±5	n.s.	Aspartame sweetened strawberry flavoured gelatin with added dairy fat as a mousse	MSF	2min	Day1	=	⇧*	n.a.
							Day2	=	⇧*	n.a.
							Day3	=	⇧*	n.a.
Le Blanc, 1991 [84]	6M/0F	21-30	22.1±n.s.	Protein vs carbohydrate; sugar pie vs haddock fish	Intake	4 min	Sugar pie	⇨	⇧⇧*	n.a.
							Haddock fish	=	⇧⇧*	n.a.
Broberg, 1989 [41]	0M/4F	33±n.s.	n.s.	Seeing and description of a cinnamon roll	thought and viewing	10 min	View and thought Cinnamon rol	n.a.	=	n.a.
Rini, 1987 [104]	5M/5F	33±8	n.s.	Two saccharine tablets	Suck on tablet	5 min	Suck on two saccharine tables	n.s.	⇨*	n.a.

Study	N	Age, y Mean±SD or range	BMI, kg/m² Mean±SD or range	Population and food cue specifics	Exposure to Food cue	Exposure duration	Conditions	Effect on		
Healthy normal weight participants								Glu	Ins	PP
Lucas, 1987 [71]	3M/2F	21-27	n.s.	Onion tart or tuna tart or combination; repeated measures	Food intake	Freely	Day1	⇨	⇧⇧	n.a.
							Day 2	⇨	⇧⇧	n.a.
Simon, 1986 [66]	2M/3F	26.7±1.3	20.2±0.5	visual and smelling cue of a meal; raw carrots, fried chicken, spaghetti and cookies	Visual and olfactory cue	15 min	Visual and smelling mixed meal	=	⇧*	n.a.
Osuna, 1986[105]	0M/5F	22.5±7	23.7±n.s.	Mixed meal breakfast; white coffee, butter toast, Danish roll	Visual and olfactory stimulation	5 min	Visual and olfactory exposure breakfast meal	=	⇧*	n.a.
Bellisle, 1983[106]	3M/4F	20-25	n.s.	mixed meal, low preferred item, high preferred food item; sandwich with crab, anchovies, liver pate, port pate and butter	Food intake (ad libitum)	Freely	Average of 42 meals	⇨	⇧⇧*	n.a.
Sjostrom, 1980 [44]	0M/22F	35±6	21.23±n.s.	tease meal (visual and smell cue) steak with fried onions, potatoes, vegetables, beer.	Visual and olfactory stimulation	15 min	Visual and olfactory exposure mixed meal	n.s.	n.s.	n.a.

Study	N	Age, y Mean±SD or range	BMI, kg/m^2 Mean±SD or range	Population and food cue specifics	Exposure to Food cue	Exposure duration	Conditions	Effect on		
Overweight participants								Glu	Ins	PP
Eliasson, 2017[69]	15M/0F	44.4±8.4	26.2 ± 3.7	Vanilla caramel muffin	Intake	5 min max.	-	⇨	⇧⇧	n.a.
Buss, 2012 [6]	39M/0F	23.4±0.5	23.2±2.5	Sugar free chewing gum	MSF	15 min	With or without visual +odor cue favorite breakfast	=	=	n.a.
Joosten, 2010 [92]	0M/22F	55.8±2.7	26.3±2.6	sham feeding pound cake, white wine or water	MSF	6 min	cake	=	⇧	⇨
							water	=	=	⇨
							white wine	=	=	⇨
Simon, 1986 [66]	7M/8F	33.3±3.3	34.3±1.4	visual and smelling cue of a meal; raw carrots, fried chicken, spaghetti and cookies	Visual and olfactory cue	15 min	Visual and smelling mixed meal	=	⇧*	n.a.

Study	N	Age, y Mean±SD or range	BMI, kg/m² Mean±SD or range	Population and food cue specifics	Exposure to Food cue	Exposure duration	Conditions	Effect on		
Obese participants								Glu	Ins	PP
Dhillon, 2017[47]	23M/41F	27±n.s.	31.2±n.s.	Overweight and obese participants. MSF Gelatin or drink with sucralose or sucrose	Rinse and MSF	15 sec. every 2 min for 14 min	Nutritive solid-RS	=	⇧*	n.a.
							Nutritive solid-NR	=	=	n.a.
							Low cal. solid-RS	=	⇧*	n.a.
							Low cal. solid-NR	=	=	n.a.
							Nutritive liquid-RS	=	⇧*	n.a.
							Nutritive liquid-NR	=	=	n.a.
							Low cal. liquid-RS	=	⇧*	n.a.
							Low cal. Liquid-NR	=	=	n.a.
Morricone, 2000 [39] study 1	3M/9F	43. 2±4.2	39.1±2.5	20 mg/10 ml saccharin/water solution and 5 ml lemon juice in 10 ml water. Control; water	Rinse	2 min	Saccharine, lemon, water	=	=	n.a.
Morricone, 2000 [39] study 2	1M/4F	40.3±3.1	38.4±4.3	spaghetti, tomatoes, cheese, meat, salad, apple	sight+smell, sight, smell alone	2 min	Only sight, only smell, sight& smell combined	=	=	⇧*
Karhunen, 1997 [107]	0M/10F	46.2±11.3	33.1±4.0	Non-binge obese, mixed breakfast meal;ham, cheese, sausage, veggies, marmalade, artificially sweetened juice, coffee, tea	Anticipation and exposure, seeing breakfast & tasting juice	5 min anticipation 17.5 min food exposure	Mixed breakfast meal	=	⇨	n.a.

Study	N	Age, y Mean±SD or range	BMI, kg/m² Mean±SD or range	Population and food cue specifics	Exposure to Food cue	Exposure duration	Conditions	Effect on		
Obese participants								Glu	Ins	PP
Karhunen, 1996 [108]	8M/30F	44.2±1.8	31-41	breakfast meal; coffee/tea orange juice, 4 sandwiches with ham cheese and veggies and two chocolate cookies	Visual and olfactory cue	15 min	Pre WL RS	=	⇧	n.a.
							Pre WL INT	=	=	n.a.
							Pre WL NR	=	=	n.a.
							Post WL RS	=	⇧	n.a.
							Post WL INT	=	=	n.a.
							Post WL NR	=	=	n.a.
Lieverse, 1994[51]	2M/12F	38±2	42.4±1.3	mixed nutrient meal; hamburger, bread, mayonnaise	Intake	30 min	Mixed nutrient meal	⇨	n.a.	⇨
Osuna, 1986 [105]	0M/10F	31.6±4.8	33±n.s.	Mixed meal breakfast; white coffee, butter toast, Danish roll	Visual and olfactory stimulation	5 min	Visual and olfactory exposure breakfast meal	=	=	n.a.
Sjostrom, 1980[44]	0M/25F	45±12	36.3±n.s.	tease meal (visual and smell cue) steak with fried onions, potatoes, vegetables, beer.	Visual and olfactory stimulation	15 min	Visual and olfactory exposure mixed meal	n.s.	n.s.	n.a.
Parra-Covarrubias, 1971 [43]	4M/2F	13.1±n.s.	30-52	Obese to morbid obese children. Sight and smell of a breakfast meal of own choosing	Sight and smell	15 min	Sight and smell of preferred breakfast meal	=	⇧*	n.a.

Study	N	Age, y Mean±SD or range	BMI, kg/m^2 Mean±SD or range	Population and food cue specifics	Exposure to Food cue	Exposure duration	Conditions	Effect on		
Diabetic participants								Glu	Ins	PP
Eliasson, 2017 [69]	16M/0F	44.4±8.4	26.2 ± 3.7	1st relative had diabetes, otherwise healthy participants. Food: muffin	Intake	5 min max.	Vanilla caramel muffin	⇨	⇧⇧	n.a.
LeBlanc, 1998 [98]	4M/3F	45±4	29.3±2.2	Non-insulin dependent diabetic participants 250 gram steak	Intake	Freely	Intake of steak	=	⇧⇧*	n.a.
Glasbrenner, 1995 [65]	14M/10F	43±n.s.	n.s.	DM with and without neuropathy. preparation of a sandwich and MSF a sandwich with butter bacon and two scrambled eggs	Visual & smell, intake	10 min visual 20 min MSF	Visual-DM	n.s.	n.a.	=
							Visual-NP	n.s.	n.a.	=
							MSF-DM	n.s.	n.a.	⇧
							MSF-NP	n.s.	n.a.	⇧

Study	N	Age, y Mean±SD or range	BMI, kg/m^2 Mean±SD or range	Population and food cue specifics	Exposure to Food cue	Exposure duration	Conditions	Effect on		
Participants with an eating disorder								Glu	Ins	PP
Bello, 2010 [50]	0M/22F	23.8±4.6	21.9±1.8	Bulimic participants low fat, half fat and full fat yoghurt with added fat free cocoa	MSF	3 min	non-fat placebo, fat placebo, fat naltrexone	=	=	*data not shown*
Karhunen, 1997[107]	0M/11F	44.6±9.7	32.8±4.2	Binge eating obese, mixed breakfast meal ham, cheese, sausage, vegetables, marmalade, artificially sweetened juice, coffee, tea	Anticipation and exposure, seeing breakfast & tasting juice	5 min anticipation 17.5 min food exposure	Mixed breakfast meal	=	⇨	n.a.
Johnson, 1994 [40]	8F/0M	27.5±n.s.	20.4±n.s.	Bulimic participants mental imagery and viewing cookies and milk and induced purge	Thinking of and viewing	2/2 min	Cookies and milk	=	=	n.a.
Broberg, 1989 [41]	0M/4F	25.5±n.s.	n.s.	Anorexic participants Seeing and description of a cinnamon roll	thought and viewing	10 min	View and thought Cinnamon roll	n.a.	⇧*	n.a.
Moyer, 1993 [102]	0M/11F	n.s.	19-25	Bulimic participants Before and after lunch meal. Visual exposure and intake of chocolate chip cookies	Viewing and intake (1 cookie)	4 min visual 8 min intake	Before lunch	n.s.	=	n.a.
							After lunch	n.s.	⇧⇧*	n.a.

⇧ cephalic peak, ⇨ post absorptive peak, ⇧⇧ pre-absorptive peak (≤10 min) and post absorptive peak, = no change from baseline, * significant different from baseline ($p<0.05$), n.a. not assessed,. n.s. not specified. Conditions of the same study with the same outcome are summarized in one line. Other abbreviations: RS=responder NR=non-responder, INT= intermediate responder, Cal=caloric, DM=Diabetes mellitus, NP= neuropathy, WL=weight loss

Supplement III- overview of included studies, sub-groups and treatments

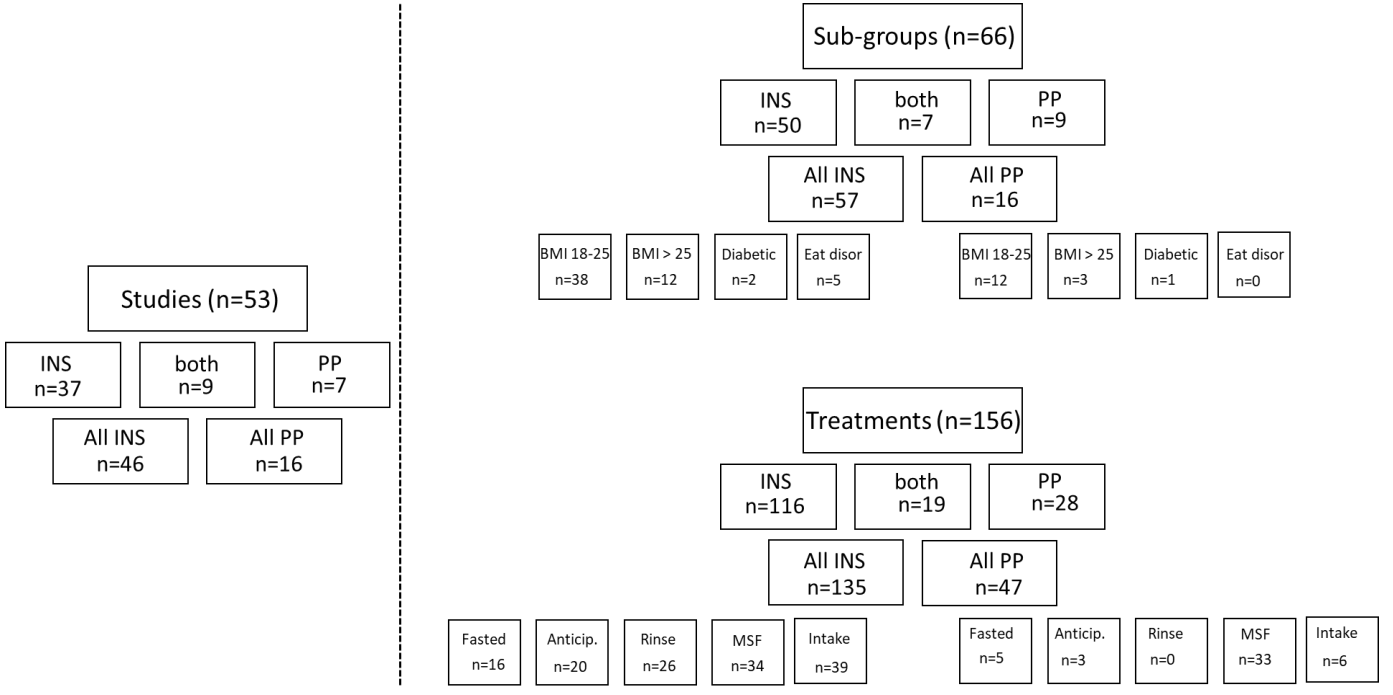

Figure 1. Overview of number of studies, subgroups and treatments included.

Supplement IV – Cochrane risk of bias graph

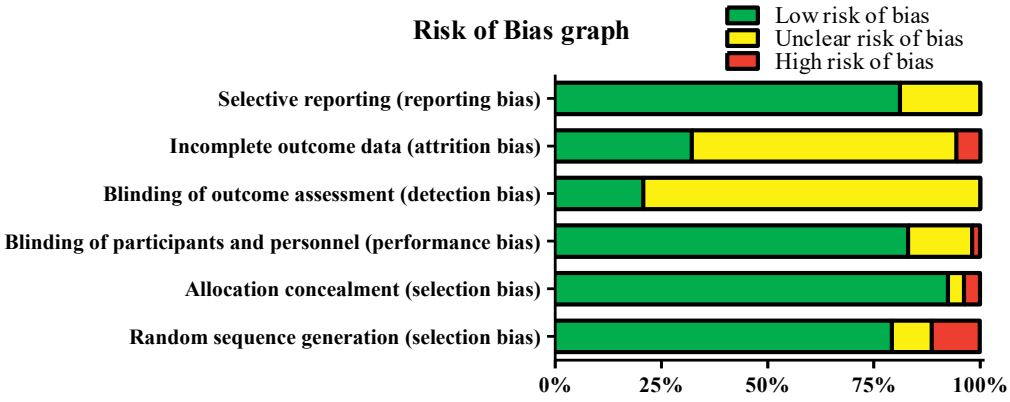

Figure 1. Cochrane risk of bias assessment of included studies.

Supplement V. Tables belonging to Figures describing insulin cephalic phase (Figure 3,4,5)

Table 1. Insulin mean±SEM change from baseline per study treatment of Figure 2.

Author	Year	Treatment	Change from baseline (μIU/mL)	SEM
Covarrubias	1971	Breakfast	15	4.7
*Bellisle	1983	Mixed meal	2.1	2.7
*Simon	1986	Normal weight	0.8	0.7
*Simon	1986	Overweight	2.5	3.4
Osuna	1986	Normal weight	8.1	-
Lucas	1987	Day 1	11.7	-
Lucas	1987	Day 2	4.8	-
Broberg	1989	Anorexic	1.4	1
LeBlanc	1991	Carbohydrate	2.4	0.6
LeBlanc	1991	Protein	4.1	0.6
*Teff	1991	Day1	3.1	1.5
*Teff	1991	Day2	2.3	1.3
*Teff	1991	Day3	2.2	0.8
*Moyer	1993	Bulimic	4	-
*Moyer	1993	Healthy	10.1	-
*Teff	1993	MSF	7.06	1.8
*Teff	1993	Intake	6.36	1.9
Secchi	1995	Pizza intake	7	0.8
*Teff	1995	1 min	1.2	0.4
*Teff	1995	3 min	2.2	0.4
Karhunen	1996	Pre-weightloss	1.9	0.3
Karhunen	1996	Post-weightloss	1.4	0.4
Teff	1996	Palatable	1.4	0.6
Teff	1996	Unpalatable	1.3	0.8
Teff	1996	Infusion	4.5	3.8
Teff	1996	Intake	4.5	3.8
*Le Blanc	1998	Diabetic	4.2	0.9
*Le blanc	1998	Control	4	1.1
Robertson	2001	MSF	7.8	3.8
Ahren	2001	Experiment 1	3.4	4.1
Ahren	2001	Experiment 2	4.5	5.6
Smeets	2005	Glucose	2.5	2.3
Smeets	2005	Maltodextrin	4.6	2.3
* Just	2008	Sucrose	1.5	0.8
* Just	2008	Saccarine	0.9	1.0
* Just	2008	Starch	2.5	2.6
* Just	2008	QHCL bitter	1.6	0.7
* Just	2008	Distilled water	1.6	4

Author	Year	Treatment	Change from baseline (µIU/mL)	SEM
Joosten	2010	MSF cake	1.03	0.8
* Dušková	2013	Sucrose	0.5	0.34
Zhu	2014	Cereal	0.9	0.35
Mannella	2015	Sweet	2.5	2.2
Manella	2015	Tasteless	2.5	2.2
Morey	2016	Soup	5.2	1.6
*Dhillon	2017	Caloric solid	3.8	2.1
*Dhillon	2017	Low cal solid	3.5	1.3
*Dhillon	2017	Caloric liquid	1.7	1.6
*Dhillon	2017	Low cal liquid	1.9	1.6
Eliasson	2017	Muffin	4	0.23

Table 2. Median(IQR) per curve specific for insulin and glucose (in- and excluding intake treatments, Figure 3.

	Insulin incl. all			Insulin excl. Intake			Glucose incl. all			Glucose excl. intake		
	Median	25th	75th	Median	25th	75th	Median	25th	75th	Median	25th	75th
Baseline	8.33	12.3	4.9	8.63	12.58	5.28	4.8	5.26	4.49	4.9	5.3	4.47
Early increase ≤10 min	11	14.85	6.45	9.4	14.85	6.4	4.8	5	4.49	4.8	5.1	4.48
Sign. early peak ≤10 min	13.3	16.9	8.4	14.05	19.83	9.4	4.71	5.1	4.46	4.8	5.25	4.46
Late increase >10 min	14.3	25.8	7.9	7.9	15.12	4.25	5.5	6.25	4.68	4.56	5.2	4.27

Table 3. Number of observations per specification of the insulin and glucose curve (baseline, increase, peak, postprandial) of Figure 3.

Curve specific	Insulin incl. all	Insulin excl. intake	Glucose incl. all	Glucose excl. intake
Baseline	95	64	74	47
Early increase ≤10 min	89	57	61	34
Sign. early peak ≤10 min	51	28	42	22
Late increase >10 min	40	13	33	9

Table 4. Number of observations, median(IQR) and percentage contribution per treatment per time bin, Figure 4.

	≤3	4-6	7-10	11-15	16-20	≤20
Num. observation	20	34	17	12	11	22
Median µIU/mL	2.95	2.4	4.6	5.7	5.93	26
25th perc. µIU/mL	1.75	1.7	2.6	1.4	4.1	13.6
75th perc. µIU/mL	4.1	4.5	10.8	18.8	16	39.7
Anticipation %	20	9	24	8	9	9
Rinse %	10	15	0	0	0	0
MSF %	35	32	18	17	64	0
Intake %	35	44	59	75	27	91

Supplement VI. Tables belonging to figures describing PP Cephalic phase (Figure 6,7,8)

Table 1. PP mean±SEM change from baseline per study treatment of Figure 5.

Author	Year	Treatment	Change from baseline (µIU/mL)	SEM
*Lam	1993	Mixed meal	12.13	8.4
Lieverse	1994	Obese	38.9	22.6
Witteman	1994	Fat solution	211.3	78
Witteman	1994	Codfish	58.8	28.5
Witteman	1994	Walnut	51.9	28.5
*Yegen	1995	Mixed meal	106.2	51.5
Glasbrenner	1995	Healthy	136.6	19
Glasbrenner	1995	Diabetic(-)	54	20
Glasbrenner	1995	Diabetic(+)	13.8	18
*Morricone	2000	Sight and Smell	225	34.6
*Morricone	2000	Sight	155.7	34.6
*Morricone	2000	Smell	143.2	34.6
Hoentjen	2001	Mixed meal	99.2	47.3
Ahren	2001	Mixed meal	26.3	20.8
*Robertson	2001	MSF	169	112
*Robertson	2001	Intake	485	108
* Crystal	2006	High fat cake	21.2	8.8
Crystal	2006	Non fat cake	12.1	8.8
*Mennella	2015	Sweet	126	9.5

Table 2. Median(IQR) per curve specific for PP pg/mL (in- and excluding intake treatments, Figure 6.

	PP incl. all				PP excl. intake			
	median	75th	25th	Num. obsv	median	75th	25th	Num. obsv
Baseline	110	143	72	37	110.5	143	72	32
Early increase ≤15 min	185	239	62.2	19	168	198	60	16
Sign. early peak ≤15 min	62.2	253	59.7	7	62.2	158	56	5
Late increase >15 min	218.4	394.4	162.5	17	218	394	162	13
late sign. Peak >15 min	394.3	456.4	266.8	8	394	534	242	6

Table 3. Number of observations, median(IQR) and percentage contribution per treatment per time bin, Figure 7..

	≤3	4-6	7-15	≤20
Num. observation	9	15	29	20
Median pg/mL	123	125	185	236
25 perc. pg/mL	77.3	62.2	140.5	174
75 perc. pg/mL	139.5	198.9	231.6	384
Rinse/MSF %	89	100	69	80
Intake %	11	-	31	20



Chapter 6

A full brain: Brain(stem) responses to chocolate milk over the course of satiation

To be submitted: Lasschuijt MP, Mars M, Duif I, Camps G, de Graaf C, Smeets PAM.
A full brain: Brain(stem) responses to chocolate milk over the course of satiation.

Abstract

Satiation develops from the integration of taste and gastric signals in the brainstem and hypothalamus. However, the transition from a hungry to a sated state and the role of the brainstem in this process is poorly understood. The aim of this study was to determine differences in brain(stem) responses to chocolate milk over the process of becoming satiated.

Using functional MRI, brain responses of 36 right-handed participants (13M/23F, 24±4 y, BMI 22±2 kg/m2) to sips of a neutral taste stimulus and to high and low sweet chocolate milk were measured in a hungry, a 50% and a 100% sated state. Additionally, gastric volume was measured at every state using MRI. Participants drank chocolate milk between runs to induce satiation. Satiation state modulated brain activation related to flavour (chocolate milk – neutral) in the bilateral (anterior) insula, putamen, amygdala and parabrachial nuclei (PBN). Activation in these areas decreased as participants became satiated. This High and lower activation in the hungry and 100% satiated state, respectivecly could completely be attributed to gastric volume, which was not the case in the 50% satiated state. Additionally, higher activation to sweet taste specifically was found in the PBN only when comparing the hungry with the 100% satiated stated.

To conclude, taste-related activation in the PBN, (anterior) insula, amygdala and putamen progressively decreases as satiation increases. In the hungry and 100% satiated state, the hungry/satiation effect on brain reactivity to a taste stimulus could completely be explained by gastric filling. In the 50% satiated state the decrease could not be explained by gastric filling. Therefore, satiation due to sensory stimulation may be especially prominent early in the meal, and is overruled by gastric distention towards the end of the meal when consuming till complete fullness.

Introduction

The amount of food we eat within a meal is determined by homeostatic, hedonic, cognitive and emotional feedback systems that ultimately affects appetite and body weight (1, 2). However, these feedback systems often drive towards a positive energy balance, for which confirmatory evidence can be found in the growing number of obese and overweight people worldwide (3, 4). Understanding the role of satiation in the control of food intake is needed to determine strategies to prevent overconsumption and weight gain (5, 6).

Over the course of a meal, the in-mouth perception of food, also referred to as 'oro-sensory exposure', is important for reaching satiation, i.e. meal termination. The effect of oro-sensory exposure on satiation has been found independent of the energy density and palatability of the food eaten and eating rate (7-14). Accordingly, increased oro-sensory exposure independently reduces meal size (12-14). Reversely, so-called fast foods, like caloric beverages, promote overconsumption because of their low degree of sensory stimulation relative to their energy content (9). A mechanism through which oro-sensory exposure may affect satiation is sensory(-specific) satiation. Sensory specific satiation has been defined in terms of a decrease in pleasantness of an eaten food, relative to an uneaten food within the time-span of a meal (15). When oro-sensory exposure is increased either in duration or taste intensity, sensory satiation occurs faster compared to foods that induce lower oro-sensory exposure (16-18). This presumably leads to earlier satiation and a smaller meal size (19-23).

Besides taste signals, gastrointestinal signals play a role in satiation (24). Notably, stomach distension activates stretch and mechanoreceptors that send satiety signals to the brain. Together with stomach stretch, gastric emptying of nutrients into the duodenum sends endocrine signals to the brain that contribute to satiation (25).

Satiation eventually evolves by the integration of taste and gastric signals in the brain. However, not much is known about central processes related to satiation, i,e. the transition from hunger to satiety, in humans. The majority of neuroimaging studies merely compared fasted- with sated brain responses (26-32), with one notable exception (33). The first important area that processes taste and gastric signals is the brainstem, but only little research has been done on the human brainstem due to technical constraints (34-36).

Based on a few human studies, but mostly on animal work, we know that the dorsal vagal complex (DVC) in the medulla and the parabrachial nucleus (PBN) in the pons play a key role in satiation. The DVC includes the nucleus of the solitary tract (NTS), the dorsal motor vagal nucleus (DMV) and area postrema (AP) and together with the PBN they are involved in integration of energy -related peripheral signals and processing taste and gastric signals (37-

41). These brainstem areas receive satiation related input at three different levels. First, neurons sense circulating metabolites and hormones such as glucose and CCK (DMV). Second, the brainstem receives vagal input from the sensory system and the gastrointestinal tract, such as taste (NTS, PBN) and gastric distention signals (PBN). Last, the brainstem receives input from the mid- and forebrain area that is integrated together with energy related signals in the area postrema (AP). The brainstem then projects these signals to hepatic and gastric efferent nerves and to other regions of the brain. Based on animal work it is thought that the pathway from the brainstem dissects into an affective and sensory pathway (42). The affective pathway includes projects to the, hypothalamus, amygdala, parahippocampal gyrus, orbitofrontal cortex (OFC), striatum, and midbrain. The sensory pathway includes the thalamus and insula. Together, these areas regulate endocrine release, appetite and food intake (33, 41). The role of these brain(stem) areas in gastric and taste processing over the course of satiation has not been investigated in humans using fMRI. Therefore, the aim of this study was to determine differences in brain(stem) responses to sweet taste over the process of becoming satiated. In addition, we studied in how far these responses to sweet taste depend on the degree of stomach filling. We expected an increase in taste activation in brainstem areas related to stomach distention. On the contrary, we expected a decrease in taste activation in taste- and reward- related areas due to sensory satiation.

Materials and methods

Participants

The study was performed at Wageningen University and at hospital 'Gelderse Vallei', Ede, The Netherlands. Potential participants were recruited from the surroundings of Wageningen using flyers and posters. In addition, emails were sent to persons in a database of volunteers who had expressed an interest in participating in nutrition studies. Healthy right-handed participants between 18-35 years old and with a BMI between 18.5-27 kg/m2 were eligible. Potential participants were screened during an information session and excluded if they: had contra-indications to MRI scanning, had difficulties with tasting, smelling, swallowing or eating in general, had allergies for one of the study products, had gained or lost >5 kg of body weight during the past two months or were on an energy restricting diet, had non-removable braces (including dental wire), used medication that could affect the study outcome, smoked (on average) 1 cigarette or more per day, drank more than 21 unites of alcohol per week, exercised more than 8 hours per week or were classified as a high restrained eaters according to the Dutch Eating Behaviour Questionnaire (DEBQ): score >2.9 men, >3.4 women. Women who were pregnant or had intention to become pregnant during the study period, or used an IUD as contraception (with exclusion of the Mirena and Kyleena IUD) were not allowed to participate. Personnel and thesis students of the Division of Human Nutrition and Health were not allowed to participate.

All participants signed informed consent prior to participation. In addition, participants had to sign consent for being informed about incidental findings of pathology through their general practitioner. Participants received a financial compensation. Prior to the study, a sample size estimation was made based on previous studies with a similar design (27, 28, 32, 43, 44). Based on this we estimated a minimum of 35 participants, assuming a 15% dropout we aimed to include 40 participants in the present study.

The study was approved by the Medical Ethical Committee of Wageningen University, The Netherlands (ABR:NL65187.081.18) and registered in the Dutch trial register under NTR7239 (http://www.trialregister.nl).

Information and training session

During the information session participants rated their liking of chocolate milk with two different sweetness levels that were used as study stimuli. Participants were excluded when they disliked the chocolate milk (defined as score <4 (neutral) on a nine point hedonic Likert scale) or had a stronger preference for one of the sweetness levels (defined as >2 point difference on a nine point hedonic Likert scale).

After the information session, participants came for a training visit during which their weight and height was measured to calculate their BMI. During the training participants were familiarized with the experimental task in a dummy scanner. While in the dummy scanner participants received sips of chocolate milk at fixed times (swallow, taste) through a tube that was attached to a syringe pump. Before and after practicing, participants were asked to drink chocolate milk in a step-wise satiation method. At the end of the training participants answered questions regarding their behavioural characteristics and physical activity (HTAS, BIS-11, BIS/BAS, IPAQ). After the training participants were excluded if they indicated to be uncomfortable with the study procedure (lying in the (dummy) scanner) or when their BMI was outside the inclusion range (18.5-27 kg/m^2).

In total, 182 participants joined the information session of which 45 potential participants were eligible and completed the training; 5 participants were excluded after the training (Supplement I). Finally, 40 participants were eligible for the test session. Two participants did not finish the test session. The final data for analysis included 36 participants (23 women), who were on average 24±4 years old, had a BMI of 22±2 kg/m2 and an average DEBQ restraint score (32) of 2.0±0.6 (men) and 2.4±0.5 (women), see Supplement II for participant characteristics.

Study design

The study had a within-participant design (Figure 1). Participants were scanned on one occasion by means of (f)MRI in a hungry (run 1), 50% satiated (run 2) and 100% satiated state (run 3). Participants arrived at the test location in a hungry state and drank chocolate milk in between scan runs to reach the 50% and 100% satiated states. Each run included one or two gastric volume scans, and a taste fMRI scan. At the start of the session an anatomical brain scan was made. Each run also included a resting state fMRI scan but these are outside the scope of this paper.

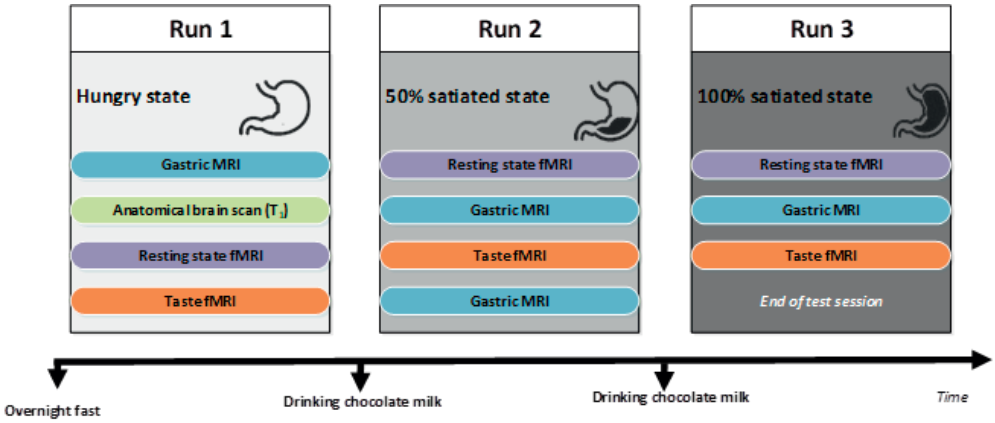

Figure 1. Schematic overview of a study test session and study conditions (hungry, 50% and 100% satiated). In between runs participants drank chocolate milk to reach a 50% or 100% satiated state. Duration of a Gastric MRI scan was 18s (5 min including set up), resting state fMRI 9 min (outside the scope of this paper) and taste fMRI scans took 15 min.

Experimental procedures

Participants were instructed to eat a similar amount of the same meal (of their own choice) the evening preceding the test session. They were instructed to eat their meal between before 8.00 PM and not to eat or drink anything except water between 10.00 PM and their standardized breakfast. Participants received a breakfast yoghurt (200 g, strawberry flavoured 'Breaker' Melkunie, The Netherlands) that they had to consume at home 3 h prior to the start of the training and test sessions. Participants were instructed not to eat or drink anything but water after their breakfast yoghurt. They were instructed to bring the empty package with them to the lab. The yoghurt package was weighed to determine intake (mean±SD, 195±5 g). Participants

were also instructed to avoid exercise and alcoholic drinks 24 h preceding the test. To assess compliance, participants were asked to keep a 24-hour food and exercise diary.

Test sessions started between 10.30 AM - 12.30 PM. Upon arrival participants changed into MRI compatible clothing and the researcher would place ECG electrodes on their chest. The test session consisted of three runs that took approximately 40 min each (Figure 1). Between each run participants drank chocolate milk according to the step-wise satiation method described previously. Before, between and after each run participants filled in an appetite, sensory and well-being questionnaire. After drinking till 100% fullness participants chose their main reason to stop drinking from a multiple choice question.

The majority (80%) indicated that they stopped drinking because they felt full, 1 indicated that the reason to stop drinking was boredom with the chocolate taste, 1 got bored with the sweet taste and 5 participants (14%) indicated that they stopped drinking because they felt nauseous. At the end of the test session participants were asked what they thought the main aim of the study was. This was an open question and 67% of the participants (n=24) indicated that the aim of the study was related to satiation.

Step-wise satiation method

After the first run, participants received 150-ml white plastic cups with 50 g of chocolate milk at room temperature (Full fat chocolate milk, 1.7% cacao, Friesland Campina, The Netherlands). Cups were given one by one and after each cup participants rated fullness on a 100-mm visual analogue scale (VAS) anchored ‘not at all’ and ‘extremely’ at the extremes and with a ‘half full’ mark at 50 mm. The researcher kept giving the participant chocolate milk cups until he/she rated fullness over the 50-mm mark. After that, participants rated their appetite and well-being and filled in a sensory questionnaire and the second scanning run began. Participants were not able to see the number of cups of chocolate milk they drank. Intake in mL was determined by adding up the number of 50 mL cups the participant drank. After the second run, the participant repeated this step-wise satiation method but was now instructed to continue drinking until he/she felt completely full and did not want to drink anymore. Special attention was given to the fact that their stomach had to feel very full and that they should not stop consumption because they did not like the taste of chocolate anymore. Participants were warned not to drink until they felt uncomfortable full, sick or nauseous.

This method was pre-tested in series of pilot studies. First participants (n=10) were asked to drink until they felt half full and thereafter to drink till complete fullness by drinking chocolate milk with a straw from a large can. Participants were not able to see the can or the amount they

drank. Using this method, the amount that participants drank and how full they felt after drinking was not re-producible, besides, participants easily drank too much (feeling nauseous). Because of this, 50-mL cups were given instead of the large can in the second pilot study (n=5). After each cup, participants indicated their feeling of fullness on a 100 mm VAS scale anchored at 0 mm with 'not full', at 50 mm with ' half-full' , and at 100 mm with 'extremely full'. Participants were more aware of how full they felt compared to the first pilot and this prevented them from drinking chocolate milk till they were nauseous. The amount that participants drank and how full they felt was also better reproducible compared to the first pilot.

However, we concluded that there was a steep learning curve for the step-wise test (pilot 2) when done multiple times. Participants seemed to be better able to estimate when they felt half full and completely full the second time they did the test. For example, when the participants felt nauseous after the first time they did the test, they would drink less the second time they did it. On the contrary, if they felt hungry after the first time they did the step-wise satiation test, they would increase their intake the second time.

For this reason we decided to do the step-wise satiation test during the training, such that participants would be better in estimating their fullness during the test session.

Study measures

- Taste fMRI scan

A taste fMRI task (Figure 2) was used to measure taste-related brain activation upon consumption, and reward-related activation during anticipation. This paradigm was based on Stice *et al.* (45). During the task participants received sips of a high- or low sweet chocolate milk or a neutral taste solution. Within the task, the order in which participants received the stimuli was semi-randomized such that participants never tasted the chocolate milk stimuli more than twice in a row. Three variants were designed in which the participants received the stimuli in different orders. These three were randomized across participants and runs (hungry, 50 and 100% satiated).

Stimuli were administered through tubes connected to programmable syringe pumps (New Era Pump Systems Inc, Wantagh, NY). One taste run consisted of 36 trials: 18 chocolate milk exposures (9 high-, 9 low sweet) and 18 neutral taste solution exposures. Through a mirror participants could see instructions on a screen that had a grey background. Images were presented for 2-7 s using Presentation® (Neurobehavioural Systems Windows). First, a glass of chocolate milk or water was shown (2-s cue), indicating that they would receive a chocolate

milk (low or high sweet) or the neutral taste solution, respectively. The cue was followed by an anticipation period (1-7 s). At the 'taste' cue (2 s) 1 mL of one of the stimuli was administered through a 2.6 mm diameter tube (1 per stimulus). Stimuli were pumped using four programmable 50mL syringe pumps controlled by Presentation®. Tubes were bundled and positioned between the front teeth. After this, participants were instructed to hold the liquid in their mouth and to focus on the taste and not to move their tongue. The duration of this 'hold' event was jittered (2-7s). Then, participants saw a 'swallow' cue (2s), followed by a 'rinse' cue (2-4 s) and another swallow cue. Last, a white crosshair appeared for 2-4 s before the next trial began. The duration of the taste fMRI task was 15 min.

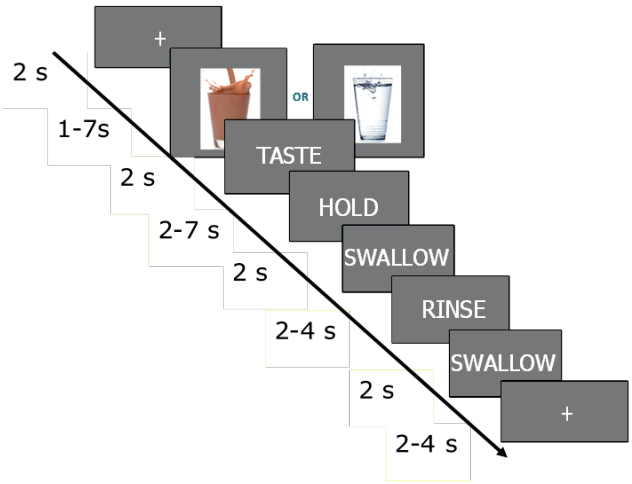

Figure 2. Schematic overview of a taste trial. After a picture cue participants received either high- or low sweet chocolate milk or the neutral taste solution. Participants were instructed to hold the liquid in their mouth, until the swallow cue. Duration of the anticipation, hold, water rinse and crosshair conditions were jittered. This taste trial is based on the paradigm described by Stice *et al.* (45).

– Taste fMRI Stimuli

High and low sweet chocolate milk were used. Based on a sensory pilot study (n=40) high and low chocolate milk recipes were developed that were equally far from optimum sweetness and thus equally liked. The final recipe for the high and low sweet chocolate milk consisted of 64 % dark chocolate milk (chocomel dark, 2.9% cacao, Friesland Campina, The Netherlands), 35% regular cow milk (full-fat, Albert Heijn, The Netherlands), 1% sweetener was added to the high sweet and 1% spring water (Spa Reine, Spa, Belgium) to the low sweet chocolate milk. Both chocolate milks contained 75 kcal/314 kJ, 3.5 g protein, 3.0 g fat, 8.1 g carbohydrates per 100 g. The neutral taste stimulus, mimicking saliva, consisted of water (SPA Reine, Spa, Belgium), 25 mM KCl (E509) and 2.5 mM NaHCO3. This has been used in many other studies to distinguish gustation from somatosensory perception (e.g. 87). All stimuli were taken out of the refrigerator the day before the session and were shaken and then served at room temperature. Tap water was used to rinse.

An overview of all sensory ratings (done during the information meeting, n=116) of the test foods can be found in Supplement III. High and Low sweet chocolate milk were rated 48±1 and 43±1 on liking (100 mm VAS), respectively. High sweet chocolate milk was rated 78±1 and low sweet 32±1 on sweet taste intensity. On the just about right scale (JAR, optimum sweet=0 mm on the scale), participants rated the high at 27±1 mm and the low sweet chocolate milk at -17±1 mm.

– Physiological measurements

During the taste scans heart rate was measured using electrocardiography (ECG) with three MRI conditional radio translucent electrodes (EL508, BIOPAC Systems, Inc.). To create a good conductive path between the electrode and skin surface the skin was lightly rubbed with a scouring pad and cleaned with alcohol sanitizer, before placement of the electrode. In addition, breathing patterns were measured with a pneumatic belt that was placed at the lower part of the participant's ribcage, above the belly button. ECG and breathing data were acquired with an MP150 data acquisition system (BIOPAC Systems, Inc.). ECG and breathing signals were recorded at a sampling rate of 5000 HZ together with scan acquisition time using AcqKnowledge® Software (Version 4.4).

– Appetite, well-being and sensory questionnaires

Participants rated the following appetite parameters on a 100-mm VAS (46, 47): fullness, hungry, desire to eat (DTE) before and after each scan run. Due to time constrains, DTE sweet, DTE savoury, feeling satiated, thirsty and nauseous and 'how much are you able to eat' together with well-being parameters: alertness, anxiousness, feeling light-headed and general wellbeing, were only questioned at the start of the session and after each run.

Participants also rated hedonics and sensory characteristics of the stimuli. At the start of the test session and after each scan run, participants rated each stimulus (including neutral taste solution and water) on liking and wanting on a 100-mm VAS. In addition, they rated sweetness on a just about right scale (JAR) anchored with not sweet enough at -50 mm, just about right at 0 mm and too sweet at 50mm.

Participants also rated, expected satiation, sweetness, bitterness, saltiness, flavour intensity, thickness, creaminess and how filling they thought the sample was on a 100-mm VAS, at the start and end of the test session. The order in which the participants rated the stimuli was randomized. Participants tasted the stimuli using 1-mL pipets, to minimize intake and to mimic the administration through tubes in the scanner.

MRI data acquisition

A 3 Tesla Siemens Magnetom Verio (Siemens, Erlangen, Germany) was used. The participant's head was fixated using foam paddings and a tape across the forehead (providing tactile feedback) to minimize head movements. The functional taste runs consisted of 273 functional volumes acquired using a 32-channel head coil and a 2D echo-planar imaging (EPI) sequence (TR=2140 ms, TE=25 ms, 90 degree flip angle, FOV=192 x 192 mm, 43 axial 2.5-mm slices, interleaved ascending order, voxel size 1.75 x 1.75 x 3.85 (2.5 + 10% gap) mm. Additionally, a T_1- weighted anatomical scan was acquired (MPRAGE, TR=2300 ms, TE=2.98ms, 90 degree flip angle, FOV=256 x 256 mm, 192 sagittal slices, voxel size 1.0 x 1.0 x 1.0 mm).

Gastric volume was measured using a thorax coil and a T2-weighted spin echo sequence (TR=850 ms, TE=87 ms, 90 degree flip angle, FOV=192 mm x 192 mm, 24 6-mm axial slices, voxel size 1.5 x 1.2 x 8.4 mm (6.0 + 2.4 mm gap). During this scan, participants were asked to hold their breath upon expiration for 18s to fixate the position of the diaphragm and stomach and prevent movement artefacts.

Data analysis

- (f)MRI data preprocessing

BIDScoin version 2.3.1, an open source python toolkit was used to convert raw DICOM datasets to nifti/json data sets following the Brain Imaging Data Structure (BIDS) standard (v2.3.1 https://github.com/Donders-Institute/bidscoin Marcel Zwiers *et al.*). The BIDS datasets were then pre-processed using the fMRIPrep BIDS App (v1.4.1 https://github.com/poldracklab/fmriprep) (48) a Nipype based tool (49). For a detailed description of the pre-processing steps of fMRIprep see Supplement IV. Brain segmentation of cerebrospinal fluid (CSF), white-matter (WM) and gray-matter (GM) was done using fast (FSL 5.0.9, RRID:SCR_002823 (50). After that scans were spatially normalized to the MNI ICBM 152 Nonlinear Asymmetrical template version 2009c in original BOLD resolution (1.75 x 1.75 x 3.85 mm) (51).

Functional scans were corrected for susceptibility distortions using field maps. Head-motion parameters (transformation matrices, and six corresponding rotation and translation parameters) were estimated before any spatiotemporal filtering using mcflirt (FSL 5.0.9) (52). Thereafter, scan series were slice-time corrected (53), and time-series were resampled to surfaces in fsaverage5 space. These scans were then resampled onto their original, native space by applying a single, composite transform to correct for head-motion and susceptibility distortions. After removal of non-steady state volumes and after spatial smoothing with an isotropic, Gaussian kernel of 6 mm FWHM (full-width half-maximum), automatic removal of motion artefacts was done using independent component analysis (ICA-AROMA, non-aggressive (54).

- Retrospective Image Correction (RETROICOR)

BOLD fMRI measures in the brainstem are prone to physiological noise (34-36). The brainstem is located near major arteries and is close to the heart and lungs. Cardiac and respiratory cycles change cerebral blood flow and volume resulting in a poor signal to noise ratio. Another challenge for measuring task-related activation in the brainstem is the small size of the nuclei compared to cortical structures (55). Also, brainstem nuclei with different functions lie in close proximity of each other (36, 55).

Measuring task-related activity in this area is difficult, nevertheless, previous studies have been successful in measuring brainstem activity related to breathing (56) pain (57) and visually triggered migraine (58). These studies used a retrospective image correction method (RETROICOR) to correct for physiological noise (56, 59). The RETROICOR method assigns

a cardiac and respiratory phase to each image in the time series. Then, noise regressors are calculated by extracting cardiac and respiratory related noise effects from the BOLD signal.
In this study, physiological waveforms (ECG, Breathing pattern) together with the scan acquisition time were pre-processed (peak detection) using the PhysIO Toolbox. PhysIO Toolbox then uses the pre-processed time series for RETROICOR to determine the noise correction model (60). The calculated noise regressors from this model (6 cardiac, 8 respiratory and 4 interaction model regressors, n=18 total) were added to the participant level model in SPM. See Supplement VI for an overview of cardiac, respiratory and interaction regressors.

- Functional MRI data analysis

FMRI data analysis was done using the SPM12 software (Wellcome Department of Imaging Neuroscience, London, UK) in MATLAB (Mathworks, Inc., Sherborn, MA version R2018b).
Seven conditions were modelled; tasting (taste + hold) of 1) low- and 2) high sweet chocolate milk and 3) neutral taste solution, anticipation (cue + anticipation crosshair) of 4) chocolate milk and 5) water and 6) rinse and 7) swallow. Responses to swallowing and rinsing were neglected in further analysis. Individual contrast maps were constructed to compare the activations within each participant. Contrasts of interest were: 'flavour' (tasting chocolate milk > tasteless), 'sweetness' (high sweet > low sweet chocolate milk), 'flavour anticipation' (chocolate milk cue > water cue). Physiological noise-related regressors (n=18) were added as covariates to filter out physiological noise.
To determine main effects and interaction effects of taste and anticipation between study conditions (hungry, 50% and 100%) a group level within participant 2x3 flexible full factorial design was used for each of the contrasts described above. Additionally, we wanted to assess the dependence of the observed taste activation on the degree of stomach distention. To this end we added stomach distention as a covariate to the group-level model. For all analysis a cluster-forming threshold of $p<0.001$ (uncorrected) was used. A cluster level family-wise-error corrected $p<0.05$ was considered statistically significant.
Additionally, Regions of Interest (ROI) masks were created using the WFU Pickatlas (61). A brainstem mask was made including the following a priori ROIs: dorsal vagal complex (DVC, at MNI [X, Y, Z; mm]: 2, -36, -45) (62), nucleus tractus solitarius (NTS, at MNI [X, Y, Z; mm]: -4, -43, -45) (63, 64), parabrachial nucleus (PBN, at MNI [X, Y, Z; mm]: -8, -36, -22) (65). For each of the brain stem regions a 3 mm sphere around the MNI coordinate was included (64). There are no known MNI coordinates of the dorsal motor vagal nucleus (DMV) and area postrema (AP), however these regions are small and fall within the DVC. Therefore no separate

mask for these regions was made (55). Figure 3. shows a graphical representation of the ROI in the brainstem based on Duvernoy's Atlas of the human brain stem (66). A mask was made including taste-related areas; hypothalamus, insula, thalamus, and a mask including reward-related areas; striatum (nucleus accumbens, Putamen), amygdala, hippocampus, midbrain (Ventral tregmental area (VTA), substantia nigra) and medial pre frontal cortex. Clusters were considered significant at peak FWE $p<0.05$ for taste and reward ROIs.

The regionally averaged beta weights of the identified clusters in the ROIs that were significantly different between hungry, 50 and 100% sate at whole brain analysis were extracted with the use of the MarsBaR toolbox (http://marsbar.sourceforge.net/). To determine differences in activation between the satiated states and conditions the extracted beta weights were analysed using a mixed model with posthoc Bonferroni-corrected T-tests to determine differences between states (hungry, 50 and 100%). These analysis were done using SAS (version 9.3; SAS Institute Inc., Cary, NC, USA).

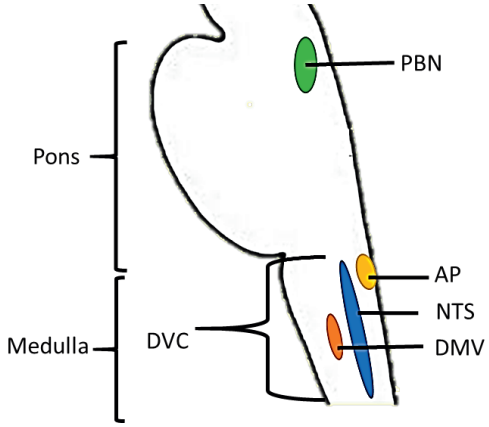

Figure 3. Graphical representation of a sagittal section of the brainstem. Depicted areas are involved in processing taste- and gastric signals and energy -related peripheral signals (hormones and metabolites). AP= Area postrema, DVC =dorsal vagal complex, DMV=Dorsal motor vagus, NTS= nucleus of solitary tract, PBN=Parabrachial nuclei. Based on Duvernoy's Atlas of the human brain stem (66).

– Gastric volume data analysis

A manual segmentation tool in MeVisLab (MeVis Medical Solutions AG, Bremen, Germany v2.5) was used to delineate gastric fluid and air content on every stomach scan (67). Gastric content volumes in mL were calculated by multiplying the gastric surface area of every MRI scan with the slice thickness, and summing up these volumes per slice. Total gastric content

was calculated by adding up the fluid and air content volume. Manual delineation was done by two observers. When total content differed by more than 50 mL the scans were re-examined and if needed delineated again till both observers agreed.

To determine the average stomach content over the course of one run (30 min) we assumed a linear decrease in gastric volume over time (68, 69). For run 1 stomach content of the first stomach scan was taken, assuming no to very small decrease in stomach content when (almost empty). Stomach content for run 2 was calculated by taking the average of the two stomach scans made before and after the fMRI taste task. For the third run, stomach content was calculated by adding up the amount that was in the stomach before drinking till fullness plus the amount that the participant drank till 100% fullness.

To study sensory satiation we adjusted the flavour-related brain activation (chocolate milk > neutral) per run (hungry, 50% and 100% satiated) for stomach content, by adding stomach content as a covariate in the group level model. Stomach content in this model was expressed as a percentage of maximum fullness, which we defined as the total content in the stomach before drinking till fullness, plus the amount that they drank till 100% fullness.

- Behavioural data

Participant characteristics (mean±SD) were calculated using PROC univariate in SAS. Sensory analysis was done using a mixed model ANOVA (SAS, PROC mixed), with sensory characteristic as dependent, stimuli as fixed independent factor, participant as a random factor and an unstructured covariance. A t-test with Bonferroni multiple-comparison correction was used as a post-hoc test. A $p<0.05$ was considered significant. The same model was used to determine intake differences between the test and training session. Parameter estimates, appetite and well-being factors were analyzed using the same mixed model with 'run' added as fixed factor and covariate structure (CV) as covariate matrix. To correct for stomach distention we added the % of max total stomach content as a covariate to this model. We found no effect of the order in which participants rated the sensory characteristics. Outcomes are reported as Mean ± SEM unless otherwise stated. Pearsons' correlations between behavioural data (appetite, sensory ratings) and parameter estimates of brain areas were calculated using partial correlations (corrected for participant dependency) in SPSS (BM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp).

Results

Chocolate milk intake and gastric volume

During the test session participants drank on average 370±31 mL to feel half-full (50% full run) (Figure 4). This amount was equal to 18% of their estimated energy expenditure. To feel completely full (100% full run), participants drank another 632±45 mL equal to 30% of their estimated energy expenditure. In total participants drank 1003± 68 mL, corresponding to 47±3% of their estimated daily energy expenditure. Participants drank in total 242±62 mL less during the training compared to the test session ($p<0.001$).

At the hungry state, participants had on average 45±4 mL in their stomach, directly after drinking till they were 50% satiated this was 294±23 mL (Figure 5). This remained the same over the course of the ' half full' – run 2. At pre- 100% satiated state the amount in the stomach was 260±21 mL and directly after drinking till 100% satiated this was 637±45 mL.

Appetite and well-being ratings.

In the hungry state, the high sweet chocolate milk was rated 13±3 mm higher ($p=0.003$) on liking compared to the low sweet chocolate milk and 23±3mm higher ($p<0.001$) compared to the neutral taste solution and water rinse (Figure 6**)**. Liking of the high sweet chocolate milk decreased when half full (-11±3 mm) and completely full (-43±3 mm) ($p\leq0.03$).

The slw sweet chocolate milk was equally liked as the neutral taste solution and the tap water in all of the three runs. Liking of the low sweet chocolate milk did decrease (-20±3 mm) when participants were completely full compared to the hungry state ($p<0.001$). Liking of the neutral taste solution and tap water remained the same over the three runs, i.e. independent of satiation state. Besides liking we asked participants how much they wanted to drink each of the solutions at each run, wanting ratings showed the same pattern as described above for liking (Supplement V). Pre- and post- run appetite and well-being scores are shown in Supplement VI. In the hungry state participants rated their fullness at 12±3 mm (100 mm VAS), in the completely full state participants rated their fullness 99±2 mm. Hunger and desire to eat were rated accordingly. Desire to eat sweet decreased 46±3 mm over the course of the 3 runs, desire to eat savoury decreased; with 29±3 mm. Thirst decreased 35±4 mm over the course of the 3 runs. Alertness and Anxiousness remained the same during the session. Sleepiness, feeling light headed, general wellbeing and feeling nauseous all increased with 10±4 mm over the course of the test-session.

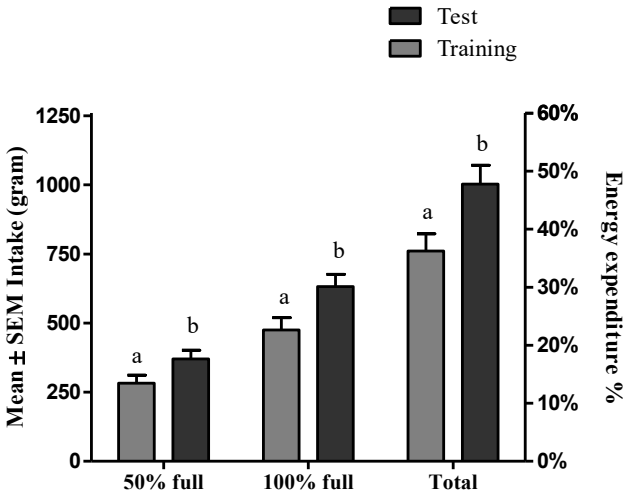

Figure 4. Chocolate milk intake during the training and test session in grams and as percentage of estimated daily energy expenditure.

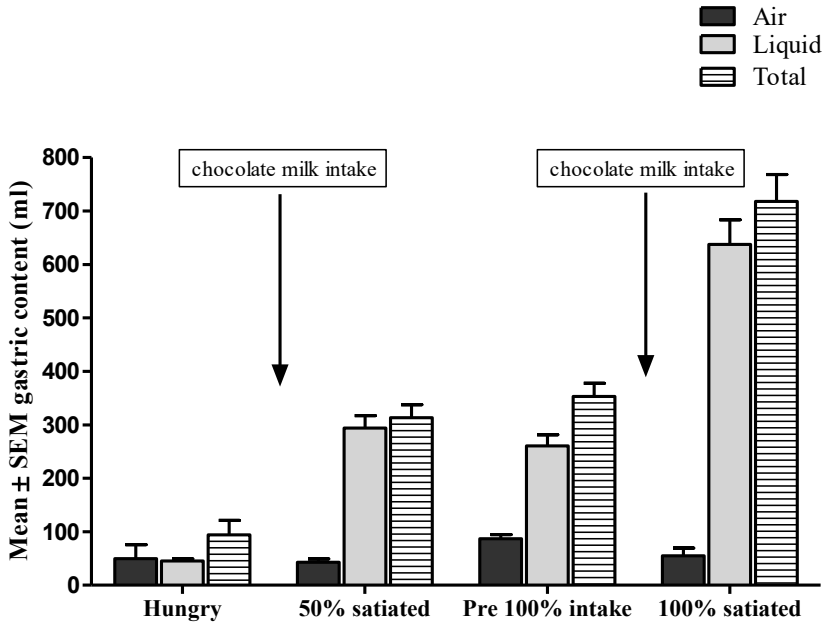

Figure 5. Mean ± SEM gastric content over the course of the test session (measured by MRI).

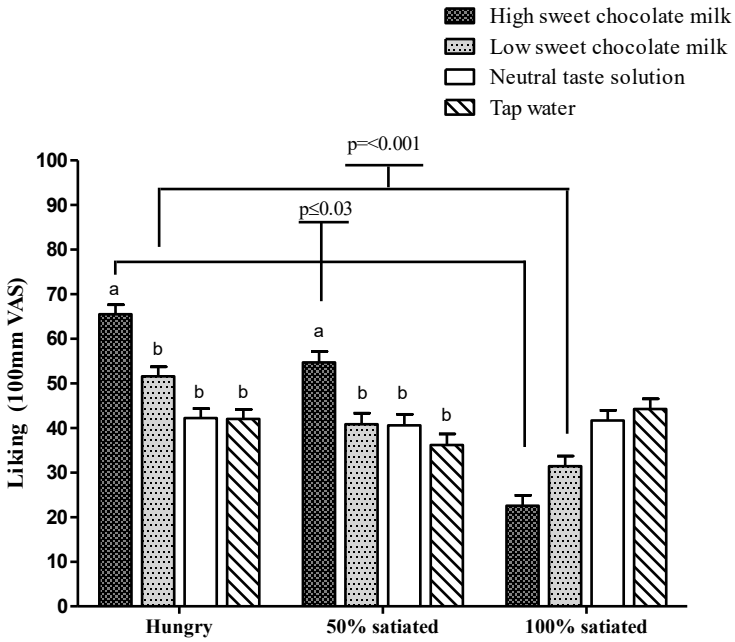

Figure 6. Mean ± SEM liking of the study stimuli when participants were in the hungry, 50% and 100% satiated state. Within each state (hungry, 50% and 100% satiated) significant differences are indicated by different letters.

Neuroimaging results

See Supplement VII for main effects of anticipation, flavour, and sweetness and main effects of satiation state and their interactions on brain activation.

Overall (over the 3 runs) there was flavour related activation (chocolate milk > neutral taste) in the inferior frontal-, postcentral-, supramarginal-, fusiform-, and precentral gyrus, anterior insula, and putamen. Furthermore, we found a main effect of satiation on flavour-related activation in the inferior frontal gyrus, bilateral caudate, and for sweetness-related activation (high > low sweet) in the inferior frontal gyrus and putamen. We did not find any overall brain activation for sweetness (high > low sweet) and flavour anticipation (chocolate cue > water cue).

ROI analysis showed, that over all runs, tasting chocolate milk compared to tasting a neutral taste solution evoked more activation in the bilateral (anterior) insula and putamen. Additionally, greater activation to chocolate milk was found in the left amygdala and parabrachial nuclei (PBN). In all these areas a gradual decrease in activity over the runs (from

hungry> 50% >100% satiation) was observed. This decrease was significant in the putamen (bilateral) and PBN (Figure 7 and 8 and Table 1).

Furthermore we found sweet taste specific activation in the PBN but only for the hungry-100% satiated state) contrast (see Supplement VIII).

To study in how far these responses to sweet taste depend on the degree of stomach filling we corrected for gastric volume (% of max). We found flavour activation in the same brain regions (chocolate milk > neutral taste). Adding stomach filling as a covariate reversed the effect such that there was a gradual increase (instead of decrease) in flavour activation from hungry to a 100% satiated state in these brain areas (Table 2). Activity in the 50% satiated state did not change when correcting for stomach filling. Parameter estimates of the PBN (chocolate milk> neutral taste) correlated negatively with gastric volume r-0.28, p=0.004 (partial correlation corrected for participant) (Figure 9).

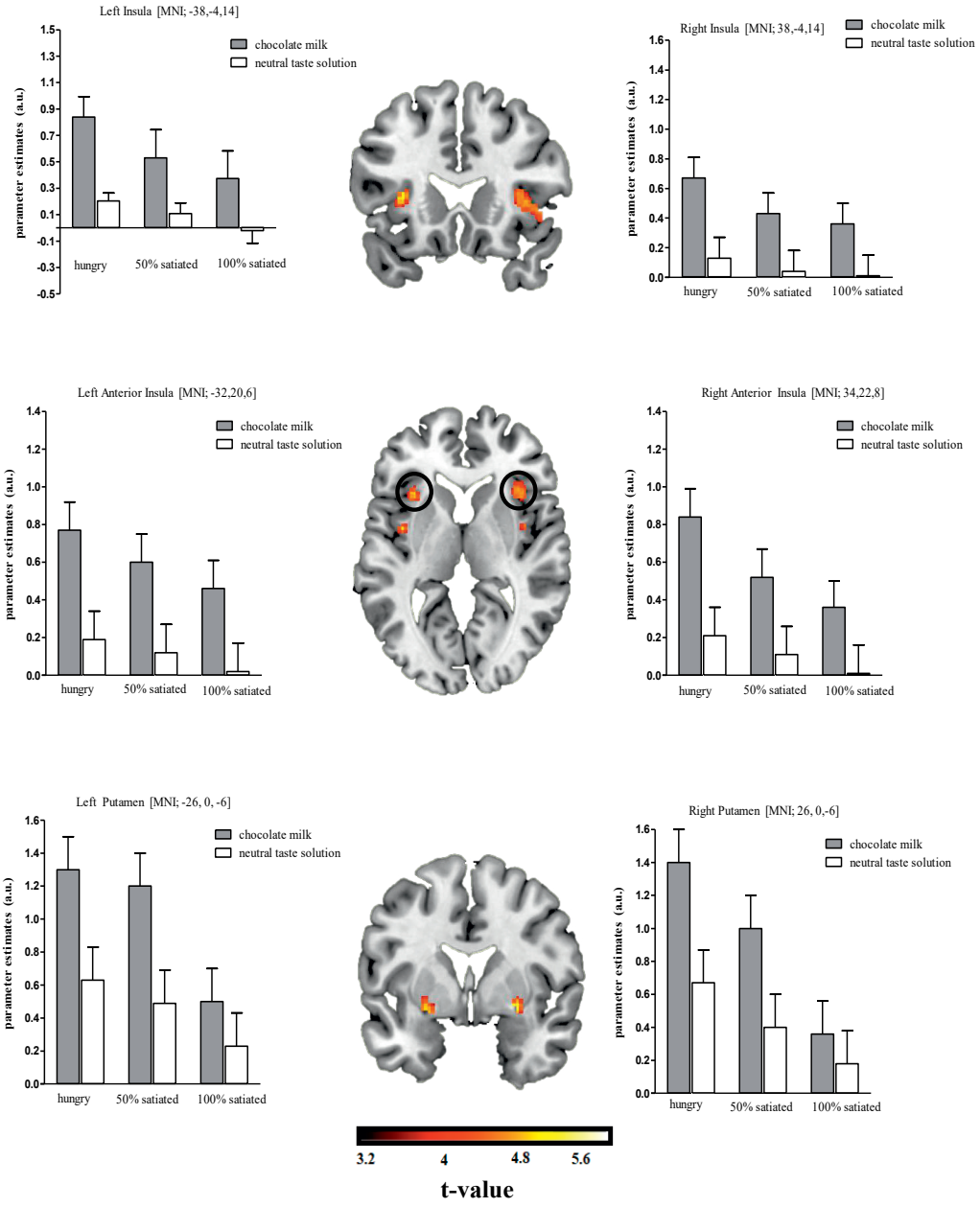

Figure 7. Significant brain activation [chocolate milk – neutral taste solution] in the bilateral (anterior) insula and putamen, taste and reward ROIs analyses, peak P-FWE<0.05. Shown are t-maps overlaid on WFU-pickatlas insula and putamen masks. Mean±SEM parameter estimates per satiation state for chocolate milk and neutral taste solution are shown in bar graphs.

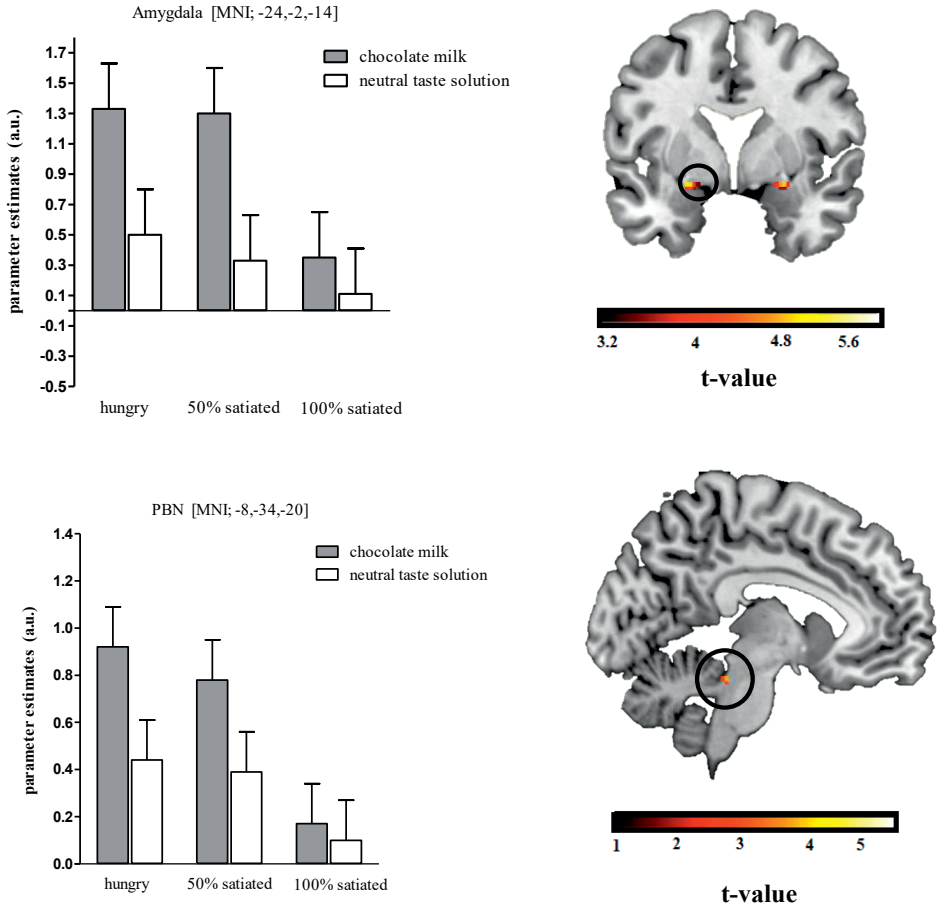

Figure 8. Significant brain activation [chocolate milk – neutral taste solution] in the bilateral (anterior) insula and putamen, taste and reward ROIs analyses, peak P-FWE<0.05. Shown are t-maps overlaid on WFU-pickatlas amygdala mask and 3mm-sphere MNI-coordinate PBN mask. Mean±SEM parameter estimates per satiation state for chocolate milk and neutral taste solution are shown in bar graphs.

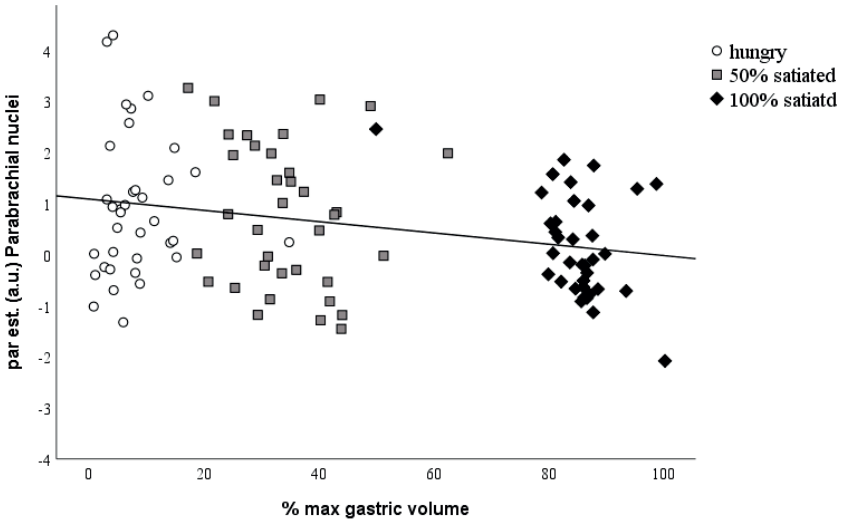

Figure 9. Correlation parameter estimate (a.u) of the PBN taste activation (chocolate milk > neutral) and % of maximum gastric volume r=-0.28, p=0.004 (partial correlation; corrected for participant).

Table 1. Mean ± SEM parameter estimates per brain region, at hungry, 50% and 100% satiated state, per stimuli. (FWE=0.05) – overall effect of flavour (chocolatemilk> water).

Region	MNI coordinate			stimuli	Hungry state	50% satiated state	100% satiated state	ANOVA fixed effect (stimuli) p-value		
	X	Y	Z					Condition	Run	C*R
Left Anterior insula	-32	20	6	Chocolate	0.77±0.15	0.60±0.15	0.46±0.15	<0.001	0.31	0.91
				Neutral	0.19±0.15	0.12±0.15	0.02±0.15			
Right Anterior insula	34	22	8	Chocolate	0.84±0.15	0.52±0.15	0.36±0.14	<0.001	0.06	0.66
				Neutral	0.21±0.15	0.11±0.15	-0.01±0.15			
Right insula	38	-2	14	Chocolate	0.67±0.14	0.43±0.14	0.36±0.14	<0.001	0.27	0.77
				Neutral	0.13±0.14	0.04±0.14	0.01±0.14			
Left insula	-38	-4	14	Chocolate	0.49±0.14	0.40±0.14	0.34±0.14	0.002	0.87	0.85
				Neutral	0.03±0.14	0.03±0.14	0.03±0.14			
Left putamen	-26	0	-6	Chocolate	1.3±0.2	1.2±0.2	0.5±0.2	0.004	0.03	0.64
				Neutral	0.63±0.2	0.49±0.2	0.23±0.2			
Right putamen	26	0	-6	Chocolate	1.4±0.2[a]	1.0±0.2	0.36±0.2[b]	0.007	0.002	0.42
				Neutral	0.67±0.2	0.4±0.2	0.18±0.2			
Left Amygdala	-24	-2	-14	Chocolate	1.27±0.3	1.27±0.3	0.35 ±0.3	0.008	0.05	0.46
				Neutral	0.50±0.3	0.33±0.3	0.11±0.3			
Brainstem-PBN	-8	-34	-20	Chocolate	0.92±0.17	0.78±0.17	0.17±0.17	0.03	0.004	0.44
				Neutral	0.44±0.17	0.39±0.17	0.10±0.17			

Significant letters indicate significant differences between time points (Bonferroni corrected)

Table 2. Mean ± SEM parameter estimates of flavour (chocolate milk> neutral) corrected for total gastric volume (liquid +air), per brain region, at a hungry, 50% and 100% satiated state (P-FWE<0.05)

Region	MNI coordinate			stimuli	Hungry state	50% satiated state	100% satiated state	ANOVA fixed effect (stimuli) p-value			
	X	Y	Z					Condition	Run	C*R	covariate
Left Anterior insula	-32	20	6	Choco	0.24±0.30	0.42±0.16	1.1±0.0.35	<0.001	0.29	0.86	0.05
				Neutral	-0.33±0.30	-.02±0.16	0.64±0.35				
Right Anterior insula	34	22	8	Choco	0.58±0.30	0.46±0.16	0.67±0.35	<0.001	0.72	0.66	0.34
				Neutral	-0.04±0.30	0.04±0.16	0.30±0.35				
Left Insula	-38	-4	14	Choco	0.18±0.28	0.30±0.14	0.95±0.33	<0.001	0.22	0.76	0.04
				Neutral	-0.36±0.28	-.08±0.15	0.60±0.33				
Right insula	38	-2	14	Choco	0.10±0.28	0.32±0.15	0.80±0.33	0.002	0.38	0.85	0.11
				Neutral	-0.36±0.28	-.05±0.14	0.5±0.33				
Left putamen	-26	0	-6	Choco	0.34±0.43	0.98±0.23	1.7±0.5	0.003	0.20	0.63	0.01
				Neutral	-0.3±0.44	0.27±0.23	1.4±0.5				
Right putamen	26	0	-6	Choco	0.60±0.43	0.82±0.23	1.5±0.51	0.006	0.37	0.42	0.02
				Neutral	-0.16±0.43	0.22±0.23	1.3±0.51				
Left Amyg-dala	-24	-2	-14	Choco	0.54±0.6	1.1±0.3	1.3 ±0.7	0.008	0.62	0.45	0.13
				Neutral	-0.28±0.6	0.14±0.32	0.1±0.7				
Brainstem- PBN	-8	-34	-20	Choco	0.23±0.34	0.61±0.18	1.0±0.40	0.03	0.30	0.43	0.02
				Neutral	-0.25±0.34	0.23±0.18	0.95±0.40				

Discussion

We investigated differences in brain(stem) responses to sweet taste over the process of satiation. We found taste-related activation in the parabrachial nucleus (PBN), which decreased over the course of satiation. In line with this, PBN taste responses negatively correlated with gastric volume. Additionally, greater activation to sweetness was found in the PBN when comparing the hungry state with 100% satiated stated.

The decrease in PBN activity over the course of satiation is in line with studies that show that the brainstem plays an important role in sensory satiation (70). The most prominent evidence for this comes from decerebrate rats that still reject taste stimuli when sated (71). In line with our findings the PBN in the brainstem has been shown to be involved in processing taste and gastric signals (39, 72-74). In rodents, an increase in PBN activity (increased and prolonged spike output) has been found when increasing stomach distension (73). This is contrary to our findings in humans as we found a decrease in flavour-related activation of the PBN when participants were in the complete satiated state (full stomach). However, our findings are in line with findings of Baird *et al.* who showed in rats that the PBN can modulate taste response of different basic tastes based on gastric distension (39). Additionally, Small *et al.* showed that greater taste intensity led to increased activation in the parabrachial region of the pons (72). Which is in line with our finding of greater activation specifically to sweet taste intensity in the PBN, in a hungry compared to fully satiated state. Cell studies have shown that taste- and gastric stretch responsive cells overlap within the PBN (74). These findings together with ours confirm the idea that integration of gastric and taste signals may take place in the PBN to induce satiation.

Gastric and taste signals are thought to project to the PBN through the NTS (74). However, we did not find NTS activation to tasting chocolate milk. Other studies have found activation, tasting a sour-sweet-salt-bitter mixture (75) and upon tasting a sucrose solution compared to tasting water (76). A reason why we did not find activation in the NTS might be the atlas that was used in the previous study was a histology atlas whereas we used MNI coordinates reported in previous studies. Additionally, we did a group analysis whereas the study of Topolovec et al. looked at the brainstem on an individual level (76). Because there is no detailed brainstem atlas, deformations to MNI space may not be as accurate for the brainstem compared to higher cortical regions. This could have led to filtering out an effect on group level due to individual variation, which may occor easily as activation in this area can consist of activity in as few as two brainstem neurons (77).

Based on gustatory pathways identified in rodents it is thought that from the PBN the pathway dissects into an affective and a sensory pathway. The affective pathway includes projections to the hypothalamus, striatum and amygdala, while the sensory pathway includes the thalamus, and the insula (42). We found areas associated with both pathways; satiation state modulated brain activation related to chocolate milk flavour in the bilateral (anterior) insula, putamen and amygdala. Activation in these areas declined as participants became satiated.

The decrease in activation in the putamen over the course of satiation is in line with previous studies that showed a decrease in putamen activation when participants were satiated in line with a decrease in the desirability of the food item (78, 79). In the present study we also observed a decrease in liking over the course of satiation for chocolate milk compared to the other stimuli. This decrease in liking of sweet is also reflected in the stronger decrease in desire to eat sweet compared to the desire to eat savoury when participants became satiated. Therefore, part of 'feeling satiated' can be attributed to chocolate milk and sweetness sensory satiation.

Additionally, the gradual decrease in (anterior) insula responses over the course of satiation is what we expected based on previous studies that found that internal state modulates insula activation and that this is associated with decreases in affective value or pleasantness of the taste stimuli (33, 72, 80, 81).

The decrease in activation in the amygdala from hungry to the 100% satiated state could reflect a decrease in pleasantness of chocolate milk in line with the subjective ratings. Previous studies have shown that amygdala activation is associated with perception of both positive and negative verbal, visual, odor and taste stimuli (82-87), we hypothesize that the amygdala signals both pleasure and aversion of taste stimuli, dependent of satiation state. When in a hungry state, amygdala activation may reflect postitive valence of the stimuli, which declined when participants felt completely full. This is in line with findings of La Bar *et al.* and Morris *et al.* that found enhanced responses to food pictures in a hungry state compared to satiated state (88, 89). However, findings in literature are unambiguous as some studies show that amygdala activation reflects odor and taste stimulus intensity, irrespective of valence (72, 90). An explanation for this difference in finding may be that the role of the amygdala in taste and odor processing is different, furthermore the simple taste solutions used in some studies may not have been as salient compared to the chocolate milk used in this study.

When corrected for activation due to stomach distension, we found that satiation state still modulated flavour-related activation in the same areas (anterior insula, putamen, amygdala, PBN). Stomach filling could explain all of the variance in the hungry and 100% satiated state, such that the found effect of fullness on flavour activation in these brain areas reversed. Instead

of a decrease in flavour related activation over the process of satiation, we found an increase when correcting for stomach filling. This suggests that flavour activation in these areas is dependent of stomach distension, which is in line with previous research (2, 26). However, flavour related activation in the 50% satiated state remained the same and subjective 'feeling half- full' seems therefore independent of gastric filling. This could be due to mere exposure to chocolate taste i.e., so-called sensory satiation (91). In this study we instructed participants to drink until they felt completely full (focussing on their stomach) and not to stop simply because they did not like chocolate milk anymore. This was done to ensure participants would be in the same state in the 100% satiated state as sensitivity to internal cues is highly variable (69). Because of this, all variation in the 100% satiated state can be explained by gastric volume and no added effect due to taste is observed.

Strengths of the current study are the comprehensive approach to study satiation related brain activation and the optimized fMRI settings to measure task-related activation in the brainstem. The size and complexity of the brainstem make it hard to probe with current fMRI approaches due to limitations in resolution and confounding by physiological noise and baseline susceptibility artefacts which are due to their location. Therefore we measured heart rate and breathing during the task to correct for physiological noise due to breathing and heart rate. fMRI is the best existing method to study brainstem activity in humans, a 7T scanner may improve specificity however this would also increase the noise/signal ratio which makes it difficult to distinguish task related activation from physiological noise (35). Better resolution would be beneficial as even within these small brainstem areas functionality of parts of these area may differ. Nuclei within the same brainstem area are involved in both taste and gastric distention processes (73, 77). Furthermore, a study in monkeys found that the posterior NTS is most responsive to glucose whereas the anterior NTS most responsive to NaCL. This underlines the heterogeneity of nuclei even within these small brainstem areas (92).

This study is one of the few studies that investigated sensory related activation in the human brainstem. To the best of our knowledge only two other studies have investigated this and they used a small number of participants (n=7-8) (75, 76). Our sample size of 36 participants is considerably larger. Additionally, we were successful in studying complete fullness in participants as they consumed a larger amount relative to their daily estimated caloric need (approximately 50%). Furthermore, they increased their intake compared to the training session suggesting that they learned from this experience and were motivated to drink more during the test session. Only 5 participants indicated that they stopped drinking because they felt nauseous, others indicated that they stopped drinking because they felt full. The high and low sweet

chocolate milk used as stimuli were equally liked when people were satiated and were equally far from 'optimum sweetness' as rated on the 'just about right' scale. Therefore our findings cannot be attributed to a general difference (independent of satiation state) in liking between the high and low sweet chocolate milk.

Chocolate milk is generally not consumed until fullness, as goes for most drinks. Future studies may use the same study design but vary the oro-sensory exposure to food (solid vs liquid) to satiate participants. Further research may study these responses more gradually within an ongoing consumption paradigm; however, this poses a challenge for measuring brain (stem) activation over the course of satiation and lowers the control over the degree of stomach filling. In such a study setting participants would not have visual cues on how much they drank, which, in our pilot studies often led to overconsumption till and nausea. Additionally, to measure satiation gradually over time, participants would have to drink more and larger sips during the fMRI task until fullness. Larger sips require more tongue movement and swallowing effort, which is also processed in the brainstem. To be able to distinguish these responses from taste activation in the brainstem, a brainstem atlas suitable for fMRI and optimized normalization of the brainstem is needed. Currently the make of such an atlas is work in progress. The lack of detailed human brain stem atlases covering all different nuclei in the brainstem makes it difficult to locate activation areas. Future studies may optimize measures in the brainstem area together with a brainstem atlas. Once this has been done, future studies may focus on brainstem areas specifically involved in processing gastric distention and different tastes. In the current study we used a non-caloric sweetener to study the effect of sweet independent of caloric content. Future studies may investigate whether areas in the brainstem process caloric content differences related to sweet taste (using sucrose instead of artificial sweetener). Eventually, the process of satiation could be investigated in eating disorders- obesity- and diabetic patients groups. For example, obese individuals are thought to be more responsive to the reward during hunger than to the signals during satiation, compared to lean (29). Comparing these findings with healthy individuals may give insight in the altered food intake regulation within a meal within such groups.

Conclusions

Based on the findings of this study we conclude that taste-related activation in the PBN, (anterior) insula amygdala, and putamen progressively decreases as satiation increases, in line with the decrease in pleasantness of the stimuli. Furthermore, the PBN may be involved in signalling intensity of sweetness, specifically in a the hungry state. The observed decrease in activation of the (anterior) insula, putamen and PBN due to satiation could be completely attributed to gastric volume in the hungry and 100% satiated state but not in the 50% satiated state. Therefore, satiation due to sensory stimulation may be especially important early in the meal, and is overruled by gastric filling towards the end of the meal when consuming till complete fullness.

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The authors declare no conflict of interest.

ML, PAM, MM, CG designed the research (project conception, development of overall research plan, and study oversight)

ML PAM MM wrote paper

ML conducted the research (hands-on conduct of the experiments and data collection)

PAM ID and GC gave advice on and helped with (f)MRI data analysis

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Supplement I- Flow chart of in- and excluded participants of the study

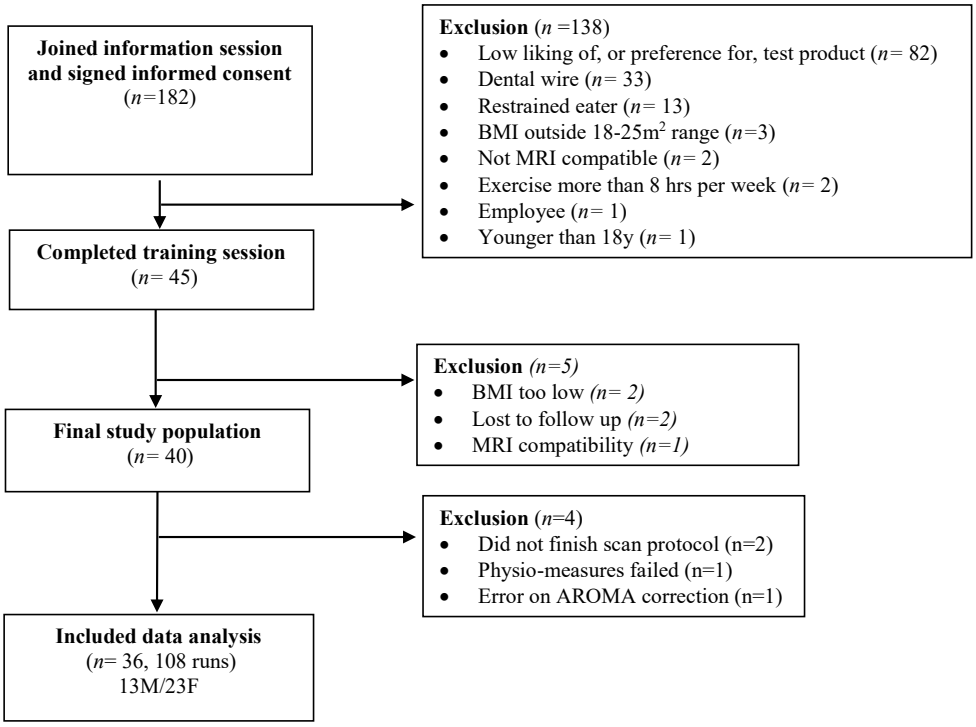

Figure 1. Flow chart of in- and excluded participants.

Supplement II – Participant characteristics (n=36)

Characteristic *N(%)*	
Gender M/F	13/23 (36/64 %)
Ethnicity	
Dutch	14 (39%)
Asian	7 (19%)
Other European	6 (17%)
American (north &south)	6 (17%)
African	3 (8%)

Characteristic (*Mean±SD)*			
	Male	Female	Total
Age (y)	24.5±4	22.9±3.2	23.5±3.7
Weight (kg)	67±10	58±8	63±9
BMI (kg/m^2)	22.2±2.6	22.0±1.9	22.0±2.1
Estimated energy expenditure (kcal/day)	2104±808	1774±238	1893±534
DEBQ			
Emotional eating	1.9±0.6	2.3±0.5	2.2±0.6
External eating	3.1±0.4	3.1±0.4	3.1±0.4
Restrained eating	2.0±0.6	2.4±0.5	2.3±0.6
BIS/BAS			
Drive	2.4±0.5	2.3±0.5	2.3±0.5
Fun seeking	1.8±0.4	2.2±0.5	2.1±0.5
Reward responsiveness	1.5±0.3	1.5±0.4	1.5±0.3
HTAS			
General health interest	4.2±0.5	4.0±0.3	4.1±0.4
Light product interest	3.7±0.5	4.0±0.7	3.9±0.6
Nature product interest	3.7±0.6	3.7±0.5	3.7±0.5
Craving sweet products	4.5±1.1	5.0±1.3	4.9±1.3
Reward	3.8±0.7	4.2±0.5	4.1±0.6
Pleasure	5.0±0.5	5.0±0.7	5.0±0.6
BIS			
Attention	2.9±0.4	3.0±0.3	2.9±0.3
Cognitive instability	2.9±0.5	2.8±0.5	2.8±0.5
Motor skills	3.0±0.3	3.0±0.4	3.0±0.3
Perseverance	2.8±0.6	2.8±0.3	2.8±0.4
Self-control	2.3±03	2.4±0.3	2.4±0.3
Cognitive complexity	2.7±0.3	2.4±0.4	2.5±0.4

Supplement III – Sensory characteristics of the study stimuli (N=116)

Table 1. Mean ± SEM Sensory characteristics of the stimuli (100 mm VAS ratings)						
	Chocolate milk			Control solutions		Main effect (p-value)
	Low sweet	Regular sweet[1]	High sweet	Neutral taste solution	Tap Water [2]	
liking	43±1.4[a]	63±1.4[b]	48±1.4[c]	45 ±1.5	48±1.5	<0.001
wanting	39±1.4[a]	58±1.4[b]	42±1.4[a]	37±1.7*	42±1.7	<0.001
filling	46±1.3[a,b]	57±1.3[b]	55±1.3[c]	22±1.6	21±1.6	<0.001
Expected satiation	46±1.5[a]	55±1.5[b]	44±1.5[a]	59±1.9*	64±1.9	<0.001
sweetness (JAR)	-17±1.1[a]	5±1.1[b]	27±1.1[c]	n.a.	n.a.	<0.001
sweetness (VAS)	33±1.0[a]	57±1.0[b]	78±1.0[c]	n.a.	n.a.	<0.001
bitterness	37±1.4[a]	21±1.4[b]	15±1.4[c]	15±1.5	15±1.5	<0.001
saltiness	25±1.4	19±1.4	20±1.4	13±1.4	13±1.4	<0.001
flavour intensity	48±1.3[a]	56±1.3[b]	66±1.3[c]	19±1.7	17±1.6	<0.001
thickness	33±1.4[a]	50±1.4[b]	38±1.4[c]	13±1.5	11±1.5	<0.001
creaminess	36±1.3[a]	54±1.3[b]	44±1.3[c]	10±1.3	8±1.3	<0.001

Ratings are based on sensory ratings done at information meeting.

Different letters indicate a significant difference between chocolate milks on that attribute (Bonferroni-corrected post hoc t-test, $p < 0.05$).

* indicates a significant difference between neutral taste solution and Tap water ($p < 0.05$)

[1] chocolate milk that participants drank between runs to reach satiated states.

[2] used as a rinse and added in the sensory test as reference to the neutral taste solution.

Supplement IV – Detailed description of fMRI data pre-processing

Results included in this manuscript come from preprocessing performed using fMRIPrep 1.4.1rc4 (48, 93); RRID:SCR_016216), which is based on Nipype 1.2.0 (93); RRID:SCR_002502).

Anatomical data preprocessing

The T1-weighted (T1w) image was corrected for intensity non-uniformity (INU) with N4BiasFieldCorrection (94), distributed with ANTs 2.2.0 (95), RRID:SCR_004757), and used as T1w-reference throughout the workflow. The T1w-reference was then skull-stripped with a Nipype implementation of the antsBrainExtraction.sh workflow (from ANTs), using OASIS30ANTs as target template. Brain tissue segmentation of cerebrospinal fluid (CSF), white-matter (WM) and gray-matter (GM) was performed on the brain-extracted T1w using fast (FSL 5.0.9, RRID:SCR_002823, (96)). Volume-based spatial normalization to two standard spaces (MNI152NLin2009cAsym, MNI152NLin6Asym) was performed through nonlinear registration with antsRegistration (ANTs 2.2.0), using brain-extracted versions of both T1w reference and the T1w template. The following templates were selected for spatial normalization: ICBM 152 Nonlinear Asymmetrical template version 2009c [Fonov *et al.* (2009) (51), RRID:SCR_008796; TemplateFlow ID: MNI152NLin2009cAsym], FSL's MNI ICBM 152 non-linear 6th Generation Asymmetric Average Brain Stereotaxic Registration Model [Evans *et al.* (2012)(97), RRID:SCR_002823; TemplateFlow ID: MNI152NLin6Asym].

Functional data preprocessing

For each of the 7 BOLD runs found per participant (across all tasks and sessions), the following preprocessing was performed. First, a reference volume and its skull-stripped version were generated using a custom methodology of fMRIPrep. A deformation field to correct for susceptibility distortions was estimated based on a field map that was co-registered to the BOLD reference, using a custom workflow of fMRIPrep derived from D. Greve's epidewarp.fsl script and further improvements of HCP Pipelines (98). Based on the estimated susceptibility distortion, an unwarped BOLD reference was calculated for a more accurate co-registration with the anatomical reference. The BOLD reference was then co-registered to the T1w reference using flirt (FSL 5.0.9,(99)) with the boundary-based registration (100) cost-function. Co-registration was configured with nine degrees of freedom to account for distortions remaining in the BOLD reference. Head-motion parameters with respect to the BOLD reference (transformation matrices, and six corresponding rotation and translation parameters) are estimated before any spatiotemporal filtering using mcflirt (FSL 5.0.9,) (52). BOLD runs were

slice-time corrected using 3dTshift from AFNI 20160207 (, RRID:SCR_005927) (53). The BOLD time-series (including slice-timing correction when applied) were resampled onto their original, native space by applying a single, composite transform to correct for head-motion and susceptibility distortions. These resampled BOLD time-series will be referred to as preprocessed BOLD in original space, or just preprocessed BOLD. The BOLD time-series were resampled into several standard spaces, correspondingly generating the following spatially-normalized, preprocessed BOLD runs: MNI152NLin2009cAsym, MNI152NLin6Asym. First, a reference volume and its skull-stripped version were generated using a custom methodology of fMRIPrep. Automatic removal of motion artifacts using independent component analysis (ICA-AROMA, (54)) was performed on the preprocessed BOLD on MNI space time-series after removal of non-steady state volumes and spatial smoothing with an isotropic, Gaussian kernel of 6mm FWHM (full-width half-maximum). Corresponding "non-aggresively" denoised runs were produced after such smoothing. Additionally, the "aggressive" noise-regressors were collected and placed in the corresponding confounds file. Several confounding time-series were calculated based on the preprocessed BOLD: framewise displacement (FD), DVARS and three region-wise global signals. FD and DVARS are calculated for each functional run, both using their implementations in Nipype (following the definitions by Power *et al.* 2014 (101)). The three global signals are extracted within the CSF, the WM, and the whole-brain masks. Additionally, a set of physiological regressors were extracted to allow for component-based noise correction (CompCor, Behzadi *et al.* 2007(102)). Principal components are estimated after high-pass filtering the preprocessed BOLD time-series (using a discrete cosine filter with 128s cut-off) for the two CompCor variants: temporal (tCompCor) and anatomical (aCompCor). tCompCor components are then calculated from the top 5% variable voxels within a mask covering the subcortical regions. This subcortical mask is obtained by heavily eroding the brain mask, which ensures it does not include cortical GM regions. For aCompCor, components are calculated within the intersection of the aforementioned mask and the union of CSF and WM masks calculated in T1w space, after their projection to the native space of each functional run (using the inverse BOLD-to-T1w transformation). Components are also calculated separately within the WM and CSF masks. For each CompCor decomposition, the k components with the largest singular values are retained, such that the retained components' time series are sufficient to explain 50 percent of variance across the nuisance mask (CSF, WM, combined, or temporal). The remaining components are dropped from consideration. The head-motion estimates calculated in the correction step were also placed within the corresponding confounds file. The confound time series derived from head motion estimates and global signals

were expanded with the inclusion of temporal derivatives and quadratic terms for each (103). Frames that exceeded a threshold of 0.5 mm FD or 1.5 standardised DVARS were annotated as motion outliers. All resamplings can be performed with a single interpolation step by composing all the pertinent transformations (i.e. head-motion transform matrices, susceptibility distortion correction when available, and co-registrations to anatomical and output spaces). Gridded (volumetric) resamplings were performed using antsApplyTransforms (ANTs), configured with Lanczos interpolation to minimize the smoothing effects of other kernels (104). Non-gridded (surface) resamplings were performed using mri_vol2surf (FreeSurfer).

Supplement V – Sensory ratings of stimuli at baseline, 50% and 100% satiated state

Table 1. Mean ± SEM sensory characteristics (100-mm VAS) at baseline, 50% and 100% satiated state. Significant letters indicate significant differences between time points (Bonferroni correction)

Sensory characteristic	Product	Baseline – hungry state	50% satiated state	100% satiated state	ANOVA fixed effect (run) p-value
Liking	High sweet	67±2[a]	55±3[b]	28±3[c]	<0.001
	Low sweet	51±2[a]	41±3[a,b]	29±3[b]	
	Neutral	42±2	40±3	40±3	
Wanting	High sweet	69±2[a]	47±3[b]	23±3[c]	<0.001
	Low sweet	51±2[a]	36±3[b]	21±3[c]	
	Neutral	53±2[a]	36±3[b]	26±3[b]	
Filling	High sweet	50±3	n.a.	52±3	0.43
	Low sweet	45±3	n.a.	50±3	
	Neutral	23±3	n.a.	23±3	
Sweetness (JAR)	High sweet	9±3	14±3	15±3	0.06
	Low sweet	-9±2	-14±3	-3±3	
Sweetness	High sweet	69±2	n.a.	70±2	0.37
	Low sweet	37±2	n.a.	43±2	

Supplement VI – Appetite and well-being ratings before and after each run

Table 1. Mean±SEM Appetite and well-being ratings (100-mm VAS) per time point (Hungry state, pre- and post-drinking till 50% satiated, pre- and post-drinking till 100% satiated and at the end of the session. different letters on the same row indicate significant differences between time points.

	Baseline – hungry state	Pre 50% fullness	Post 50% fullness	Pre 100% fullness	Post 100% fullness	End of session	ANOVA fixed effect (pre-post run) P-value
Fullness	12±2[a]	25±2[b]	52±2[c]	51±2[c]	99±2[d]	84±2[e]	<0.001
Hungry	71±3[a]	66±3[a]	51±3[b]	51±3[b]	19±3[c]	26±3[c]	<0.001
Desire to eat	69±3[a]	70±3[a]	56±3[b]	55±3[b]	28±3[c]	29±3.5[c]	<0.001
Desire to eat sweet	57±3[a]	n.a.	37±3[b]	n.a.	10±3[c]	11±3[c]	<0.001
Desire to eat savoury	67±4[a]	n.a.	63±4[a]	n.a.	40±4[b]	38±4[b]	<0.001
Satiated	18±3[a]	n.a.	50±3[b]	n.a.	78±3[c]	74±3[c]	<0.001
How much able to eat	73±3[a]	n.a.	54±3[b]	n.a.	22±3[c]	27±3[c]	<0.001
Thirsty	56±4[a]	n.a.	33±4[b]	n.a.	22±4[b]	21±4[b]	<0.001
Nauseous	17±3[a]	n.a.	19±3[a,c]	n.a.	29±3[c,d]	28±3[c]	<0.001
Sleepy/tired	30±4[a]	n.a.	28±4[a]	n.a.	34±4[a,b]	41±4[b]	<0.001
Alertness	47±4	n.a.	n.a.	n.a.	n.a.	42±4	0.19
Anxiousness	17±3	n.a.	n.a.	n.a.	n.a.	12±4	0.10
Light-headed	24±4[a]	n.a.	n.a.	n.a.	n.a.	36±4[b]	0.008
General wellbeing	77±3[a]	n.a.	n.a.	n.a.	n.a.	68±3[a]	0.005

Supplement VI – Cardiac, Respiratory and interaction regressors

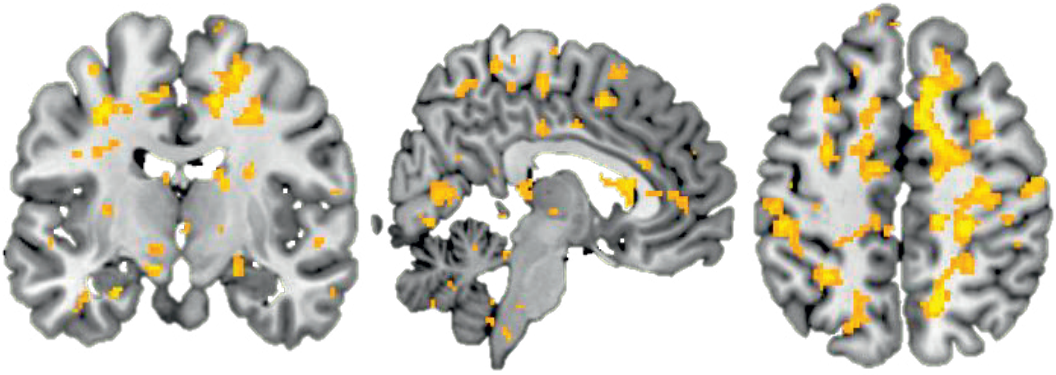

Figure 1. Cardiac regressors.

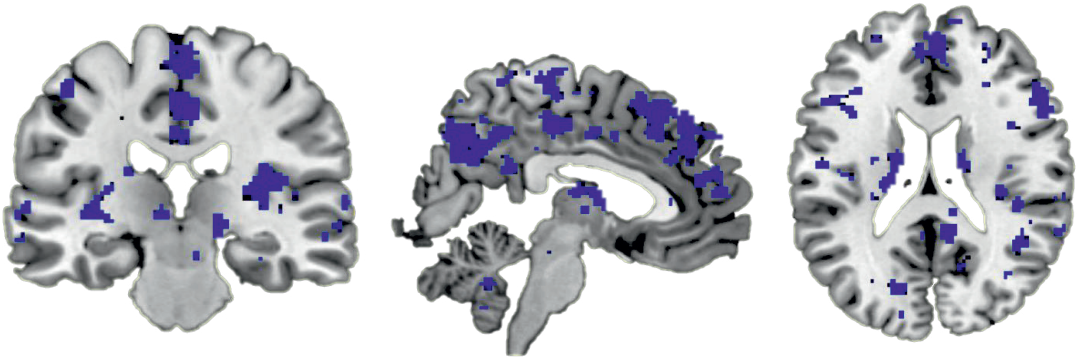

Figure 2. Respiratory regressors

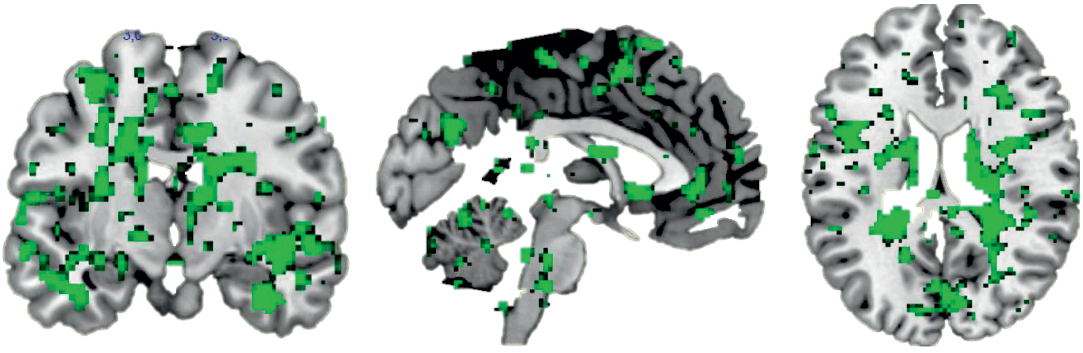

Figure 3. Cardiac and respiratory interaction regressors.

Supplement VII – Whole brain main effects of condition (flavour, sweet, anticipation) and main effect of satiation (hunger, 50% and 100% satiated) and their interaction effects.

Table 1. Main effect of condition (whole brain analysis, sig. <0.001, k8 $FWE_{corrected}$ 0.05).

Main effect of condition:	Region		Peak voxel coordination of fROI			Cluster size (voxels)	z-score	p-value FWE-cor
			X	Y	Z			
Flavour choco >neutral	L	Inferior frontal gyrus	-52	6	18	374	7.69	<0.001
	R	Postcentral gyrus somatosensory	60	-16	30	677	7.39	<0.001
	L	Supramarginal gyrus somatosensroy	-60	-22	40	1620	7.29	<0.001
	L	Cerebellum exterior	-16	-78	-32	468	7.29	<0.001
	R	Precentral gyrus Premotor cortex	58	12	18	273	7.05	<0.001
	L	Fusiform gyrus	-20	-94	-6	138	6.97	<0.001
	R	Cerebellum exterior	24	-68	-24	216	6.89	<0.001
	R	Cerebral white matter	26	-48	18	125	6.66	<0.001
	R	Fusiform gyrus	26	-92	-4	62	6.08	<0.001
	L	Anterior insula	-32	20	6	15	5.43	0.003
	L	Anterior insula	-38	-4	14	12	5.42	0.005
	L	Middle temporal gyrus	-50	-58	8	12	5.28	0.005
	L	Superior partietal lobule	-12	-58	60	16	5.18	0.003
	L	Superior parietal lobule	-10	-70	56	30	5.17	0.001
	L	Putamen	-24	0	-8	15	5.17	0.003

	L	Cerebral white matter	-24	-50	20	11	5.07	0.006
	R	Anterior insula	38	18	2	16	4.93	0.003
Sweet high sweet > low sweet	-	-	-	-	-	-	-	-
anticipation choco > water	-	-	-	-	-	-	-	-

Table 2. Main effect of satiation (hungry, 50% and 100% fullness) per condition

Main effect of satiation:	Region		Peak voxel coordination of fROI			Cluster size (voxels)	z-score	p-value FWE-cor	p-value uncorr
			X	Y	Z				
Flavour choco > neutral	R	Inferior frontal gyrus	58	20	18	151	4.68	0.04	
	L	caudate	-16	10	4	153	4.53	0.04	
	R	putamen	26	4	-8	97	3.96	0.16	0.01
	R	caudate	10	8	4	69	3.81	0.35	0.03
Sweet high sweet > low sweet	R	Inferior frontal gyrus	58	20	18	133	4.36	0.04	
	R	Putamen	24	0	-6	49	3.84	0.53	0.045
anticipation choco > water	-	-	-	-	-	-	-	-	-

Table 3. Main interaction effect condition*satiation (hungry, 50% and 100% fullness)

Main effect of satiation:	Region		Peak voxel coordination of fROI			Cluster size	z-score	p-value FWE-cor	p-value uncorr
			X	Y	Z				
Flavour choco > neutral	-	-	-	-	-	-	-	-	-
Sweet high sweet > low sweet	L	Superior temporal gyrus	-54	-50	20	47	3.84	0.5	0.036
anticipation choco > water	R	Inferior frontal gyrus	50	38	12	48	4.01	0.43	0.03

Supplement VIII – Whole brain contrast effects of condition (flavour, sweet, anticipation)

Table 1. Mean ± SEM parameter estimates per brain region, at baseline, 50% and 100% satiated state, per stimuli. (FWE=0.05)- contrasts where hungry> 100%

Factor of interest	Region	MNI coordinate			stimuli	Hungry state	100% satiated state
		X	Y	Z			
Flavour	Putamen	26	2	-6	Choco	1.3±0.2*	0.83±0.2
					Neutral	0.30±0.2*	0.07±0.2
	striatum	10	8	2	Choco	1.1±0.18	0.55±0.18
					Neutral	0.16±0.17	-0.13±0.17
	striatum	-18	12	4	Choco	0.65±0.12	0.23±0.12
					Neutral	0.07±0.12	-0.10±0.12
	caudate	-10	2	12	Choco	1.3±0.2*	0.51±0.2
					Neutral	0.23±0.2*	0.04±0.2
	caudate	-12	8	20	Choco	0.86±0.2	0.18±0.2
					Neutral	0.12±0.2	-0.12±0.2
	PBN	-8	-34	-20	Choco	0.74±0.14	0.08±0.14
					Neutral	0.36±0.14	0.06±0.14
	Midbrain	-10	-28	-16	Choco	1.1±0.14	0.04±0.14
					Neutral	0.49±0.14	0.0±0.14
Sweet ness	PBN	-8	-34	-20	High sweet	0.92±0.17	0.17±0.17
					Low sweet	0.44±0.17	0.10±0.17
Anticip ation	-	-	-	-	-	-	-

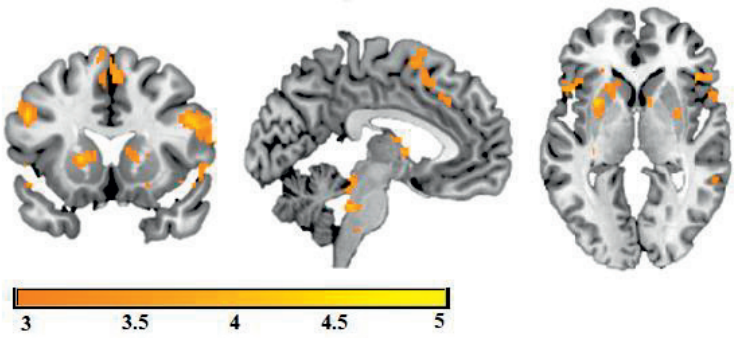

Figure 1. Flavour (chocolate milk > neutral) where hungry> 100% satiated.

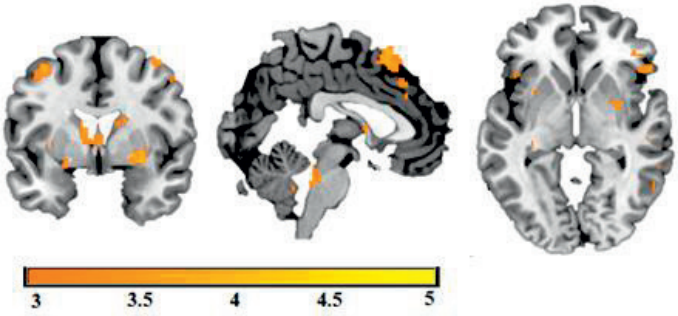

Figure 2. Sweet (high sweet > low sweet) where hungry> 100% satiated.



Chapter 7

General Discussion

General Discussion

The overall aim of this thesis was to better understand the role of sensory signals in the physiological processes underlying satiation. This chapter describes the main findings and puts them in context of previous research and reflects on the methods used. Furthermore, the main conclusions are stated together with their implications and directions for further research.

Main findings

Figure 1 depicts the sensory, peripheral, and brain processing factors studied in this thesis. The factors that were found to affect satiation or to play a role in the physiology of satiation are highlighted in green.

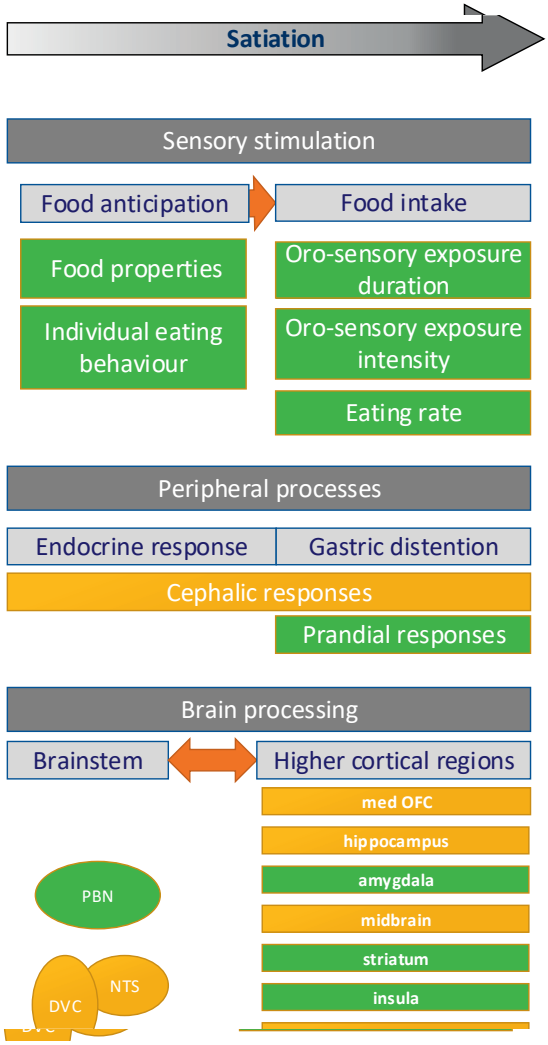

Figure 1 Diagram showing satiation as the relationship between the three levels: sensory stimulation, peripheral processes and brain processing. Figure is partly based on Hopkins and Blundell et al. (1, 2). In green the factors that were shown to be involved in satiation within this thesis.

Sensory effects on satiation

Table 1. shows the effect of the sensory and eating behaviour manipulations on satiation, that is the food intake, found in this thesis. Longer oral processing duration led to a lower intake of the model food, whereas no significant effect of sweetness intensity was found on intake (chapter 2). In chapter 4, we found that a reduced eating rate (only in the high oro-sensory exposure condition) and increased oro-sensory exposure decreased food intake, depending on the portion size given.

Table 1. Effect sizes of sensory and eating behaviour manipulations on satiation.

Sensory or eating behaviour variable	Factor (difference between manipulations)	Decrease in intake (%)	Manipulation	Chapter
Oro-sensory exposure duration	2	21%*	Texture hardness	2
	2	2.5% (small portion)	Adding chewy condiments to a semi-solid	4
	2.6	12%* (large portion)		
Oro-sensory exposure intensity	1.5	11% ns.	Sweetness intensity	2
Eating rate	1.6	10%** (small portion)	Breaks between bites	4
	1.8	5% ns. (large portion)		

* significant effect compared to control

** significant but only in the high oro-sensory exposure condition.

Overall, we found that the effect of texture on food intake is larger compared to taste. The 10% reduction in food intake due to sweet taste intensity is however comparable to the effect size of salt intensity on food intake (3). Furthermore, this finding is in line with a review by McCrickerd *et al.* (4) which argues that texture effects on food intake are larger (30 %) than taste effects (10%).

We hypothesized that the direct effect of OSE duration and OSE intensity on sensory satiation is of equal magnitude, see Figure 2. However, the larger effect of oro-sensory exposure duration might be due to an indirect effect, as increased oro-sensory exposure duration also reduces eating rate. Previous studies have shown that a reduced eating rate leads to a decrease in food intake, which we confirmed in chapter 4 (5-7).

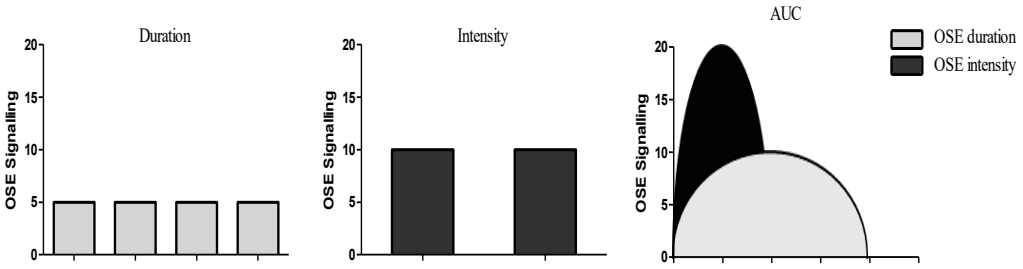

Figure 2. Oro-sensory exposure duration and intensity effects on sensory signalling involved in satiation

Even though participants ate less in the conditions where oro-sensory exposure was increased they did not report to feel less satiated or more hungry after the meal compared to the low oro-sensory exposure conditions (chapter 2). This is in accordance with previous studies reporting decreases in intake due to texture hardness, but that did not find differences in appetite ratings (8, 9). In chapter 4 participants even reported feeling more full and less hungry in the high- compared to the low oro-sensory exposure condition.

In chapter 2, model foods were used to study the effects of hard texture and sweet taste on satiation or food intake. In contrast to commercially available foods these model foods led us to only vary texture and taste, while having other food properties such as appearance, and macronutrients and energy content similar. In this way we could clearly show the effects of these food properties on intake. Additionally, we were successful in manipulating texture and taste properties while the food stimuli were perceived similar in palatability. This is rather difficult as texture and taste are both drivers of palatability and food acceptance (4, 10).

Part of the reason why the effect of texture is larger compared to the effect of taste is because there is a much narrower range of sweetness that can be manipulated without it effecting the palatability of the food item, compared to texture hardness. Furthermore, it is not known whether this 'higher' sweet taste intensity should be relative to participants habitual sweetness level to affect intake. A systematic review paper by Appleton *et al.* suggests that there is evidence from controlled studies that a higher sweet taste intensity leads to reduced preferences for sweetness on the short term but that there is little evidence for effects on the long term (11). Prospective research is needed to determine whether people habituate to higher sweet taste intensities and whether this leads to changes preferences for sweet tasting products. Within the research described in this thesis the focus was on sweet taste intensity but the effect on food intake may be translated to flavour intensity, as shown for savoury by Forde *et al*. (3).

In both chapters we studied the effects of oro-sensory exposure duration, eating rate and sweet taste intensity separately. However, this would never occur in a normal eating situation. When separating these affects it also interferes with other eating behaviour characteristics. For example, when participants are aware of the eating behaviour manipulation this will lead to compensatory eating behaviour such as increasing bite size and meal duration as shown in chapter 4. Studies not interested in the underlying physiology but solely in the effect of eating behaviour on food intake may keep eating situations as normal as possible and study these effects as a whole.

Although texture and eating rate effects on food intake have been shown to be quite robust in studies that studied these effects in a more real life setting (12, 13). Within the context of daily life food intake is dependent on many factors including, but not limited to; packaging, portion size, social norms, (memory of) previously eaten foods and distraction while eating (14-17). Furthermore, in all of the studies within this thesis healthy normal-weight participants were included. Overweight or obese people may be insensitive to internal food cues and when eating highly palatable foods the oro-sensory exposure duration effect on satiation may be overruled by hedonic eating (18). Extrapolating these findings to a real life context or to overweight or obese people should therefore be done with caution.

To conclude, texture manipulations have a larger effect on food intake compared to taste intensity as they have a direct effect on sensory satiation and an additional indirect effect by slowing down eating rate. In overall, mere oro-sensory exposure to food and a slow ingestion rate helps to control food intake.

Peripheral processes underlying satiation

In chapter 3, 4 , 5 and 6 we investigated the role of peripheral processes in satiation. In chapter 3 and 4 we hypothesized that the physiological mechanism underlying the oro-sensory exposure effect on satiation were insulin, pancreatic polypeptide (PP) and ghrelin cephalic phase responses. Contrary to our hypothesis no typical cephalic phase peak response was found for any of the hormones in any of the conditions (chapter 3). In chapter 4, we studied the same endocrine responses but instead of modified sham feeding, participants ate a meal. Similar to findings of chapter 3 we found no typical cephalic phase peak. However, OSE did increase prandial insulin but not PP responsiveness. In contrast, PP responses were greater when oro-sensory exposure was low and eating rate fast. Together, insulin and PP prandial responses may mediate the effects of OSE and eating rate on satiation.

Finally in chapter 5 we summarized the current literature on cephalic insulin and PP responses. A cephalic phase insulin and PP increase was observed in about half of all included treatments. Only about one fifth of the treatments induced a significant increase in PP or insulin concentrations from baseline. Based on this we concluded that there is little evidence for physiologically relevant cephalic insulin or PP responses in daily life.

Part of the reason why cephalic phase responses are consistently found in rodents and not in humans might be because in rodents taste signals are directly projected to reward areas whereas in humans these signals are modulated in the insula and hypothalamus. Here, the initially same taste signals (as in rodents) are changed depending on internal state (hungry or satiated) and depending on whether or not they are confirm the expectations. Therefore it may not be surprising that the way food cues trigger cephalic responses is fundamentally different in rodents compared to humans (19).

To study cephalic phase responses we set up a strict standardized experimental protocol to be able to measure small changes in insulin, pancreatic polypeptide and ghrelin concentrations. However, such a laboratory setting makes it difficult to study cephalic phase responses as these responses are thought to be dependent on 'wanting' to eat the presented food item (20). Even though studies may use food items that are generally liked (such as the chocolate custard used in chapter 4), the person eating the food may not find the food palatable at that moment and in that situation (low 'wanting'). A laboratory setting and simultaneous blood sample collection may decrease the reward value of the food. This makes inherently difficult to measure cephalic responses in the laboratory, which would explain the lack of findings in about half of the studies and the variable responses within the same participant presented to- or eating the same food item (21). The small response size in combination with the unpredictability make cephalic endocrine responses difficult to grasp and therefore it seems nearly impossible to relate these responses to food properties such as texture or taste. Especially taking into consideration that based on a statistical approach dynamic hormone and glucose changes, which are much larger than CPRS, are thought to only explain between 40-70% of the variation to predict intake or appetite (22, 23).

A clear definition of a cephalic phase insulin and pancreatic polypeptide response is necessary to be better able to compare findings of studies. This definition should take baseline variation into consideration to conclude whether a peak increase is due to the presented food cue or due to natural fluctuations (24). Furthermore the definition should include a strict time range in which the cephalic peak response should take place. As insulin is dependent on blood glucose

levels, for insulin the definition should be food cue- specific (i.e. seeing, smelling, modified sham feeding (chew and spit out), or food intake). Examples of such definitions are:

- An insulin cephalic phase response is a peak response the size of 2 times the standard deviation of the baseline mean (based on at least 2 baseline measures) that has to occur within the first 10 min after a food cue (visual/smell), within 8 min after modified sham feeding and within the first 5 min after actual food intake.
- A pancreatic polypeptide cephalic phase response is a peak response of 100±20 pg/ml above baseline (based on at least 2 baseline measures) within the first 10±5 min after food cue exposure.

Based on our findings endocrine cephalic phase responses are not the physiological mechanism underlying the oro-sensory exposure effect on satiation. An alternative or additive mechanism through which oro-sensory exposure may affect satiation is through cognitive believes, experience and learned associations. For example, foods that are perceived as chewy or as thick (semi-solids) are believed to be more filling (25, 26). This has also been shown by studies that found associations between eating rate and expected satiation and meal size (27-30). Furthermore, food structure has been shown to affect gastric kinetics such that larger pieces are retained in the stomach until sufficiently small. By inhibiting gastric emptying, the stomach distends more which may also induce satiation (31). We studied gastric volume associated brain activity in chapter 6. these findings are discussed in the following paragraph (brain processing). Based on the findings in chapter 3,4 and 5 we conclude that cephalic phase insulin, pancreatic polypeptide and ghrelin responses do not mediate the effect of oro-sensory exposure on satiation. Instead, we hypothesized that increased oro-sensory exposure affects satiation directly through sensory satiation and indirectly affects endocrine responses by reducing eating rate. By prolonging the duration of the meal there is more time for hormones to be secreted and to induce satiation and consequently meal termination, before larger quantities are eaten (Figure 3). A large population level study where insulin and PP concentrations can be measured continuously throughout everyday life is needed to confirm the conclusions made in this thesis.

Brain processing

In chapter 6 we investigated how oro-sensory exposure or taste signals are processed to affect satiation in the brain. We performed an fMRI study where we measured neural reactivity in the brain(stem) to chocolate milk over the course of satiation. Additionally, we measured gastric volume such that we could identify regions that respond solely to taste (independent of gastric distention). We found that activity in the parabrachial nuclei (PBN) in the brain stem, and

bilateral (anterior) insula, putamen and amygdala gradually decreased as satiation increased, in line with the decrease in affective value of the chocolate milk stimuli. This is the first human study to show a decrease in PBN activation as satiation increases. This finding is in line with rodent and cell-line studies as the PBN contains both taste and gastric cells, that overlap; suggesting integration of both signals within the PBN (32-34).

The observed decrease in activation of the PBN, (anterior) insula, amygdala and putamen due to satiation could be completely attributed to gastric filling in the hungry and 100% satiated state but not in the 50% satiated state. Indicating that satiation due to sensory stimulation may especially be prominent early on in the meal and is overruled by gastric satiation towards the end of the meal when drinking till complete fullness.

To determine changes in brain reactivity to chocolate milk over the course of satiation, continues measures would be most favorable, this can be done using fMRI. However, a large amount of chocolate milk need to be consumed to feel satiated, therefore larger sips (than 2 ml) are needed to speed up the process, however, taking larger sips causes distortions in the brainstem area and is therefore not a suitable method to study these responses in this area. In chapter 6, participants received sips of chocolate milk through a tube while lying inside the MRI scanner bore. As with all laboratory experimental studies, the setting is far from a 'normal' chocolate milk consumption situation therefore findings may not be directly translational for what happens in real life.

Based on this chapter we conclude that the PBN, (anterior) insula, amygdala and putamen are involved in sensory satiation modulated by gastric volume. Sensory satiation may especially of importance early on in the meal.

Future studies may investigate how sensory signals are processed over the course of satiation in other population groups such as in obese of overweight people do determine whether the responses in the areas found in this chapter are differential in these populations. Additionally, future studies may focus on translating satiation related neurophysiological findings in rodents to humans, to further elucidate the role of the brainstem in (sensory) satiation.

Main conclusions

To conclude, as a proof of principle we confirmed that increased oro-sensory exposure decreases food intake. Insulin, PP or ghrelin cephalic phase responses do not mediate this effect. Instead, we hypothesize that the underlying mechanisms are; 1) a direct effect of oro-sensory exposure on satiation through attenuated hedonic reactions to the food's taste, inducing sensory satiation and 2) an indirect effect of increased oro-sensory exposure duration which also slows down the rate at which the food is consumed. This gives more time for hormones to be secreted and for stomach distention signals and early uptake of nutrients to be processed by the brain, inducing satiation (Figure 3).

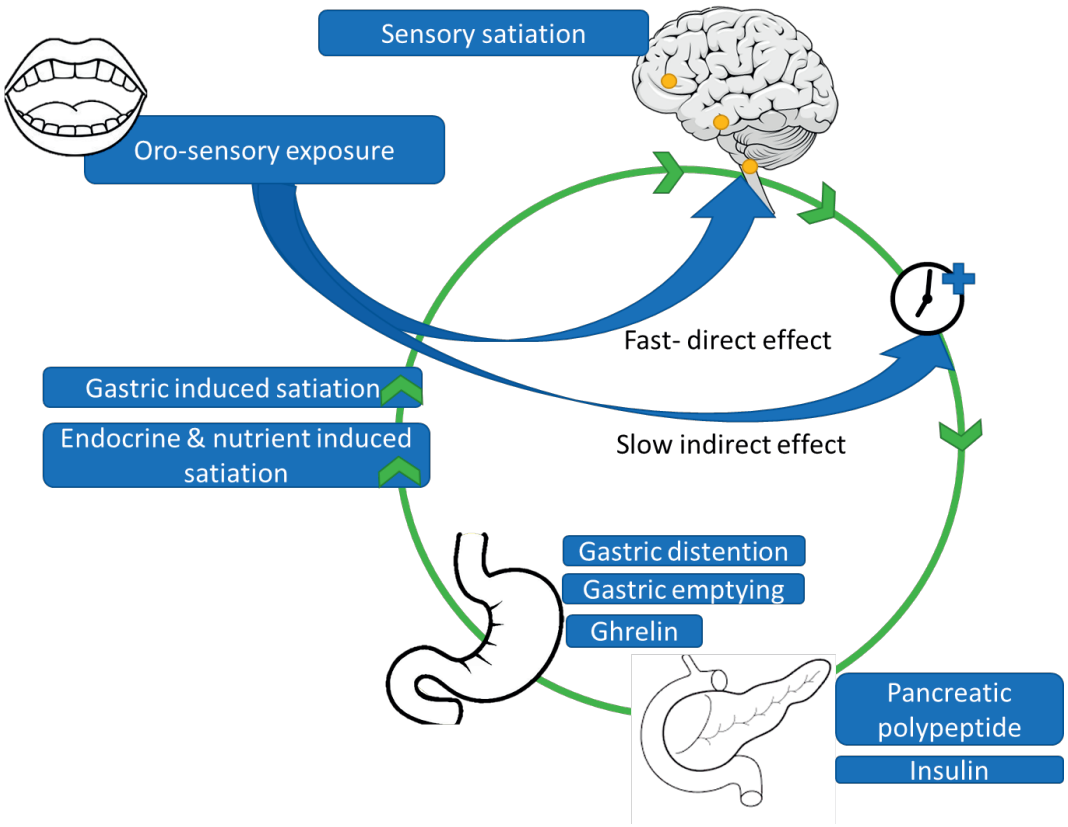

Figure 3. Proposed underlying physiological mechanism of the oro-sensory exposure effect on satiation

Implications and suggestions for future research

Based on the findings of this thesis, and previous research we can conclude that increased oro-sensory exposure and a slowed down eating rate, helps control food intake (7, 38). Harder food textures (i.e. foods that require oral processing effort such as chewing) can be used as a strategy to counterbalance over eating due to the high energy density and palatability of the foods in our food environment. Not all calories are the same, as foods with the same caloric content can have markedly different satiating capacities. Because of this, food industries may develop new (versions of existing) food products with a harder texture to help consumers feel satiated without consuming too many calories relative to their energy expenditure. However, it should be taken into account that multiple texture dimensions within a food product may increase intake due to the increase in sensory variety. Future research should define whether there is 'texture specific satiation' (i.e. boredom of texture of the food eaten relative to an un eaten food with a different texture) and whether foods that have different texture layers lead to an increase intake (39). For example, eating semi solids throughout the day may increase wanting of foods with a harder food texture, as is observed in patients who receive tube feeding who report lack of chewing as one of the most common sensations related to psychosensory deprivation (40). When this is true, sensory rich foods such as those that have different textures within the food product may increase food intake as they may not lead to texture specific satiation as fast as 'single' texture type foods due to increased variety.

In the current food environment two process are likely to be activated; hedonic and homeostatic processes (18). The amount that is eaten is determined by these control systems and their interaction, which is affected by environmental, economic, and social factors. Therefore, whether or not individuals act on feeling full is dependent on 'satiety responsiveness' (41). For example, individuals may continue eating beyond satiation when the food that is eaten is highly palatable. Based on the research described in chapter 6 we noticed that it is particularly difficult for participants to determine when they feel half-full and completely full. Increasing satiety responsiveness may be beneficial for weight balance. Intervention studies in children at a young age are needed to see whether training on recognizing of feeling full or satiated may lead them to better resist the hedonic drive to eat when they become adults.

For weight management it would be beneficial to eat foods that have a high satiation/energy density ratio. A recommendation to eat foods with a harder texture should therefore be part of dietary guidelines to maintain a healthy body weight.

Currently the Dutch nutrition center ('voedingscentrum' the Dutch equivalent to the US MyPlate a federal food program of the health & Human services (HHS) and department of food

and agriculture (FAO) already mentions that smoothies do not count as a daily portion of fruit as you feel less satiated after a smoothie compared to eating whole pieces of fruit (42). Besides, in the Dutch dietary guidelines it is stated to limit sugar containing beverages (43). Similarly the HHS recommends Fruits, especially whole fruits (42). This is already a step in the right direction however to these recommendations clear recommendations about eating 'slow hard textured foods' should be added.

Recommendations concerning food texture may be more effective than general advice to eat slow as this is more difficult and people may change their bite size and meal duration to compensate for the slowed down eating rate. For example, the ' Fletcherism' diet trend in the 80's advised diet-followers to chew at least 30 times per bite. However, this introduces disgust of the food that is eaten and is therefore difficult to maintain (44). Eating rate is a trait of the individual and might therefore be difficult to change. Some individuals always eat fast whereas there are individuals who will eat slow in comparison to others, independent of the food eaten (7). Devices such as the slow control fork© or mandometer© may be useful to decrease eating speed additionally to reduce eating rate as a weight loss strategy (5, 45).

Why some people simply eat faster than others may be related to energy expenditure and FFM as this is the main driver of daily food intake and meal size (46). However, this within participant fixed eating speed seems to be acquired at a young age. Four to five year old children can already be classified as slow and fast eaters and a fast eating rate at a young age has been associated with a higher BMI (47-49). Eating speed is inheritable, however children's' eating rate is also affected by parental feeding strategies and might be affected by early exposure to hard texture foods (50, 51). Early exposure to different types of textures helps the development of oral physiology which partly determines eating rate and texture acceptability (52). A study of Gisel *et al.* showed that oral processing of semi-solid foods (applesauce) is already acquired at 6 months old and the number of processing cycles does not change from when the infant was 2 years old (53) Eating behaviour or feeding skill of solid foods is not established until the infant is 18 months where jaw closing speed starts to dependent on the food that is eaten. Already at this age, infants learn to adapt their bite force to the texture of foods (54). However, how early exposure to different texture foods influences eating rate at an older age is not investigated yet.

Besides weight maintenance, future research is needed to determine whether recommending eating hard texture foods on top of a low calorie diet may lead to sustained weight loss in people that are overweight or obese. First a long term study, of at least one month, is needed to investigate whether people will decrease their daily energy intake and lose weight when eating

hard texture foods over this period of time. The food texture hardness should be balanced such that intake is lowered, but the hedonic value and consumer acceptance of a food remains unchanged. Next, an implementation study may be done where eating hard texture foods are recommended next to healthy diet advice to see if such effects are sustained over the course of a at least half a year, as weight intervention studies on average result in 5- 9% weight loss within 6 months (8, 55, 56). Such an intervention may be considered successful at 10% weight loss, although this depends on the aim as 5% weight loss already lowers the risk of cardiovascular diseases and diabetes (57-60). Special care should be taken for people using condiments such as sauce which would increase both eating speed and energy content of the meal eaten (61).

To summarize, future research may focus on the development of palatable hard texture food products as replacement of existing soft texture products. Furthermore, research to better understand how eating rate is established as an individual trait is needed to develop strategies and feeding recommendations for children at a young age to slow down eating rate. Future research will have to determine whether hard texture foods in combination with a low caloric diet will lead to sustained weight loss in overweight or obese people. Last, clear recommendations in the Dutch dietary guidelines are needed concerning food texture.

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Summary

Satiation is a process that occurs during eating and involves inhibitory signals at a sensory, hormonal, digestive and cognitive level. The taste and texture of food influence this process. Harder foods with an intense taste are more satiating, i.e. we need to eat less of these foods to feel full, compared to softer foods with lower taste intensity. Oro-sensory exposure (OSE), the in-mouth taste perception of food, thus plays an important role in satiation and influences meal size. However, how oro-sensory signals affect satiation is not fully understood. Therefore, the main aim of this thesis was to better understand the role of sensory signals in the physiological processes underlying satiation. To study this we investigated the effect of OSE (duration and intensity) and eating rate on food intake, associated (cephalic) endocrine responses and brain reactivity.

We started by investigating the independent contributions of oral processing duration and taste intensity and their combined effect on satiation (**chapter 2**). This was done by manipulating texture and sweet taste intensity of model foods (2x2 study design, soft and hard vs. sweet and high sweet). Hard textured model foods decreased food intake through increased OSE duration, whereas sweetness intensity did not affect intake.

To better understand the physiology underlying the effects of oro-sensory exposure on satiation we studied the effect of texture and sweetness intensity on endocrine cephalic phase responses (**chapter 3**). Cephalic phase responses are neurally mediated anticipatory and conditioned responses to food cues and are considered the first phase of digestion. Insulin, pancreatic polypeptide and ghrelin responses were measured while participants modified sham fed (chew and spit) the same model foods as used in chapter 2. We expected a cephalic peak increase in these hormones 5-15 min after food exposure. However, we did not find a typical cephalic phase peak response for any of the hormones. Insulin levels tended to be higher 5 min after starting to chew but this increase could not be not be attributed to the texture or taste manipulations. In addition, we found that pancreatic polypeptide was more responsive to sweetness. Ghrelin concentrations were higher when chewing the hard texture compared to the soft texture model foods.

Based on the results from chapter 2 and 3 we hypothesized that part of the mechanism behind the oro-sensory exposure duration effect on satiation might be eating rate. When the duration of oro-sensory exposure is increased, eating rate is slowed down. Previous studies have shown that a reduced eating rate also leads to a decrease in food intake. Additionally, we hypothesized that part of the reason why we did not find typical cephalic responses in chapter 2 was because

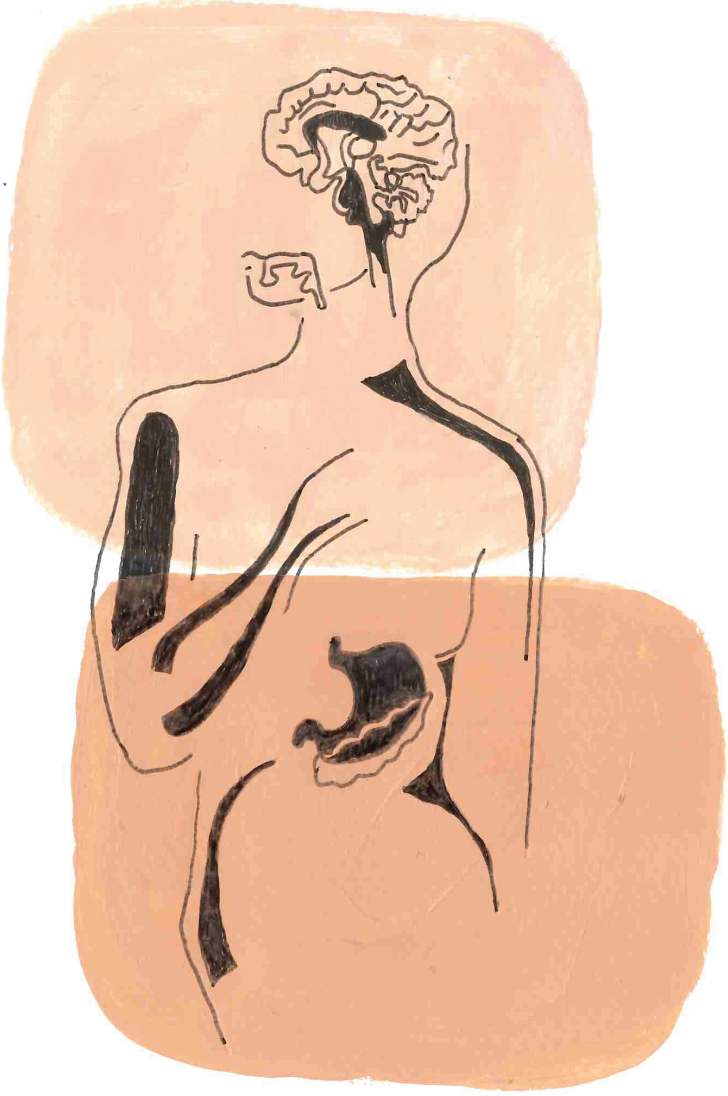
the model foods scored 'neutral' on liking, whereas palatability may be important to trigger a cephalic response. We therefore investigated the effect of OSE duration and eating rate on food intake (palatable chocolate custard) and associated endocrine responses (**chapter 4)**. Two studies were set up. In both studies subjects ate until fullness of chocolate custard with and without fudge pieces (low or high oro-sensory exposure) at two different eating rates (slow or fast eating rate). In study 1 participants received a small portion and in study 2 a larger portion. Additionally, blood samples were collected during the meal in study 2. We found that a reduced eating rate (ER) (only in the high oro-sensory exposure condition) (study 1) and increased oro-sensory exposure (study 2) decreased food intake but that this was dependent on the portion size. Eight minutes after starting to eat, insulin concentrations increased for all treatments compared to control. At the end of the meal insulin concentrations were higher in the high OSE, slow ER compared to the low OSE, fast ER condition. Pancreatic polypeptide increased at 5 min after meal onset in the low OSE, fast ER condition. There were no changes in ghrelin concentration. Greater OSE thus increases insulin responsiveness. In contrast, PP responses are stronger when OSE is reduced and ER is fast. Prandial Insulin and PP responses may mediate the independent effects of OSE and ER on food intake.

To determine whether typical cephalic phase responses were specific to certain food cues or sub-populations we quantified outcomes of existing literature on cephalic insulin and pancreatic polypeptide responses in a systematic review (**chapter 5**). In addition, we aimed to quantify the hypotheses made by previous qualitative cephalic phase reviews that cephalic responses allow for larger meal sizes, induce satiation earlier on in the meal and improve postprandial glucose homeostasis. A cephalic phase insulin and pancreatic polypeptide increase was observed in about half of all included treatments. About one fifth of the treatments induced a significant increase from baseline. The size of the cephalic insulin increase relative to spontaneous fluctuations was small and there was substantial variation in magnitude and onset time of cephalic insulin and pancreatic polypeptide responses between food cues and individuals. Based on this we concluded that cephalic phase insulin responses are small compared to spontaneous fluctuations. Although cephalic pancreatic polypeptide responses are of a larger magnitude, both show substantial variation in magnitude and onset time. Based on the current evidence, we refute the hypotheses that CPRs improve satiation and glucose homeostasis in daily life

Finally, to determine how OSE or taste signals are processed in the brain to affect satiation we performed an fMRI study in which we measured neural reactivity to chocolate milk in the brain(stem) over the course of satiation (**chapter 6**). Additionally we measured gastric volume

such that we could identify regions that respond solely to taste, independent of gastric distention. We found that taste activation in the parabrachial nuclei (PBN) in the brain stem, and bilateral (anterior) insula, amygdala and putamen gradually decreased as satiation increased, in line with the decrease in affective value of the chocolate milk stimuli. When subjects were hungry and completely satiated this effect could completely be explained by gastric filling, whereas this was not the case when subjects felt half full. Responses of these brain regions are thus modulated by gastric volume and sensory satiation seems especially important early in the meal when not completely satiated.

To conclude, we confirmed that increased OSE can decrease food intake. Insulin, PP and ghrelin cephalic phase responses do not mediate the oro-sensory exposure effect on satiation. Instead, we hypothesize that the underlying mechanisms are 1) a direct effect of oro-sensory exposure on satiation through sensory satiation and 2) an indirect effect of increased oro-sensory exposure duration which also slows down ingestion rate. This allows more time for stomach distention signals, hormone secretion, and early uptake of nutrients which are processed by the brain and induce satiation.



Dankwoord

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Overview of completed training activities

Dankwoord

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About the author



Marlou Pascalle Lasschuijt was born on November 18th, 1990 in Den Helder, The Netherlands. After completing secondary school at Bonhoeffer college in Castricum, she completed her Bachelor in Nutrition and Dietetics at the University of Applied sciences in Amsterdam. As part of her Bachelor program she did the Erasmus program in Leuven, Belgium. As part of this program she gave dietetic advice to refugees and taught nutrition classes to children with autism.

After her bachelor she enrolled in the MSc specialisation Nutrition and Health. She wrote her MSc thesis on taste and smell dysfunctions after intensive care stay and an extra curriculum thesis on cultural differences in smell perception.

After her thesis she interned at Penn State University in the US where she researched the effect of portion size on children's eating behaviour and brain activity. After receiving her master's degree, she was a research assistant in the Sensory Science and Eating behaviour group at Wageningen University and Research.

In 2015 Marlou was appointed as a PhD candidate at the chair of Sensory Science and Eating Behaviour, part of the Division of human nutrition & Health at Wageningen university and Research. Her research focussed on the role of sensory signals in the physiological processes underlying satiation. During her PhD project Marlou attended several (international) conferences and courses. Next to this, she was involved in teaching and supervising Bachelor's and Master's students. Furthermore, she was a member and interim chair of the organizing committee of the PhD study tour to the UK in 2017. In 2018, she was selected for the Essentials programme of the European Nutrition Leadership Platform (ENLP).

List of publications

Publications in peer reviewed journals

Lasschuijt MP, Mars M, de Graaf C, Smeets PAM. Exacting Responses: Lack of Endocrine Cephalic Phase Responses Upon Oro-Sensory Exposure. Front Endocrinol (Lausanne) 2018;9:332. doi: 10.3389/fendo.2018.00332. (this thesis)

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English L, **Lasschuijt MP**, Keller KL. Mechanisms of the portion size effect. What is known and where do we go from here? Appetite 2015;88:39-49. doi: 10.1016/j.appet.2014.11.004.

Submitted Papers

Lasschuijt MP, Mars M, de Graaf C, Smeets PAM. How oro-sensory exposure and eating rate affect satiation and associated endocrine responses- a randomized trial. (this thesis)

Lasschuijt MP, Mars M, de Graaf C, Smeets PAM. Endocrine cephalic phase responses to food cues – A systematic review. (this thesis)

Papers in preparation for submission

Lasschuijt MP, Mars M, Duif I, Camps G, de Graaf C, Smeets PAM. A full brain: Brain(stem) responses to chocolate milk over the course of satiation. (this thesis)

Abstracts and presentations

Lasschuijt MP, Mars M, Stieger M, Miquel-Kergoat S, de Graaf C, Smeets PAM. Effects of orosensory exposure manipulations on satiation. 4th International Conference on Food Oral Processing, Lausanne, Switzerland, 2016. (oral presentation)

Lasschuijt MP, Mars M, Stieger M, Miquel-Kergoat S, de Graaf C, Smeets PAM. Effects of orosensory exposure manipulations on satiation. Society for the Study of Ingestive Behavior, Porto, Portugal, 2016. (Gerard P. Smith Award for best oral presentation)

Lasschuijt MP, Mars M, Miquel-Kergoat S, de Graaf C, Smeets PAM. Effects of oro-sensory exposure on cephalic phase responses and satiation. The British Feeding and Drinking Group Annual Meeting, Reading, UK, 2017. (poster presentation)

Lasschuijt MP, Mars M, Miquel-Kergoat S, de Graaf C, Smeets PAM. Effects of oro-sensory exposure on cephalic phase responses and satiation. Eetprikkel, Utrecht, NL, 2018. (nominated best poster presentation)

Lasschuijt MP, Mars M, de Graaf C, Smeets PAM. Oro-sensory exposure or eating rate? Effects on satiation and associated endocrine responses. Society for the Study of Ingestive Behavior, Bonita Springs, USA, 2018. (New Investigator Travel Award, oral presentation).

Lasschuijt MP, Mars M, de Graaf C, Smeets PAM. Endocrine cephalic phase responses to food cues – weighing the evidence. Society for the Study of Ingestive Behavior, Utrecht, NL, 2019. (poster presentation)

Lasschuijt MP, Camps G, Koopman Y, Smeets PAM. Unaware of the amount consumed: systematic error in food- and drink intake estimation. Society for the Study of Ingestive Behavior, Utrecht, NL, 2019. (poster presentation)

Lasschuijt MP, Mars M, de Graaf C, Smeets PAM. A full brain: Brain(stem) responses to tasting chocolate milk over the course of satiation. Society for the Study of Ingestive Behavior, Utrecht, NL, 2019. (poster presentation)

Overview of completed training activities

Discipline specific courses and activities		
Nutritional Science days (NAV)	Deurne, NL	2015
Food Oral processing conference	Laussane, Swiss	2016
Society for the Study of Ingestive Behavior	Porto, Portugal	2016
The British Feeding and Drinking Group Annual Meeting	Reading, UK	2017
Society for the Study of Ingestive Behavior	Bonita Springs, US	2018
Basic and advanced (resting state) fMRI course	Utrecht, NL	2018
Eetprikkel congres (voeding Nederland)	Utrecht, NL	2018
Sensory perception and food preferences (VLAG)	Wageningen, NL	2018
Society for the Study of Ingestive Behavior	Utrecht, NL	2019
General courses and activities		
PhD introduction week (VLAG)	Baarlo, NL	2015
Pitch Perfect (NWO)	Utrecht, NL	2016
Scientific writing (VLAG)	Wageningen, NL	2016
Ethics (Human Nutrition and Health department)	Wageningen, NL	2016
Speed reading (VLAG)	Wageningen, NL	2017
Wetenschapper voor de klas (wetenschapsknooppunt)	Wageningen, NL	2017-2018
Career perspectives (VLAG)	Wageningen, NL	2019
European nutrition leadership program (ENLP)	Luxembourg, LU	2019
Optional courses and activities		
Preparation of research proposal	Wageningen, NL	2015
Instrumental sensory science (MSc course)	Wageningen, NL	2016
Organizing and participating in PhD study tour to the UK	UK	2017
Staff seminars and chair group meetings	Wageningen, NL	2015-2019

Colophon

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