Food Texture and Food Intake

The role of oral sensory exposure

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ABSTRACT

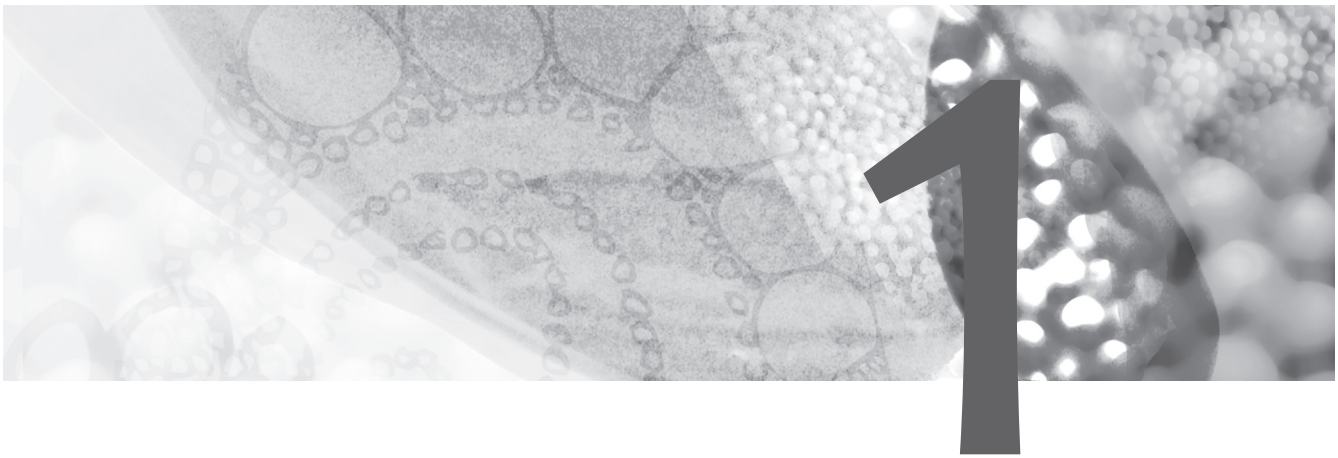
Background and objective: In view of the growing epidemic of obesity, it is important to investigate factors which influence food intake. Food texture has been shown to play a role in food intake regulation but underlying explaining mechanisms are unknown. The aim of this thesis was to determine the effect of food texture on satiation (assessed as *ad libitum* food intake) and to investigate the mediating role of oral sensory exposure and gastro-intestinal physiology in this effect.

Methods: We started by investigating the effect of food viscosity on *ad libitum* food intake (*n*=108). Next, we investigated whether the effect of viscosity could be related to eating effort and/or eating rate (*n*=49) or to the release of the gastro-intestinal hormones ghrelin, CCK and GLP-1 (*n*=33). To further explore the role of oral sensory exposure, we investigated the effect of changing bite size and oral processing time of a semi-solid food on satiation (*n*=22), and the effect of changing food texture of three pairs of solid foods on satiation (*n*=106). Finally, we observed eating behavior and retro-nasal aroma release in normal weight and overweight subjects (*n*=54).

Results: Viscosity of food had a clear effect on food intake; increasing viscosity significantly decreased *ad libitum* food intake and the difference in intake between the liquid and semi-solid product ranged from 29% to 34% in different settings. Eating rate played an important role; the liquid product was consumed significantly faster than the semi-solid product and a standardized eating rate led to similar intakes of these products. The selected gastro-intestinal hormones could not explain the effect of viscosity on food intake; a fixed amount of the liquid and semi-solid test product resulted in a similar response of the hormones. Food intake of a semi-solid food was significantly less when oral processing time was fixed to 9 s compared to 3 s (on average a difference of 42 g) and when consumed with fixed small bite sizes (≈ 5 g) compared to large bite sizes (≈ 15 g) (on average a difference of 106 g). Differences in food texture of solid foods, aimed to change oral processing time, did not affect food intake. Texture differences were probably too subtle to lead to differences in eating rate and subsequently to differences in food intake. Significant positive associations between food intake and eating behaviors such as eating rate and bite size were observed. Small to no differences in eating behavior and retro-nasal aroma release were found between normal weight and overweight subjects.

Conclusion: The results of this thesis show that food viscosity has a direct effect on food intake. Oral sensory exposure plays a major role in this since changing eating rate, oral processing time and bite size affected food intake. These factors contribute to the effect of food texture on food intake. In addition, eating rate and bite size were characteristics of the eating style of an individual.

CONTENTS



1

Introduction

INTRODUCTION

The evolution of the human diet over the past 10,000 years has resulted in large changes in food patterns [1]. The most profound changes, from a Paleolithic diet to our current food pattern, took place with the onset of the Industrial Revolution which introduced convenience and prepackaged foods [1]. Today we consume many processed foods which are high in energy density, low in fiber and have a high fat and added sugar content [2, 3]. This stands in strong contrast with most foods in the Paleolithic diet, which predominantly consisted of minimally processed plant-based foods and foods from animal origin [4-6].

Aside to changing food patterns, our beverage consumption has also changed dramatically. Until 10,000 years ago, water and breast milk were the only types of beverages assumed to have been consumed [7, 8]. From that point on, humans also began to consume other beverages [7, 8]. In the past decades, the consumption of energy-containing beverages and mainly sugar-sweetened beverages has increased considerably [7, 9-11]. The latter includes, among others, soda sweetened with sugar, corn syrup, or other caloric sweeteners, and other carbonated and uncarbonated drinks, such as sports and energy drinks. The proportion of energy obtained from calorically sweetened soft drinks and fruit drinks has increased 3-fold in the United States; from 50 kcal/day to 144 kcal/day between 1977 and 2001 [11]. The intake of soft drinks per capita increased with 60% in the Netherlands between 1980 and 2007; from 59 liter to 96 liter [12]. The latter consisted of 66 liter per capita of regular energy containing soft drinks and 30 liter per capita of light soft drinks [12]. Some studies indicate that liquid foods and especially energy containing beverages have a weaker satiety value compared to solid foods [13-22]. Additionally, studies indicate that a higher intake of sugar-sweetened beverages is associated with weight gain and increased risk of obesity [23-25]. Weight gain is a consequence of a long-term positive energy balance in which energy intake, i.e. food intake, exceeds energy expenditure [26].

The shift of our food pattern to processed foods and the increasing consumption of energy containing beverages has led to a food pattern with 'easy-to-consume foods', which can be eaten fast with little effort and a minimal amount of chewing. Nowadays, these are possible relevant factors in food intake regulation. The weaker satiety responses to liquid foods as compared to solid foods indicates that food texture is potentially an important factor affecting food intake. Food texture can be defined as "the sensory and functional manifestation of the structural, mechanical and surface properties of foods, detected through the senses of vision, hearing, touch and kinesthetics" [27]. It is unclear how the texture of food affects satiety responses and if food texture can have a direct effect on food intake. In view of the growing epidemic of obesity [26], it is important to further investigate factors influencing food intake.

The research described in this thesis investigated the role of food texture in food intake regulation and possible underlying factors involved in the effect. To understand how food

texture can affect food intake regulation, it is important to have a general understanding of the latter. Therefore, this introduction will start with a brief overview of food intake regulation. Important aspects that will be introduced are the difference between satiation and satiety and the role of gastro-intestinal factors. This will be followed by a section on the definition of food texture, as well as its important elements relevant for the studies in this thesis. Since we do not know which factors are responsible for the effect of food texture on food intake regulation, a large part of the studies described in this thesis focused on possible underlying responsible factors. Both the role of oral sensory exposure and gastro-intestinal hormones as possible underlying factors will be addressed. Finally, the rationale and thesis outline will be presented.

Food intake regulation

Food intake is an episodic activity that is expressed as a pattern of eating moments, i.e. meals. The next section will introduce the distinction between satiation and satiety, followed by a section on gastro-intestinal regulation.

Satiation and satiety

The biological drive to eat can be linked with the satiating power of food. Food brings about this satiating effect by mediating processes classified as sensory, cognitive, post-ingestive and post-absorptive. These processes have been referred to as the "satiety cascade" (**Figure 1.1**) [28-30]. Important is the distinction between satiation and satiety. Satiation refers to the processes which bring a period of eating to an end, and thus refers to within meal

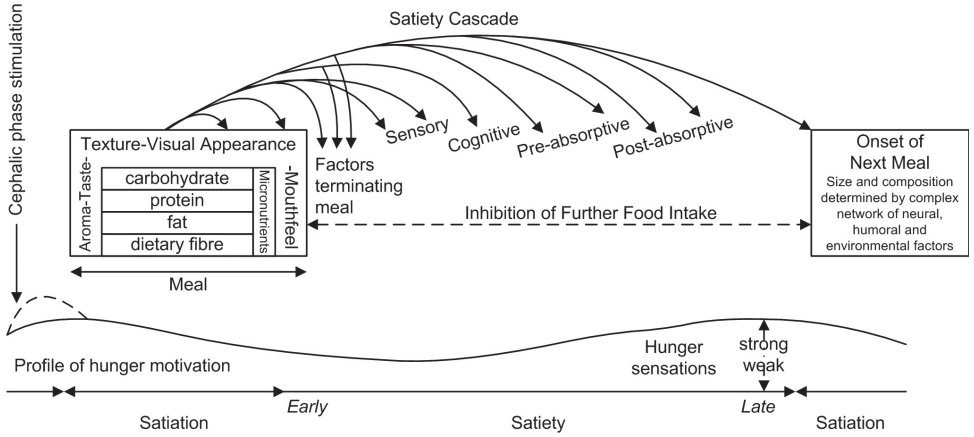


Figure 1.1 The satiety cascade. Illustration of the contributions of sensory, cognitive, post-ingestive and post-absorptive processes to the time course of satiation and satiety (from [47]).

processes and meal termination [28-30]. Satiety refers to the inhibition of hunger and further eating, which arises as a consequence of food ingestion [28-30]. Satiety thus refers to between meal processes and meal initiation (**Figure 1.1**).

Satiation is influenced by several aspects of food, such as energy density, portion size, and sensory aspects such as taste, texture and palatability [31]. Recent studies have shown that when either the energy density or the portion size of a specific food increases, energy intake also increases [e.g. 32-35]. Even changing the volume of food by incorporating air has been shown to affect food intake [36]. Palatability is one of the main drivers of food choice and several studies have shown that food intake increases as palatability increases [37-39]. With regard to macronutrient composition, a hierarchy has been observed for the satiating effects of protein, carbohydrate and fat, with protein as most satiating and fat the least [e.g. 40-44].

Appetite, i.e. the drive to eat, can be measured by psychological, behavioral and biological markers and together these contribute to the measurement of satiation and satiety [45, 46]. Psychological markers are subjective sensations such as hunger, fullness, desire to eat and prospective consumption, which can be translated into quantitative data by the use of rating scales. Behavioral markers include actual food intake. Biological markers include, among others, blood levels of gastro-intestinal hormones [45, 46].

Gastro-intestinal regulation

The response of the gastro-intestinal tract to the consumption of food is divided into cephalic, gastric and intestinal phases [48, 49]. The cephalic phase response of the body is elicited by exposure to sensory properties of food, e.g. sight, smell, taste, texture, as well as by the thought of eating, resulting in a cascade of physiologic processes at multiple sites in the body, e.g. salivary glands, gastrointestinal tract, endocrine parts of the pancreas [49, 50]. Studies investigating the cephalic phase response have shown that among others the release of the hormone insulin is stimulated and ghrelin suppressed after modified sham feeding [49-54]. Modified sham feeding is a well established technique in which nutrients are smelled, chewed, and tasted, but not swallowed.

In the gastro-intestinal tract, the ingested food is digested and absorbed. Ingested food evokes satiation and satiety via processes such as gastric distension and release of peptides from endocrine cells [55, 56]. Studies have demonstrated that there is a direct inverse relation between gastric distension and appetite [46, 56]. Important peptide hormones being released after food ingestion are among others ghrelin, cholecystokinin (CCK) and glucagon-like peptide 1 (GLP-1) [46, 55, 56]. Ghrelin is a hormone mainly synthesized in the stomach and concentrations have been shown to increase before meals and decrease with eating [46, 57]. Ghrelin is presumably involved in meal initiation and is considered the only orexigenic hormone [46, 57]. CCK is released in the blood when nutrients enter the duodenum. It stimulates gallbladder contraction, gastric and pancreatic acid secretion, and has a direct

effect on gastric emptying [46, 55, 56, 58]. GLP-1 is released when nutrients enter the ileum and colon and is involved in the ileal-break mechanism, i.e. the feed-back mechanism that slows stomach emptying and gut motility after food ingestion [46, 55]. CCK and GLP-1 are so called anorexigenic hormones since they reduce appetite. The above mentioned peptides are part of pre-absorptive signaling. Blood levels of metabolites such as glucose, free-fatty acids and amino-acids are considered post-absorptive signals [45]. Signals from the gastro-intestinal tract, together with other signals, for instance leptin levels reflecting body adiposity and energy balance, are integrated by peripheral nerves and brain centers [59].

Food texture

Food texture is a multi-parameter attribute and is described by a large number of words, of which hardness, cohesiveness and thickness are examples [27, 60]. A general definition of food texture states that "texture is the sensory and functional manifestation of the structural, mechanical and surface properties of foods detected through the senses of vision, hearing, touch and kinesthetics" [27]. Texture is an essential part of the sensory properties of a food. Certain parameters of texture can be detected and quantified by texture testing instruments [27, 60].

Characteristics of texture which are relevant in the context of this thesis are viscosity, hardness and chewiness. Thickness is generally thought to be related to the viscosity of products [61]. A physical definition of *viscosity* is "the rate of flow per unit force" and a sensory definition is "force required to draw a liquid from a spoon over the tongue" [27]. A physical definition of *hardness* is "force necessary to attain a given deformation" and a sensory definition "force required to compress a substance between molar teeth or between tongue and palate". A physical definition of *chewiness* is "energy required to masticate a solid food to a state ready for swallowing: a product of hardness, cohesiveness and springiness". The sensory definition is "length of time required to masticate the sample, at a constant rate of force application, to reduce it to a consistency suitable for swallowing" [27].

The role of food texture in food intake regulation

A number of studies show that energy-yielding liquid foods elicit weaker suppressive appetitive responses compared to solid foods. Postprandial hunger scores are significantly lower and/or satiety scores significantly higher after ingestion of more viscous/solid foods compared to liquid foods [13-18]. Furthermore, compared to solid foods, liquid foods seem to elicit a weak compensatory response in balancing energy intake of a test meal or throughout the day [17, 19-21]. This means that calories from liquid foods are not well compensated for by reducing other food intake, and add to total daily calorie intake.

This effect is not only apparent in short term preload studies, but is also observed in longer term intervention studies. In a 4-week cross over study [22], subjects daily consumed a liquid or a solid carbohydrate food load for 4 weeks. Free energy intake during the period that the solid was consumed was significantly lower than prior to this period. No decrease in energy intake occurred during the period that the liquid load was consumed. Total 24 h energy intake even increased by an amount equal to the energy content of the liquid food load. Also other long term studies have shown that energy ingested from energy yielding liquids add to the total energy intake during the day [62-64]. These data suggest that a substantial consumption of liquid calories (especially from energy containing beverages) may lead to a positive energy balance and subsequent weight gain. This is confirmed by studies indicating that a higher intake of sugared beverages is associated with weight gain and increased risk of obesity [23-25]. Since liquid foods elicit lower satiety responses, it has been suggested that it is probably not the sugar as such which leads to the possible overconsumption, but the fact that it is contained in the form of a beverage [65].

Some studies found no difference in satiety response between liquids and solids or found liquids to be more satiating [66-70]. A number of these studies included soup as test product [67, 68, 70], and soup seems to be an exception.

Most studies investigating the role of food texture were so called preload-test meal studies. In these types of studies, subjects consume a fixed amount of the test products under study, usually equal in energy content, followed by an *ad libitum* meal of other food products. Appetite ratings after the fixed load and intake at the next meal serve as main research parameters [31, 71]. To our knowledge, no study investigated the effect of food texture directly on food intake yet. Whereas preload-test meal studies may be more effective in cases where food intake is expected to be mainly influenced by post-ingestive signals, *ad libitum* intake studies may be more effective when intake is expected to be mainly influenced by stimulation of the oral cavity [68], which is largely the case with sensory properties of food. Furthermore, in some studies, data is derived from foods differing in many characteristics other than texture. For instance the study of Almiron-Roig et al. [66] compared cola with cookies and the study of Haber et al. [16] compared fruit to fruit juice. These other characteristics such as energy density, palatability or macronutrient composition may have affected the interpretation of liquid-solid satiety differences. Additionally, underlying mechanisms involved in the weaker satiety responses to liquid foods are unknown.

Possible factors involved in the effect of food texture

Oral sensory exposure

A possible factor involved in the weaker satiety responses of liquid foods versus solid foods might be oral sensory exposure. Solid foods need to be orally processed before swallowing and remain in the mouth for a longer period of time. Solid foods are therefore eaten much slower compared to liquid foods which require little to no oral processing and can be swallowed almost immediately. A longer oral processing time (also referred to as oral transit time), which we see as the time food enters the mouth until swallowing, may be related to a longer sensory exposure time in the mouth.

Oral sensory stimulation is an important requirement for appetite suppression, as has been clearly shown by a number of studies comparing normal eating of nutrients to infusion of nutrients directly in the stomach, thereby bypassing oral stimulation [72-74]. Additional indications that oral exposure is important, can be obtained from the fact that it is an important part of the cephalic phase response [49, 50].

The study of Lavin et al. [75] indicates that oral processing time might play an important role in appetite regulation. When subjects had to chew and consume several preloads within a different amount of time, intake from a test lunch was significantly reduced after consuming sucrose-containing-pastilles within 10 min compared to the consumption of plain water and a sucrose containing drink within 2 min. The pastilles reduced energy intake even more than compensating for the energy content of the preload, while the sucrose containing drink produced a small increase in energy intake. This is in agreement with the concept that the compensatory response in energy is weaker after liquids than after solids.

Next to food texture, behavioral eating parameters such as eating fast, eating with large bite sizes or eating with a minimal amount of chewing can also result in a faster oral processing time. A number of studies have shown that a lower eating rate resulted in less food intake [e.g. 76-78] and also decreasing bite size seems to reduce food intake [79-81], although some studies found no effect [82, 83]. Cross-sectional studies showed positive relations between BMI and self-reported eating rate [e.g. 84-86], eating quickly was associated with overweight.

Currently there is no direct objective measure for oral sensory exposure available, but a novel interesting measure is the extent of *in vivo* retro-nasal aroma release [87, 88]. Retro-nasal aroma release is an important aspect of the sensory perception of a food. The transfer of aroma via the retro-nasal route is also related to the texture and the volume (i.e. bite size) of food in the mouth [89]. Additionally, different eating styles, related to chewing force, chewing rate and number of chews, have been shown to result in differences in aroma release [90]. Possibly, the extent of retro-nasal aroma release could function as a marker for (part of) oral sensory exposure.

Gastro-intestinal hormones

Ingested food evokes satiation and satiety via gastric distension and release of peptides from endocrine cells [55, 56]. It is possible that the effect of texture on food intake regulation operates via the release of certain gastro-intestinal hormones. At the starting point of this thesis, few studies had investigated the role of food texture on gastro-intestinal hormone release.

RATIONALE AND THESIS OUTLINE

Food texture has been suggested to be an important factor affecting regulation of food intake. When starting this thesis, to the best of our knowledge, no study had investigated the effect of food texture directly on *ad libitum* food intake yet. Furthermore, in some studies, data was derived from foods differing in many characteristics other than texture which may have affected the interpretation of liquid-solid differences. Additionally, underlying explaining factors how food texture may affect satiation and satiety responses were unknown. Therefore, the aim of this thesis is:

"*to determine the effect of food texture on satiation and to investigate the mediating role of oral sensory exposure and gastro-intestinal physiology in this effect*".

Figure 1.2 displays our conceptual model how food texture may affect food intake. It is possible that the effect of food texture works via sensory aspects and in that case texture affects satiation. Satiation was assessed as *ad libitum* intake in this thesis. Besides sensory aspects, also physiological aspects could play a role in a possible explaining mechanism and thereby affect satiation or satiety.

We started by first focusing on the effect of viscosity on food intake (study 1, Chapter 2), using specially developed products differing in viscosity but similar in palatability, macronutrient composition and energy density. To further investigate underlying factors responsible for the effect of viscosity on food intake, we investigated the role of eating effort and/or eating rate (study 2, Chapter 2), and the role of gastro-intestinal hormone release of ghrelin, CKK and GLP-1 (Chapter 3).

Thereafter, we further explored our work on the role of oral sensory exposure by determining the effect of changing bite size and oral processing time of a semi-solid food on food intake (Chapter 4), and the effect of oral processing differences within solid foods on food intake (Chapter 5). So far, studies investigating the effect of food texture mainly focused on (semi)liquid products or compared liquid to solid products.

In the last study described in this thesis (Chapter 6) we investigated eating behavior and retro-nasal aroma-release in normal weight and overweight subjects. In the final chapter (Chapter 7), we summarize the presented results of the studies, discuss our findings, and implications and directions for further research are given.

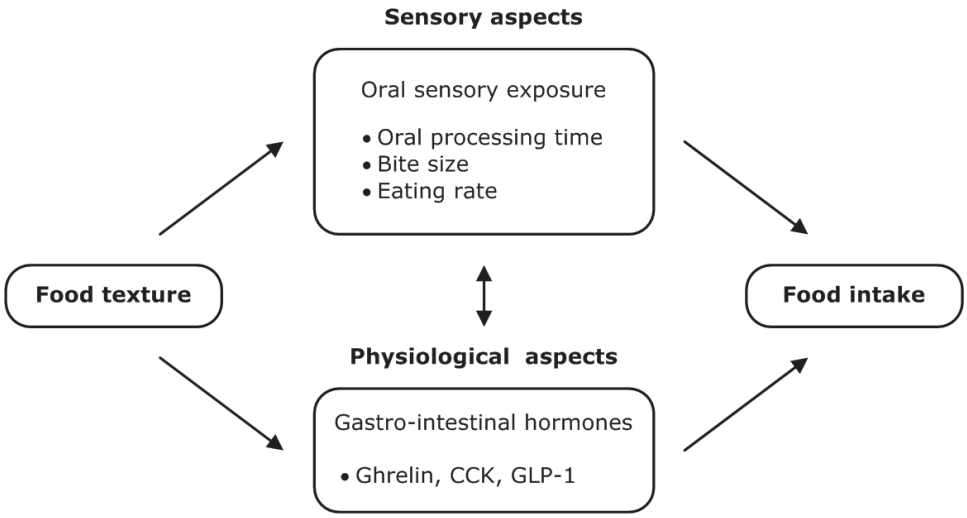


Figure 1.2 Conceptual model regarding the relation between food texture and food intake, as has been studied in this thesis. It is hypothesized that food texture can affect food intake via sensory and/or physiological aspects. The role of oral sensory exposure was explored by investigating the effects of oral processing time, bite size and eating rate on ad libitum food intake. The role of gastro-intestinal hormones was explored by investigating the concentration of ghrelin, CCK and GLP-1 in blood plasma.

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2

The effect of viscosity on *ad libitum* food intake

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ABSTRACT

Background: Energy-yielding liquids elicit weak suppressive appetite responses and weak compensatory responses, suggesting that liquid calories might lead to a positive energy balance. However, data is often derived from foods differing in many characteristics other than viscosity.

Objective: To investigate the effect of viscosity on *ad libitum* food intake in real-life setting and to investigate whether a difference in *ad libitum* intake is related to eating rate and/or eating effort.

Design: In real-life setting 108 nonrestrained subjects (26 ± 7 years, BMI 22.7 ± 2.4 kg/m^2) received a chocolate flavored liquid, semi-liquid and semi-solid milk-based product, similar in palatability, macronutrient composition and energy density. In laboratory setting 49 nonrestrained subjects (24 ± 6 years, BMI 22.2 ± 2.3 kg/m^2) received the liquid or semi-solid product. Effort and eating rate were controlled by means of a peristaltic pump.

Results: In real-life setting the intake of the liquid (809 ± 396 g) was respectively 14% and 30% higher compared to the semi-liquid (699 ± 391 g) and semi-solid product (566 ± 311 g; $P<0.0001$). In laboratory setting, removing eating effort resulted in a 29% ($P<0.0001$) intake difference between liquid (319 ± 176 g) and semi-solid (226 ± 122 g). Standardizing eating rate resulted in 12% difference between liquid (200 ± 106 g) and semi-solid (176 ± 88 g; $P=0.24$). If not controlled, the difference in intake between liquid (419 ± 216 g) and semi-solid (277 ± 130 g) was comparable to the real-life setting (34%; $P<0.0001$).

Conclusions: Products different in viscosity but similar in palatability, macronutrient composition and energy density lead to significant differences in intake. This difference is partially explained by the higher eating rate of liquids.

INTRODUCTION

Several studies have shown that energy-yielding liquids seem to elicit weaker suppressive appetitive responses compared to solids. Postprandial hunger scores are significantly lower and/or satiety scores significantly higher after ingestion of more viscous/solid foods compared to liquid foods [1-4]. Furthermore, compared to solids, liquids seem to elicit a weak compensatory response in balancing energy intake throughout the day [5, 6]. This effect is not only apparent in short-term preload studies but is also observed in longer term intervention studies. In a 4-week crossover study [7] subjects consumed daily a liquid or a solid carbohydrate load for four weeks. Free energy intake during the period that the solid was consumed was significantly lower than prior to this period. No decrease in energy intake occurred during the period that the liquid load was consumed. Total 24 h energy intake even increased by an amount equal to the energy content of the liquid food load. Also other long-term studies have shown that energy ingested from energy-yielding liquids add to the total energy intake during the day [8-12]. These data suggest that liquid calories may lead to a positive energy balance and subsequent weight gain.

The underlying mechanism responsible for the difference in satiety responses between liquids and solids is still unclear. A possible mechanism that has been suggested in earlier studies is that the act of chewing the more solid foods may give a satiety signal which is not induced by swallowing a liquid [4, 5]. This may be related to the notion that the faster transit of fluids compared to solids leads to smaller sensory exposure time in the mouth.

The objective of our study is first to investigate whether products differing in viscosity lead to differences in *ad libitum* intake and furthermore to investigate the hypothesis that the difference in *ad libitum* intake between products with different viscosities might be due to differences in oral exposure time. This implies that this effect operates through sensory mechanisms. A thicker product is probably eaten more slowly than a more liquid product. We assume that eating slower results in a longer stay of the product in the oral cavity and therefore in a longer oral exposure time.

We hypothesize that the effect of viscosity on food intake runs via sensory mechanisms. As explained in the satiety cascade of Blundell [13], sensory processes have an early onset during the course of eating and primarily affect satiation. Satiation is the process that eventually brings the period of eating to an end and thus is responsible for meal termination. The importance of sensory aspects in meal termination has also been shown in the study of Hetherington [14]. Therefore in the design of our studies we chose to focus on satiation/meal termination, which we defined as *ad libitum* food intake.

To achieve our objective we performed two studies. The aim of study 1 was to investigate the effect of viscosity on *ad libitum* food intake in a real-life setting. In this study it was found that subjects consumed more from more liquid products. Two possible explanations

were differences in eating rate and differences in the effort to get the product in the mouth. Therefore the aim of study 2 was to investigate whether the observed difference in *ad libitum* intake could be related to eating rate and/or eating effort. Since interpretation of studies investigating viscosity may be hampered by the use of foods that differ along other dimensions than viscosity, we used foods with different viscosities but with the same energy content, energy density, nutrient composition and similar palatability.

SUBJECTS AND METHODS

Subjects

Subjects were recruited in the surroundings of Ede and Wageningen (the Netherlands) via flyers, advertisement in local papers and email. Subjects had to be healthy, aged 18–50 years, normal weight (BMI 18.5–30.0 kg/m^2) and had to like chocolate flavored dairy products. Exclusion criteria were restrained eating (Dutch Eating Behaviour Questionnaire (DEBQ) men: score>2.37, women: score>3.24 [15]), lack of appetite for any (unknown) reason, following an energy restricted diet during the last 2 months, change in body weight of >5 kg during the last 2 months, stomach of bowel diseases, diabetes, thyroid disease or any other endocrine disorder, or hypersensitivity for milk components.

Subjects were not aware that the primary outcome of the studies was *ad libitum* food intake, as this could affect the outcome of the study. Subjects of study 1 were informed that the aim of the study was to test the palatability of chocolate flavored milk products while watching a movie. Subjects of study 2 were informed that the aim of the study was to investigate the effect of certain factors, such as eating rate and effort to consume a product, on taste experience. The studies were approved by the Medical Ethical Committee of the Wageningen University and all subjects gave their informed consent. We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research.

A total of 132 subjects participated in study 1, of which 108 subjects were included in the

Table 2.1 Descriptive variables of the subjects who participated in two studies

	Study 1 (*n*=108)	Study 2 (*n*=49)
Gender (male/female)	36 (33.3 %) / 72 (66.7 %)	14 (29%) / 35 (71%)
Age (years) mean ± SD	26 ± 7	24 ± 6
BMI (kg/m^2) mean ± SD	22.7 ± 2.4	22.2 ± 2.3
Restraint score DEBQ[a]	2.03 ± 0.64	2.03 ± 0.69

DEBQ, Dutch Eating Behaviour Questionnaire. [a] On the restraint scale 1 = not at all, 5 = very high.

data analysis (**Table 2.1**). Subjects were excluded from data analysis because of incomplete data with respect to *ad libitum* intake (*n*=7) and mistakes in weighing the *ad libitum* intake (*n*=13). Furthermore, 4 subjects were accidentally included who were restrained eaters (*n*=2) and did not fulfill the criteria of a normal BMI (*n*=2). Fifty subjects participated in study 2, of which 1 subject was excluded from data analysis because of not adhering to the study protocol. Thirty–four subjects participated in both studies.

Test products

The test products were milk-based products with chocolate flavor specially developed for this study (NIZO food research, Ede, the Netherlands). The basis of all chocolate products was whole fat milk (68%), water (18%), sugar (6.5%), modified starch (3.5%), cream (2%), cacao (1.5 %) and carrageenan (0.05%). The type starch was varied in the products to obtain three identical products differing solely in physical state, a liquid (comparable to commercially available chocolate milk), a semi-liquid and a semi-solid product (comparable to commercially available chocolate custard).

The products were made in the following way: After analysis of the protein, fat and lactose content, the fresh milk was standardized at 2.2% protein and 3.7% fat, by adding cream. To the standardized milk a dry mix of sugar, starch mixture (Perfectamyl A3108 and/or Farinex VA85T and/or Farinex VA40), cacao and carrageenan was added. After mixing of the ingredients, the product was pasteurized (30 s, 85°C) and cooled down (<7°C). Pasteurization was performed to prolong shelf life, however products still had to be produced in several batches to maintain palatability and microbiological safety though-out the studies. Products are commercially available at NIZO food research. **Table 2.2** gives an overview of the mean liking scores and the sensory profile of the test products.

Figure 2.1 shows the viscosity measurements of the experimental products. Measurements were made with a AR 2000 small strain rheometer (TA Instruments, Etten-Leur, the Netherlands) at 5°C with shear rates increasing from 0 to 1000 per second in 5 min. Viscosity at a shear rate of 50 per second at 5°C was on average 85 mPa s for the liquid product, 233 mPa s for the semi-liquid product and 788 mPa s for the semi-solid product. For comparison a commercially available chocolate milk and chocolate custard were also measured at 42 per second and 57 per second; for the chocolate milk this was 60 and 56 mPa s, and for the chocolate custard 2360 and 1870 mPa s.

Test products were equal in energy content, energy density, volume and macronutrient composition. Macronutrient content was determined by chemical analysis; protein by the Kjeldahl method, fat by the Rose Gottlieb method, subsequently carbohydrate was calculated by subtracting moisture, ash, protein and fat from total weight. Energy content was calculated from the macronutrient composition by means of the Atwater factors. For both studies, products were produced in several batches. For the chemical

Table 2.2 Liking scores (mean ± SD, measured on a 10-point scale from very unpleasant to very pleasant) and scores sensory attributes of the test products (mean score ± SD, measured on a 7-point category scale from not strong at all to very strong) before *ad libitum* consumption (*n*=108)

	Liquid	Semi-liquid	Semi-solid	*P*-value[†]
Liking	7.5 ± 1.1[a]	7.3 ± 1.2[ab]	7.0 ± 1.6[b]	0.005
Chocolate taste	4.8 ± 1.1	4.8 ± 1.1	4.4 ± 1.3	ns
Off taste	2.5 ± 1.3	2.5 ± 1.2	2.5 ± 1.2	ns
Bitterness	2.4 ± 1.4	2.3 ± 1.3	2.7 ± 1.3	ns
Sweetness	4.4 ± 1.2[a]	4.3 ± 1.3[a]	3.9 ± 1.3[b]	<0.0001
Thickness	1.9 ± 1.1[a]	3.8 ± 1.6[b]	5.5 ± 1.4[c]	<0.0001
Creaminess	3.3 ± 1.5[a]	4.4 ± 1.4[b]	4.7 ± 1.4[b]	<0.0001
Heterogeneous	2.1 ± 1.7[a]	2.4 ± 1.7[a]	3.3 ± 1.9[b]	<0.0001
Roughness	2.1 ± 1.3[a]	2.5 ± 1.3[b]	3.4 ± 1.6[c]	<0.0001
Slimy	2.5 ± 1.4[a]	2.9 ± 1.4[b]	3.3 ± 1.6[c]	0.0002
Slippery	4.4 ± 1.7[a]	4.6 ± 1.5[a]	3.9 ± 1.4[b]	0.0016
Mouth filling / satiating	3.3 ± 1.5[a]	4.5 ± 1.4[b]	5.3 ± 1.4[c]	<0.0001

Abbreviation: ns, not significant. Numbers within a row having letter superscripts in common do not differ significantly ($P>0.05$). [†] Analyses of liking scores and sensory attributes were conducted using the General Linear Model (GLM) procedure with participant and product as independent variables. Least Squares Means were used for post hoc comparisons.

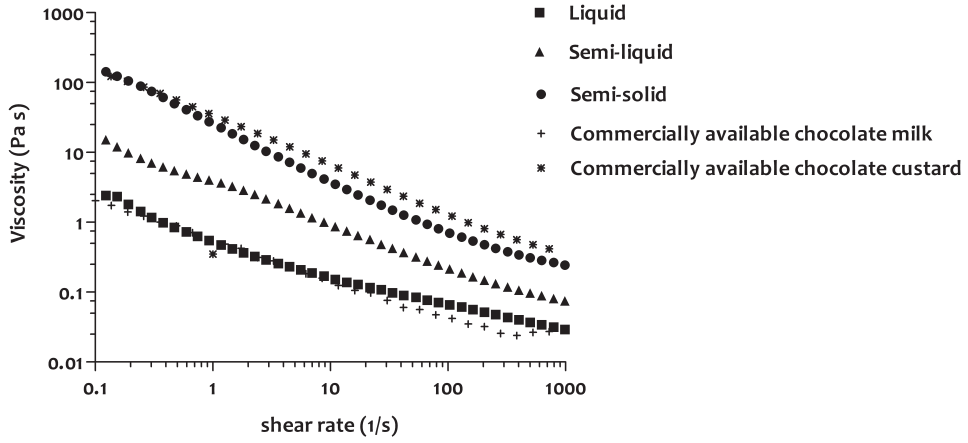

Figure 2.1 Results of the viscosity measurements in Pa s of the liquid, semi-liquid and semi-solid test product and of a commercially available chocolate milk and chocolate custard. Viscosity measurements were made on a AR 2000 small strain rheometer at 5°C with shear rates increasing from 0 to 1000 per second in 5 min.

analyses, food samples were taken from a homogenous mixture of all batches within a study. Data from the different studies were averaged and are shown in **Table 2.3**. In study 1, the liquid, semi-liquid and semi-solid products were used and in study 2, the liquid and semi-solid products.

Experimental procedure

The studies were randomized crossover experiments. The order of test products and conditions were randomized within subjects. The time between sessions was at least one day. *Ad libitum* intake was the primary outcome of both studies and was calculated from the weight of the products before and after consumption. Products were weighed on a digital scale with a precision of 0.1 g (model XP-3000, Denver Instruments, Göttingen, Germany and model 1203-MP, Sartorius, Goettingen, Germany). Subjects were not aware of the weighing. Products were served chilled (temperature varying from 5–13°C in study 1 and 3–5°C in study 2).

Study 1: *Ad libitum* intake in a real-life setting

To create a real-life setting and to distract subjects from visual and weight cues, study 1 was performed in a cinema. Each subject participated in three sessions, which were chosen out of total of six available test days. Each test session started at 1800 hours. On each test day a different movie was shown, however the type of movie was similar (romantic comedy). During the movie, subjects were instructed to eat as little or as much of the test product as they wanted. In order to keep any visual or weight cues from the subjects and to stimulate *ad libitum* intake, a surplus of the test product was served in white carton 'bag-in-boxes' (max content of 2 l, height 23 cm, width 12 cm, depth 8.5 cm) and consumed with a thick straw (length 26 cm, diameter 0.9 cm). The total duration of each movie was set at 90 min. The movie was divided into three parts of 30 min with breaks of 15 min

Table 2.3 Mean values of the chemical analysis of protein, fat and carbohydrate in g per 100 g of test product and in energy percentages, and the calculation of energy content in kilojoules per 100 g for the liquid, semi-liquid and semi-solid test product

	Liquid	Semi-liquid	Semi-solid
Protein	2.8 (12 en%[a])	3.2 (13 en%)	2.9 (12 en%)
Carbohydrate	13.9 (58 en%)	13.5 (55 en%)	14.0 (57 en%)
Fat	3.4 (30 en%)	3.7 (32 en%)	3.6 (32 en%)
Energy	408	421	420

[a] Percentage of energy derived from the specific nutrient, compared to the total energy content of the test product.

in between. **Table 2.4** gives an overview of the time schedule of a test session. During the break, subjects left the theater and handed in the box with test product. Before the restart of the film, they received a new box with test product. On average three times 1501 ± 15 g of product was offered (total session 4504 ± 34 g). At the beginning and end of the movie, satiety parameters and sensory attributes were rated.

Study 2: *Ad libitum* intake during controlled eating rate and/or effort

In study 2, subjects returned for six sessions. All test sessions were held during dinnertime. Subjects could choose out of three starting times (16.15, 17.15 or 18.15 h) which had to be the same for all six sessions. After arrival (t=0 min) subjects received a preload (see 'Standardization of satiety state before *ad libitum* intake'). After 45 min (t=45 min) subjects received the test product.

This study took place in the sensory cabins of the Department of Human Nutrition, Wageningen University. There were three experimental conditions: (1) free eating rate, different effort; (2) free eating rate, no effort and (3) fixed eating rate, no effort. During all conditions subjects were instructed 'to eat from the test product until pleasantly satiated'. It was not required to remain in the laboratory for a set period of time.

During condition 1 (control), subjects consumed from the blinded box by means of a thick straw, identical to the manner of consumption in study 1. In this condition, on average 1498 ± 23 g of product was offered per session. During condition 2, an electric peristaltic pump (Watson-Marlow, type 323Dz and 505s, Watson-Marlow pumps Bredel, USA/Canada) with a silicon tube (length 2 m, diameter 4.8 mm) (Rubber BV, Hilversum, the Netherlands)

Table 2.4 Time schedule of a test session of study 1, *ad libitum* intake in a real-life setting

Time (t) (min)	Activity	Procedure
0 (1800 hours)	Arrival	Receiving preload (pizza)
45 - 60		Receiving box with test product, rating satiety parameters and test product
60	Start movie	Eating *ad libitum* from test product
90	Break	Handing in product
105	Restart movie	Receiving new box with test product and eating *ad libitum* from the product
135	Break	Handing in product
150	Restart movie	Receiving new box with test product and eating *ad libitum* from the product
180	End movie	Rating satiety parameters and handing in product

was used to deliver the product in the subject's mouth, so that no effort was required to consume the product. Subjects still needed to orally process the product and swallow.

Subjects could control the pump themselves (minimum speed that was used: 12 g/min, maximum speed: 224 g/min). During condition 3, the peristaltic pump was set at a standardized rate to control eating rate. The pump was adjusted to pump for 10 s, hereafter to stop for 10 s and to repeat this cycle, to allow subjects to swallow and to imitate a natural drinking situation. The pump was set at a fixed rate of 100 g/min for men and 80 g/min for women. Since the pump was set to pump the product every other 10 s the total intake of the subjects was fixed to 50 g/min for men and 40 g/min for women. Flow rates were higher for men since they have a higher energy need compared to women. During pre-testing, the chosen rates were experienced as convenient. In this condition the pump was not visible to the subject.

In all conditions total time of consumption was measured. In condition 1, *ad libitum* intake was calculated by weighing the boxes before and after consumption and in condition 2 and 3, *ad libitum* intake in grams was registered every 30 s. This was performed by placing the box with test product on the digital balance and recording the number on the display every 30 s. Subjects were not aware of the weighing.

At the beginning and end of the test session subjects rated satiety parameters, and before *ad libitum* intake sensory attributes were rated.

Standardization of satiety state before *ad libitum* intake

To standardize the individual state of satiety, subjects in study 1 and 2 were instructed to eat the same breakfast and lunch during all test days and record their food and drink consumption in a diary. Furthermore, the individual state of satiety was standardized by means of a preload, which was a piece of commercially available mini pizza (1130 kJ per mini pizza, brand Chaupain, Kreko, Dordrecht, the Netherlands). The amount of pizza was based on individual energy needs, estimated by means of the Schofield I equation [16], taking into account age, weight and a physical activity level (PAL) of 1.6. About one sixth of energy of the daily estimated energy needs was provided by the preload, this is about half the amount of energy provided by the evening meal in the Netherlands [17]. For logistic reasons three energy groups were formed; group 1 (estimated energy needs ≤8.5 MJ): 1 mini pizza, group 2 (estimated energy needs between 8.5-11.9 MJ): 1.5 mini pizzas, and group 3 (estimated energy needs ≥11.9 MJ): 2 mini pizzas. In study 1, 7 subjects received 1 mini pizza, 78 received 1.5 mini pizzas and 23 received 2 mini pizzas. In study 2, 4 subjects received 1 mini pizza, 37 received 1.5 mini pizzas and 8 received 2 mini pizzas.

During the test session, subjects were not allowed to eat or drink anything other than the preload and the test products.

Satiety parameters

In both studies, before and after *ad libitum* intake, subjects rated hunger, fullness, desire to eat, appetite for something sweet, appetite for something savory, prospective consumption and thirst on 10-point category scales. Scales were anchored from not at all/ very little until very much. Changes in scores were calculated for the satiety parameters by subtracting the ratings before *ad libitum* consumption from after *ad libitum* consumption; thus negative scores indicate a decrease in rating.

Statistical analyses

Data are presented as means ± SD. Statistical analyses were performed by means of SAS (version 9.1.3; SAS Institute Inc., Cary, NC, USA). Significance was set at $P<0.05$.

In study 1, analysis on *ad libitum* intake and on the satiety parameters were conducted by one-way ANOVA (Proc GLM, SAS) with participant and product as independent variables. If type of product had a significantly effect, least squares means were used for post hoc comparisons. The liking scores before *ad libitum* intake were significantly different between products (see **Table 2.3**), therefore the analysis on the *ad libitum* intake was also performed with a correction for liking by adding the liking scores as independent variable to the model. To test whether the difference in intake between products was dependent on BMI, linear regression analysis was performed.

In study 2, the *ad libitum* intake data, the duration time of *ad libitum* eating, the rate of consumption and the satiety parameters were analyzed by two-way ANOVA (proc GLM, SAS), with participant, product and condition as independent variables including the condition x product interaction.

The analysis on *ad libitum* intake was also performed with correction for liking by adding the liking scores as independent variable. Least squares means were used for post hoc comparisons.

To test whether the difference between the liquid and the semi-solid was altered as an effect of condition, the difference between the intake of the liquid and the semi-solid was calculated for each condition (intake liquid minus intake semi-solid). These intake differences were compared among the three conditions by one-way ANOVA (proc GLM, SAS) with condition and participant as independent variable. If condition had a significant effect, least squares means were used for post hoc comparisons. To test whether the difference in intake between the liquid and the semi-solid was dependent on BMI, linear regression analysis was performed.

Furthermore, linear regression analysis was performed within the 'free eating, no effort' condition on the *ad libitum* intake data versus consumption rate.

RESULTS

Ad libitum intake test products in real-life setting

The intake of the three test products was significantly different from each other ($P<0.0001$; **Figure 2.2**). *Ad libitum* intake was 809 ± 396 g for the liquid, 699 ± 391 g for the semi-liquid and 566 ± 311 g for the semi-solid products. The difference between the liquid and the semi-solid products was 243 ± 304 g (30%; $P<0.0001$). The difference in intake between the liquid product and the semi-liquid product was 110 ± 299 g (14%; $P<0.0001$). The difference between the semi-liquid and the semi-solid products was 133 ± 233 g (19%; $P<0.0001$).

The intake of the products in the first half-hour of the test session were liquid 443 ± 267 g, semi-liquid 384 ± 234 g, semi-solid 316 ± 209 g. In the second half-hour intakes were liquid 187 ± 133 g, semi-liquid 161 ± 130 g, semi-solid 124 ± 88 g. In the last half-hour of the test session, mean intakes were 179 ± 115 g for the liquid, 155 ± 126 g for the semi-liquid and 126 ± 97 g for the semi-solid.

Linear regression analysis showed that the differences in *ad libitum* intake were not statistically significantly dependent on BMI.

The correction for liking did not influence the mean *ad libitum* intake data or the *P*-values. Mean *ad libitum* intakes after correction were 802 g for the liquid, 699 g for the semi-liquid and 576 g for the semi-solid.

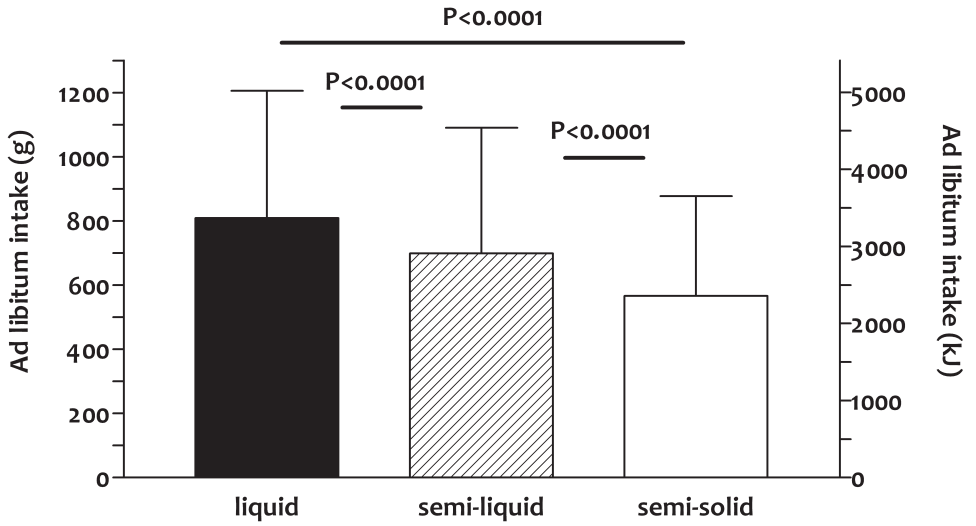

Figure 2.2 *Ad libitum* intake in grams ± SD and in energy intake (kJ) of the liquid, semi-liquid and semi-solid test products in study 1, real-life setting (n=108). For the calculation of energy intake a mean energy value of 416 kJ per 100 g was used, which is the average energy content over all test products.

Ad libitum intake test products in laboratory setting

Subjects consumed significantly more of the liquid product than of the semi-solid product (**Figure 2.3**) in the 'free eating rate, different effort' condition and in the 'free eating rate, no effort' conditions. However, the *ad libitum* intake of the liquid and the semi-solid product was not statistically significantly different in the condition where eating rate was standardized. These effects are reflected in a significant condition x product interaction (F=9.24, P=0.0001).

In the 'free eating rate, different effort condition' (condition 1), the absolute intake of the liquid and the semi-solid was the highest (419 ± 216 g and 277 ± 130 g respectively) and the difference in intake between the liquid and the semi-solid was the largest, 143 ± 200 g (34%; P<0.0001).

In the 'free eating rate, no effort condition' (condition 2) the absolute intake of the liquid and the semi-solid were smaller (319 ± 176 g and 226 ± 122 g respectively) and the difference between the products was also smaller, 93 ± 137 g (29%; P<0.0001). However, this difference of 93 g was not significantly different from the difference of 143 g in the 'free eating rate, different effort'. So, controlling for effort had no statistically significant effect on the *ad libitum* intakes.

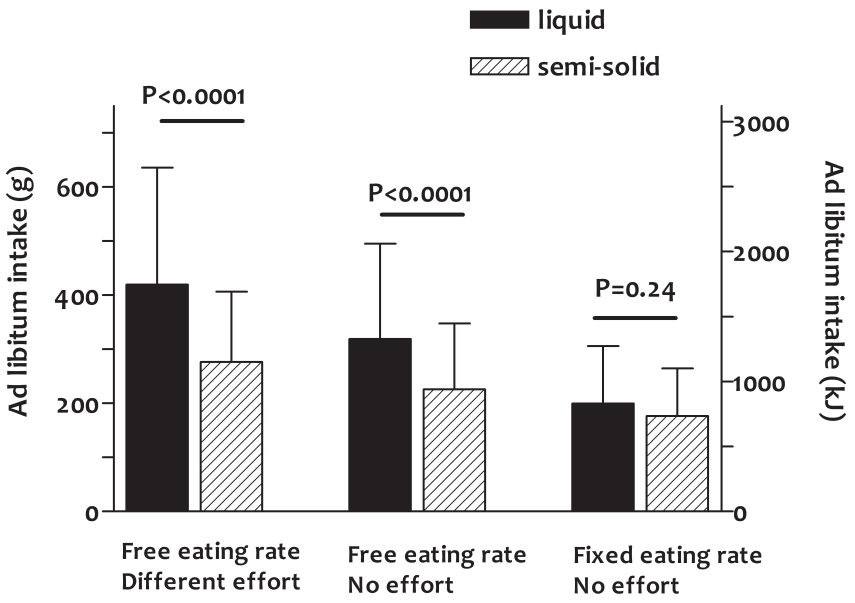

Figure 2.3 *Ad libitum* intake in grams ± SD and in energy intake (kJ) of the liquid and semi-solid test products under the different experimental conditions in study 2 (n=49). For the calculation of energy intake a mean energy value of 416 kJ per 100 g was used, which is the average energy content over all test products.

In the 'fixed eating rate, no effort' condition (condition 3) the absolute intake of the liquid and the semi-solid were the smallest and not significantly different from each other (200 ± 106 g and 176 ± 88 g respectively; *P*=0.24). The difference between the intake of the liquid and the semi-solid was also the smallest, 23 ± 78 g (12%). This difference was significantly different from the difference in intake between the liquid and the semi-solid in condition 1 and 2 (*P*<0.0001 and *P*=0.01, respectively). So controlling for effort and eating rate has a significant effect on *ad libitum* intakes.

Linear regression analysis showed that the differences in *ad libitum* intake between the liquid and the semi-solid product were not statistically significantly dependent on BMI.

After correction for liking, the means of the *ad libitum* intake were not changed and significant results remained the same. Mean *ad libitum* intakes after correction were 419 g for the liquid and 278 g for the semi-solid in the 'free eating rate, different effort' condition, 316 g for the liquid and 229 g for the semi-solid in the 'free eating rate, no effort' condition and 197 g for the liquid and 177 g for the semi-solid in the 'fixed eating rate, no effort' condition.

Eating time and eating rate in laboratory setting

In study 2, the total time the subjects took for *ad libitum* intake (without the time of the sensory rating of the product) was not significantly different between the liquid and semi-solid in the three testing conditions. The overall median time was 2.6 min for the liquid product and 2.8 min for the semi-solid product.

Figure 2.4 shows the cumulative consumption over time for test conditions 2 and 3. This figure stops at 4 min which is the 75 percentile value for both the liquid and semi-solid products. In condition 1, the 'free eating rate, different effort' condition cumulative

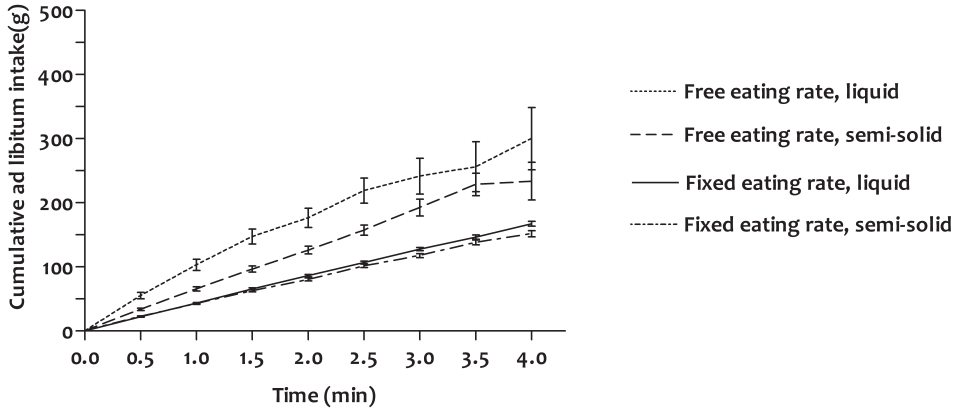

Figure 2.4 Cumulative *ad libitum* intake (grams ± SEM) over time (min) of the liquid and semi-solid product in study 2 under the experimental conditions 'free eating rate, no effort' and 'fixed eating rate, no effort'.

consumption could not be measured. In the 'fixed eating rate, no effort' condition the mean consumption rate overall was 43.7 ± 5.8 g/min for the liquid product and 41.5 ± 6.5 g/min for the semi-solid product. The mean consumption rates in the 'free eating rate, no effort' condition were significantly higher, 89.5 ± 50.1 g/min for the liquid product and 56.7 ± 20.2 g/min for the semi-solid product ($P<0.001$).

Furthermore, regression analysis within the 'free eating rate, no effort' condition showed that there is a significant positive relation between *ad libitum* intake and consumption rate; for the liquid product R-square=0.43 and $P<0.0001$ and for the semi-solid product R-square=0.44 and $P< 0.0001$.

Satiety parameters

Table 2.5 gives an overview of the satiety ratings before and after *ad libitum* intake in the real life setting (study 1). Before *ad libitum* intake there were no significant differences in ratings between products. After *ad libitum* intake, change in thirst scores was significantly different between the products ($P=0.007$). The liquid product decreased thirst more than the other products. Similar ratings were obtained in the laboratory study (study 2; data not shown).

Table 2.5 Before and after ad libitum intake scores on satiety parameters in mean ± SD, measured on a 10-point category scale from not at all/very little until very much

		Liquid	Semi-liquid	Semi-solid	*P*-value
Hunger	Before	5.3 ± 2.3	5.2 ± 2.2	5.5 ± 2.1	ns
	After	3.1 ± 1.8	2.9 ± 1.8	2.9 ± 1.8	ns
Fullness	Before	4.7 ± 2.1	4.8 ± 1.9	4.9 ± 1.9	ns
	After	7.0 ± 2.1	7.1 ± 2.0	6.9 ± 2.0	ns
Desire to eat	Before	5.8 ± 2.3	5.5 ± 2.2	6.0 ± 2.2	ns
	After	3.4 ± 2.0	3.4 ± 2.0	3.4 ± 2.0	ns
Appetite for something sweet	Before	5.6 ± 2.2	5.5 ± 2.2	5.7 ± 2.2	ns
	After	2.6 ± 1.7	2.6 ± 1.8	2.6 ± 1.8	ns
Appetite for something savory	Before	5.4 ± 2.4	5.2 ± 2.4	5.2 ± 2.4	ns
	After	4.5 ± 2.5	4.6 ± 2.5	4.5 ± 2.5	ns
Prospective consumption	Before	5.2 ± 2.0	5.2 ± 1.9	5.4 ± 2.0	ns
	After	3.2 ± 1.6	3.0 ± 1.6	3.2 ± 1.7	ns
Thirst	Before	6.3 ± 2.1	6.6 ± 1.9	6.6 ± 2.1	ns
	After	5.9 ± 2.3[a]	6.8 ± 2.1[b]	7.0 ± 2.0[b]	<0 .0001

Abbreviation: ns, not significant. Results of study 1, cinema setting (n=108). Numbers within a row having letter superscripts in common do not differ significantly ($P>0.05$).

DISCUSSION

The results of our studies show that food differing in viscosity leads to clear differences in *ad libitum* intake, both in the realistic setting as well as in the laboratory setting. As far as we know these are the first studies that demonstrated such a clear effect of viscosity on *ad libitum* intake/meal termination. This effect was not due to differences in energy content, energy density or macronutrient content as they were all identical. Thus the potential confounders in the interpretation of liquid-solid differences in satiety were controlled for. The effect was, furthermore, not due to the effort to get the product in the mouth. In the present studies the effect was explained by the faster eating rate of the liquid compared to the semi-solid product.

In our study, we found that the eating rate of the liquid product was significantly higher than the eating rate of the semi-solid product. Other studies which measured eating rate also showed that the consumption rate of liquids is much higher than that of solids, which is in agreement with our results. In the study of Haber et al. [4] subjects had to consume a fixed amount of apples, apple purée or apple juice. The apple juice was eaten more than ten times faster than the apple and nearly three times faster than the puree. Also in the study of Kissileff et al. [18] it was found that the liquefied version of the food was eaten faster (108 g/min) than the solid version (71 g/min) when both foods had to be eaten with a spoon.

The finding that the differences in intake between the liquid and the semi-solid are explained by eating rate, suggests that the transit time in the oral cavity plays an important role in this respect. With eating rate we mean the consumption volume per minute rather than the total time available for an eating episode. A liquid is eaten at a much higher rate and does not stay in the mouth for a long time, while a thick product is eaten more slowly and stays in the mouth much longer. This could be an important factor in the explanation of differences in satiety responses between liquids and solids. When a product stays in the mouth for a longer time, the exposure time to sensory receptors in the oral cavity is longer and there is more opportunity for more exposure to taste, smell, texture etc.

In our second study in the laboratory setting we found that the *ad libitum* intake in the conditions with a pump was lower compared to the intake by means of a straw. Additionally, we found that the total eating time in this study was low. This could have been a result of the fact that subjects were not instructed to remain in the laboratory for a fixed period of time. Perhaps in future studies it would be better to set a fixed time of presence in the laboratory, to make sure subjects are eating normally. Furthermore, in this second study we were able to remove the effort to get a product into the mouth by using a pump. We did not remove the effort to orally process the product. However we assume that these factors did not have a large effect on our data since the products used in our studies required no chewing. Nevertheless, further studies in this area are needed. Eating rate and oral physiology are also subjects of our future studies.

Other factors that could have influenced the *ad libitum* intake in study 2 is that drinking by means of a peristaltic pump was unnatural or that the fixed eating rate of 50 g/min (men) or 40 g/min (women) was low and the cycle of the pump to stop every 10 s was unpleasant or became boring. However, the satiety ratings were not significantly different between the different test conditions. Apparently subjects did consume the products until satiated.

The finding that there were no differences in satiety parameters after *ad libitum* intake in our studies, despite the considerable differences in intake, is interesting. Apparently subjects did not feel less hungry or fuller after the larger consumption of the liquid product. This was also seen in the study of Wansink et al. [19] in which subjects ate significantly more soup from self-refilling bowls than subjects eating from normal bowls. Although they consumed 73% more compared to the subjects eating from normal bowls, they did not feel more satiated. Some other studies investigating satiety responses to liquids and solids did find differences in satiety ratings. Mattes and Rothacker [2] performed a study with a thin and thick version of a shake with equal volume, energy content and macronutrient composition. They found that hunger ratings were significantly lower after ingestion of the more viscous shake. Also Hulshof et al. [1] found clear differences in appetite ratings between three preloads differing in physical states, with the solid preloads suppressing appetite ratings the most. In the study of Tsuchiya et al. [3] it was found that satiety ratings following a yoghurt preload were significantly higher than following a preload of fruit drink or dairy fruit drink. No difference however was found between the two yoghurts, which were a semi-solid yoghurt and the same yoghurt homogenized to liquid form. However, the yoghurts contained more protein than the beverages and it has been shown that of the macronutrients, protein is more satiating [20, 21].

All of the above mentioned studies that did find differences in satiety ratings were preload studies. In these types of studies, subjects consume a fixed amount of the several test products, usually equal in energy content. In our study, subjects could eat *ad libitum* until satiated. Apparently energy intake and feelings of satiety do not correspond with each other between liquids and solids. When the same amounts of calories are consumed, the subjective feelings of satiety are different and when the subjective feelings of satiety are the same, the amount of calories consumed is different. That hunger ratings are not accurate predictors of energy intake has already been shown in earlier studies, e.g. Mattes [22]. Perhaps the human appetite system is not well equipped to sense liquid calories. In nature, calories in liquid form do not occur, except for milk during infancy, which is a period of rapid growth. During this period there is a largely direct relationship between food viscosity and caloric content since the viscosity and the caloric density of human breast milk appear to vary together [23, 24]. Breast feeding may provide an important initial exposure to a general rule that thicker substances contain more calories than thinner substances [24, 25]. This could mean that the difference in satiety responses between liquids and solids is

based on learned behavior. Furthermore that solely the mouth feel of a product already could have an effect on the relationship between viscosity and intake.

So far, based on the studies described in this article, we conclude that food intake increases with decreasing viscosity and that the mechanisms through which viscosity affects food intake work (partly) through a higher/longer sensory exposure time and/or a longer transit time of the product in the oral cavity.

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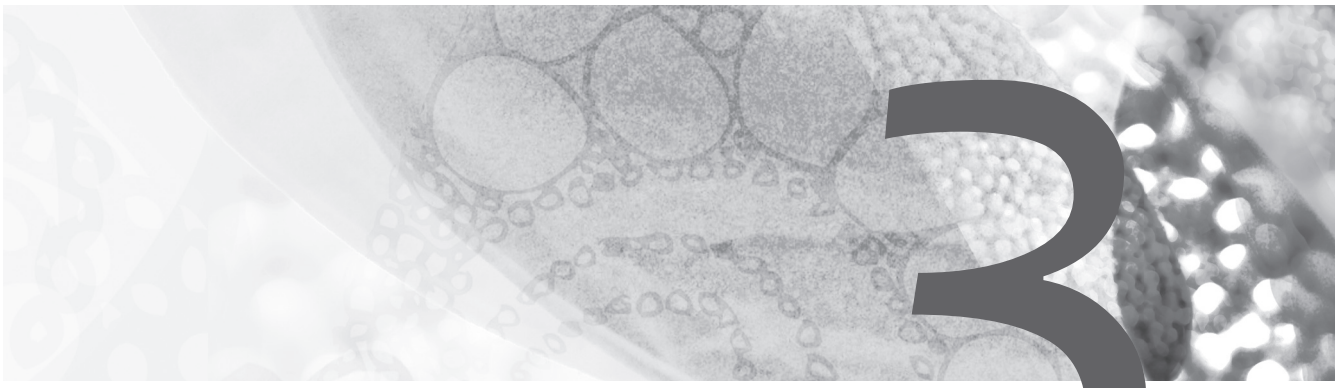

3

Effect of viscosity on appetite and gastro-intestinal hormones

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ABSTRACT

In previous studies we showed that higher viscosity resulted in lower *ad libitum* intake and that eating rate is an important factor. In this study we aimed to explore the effect of viscosity on the gastro-intestinal hormones ghrelin, CCK-8 and GLP-1. Thirty-two subjects (22 ± 2 years, BMI 21.9 ± 2.2 kg/m^2) participated in this crossover study. Subjects received a fixed amount of a chocolate flavored milk-based liquid or semi-solid product similar in energy density and macronutrient composition. Before intake and 15, 30, 60 and 90 min thereafter, appetite was rated and blood was drawn to determine glucose, CCK-8, active ghrelin, desacyl ghrelin and GLP-1 concentrations. After the last blood withdrawal, subjects were offered a chocolate cake meal to consume *ad libitum*. In the appetite ratings we observed a small effect showing that the semi-solid product is apparently considered as more satisfying than the liquid. There was a significant product effect for fullness (P=0.03), desire to eat (P=0.04), appetite something sweet (P=0.002) and prospective consumption (P=0.0009). We observed no clear effect of viscosity on gastro-intestinal hormones. Only for desacyl ghrelin there was a significant product effect (P=0.004). Concentrations were consistently higher after intake of the semi-solid product. *Ad libitum* intake of the chocolate cake was 102 ± 55 g after the liquid and 96 ± 46 g after the semi-solid product (ns). The results of our study show a similar response of the gastro-intestinal hormones CCK-8, ghrelin and GLP-1 after a fixed preload of a liquid and semi-solid product similar in energy- and macronutrient composition.

INTRODUCTION

Texture plays an important role in food intake regulation. Several studies have shown that liquid foods elicit weaker suppressive appetite responses [e.g. 1-3] and a weaker compensatory response in energy intake [e.g. 4-8] than solid or semi-solid foods. These findings imply that ingestion of liquid calories may lead more rapidly to a positive energy balance and subsequent weight gain than calories from solid foods.

In both our previous studies we showed a clear effect of viscosity on *ad libitum* food intake, regardless of the palatability, energy density and macronutrient composition of the products [9]. In the first study in a real-life setting subjects (n=108) consumed 30% more of a liquid product (mean intake 809 ± 396 g) compared to a semi-solid product (mean intake 566 ± 311 g) (P<0.0001). In the second follow-up study, again subjects consumed 34% more of the liquid product (419 ± 216 g) than the semi-solid product (277 ± 130 g). Although these results are very clear, we are not sure which underlying factors can explain these results. We did show in the second study that eating rate is one of the important factors in this; it was observed that the liquid was eaten at a higher rate (90 ± 50 g/min) than the semi-solid product (57 ± 20 g/min) which led again to significantly higher *ad libitum* intakes for the liquid (319 ± 176 g) compared to semi-solid product (226 ± 122 g) (P<0.001). However, besides eating rate other factors are likely to have played a role. Among others, several gastro-intestinal hormones have shown to be an important factor in food intake regulation [10, 11].

The present study aimed to explore the effect of viscosity on three gastro-intestinal hormones that have proven to be important in food intake regulation; ghrelin, CCK and GLP-1 [10, 11]. These three hormones are secreted by endocrine cells in different parts in the gastro-intestinal tract and have different functions; ghrelin is mainly synthesized in the stomach and increases gastro-intestinal motility and is presumably involved in meal initiation; CCK is among others released in the blood when nutrients enter the duodenum, it stimulates gallbladder contraction and gastric and pancreatic acid secretion; GLP-1 is released when nutrients enter the ileum and colon and is involved in the ileal-break mechanism, i.e. the feed-back mechanism that slows stomach emptying and gut motility after food ingestion [10, 11].

In the current study, a fixed amount of the products identical to those in our previous studies was offered to subjects. Before and after consumption of the products, we measured appetite responses and gastro-intestinal hormone concentrations and furthermore the *ad libitum* intake of a second meal was measured. We hypothesize that the appetite ratings will show similar results as our previous findings, i.e. that the semi-solid product would have a higher satiating capacity compared to the liquid product and thus lead to lower appetite ratings after consumption of the semi-solid product. Next, we investigated whether or not differences in subjective satiety are reflected in the hormonal

responses. Finally, we investigated whether or not subjects would consume less of the test meal after consumption of the semi-solid product than after consumption of the liquid product.

SUBJECTS AND METHODS

Subjects

The study was performed at the Wageningen University, Wageningen, the Netherlands. A total of 32 subjects were recruited. Subjects had to be healthy, 18–50 years, normal weight (BMI 18.5 – 30.0 kg/m^2) and they had to consume breakfast regularly. Exclusion criteria were: restrained eating (Dutch Eating Behaviour Questionnaire, men: score > 2.37, women: score > 3.24 [12]), lack of appetite for any (unknown) reason, following an energy restricted diet during the last 2 months, change in body weight > 5 kg during the last 2 months, stomach or bowel diseases, diabetes, thyroid disease or any other endocrine disorder or hypersensitivity for milk components. Furthermore, subjects were excluded if they had experienced any problems with drawing blood in the past, had anemia (men: Hb< 8.5 mmol/l, Ht<0.41; women Hb< 7.4 mmol/l, Ht<0.36), fasting glucose levels >5.8 mmol/l, or when their antecubital veins were considered not suitable for blood drawing by means of a catheter. Potential subjects were invited for a screening visit to the Wageningen University during which blood was drawn and body weight and height were measured. Subjects were informed on the aim of the study, the influence of food viscosity on appetite regulation. The study was approved by the Medical Ethical Committee of the Wageningen University and all subjects gave their informed consent. A total of 32 subjects (12 men, 20 women) aged 22 ± 2 years, with a mean BMI of 21.9 ± 2.2 kg/m^2 and with a mean DEBQ restraint score of 2.09 ± 0.70 participated in the study.

Test products

The test products were milk based products with chocolate flavor specially developed for this study and our previous studies [9] by NIZO food research in Ede, the Netherlands. The basis of all chocolate products was whole fat milk (68%), water (18%), sugar (6.5%), modified starch (3.5%), cream (2%), cacao (1.5 %) and carrageenan (0.05%). The type of thickening agents (starch) was varied in the products to obtain identical products differing solely in physical state, a liquid (comparable to commercially available chocolate milk) and a semi-solid product (comparable to commercially available chocolate custard). Test products were similar in energy content and energy density, volume and macronutrient composition.

Chemical analysis resulted in the following values per 100 g: liquid 408 kJ, 2.8 g protein, 13.9 g carbohydrate, 3.4 g fat ; semi-solid 420 kJ, 2.9 g protein, 14.0 g carbohydrate, 3.6

g fat. More details on production, analysis and viscosity of the products can be found in our previous article [9].

Test products were served in a white non-transparent plastic cup with a maximum content of 0.5 l, covered with a lid, through which a thick straw of 26 cm and a diameter of 0.9 cm was placed. Subject were not able to see the content of the cup.

Experimental procedure

The study was a randomized crossover experiment. All subjects participated in 2 test sessions, once receiving the liquid product and once receiving the semi-solid product. The washout period between test sessions was at least 2 days.

Subjects arrived between 8.00 and 9.00 a.m. after an overnight fast which was defined as no eating or drinking except for water, tea and coffee without sugar or milk after 20.00 p.m. and no drinking except for water after 22.00 p.m. To standardize individual satiety levels, subjects were instructed to eat the same products on the day preceding a test session and to record their consumption in a food diary. After arrival, a catheter was inserted in the antecubital vein and subjects had to sit calmly for at least 15 min before the first blood sample was taken (t=0). Hereafter, subjects received a fixed amount of the test product. Products were prepackaged delivered in the plastic cup weighing 500 g (including weight of the plastic cup of 15 g) for men and 400 g (including weight of the plastic cup of 15 g) for women. Subjects were instructed to consume the test product within 10 min. After consumption we weighed all the cups to determine the actual intake, this was as follows: men, liquid product 480 ± 2 g, semi-solid product 460 ± 6 g; women, liquid product 380 ± 1 g, semi-solid product 358 ± 6 g.

During the first sip of the test product, pleasantness of the product and several sensory attributes were rated by means of a questionnaire (results, see **Table 3.1**). After consumption of the test product, subjects were not allowed to eat or drink anything during 1.5 h. Blood samples were taken again 15, 30, 60 and 90 min after the first fasting blood sample. During each blood sampling subjects rated subjective appetite on a questionnaire.

At the end of the experiment after taking the last blood sample (between 10 and 11 a.m.), the subjects were offered a test meal consisting of a plate with a large amount of slices of chocolate cake (± 450 g, Albert Heijn, Zaandam, the Netherlands. Per 100 g 1620 kJ, 5.5 g protein, 44 g carbohydrate, 21 g fat). The chocolate cake was offered *ad libitum* and subjects were instructed to consume as much of the cake as they wanted. The plates with the chocolate cake were weighed before and after *ad libitum* intake. Chocolate cake was chosen because cake is a common food to eat in the Netherlands during the morning break. The chocolate flavor was chosen to determine if satiety for chocolate taste in general would occur since the test products also have a chocolate taste.

Table 3.1 Liking responses, desire to eat the test product and prospective consumption of the test product (mean ± SD, measured on a 10-point scale) and rating sensory attributes of the test products (mean score ± SD, measured on a 7-point scale from not strong at all to very strong) (*n*=32)

	Liquid	Semi-solid	*P*-value[a]
Pleasantness	6.9 ± 1.3	6.4 ± 1.5	ns
Desire to eat the test product	6.3 ± 1.4	5.9 ± 1.6	ns
Prospective consumption of the test product	6.3 ± 1.4	5.6 ± 1.7	0.03
Chocolate taste	5.3 ± 0.9	5.0 ± 1.1	ns
Sweetness	4.7 ± 1.1	4.4 ± 0.9	ns
Thickness	3.4 ± 1.4	6.0 ± 0.9	< 0.0001

ns = not statistically significant.
[a] Analyses of scores between the liquid and the semi-solid product were conducted using a paired *t*-test.

Blood sampling and biochemical analysis

To prevent the catheter from clogging, physiological salt solution (NaCl 0.9%) was injected in the catheter after each sample. With each blood sample, one extra tube was drawn and rejected to remove the physiological salt solution from the catheter. All samples were divided over aliquots which were immediately frozen and stored at -80°C until analysis. All samples of one subject were analyzed in one assay run and means of duplicate measures were used.

Blood samples for the glucose analysis were collected in tubes containing NaF and centrifuged for 10 minutes at 1100 x g / 2600 rpm at 4°C. Plasma glucose was measured by bichromatic endpoint assay (GluFlexTm reagent). Blood samples for the desacyl ghrelin and active ghrelin analysis were collected in glass plasma tubes containing Aprotinin. After collection, samples were centrifuged for 10 min at 1500 x g / 2900 rpm at 4 °C. Immediately after centrifugation 1M HCL was added.

Active ghrelin and desacyl ghrelin were measured using commercially available human ELISA kits (Linco Research Inc., St.Charles, MO, USA). The lowest level of active ghrelin that could be detected by this kit was 2.5 fmol/mL. The intra-assay CV of our own measurements was 10% and 6% at mean concentrations of 5.0 fmol/ml (expected range 3.3-6.8 fmol/ml) and 26.8 fmol/ml (expected range 16.6-34.4 fmol/ml) respectively. The inter-assay CV at the same mean concentrations was 27% and 13%. The lowest level of desacyl ghrelin that could be detected by the kit was 12.5 fmol/mL. The intra-assay CV of our own measurements was 11% and 5% at mean concentrations of 15.7 fmol/ml (expected range 24.9-51.8 fmol/ml) and 104 fmol/ml (expected range 118.0-245.0 fmol/ml) respectively. The inter-assay CV at the same mean concentrations was 31% and 17%. Blood samples for the CCK-8 analysis were also collected in glass plasma tubes containing Aprotinin. After collection, samples were

centrifuged for 10 min at 1500 x g / 2900 rpm at 4°C. Plasma CCK-8 concentrations were measured using an optimized and validated commercial human RIA kit (Euro-Diagnostica, Malmö, Sweden). This improved assay system has been optimized to reach a very high sensitivity of 0.05 pmol/l and no cross-reactivity toward gastrin-17 and sulfated gastrin. Validation data showed that the intra-assay CV was 8.9% at a concentration of 0.85 pmol/l and 4.9% at a concentration of 2.04 pmol/l. The inter-assay CV was 9.5% at a concentration of 0.85 pmol/l and 4.9% at a concentration of 2.04 pmol/l.

Blood samples for the total GLP-1 analysis were collected in plasma tubes. Immediately after sampling, Dipeptidyl Peptidase (DPP)-IV inhibitor was added. After collection, samples were centrifuged for 10 min at 1100 x g / 2600 rpm at 4°C. Total GLP-1 was measured by radioimmunoassay after extraction of plasma with 70% ethanol (vol/vol, final concentration) using antiserum 89390 which has an absolute requirement for the intact amidated carboxy-terminus of GLP-1 7-36amide and reacts equally well with GLP-1 9-36amide, the primary metabolite of dipeptidyl-peptidase 4 mediated degradation. The sum of the two components (total GLP-1 concentration) reflects the rate of secretion of the L-cell [13]. This assay has a detection limit of ~ 1 pmol/l. The intra- and inter-assay CV were <6% and <15% respectively.

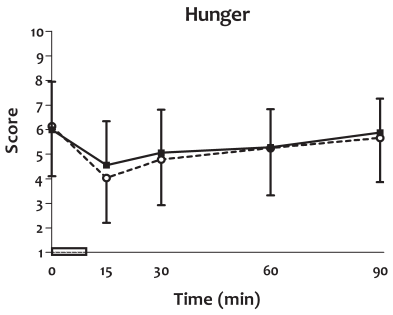
Appetite ratings

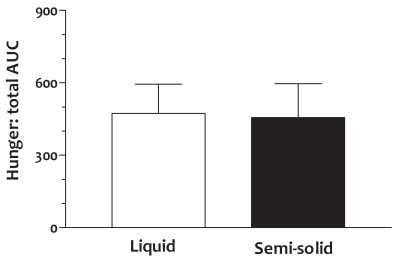
Simultaneously with each blood sample, subjects rated hunger, fullness, desire to eat, appetite for something sweet, appetite for something savory, prospective consumption and thirst on a 10-point category scale, with statements expressing extremes (not at all/ very little until very much) anchored at each end [14].

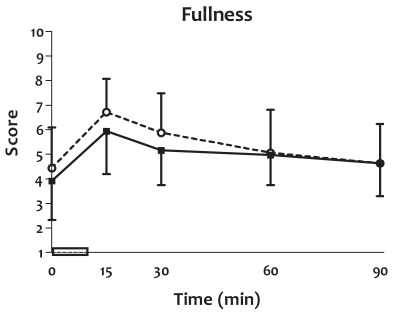
Statistical analyses

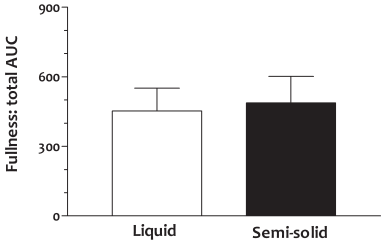
Data are presented as means ± standard deviations (SD). Statistical analyses were performed by means of SAS (version 9.1.3; SAS Institute Inc., Cary, NC, USA). Significance was set at $P<0.05$. Differences in palatability and other sensory characteristics of the test products were analyzed by means of paired t-tests.

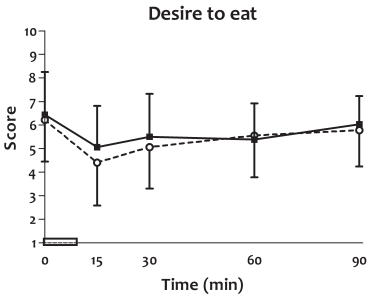
Appetite ratings over time were tested by means of a mixed model ANOVA (proc Mixed, SAS) for an overall treatment (= product) effect, time effect and treatment x time interaction. To control for differences at baseline, the baseline values were added to the model. Participant was added as random variable. If the treatment x time interaction was statistically significant then least square means were used for post hoc comparisons at different time points. The same procedure was performed for the blood parameters, glucose, CCK, active and desacyl ghrelin and GLP-1. Since all blood parameters were not normally distributed, they were log-transformed with a natural logarithm before testing.

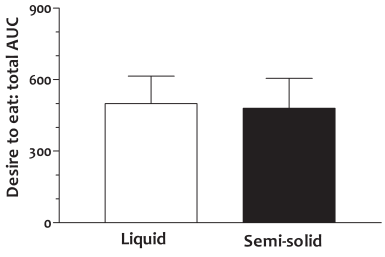

Hunger
Score
Time (min)
0
15
30
60
90

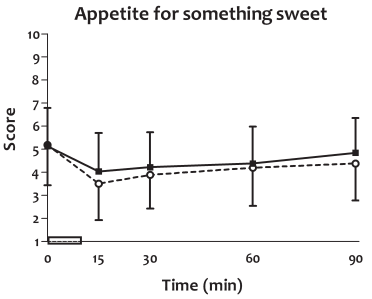

Hunger: total AUC
900
600
300
0
Liquid
Semi-solid

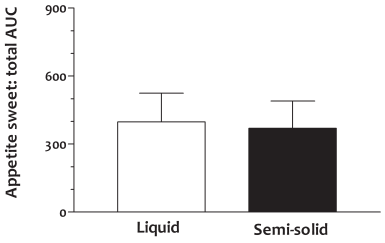

Fullness
Score
Time (min)
0
15
30
60
90


Fullness: total AUC
900
600
300
0
Liquid
Semi-solid


Desire to eat
Score
Time (min)
0
15
30
60
90


Desire to eat: total AUC
900
600
300
0
Liquid
Semi-solid


Appetite for something sweet
Score
Time (min)
0
15
30
60
90


Appetite sweet: total AUC
900
600
300
0
Liquid
Semi-solid

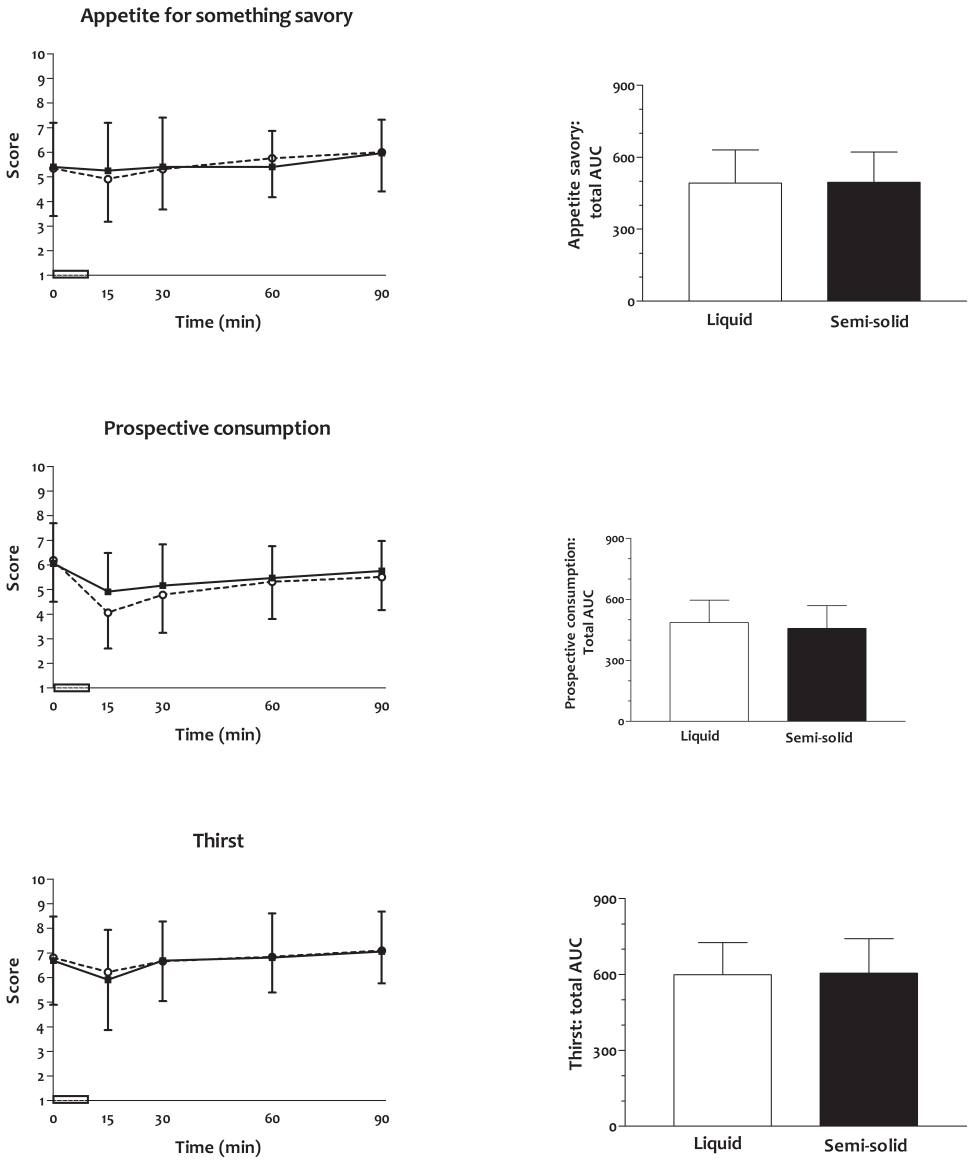


Figure 3.1 On the left, mean (± SD) scores for the appetite parameters: hunger, fullness, desire to eat, appetite for something sweet, appetite for something savory, prospective consumption and thirst for the liquid product (—■—) and the semi-solid product (--○--). The small block on the first 10 min of the x-axis represents the consumption of the product. On the right: Mean total areas under the curve for the satiety parameters for the liquid and semi-solid product.

Total areas under the curves were calculated for the appetite ratings and all blood parameters (Proc Expand, SAS). Comparison of the area under the curve for the liquid and semi-solid product were performed by means of one-way ANOVA (Proc GLM, SAS) adjusted for participant and baseline values of the specific appetite rating or blood parameter.

Differences in *ad libitum* intake of the chocolate cake were analyzed by means of a paired *t*-test.

RESULTS

Subjective appetite ratings

As expected, fullness ratings significantly increased after intake (time effect $P<0.0001$) and all other appetite ratings significantly decreased (time effect $P<0.0001$). All ratings showed a peak at 15 min, approximately 5 min after consumption of the product (**Figure 3.1**).

In **Figure 3.1** it can be seen that for desire to eat, appetite for something sweet and prospective consumption the overall values after the intake of the semi-solid product are lower compared to the values after the intake of the liquid product and for fullness the overall values after the semi-solid product are higher compared to the liquid product; meaning that the semi-solid product decreased the subjective feelings of appetite more than the liquid product. This is also reflected in a statistically significant product effect for the above mentioned attributes (desire to eat: $F(1,216)=4.22$, $P=0.04$; appetite something sweet: $F(1,216)=10.09$, $P=0.002$; prospective consumption: $F(1,216)=11.33$, $P=0.0009$ and fullness: $F(1,216)=4.51$, $P=0.03$). Approximately 90 min after consumption, appetite levels returned to fasting levels. However, there were no statistically significant product x time interactions for any of the appetite ratings or thirst. Moreover, the areas under the curve of the appetite ratings were not significantly different between the liquid and the semi-solid product.

Blood parameters

The glucose and the gastro-intestinal hormone concentrations are presented in **Figure 3.2**. All blood parameters showed a significant time effect ($P<0.0001$). After intake of the test products, glucose concentrations rose and peaked at 30 min. Glucose curves were similar for the liquid and the semi-solid product; there was no statistically significant product effect. Desacyl ghrelin showed an initial rise with a small peak at 15 min, thereafter desacyl ghrelin decreased below fasting levels. In **Figure 3.2** it can be seen that the blood values of desacyl ghrelin are higher after intake of the semi-solid product than after the intake of the liquid product. This is also expressed in an overall statistically significant product effect ($F(1,214)=8.67$, $P=0.004$). There was no significant product x time interaction. Active ghrelin decreased after the intake of the test product and no differences in plasma concentrations

were observed over time points between the liquid and the semi-solid test product; no significant product effect was shown. CCK levels increased after intake of the products, but there was also no statistically significant product effect. GLP-1 values also rose after intake of the products. The mean values after the consumption of the liquid product seemed to be higher compared to the values after the consumption of the semi-solid product, but there was no statistically significant product effect. Furthermore none of the blood parameters had a statistically significant difference in AUC between the liquid and the semi-solid product (**Figure 3.2**).

Ad libitum intake chocolate cake

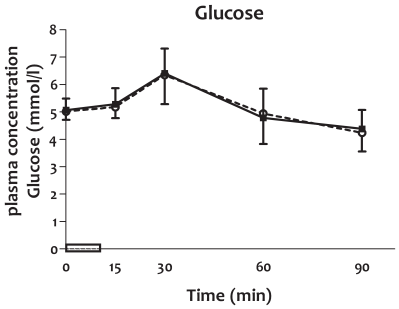
At the end of a test session subjects were offered an *ad libitum* meal of chocolate cake. Mean liking for the chocolate cake was 7.0 ± 1.2 after the intake of the liquid product and 7.2 ± 0.9 after the intake of the semi-solid product (measured on 10-point category scale ranging from not at all liked until liked very much). *Ad libitum* intake of the cake after the fixed preload of the liquid product was 102 ± 55 g and after the semi-solid product 96 ± 46 g (ns).

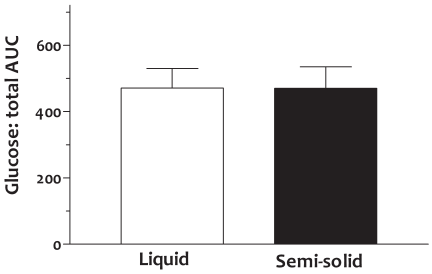
DISCUSSION

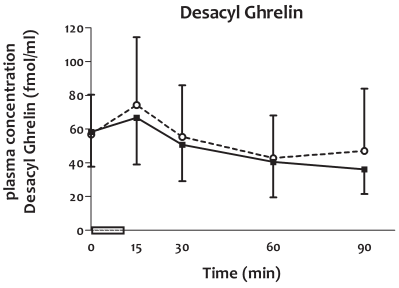
The present study shows that the viscosity of chocolate dairy products similar in energy density, macronutrient composition and volume had a small effect on postprandial appetite ratings, i.e. after consumption the semi-solid product showed a significantly lower appetite for desire to eat (P=0.04), appetite for something sweet (P=0.002) and prospective consumption (P=0.0009) and a higher rating for fullness (P=0.03). However, the viscosity of the products did not show a distinct effect on the postprandial responses of ghrelin, CCK and GLP-1 in plasma. Nor was there an effect of viscosity on the *ad libitum* intake of a test meal of chocolate cake 90 min after the intake of the test products.

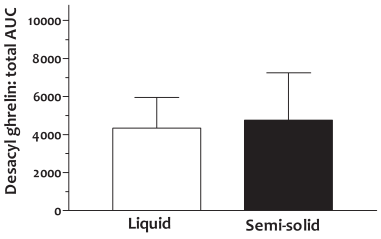
The effect that we found in the appetite ratings; i.e. that the semi-solid product is more satiating, is in agreement with our previous studies in which the *ad libitum* intake of the same semi-solid product was significantly lower than the intake of the liquid product, with a difference in intake of 30% [9]. However, given the fact that the effect of viscosity in the previous studies was so clear, the results on appetite ratings in the present study are relatively small. We had expected to see a larger effect and especially at t=15 min immediately after intake. Other studies investigating the effect of texture on satiety also showed lower appetite ratings after a fixed preload of a solid / more viscous product compared to liquid / less viscous products [e.g. 1-3]. Some of these studies also showed significant, but small differences in ratings between the different test products as well.

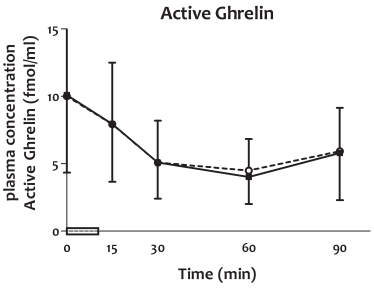
Although we investigated if the higher satiation capacity of the semi-solid product would

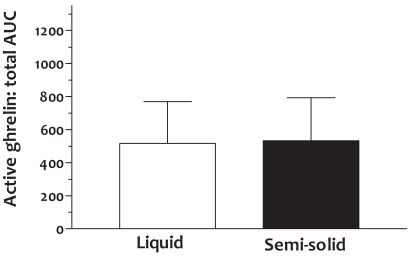

Glucose
plasma concentration
Glucose (mmol/l)
8
7
6
5
4
3
2
1
0
0
15
30
60
90
Time (min)


Glucose: total AUC
600
400
200
0
Liquid
Semi-solid


Desacyl Ghrelin
plasma concentration
Desacyl Ghrelin (fmol/ml)
120
100
80
60
40
20
0
0
15
30
60
90
Time (min)


Desacyl ghrelin: total AUC
10000
8000
6000
4000
2000
0
Liquid
Semi-solid


Active Ghrelin
plasma concentration
Active Ghrelin (fmol/ml)
15
10
5
0
0
15
30
60
90
Time (min)


Active ghrelin: total AUC
1200
1000
800
600
400
200
0
Liquid
Semi-solid

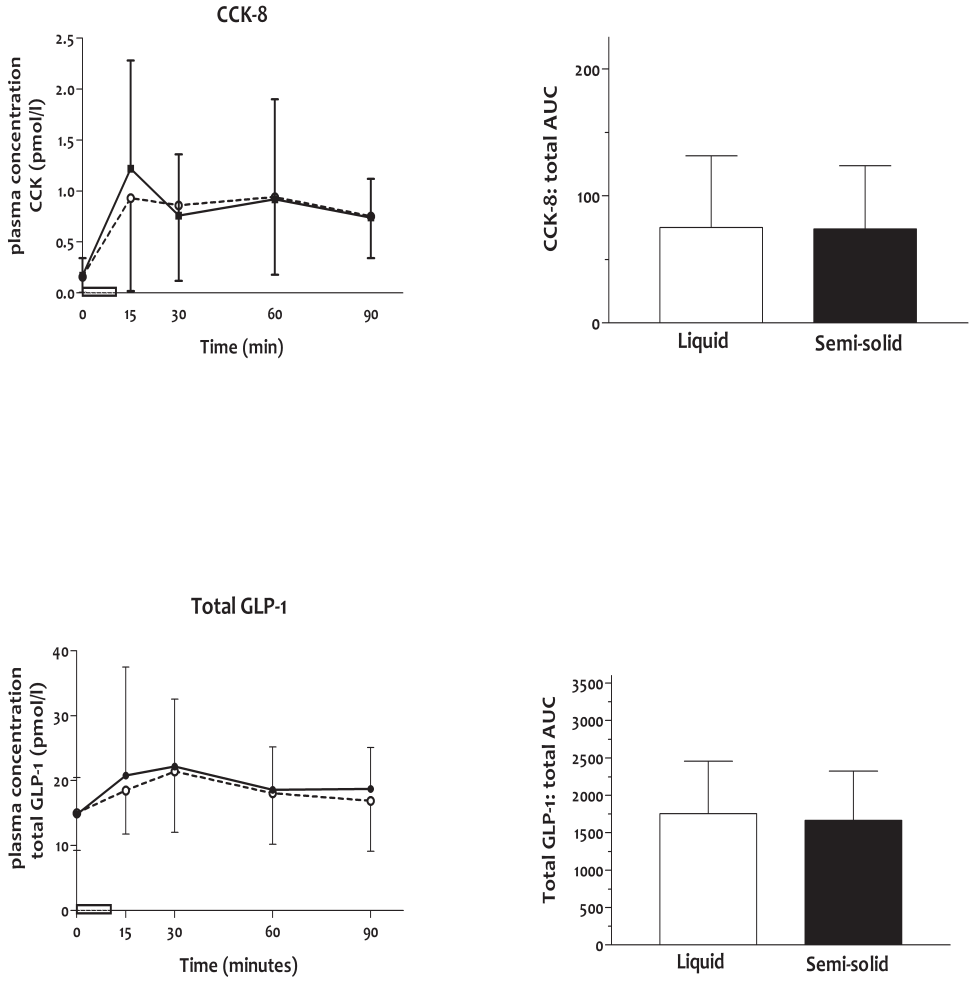


Figure 3.2 On the left, mean (± SD) values of glucose, desacyl ghrelin, active ghrelin, CCK-8 and total GLP-1 for the liquid product (—■—) and the semi-solid product (--○--). The small block on the first 10 min of the x-axis represents the consumption of the product. On the right: Mean total areas under the curve for the blood values for the liquid and semi-solid product.

be reflected in the hormonal responses, this was not the case. We have observed no clear effect of viscosity on glucose, active ghrelin, CCK-8 or GLP-1. We did show a statistically significant product effect for desacyl ghrelin. The values after consumption of the semi-solid product were consistently higher than the values after the liquid product.

Two factors could have influenced our results. First, the composition and ingredients of our test products. Our test products had a similar nutrient composition and viscosity was changed by using different types of starch and not the amount of starch. It is of course a strong point that it was possible to make such products which are so similar in many characteristics except for viscosity. However, it could also be the reason why we did not find distinct effects since the nutrients that entered the gastro-intestinal tract were the same, and thus evoked the same hormone response.

The second factor that could be important is the gastric emptying rate of the test products. The gastric emptying rate of the liquid product could have been faster compared to the semi-solid product. Studies have shown that increasing the viscosity of food decreases the gastric emptying rate [e.g. 15, 16]. If indeed the gastric emptying rate of the more viscous product was slower, than it is possible that it took more time before nutrients of the semi-solid product reached the duodenum and the rest of the small intestine. Since ghrelin is produced primarily by the stomach and proximal small intestine [10, 11], this could explain why the levels for desacyl ghrelin are higher after intake of the semi-solid product. This is also in agreement with the study of Blom et al. [17] which showed that postprandial ghrelin responses are inversely associated with gastric emptying rate. However, we do not know if the gastric emptying rate was indeed different between the products. The products differed only in the type of starch and not in the amount of starch. It is therefore also possible that the amount of starch was already broken down by amylase in saliva and that the viscosity that the products had in the stomach was the same. If this was the case, than this could explain why we did not find an effect on the hormones that we measured in this study. Unfortunately, we have no data on the real gastric emptying times of these specific products.

At the end of a test session 90 min after the test product, subjects were offered a plate with chocolate cake. We did not find a significant difference in intake. This has probably to do with the fact that we only found a small effect of the products on appetite ratings. We most likely cannot expect that this small effect, mainly directly after the intake of the products at *t*=15 min, would last until 90 min after intake. Furthermore it is possible that the time between intake and test meal was too long and the effect of viscosity is not that long lasting. Some studies investigating the time between preload and test meal have shown that results can disappear or diminish when the time between preload and test meal is longer [e.g. 18-20].

A comparable study to ours is the study of Tieken et al. [21], which has also investigated the

effect of texture of food on appetite hormones and appetite ratings. Whereas in our study the difference in texture between the liquid and semi-solid product was small, in the Tieken study the difference in texture was more pronounced, i.e. a liquid product versus a solid product. As expected, a larger difference in texture also resulted in larger differences in appetite ratings and for a longer period of time. Hunger and desire to eat ratings were more suppressed by the solid product than by the liquid product, although no effect on fullness was shown. Consistent with the appetite data, they observed a greater and more prolonged reduction of plasma total ghrelin concentrations following the solid product. Interestingly, also in this study, no differences in CCK responses between the products was shown, despite the higher hunger and desire to eat ratings after the liquid product.

Based on the results of the present study and also the results of our previous studies [9], we believe that viscosity is probably more involved in the process of satiation / meal termination than in the process of satiety and thus that the fixed preload- test meal paradigm is not the best way to measure the satiating effect of viscosity. Fixed preload studies are appropriate to measure the effect of a food product on satiety (between meals) whereas *ad libitum* intake measures give an indication of meal size and thus satiation. The difference between satiation and satiety is clearly depicted in the satiety cascade of Blundell [22]. As Kissileff suggested several years ago, attributes which influence intake primarily by post-ingestive signals can be studied more effectively by preloading procedures, while attributes which influence intake primarily by stimulating the oropharynx are more effectively studied by measuring *ad libitum* intake of a single meal [23]. We agree with this point and we believe that we must not search for an explaining mechanism in the area of hormonal processes but more in the area of oral sensory processes. Oral sensory exposure may be an import factor in satiation since oral factors are operational during the process of eating and not thereafter.

In conclusion, the results of our study show a similar response of the gastro-intestinal hormones CCK-8, ghrelin and GLP-1 after a fixed preload of a liquid and semi-solid product similar in energy- and macronutrient composition. We believe that these hormones do not play a key role in the explaining mechanism why liquid products are less satiating than semi-solid products. We suggest that further research on the effect of texture on food intake regulation should focus on the influence of oral sensory exposure.

ACKNOWLEDGEMENTS

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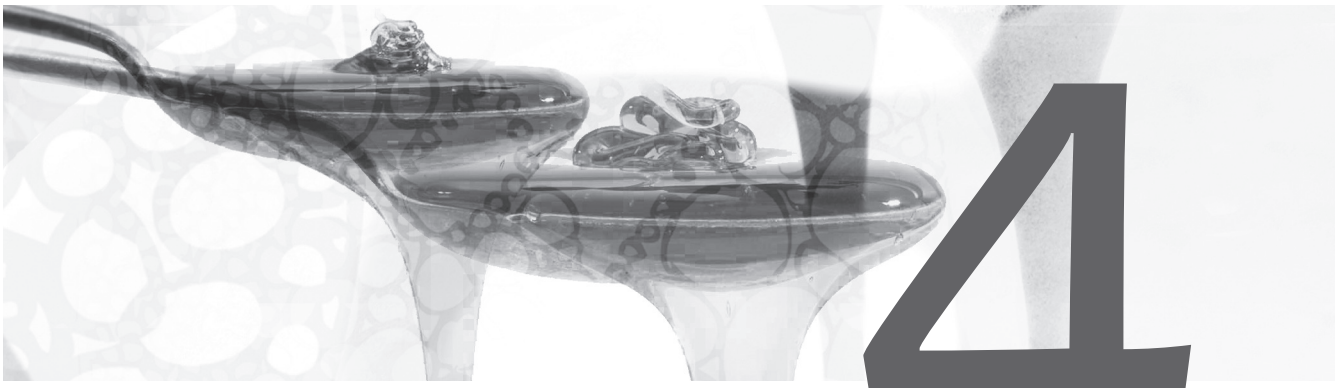

4

Effect of bite size and oral processing time of a semisolid food on satiation

Nicolien Zijlstra
René A de Wijk
Monica Mars
Annette Stafleu
Cees de Graaf

American Journal of Clinical Nutrition 2009;90:269-275

ABSTRACT

Background: Food texture plays an important role in food intake regulation. In previous studies we showed a clear effect of viscosity on *ad libitum* food intake and found indications that eating rate, bite size, and oral processing time (OPT) could play a role.

Objective: The objective was to determine the effect of bite size and OPT of a food on satiation, defined as *ad libitum* food intake.

Design: Twenty-two healthy subjects participated in all 7 test conditions. Bite sizes were free or fixed to small bite sizes (≈5 gram) or large bite sizes (≈15 gram). OPT was free (only in combination with free bite size) or fixed to 3 or 9 s. Subjects consumed chocolate custard through a tube, which was connected to a peristaltic pump. Sound signals indicated OPT duration.

Results: Subjects consumed significantly more when bite sizes were large than when they were small (bite size effect: $P<0.0001$) and when OPT was 3 s rather than 9 s (OPT effect: $P=0.008$). Under small bite size conditions, mean (± SD) *ad libitum* intakes were 382 ± 197 g (3-s OPT) and 313 ± 170 g (9-s OPT). Under large bite size conditions, *ad libitum* intakes were much higher: 476 ± 176 (3-s OPT) and 432 ± 163 g (9-s OPT). Intakes during the free bite size conditions were 462 ± 211 (free OPT), 455 ± 197 (3-s OPT) and 443 ± 202 g (9-s OPT).

Conclusion: This study shows that greater oral sensory exposure to a product, by eating with small bite sizes rather than with large bite sizes and increasing OPT, significantly decreases food intake.

INTRODUCTION

The prevalence of overweight and obesity has increased rapidly in recent years [1]. Overweight and obesity result from a long-term positive energy balance, in which energy intake via food intake is larger than energy expenditure. The texture of food plays a role in food intake regulation. Studies have shown that liquid foods elicit weaker suppressive appetite responses [e.g. 2-4] and a weaker compensatory response in energy intake [e.g. 5-9] than do solid or semi-solid foods. In our previous studies we additionally showed that the viscosity of food also affects *ad libitum* food intake; subjects consumed 30% more of a liquid product than of a semi-solid product similar in palatability, energy density, and macronutrient composition [10, 11].

It is important to identify possible mechanisms that could explain the finding of differences in satiety responses between liquids and solids. So far, underlying mechanisms responsible for differences are still unclear. In our previous studies we showed that eating rate is an important factor; the liquid product was consumed at a higher rate than the semi-solid product, which also led to a significantly higher *ad libitum* intake. More importantly, a standardized eating rate led to nonsignificant differences in intake [11]. Also other studies have shown that eating slower decreases food intake [12, 13]. In our third study we found that not only the intake of the liquid product was higher, but also the "bite" or "sip" sizes for the liquid product were higher than those of the semi-solid product. Furthermore, we found indications that the oral processing times (OPTs) of the liquid and semi-solid products were different [10]. This has been confirmed by other studies that also showed that bite size is an important factor in food intake regulation, and larger bite sizes led to a higher ingestion rate, but there was no effect on total food intake [14, 15].

On the basis of our previous results, we hypothesized that oro-sensory exposure (i.e. the time a product stays in the mouth) is an important factor that could explain the difference in intake between liquid and solid products (see also our previous article [11]). When a product stays in the mouth for a longer time, the exposure time to sensory receptors in the oral cavity is longer and there is more opportunity for more exposure to taste, smell, texture, and other properties of food. We hypothesized that this will lead to earlier sensory satiation and thus a smaller meal size. The importance of oro-sensory stimulation for appetite suppression has been clearly shown by a number of studies that compared normal eating of nutrients with infusion of nutrients and thereby bypassing oro-sensory stimulation [16-19]. Furthermore, the study of Lavin et al. [20] especially supports our hypothesis that oral exposure time plays an important role in food intake regulation. They showed a significant reduction in the intake of a test lunch after a preload of sucrose pastilles was chewed and consumed within 10 min than when a sucrose drink with the same nutrient composition was consumed within 2 min.

In the present study, we wanted to further explore our hypothesis by determining the

effect of bite size and OPT of a food on satiation, defined as *ad libitum* food intake. We hypothesized that both a smaller bite size and a longer OPT would lead to greater oral exposure to the product and thus would lead to earlier sensory satiation and a lower *ad libitum* food intake.

SUBJECTS AND METHODS

Subjects

The study was performed at Wageningen University, Wageningen, the Netherlands. The subjects had to be healthy, be aged 18 – 30 years, be of normal weight [body mass index (in kg/m^2): 18.5 – 25.0], and like chocolate custard. Exclusion criteria were as follows: restrained eating (Dutch Eating Behaviour Questionnaire score of >2.89 for men and >3.39 for women [21]); lack of appetite for any reason; following an energy restricted diet during the past 2 months; change in body weight >5 kg during the past 2 months; stomach or bowel diseases, diabetes, thyroid disease, or any other endocrine disorder; difficulties with eating or swallowing; hypersensitivity to the test product; or participation in our previous studies. Menstrual cycle was not controlled for; however, because the order of test conditions was randomized within subjects, we expect this to have a minimal or no influence on our results.

Potential subjects were invited for a screening visit at Wageningen University, during which body weight and height were measured. Subjects were not aware that the aim of the study was to measure *ad libitum* food intake, but were informed that the study was performed to measure the effect of bite size and eating rate on the pleasantness of chocolate custard. The study was approved by the Medical Ethical Committee of Wageningen University, and all subjects gave their informed consent. Recruitment started 31 May 2007. A total of 22 subjects (8 men and 14 women) aged 21 ± 2 years, with a mean (± SD) body mass index of 21.9 ± 1.5 kg/m^2 and a mean Dutch Eating Behaviour Questionnaire restraint score of 2.3 ± 0.6 participated in the study.

Test product

The test product was commercially available milk-based chocolate custard (Royal Friesland Foods, Deventer, the Netherlands). To determine solely the effect of the factors bite size and OPT on food intake, without introducing possible effects due to food properties, we chose to use only one food product in all test conditions. The test product is comparable with the semi-solid product used in our previous studies. The ingredients of the custard were milk, whey, thickening agents, cacao, coloring agents, salt and aroma. Per 100 mL, the product contained 395 kJ (94 kcal), 2.3 g protein, 14.7 g carbohydrate and 2.8 g fat.

Experimental conditions

The study was a randomized crossover experiment in which all subjects participated in the following test conditions: 1) free bite size, free OPT; 2) free bite size, 3-s OPT; 3) free bite size, 9-s OPT; 4) small bite size (≈ 5 g), 3-s OPT; 5) small bite size (≈ 5 g), 9-s OPT; 6) large bite size (≈ 15 g), 3-s OPT; 7) large bite size (≈ 15 g), 9-s OPT. Condition 1 was a control condition to observe intake when both bite size and OPT were free.

Control of bite sizes and oral processing time

To measure and control bite sizes, subjects consumed the test product through a silicon tube (diameter: 6.4 mm) that was connected to a peristaltic pump (Watson-Marlow, type 503s; Watson-Marlow Bredel, Wilmington, MA). The sequence of events during a test session is shown schematically in **Figure 4.1**. At 20-s intervals, an auditory bleep signaled the subject to take a sip or bite (referred to as "bite" hereafter) from the test product. In conditions 1-3, in which the bite size was free, the subjects stopped each presentation of test product via a push button, enabling them to control the size of the bite. Pump rate was set at ≈100 g/min. In the other conditions, bite sizes were fixed to either ≈5 or ≈15 g. This amount was delivered into the subjects' mouths in a period of 5 s.

After the pump was stopped (either by the subject or automatically), another auditory bleep signaled the start of oral processing, which was followed either 3 or 9 s later by a higher pitch auditory bleep to signal swallowing. The subjects were instructed to move the product in their mouth during the OPT and to try to mimic normal oral

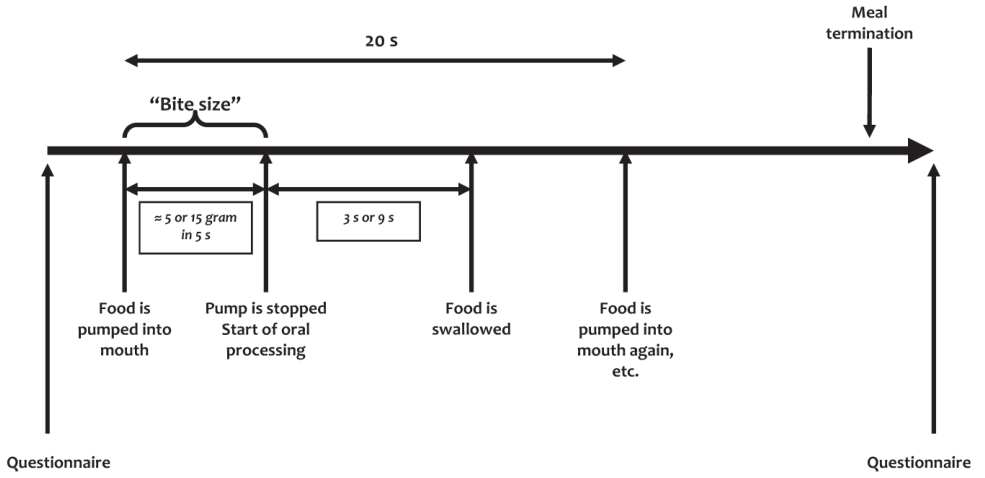

Figure 4.1 Schematic sequence of events during the "fixed bite size" conditions (conditions 4-7; see Experimental conditions). In the "free bite size" conditions (not shown in this figure), the pump was stopped by the subject via a push button, and oral processing time was free, 3 s or 9 s.

processing of this product. To quantify oral movements during food processing, we have used vibromyography. Six piezo-electric disc transducers were placed on the faces of the subjects. Saliva was collected at the beginning and end of a test session. The vibromyography and saliva data are not discussed in the present article but are only mentioned to give an overview of the experimental setting.

Ad libitum intake

The subjects were instructed to consume as much of the product until pleasantly satiated. The test product was placed on a computerized scale (XP6002S DeltaRange; Mettler-Toledo Inc, Columbus, OH); thus intake was measured continuously. The subjects were not aware of the weighing and received no visual cues on the amount of food ingested.

Duration of conditions

In the first 3 conditions the pump continued for ≥ 30 min. If a subject was satiated before the 30 min were finished, he or she was instructed to stop the pump by pushing the button immediately after the auditory bleep that signaled the start of the pump. In the other conditions the pump was stopped by the researchers, when the subject indicated that he or she was full. In those conditions, instead of continuing the pump for 30 min, which was not possible because the subjects had no push button to stop the pump, the subjects were instructed to remain in the sensory cabin for ≥ 30 min. The subjects were informed that the pump could be restarted if they wanted to consume more of the product, but none of the subjects chose for this option. The subjects were allowed to read during all test conditions, even during consumption of the product, to make the stay in the sensory cabin more comfortable. The period of 30 min was chosen to prevent subjects from leaving the research area early for other reasons than being satiated with the product. If a subject wanted to consume the product for a longer period of time than 30 min, the session was continued. This was the case for 5 subjects, 3 of whom wanted to continue both "small bite size" conditions and 2 of whom wanted to continue one of the "small bite size" conditions.

Standardization of satiety state

The test sessions took place around lunchtime (between 1130 and 1430) or around dinnertime (between 1630 and 1930). Each subject was tested at the same time of day, with at least one nontesting day between test sessions. The subjects were instructed not to eat or drink anything except for water, tea or coffee without sugar and milk in the 3 h before the start of a test session and not to consume anything except water in the past hour before the start. Until 1 h after a test session, subjects were not allowed to eat or drink anything except water, tea or coffee without sugar and milk. This last instruction

was given to motivate subjects to consume until pleasantly satiated. This instruction was not controlled.

Satiety ratings and questionnaires

Before and after *ad libitum* intake, the subjects rated hunger, fullness, desire to eat, appetite for something sweet, appetite for something savory, prospective consumption and thirst on 10-point category scales. The scales were anchored from "not at all/very little" to "very much".

Furthermore, before and after intake, the subjects were offered a small portion of the test product (≈20 g) and asked to grade the product on a 10-point scale and to rate the product on several sensory aspects (data not shown). Finally, at the end of a test session, the subjects were asked for the reason they stopped eating. This was done by asking the subjects to what extent they agreed with the proposition "I stopped consuming the product because…" and then the following answers were given: "the product tasted less pleasant", "I was full", "the manner of eating was not pleasant", "the pace of eating was too slow", "the pace of eating was too fast", "I got tired of the product", "drinking from a tube is not pleasant", or "other" (open answer which could be filled in). The proposition had to be answered on a 5-point scale ranging from "totally disagree" to "completely agree".

Statistical analysis

Data are presented as means ± SDs. Statistical analyses were performed by using SAS (version 9.1.3; SAS Institute Inc, Cary, NC). Significance was set at $P<0.05$.

To determine whether the *ad libitum* intakes in the 7 test conditions differed significantly from one another, the intake data were first analyzed by one-factor analysis of variance (ANOVA) (PROC GLM, SAS) with condition and subject number as fixed factors. Because intake increased as the number of completed sessions increased (independent from condition), the order of days was also included in the model statement. To determine the effect of bite size and OPT on intake, the *ad libitum* intake data were further analyzed by two-factor ANOVA (PROC GLM, SAS), with subject number, bite size, OPT and order of days as independent variables and including the interaction term bite size x OPT. Least-squares means with Bonferroni correction for multiple testing were used for post-hoc comparisons. The "free bite size, free OPT" condition was excluded from this analysis because this condition was a control condition to observe intake when subjects were free in both factors.

Intake per bite was defined as the amount of product that was pumped into the subjects mouth in a 20-s interval. These intakes were calculated by subtracting the end value of the

weighing scale in the 20-s interval from the begin value. In the free bite size conditions, the pump continued for a period of 30 min and when satiated, the subjects had to push the button to stop the pump. Occasionally, a subject was not fast enough in pushing the button and still received a small amount of test product. The data for intake per bite over time showed a sudden drop from normal bite sizes to very small bite sizes (e.g. <1 or 2 g). If, at a certain time point, >5 consecutive bites sizes became smaller than 25% of the mean bite size of the preceding bites, the remaining bites were deleted from the data and thus not taken into account in the calculation of mean bite size values or in the statistical analysis of bite size over time. It was assumed that a subject was satiated with the product and that the small bite sizes were due to not pushing the stop button efficiently. For the calculation of total *ad libitum* intake, the intake from the remaining bites was taken into account.

To determine whether the intakes per bite over time in the free bite size conditions differed between the first 3 conditions or changed over time, intakes per bite over time were tested with a mixed-model ANOVA (PROC MIXED, SAS) for an overall effect of condition and time effect (bite number). A condition x time interaction was first included, but was not significant and thus left out of the model. Order of days was included in the model statement and bite number was included in the repeated statement in which subject number was also added to identify the subjects in the model. Compound symmetry was used as covariance structure. If the main effects were statistically significant, least-square means were used for post hoc comparisons between test conditions.

To investigate whether the mean bite size in the free bite size conditions was related to the *ad libitum* intake (i.e., to determine whether subjects who take large bite sizes also have a higher *ad libitum* intake), a mean bite size was calculated per subject per condition and these values were analyzed by mixed-model regression (PROC MIXED SAS). Condition was also included in the model, and the random statement included intercept and subject number to identify the subjects in the model.

Differences between the 7 test conditions in satiety ratings, liking scores, and reasons for stopping with eating were analyzed by one-factor ANOVA (PROC GLM, SAS) with condition, subject number and order of days as fixed factors. Least-squares means adjusted with Bonferroni correction for multiple testing were used for post-hoc comparisons. To test whether the satiety ratings scores before *ad libitum* intake differed from those after *ad libitum* intake, a paired *t*-test was performed.

RESULTS

Ad libitum intake

The *ad libitum* intake of the chocolate custard was significantly different for the different test conditions ($P<0.001$; **Figure 4.2**). Mean intake in the "free bite size, free OPT" condition was 462 ± 211 g, in the "free bite size, 3-s OPT" condition 455 ± 197 g and in the "free bite size, 9 sec OPT" condition 443 ± 202 g. *Ad libitum* intakes in the small bite size conditions were much lower than in the other conditions: 382 ± 197 g in the "small bite size, 3-s OPT" condition and 313 ± 170 g in the "small bite size, 9-s OPT" condition. In the large bite size conditions, the mean intakes were also larger than in the small bite size conditions: 476 ± 176 g in the "large bite size, 3-s OPT" condition and 432 ± 163 g in the "large bite size, 9-s OPT" condition.

The subjects consumed significantly more when bite sizes were large than when small (bite size effect: $P<0.0001$) and when OPT was 3 s rather than 9 s (OPT effect: $P=0.008$). Post hoc analysis showed that intakes in the small bite size conditions differed significantly from intakes in the free and large bite size conditions ($P<0.0001$). There was no significant bite size x OPT interaction.

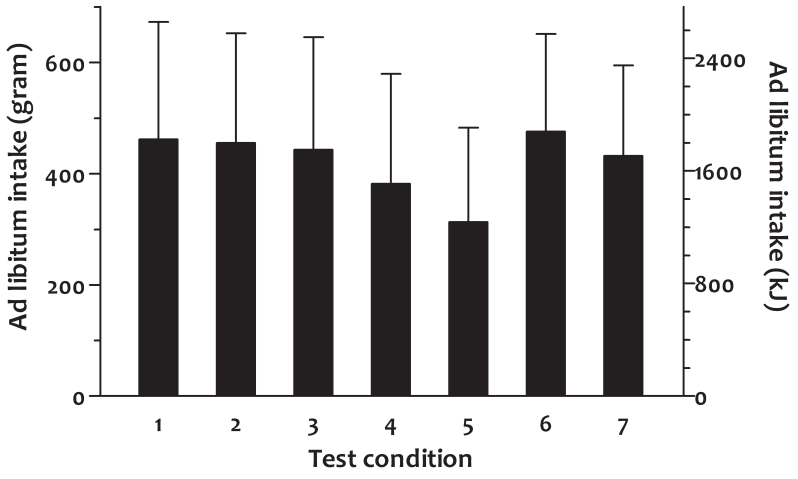

Figure 4.2 Mean (± SD) *ad libitum* intakes of the semi-solid custard in the 7 different test conditions ($n=22$): 1) free bite size, free oral processing time; 2) free bite size, 3-s oral processing time; 3) free bite size, 9-s oral processing time; 4) small bite size (≈ 5 g), 3-s oral processing time; 5) small bite size (≈ 5 g), 9-s oral processing time; 6) large bite size (≈ 15 g), 3-s oral processing time; 7) large bite size (≈ 15 g), 9-s oral processing time. *Ad libitum* intakes differed significantly between conditions ($P<0.0001$). There was a significant main effect of bite size ($P<0.0001$) and oral processing time ($P=0.008$), and there was no significant bite size x oral processing time interaction (Two-factor ANOVA excluding condition 1 because this was a control condition).

Mean overall bite size and bite sizes over time

Mean overall bite size in the free bite size conditions was 10.3 ± 4.0 g when OPT was free, 9.9 ± 4.8 g when OPT was 3 s and 10.1 ± 4.8 when OPT was 9 s. In the small and large fixed bite size conditions, we aimed to fix the bite size to either ≈5 or 15 g. In the end, mean overall bite sizes were 5.2 ± 0.5 g in both small bite size conditions. In the large bite size condition, mean overall bite size was 14.2 ± 1.9 g when OPT was 3 s and 14.8 ± 1.9 g when OPT was 9 s.

The mean bite size versus bite number in all test conditions is represented in **Figure 4.3**. Considering only the free bite size conditions, the average bite size decreased with increasing bite number in all three conditions (bite number effect: $P<0.0001$). There was a significant difference in mean bite intake between conditions (condition effect: $P= 0.0003$). Post hoc analyses showed that "free bite size, free OPT" condition differed significantly from the "free bite size, 3-s OPT ($P=0.007$) and from the "free bite size, 9-s OPT" ($P=0.0004$).

Subjects who consumed larger bites on average in the free bite size conditions also had a higher *ad libitum* intake (**Figure 4.4**). Regression analysis on *ad libitum* intake showed a significant contribution of mean bite size to the model ($\beta=27.1$ and $P=0.0001$). Based on the regression coefficient in this model, an increase in bite size by 1 g would lead to an increase in *ad libitum* intake of 27 g.

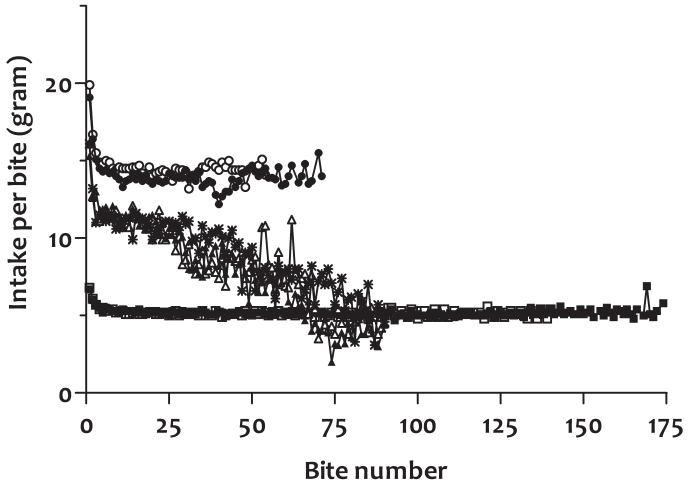

Figure 4.3 Mean intake per bite of the semi-solid custard versus bite number in the 7 different test conditions; free bite size, free oral processing time (solid asterisks); free bite size, 3-s oral processing time (solid triangles); free bite size, 9-s oral processing time (open triangles); small bite size, 3-s oral processing time (solid squares); small bite size, 9-s oral processing time (open squares); large bite size, 3-s oral processing time (solid circles), and large bite size, 9-s oral processing time (open circles). At the start of the first bite, each group consisted of 22 subjects. Bite sizes were fixed in the small and large bite size conditions. Free bite size conditions: bite sizes were significantly different between conditions (condition effect: $P<0.0001$) and bite sizes significantly decreased over time (time effect: $P<0.0001$) (mixed model ANOVA).

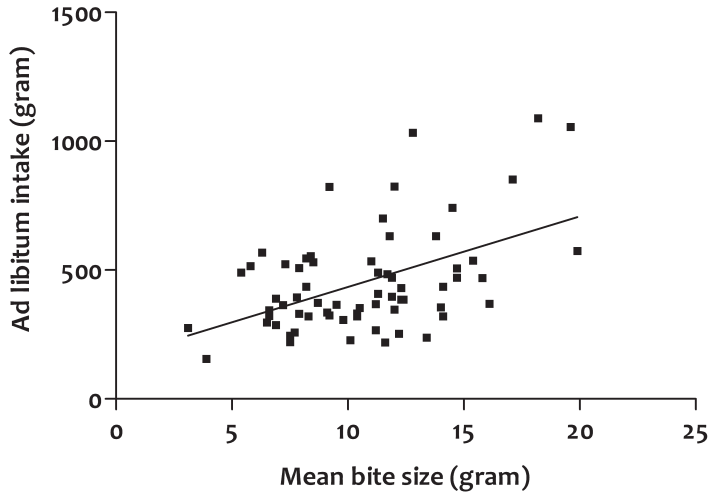

Figure 4.4 Mean bite size versus *ad libitum* intake in all three free bite size conditions for all 22 subjects. Regression analysis showed a significant contribution of mean bite size to the model (β=27.1, *P*=0.0001; mixed model regression).

Table 4.1 Satiety ratings before and after ad libitum intakes in all conditions (*n*=22), measured on a 10-point category scale from "not at all/very little" until "very much" [1]

	Before ad libitum intake ratings mean ± SD [2]	After ad libitum intake ratings mean ± SD
Hunger	6.9 ± 1.4	2.9 ± 1.5 [3]
Fullness	3.1 ± 1.4	7.6 ± 1.5
Desire to eat	7.5 ± 1.3	3.1 ± 1.5 [3]
Appetite for something sweet	6.7 ± 1.3	2.4 ± 1.2
Appetite for something savory	7.1 ± 1.5	4.2 ± 1.9 [4]
Prospective consumption	6.8 ± 1.1	3.0 ± 1.3 [5]
Thirst	6.1 ± 1.7	5.6 ± 1.8

[1] All values are means ± SDs. [2] Ratings were not significantly different between the 7 test conditions (ANOVA) [3] Ratings differed slightly between conditions for hunger (*P*=0.01) and desire to eat (*P*=0.02). Post hoc analysis showed no statistically significant differences (ANOVA, post hoc test with Bonferroni correction). [4] Ratings differed slightly between conditions (*P*=0.01). The "small bite size, 3-s oral processing time" condition differed significantly from the "large bite size, 3-s oral processing time" (*P*=0.047) and the "large bite size, 9-s oral processing time" (*P*=0.013) conditions (ANOVA, post hoc test with Bonferroni correction). Mean scores for these conditions were 4.9±1.8, 3.7±1.9, and 3.6±1.4, respectively. [5] Ratings differed slightly between conditions (*P*=0.001). The "small bite size, 3-s oral processing time" condition differed significantly from the "free bite size, 3-s oral processing time" (*P*=0.046), the "large bite size, 3-s oral processing time" (*P*=0.0003), and the "large bite size, 9-s oral processing time" (*P*=0.003) conditions (ANOVA, post hoc test with Bonferroni correction). Mean scores for these conditions were 3.6± 1.3, 2.9±1.2, 2.6±1.3, and 2.7±1.2, respectively.

Satiety parameters

Fullness ratings significantly increased ($P<0.05$) after *ad libitum* intake, and the scores for all other satiety ratings decreased significantly ($P<0.05$) after intake. An overview of the satiety ratings before and after *ad libitum* intake is shown in **Table 4.1**. Mean values based on all conditions are shown. Before *ad libitum* intake, there were no significant differences in ratings between the test conditions. After intake there were small differences in ratings between conditions, which were significant for hunger ($P=0.01$), desire to eat ($P=0.02$), appetite savory ($P=0.01$) and prospective consumption ($P=0.001$).

Rating product and reasons for stopping with eating

Mean liking for the test product was 7.4 ± 0.9 before *ad libitum* intake and 6.5 ± 1.3 after *ad libitum* intake. There were no significant differences in liking before or after the *ad libitum* intakes in the different test conditions.

An overview of the mean answer scores to what extent subjects agreed with the proposition "I stopped consuming the product because…" is shown in **Table 4.2**. As can be seen in the table, answer scores for the reason "I was full" received the highest scores.

Table 4.2 Scores for the propositions of reasons for stopping eating, measured on a 5-point scale from "do not agree at all" to "completely agree" ($n=22$) [1]

	Free bite size			Small bite size		Large bite size	
	free OPT	3 sec OPT	9 sec OPT	3 sec OPT	9 sec OPT	3 sec OPT	9 sec OPT
Product not pleasant anymore	2.8 ± 1.3	2.8 ± 1.2	2.8 ± 1.2	2.6 ± 1.2	2.7 ± 1.4	2.9 ± 1.2	2.5 ± 1.3
Full [2]	4.2 ± 0.8	4.5 ± 0.8	3.9 ± 1.2	4.1 ± 0.8	3.9 ± 1.0	4.3 ± 0.9	4.5 ± 0.7
Unpleasant manner of eating [3]	2.0 ± 0.9^{ab}	2.4 ± 1.0^{ab}	2.7 ± 1.3^{b}	2.0 ± 1.0^{a}	2.5 ± 1.4^{ab}	1.9 ± 0.9^{a}	2.1 ± 1.0^{ab}
Pace of eating too slow [4]	1.6 ± 0.7^{a}	1.5 ± 0.7^{a}	1.6 ± 1.0^{a}	2.4±1.3^{b}	2.9± 1.4^{b}	1.4 ± 0.6^{a}	1.5 ± 0.7^{a}
Pace of eating too fast [4]	1.5 ± 0.7^{a}	1.7 ± 0.8^{a}	2.2 ± 1.3^{ac}	1.4 ± 0.7^{a}	1.4 ± 0.8^{a}	3.1± 1.2^{b}	3.0 ± 1.2^{bc}
Tired of the product	3.2 ± 1.2	3.2 ± 1.1	3.0 ± 1.3	3.1 ± 1.0	3.0 ± 1.0	3.1 ± 1.1	3.2 ± 1.2
Consuming from a tube is not pleasant	2.1 ± 1.1	2.0 ± 1.0	2.2 ± 1.3	2.0 ± 1.1	2.0 ± 1.2	1.7 ± 0.9	2.2 ± 1.1

[1] All values are means ± SDs. OPT, oral processing time. Values in a row with different superscript letters are significantly different, $P<0.05$ (ANOVA, post-hoc test with Bonferroni correction). [2] Significant main effect of condition ($P=0.02$); post hoc test showed no significant differences. [3, 4] Significant main effect of condition: [3] $P=0.003$; [4] $P<0.0001$.

Answer scores of this variable differed significantly between the different conditions (P=0.02) but post hoc test showed no differences. Other answer categories that differed between conditions were " the manner of eating was unpleasant" (P=0.003), " the pace of eating was too slow" (P<0.0001), and "the pace of eating was too fast" (P<0.0001). As expected, for the reason "the pace of eating was too slow" the highest scores were given in the small bite size conditions, and these conditions also differed significantly from the other conditions (post hoc analysis, P<0.05). For the reason "the pace of eating was too fast", the highest scores were given in the large bite size conditions, and these conditions differed significantly from the other conditions (post hoc analysis, P<0.05).

DISCUSSION

The results of this study show a clear effect of bite size and OPT of a food on food intake. A smaller bite size led, as hypothesized, to a lower intake. Mean intakes in the small bite size conditions were 381 g (3-s OPT) and 313 g (9-s OPT) compared with 476 (3-s OPT) and 432 g (9-s OPT) in the large bite size conditions. Also a longer OPT led to a lower food intake, although this effect was not as strong as was the bite size effect. The intake in the long OPT conditions was on average 42 g less than in the short OPT conditions.

Other studies investigating the effect of bite size on food intake regulation have shown varying results [15, 22, 23]. Spiegel et al. [15] showed that large bite sizes increased average ingestion rate (defined as meal size/meal duration), but, because total meal duration decreased as bite size increased, there was no effect on food intake. However, in the latter experiment, solid food products were used, i.e. bagels and sandwiches. As the authors mention, it is possible that these products were already eaten at a slow pace and therefore the bite size difference had no effect on meal size. However, Weijzen et al. [23] did find an effect of bite size on food intake. Snacks in nibble size were consumed with smaller bite sizes, and mean *ad libitum* intake was 12% lower than the intake of bar size snacks. Moreover, the study of Walden et al. [22] found an effect on not just one meal but on food intake of a whole day. They used a very interesting new approach to reduce bite size by giving subjects a removable dental tool (fitted for an individual) that reduced the size/space in the oral cavity, thereby potentially reducing bite size. Food intake was significantly lower on the day that the dental tool was used than on the control day, i.e. 26% lower in grams of food eaten. Interestingly, satiety and hunger ratings were unaffected. The latter two studies support our findings that eating with smaller bite sizes leads to lower *ad libitum* intakes.

The current study was set up in such a way that it was possible to investigate total food intake if you eat the same number of bites per minute but with large or small bites. As a result of this, the total eating rate was different in the different conditions. In a 20-s cycle, subjects received either 5 g or 15 g. The eating rate was thus 15 g/min or 45 g/min.

The eating rate itself could have affected our results. Studies have shown that a slower eating rate decreases energy intake. For instance, the study by Andrade et al. [12] showed that eating with a teaspoon and taking pauses between bites significantly reduced energy intake compared with eating with a soup spoon and taking no pauses. Martin et al. [13] and Kissileff et al. [24] also found that a lower eating rate resulted in less food intake in overweight and obese subjects and normal weight subjects. However, in the study of Martin et al., the effects were found in men but not in women; in the study by Andrade et al. and Kissileff et al., only women were studied. Regarding the results on eating rate, one could wonder whether we would have found different results if we had used a different approach in which small bite sizes were combined with a high bite frequency. Interestingly the study of Weijzen et al. [25] used exactly this approach and found the same results. The consumption of orangeades in small sip sizes with small intervals between bites, while eating rate was held constant, resulted in significantly lower *ad libitum* intakes than did the consumption in large sip sizes with large intervals between bites. The difference was 141 g (29%) for the regular-energy orangeades and 66 g (16%) for the no-energy orangeade.

The current study showed clear effects of bite size and OPT on food intake. The results are based on a single meal product in an experimental setting. The fact that subjects consumed more of the products after the first sessions (independent of the conditions) could have been influenced by this experimental way of eating. There was no testing day in which subjects could get acquainted with the test situation before the start of the study, because we did not want to expose subjects to the product more than necessary. The subjects already had to participate in a total of seven sessions in which they received the same product. Even though the subjects gave a fairly high score for the proposition that they were tired with the product at the end of a test session (a score of ≈ 3 on a 5-point scale), the increase in intake when sessions proceeded showed that they were probably not tired of the product in general. Furthermore, we were concerned that subjects would eat less because of the experimental setting but the results for the answers to the propositions "I stopped eating because of the unpleasant manner of eating" and "I stopped eating because consuming from a tube is not pleasant" showed that this had only a small influence; on average, these propositions were scored as 2 on a 5-point scale.

Our hypothesis that greater oro-sensory exposure would lead to earlier sensory satiation and thus to a smaller meal sizes seems to be valid. Oral exposure is an important factor in food intake regulation. This was confirmed by a number of studies that compared normal eating of nutrients with infusion of nutrients, which thereby bypassed oro-sensory stimulation [16-19]. This was also confirmed by the study of Lavin et al. [20], which showed differences in intake of a test lunch after chewing sucrose containing pastilles for 10 min compared with the same sucrose content but as a drink within 2 min. In the study by Lavin et al, next to oral sensory exposure, chewing as such could also have played a role. It is thought that the act of chewing a solid food gives a satiety signal that is not induced

by swallowing a liquid product [2, 7]. Chewing also leads to more saliva production, which is important for taste perception. In our study, chewing did not play a role because the semi-solid product used required no chewing.

Whereas the studies mentioned in the paragraph above [16-20] clearly showed effects on appetite ratings and the food intake of a second meal, our study showed direct effects on meal termination, i.e. *ad libitum* food intake. We expect these results on meal termination to be very relevant in the regulation of food intake. Considering the literature on energy intake compensation, we speculate that the extra calories acquired by eating with larger bites are probably not compensated for during the rest of the day. Studies have shown that people poorly compensate for extra calories, for instance when being overfed [e.g. 26, 27]. Furthermore, the study by Walden et al. [22] already showed food intake differences on a whole day, without differences in satiety ratings, which confirms our expectation. Thus, eating with small bites and eating slowly with more oral sensory exposure to products will probably result in a lower energy intake during the day. This advice is already given in some weight-loss programs.

In conclusion, the results of our study show that greater oral sensory exposure to a product, by eating with small bite sizes rather than with large bite sizes and increasing OPT, significantly decreases food intake. Further research is needed to extrapolate the results of this study to other food products and meals in a less experimental setting. Furthermore, it would be interesting to find a way to objectively measure how much oro-sensory exposure a person receives from a product. Although this is very difficult and differs between persons, perhaps there is a potential role for real-time atmospheric pressure chemical ionization mass spectrometry (APcI-MS), which measures *in-vivo* retro-nasal aroma release [28]. In a recent study of Ruijschop et al. [29], retro-nasal aroma release intensity and profile morphology appeared to be subject specific. Furthermore, a negative trend was observed between the extent of retro-nasal aroma release and the amount of *ad libitum* food intake (P=0.07) in a subgroup of 15 subjects. Although this technique measures just one important aspect of oral sensory exposure, namely aroma release, it seems a new and interesting approach to use in studies investigating mechanisms behind the satiation effect of food.

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Investigating the effect of texture differences on satiation in 3 pairs of solid foods

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ABSTRACT

This study aimed to determine the effect of texture differences on satiation (*ad libitum* food intake) in 3 pairs of solid foods. Test foods were specially developed luncheon meat, meat replacers and sweets. Each food consisted of a "hard" and "soft" version, expected to lead to different eating rates and consequently to differences in oral sensory exposure time. 106 non-restrained normal-weight subjects participated in 7 test sessions. During the first 6 sessions, subjects consumed the test products *ad libitum* while watching a movie in a cinema. During the 7th session, eating rate of all products was measured. *Ad libitum* intake did not differ significantly between the hard and the soft version for any of the products. Eating rate was significantly lower for the hard version of the luncheon meat than for the soft version (21 ± 10 vs. 25 ± 13 g/min); there were no differences in the other product groups. Interestingly, eating rate and intake were significantly correlated in all test products (overall: r=0.54). Texture differences between the hard and the soft version of the investigated test products may have been too subtle to lead to differences in eating rate and subsequently to differences in *ad libitum* intake.

INTRODUCTION

The texture of food plays a role in food intake regulation. Studies have shown that liquid foods elicit weaker suppressive appetite responses [e.g. 1-3] and a weaker compensatory response in energy intake [e.g. 4-8] than do solid or semi-solid foods. In our previous studies we showed a clear effect of the viscosity of food on *ad libitum* food intake; subjects consumed 30% more of a liquid product compared to a semi-solid product similar in palatability, energy density and macronutrient composition [9]. Furthermore, in another study we demonstrated that increasing oral sensory exposure to a product, by eating with small bite sizes compared to large bite sizes and increasing oral processing time, by holding the food longer in the mouth, significantly decreases food intake [10].

Based on the results of these studies, we presume that the transit time in the oral cavity plays an important role in the explanation of the effect of texture on food intake. A liquid is consumed at a much higher rate and does not stay in the mouth for a long time, whereas a solid product is eaten more slowly and stays in the mouth much longer [9]. We assume that eating more slowly, and taking more time to process the food in the mouth, results in a longer oral exposure time. Furthermore, that a longer oral exposure time will lead to earlier sensory satiation which in turn will lead to a lower *ad libitum* food intake [10].

Until now, studies investigating the effect of food texture on food intake have mainly focused on food viscosity or compared liquid products to solid products. For instance, the study of Haber et al. [1], Tournier and Louis-Sylvestre [8] and DiMeglio and Mattes [4] compared liquid products to solid products. Mattes and Rothacker [3] and also our own previous studies [9] compared products varying from liquid to semi-solid. These differences in texture/physical state are quite large. So far, we do not know if the effects of texture and our hypotheses on oral sensory exposure, also apply within the category of solid foods. On the basis of the mechanism suggested above, we hypothesize that a hard solid food, which requires a relatively longer time to process in the mouth, will lead to a slower eating rate and a reduced *ad libitum* intake because of more oral sensory exposure compared to a soft solid food, which requires a relatively shorter oral processing time.

This hypothesis was tested by investigating the effect of texture differences on satiation using 3 pairs of specially developed solid food products. Satiation was assessed as *ad libitum* food intake. Within each pair of products, the version of the product with a relatively harder texture was expected to require a longer oral processing time than the version with a relatively softer texture, resulting in a slower eating rate. Apart from texture, product pairs were similar in energy content, energy density and macronutrient composition.

SUBJECTS AND METHODS

Subjects

Subjects were recruited in the surroundings of Ede and Wageningen (the Netherlands) via flyers, advertisements in local papers, posters and emails to persons in a database of volunteers interested in participating in studies at Wageningen University. Subjects had to be healthy, 18 – 50 years, normal weight (BMI 18.5 – 25.0 kg/m^2) and they had to like the test products, which were luncheon meat, vegetarian meat replacer and chewy candy (with liking ≥3 on a 5-point scale on a questionnaire with a written description of the test products). Subjects answering never to have eaten one of the test products, but liking the other test products, were also included. This was especially the case for the meat replacer, with 57 out of the final sample of 106 subjects answering never to having eaten this product before, whereas this was the case for only one subject for the luncheon meat and one subject for chewy candy.

Subjects scoring high on restrained eating were excluded (Dutch Eating Behaviour Questionnaire, men: score >2.89, women: score >3.39) [11]. Restrained eating may be defined as the cognitive awareness of food intake and food intake in subjects with high restrained eating is less likely to be regulated by physiological processes [12-14]. Other exclusion criteria were: lack of appetite for any reason; following an energy restricted diet during the last 2 months; change in body weight >5 kg during the last 2 months; stomach or bowel diseases, diabetes, thyroid disease, or any other endocrine disorder; having difficulties with eating or swallowing; hypersensitivity to the test product; being vegetarian or vegan; or participation in our previous studies.

Potential subjects were invited for a screening visit to Wageningen University, during which body weight and height were measured. Subjects were not aware that the aim of the study was to measure *ad libitum* food intake; they were informed that the study was designed to measure the pleasantness of a number of snacks in a real-life setting. The study was approved by the Medical Ethical Committee of the Wageningen University and all subjects gave their informed consent.

A total of 116 subjects participated in the study. Only subjects with complete *ad libitum* intake data for all products were included in data analysis (although including subjects with incomplete data did not change the results). Two subjects were excluded from the data analysis because of not adhering to the study protocol. Eight subjects did not participate in all *ad libitum* intake sessions for reasons such as illness or work obligations. Consequently, the data of 106 subjects were included in the data analysis. These were 45 men and 61 women, aged 24 ± 7 years, with a mean BMI of 21.8 ± 1.7 kg/m^2 and with a mean DEBQ restraint score of 1.9 ± 0.7. Of these 106 subjects, one subject did not show up at the last test session and therefore we have no data on eating rate for this subject.

Six subjects did not consume the complete fixed amount of one or more products, and so their results for these products were excluded from the data analysis of eating rate.

Test products

The test products were luncheon meat, vegetarian meat replacer and chewy candy, all of which were specially developed for this study. Within each pair of products, one version of the product was expected to require a relatively shorter oral processing time, hereafter indicated as the "soft" version, and the other version was expected to require a relatively longer oral processing time, hereafter indicated as the "hard" version.

The luncheon meat was produced by Lutz Fleischwaren GmbH, Landsberg am Lech, Germany, and was comparable to commercially available luncheon meat. The ingredients were: pork meat, water, pork fat, salt, regulator: sodium citrate, spices, dextrose, flavourings, preservative: nitrite. The hard version also included transglutaminase, an enzyme that binds meat protein.

The vegetarian meat replacers were produced by Valess, Tilburg, the Netherlands. Ingredients were: skimmed milk, thickening agents: E401 (fibres), E461 and E509, acidity regulators E326 and E452, sunfloweroil, free-range egg protein, sundried tomatoes (2%), mediterranean herbs (a.o. 1,5% fresh basil), flavour, oat fibre, salt, spices, caramel, ferricpyrophosphate, vitamin B6. The difference in texture was achieved by an adjustment in the processing of the milk, which is the main ingredient of this product.

The chewy candies were provided by Rousselot, Isle-sur-la-Sorgue, France. Ingredients were: sugar, glucose syrup, lecithin, water, gelatin, lactic acid, flavor (blackcurrant). By using different types of gelatin, i.e. 100 Bloom and 250 Bloom, the candies differed in texture.

Each pair of test products was similar in energy content, energy density and macronutrient composition. Macronutrient content was determined by chemical analysis; protein by the Kjeldahl method, fat by the acid hydrolysis method, fibre by the Prosky method, and subsequently carbohydrate was calculated by subtracting moisture, ash, fiber, protein and fat from total weight. Energy content was calculated from the macronutrient composition by using the following energy conversion factors: protein 16.7 kJ/g; fat 37.7 kJ/g; carbohydrate 15.7 kJ/g. The luncheon meat and candies were produced in several batches. For the chemical analysis, food samples were taken from a homogenous mixture of all batches. **Table 5.1** shows the results of the chemical analysis and the energy content calculated from these data.

Rheological characteristics of the texture of the food products were determined by means of a texture instrument that applies uniaxial measurement (Texture Analyzer model TA-XT2, Stable Micro Systems, Etten-Leur, the Netherlands). Cutting measurements were

Table 5.1 Mean values of the chemical analysis of protein, fat and carbohydrate in g per 100 g of test product, and the calculation of energy content in kilojoules and kcal per 100 g for the hard and soft versions of the luncheon meat, meat replacer and chewy candy

	Luncheon meat		Meat replacer		Candy	
	Hard	Soft	Hard	Soft	Hard	Soft
Protein	11.2	11.0	14.6	14.7	0.5	0.4
Carbohydrate	1.0	0.5	3.5	3.7	79.9	79.8
Fat	24.2	23.7	3.5	3.3	12.0	12.5
Fiber	0.6	0.7	5.5	5.1	–	–
Energy (kJ)	1113	1086	429	429	1716	1732
Energy (kcal)	266	259	103	102	410	414

done with a wedge (width 2 cm, angle 3 degrees); distance of cutting was 8 mm (luncheon meat), or 10 mm (vegetarian meat replacer and candies). The force needed to compress or cut to this degree is measured in Newtons. Compression tests were performed with a 20 mm cylinder, and the distance of compression was 10 mm. Twenty measurements were performed per sample. See **Figure 5.1** for the results of the rheological characteristics of the different products. The cutting measurements can be seen as biting of the products, in which a tooth is represented by the wedge. The compression test can be seen as chewing the products between the molars. From the figure it can be observed that more force is needed in a shorter period of time to cut and compress the hard versions of the products.

Experimental procedure

The study was a randomized crossover experiment. The order of all six test products was randomized within subjects over the *ad libitum* intake sessions. The time between test sessions was at least one day. *Ad libitum* intake was the primary outcome of the study and was calculated from the weight of the products before and after consumption. Products were weighed on a digital scale with a precision of 0.1 g (model XP-3000, Denver Instruments, Göttingen, Germany). Subjects were not aware of the weighing.

To create a real-life setting and to distract subjects from visual and weight cues, the study was performed in a cinema. Each subject participated in seven sessions, which were chosen out of a total of 11 available test days. In the first six sessions, *ad libitum* intake was measured. The last available test day was the seventh session for all participants. During this session, oral processing time of fixed amounts of the products was measured to calculate eating rate. During each test day a different movie was shown, but the type

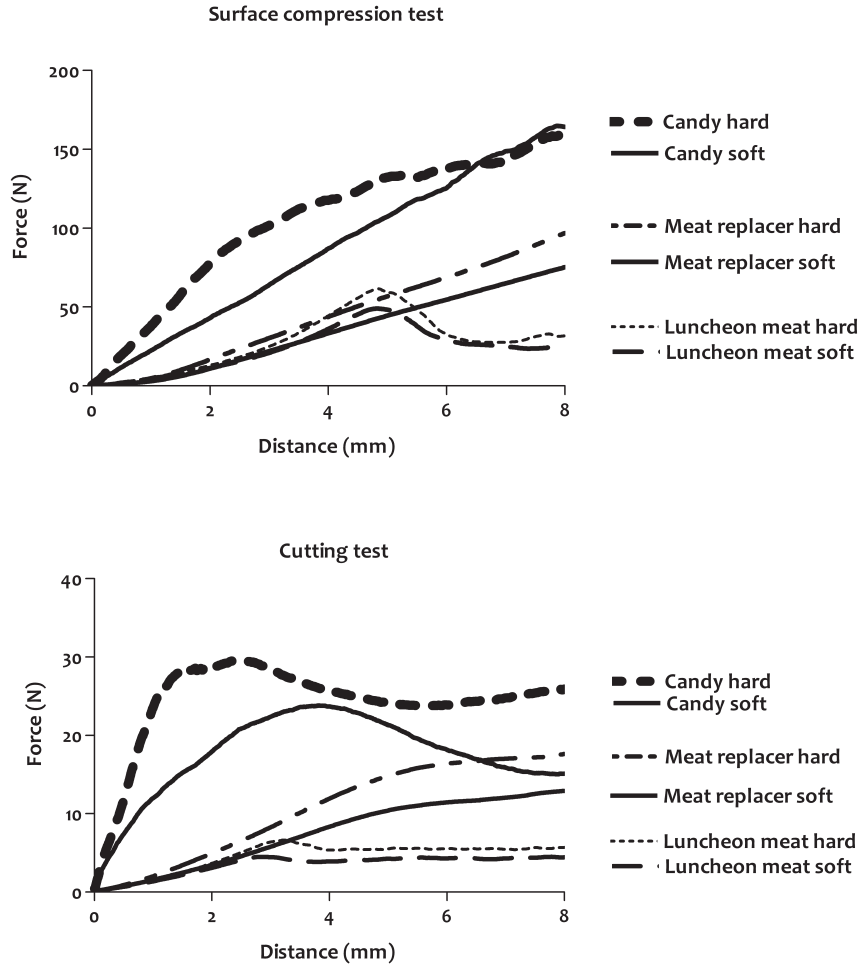

Figure 5.1 Rheological measurements performed by a Texture Analyzer on the hard and soft versions of the chewy candy, meat replacers and luncheon meat. Upper figure: surface compression test. Lower figure: cutting test.

of movie was similar (romantic comedy). The number of subjects present on the first to tenth test day varied between 50 and 84.

Overview of *ad libitum* intake sessions

Each test session started at 18.00 h. After arrival, subjects received a preload food. At 18.45 h, subjects were seated in the theater with one empty seat between each other to avoid interaction. During the movie, subjects were instructed to eat as much of the products as they wanted until they were pleasantly satiated. It was dark in the theater while the

movie was playing. In order to keep any visual or weight cues from the subject and to stimulate *ad libitum* intake, a surplus of the test product was served in cardboard isolating containers. Containers were served with a lid, which was removed by the subject once in the theater. The total duration of each movie was set at 90 min. The movie was divided into three parts of 30 min with breaks of 15 min in between. **Table 5.2** gives an overview of the time schedule of a test session. During the break, subjects left the theater and handed in the container with test product. Before the re-start of the film they received a new container with the same test product. By providing a new container with test product after the breaks, we removed cues regarding previous consumption. Subjects could talk to each other during the break, but no food was available. At the beginning and end of the movie, satiety and sensory ratings were performed.

The luncheon meat was stored in a refrigerator at approximately 3°C and removed from the refrigerator 1 h before serving. Serving temperature was approximately 10°C. Luncheon meat was cut into standardized triangles. On average, three times 426 ± 2 g of luncheon meat was offered. The vegetarian meat replacers were heated in an oven at 180°C for 8 min, cut into standardized triangles and placed in the containers in a banqueting wagon at 70°C, until the moment it was served. On average, three times 426 ± 3 g meat replacer was offered. The candies were manufactured in standardized rectangles. Candies were served unwrapped to avoid cognitive cues regarding consumption. On average, three times 375 ± 2 g of candies was offered. No subject consumed all of the provided test product.

Table 5.2 Time schedule of a test session in the cinema in which *ad libitum* food intake was measured

Time (min)	Activity	Procedure
t = 0 (18.00 h)	Arrival	Receiving preload
t = 45 – 60		Receiving container with test product, rating satiety parameters and test product
t = 60	Start movie	Eating *ad libitum* from the container with test product
t = 90	Break	Handing in the container
t = 105	Restart movie	Receiving new container with same test product and eating *ad libitum* from the container
t = 135	Break	Handing in the container
t = 150	Restart movie	Receiving new container with same test product and eating *ad libitum* from the container
t = 180	End movie	Rating satiety parameters and test product and handing in the container. If the movie was not finished, subjects were free to stay and watch the rest of the movie after handing in the container with test product.

Oral processing time session

During the oral processing time test session (the 7th test session), the movie was divided into 5 parts, each lasting 25 min. Before and after the movie, and in the 4 breaks in between, a fixed amount of food had to be consumed and time required for consumption was recorded. The fixed amounts were on average 46 ± 5 and 48 ± 4 g hard and soft luncheon meat (6 pieces); 51 ± 4 and 50 ± 4 g hard and soft meat replacer (6 pieces); 28 ± 1 g for both hard and soft candies (4 pieces).

The vegetarian meat replacer was consumed before the start of the movie. In break 1 the luncheon meat was consumed, in break 2 the candies, in break 3 the meat replacer, in break 4 the luncheon meat and after the movie the candies were consumed again. This serving order was chosen for reasons of logistical convenience. Each time, half of the subjects received the hard version and the other half of the subjects received the soft version, by random assignment. Thus, by the end of the evening every subject had consumed all versions of all products.

A projector displayed a digital clock with minutes and seconds. Subjects were instructed to record start and end time of consumption. The projector continued for at least 10 min and longer if necessary. Instructions were given to subjects to consume the several pieces one after another at their own pace, but without taking breaks between the pieces or drinking water.

Standardization of satiety state

To standardize individuals' initial state of satiety, subjects were instructed to eat the same breakfast and lunch during all 7 test days and to record their food and drink consumption in a diary. Furthermore, they were instructed not to consume anything except water or coffee and tea without milk and sugar, in the 2 h prior to the start of a session. Physical activity was not controlled.

Additionally, the individual state of satiety was standardized by means of a preload, which was a number of small wheat buns (local bakery), weighing approximately 23 g and containing approximately 226 kJ per bun. The number of buns was based on individual energy needs, estimated by means of the Schofield I equation [15], taking into account age, weight and a physical activity level (PAL) of 1.6. About 10 percent of energy of the daily estimated energy need was provided by the preload and for logistic reasons only whole buns were provided. Bread buns were to be consumed "dry" without any spread or filling, in order to keep the taste of the preload as neutral as possible because a sweet or savory filling might influence intake of the sweet or savory test product. Forty-eight subjects received 4 buns, 44 subjects received 5 buns, 14 subjects received 6 buns. Together with the preload, a 0.5 l bottle of water was provided. The water could be consumed at any

point during a test session but water bottles had to be handed in empty at the end. During a test session, subjects were not allowed to eat or drink anything other than the preload, the provided water and the test products.

Satiety ratings and questionnaires

Before and after *ad libitum* intake, subjects rated hunger, fullness, desire to eat, appetite for something sweet, appetite for something savory, prospective consumption and thirst on 100 mm VAS scales. Scales were anchored from not at all/very little to very much. In addition, subjects also rated liking and other sensory attributes of the test products on 100 mm VAS scales. At the end of a test session, subjects were asked why they stopped eating. This was done by asking the subjects to what extent they agreed with the proposition "I stopped consuming the product because…" and then the following options were given: "I had enough of the taste of the product", "I was full", "I was tired", "the product got cold", "the smell of the product was too strong", "the other persons stopped eating", "the container with test product was empty", "I had the feeling that I already consumed very much", or "other" (open answer that could be filled in). All propositions had to be answered on 100 mm VAS scales ranging from "totally disagree" to "completely agree".

Statistical analysis

Data are presented as means ± SDs. Statistical analyses were performed by means of SAS (version 9.1.3; SAS Institute Inc., Cary, NC, USA). Significance was set at $P<0.05$.

Differences in *ad libitum* intake between the 10 test days and possible order effects in eating rate during the 7th test session were analyzed by means of one-way ANOVA (SAS proc GLM). Overall *ad libitum* intake did not differ significantly among the ten test days and there was no significant product x evening interaction, so the movies did not affect food intake differently. There was also no statistically significant effect of serving order on eating rate. The eating rates of a product group did not differ significantly between the first and second time the product group was served.

Within a pair of products, differences in *ad libitum* intake between the hard and the soft version were analyzed by means of one-way ANOVA (SAS proc GLM) including presenting order of test version over test days as a covariate. Based on the oral processing times of the fixed amount of pieces and the actual weight of these pieces, an eating rate in g/min was calculated for all participants and all products. Within a product pair, differences in eating rates between the hard and soft version were analyzed by means of one-way ANOVA (SAS proc GLM) including presenting order of test version over test days as a covariate. The same ANOVA procedure was also used to analyze differences between the hard and soft version of a product pair in: liking, sensory ratings, satiety ratings and ratings of the reasons for stopping eating. Chi-square tests were used to analyze frequencies.

In addition to the analyses mentioned above, we also explored factors influencing *ad libitum* intake by performing Pearson correlations. Among others, for each test product, a correlation was calculated between *ad libitum* intake and eating rate, between liking and *ad libitum* intake and between liking and eating rate. For the overall correlation between *ad libitum* intake in the first 6 sessions and eating rate of the fixed amounts during the last session, a mean *ad libitum* intake was calculated for each subject (combining the *ad libitum* intakes of all products). In the same manner, a mean eating rate for each subject was calculated, averaging the eating rates of all products for that subject. A correlation was calculated between mean *ad libitum* intake and mean eating rate. The same procedure was used for analyzing the overall correlation between *ad libitum* intake and liking scores.

RESULTS

Liking and sensory ratings test products

Liking scores for the hard and soft version within a product pair were not significantly different. Mean liking was respectively 50 ± 24 and 51 ± 23 for hard and soft luncheon meat, 41 ± 25 and 45 ± 25 for the hard and soft version of the meat replacer and 66 ± 21 and 66 ± 19 for the hard and soft version of the candy, measured on 100 mm VAS scales.

Mean sensory ratings of the test products are given in **Table 5.3**. Texture differences between the hard and soft version of a product group were noticed by the subjects for luncheon meat and partly noticed for the meat replacer, since the hard and the soft version of the luncheon meat differed significantly from each other in firmness, smoothness and effort needed to chew the product ($P<0.05$). The hard and the soft version of the meat replacer differed in saltiness and effort needed to chew the product ($P<0.05$). The hard and the soft version of the candy did not differ significantly from each other in sensory ratings.

Satiety ratings

Before intake, there were no significant differences in satiety ratings between the hard and soft version of a product pair, except for fullness before intake of the luncheon meat. Mean values of satiety ratings for each product group are shown in **Table 5.4**. As expected, the ratings of hunger, desire to eat and prospective consumption decreased after intake and the ratings of fullness increased after intake. After *ad libitum* intake there were no significant differences in satiety ratings between the hard and soft version of a product pair.

Ad libitum intake

Mean *ad libitum* intake for the hard and soft version of the luncheon meat was 148 ± 121 and 157 ± 125 g. Mean intake for the hard and soft meat replacers was 160 ± 109 and 171 ± 111 g and mean intake for the hard and soft candies was 138 ± 83 and 143 ± 90 g

Table 5.3 Sensory ratings (mean ± SD) of the hard and soft version of luncheon meat, meat replacer and candy, measured before *ad libitum* intake on 100 mm VAS scales ranging from "not at all" to "very much"

	Luncheon meat		Meat replacer		Candy	
	Hard	Soft	Hard	Soft	Hard	Soft
Attractiveness	29 ± 23	32 ± 24	47 ± 24	50 ± 24	39 ± 23	40 ± 23
Liking	50 ± 24	51 ± 23	41 ± 25	44 ± 25	66 ± 21	66 ± 19
Strength of taste	53 ± 20	54 ± 21	52 ± 22	49 ± 22	62 ± 21	62 ± 20
Sweet					78 ± 14	78 ± 14
Sour					24 ± 22	23 ± 22
Salty	46 ± 24	45 ± 25	50 ± 23$^{\#}$	42 ± 23$^{\#}$		
Spicy	22 ± 20	23 ± 23	30 ± 23	25 ± 20		
Seasoning	32 ± 25	34 ± 25	53 ± 22	54 ± 20		
Smoothness	72 ± 22*	77 ± 17*				
Dryness			71 ± 21	66 ± 24		
Firmness	67 ± 23**	58 ± 23**	62 ± 20	62 ± 18	53 ± 23	56 ± 20
Granular	24 ± 26	21 ± 22				
Toughness					54 ± 28	54 ± 27
Stickiness					76 ± 22	76 ± 21
Soft					67 ± 20	63 ± 19
Fibrous			61 ± 21	58 ± 22		
Effort of chewing	36 ± 26***	25 ± 22***	50 ± 22$^{\#\#}$	44 ± 24$^{\#\#}$	60 ± 25	59 ± 26
Aftertaste	44 ± 26	49 ± 26	47 ± 24	48 ± 25	50 ± 24	51 ± 25

Statistical analyses are performed between the hard and soft version within the same product group.
* $F(1,104)=4.6$, $P=0.03$; ** $F(1,104)=15.6$, $P=0.0001$; *** $F(1,104)=24.1$, $P<0.0001$.
$^{\#}$ $F(1,104)=10.6$, $P=0.002$; $^{\#\#}$ $F(1,104)=6.2$, $P=0.01$.

(**Figure 5.2**). There were no statistically significant differences in intake between the hard and soft version within a product pair.

Interestingly, of the 106 subjects, a total of 67 subjects consumed more of the soft version of luncheon meat than of the hard version, as opposed to 39 subjects who consumed more of the hard version. This difference was statistically significant, Chi-square=7.4, $P=0.007$. Two-thirds of these 67 subjects, i.e. 45 subjects, also consumed the soft luncheon meat faster than the hard version. For the other food groups, the number of subjects consuming more of the soft version (meat replacer 61 subjects, candy 55 subjects) was not significantly different from the number of subjects consuming more of the hard version (meat replacer 45 subjects, candy 51 subjects).

Table 5.4 Satiety ratings (mean ± SD) before and after *ad libitum* intake of the luncheon meat, meat replacer and candy, measured on 100 mm VAS scales ranging from "not at all" to "very much". Mean values per product group are given which are average ratings of the hard and soft version.

	Luncheon meat		Meat replacer		Candy	
	Before intake	After intake	Before intake	After intake	Before intake	After intake
Hunger	59 ± 22	35 ± 27	61 ± 22	31 ± 29	58 ± 23	30 ± 25
Fullness	32 ± 23*	50 ± 27	30 ± 21	54 ± 30	32 ± 21	58 ± 25
Desire to eat	66 ± 22	42 ± 26	68 ± 22	38 ± 31	67 ± 21	39 ± 27
Appetite sweet	49 ± 27	54 ± 30	49 ± 27	52 ± 31	55 ± 26	14 ± 16
Appetite savory	62 ± 24	34 ± 29	65 ± 24	30 ± 31	62 ± 25	55 ± 31
Prospective consumption	58 ± 20	36 ± 22	61 ± 20	33 ± 26	58 ± 19	32 ± 22
Thirst	67 ± 21	50 ± 29	62 ± 22	54 ± 27	62 ± 23	46 ± 27

* Fullness ratings before intake of the luncheon meat differed significantly between the hard (30±23) and the soft version (35±22), ($F(1,104) = 5.1$, $P=0.03$).

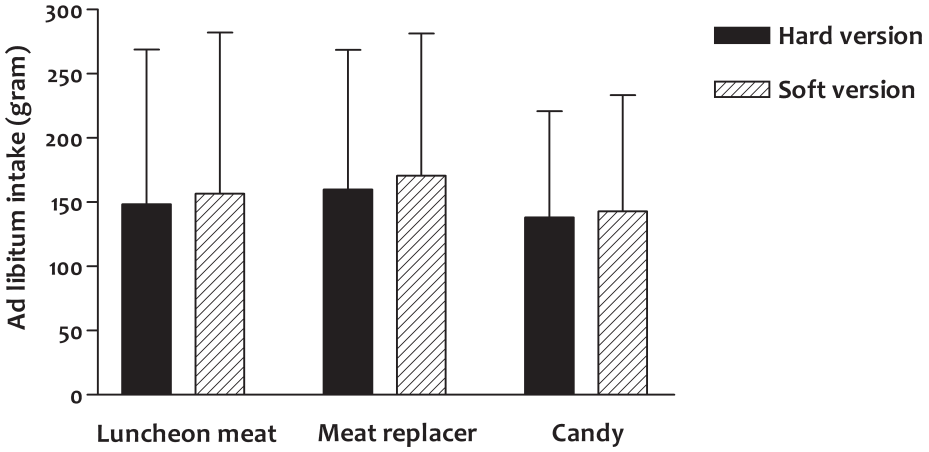

Figure 5.2 *Ad libitum* intake (mean ± SD) of the hard and soft version of luncheon meat, meat replacer and candy in gram.

Eating rate

The eating rate of the hard luncheon meat, 21 ± 10 g/min, was significantly slower than the eating rate of the soft luncheon meat, 25 ± 13 g/min ($F(1,98)=21.4$, $P<0.0001$). Eating rates of the hard and soft version were not significantly different within the other food groups. Mean eating rate for the hard and soft meat replacers was 19 ± 9 and 19 ± 16 g/min and mean eating rate for both the hard and soft version of the candies was 8 ± 4 g/min (**Figure 5.3**).

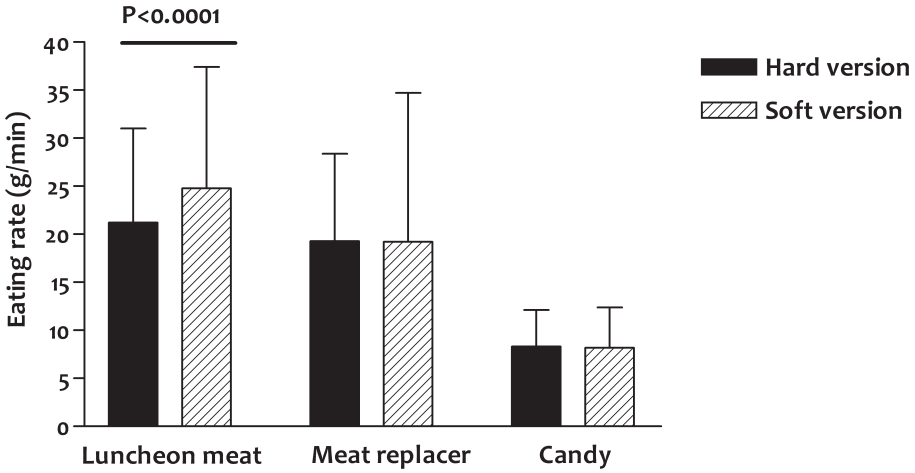

Figure 5.3 Eating rate (mean ± SD) of the hard and soft version of luncheon meat, meat replacer and candy in g/min.

Correlations between liking, eating rate and intake

Liking scores were significantly correlated with *ad libitum* intake, overall (r=0.56; *P*<0.0001) and for all test products separately (*P*<0.05) with correlation coefficients varying from r=0.44 to r=0.56. Liking scores were also significantly correlated with eating rate (*P*<0.05) but only for the product groups luncheon meat (hard r=0.30 and soft r=0.29) and meat replacer (hard r=0.22 and soft r=0.24).

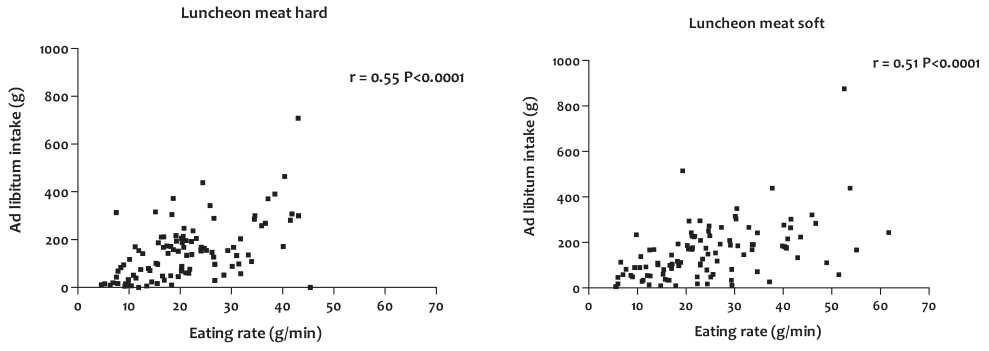

Figure 5.4 Correlation between eating rate (g/min) and *ad libitum* intake (g) for the hard and soft luncheon meat.

Eating rate of the fixed amounts of products in the 7th test session was significantly correlated with *ad libitum* intake in the earlier test sessions for all test products ($P<0.05$), with the strongest correlation in the luncheon meat products (**Figure 5.4**). Correlation coefficients were: luncheon meat: hard r=0.55, soft r=0.51; meat replacer: hard r=0.36, soft r=0.28; candy: hard r=0.34, soft r=0.22. The overall correlation between eating rate and *ad libitum* intake was r=0.54 ($P<0.0001$). This correlation was approximately the same in men (r=0.49 and $P=0.0007$) and women (r=0.50 and $P<0.0001$).

Correlations in eating rate between products and within subjects

The eating rate of the hard versions of a product pair was strongly correlated with the soft version of the product pair ($P<0.0001$); luncheon meat r=0.76; meat replacer r=0.40 and candy r=0.83. Since the eating rate within a product pair was strongly correlated, we calculated a mean eating rate per person for each product group (averaging the eating rate of the hard and soft version). To investigate if the subjects who consumed items of one product group faster also consumed items of another product group faster, we correlated the mean eating rate of luncheon meat with the mean eating rate of meat replacer (r=0.40 and $P<0.0001$), luncheon meat with candy (r=0.37 and $P=0.0002$), meat replacer with candy (r=0.29 and $P=0.003$).

Reasons to stop eating

Table 5.5 gives an overview of the mean answer scores to what extent subjects agreed with the propositions of the reasons for stopping eating. There were no significant differences in scores for the hard and soft version of a product pair. As can be seen from this table,

Table 5.5 Ratings (mean ± SD) proposition reasons stopping with eating, measured on 100 mm VAS scales ranging from "totally disagree" to "agree completely"

	Luncheon meat		Meat replacer		Candy	
	Hard	Soft	Hard	Soft	Hard	Soft
Enough of taste	80 ± 21	77 ± 25	82 ± 21	81 ± 20	67 ± 30	69 ± 28
Full	40 ± 32	40 ± 32	43 ± 34	46 ± 35	51 ± 33	45 ± 31
Tired	12 ± 19	13 ± 17	15 ± 22	15 ± 21	14 ± 20	17 ± 22
Product cold	3 ± 7	4 ± 10	17 ± 25	21 ± 28	4 ± 9	4 ± 8
Smell too strong	32 ± 33	33 ± 33	47 ± 35	48 ± 33	9 ± 15	10 ± 17
Others stopped eating	4 ± 10	6 ± 12	6 ± 10	5 ± 10	5 ± 9	5 ± 9
Portion was finished	2 ± 2	3 ± 10	2 ± 3	2 ± 3	2 ± 3	2 ± 3
Consumed too much	23 ± 26	28 ± 28	29 ± 31	31 ± 31	39 ± 34	39 ± 32

having enough of the taste of the products receives the highest ratings, followed by feeling full. The most frequent answers given in the open-ended answer category had to with: not liking the product, becoming nauseated, finding the product and/or texture unusual, being fed up with the product, being thirsty and/or not having enough water, and being distracted by the movie.

DISCUSSION

This study investigated the effect of texture differences on satiation in three pairs of specially developed solid foods, to determine if solid food products that require a relatively longer oral processing time ("hard" version), would lead to a lower *ad libitum* intake compared to products that require a relatively shorter oral processing time ("soft" version). No significant parametric differences in *ad libitum* intake were found between the hard and the soft versions of the products. However, there were only small to no differences in eating rate between the hard and soft versions.

On the basis of texture differences, it was expected that one version of a food pair would require a relatively longer oral processing time than the other version, leading to a slower eating rate. Although the rheological measurements indicate that indeed more force was needed to cut and compress the hard versions of the products than the soft versions, this difference was not reflected in the subjective measurements of attributes such as firmness, toughness, fibrousness or effort needed for chewing, for all products. In the product group luncheon meat the difference between the hard and soft version was noticed, since this version was rated as less smooth, more firm and needing more effort to chew. Additionally, the texture differences in this food group also led to a difference in eating rate; the soft version of the luncheon meat was eaten faster than was the hard version (25 g/min vs. 21 g/min). This difference was probably not large enough to lead to differences in *ad libitum* intake, as we had expected based on our hypothesis. However, in line with our hypothesis, significantly more subjects consumed more of the soft version of luncheon meat than the hard version (67 vs. 39). For the other two food groups, the meat replacer and the candies, our assumption that texture differences would lead to a difference in eating rate was not met. This probably explains why we found no difference in either *ad libitum* intake or in number of subjects eating more of the soft version of these products.

It is important to note that in choosing the products for this study we were bound by technical constraints; it was very difficult to create solid products equal in energy content, macronutrient composition and liking but different in texture. The products chosen for this research were among the few for which it was possible to manipulate texture while keeping other food characteristics similar. As soon as texture differences were made more extreme, many other characteristics of the products changed and so many other factors

influencing food intake could not be controlled for. Perhaps at this time it is technically not possible to create the products needed for this kind of research.

This study was performed in a cinema to create a real-life setting and to distract subjects from visual and weight cues. A strength of this study was the large number of subjects, and the cinema setting made it possible to test a large number of subjects at the same time. However, the setting could also have influenced the results. Although it was dark in the cinema and subjects were not seated next to each other to avoid interaction while eating, subjects talked to each other in the breaks. Furthermore, the distraction of watching a movie could have led to higher food intakes than in an other setting. Social situation and setting can affect eating behavior, as has been shown by a number of studies [e.g. 16-19]. To what extent our results were influenced by the setting and the social situation is unknown. However, any possible effect that might have influenced the data, probably influenced the data of all food products and on all test evenings to the same degree, since overall *ad libitum* intake was not significantly different between the different test days.

In addition to the texture of the products, liking of the products is also very important. In this study the liking for the test products was relatively low, especially for the luncheon meat and the meat replacer (around 40 – 50 mm on 100 mm VAS scales). Although, a lower liking for "experimental" products is not very uncommon, this might have influenced our results. On the "reasons to stop eating" questionnaire, "having enough of the taste of the products" received higher ratings than the reason "I was full". Probably an increased liking for a product increases your eating rate and a decreased liking will decrease eating rate. In the present study there was a significant positive correlation between liking and eating rate for the food groups luncheon meat and meat replacer. Yeomans et al. have also shown that enhancing the palatability of foods indeed increases eating rate [20].

In a recent study, Viskaal-van Dongen et al. [21] measured the eating rate of an array of 27 frequently consumed food products varying in texture from liquid to solid. Eating rate differed enormously, varying from 9 g/min (a solid product) to 334 g/min (a liquid product). Interestingly, in that study there was also a strong correlation between eating rate (g/min) and intake (g), r=0.58, $P<0.001$. This is in agreement with the overall correlation coefficient of 0.54 between eating rate and intake in the present study. The strong correlation between eating rate and food intake is very interesting and suggests that texture differences in foods, which would lead to differences in eating rate, can potentially have a large effect on intake.

The importance of eating rate in food intake behavior has already been shown by a number of other studies. For example Andrade et al. have shown that *ad libitum* intake of a meal was lower and satiety ratings were higher, when subjects were instructed to eat slowly [22]. Martin et al. have shown similar results but only for men and not for women [23]. Also our previous study showed that eating with small bite sizes and a longer oral processing time,

thus at a slower eating rate, decreased *ad libitum* intake [10]. It would be interesting to know the biological relevance of this relation between eating rate and intake. Eating rate seems to be a characteristic of a person since individual behavior was strongly correlated in this study. Subjects who consumed one product faster also consumed the other products faster. It would be interesting to find out more about the factors determining a person's eating rate. One contribution to this question is provided by study of Llewellyn et al. showing a higher correlation of eating rate within monozygotic twin pairs compared to dizygotic twin pairs, suggesting a strong genetic contribution to eating rate [24]. Further research on the individuality of eating rate is necessary.

Although we did not find differences in intake between the hard and soft version of the test products in this study, we still maintain our concept that eating more slowly and taking more time to orally process the food will result in a longer oral exposure time, which will lead to a lower *ad libitum* food intake. The significant correlation between eating rate and *ad libitum* intake, and the data on the luncheon meat, provides support for this concept. In the luncheon meat we see the largest differences in eating rate, the strongest correlations between intake and eating rate and significantly more subjects consuming the soft version. Our concept is additionally confirmed by studies such as that of Weijzen et al. [25], showing that increased oral sensory exposure per unit of consumption can lower intake of sweet drinks. Further support derives from our previous study in which we showed that more oral sensory exposure to a product, through small bite sizes and increased oral processing time due to holding the product longer in the mouth, significantly decreases food intake [10]. We expect that factors such as oral processing and oral sensory exposure are also very relevant in solid foods, but that the relation with intake is more difficult to show in an experiment. As far as we know, we are the first to investigate the effect of texture differences of solid foods on satiation, and although texture differences in our study were too small to lead to differences in eating rate and intake, the study does provide valuable information by showing that texture differences in foods need to be more dramatic to affect eating rate and food intake.

In conclusion, so far the effect of texture differences of solid foods on satiation is not yet clear. Differences in texture of the hard and soft version of the test products may have been too subtle to lead to differences in eating rate. However, we still believe that oral processing and oral sensory exposure are very relevant factors influencing intake of solid foods and that the challenge for future research lies in creating solid food products that are similar in liking and macronutrient composition but different enough in texture to lead to clear differences in eating rate.

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6

Eating behavior and retro-nasal aroma release in normal weight and overweight subjects

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Submitted

ABSTRACT

Eating rate and bite size are shown to be important factors affecting food intake and we hypothesize that oral sensory exposure plays an underlying role herein. Currently no objective parameters are available to measure oral sensory exposure, but an interesting novel measure is extent of *in vivo* retro-nasal aroma release. It is thought that overweight subjects differ from normal weight subjects in eating behavior, but literature is ambiguous. Consequently the aims were to investigate 1) if eating behavior (food intake, eating rate, bite size, number of bites, meal duration) relates to weight status and 2) whether extent of retro-nasal aroma release relates to eating behavior and weight status. A matched group (on gender, age and dietary restraint score) of 27 normal weight (BMI 21.8 ± 1.6 kg/m^2) and 27 overweight/obese subjects (BMI 30.5 ± 5.8 kg/m^2), consumed a spiced rice meal and apple-pie yoghurt *ad libitum* on separate test days. Extent of retro-nasal aroma release was measured on a third test day. Mean bite size for spiced rice was significantly (P=0.03) larger in the overweight/obese (10.3 ± 3.2 g) versus the normal weight subjects (8.7 ± 2.1 g). There were no other significant differences in eating behavior or extent of retro-nasal aroma release between the weight groups. None of the eating behaviors were correlated with BMI or to extent of retro-nasal aroma release. Subjects showed consistent eating behavior for both products. Eating behavior might be a characteristic of an individual but not by definition a characteristic for a group of people based on their weight.

INTRODUCTION

Given the growing rate of obesity [1], we need to know more about factors influencing food intake. The rate of eating and bite size are important factors shown to affect intake [2-6]. Food characteristics such as food texture have an effect on these factors. A recent study of Viskaal-van Dongen and de Graaf showed that eating rates differed enormously between a number of frequently consumed foods [7]. In our previous studies we showed that a liquid product was eaten faster and with larger bite sizes than the same product in semi-solid form [2, 8]. Next to food characteristics, individual aspects determine eating behavior. In a previous study we observed that subjects who consumed one product faster, also consumed the other products faster [9]. Spiegel et al. observed that subjects who ate the most, paused the most or chewed the fastest in one condition also did this in the other conditions [10]. Westerterp et al. also showed consistency in individual eating behavior for chewing time per bite and bite interval [11].

Based on our previous studies [2, 6], we expect that oral sensory exposure is an important underlying factor involved in the relation of eating rate and bite size to food intake. Eating slower and taking more time to process the food in the mouth results in longer oral exposure time. This longer oral exposure time may lead to earlier sensory satiation and a lower *ad libitum* food intake. Additionally, eating with small bite sizes may lead to relatively more oral sensory exposure to a product. Currently there is no direct objective measure for oral sensory exposure available, but a novel interesting measure is the extent of *in vivo* retro-nasal aroma release [12, 13].

Retro-nasal aroma release is an important aspect in the sensory perception of a food. The transfer of aroma via the retro-nasal route is also related to the texture and the volume (i.e. bite size) of food in the mouth [14]. Additionally, different eating styles related to chewing force, chewing rate and number of chews, have been shown to result in differences in aroma release measurements [15]. Furthermore, in a recent study of Ruijschop et al. the extent of retro-nasal aroma release appeared to be subject specific and an inverse trend was observed between aroma release and *ad libitum* food intake (P=0.07) [16]. Together, the above mentioned information indicates that *in vivo* retro-nasal aroma release might be a possible marker for oral sensory exposure. Although this technique measures aroma release and not all of oral sensory exposure, it is a novel and interesting measure to investigate.

Though data are ambiguous, studies indicate that overweight and/or obese subjects may have a different eating behavior compared with normal weight subjects [17-20]. These groups might thus potentially also differ in extent of oral sensory exposure and in extent of in vivo retro-nasal aroma release.

Consequently, the aims of the current study were to investigate 1) if eating behavior (i.e. food intake, eating rate, bite size, number of bites, meal duration) relates to weight status

and 2) whether the extent of retro-nasal aroma release relates to eating behavior or weight status. To study these aims, eating behavior was investigated in a controlled manner of observation (including weighing scale and video-observations) but in an uncontrolled manner of eating (without giving instructions on eating rate and bite size) and the extent of *in vivo* retro-nasal aroma release was measured on a separate day. As far as we know, we are one of the first to investigate the extent of retro-nasal aroma release in a large group of subjects with different BMIs and relate this directly to eating behavior such as *ad libitum* intake and eating rate.

SUBJECTS AND METHODS

Subjects

Subjects were recruited in the surroundings of Ede and Wageningen (the Netherlands) via flyers, advertisements in local papers, posters and emails to persons in a database of volunteers willing to participate in studies of Wageningen University. Furthermore, a part of the subjects was recruited via a recruitment agency who emailed potential overweight and obese subjects in their database. Subjects had to be healthy, 18 – 55 years, normal weight (BMI 18.5 – 25.0 kg/m^2) or overweight/obese (BMI >25.0 kg/m^2) and they had to like the test products (scoring liking ≥5 on a 9-point scale with a description of the test products). Exclusion criteria were: lack of appetite for any reason; following an energy restricted diet during the last 2 months or planning to start an energy restricted diet during the time of the study; change in body weight >5 kg during the last 2 months; stomach or bowel diseases, diabetes, thyroid disease, or any other endocrine disorder; having difficulties with eating or swallowing; or hypersensitivity for the test products.

Potential subjects were invited for a screening/training visit to Wageningen University, during which body weight and height were measured. Furthermore, subjects practiced with the computer program which was going to be used for satiety and sensory ratings on Visual Analogue Scales (VAS) and for a computerized food preference questionnaire. No food was provided during the training session.

Subjects were not aware that the aim of the study was to measure eating behavior, but were informed that the study was designed to measure the pleasantness of different products and to investigate if this correlates with the extent of retro-nasal aroma release. The study was approved by the Medical Ethical Committee of Wageningen University and all subjects gave their informed consent.

Overweight/obese subjects were individually matched to normal weight subjects, based on gender, age and restraint eating score (Dutch Eating Behaviour Questionnaire) [21]. The data of 54 subjects was included in data analysis of eating behavior, which included 27 overweight/obese subjects (6 men, 21 women, mean BMI 30.5 ± 5.7, mean age 36 ± 14

years and mean restraint score 2.9 ± 0.7) matched with 27 normal weight subjects (6 men, 21 women, mean BMI 21.8 ± 1.6, mean age 36 ± 14 years and mean restraint score 2.9 ± 0.8). The overweight/obese subject group will hereafter be referred to as 'overweight group' and data of both the overweight and obese subjects is included in the results of this group. Retro-nasal aroma release data of 24 matched pairs (n=48) were available for data analysis.

Experimental design

The study had a randomized crossover design in which all subjects came twice to Wageningen University (Wageningen, the Netherlands) for a sensory test session. Subjects participated in a third session at NIZO food research (Ede, the Netherlands) during which the extent of *in vivo* retro-nasal aroma release was measured.

Test products

A simple spiced rice (Dutch: nasi) was chosen as neutrally liked meal product and a sweet, creamy apple-pie yoghurt with currents as highly liked product. Data of the sensory sessions showed that mean liking was 67 ± 16 for the rice and mean liking was 83 ± 13 for the yoghurt (100 units VAS scales, no differences between the weight groups, P<0.001 between products). Other sensory ratings (100 units VAS scale) were: rice mean saltiness 49 ± 20 and spiciness 28 ± 21; yoghurt mean sweetness 69 ± 16 and creaminess 76 ± 13.

One hour before each sensory test session, the spiced rice was prepared in a standardized manner with white rice (AH private label, Albert Heijn, Zaandam, the Netherlands) and nasi flavourings (Knorr, Unilever, Rotterdam, the Netherlands). The prepared product was stored au bain-marie at 80°C. The serving temperature was measured between 67 and 71°C. The apple-pie yoghurt was a commercially available product (Mona, FrieslandCampina Consumer Products Europe, Amersfoort, the Netherlands). The yoghurt was stored in a refrigerator between 3-5°C and removed at a standardized time before the test session. Serving temperature was measured between 8.5 and 10°C.

For the retro-nasal aroma release session, the spiced rice was prepared beforehand in the same manner as described above and afterwards deep-frozen. Before a test session, the rice was defrosted, heated in a microwave and kept warm in a water bath of 37°C before serving. The apple-pie yoghurt was stored in a refrigerator at 4°C and removed at a standardized time before the start of measurements.

Macronutrient content was determined by chemical analysis; protein by the Kjeldahl method, fat by the acid hydrolysis method, fibre by the Prosky method; subsequently carbohydrate was calculated by subtracting moisture, ash, fiber, protein and fat from total weight. Energy content was calculated from the macronutrient composition by using the

following energy conversion factors: protein 16.7 kJ/g; fat 37.7 kJ/g; carbohydrate 15.7 kJ/g. Per 100 g, the spiced rice contained 563 kJ (134 kcal), 3.2 g protein, 27.3 g carbohydrates, 2.2 g fat and 1.6 g fiber. Per 100 g, the apple-pie yoghurt contained 522 kJ (125 kcal) and 2.3 g protein, 16.4 g carbohydrates, 6.0 g fat and 0.3 g fiber.

Procedure sensory test sessions

The two sessions at Wageningen University were separated by at least one day and were held in the sensory cabins around lunch time, starting at 11.30 a.m., 12.30 p.m. or 1.30 p.m. For every subject, the second test session started at the same time as the first test session. The order of the test products was randomly allocated to the subjects. However, the rice and yoghurt were never given to different subjects in the same session, since the smell of the rice might influence eating behavior of the apple-pie yoghurt.

At the beginning of the session, subjects rated hunger, fullness, desire to eat, appetite for something sweet, appetite for something savory, prospective consumption and thirst on 100 unit VAS scales on a computer screen. Scales were anchored from "not at all/very little" to "very much".

When finishing the ratings, explicit and implicit aspects of food choice were assessed by means of a computerized food preference questionnaire (FPQ, paradigm of Finlayson et al. [22, 23], data not shown in this paper). Next, subjects received a small amount of the test product (on average 31 ± 4 g spiced rice or 47 ± 4 g apple-pie yoghurt) to rate liking and other sensory attributes of the test products on 100 unit VAS scales. After that, subjects received a large bowl of test product and were instructed to eat as much of the products as they wanted until they were pleasantly satiated. In order to try to keep any visual or weight cues from the subjects and to stimulate *ad libitum* intake, a surplus of the test product was served; on average 901 ± 7 g spiced rice and 1301 ± 1 g apple-pie yoghurt. The offered amount in weight was different between the products because of differences in energy density, but visually the bowls were filled with approximately the same volume. After *ad libitum* intake, subject had to rate their degree of hunger and satiety again by the same questions as in the beginning of the session, received a small amount of test product (on average 32 ± 4 g spiced rice or 47 ± 3 g apple-pie yoghurt), had to rate liking and sensory attributes again and the FPQ was rerun.

To prevent subjects leaving early or stopping with eating because of other reasons than being satiated, they were instructed to stay at least 45 min in the sensory cabins. Additionally, we wanted to prevent subjects distracting each other when finished, and this time period allowed everyone to finish eating at their own pace. Reading material was provided when subjects were finished.

Measurements of eating behavior

Food intake was measured via hidden electronic scales (KERN 440, ATP-Messtechnik, Ballingen, Germany) in the tables of the sensory cabins. Data was saved by specially developed software. During eating, weight of the offered test meal was registered every 0.5 s. In addition, the product was weighted before the meal was offered and immediately after the subjects returned the bowl, which was done on a different electronic scale (model 1203-MP, Sartorius, Goettingen, Germany).

The test sessions were videotaped via a camera. The video recordings were compared with the data of the electronic scales to determine total *ad libitum* intake, meal duration, eating rate, number of bites and bite size in an accurate way. To reduce errors in weighing, participants were given instructions to only take food on their spoon until ready to ingest it, not to leave the spoon in the bowl during eating and to leave the spoon next to the food bowl when completing ratings or finished eating. Intake, meal duration, eating rate, number of bites and bite size were measured in the following way:

Total ad libitum intake – The difference between the weight of the product before the meal was offered and the weight after the subject returned the bowl.

Meal duration – The by the subject defined eating period. In the computer program the subjects had to press an "I start with eating" button and an "I am satisfied" button. The time difference between these two moments was defined as the meal duration. For seven of the 108 measurements, the actual meal duration was longer since the subject took one or more bites before or after this time period, which was observed on the video recordings. Using these recordings we corrected meal duration; the time between the spoon touching the food and the last chewing movements outside the self-defined period was added, to approach the true meal duration. For one subject the actual meal duration was shorter since this person forgot to press the "I am satisfied button". Meal duration was again corrected by means of the video recordings.

Eating rate – The total *ad libitum* intake in gram divided by meal duration in minutes. Initial eating rate and deceleration rate were calculated from curve fitting the cumulative intake curves (see section on statistical analysis).

Number of bites – The number of bites counted from the video recordings. A bite was defined as a contact with the food followed by chewing or swallowing. When subjects took two bites from one spoonful, which were both followed by a period of chewing and swallowing, this was counted as two bites.

Bite size – Total *ad libitum* intake divided by the number of bites.

Standardization of satiety state for the sensory test sessions

To standardize individual's initial state of satiety, subjects were instructed to eat the same breakfast at home on both test days and to record their food and drink consumption in a diary. Furthermore, they were instructed not to consume anything except water after 10 a.m. and until 1 h after the test session. This last instruction was not controlled, but was given to promote subjects consuming until satiated from the test products.

Procedure and measurements of *in vivo* retro-nasal aroma release

The retro-nasal aroma release session took place between 8.30 a.m. and 4.00 p.m. Subjects were instructed not to consume coffee in the 2 h before the test session and not to use strong perfumes or deodorants on the day of the measurement.

Prior to the *in vivo* retro-nasal aroma release measurements, the aroma compounds with the highest response in air released from an artificial mouth were monitored by on-line sampling by an Atmospheric Pressure Chemical Ionisation Gas-Phase Analyzer (APcI-GPA) attached to a VG Quattro II mass spectrometer (MS-Nose; Micromass UK Ltd., Manchester, UK) [12, 13, 24, 25]. Compounds were ionized by a 3.0 kV discharge (source and probe temperature were 80°C) and scanned for m/z 50-250. M/z values (i.e. the ion mass to charge ratio of a specific aroma component) with the highest response were selected (**Table 6.1**).

During the test session, *in vivo* retro-nasal aroma release was assessed in the exhaled breath of the subjects using real-time atmospheric pressure chemical ionization mass spectrometry (APcI-MS). Subjects consumed three consecutive spoonfuls of test product (fixed amount, on average 12 ± 2 g for the spiced rice and 19 ± 1 g of apple-pie yoghurt). During eating, subjects breathed in and out via the nose while one nostril was placed over a small disposable plastic tube. Subjects could breath and eat normally, no chewing/swallowing instructions were given. Aroma compounds in the air released from the exhaled

Table 6.1 Test products used during the *in vivo* retro-nasal aroma release session, their serving and weight and with their m/z-value with the highest response in atmospheric pressure chemical ionization mass spectrometry (APcI-MS) measurement

Product	Serving per measurement	Weight per serving spoon	Ion mass (m/z value)	Aroma component
Spiced rice	1 spoonful	12 ± 2 g	73	2-Butanone
			87	Diacetyl Pentatone
Apple-pie yoghurt	1 spoonful	19 ± 1 g	137	Limonene Terpinene
			149	Cumic Aldehyde

breath of the subjects was monitored by on-line sampling of the exhaled air directly into the APcI-MS via the tube. The air was sampled (75 mL/min) through a capillary tube (0.53 mm internal diameter, heated to 100°C). The compounds were monitored in selected ion mode (0.08 s dwell on each ion), in two independent sets. The cone voltage was 20V.

The two food products were measured separately. First the spiced rice was measured followed by the apple-pie yoghurt. Subjects consumed a plain cracker and rinsed their mouth with water between the two test products. Blank experiments with water were recorded before the consumption of the test products, according to the same protocol.

Acetone, present in human breath, was measured at m/z 59 (19 V) as an indicator for the breathing pattern [13, 26]. The area of the resulting breath peaks in the aroma signal was taken as a measure of in vivo retro-nasal aroma release. Since we were interested in comparative retro-nasal aroma release between subjects, expression of the extent of retro-nasal aroma release in arbitrary units (A.U.) was sufficient to analyze differences [13]. The following parameters were extracted from each individual retro-nasal aroma release curve:

- I_{max} = the maximum intensity (A.U.)
- T_{max} = time at which maximum intensity occurs (min)
- AUC = total Area Under Curve (A.U. x min)

The characteristic retro-nasal aroma release parameters (T_{max}, I_{max} and AUC) were averaged for each subject, for each product from the three consecutive bites. This procedure is allowed, since subjects are reproducible in their extent of retro-nasal aroma release during consumption of a specific food product [14, 16]. Due to the lower signal-to-noise ratio for the m/z values 137 (spiced rice) and 87 (apple-pie yoghurt) compared to the m/z values 149 (spiced rice) and 73 (apple-pie yoghurt), the background noise, as measured with the blank experiments, was subtracted from the measurements with the two test products per subject for all m/z values.

After consuming the test products for the retro-nasal aroma release measurements, subjects rated intensities of smell, taste, and aftertaste of the product on 100-mm VAS, anchored from "not at all intense" to "very intense" (data not shown, but no significant differences between weight groups).

Statistical analysis

Data are presented as means ± standard deviations (SD). Statistical analyses were performed by means of SAS (version 9.1.3; SAS Institute Inc., Cary, NC, USA). Significance was set at $P<0.05$.

Differences in liking, sensory ratings, eating behaviors and satiety ratings between the group of normal weight subjects and the group of overweight subjects were analyzed with paired student *t*-tests. A paired student *t*-test was chosen because subjects were

individually matched on gender, age and restraint score. Correlation analyses on eating behavior were performed by means of Pearson correlation analysis.

The cumulative food intake was fitted per person per product to a quadratic equation: $y = a + bt + ct^2$, where b = constant slope of the curve over time, i.e. initial eating rate and c = change in the slope of the curve over time, i.e. rate of deceleration [27, 28]. Differences in initial eating rate and deceleration rate between the normal weight and overweight group were analyzed by means of a paired student *t*-test.

Rank scores were calculated for all retro-nasal aroma release parameters (T_{max}, I_{max} and AUC) for each test product, since the two food products had a different magnitude of the characteristic retro-nasal aroma release parameters and the data was not normally distributed. Furthermore, a combined sum rank of rank I_{max} and rank AUC was calculated per product. This combined ranking is a value with respect to retro-nasal aroma release intensity and will hereafter be referred to as "intensity". Also, a combined sum rank of rank I_{max} and rank T_{max} was calculated per product. This combined ranking is a value with respect to retro-nasal aroma release morphology and will hereafter be referred to as "morphology" [16].

The different characteristic retro-nasal aroma release parameters were compared between the group of normal weight and overweight subjects by means of Wilcoxon signed rank sum test. Furthermore, retro-nasal release parameters were correlated with BMI and eating behaviors (values obtained from the sensory sessions) by means of Spearman correlation analysis.

RESULTS

Eating behavior and weight status

Table 6.2 gives an overview of the mean results on eating behavior for both normal weight and overweight subjects and both test products. There were no statistically significant differences in *ad libitum* intake, meal duration, eating rate or number of bites between the normal weight and overweight group for either test product. The eating rate of the apple-pie yoghurt in the overweight group was on average higher compared to the normal weight group but this was not significant. The same applies to the mean bite size of the apple-pie yoghurt, which also tended to be higher in the overweight group. There was a significant difference in mean bite size for spiced rice; the overweight subjects consumed this product on average with larger bite sizes than the normal weight subjects ($t(26)=-2.26$, $P=0.03$).

Ad libitum intake and eating rate were significantly higher ($P<0.05$) in men compared to women, for both test products. Mean bite size and meal duration were also significantly higher ($P<0.05$) in men, but only for the apple-pie yoghurt (data not shown).

Initial eating rate and deceleration rate were not significantly different between the normal weight and overweight group for either product. Initial eating rate for the spiced

Table 6.2 *Ad libitum* intake, meal duration, eating rate, number of bites and bite size (all mean ± SD) of the normal weight subjects and overweight subjects for the test products spiced rice and apple-pie yoghurt

	Spiced rice		Apple-pie yoghurt	
	Normal weight *n*=27	Overweight *n*=27	Normal weight *n*=27	Overweight *n*=27
Ad libitum intake (g)	281 ± 148	276 ± 124	458 ± 216	480 ± 201
Meal duration (s)	457 ± 189	456 ± 151	315 ± 105	306 ± 106
Eating rate (g/min)	37 ± 10	38 ± 15	89 ± 32	100 ± 43
Number of bites	34 ± 21	28 ± 13	36 ± 17	33 ± 12
Bite size (g) [1]	8.7 ± 2.1*	10.3 ± 3.2*	13.6 ± 4.5	16.2 ± 7.4

* Significantly different from each other, t (26) = -2.26, P= 0.03.

rice was 0.50 ± 0.65 g/s (converted 30 ± 39 g/min) for the normal weight and 0.58 ± 0.21 g/s (converted 35 ± 13 g/min) for the overweight group. Initial eating rate for the apple-pie yoghurt was somewhat higher in the overweight group, 2.11 ± 1.52 g/s (converted 127 ± 91 g/min) versus the normal weight group 1.51 ± 1.19 g/s (converted 91 ± 71 g/min) but this was not significant (t(26)=-1.68, P=0.10).

BMI had no significant correlation (P>0.05) with *ad libitum* intake or any other eating behavior (correlation coefficients varied from -0.09 to 0.21).

Correlations between eating behaviors

Many of the eating behaviors were significantly correlated (P<0.05) with each other. *Ad libitum* intake significantly positively correlated with meal duration (rice r=0.71; yoghurt r=0.52), eating rate (rice r=0.56; yoghurt r=0.60), number of bites (rice r=0.74; yoghurt r=0.46) and bite size (rice r=0.27; yoghurt r=0.48). Bite size was also positively correlated with eating rate (rice r=0.37; yoghurt r=0.71) and negatively correlated with number of bites (rice r=-0.30; yoghurt r=-0.48). Number of bites was positively correlated with meal duration (rice r=0.54; yoghurt r=0.64) and to eating rate but only for rice (r=0.36). Eating rate was additionally correlated with meal duration but only for the yoghurt product (r=-0.33).

Satiety ratings

All satiety ratings decreased significantly after *ad libitum* intake for both products (P<0.05), except for fullness which, as expected, increased significantly after intake (P<0.05). There were no significant differences in satiety ratings between the normal weight group and the overweight group for both products. Thirst was significantly (t(26)=-3.68, P=0.001) lower after intake of the apple-pie yoghurt in the normal weight group (36 ± 25) compared

to the overweight group (58 ± 24). **Table 6.3** gives an overview of the satiety and thirst ratings per product, averaged over all subjects.

Retro-nasal aroma release and weight status

The parameters of retro-nasal aroma release, rank I_{max}, rank AUC and rank T_{max}, were not significantly different between the normal weight and overweight group for either test product. Also the combined ranking for intensity and morphology were not statistically significantly different between the two groups for either test product.

BMI was not correlated with the ranks of T_{max}, AUC and I_{max} for either test product, nor with the sum ranks for intensity or morphology (correlation coefficients varied between -0.24 to 0.11).

Retro-nasal aroma release and eating behaviors

None of the retro-nasal aroma release parameters (rank T_{max}, rank AUC and rank I_{max}) nor the sum ranks for intensity or morphology, were significantly correlated with *ad libitum* intake, meal duration, eating rate, number of bites or mean bite size for either test product (correlation coefficients varied between -0.19 to 0.24).

Eating behavior and retro-nasal aroma release within an individual

Subjects showed consistent eating behavior for both products; *ad libitum* intake and the other eating behaviors of the spiced rice were stongly correlated with the intake and eating behaviors of the apple-pie yoghurt (**Figure 6.1**).

Table 6.3 Average satiety ratings before and after ad libitum intake of the spiced rice and apple-pie yoghurt. Mean scores ± SD, averaged over all subjects (*n*=54)

	Spiced rice		Apple-pie yoghurt	
	Before *ad libitum* intake	After *ad libitum* intake	Before *ad libitum* intake	After *ad libitum* intake
Hunger	67 ± 21	10 ± 11	68 ± 18	13 ± 12
Fullness	22 ± 17	76 ± 16	23 ± 17	77 ± 19
Desire to eat	75 ± 19	21 ± 16	75 ± 16	19 ± 17
Appetite for something sweet	56 ± 24	41 ± 29	61 ± 22	12 ± 12
Appetite for something savory	74 ± 21	15 ± 19	71 ± 22	34 ± 27
Prospective consumption	65 ± 16	22 ± 14	65 ± 14	22 ± 18
Thirst	63 ± 21	79 ± 19	57 ± 24	47 ± 27*

* Average thirst ratings after intake of the apple-pie yoghurt were significantly lower ($t(26) = -3.68$, $P=0.001$) in the normal weight group (36 ± 25) compared with the overweight group (58 ± 24).

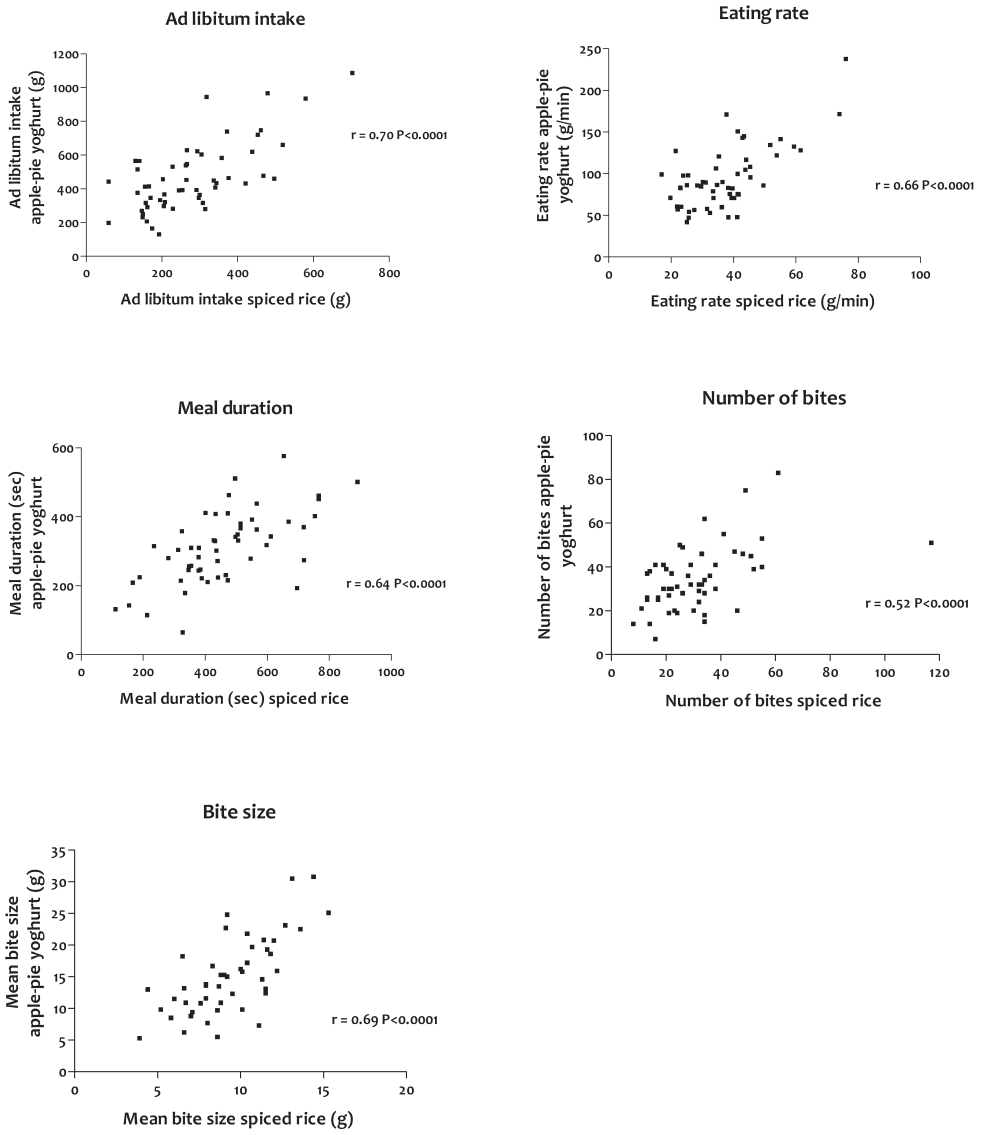

Figure 6.1 Associations between eating behaviors for the two test products spiced rice and apple-pie yoghurt, (within subjects *n*=54). r = Pearson's correlation coefficient.

Within subjects, rank numbers of T_{max} and AUC for the spiced rice were significantly correlated with the rank numbers of T_{max} and AUC for the apple-pie yoghurt (r=0.29, *P*=0.049 and r=0.54, *P*<0.0001 respectively). Also the sum rank for intensity and morphology for spiced rice were significantly correlated with that for apple-pie yoghurt (intensity r=0.39, *P*=0.006; morphology r=0.44, *P*=0.002). Only the ranks of I_{max} were not significantly correlated (r=0.21, *P*=0.15) between the spiced rice and apple-pie yoghurt.

DISCUSSION

The aim of this study was to investigate if eating behavior or extent of retro-nasal aroma release relates to weight status. Furthermore, to relate differences in eating behavior, assumed to affect oral sensory exposure, to extent of retro-nasal aroma release. We found a significant difference in bite size of the spiced rice product between the weight groups; the overweight subjects took on average larger bite sizes than the normal weight subjects. We found no significant differences between the weight groups in either *ad libitum* intake, other eating behavior or extent or retro-nasal aroma release. Extent of retro-nasal aroma release was not correlated with eating behavior such as *ad libitum* intake and eating rate. The overweight subjects showed comparable eating behavior to that of normal weight subjects. Overall we found that eating behavior is very consistent within individuals.

Eating behavior between weight groups was investigated for two types of test products, i.e. a more neutral spiced rice meal type product and a highly liked apple-pie yoghurt. This enabled us to investigate if possible effects were consistent for two different products. We expected beforehand that differences in eating behavior would probably be more pronounced in highly liked products. Laesle et al. [18] found clear differences in food intake, spoonful size and initial eating rate between normal weight and obese subjects, using chocolate mousse, which is a highly liked product. Spiegel et al. showed that mean rate of intake for obese women was higher than that of lean women when the food was of high palatability and the obese women also tended to eat more of the high palatability food [29]. An earlier study also found that obese subjects ate more high preference food and less low preference food than the non-obese subjects [30]. In contrast to our original expectation, we only found a difference in bite size, and interestingly only for the product type spiced rice and not for the apple-pie yoghurt.

Eating behavior in the present study was measured in a laboratory setting. This setting was chosen to be able to measure eating behavior accurately. A strength of our study is the combination of using both hidden weighing scales and video observations. Via the video observations we observed many individual styles of eating and only with these observations (or life observations) is it possible to count the number of bites accurately. Some people have a tendency to consume several bites from one spoonful and if you would only investigate changes in weighing scale data, you would draw different conclusions on number of bites and bite size. Unfortunately, these video observations also have the disadvantage that subjects can feel observed and possibly adapt their eating behavior to what they expect is socially desirable. To what extent the laboratory setting with video observations influenced our results is unknown.

Whereas experimental studies have the advantage of being able to measure behavior very accurately, they do have the disadvantage of having lower external validity. So far, most studies have been performed in laboratory settings but an interesting study in real-life

setting is the study of Llewellyn et al. [31]. They observed eating rate of 254 twin-children at home. They found a significant association between eating rate (bites/min) and adiposity. Studies in adults in real-life settings are much older and the methods used were not very accurate. For instance, weight status, bite size and food intake were estimated by an observer without any weighing scale or predefined bites [32-35]. More studies in real-life might be necessary to investigate if and how obese and overweight subjects differ in eating behavior from normal weight subjects.

The characteristics of the study population and the number of subjects are important factors influencing results. A strength of our study was the fact that overweight subjects were individually matched to normal weight subjects based on gender, age and dietary restraint score. We included subjects who were currently not on a diet. Unfortunately, we have no information on their diet history. It is possible that subjects were currently not on a diet but have been on many diets before, which may have affected their current eating behavior. Additionally, weight stable subjects were included, but if a overweight subject is truly weight stable this could mean that he or she is comparable to a normal weight subject in eating behavior, as also suggested by others [36]. Although many methodological challenges go together with investigating dieting, non-weight stable overweight subjects, this might be the most interesting group to investigate.

For a study investigating retro-nasal aroma release our study was relatively large, but where eating behavior is concerned, our study was relatively small and the power to detect relevant differences in eating rate and *ad libitum* intake may have been too low. The variation was larger than expected and sample size calculations using the observed variation showed that we needed several dozens of subjects per weight group to find differences in eating rate of >10% between groups and several hundreds of subjects per weight group to find differences in *ad libitum* intake of >10% [37]. Of course not all of these subjects would have to be included because the variation within groups will decrease as groups will become more homogeneous, nevertheless this raises the question if relevant differences in *ad libitum* intake or eating rate can be found with these numbers of subjects. Nonetheless, cross-sectional studies investigating several hundreds to thousands of subjects, do show positive relations between BMI and self-reported eating rate. In these studies eating quickly was associated with overweight [38-40] and reporting to eat faster was an independent predictor of weight change [41]. However, no conclusions on a causal relation can be drawn from these cross-sectional studies. Although the existence of an obese eating style is a concept widely assumed to be present, data from literature on experimental studies is ambiguous. Some studies found differences in eating behaviors such as intake, eating rate, bite sizes or meal duration between normal weight and overweight subjects [e.g. 17-20], whereas others did not [e.g. 10, 42, 43]. It is possible that differences in eating behavior might be very subtle and that we need much larger studies, preferably in real-life settings, to find systematic and consistent differences.

A second aim of our study was to relate extent of retro-nasal aroma release to eating behavior and to weight status. Eating faster and taking larger bites most likely leads to shorter oral transit times and we expect this to lead to lower oral sensory exposure of products [2, 6, 44]. Furthermore, we expected that these types of behavior would be more present in the overweight group of subjects. A recent study of Ruijschop et al. [16] showed that *ad libitum* intake might correlate with retro-nasal aroma release. If retro-nasal aroma release represents (part of) oral sensory exposure, then based on our expectations, this would be correlated with behavior such as eating rate.

We found no differences in retro-nasal aroma release between the weight groups, which is probably explained by the fact that we also found no clear differences in eating behavior between the groups. We did observe that eating behavior differed largely between subjects, for instance in eating rate. Some subjects ate reasonably slow whereas others ate reasonable fast. Therefore, aside from weight status, we expected that eating behavior and especially eating rate would be correlated with retro-nasal aroma release parameters. One explanation for not finding this, could be that we used fixed spoonfuls of test product to measure retro-nasal aroma release. This approach of fixed spoonfuls was a good starting point and probably sufficient to detect differences between persons based on factors such as nasal anatomy, breathing or saliva flow, which are shown to affect aroma release measurements [24]. However, this consumption of a standardized spoonful which was not based on individuals' normal bite size, might not be sufficient enough to relate eating behavior such as eating rate and bite size to aroma release during an entire meal. Another explanation could be related to the combination of the physical state of the test products and the fixed bite size. Studies have shown that there is a relation between retro-nasal aroma release and chewing behavior [15, 45]. Our products could be relatively easily consumed and did not require extensive oral processing such as chewing, before they could be swallowed. This could have resulted in too little variation in eating behavior between subjects during consumption of fixed bite sizes. Therefore, a suggestion for further research would be to measure retro-nasal aroma release in a large group of subjects while consuming test foods which require a relatively long oral processing time, at subjects own preferred eating rate and sizes of bites.

We found no differences in eating behavior between the weight groups, however, we do observe differences between the test products. Interestingly the *ad libitum* intake of the apple-pie yoghurt is significantly higher compared to the spiced rice. On average the apple-pie yogurt also received higher liking ratings, was eaten significantly faster, with larger bite sizes, and in a shorter period of time. Satiety ratings after intake of both products are quite similar while in grams and calories, subjects consumed significantly more of the yoghurt. Although this is speculative, it is possible that subjects passively overconsumed from the yoghurt and that this has to do with the manner of eating. Within products, eating behavior such as eating rate and bite size were correlated with *ad libitum* intake.

Although important to note here is that careful interpretation of these correlations is necessary since some of the behaviors are partly calculated from each other in this study. Nevertheless, this correlation between eating rate and bite size with *ad libitum* intake was also shown in our previous studies [6, 9]. Other studies have shown that eating faster and eating with larger bite sizes leads to a higher food intake [3-5, 46, 47].

Instead of trying to find an obese eating style as a group behavior, we should focus more on individual behavior. Subjects showed consistent eating behavior for both test products. Subjects who consumed a large amount of one product also consumed a large amount of the other product. The same holds for the other eating behaviors. Since eating rate and bite size have been shown to affect food intake, it seems important to further investigate if decreasing someone's eating rate, bite size or other important parameter, would be an effective strategy to decrease food intake and not focus or quantify the level it is now. This seems to have effect as shown for instance by the study of Walden et al. [47], Andrade et al. [3] and also by our own previous study [6], but more longer term studies in real-life and non-laboratory settings are necessary.

In conclusion, this study showed no clear differences in eating behavior or retro-nasal aroma release between normal weight and overweight subjects and no correlation between eating behavior and extent of retro-nasal aroma release. Both eating behavior and retro-nasal aroma release seem subject specific. Eating behavior might be a characteristic of an individual but not by definition a characteristic for a group of people based on their weight. This study was one of the first studies to relate eating behavior to retro-nasal aroma release and more research under non-standardized eating conditions is necessary to investigate if extent of retro-nasal aroma release can be used as a marker for (part of) oral sensory exposure.

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7

General discussion

In view of the growing epidemic of obesity, it is important to investigate factors which influence food intake. Food texture has been shown to be involved in food intake regulation but underlying explaining mechanisms are unknown. Therefore, the aim of this thesis was to determine the effect of food texture on satiation (assessed as *ad libitum* food intake) and to investigate the mediating role of oral sensory exposure and gastro-intestinal physiology.

This final chapter starts with a brief overview of the main findings, followed by a section on methodological considerations. Next, a reflection of the findings and comparison with other studies is discussed. Finally, implications and recommendations for further research are provided.

MAIN FINDINGS

The main results with respect to the effect of food texture on food intake and the role of oral sensory exposure and gastro-intestinal hormone release are summarized in **Table 7.1**. Viscosity had a clear effect on food intake; increasing viscosity significantly decreased food intake and the difference in intake between the liquid and semi-solid was around 30% (Chapter 2). This was not due to the palatability, macronutrient composition or energy density of the products, nor was it related to the effort needed to get the products into the mouth via a straw. Eating rate played an important role; the liquid product was consumed faster than the semi-solid product, resulting in a higher intake (29% difference). Standardizing eating rate led to non-significant differences in *ad libitum* intake between these products (Chapter 2). The selected gastro-intestinal hormones could not explain the effect of viscosity on food intake; a fixed amount of the test products differing in viscosity resulted in a similar response of the hormones CCK, ghrelin and GLP-1 (Chapter 3). Further research focused on oral sensory exposure and it was shown that greater oral sensory exposure, achieved by eating with small bite sizes and increasing oral processing time, significantly decreased food intake of a semi-solid food (Chapter 4). Differences in texture of solid foods, aimed to change oral processing time, did not affect food intake (Chapter 5). Associations between food intake and eating behaviors such as eating rate and bite size were observed several times (Chapters 4–6), which provide support to our concept that eating slower and taking more time to orally process the food, will result in lower *ad libitum* food intake. Small to no differences in eating behavior and retro-nasal aroma release were found between normal weight and overweight subjects (Chapter 6). Eating behavior might be a characteristic of an individual but not by definition a characteristic for a group of people based on their weight.

Table 7.1 Overview of the main findings

The role of:	Investigated aspect	Results/Conclusions	Chapter
Texture	Viscosity	• Clear effect of viscosity on *ad libitum* food intake. Intake of a liquid product was respectively 14 and 30% higher compared to a semi-liquid and semi-solid product in real-life setting ($P<0.0001$) and 34 and 29% compared to a semi-solid product in laboratory setting ($P<0.0001$) in two different conditions.	2
		• A fixed preload of a semi-solid product was considered as more satiating than a liquid product. Appetite ratings: significant effect on fullness ($P=0.03$), desire to eat ($P=0.04$), appetite for something sweet ($P<0.01$) and prospective consumption ($P<0.001$) .	3
		• No effect of a fixed preload of a liquid and semi-solid product on *ad libitum* intake of a second meal of chocolate cake.	3
	Texture differences of solid foods	• Differences in food texture of solid foods, aimed to change oral processing time, did not affect *ad libitum* food intake. Texture differences of the investigated test products may have been too subtle to lead to differences in eating rate and food intake.	5
Oral sensory exposure	Oral processing time	• *Ad libitum* intake of a semi-solid food was significantly lower when oral processing time was fixed to 9 s compared to 3 s ($P=0.008$; difference on average 42 g).	4
	Bite size	• *Ad libitum* intake of a semi-solid food was significantly lower when bite sizes were fixed to small (≈ 5 g) compared to large (≈ 15 g) ($P<0.0001$; difference on average 106 g).	4
		• Significant positive associations between bite size and *ad libitum* intake were observed (correlation coefficients from 0.27-0.60)	4, 6
		• Average bite size for a rice product was significantly higher ($P=0.03$) for overweight subjects (10.3 ± 3.2 g) versus normal weight subjects (8.7 ± 2.1 g). For a yoghurt product no differences were observed. No difference in *ad libitum* intake between weight groups.	6
	Eating rate	• A liquid product was consumed faster (90 ± 50 g/min) than a semi-solid product (57 ± 20 g/min) resulting in a higher *ad libitum* intake (29% difference). Standardizing eating rate led to non-significant differences in intake between these products.	2
		• Significant positive associations between eating rate and *ad libitum* intake were observed (correlation coefficients from 0.22-0.60).	5, 6
		• Average eating rate was not significantly different between normal weight and overweight subjects for a rice meal or yoghurt product.	6
Gastro-intestinal hormones	Ghrelin, CCK, GLP-1	• A fixed amount of products differing in viscosity, i.e. liquid and semi-solid, resulted in a similar response of the hormones ghrelin, CKK and GLP-1. Only for desacyl ghrelin a small but significant product effect ($P<0.01$) was observed, concentrations were lower after the liquid product.	3

METHODOLOGICAL CONSIDERATIONS

This section discusses methodological considerations which are important to take into account when interpreting results. The studies described in this thesis aimed to investigate the role of food texture and aspects of oral sensory exposure on outcome measures such as food intake, appetite ratings and biological measures such as gastro-intestinal hormone release. Among others, the type and texture manipulation of test products determine if results can truly be attributed to an effect of texture only. Additionally, conclusions concerning the role of oral sensory exposure depend on the investigated parameters that were chosen to affect oral sensory exposure. These aspects will be discussed first. Thereafter the choice of outcome measures will be addressed, followed by a discussion on factors which can affect the interpretation of results, such as study design and subjects.

Test products

Type of test products

The type of test products and the manner of texture manipulation is essential for obtaining valid results. Product characteristics other than food texture can affect food intake regulation such as macronutrient composition [e.g. 1-5], energy density [e.g. 6-9], volume [e.g. 10], palatability [e.g. 11-13] or portion size [e.g. 7, 8, 14]. Some of these characteristics may have interfered with the results of previous studies investigating the role of food texture in food intake regulation. During the developmental stage of our test products, the current knowledge and expertise of both science and industry were applied. A major strength of our test products (Chapters 2, 3 and 5) is the fact that they were different in texture (measured both objectively via rheological measurements and subjectively via ratings on questionnaires) but similar in macronutrient composition, energy density, and palatability. Thereby interference of these factors was controlled for, and this enabled us to investigate a true effect of food texture.

Creating a difference in texture but keeping macronutrient composition, energy density and palatability similar, was not possible to achieve in every desired food product. Therefore, these requirements limited the potential number of food products to choose from as test products. This might have resulted in testing products outside of their normal setting. Although chocolate dairy products (Chapter 2), meat replacers and luncheon meat (Chapter 5) are common foods to consume in the Netherlands, these are normally not consumed in a cinema while watching a movie. Furthermore, consuming chocolate custard (Chapters 2 and 4), apple-pie yoghurt (Chapter 6), luncheon meat, meat replacer or chewy candy (Chapter 5) as a lunch or evening meal may not be representative for a normal meal.

Although consuming certain products as a meal or outside of their normal setting is not comparable to real life situations, we do not expect that the specific type of products or moments of consumption affected our results. The types of test products served as model products to investigate the roles of food texture and oral sensory exposure. We expect that the effect of these factors on food intake work as a concept, and are independent of type of food. In case of viscosity, this expectation is confirmed by other studies within our group, showing the same effect of viscosity on food intake for yoghurt products [15, 16]. To be certain that bite size and oral processing time can be extrapolated to other foods, our study should be repeated with other food products. Nevertheless, the observed positive associations between eating rate and intake, and bite size and intake in other studies (Chapters 5 and 6) with completely different products, support our expectation.

In the case of the solid test products (Chapter 5), our requirements of similar macronutrient composition, energy density and palatability limited the maximum texture difference that could be achieved. Texture differences between the hard and the soft version were only subtle. As soon as texture differences were made more distinct, however, many other characteristics of the products changed. Although we found no difference in intake, our study does provide insight that texture differences need to be larger. In the future, this study should be repeated for other food products with larger texture differences, but at the present it seems technically not possible to create texture differences in solid products of the same product category, needed for this kind of research.

In the study investigating differences between normal weight and overweight subjects (Chapter 6) we specifically chose for a high liking single meal item (apple-pie yoghurt) but also for a more meal type food (spiced rice). This enabled us to investigate if possible effects were consistent for two different products. We failed to show clear differences in eating behavior for either test product. Whether this was due to the choice of test products is unknown, although differences have been shown by other researchers when using chocolate mousse [17]. This suggests that the lack of effect is not because of the chosen products.

Single foods

The test products that we used in our studies were single homogeneous foods, which assured a clear investigation of the effect of food texture on food intake. We did not investigate the effect of food texture in combined meals of several products, but we expect the same findings. This is confirmed by the study of Moorhead et al. [18], which showed that adding whole carrots to a fixed lunch led to significantly lower *ad libitum* intakes of an afternoon meal compared to adding blended carrots. Gustafsson et al. [19] showed no difference in satiety scores between a mixed meal including spinach in cut or in minced form. However, in contrast to the study of Moorhead et al., the difference in texture between the spinach food forms was probably not distinct enough.

Oral sensory exposure

The findings of this thesis indicate that oral sensory exposure is an important factor contributing to regulation of food intake. To investigate the role of oral sensory exposure we have taken oral processing time, i.e. the time food enters the mouth until swallowing, as a proxy for oral sensory exposure. A longer oral processing time most likely leads to a lower eating rate, hence also eating rate was seen as a factor related to oral sensory exposure. Additionally, bite size was seen as a factor related to oral sensory exposure. A smaller bite size may lead to relatively more oral sensory exposure per gram consumed, since more small bites than large bites are needed to consume the same amount of food. Currently, there are no direct measures of oral sensory exposure available. However, based on our results and results from literature, it is plausible that oral processing time, eating rate and bite size affect oral sensory expose and we believe that our manipulations can serve as valid means to investigate the role of oral sensory exposure.

Outcome measures

Ad libitum intake

In our satiation studies we chose for actual food intake as main outcome (Chapters 2, and 4–6). Most other studies investigating the role of food texture focused on subjective sensations measured via rating scales and *ad libitum* intake of a second test meal. Subjective sensations were also measured in our studies, but only before and after *ad libitum* intake.

We expected food texture and oral sensory factors to largely affect satiation and thus directly affect meal termination. Oral sensory factors are operational during the process of eating and not thereafter. Additionally, we considered *ad libitum* intake to be a more objective measure for actual behavior in natural living conditions, than subjective measures on rating scales. *Ad libitum* intake has been shown to be highly reproducible with no significant differences between two equal test days [20, 21]. Although appetite ratings also have been shown to be reproducible and related to energy intake, they are not reliable predictors of energy intake [22-25]. Therefore, we believe that measuring *ad libitum* intake was the appropriate choice to investigate the role of food texture and oral sensory factors in food intake regulation.

To measure *ad libitum* intake, we served a surplus of test products (Chapters 2, and 4–6) to allow for individual differences and minimize visual cues on amount consumed. Offering a larger portion than a typical serving size is important to minimize the tendency of people to clean their plate and base meal size on a offered amount. This approach could, however, have led to higher absolute levels of intake compared to real life situations. Wansink et al. [26] has shown that subjects consumed significantly more soup in the absence of visual cues of consumption. Other studies have shown that increasing portion size leads to higher intakes [e.g. 14, 27]. Although *absolute* levels of food intake might have been affected in our studies, it

is important to mention that the main outcome of our studies was a *difference* in intake between products or conditions.

Markers for gastro-intestinal physiology

The study investigating the effect of viscosity on satiety responses and the role of gastro-intestinal physiology (Chapter 3) focused on gastro-intestinal hormone release of ghrelin, CCK and GLP-1. These hormones were chosen since they have proven to play a causal role in food intake regulation and are secreted by endocrine cells in different parts of the gastro-intestinal tract, i.e. ghrelin in the stomach, CCK in the duodenum and GLP-1 in the ileum and colon [28-30]. These hormones have also been shown to react on the short term [30], which was important since we expected the effect of viscosity to act on meal termination.

Measuring insulin would also have been interesting because this hormone is part of the cephalic phase response. However, our study design was not aimed at measuring cephalic phase responses and since cephalic phase increases of insulin have been shown to be lasting around 10 min [31], our design was not suitable to investigate these early responses. Additionally, in performing experiments such as this study we were bound to certain constraints. One of these is the maximum amount of blood which can be drawn from one subject. Another, related to this, is that different blood hormones require different conditions of sampling, which results in different tubes needed for collection or different inhibitors which need to be added. Due to these constraints, it was not possible to measure additional hormones. However, we do not expect that measuring other or additional hormones would alter our conclusion.

A factor which we did not measure was gastric emptying rate. Some intestinal satiation signals inhibit gastric emptying and thereby aid to limit ingestion [28-30]. In our study, it might be possible that the gastric emptying rate of the liquid product was faster compared to that of the semi-solid product. Studies have shown that increasing the viscosity of food decreases the gastric emptying rate [e.g. 32, 33]. Our test products differed only in the type of starch and not in the amount of starch. Therefore, it is possible that the amount of starch was already broken down by amylase in saliva, and that the viscosity of the products in the stomach was the same. In that case, gastric emptying would not have played a role in explaining the differences in intake between the liquid and semi-solid product. Unfortunately, we did not have the means to measure gastric emptying rate at the time of this study.

Study design

Crossover

Except for the last study, all studies in this thesis had randomized crossover designs in which all subjects participated in all test conditions. This allowed for within subject comparison of effects, controlling many unpredictable individual variables related to

food intake and making the studies statistically more powerful. In our last study we compared normal weight subjects to overweight subjects. A strength of that study was that overweight subjects were individually matched to normal weight subjects based on gender, age and dietary restraint score to reduce variability between the groups.

Standardization of satiety state

Ad libitum intake was the main outcome of almost all studies (Chapters 2, and 4–6). This outcome is affected by the state of subjects, i.e. subjects eat more when they are hungry compared with when they are full [34]. Therefore, individual state of satiety was standardized by instructing subjects to eat the same amount of food and food items during all test days and to record their food and beverage consumption in a diary (Chapters 2, and 4–6). Furthermore, individual state of satiety was standardized by giving subjects a fixed amount of preload food to eat at the beginning of a test session (Chapters 2 and 5) or by instructing subjects not to eat or drink anything other than water until several hours before the start of a test session (Chapters 4 and 6). To standardize the measurement of gastro-intestinal hormones, test sessions were preceded by an overnight fast and subjects were instructed to consume the same food on the day preceding the test session and to record their consumption in a diary (Chapter 3).

Setting

Our studies were conducted both in real-life setting (Chapters 2 and 5) and laboratory setting (Chapters 2, 3, 4 and 6). The two studies conducted in real-life setting were performed in a cinema. This setting allowed subjects to be distracted from visual and weight cues of eating. However, this setting could also have influenced intake. Although it was dark in the cinema and subjects were not seated next to each other to avoid interaction while eating, subjects talked to each other in the breaks. Furthermore, the distraction of watching a movie and the time available to eat (1.5 hour) could have led to higher absolute levels of food intake compared to other settings. Studies have shown that food intake is significantly increased when subjects watch television [35,36]. Distraction and time available to eat could explain why the absolute amounts of intake of the liquid and semi-solid product in the realistic setting were considerably higher (averages from 566 to 809 g) compared to those in the laboratory setting (averages from 176 to 419 g) (Chapter 2).

The studies in laboratory setting were conducted in sensory cabins. This setting enables accurate measurement of eating behavior without distraction from the (social) environment. Studies have shown that subjects tend to eat more in the presence of others than when alone, though the presence of others may increase or decrease intake depending on how much these other people eat or the quality of social interaction [36-39]. The sensory cabin setting makes extrapolation of results to real life situations more difficult. Sitting alone in a cubical with all focus on the food is not comparable to normal eating situations which

often take place in the company of others or while listening to music or watching television. However, the purpose of measuring *ad libitum* intake in the laboratory was not to replicate real life situations, but to study the factors of interest under controlled conditions free of interfering variables [40].

Although the settings could have affected the *absolute* levels of food intake, it is again important to mention that the main outcome of our studies was a *difference* in intake between products or conditions. Furthermore, we investigated differences within subjects, who were always tested in the same setting. The fact that we found the same effect of viscosity on intake in both realistic (30% difference) and laboratory setting (34% and 29% difference) (Chapter 2), indicates that this effect is independent of the setting. The same holds for the relation between eating rate and food intake, which has been repeatedly shown in different studies/settings (Chapters 2, 5 and 6), indicating that this is a robust effect.

To investigate the effect of bite size and oral processing time on food intake (Chapter 4), subjects had to consume the product by means of a peristaltic pump. It is possible that this manner of eating was not pleasant, lasted too long, or that subjects were bored with the procedure. Especially the small bite size conditions may have been more susceptible to these aspects. If this indeed occurred, than this may have interfered with the outcome. Therefore, this experimental manner of eating should be repeated in a more realistic manner of eating and setting. However, because the differences in intake were large and since the manner of consumption via a peristaltic pump did not change the results on viscosity (Chapter 2: consuming via straw 34% and via pump 29% difference), we expect that the effects will also be found in other settings.

In the last study (Chapter 6), the setting of the study might have interfered in such a way that the measure of interest was affected. This study investigated differences in eating behavior between normal weight and overweight subjects in a laboratory setting with video-observations. It is possible that differences between these subject groups are very subtle and easily affected in a laboratory setting. The video-observations may have the disadvantage that subjects felt observed and therefore behaved in a socially desirable manner. Intake in the laboratory was free of social and external factors such as food cues from other foods. Additionally, intake of as single meal was studied, instead of intake of several meals or snacking behavior. It is plausible that these are the factors that determine different eating behavior of normal weight and overweight subjects in real life situations and are therefore not found in laboratory settings. In case of future research into differences in eating behavior between normal weight and overweight subjects, we recommend to combine large observational studies in real life with occasional accurate measurements in the laboratory. In this way specific subjects could be studied in more detail, for instance those in the lower and upper segment of the variable of interest. Additionally, self reported data could be validated.

Subjects

Subjects scoring high on restrained eating (assessed by the Dutch Eating Behaviour Questionnaire [41, 42]) were excluded from participation in all studies, except in the last study (Chapter 6). Restrained eating is the cognitive awareness of food intake regulation in everyday life, and food intake in subjects with high restrained eating is less likely to be regulated by physiological processes [42-44]. Dietary restraint has been associated with an abnormal eating behavior under laboratory settings, although not all studies found this effect (see for a review [45]). Hence, including high restrained eaters could have affected the outcome in an unpredictable way. In our last study (Chapter 6), normal weight and overweight subjects were compared in eating behavior. Since overweight subjects are likely to score high on restrained eating [41, 42], excluding restrained subjects would not only have resulted in finding few suitable subjects, but also in a very selective group of overweight subjects. To minimize interference of an effect due to restrained eating, overweight subjects were individually matched to a normal weight subjects based on restraint eating score.

In the field of food intake regulation, most of our studies are considerably large with regard to the number of subjects included, which is a major strength. For instance, the studies in real-life setting (Chapters 2 and 5) included 108 and 106 subjects. Power calculations were performed before the start of each study in which a difference in intake of 10-15% between products was considered as relevant [34].

In the last study (Chapter 6) we found no difference in eating behavior such as *ad libitum* intake and eating rate between normal weight and overweight subjects. The variation was larger than expected and therefore power was too low to detect differences between groups for these parameters. Sample size calculations using the observed variation showed that we needed several dozens of subjects per weight group to find differences in eating rate of >10% between groups and several hundreds of subjects per weight group to find differences in *ad libitum* intake of >10%. Of course not all of these subjects would have to be included because the variation within groups will decrease as groups will become more homogeneous, nevertheless this raises the question if relevant differences in *ad libitum* intake or eating rate can be found with these numbers of subjects.

INTERPRETATION OF FINDINGS AND COMPARISON WITH OTHER STUDIES

The effect of food texture on food intake regulation

Our studies showed a clear effect of viscosity on *ad libitum* intake (Chapter 2). This effect was consistently shown in both real-life setting and laboratory setting and furthermore replicated in other studies within our group [15, 16, 46]. To our knowledge, there are no other

studies investigating the direct effect of viscosity on *ad libitum* food intake. The study of Mattes and Rothacker [47] did investigate the role of viscosity, but as a fixed preload on appetite ratings and *ad libitum* intake of a subsequent test meal. They showed that hunger ratings were significantly lower following ingestion of a 325 ml thick shake compared to a thin shake, and remained lower than baseline for a longer period of time. The authors also suggested an oral sensory effect since the difference between hunger ratings was greatest within 10 min of preload ingestion. No significant differences were seen in time to the subsequent meal, size of this meal, or 24 hour (self-reported) energy intake. To a certain degree, these results are comparable with our study on the effect of viscosity on appetite ratings (Chapter 3). We found a significant effect on fullness ratings and not on hunger ratings but both studies showed no effect on food intake of a subsequent meal. This confirms our believe that texture of food, and specifically food viscosity, largely affects satiation/meal termination and not satiety/meal initiation. The study of Vuksan et al. [48] did show an effect of preloads differing in viscosity on *ad libitum* intake of a second meal, i.e. intake was significantly lower after the high viscous preload. However, the preloads contained different types of fibers that developed into different viscosities in the gastro-intestinal tract. Sensory exposure to the texture in the mouth did not play a role in this study, which makes it difficult to compare to our studies.

There are a number of studies comparing liquid foods with (semi) solid foods in regard to food intake regulation. These studies show that a fixed preload of (semi) solid foods decrease appetite ratings more and/or lead to lower intakes of a second meal compared to liquid foods [49-51]. Other studies show a stronger compensatory response in balancing energy intake of either one test meal or throughout the day after solid foods compared with liquid foods [52-55]. Some studies found no difference in satiation response between liquids and solids or an opposite effect in which liquids were found to be more satiating [56-60]. However, a number of these studies included soup as test product [57, 58, 60], and soup seems to be an exception [61]. Mattes has shown that soups led to reductions of hunger and increases of fullness comparable to solid foods and suggested that it is likely that cognitive factors play a role in this effect [61]. Furthermore, soups may contain large food particles or have a high viscosity in case of creamy soups [62, 63], although the study by Flood and Rolls [64] showed no effect of different particle sizes in soup on test meal intake.

The test products used in the studies investigating the effect of food form had large differences in texture. Most studies compared liquid foods to semi-solid or solid foods. To the best of our knowledge, no study had investigated the effect of texture differences within solid foods on satiation. Our study (Chapter 5) failed to show difference in *ad libitum* intake between solid products differing in texture. This was probably due to the too subtle differences in texture, leading to no or small changes in oral processing time and eating rate.

In conclusion, the effect of food texture, especially viscosity, on food intake and appetite ratings has been consistently shown, i.e. liquid foods are less satiating. More research is necessary to investigate the effect of texture differences within solid foods on food intake.

Underlying mechanisms

The role of oral sensory exposure

Oral stimulation is an important requirement for appetite suppression [65-67]. Oral sensory exposure may especially be important in meal termination since oral factors are operational during the process of eating and not thereafter.

Our studies show that duration of oral sensory exposure, manipulated via oral processing time, is important since the product which was consumed faster also had a higher intake (Chapter 2); standardizing eating rate led to non-significant differences in intake between products (Chapter 2); keeping a product longer in the mouth resulted in a lower intake (Chapter 4); and eating rate was significantly positively correlated to intake (Chapters 5 and 6). Also, relatively more oral sensory exposure to a product seems important. Consuming a semi-solid product with smaller bite sizes (relatively more oral sensory exposure per unit of product because more bites are needed to consume the same amount of food) led to significantly lower food intakes than consuming with large bite sizes. In our study (Chapter 4) the delivery of the large or small bite size was given in the same time and due to this experimental setting, eating rate per minute was different. In the small bite size conditions, a subject could eat a maximum of 15 gram per minute while in the large bite size conditions this was 45 gram per minute. Interestingly, the research by Weijzen et al. [68] showed that even if eating rate is held constant, consuming with small bite sizes leads to lower intakes. Thus, also relatively more oral sensory exposure is important, a possible additional effect to the time effect of oral sensory exposure.

An additional factor which could contribute to oral sensory exposure is chewing. During chewing, food is broken down into smaller pieces in order to make swallowing possible. The act of chewing stimulates the flow of saliva and food is mixed with saliva for lubrication and to soften hard and dry foods while enzymes in saliva start the digestion of starches. Chewing increases the surface area available to digestion, and chewing also facilitates flavor release because a number of substances responsible for odor and taste sensations are released as the food is broken down [69]. In comparison to a product which only needs to be swallowed, a product which requires chewing remains in the mouth for a longer period of time leading to a longer oral sensory exposure time. Hence, chewing decreases the eating rate of a product. The results of a study by Viskaal-van Dongen et al. [70] showed that the variation in eating rate was much smaller within solid foods than within liquid and semi-solid foods. This suggests that finding a relevant effect of texture on food intake

within solid foods may be more difficult.

The act of chewing solid foods might give a satiety signal which is not induced by swallowing a liquid, as been hypothesized before [50, 53]. Chewing sucrose-containing pastilles has been shown to decrease subsequent energy intake, which was not seen by drinking an equicaloric sucrose drink or water [71]. It is important to mention, however, that also consumption time of the pastilles was much longer. Chewing on gum has been shown to suppress appetite ratings and food intake in the study of Hetherington and Boyland [72] but not in the study of Julis and Mattes [73]. Additionally, chewing on its own was shown to be effective to increase cephalic phase salivary responses [74] and chewing additionally to tasting was shown to be necessary to elicit cephalic phase insulin responses in a study by Teff et al. [75].

This connection between chewing and cephalic phase responses is interesting. Chewing is an important requirement for solid foods but not for liquid foods. Additionally, the effect of texture seems to operate via oral sensory exposure, which is an important part of the cephalic phase response [31, 76]. This suggests that cephalic phase responses might be involved in part of the underlying mechanism explaining the low satiety responses to liquid foods. Results of a recent study by Teff [77] suggest that tasting liquids does not provide adequate stimulation for vagal activation since no increases in pancreatic polypeptide (a gut hormone [78]) greater than fasting were seen. It was suggested that greater oral sensory stimulation was required and indeed oral sensory stimulation by mixed nutrient foods elicited significant increases in pancreatic polypeptide levels. These data provide indications for differences in cephalic phase responses between liquids and solids. Further investigation in the contribution of chewing to oral sensory exposure and satiation and the involvement of the cephalic phase is necessary.

Another factor which might contribute to oral sensory exposure is the coating of food in the oral cavity. After swallowing foods, a viscous salivary coating is retained on the back of the tongue [79, 80]. This coating contains particles of food and it may also contain odorants, which could lead to a prolonged perception of food aroma after the food bolus is already swallowed [80]. A product which leaves behind a coating could lengthen the time of oral sensory exposure. We did not investigate coating in our studies, but it is possible that the semi-solid product retained more coating in the oral cavity than the liquid product and that this contributed to longer oral sensory exposure to the semi-solid product (Chapter 2).

As mentioned above, the layer of coating may contain odorants. Extent of odorant release (or aroma release) is probably related to oral sensory exposure. Therefore, measuring *in-vivo* retro-nasal aroma release [81, 82] is an interesting novel measurement and a potential marker for oral sensory exposure. Retro-nasal aroma perception is part of the sense of smell, which is an important sensory aspect. In a recent study of Ruijschop et al. [83], extent of retro-nasal aroma release appeared to be subject specific and an inverse trend was observed with *ad libitum* food intake. In our last study (Chapter 6) we have investigated

retro-nasal aroma release and although this was product and subject specific, it was not correlated to *ad libitum* intake or eating rate. Extent of retro-nasal aroma release still remains an interesting measure. The study by Buetner et al. indicates that retro-nasal aroma release is related to the texture and the volume (i.e. bite size) of food, factors related to oral sensory exposure. "The more fluid the texture of the bolus and the greater its volume, the more efficient the closure of the oral cavity against the nasopharynx and dorsal oropharynx. During this closure, no transfer of odorants is possible via the retro-nasal route to the nasal cavity and the olfactory epithelium" [80]. Furthermore, they mention that "when swallowing liquid foods, retro-nasal aroma perception will be more or less reduced to one main aroma flash associated with the swallowing event itself. When masticating solid foods, there is a series of retro-nasal aroma perceptions, mainly related to the swallowing of small portions of the food material as well as portions of saliva" [80]. Interestingly, different eating styles related to chewing force, chewing rate and number of chews have been shown to result in differences in aroma release [84]. These data indicate a potential role for retro-nasal aroma release as a marker for (part of) oral sensory exposure and further investigation is needed.

In conclusion, we have investigated the role of oral sensory exposure by manipulating different aspects of eating behavior since true objective measurements are unavailable. Our studies suggest that duration of oral sensory exposure and relatively more oral sensory exposure per unit of product are important in affecting food intake. Further investigation of aspects of oral sensory exposure important in affecting food intake, factors contributing to oral sensory exposure, and techniques to measure oral sensory exposure is necessary.

The role of gastro-intestinal hormones

We found a similar response of the gastro-intestinal hormones CCK-8, ghrelin and GLP-1 after fixed preloads differing in viscosity (Chapter 3). Only for desacyl ghrelin a systematic lower overall response was observed after the liquid product, which was opposite to expected. We believe that these hormones do not play a key role in the explaining mechanism of viscosity. However, we cannot exclude that physiological factors play a role in the effect of food texture in general.

Tieken et al. [85] showed that hunger and desire to eat ratings were more suppressed by a solid meal-replacement product than by a liquid meal-replacement product, although no effect on fullness was shown. Consistent with the appetite ratings, they observed lower concentrations of plasma total ghrelin and insulin following the solid product, but no differences in CCK or leptin responses. Besides food texture, the test products also differed in macronutrient composition and fiber content, which might have influenced the results. In a subsequent study [86], they used a liquid and solid meal replacement product with the same energy content and macronutrient composition and no dietary fiber. They showed that the liquid product resulted in greater hunger and reduced satiety with accompanying

lower concentrations of glucose and insulin and higher concentrations of ghrelin. However, the effects of food form on these hormones were not significant anymore after adjusting for age. No differences between the liquid and solid product were seen on CCK or GLP-1.

The studies of Juvonen et al. [87] and Santangelo et al. [59] showed effects in the opposite direction as we would expect. In the study of Juvonen et al. a low-viscosity oat bran beverage induced a greater postprandial increase in satiety and plasma glucose, insulin, CCK, GLP-1 and PYY and a greater decrease in ghrelin, than a high-viscosity oat bran beverage. In the study of Santangelo et al. a meal in homogenized form was significantly more satiating than the same meal in solid-liquid form and although no overall significant differences in CCK were observed between the meals, the CCK peak occurred later after the homogenized meal. In both studies, gastric emptying rate was faster after the low viscosity beverage and the homogenized meal, which is consistent with others studies showing increasing viscosity delays gastric emptying [e.g. 32, 33]. An earlier availability of food in the intestinal tract, might explain the differences in hormonal responses.

One of the first studies to show differences in physiological responses for eating rate, are the studies by Kokkinos et al. [88] and Sobki et al. [89]. In the study by Kokkinos et al., eating the same meal over 30 min instead of 5 min, led to higher concentrations of the hormones PYY and GLP-1. No effect was seen on glucose, insulin or ghrelin. There was a trend for higher fullness ratings after the 30 min meal but no differences in hunger ratings. Contradictory, the study by Sobki et al. observed significantly higher ghrelin concentrations after eating slower (meal duration 22 ± 1.4 min) in stead of eating the same meal at a normal rate (meal duration 10 ± 0.4 min).

In conclusion, our study showed no clear role for gastro-intestinal hormones in the explanation of the effect of viscosity on food intake. Nevertheless, other studies do show indications for a role of certain hormones in the effect of food texture and eating rate on satiety, but data are equivocal.

Other factors: learning and texture specific satiety

Although not studied in this thesis, dietary learning has been shown to play an important role in eating behavior. Meal size in humans is partly based on previous experience with foods, in which sensory properties and post-ingestive consequences play an important role [90-92]. It is possible that differences in satiety responses between products differing in texture is partly based on learned behavior, which may lead to different anticipated satiety responses for products with different textures. An interesting notion related to this is that the viscosity and the caloric density of human breast milk appear to vary together [93, 94]. As suggested by Davidson and Swithers "breast feeding may provide an important initial exposure to a general rule that thicker substances contain more calories than thinner substances" [95]. Additionally, cephalic phase responses may possibly be learned behaviors. This has also been suggested in the paper of Teff [77]. Cephalic phase pancreatic polypeptide

responses were not shown after subjects chewed non-nutritive sweetened flavored and non flavored gum. It was suggested that the typically cephalic phase reflexes that would be expected after chewing a sweet flavored stimulus might have been extinguished because of subjects previous experience of chewing gum having no caloric load.

Texture-specific satiety may also affect the intake of products differing in texture. When a food is eaten to satiety, the pleasantness of that food is decreased in comparison to foods that have not been eaten, this is called sensory-specific satiety [96]. Sensory specific satiety is mainly related to the sensory stimulation of ingesting food instead of to the post-absorptive consequences, since the changes in pleasantness occur immediately after ingestion of the foods and the magnitude of the changes does not increase over time [97] and sensory specific satiety has also been shown to occur in the absence of post-ingestive feedback via the use of the modified sham feeding procedure [e.g. 98, 99].

Sensory specific satiety has been clearly shown for the taste of food but it can also result from the texture of food. Guinard and Brun [100] showed that pleasantness of the texture and desire to eat hard test foods decreased after eating a hard lunch food, and similarly for one of the soft foods. Rolls et al. [101] showed that changes in the shape of pasta, which affected both the appearance and the mouth feel of pasta, led to specific decrease in the pleasantness of the shape eaten. This decrease in pleasantness could contribute to earlier satiation for foods of certain textures. In the studies in this thesis, liking of the products differing in texture, decreased to the same degree (Chapters 2 and 5).

CONCLUSIONS

The research described in this thesis shows a role for food texture in the regulation of food intake. Viscosity of food had a direct effect on food intake; increasing viscosity significantly decreases food intake. Food texture differences within solid foods can potentially have an effect on food intake but texture differences within the studied solid foods were too subtle to show effects.

Gastro-intestinal hormone release could not explain the effect of viscosity on food intake. Oral sensory exposure is shown to play a major role in the effect of food texture on food intake. Influencing oral sensory exposure by changing eating rate, oral processing time and bite size were shown to affect food intake. A low viscosity product was eaten faster and resulted in higher food intakes than a high viscosity product. Standardizing eating rate led to non-significant differences in intake. Increasing oral processing time by holding the food longer in the mouth and eating with smaller bite sizes, decreased intake of a semi-solid food. These factors contribute to the effect of food texture on food intake. Eating rate and bite size were also characteristics of the eating style of an individual.

IMPLICATIONS

The knowledge that food texture can have a direct effect on food intake has implications for every day life. Liquid foods are less satiating compared to (semi) solid foods. This suggests that energy from liquids may easily lead to passive overconsumption. Perhaps the human appetite system is not well equipped to sense liquid calories since in nature calories in liquid foods hardly occur and breast milk and water were the only beverages assumed to be consumed during the greater part of our evolutionary history [102]. Regulating intake of liquid foods may be difficult if our body is not well equipped for liquid calories. Therefore, a cognitive awareness of type and amount of consumption of energy containing liquid foods is important.

The information that liquid foods are less satiating than (semi) solid foods, is important information to take into account for persons who want to control their food intake. On the one hand this information is useful for those who want to decrease food intake, on the other hand this information can also be useful for those who need to increase food intake. The latter could be relevant in the clinical setting. Patients who need extra calories are probably better of with extra energy in the form of drinks and shakes, which facilitates consumption of the required amount. This is currently applied in the clinical setting and our results support this approach.

Our studies show that taking a longer time to orally process the food and eating slowly and taking small bites are important aspects to decrease food intake. While this is possibly already part of common belief, our studies provide scientific evidence. In view of product development, this information can be used to develop products which require more oral processing and remain in the mouth for a longer period of time and thereby are potentially more satiating. A simple approach to accomplish this is to add pieces such as fruit to food products. In view of behavior, it is important to encourage people to eat slowly, extend oral sensory exposure time by taking time to chew and orally process the food in the mouth, and also to eat with small bite sizes. These strategies might help people to be satisfied with less food during a meal.

RECOMMENDATIONS FOR FURTHER RESEARCH

Our research showed a clear role of viscosity on food intake of a single meal. The *ad libitum* intake of the liquid product was significantly higher compared to those of the semi-liquid and semi-solid products. It is necessary to further investigate compensation behavior on a longer term to determine if this effect contributes to a change in total daily energy intake. For instance, do people compensate for the extra calories of the liquid product during the remainder of the day? Studies using a fixed amount of test products indicate that calories from liquid foods are not well compensated for. Long term compensation

after *ad libitum* intake has not been investigated yet. When a reduction of daily energy intake is demonstrated, further examination of the effect on body weight is important.

The effect of texture differences within solid foods on food intake also needs further attention. The challenge lies in creating suitable test products similar in palatability and macronutrient composition but differing in texture to such an extent that oral processing time and eating rate are affected. Ideally, prior to investigating intake, oral processing times should be measured for every subject. Techniques which measure the time a product remains in the mouth before swallowing need to be used. When clear differences in oral processing time have been established, *ad libitum* intake of these products can be investigated. If those food products with a longer oral processing time indeed lead to lower *ad libitum* intake, then it would be of further interest to investigate the relation between the quantity of oral processing time and intake, and to study oral processing behavior in more detail.

More research is needed with respect to the role of oral sensory exposure in food intake regulation, focusing on manners to objectively measure oral sensory exposure. An important aspect would be to determine how many taste and smell molecules actually reach the peripheral sensory cells. Does this differ for products differing in texture, or via aspects of eating behavior such as eating rate and bite size? This would confirm our assumptions that the effect of texture on food intake operates via oral sensory exposure. *In-vivo* retro-nasal aroma release remains a potential candidate as marker for oral sensory exposure. Extent of retro-nasal aroma release needs to be investigated during a longer period, for instance during a meal, while subjects consume foods at their own preferred eating rate and sizes of bites. Additionally, more research is needed to determine which factors affect oral sensory exposure. Aspects of interest are the role of chewing and the coating a product leaves behind in the oral cavity. Chewing has also been shown to elicit cephalic phase responses and the relation between chewing and oral sensory exposure, and the relation between oral sensory exposure and cephalic phase responses also needs further investigation.

Cognitive aspects in relation to food texture have not been investigated in this thesis. However, it is possible that the expected satiating capacity of a food is different for foods differing in texture. In other words, people may expect liquid foods to be less satiating than solid foods and this may affect their food intake. This expectation could be based on learned behavior. Further research is needed to investigate if expected satiation is indeed different for similar products differing only in texture. New methods to measure expected satiation could be useful, for instance that of Brunstrom et al. [103].

Together with our research, it has been shown that decreasing eating rate and bite size decreases food intake of a single meal. Although eating slow and decreasing bite size are already part of advice in weight loss programs, few studies have investigated the

effectiveness of this advice on longer term energy intake and weight status. Further research is necessary to investigate if it is possible to learn someone to develop a new eating style of eating slower and with smaller bite sizes. Very important herein is to investigate if this new eating style will be maintained on the long term and if this eventually will decrease energy intake and ultimately decrease body weight. A recent long term study in obese adolescents [104] has shown significant changes in body mass index standard deviation score, in those subjects using a computerized feedback device to slow down eating. This is a promising first result and more research in this field is necessary.

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Summary

In the last thousand of years our food pattern has shifted from minimally processed foods to a pattern with convenient and processed foods and with an increased consumption of energy containing beverages. This shift has led to an increase of 'easy-to-consume foods', which can be eaten quickly, with little effort and a minimal amount of chewing. These factors are possibly relevant for the regulation of food intake. Research has shown that liquid foods have a weaker satiety value compared to solid foods, indicating that food texture is potentially an important factor affecting food intake. Food texture, with characteristics such as hardness and thickness, is an essential part of the sensory profile of a food. The underlying mechanisms involved in the weaker satiety responses to liquid foods are unknown and it is unclear whether or not food texture has a direct effect on food intake. So far, studies investigating the role of food texture were so called "preload-test meal" studies in which subjects first consume a fixed amount of the test products under study, followed by an *ad libitum* meal of other food products. Almost no study investigated the effect of food texture on *ad libitum* food intake of the food itself (meal termination). In many studies, foods did not only differ in texture, but also in other characteristics such as energy density, macronutrient composition or palatability. This may have affected the interpretation of the effect of food texture on satiety responses. Therefore, the aim of this thesis is to determine the effect of food texture on satiation (assessed as *ad libitum* food intake) and to investigate the mediating role of oral sensory exposure and gastro-intestinal physiology in this effect.

We started our research by focusing on the effect of viscosity on food intake (Chapter 2). In a real-life setting, 108 subjects received a chocolate flavored liquid, semi-liquid or semi-solid milk based product. These products were specially developed for this study and differed in viscosity but were similar in palatability, macronutrient composition and energy density. Subjects consumed one of the products *ad libitum* while watching a movie in a cinema. Clear significant differences in intake were observed; the intake of the liquid product was 14% higher ($P<0.0001$) compared to the semi-liquid and 30% higher ($P<0.0001$) compared to the semi-solid product. The aim of a second study (Chapter 2) was to investigate whether the observed differences in intake could be related to eating rate and/or eating effort to get the product in the mouth via a straw. In a laboratory setting, 49 subjects received the liquid and semi-solid product while effort and eating rate were controlled by means of a peristaltic pump. A tube was connected to this pump, which delivered the product into the subjects' mouth. It was shown that the difference in intake of the liquid and semi-solid product was not related to the effort needed to get the products into the mouth; controlling for effort still resulted in a 29% higher intake for the liquid product ($P<0.0001$). Eating rate played an important role; the liquid product was consumed significantly faster than the semi-solid product and standardizing eating rate led to non-significant differences in *ad libitum* intake between these products. When subjects were instructed to consume the products via a straw instead of via a pump, the

findings of the real-life setting were replicated; the intake of the liquid product was 34% higher compared to the semi-solid product ($P<0.0001$).

To further investigate underlying factors responsible for the effect of viscosity on food intake, the role of gastro-intestinal hormones was investigated. The aim of the study was to explore the effect of a fixed preload of the liquid and semi-solid test product on the release of ghrelin, CCK and GLP-1 (Chapter 3). These hormones play a causal role in food intake regulation and are released in different parts of the gastro-intestinal tract. Results showed that the selected gastro-intestinal hormones could not explain the effect of viscosity on food intake; a fixed amount of the liquid and semi-solid test products resulted in similar responses of the hormones ghrelin, CCK and GLP-1. Based on these results in Chapter 2 and 3, we decided to pursue the role of oral sensory factors, and not gastro-intestinal factors, in explaining the effect of texture on food intake.

The study described in Chapter 4 assessed the role of oral sensory exposure by investigating the effect of bite size and oral processing time of a semi-solid product on *ad libitum* food intake. Oral processing time, i.e. the time between the food entering the mouth and swallowing, was considered a proxy for oral sensory exposure. Additionally, bite size was seen as a factor related to oral sensory exposure; a smaller bite size may lead to relatively more oral sensory exposure since more small bites than large bites are needed to consume the same amount of food. A total of 22 subjects consumed chocolate custard via a peristaltic pump under different test conditions. Bite sizes were free, or fixed to small bite sizes (≈ 5 g) or large bite sizes (≈ 15 g). Oral processing time was free (only in combination with free bite size) or fixed to 3 or 9 seconds. Food intake was significantly less when oral processing time was fixed to 9 s compared to 3 s (on average a difference of 42 g; $P=0.008$) and when consumed with fixed small bite sizes compared to large bite sizes (on average a difference of 106 g; $P<0.0001$). Additionally, there was a significant relationship between bite size in the free bite size conditions and *ad libitum* intake in those conditions; subjects who consumed with larger bite sizes also had a higher intake. The results of this study reinforce the important role of oral sensory exposure.

The obtained results of all studies so far show that the transit or processing time in the oral cavity plays an important role in the explanation of the effect of food texture on food intake. A liquid product is eaten at a higher eating rate and does not stay in the mouth for a long time, while a solid product is eaten more slowly and stays in the mouth much longer. Eating slower and taking more time to orally process the food in the mouth, results in a longer oral sensory exposure time, leading to a lower *ad libitum* food intake. Until now, studies investigating the effect of food texture mainly focused on (semi-)liquid products or compared liquid to solid products. It is unclear whether or not texture differences and the role of oral sensory exposure are also relevant within solid foods. Therefore, the study described in Chapter 5 investigated the effect of texture differences within solid foods

on *ad libitum* intake. Specially developed test foods were used i.e. luncheon meat, meat replacers and sweets. Each food had a hard and soft version with a similar energy content, macronutrient composition and palatability. Texture differences between the hard and the soft version were expected to lead to differences in oral processing time and eating rate and consequently to differences in oral exposure time. This study was again performed in a real-life setting and a total of 106 subjects consumed the test products *ad libitum* while watching a movie in a cinema. During the final test session, eating rate of all products was measured. In contrary to our expectation based on rheological measurements, eating rate was only significantly lower for the hard version of the luncheon meat than for the soft version (21±10 g/min vs. 25±13 g/min; $P<0.0001$); there were no differences in eating rate between the hard and soft version in the other products. *Ad libitum* intake was not significantly different between the hard and the soft version for any of the products. We believe that texture differences between the hard and the soft versions were probably too subtle to lead to differences in eating rate and subsequently to differences in food intake. Interestingly, eating rate and intake were significantly correlated in all test products (overall r=0.54). In addition, in the luncheon meat the largest differences in eating rate and the strongest correlations between eating rate and intake were observed. Furthermore, a larger number of subjects consumed more of the soft version of luncheon meat than of the hard version (67 vs 39; $P<0.01$). These data provide support to our idea that eating more slowly and taking more time to orally process a food will result in a longer oral exposure time and eventually lead to a lower *ad libitum* food intake. However, further research within solid foods is needed.

Currently no objective parameters are available to measure oral sensory exposure, but a possible marker is the extent of *in vivo* retro-nasal aroma release. Retro-nasal aroma release has been shown to be related to the texture and bite size of food in the mouth, similar to oral sensory exposure. In the last study, eating behavior and retro-nasal aroma release were observed in normal weight and overweight subjects (Chapter 6). The aims of the study were 1) to investigate if eating behavior (*ad libitum* intake, eating rate, bite size, number of bites, meal duration) relates to weight status, and 2) whether extent of retro-nasal aroma release relates to eating behavior and weight status. A group of 27 normal weight and a group of 27 matched (on gender, age and degree of restraint) overweight subjects consumed a spiced rice meal or apple-pie yoghurt during two separate test sessions. Extent of retro-nasal aroma release was measured on a separate third test day. The results showed small to no differences in eating behavior and retro-nasal aroma release between the weight groups. None of the eating behaviors were correlated to retro-nasal aroma release. Subjects showed consistent eating behavior for both products; *ad libitum* intake and the other eating behaviors of the spiced rice were strongly correlated with the intake and eating behaviors of the apple-pie yogurt (correlation coefficients from 0.52 to 0.70). Eating behavior might be a characteristic of an individual but not by definition a

characteristic for a group of people based on their weight.

In the final Chapter (Chapter 7), the main findings, methodological considerations, interpretation of findings and comparison with other studies are discussed. Additionally, implications and suggestions for further research are given. Our studies show a consistent effect of food texture on food intake and satiety, i.e. more liquid foods are less satiating. More research is necessary to investigate the effect of texture differences within solid foods on food intake. Further investigation of the role of oral sensory exposure in food intake regulation is also needed, focusing on methodology to objectively measure oral sensory exposure. Additionally, it is important to determine which factors affect oral sensory exposure. Finally, it is important to investigate long term energy intake compensation after *ad libitum* intake of products differing in texture, to determine if the effect of food texture on food intake contributes to changes in energy balance.

In conclusion, the results of this thesis show that food viscosity has a direct effect on food intake. Oral sensory exposure plays a major role herein as changing eating rate, oral processing time and bite size affected food intake. These factors contribute to the effect of food texture on food intake.

Samenvatting
(Summary in Dutch)

In de afgelopen duizenden jaren is ons voedingspatroon veranderd van een patroon met nauwelijks bewerkte voedingsmiddelen naar een patroon met veel industrieel bewerkte producten en een toegenomen aandeel van energiehoudende dranken. Deze verschuiving heeft geleid tot een toename van 'eenvoudig te consumeren' voedingsmiddelen, die snel, met weinig moeite en met minimale kauwbewegingen kunnen worden geconsumeerd. Deze veranderingen in voedingspatroon zijn mogelijk relevant in de regulatie van voedselinname. Onderzoek heeft aangetoond dat voedingsmiddelen in vloeibare vorm minder verzadigend zijn dan in een vaste vorm. Dit wijst erop dat de textuur van voedingsmiddelen een potentieel belangrijke factor is in de regulatie van voedselinname. De textuur van voedingsmiddelen, met karakteristieke eigenschappen zoals hardheid en viscositeit, is een essentieel onderdeel van het sensorisch profiel van voedsel. Het is onbekend welke onderliggende mechanismen een rol spelen bij de lagere verzadigende werking van vloeistoffen ten opzichte van vaste voedingsmiddelen. Daarnaast is het onduidelijk of de textuur van voedingsmiddelen een direct effect heeft op de hoeveelheid die men eet. Eerdere onderzoeken naar het effect van textuur op voedselinname hebben veelal gebruik gemaakt van de zogenaamde "preload-testmaaltijd" opzet. Hierbij consumeren deelnemers eerst een bepaalde hoeveelheid van een testproduct (preload), na enige tijd gevolgd door het naar believen consumeren van een maaltijd bestaande uit andere voedingsmiddelen (testmaaltijd). Ondertussen worden honger- en verzadigingsgevoelens bijgehouden via vragenlijsten en wordt gemeten hoeveel de deelnemers van de testmaaltijd consumeren. Er zijn echter vrijwel geen onderzoeken gedaan naar het effect van de textuur op de inname van het testproduct zelf (maaltijdbeëindiging). Bovendien waren de testproducten vaak niet alleen verschillend in textuur maar ook in andere eigenschappen zoals energiedichtheid, smakelijkheid of macronutriëntensamenstelling (de verhouding van koolhydraten, eiwitten, vetten). Het verschil in deze eigenschappen heeft mogelijk de interpretatie van het effect van textuur op verzadiging beïnvloed.

Het doel van dit proefschrift is het vaststellen van het effect van de textuur van voedingsmiddelen op voedselinname, en de rol van orale sensorische blootstelling en maagdarmfysiologie bij dit effect. Het effect op voedselinname wordt hierbij bepaald door het effect van textuur op maaltijdbeëindiging vast te stellen en de directe inname van de testproducten te meten.

In het eerste onderzoek hebben we het effect van viscositeit op voedselinname onderzocht (Hoofdstuk 2). Tijdens dit onderzoek consumeerden 108 deelnemers zuivelproducten met chocoladesmaak in een vloeibare, halfvloeibare of halfvaste vorm. Deze zuivelproducten waren speciaal ontwikkeld voor dit onderzoek; de producten verschilden in viscositeit maar waren gelijkwaardig in energiedichtheid, macronutriëntensamenstelling en smakelijkheid. Het onderzoek vond plaats in een bioscoop om een alledaagse situatie na te bootsen. Tijdens het kijken van een film consumeerden de deelnemers de testproducten totdat ze

prettig verzadigd waren. Door de testproducten voor en na consumptie te wegen, kon worden vastgesteld hoeveel elke deelnemer had geconsumeerd. We vonden duidelijke statistisch significante verschillen in inname; de inname van het vloeibare product was 14% hoger ten opzichte van het halfvloeibare (P<0,0001) en 30% hoger ten opzichte van het halfvaste product (P<0,0001). Het doel van het tweede onderzoek (Hoofdstuk 2) was onderzoeken of het gevonden verschil in inname gerelateerd was aan eetsnelheid en/of de moeite die het deelnemers kostte om het product in de mond te krijgen via een rietje. In een gecontroleerde onderzoeksomgeving ontvingen 49 deelnemers dezelfde vloeibare en halfvaste producten als in het eerste onderzoek. Zowel eetsnelheid, als de moeite om het product in de mond te krijgen, werden gecontroleerd door middel van een pompje. Het testproduct werd in de mond van de deelnemers toegediend via een slang verbonden aan dit pompje. De resultaten van het onderzoek lieten zien dat het eerder gevonden verschil in inname niet gerelateerd was aan de moeite die het kostte om het product in de mond te krijgen; het weghalen van deze moeite door middel van het pompje resulteerde nog steeds in een 29% hogere inname voor het vloeibare product ten opzichte van het halfvaste product (P<0,0001). Eetsnelheid daarentegen speelde wel een belangrijke rol; het product in vloeibare vorm werd significant sneller gegeten dan het product in halfvaste vorm. Daarnaast waren er geen significante verschillen in inname tussen de twee producten wanneer eetsnelheid werd gestandaardiseerd. Wanneer de deelnemers waren geïnstrueerd om de producten via een rietje in plaats van via de pomp te consumeren, resulteerde dit in dezelfde bevindingen als in het eerste onderzoek; de inname van het vloeibare product was 34% hoger ten opzichte van het halfvaste product (P<0,0001).

Om de onderliggende factoren die verantwoordelijk zijn voor het effect van viscositeit op voedselinname verder te achterhalen, werd de rol van maagdarmhormonen onderzocht. Na het consumeren van een afgemeten hoeveelheid van de vloeibare en halfvaste testproducten werd de respons op de hormonen ghreline, CCK en GLP-1 vastgesteld (Hoofdstuk 3). Deze hormonen spelen een causale rol in voedselinnameregulatie en komen vrij in verschillende delen van het maagdarmstelsel. De resultaten lieten zien dat de onderzochte hormonen het effect van viscositeit op voedselinname niet konden verklaren; een afgemeten hoeveelheid van het vloeibare en halfvaste testproduct resulteerde in een vergelijkbare respons van de hormonen ghreline, CCK en GLP-1. Aan de hand van de gevonden resultaten in Hoofdstuk 2 en 3 werd besloten om verder in te gaan op de rol van orale sensorische factoren om het effect van textuur op voedselinname te onderzoeken.

In Hoofdstuk 4 hebben we de rol van orale sensorische blootstelling op de voedselinname nader onderzocht. Dit werd gedaan door zowel de hapgrootte als de verblijfsduur in de mond te varieren (Hoofdstuk 4). Verblijfsduur werd beschouwd als een schatter voor het concept orale sensorische blootstelling. Daarnaast werd hapgrootte beschouwd als een

aan orale sensorische blootstelling gerelateerde factor; een kleinere hapgrootte leidt mogelijk tot relatief meer orale sensorische blootstelling, omdat meer kleine dan grote happen nodig zijn om dezelfde hoeveelheid voedsel te consumeren. Onder verschillende testcondities consumeerde een groep van 22 deelnemers chocoladevla via een pompje. Hierbij was de hapgrootte klein (≈5 g), groot (≈15 g) of konden de deelnemers de hapgrootte zelf bepalen. De orale verwerkingstijd was 3 of 9 seconden, of zelf te bepalen door de deelnemer (alleen in combinatie met zelf te bepalen hapgrootte). De resultaten lieten zien dat voedselinname significant lager was bij een orale verwerkingstijd van 9 seconden ten opzichte van 3 seconden (gemiddeld een verschil van 42 g; *P*=0,008) evenals bij het consumeren met grote happen ten opzichte van kleine happen (gemiddeld een verschil van 106 g; *P*<0.0001). Daarnaast was er een significante relatie tussen de zelf te bepalen hapgrootte en voedselinname; deelnemers die het testproduct met grotere happen consumeerden hadden ook een hogere voedselinname. De resultaten van dit onderzoek bevestigen de belangrijke rol van orale sensorische blootstelling.

De tot hier toe gevonden onderzoeksresultaten laten zien dat de orale verwerkingstijd een belangrijke rol speelt in de verklaring van het effect van textuur op voedselinname. Een voedingsmiddel in vloeibare vorm wordt sneller geconsumeerd en verblijft niet lang in de mond, terwijl een voedingsmiddel in vaste vorm langzamer wordt geconsumeerd en veel langer in de mond verblijft. Langzaam eten en meer tijd nemen voor het verwerken van voedingsmiddelen in de mond, leidt tot langere orale sensorische blootstelling en als gevolg hiervan tot een eerdere beëindiging van de maaltijd. Eerdere onderzoeken naar het effect van de textuur van voedingsmiddelen waren voornamelijk gericht op voedingsmiddelen in (half)vloeibare vorm, of vergeleken vloeibare voedingsmiddelen met voedingsmiddelen in vaste vorm. Het was nog niet onderzocht of de textuur van voedingsmiddelen en de rol van orale sensorische blootstelling al dan niet relevant zijn binnen de groep van vaste voedingsmiddelen. Daarom was het onderzoek, beschreven in Hoofdstuk 5, gericht op het onderzoeken van het effect van textuurverschillen van vaste voedingsmiddelen op voedselinname. Speciaal ontwikkelde boterhamworst, vleesvervanger en snoep werden als testproducten voor dit onderzoek gebruikt. Van ieder testproduct bestond zowel een harde als zachte variant, beide met een gelijkwaardige energiedichtheid, macronutriëntensamenstelling en smakelijkheid. De verwachting was dat de textuurverschillen van de harde en zachte varianten zouden leiden tot een verschil in orale verblijfsduur en eetsnelheid, en dus tot verschillen in de duur van orale sensorische blootstelling. Ook dit onderzoek werd uitgevoerd in een bioscoop om een alledaagse situatie te creëren. Tijdens het kijken naar een film consumeerden 106 deelnemers de testproducten totdat ze prettig verzadigd waren. Gedurende de laatste testsessie werd de eetsnelheid van alle testproducten gemeten. In tegenstelling tot onze verwachting was eetsnelheid alleen significant lager voor de harde boterhamworstvariant versus de zachte boterhamworstvariant (21±10 g/min vs. 25±13 g/min; *P*<0,0001). Er waren geen verschillen

in eetsnelheid tussen de harde en zachte varianten voor de overige twee testproducten. Voedselinname verschilde niet significant tussen de harde en zachte varianten van alle testproducten. We denken dat de verschillen tussen de harde en zachte varianten van de testproducten te subtiel waren om verschillen in eetsnelheid en dus verschillen in voedselinname teweeg te brengen. Wel vonden we een significante correlatie tussen eetsnelheid en voedselinname (over het geheel een correlatiecoëfficiënt van r=0,54), wat betekent dat een hogere eetsnelheid samenhing met een hogere inname. Daarnaast werden de grootste verschillen in eetsnelheid en de sterkste correlatie tussen eetsnelheid en voedselinname gevonden voor boterhamworst. Ook consumeerde een groter aantal deelnemers meer van de zachte dan van de harde boterhamworstvariant (67 tegenover 39; *P*<0,01). Deze gegevens ondersteunen het idee dat langzamer eten en meer tijd nemen voor het verwerken van voedingsmiddelen in de mond, leidt tot langere orale sensorische blootstelling en uiteindelijk tot een lagere voedselinname. Echter, meer onderzoek binnen de groep van voedingsmiddelen in vaste vorm is nodig.

Tot op heden zijn er geen objectieve parameters beschikbaar voor het meten van orale sensorische blootstelling. Echter, een mogelijke indicator is de mate van *in vivo* retro-nasale aroma afgifte, ofwel de mate van interne overdracht van aroma's via de mond naar de neus gedurende voedselconsumptie. Onderzoek heeft aangetoond dat er een relatie bestaat tussen de retro-nasale aroma afgifte en de textuur en hapgrootte van voedingsmiddelen, in overeenstemming met orale sensorische blootstelling. In ons laatste onderzoek hebben we eetgedrag en retro-nasale aroma afgifte onderzocht bij deelnemers met een normaal gewicht en overgewicht (Hoofstuk 6). De doelen van dit onderzoek waren 1) het onderzoeken of eetgedrag (voedselinname, eetsnelheid, hapgrootte, aantal happen, maaltijdduur) gerelateerd is aan gewichtsstatus, en 2) of de mate van retro-nasale aroma afgifte gerelateerd is aan eetgedrag en gewichtsstatus. Een groep van 27 deelnemers met een normaal gewicht afgestemd (op basis van geslacht, leeftijd en graad van eetrestricties) op een groep van 27 deelnemers met overgewicht, consumeerden een gekruide rijstmaaltijd of yoghurt met appeltaartsmaak gedurende twee aparte testsessies. De mate van retro-nasale aroma afgifte werd gemeten tijdens een derde testdag. De resultaten lieten weinig tot geen verschillen in eetgedrag en retro-nasale aroma afgifte zien tussen de twee gewichtsgroepen. Geen van de aspecten van eetgedrag correleerde met retro-nasale aroma afgifte. De deelnemers vertoonden consistent eetgedrag voor beide testproducten; voedselinname en de andere aspecten van eetgedrag voor de gekruide rijstmaaltijd correleerden sterk met de voedselinname en aspecten van eetgedrag van de appeltaartyoghurt (correlatiecoëfficiënten van r=0,52 tot r=0,70). Eetgedrag lijkt een kenmerk van een individu te zijn, maar niet per definitie een kenmerk van een groep mensen gebaseerd op hun gewicht.

Hoofdstuk 7 bespreekt de belangrijkste bevindingen, methodologische overwegingen, interpretaties van de bevindingen en vergelijking met andere onderzoeken. Ook worden

hier implicaties en suggesties voor verder onderzoek gegeven. Onze onderzoeken tonen consistent het effect van de textuur van voedingsmiddelen op voedselinname en verzadiging aan; voedingsmiddelen in vloeibare vorm zijn minder verzadigend. Meer onderzoek is nodig om het effect van de textuurverschillen van vaste voedingsmiddelen op voedselinname te bepalen. Daarnaast is er ook meer onderzoek nodig om de rol van orale sensorische blootstelling in voedselinnameregulatie te bepalen, waarbij de nadruk zal moeten liggen op het vinden van manieren om orale sensorische blootstelling objectief te kunnen meten. Verder is het belangrijk om te bepalen welke factoren van invloed zijn op orale sensorische blootstelling. Tot slot is het belangrijk om lange termijn energiecompensatie na vrije inname van voedingsmiddelen met verschillende texturen te onderzoeken, om te bepalen of het effect van de textuur van voedingsmiddelen op voedselinname bijdraagt aan een verandering in de energiebalans.

Concluderend, de resultaten van dit proefschrift tonen aan dat de viscositeit van voedingsmiddelen een direct effect heeft op voedselinname. Orale sensorische blootstelling speelt hierbij een belangrijke rol, omdat het veranderen van eetsnelheid, orale verwerkingstijd en hapgrootte een effect heeft op voedselinname. Deze factoren dragen bij aan het effect van de textuur van voedingsmiddelen op voedselinname.

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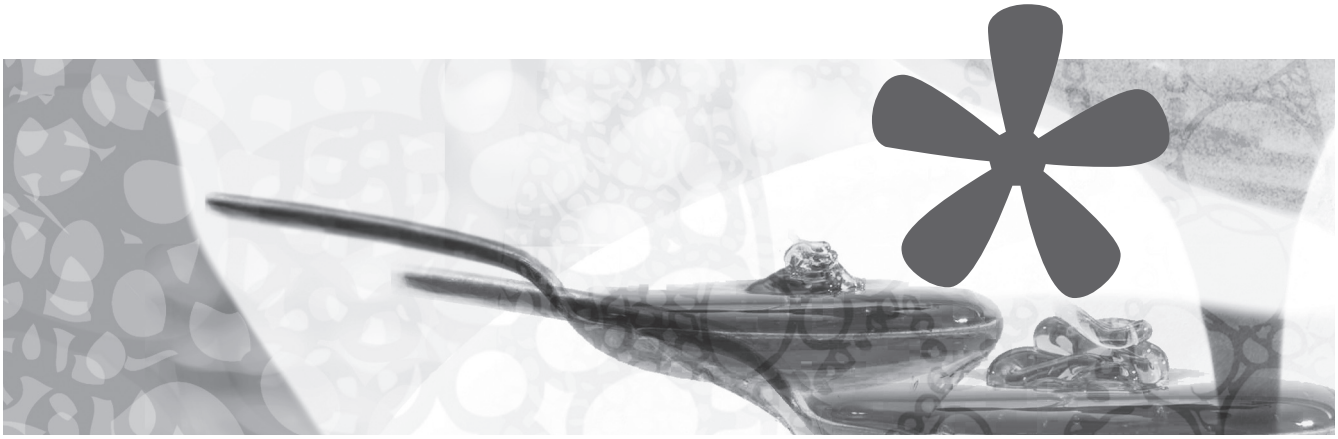
Nicolien



About the author

CURRICULUM VITAE

Nicolien (Nieske) Zijlstra was born on February 24, 1981 in Hoogezand-Sappemeer, the Netherlands. After passing secondary school (Voorbereidend Wetenschappelijk Onderwijs) at Augustinus College in Groningen, she started the Bachelor program Nutrition and Dietetics at Hanzehogeschool Groningen. She obtained her Bachelor's degree in 2003 and enrolled in the Master program Nutrition and Health at Wageningen University. For her Master thesis she performed a research study at the University Medical Centre in Utrecht, which resulted in a thesis and published paper entitled "24-hour indirect calorimetry in mechanically ventilated critically ill patients". In 2005 she obtained her Master's degree and immediately started working as a research assistant at the Division of Human Nutrition and Top Institute Food and Nutrition (at that time known as Wageningen Centre for Food Sciences) for the project "Sensory satiety, metabolic satiety and food intake regulation". In September 2006 the project extended and was renamed "Effects of physical chemical properties of food on sensory satiety, metabolic satiety, reward and food intake regulation". From that moment on, Nicolien was appointed as PhD fellow. Her research focused on the role of food texture in the regulation of food intake. During her PhD project Nicolien joined the educational program of the graduate school VLAG, she attended several (international) conferences and courses and was involved in teaching. Additionally, she was a member of the organizing committee of the PhD study tour to the United States in 2007 and a member of the daily board of the committee of temporary scientific staff within the Division of Human Nutrition from 2008 to end 2009. For her first paper she won the Top Institute Food and Nutrition Publication award of 2008. She was selected and joined the European Nutrition Leadership Programme in April 2010. In the end of 2009 she worked for several months as organizer, coordinator and teacher of the new Master course "Sensory Science I: Principles of Sensory Science" which is part of the new Master program Sensory Science of Wageningen University.

LIST OF PUBLICATIONS

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Zijlstra N, Wijk RA de, Mars M, Stafleu A and Graaf C de. Effect of bite size and oral processing time of a semisolid food on satiation. Am J Clin Nutr 2009; 90: 269-275.

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Zijlstra N, Bukman AJ, Mars M, Stafleu A, Ruijschop RMAJ and Graaf C de. Eating behavior and retro-nasal aroma release in normal weight and overweight subjects. Submitted.

Ruijschop RMAJ, Zijlstra N, Boelrijk AEM, Dijkstra A, Burgering MJM, Graaf C de and Westerterp-Plantenga MS. Effects of bite size and duration of oral processing on retro-nasal aroma release – features contributing to meal termination. Submitted.

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Zijlstra N, Bukman AJ, Mars M, Stafleu A, Ruijschop RMAJ and Graaf C de. Eating behavior and retro-nasal aroma release in normal weight and overweight subjects. In: abstract book British Feeding and Drinking Group meeting, 25-26 March 2010, Maastricht, NL (Oral presentation).

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Zijlstra N, Mars M, Stafleu A, Wijk RA de, Prinz JF, Hück NL and Graaf C de. Effect of bite size and oral processing time on satiation (abstract European Congress on Obesity, 6-9 May 2009, Amsterdam, the Netherlands). Obesity Facts 2009;2(suppl 2):165 (Poster presentation).

Zijlstra N, Mars M, Stafleu A, Wijk RA de, Prinz JF, Hück NL and Graaf C de. Effect of bite size and oral processing time on satiation (ad libitum food intake) (abstract Wageningen Nutritional Sciences Forum, 4-6 March 2009, Wageningen, the Netherlands). European Journal of Clinical Nutrition 2009; 63: S5–S23 (Poster presentation).

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Zijlstra N, Mars M, Stafleu A, Wijk RA de, Prinz JF, Hück NL and Graaf C de. Effect of bite size and oral processing time of food on satiation (abstract British Feeding and Drinking Group meeting, 26-27 March 2008, Liverpool, UK). Appetite 2008; 51: 753 (Oral presentation).

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Zijlstra N, Mars M, Wijk RA de, Westerterp-Plantenga MS and Graaf C de. Liquid foods result in higher ad libitum food intake than semi-solid foods because of a higher eating rate (Abstract European Congress on Obesity, 22 – 27 April 2007, Budapest, Hungary). International Journal of Obesity 2007; 31: S21–S26 (Oral presentation).

Zijlstra N, Mars M, Wijk RA de, Westerterp-Plantenga MS and Graaf C de. Liquid foods result in higher ad libitum food intake than semi-solid foods because of a higher eating rate (Abstract British Feeding and Drinking Group meeting, 2-3 April 2007, Newcastle upon Tyne, UK). Appetite 2008; 50, Issues 2-3; 567 (Oral presentation).

OVERVIEW OF COMPLETED TRAINING ACTIVITIES

Description	Organizer & location	Year
DISCIPLINE SPECIFIC ACTIVITIES		
Courses and workshops		
Course "Regulation of food intake and its implications for nutrition and obesity"	Graduate School VLAG, Wageningen (NL)	2006
Course "Food perception and preference"	Graduate School VLAG, Wageningen (NL)	2007
Workshop "The Influence of Sensory and Normative Cues on Human Food Intake"	Behavioural Science Institute, Radboud University Nijmegen, Nijmegen (NL)	2008
Course "New directions of sensory food research"	ABS Finnish Graduate School, Helsinki, Finland	2009
Conferences and meetings		
Meetings werkgroep Voedings-gewoonten (Nutritional Habits)	WEVO	2006 - 2008
Second European Conference on Sensory Consumer Science of Food and Beverages	European Sensory Network The Hague (NL)	2006
Annual meeting of the British Feeding and Drinking Group	BFDG Newcastle upon Tyne (UK) Liverpool (UK) Swansea (UK) Maastricht (NL)	 2007 2008 2009 2010
15th and 17th European Congress on Obesity (ECO)	EASO Budapest (H) Amsterdam (NL)	 2007 2009
ECO Satellite meeting "Nutrition & the brain"	EASO Budapest (H)	 2007
5th NIZO Dairy Conference "Prospects for Flavour Formation and Perception"	NIZO food research Papendal (NL)	 2007
Annual meeting of the Society of the Study for Ingestive Behavior (SSIB)	SSIB Steamboat Springs (USA)	 2007
7th and 8th Pangborn Sensory Science Conference	Elsevier Minneapolis (USA) Florence (IT)	 2007 2009

Symposium "Early development and obesity: Food preferences, diet and appetite regulation"	Association for the Study of Obesity (ASO) Liverpool (UK)	2008
Wageningen Nutritional Sciences Forum	Division of Human Nutrition, WUR Arnhem (NL)	2009
GENERAL COURSES AND WORKSHOPS		
PhD workshops	Wageningen Centre for Food Sciences (WSFS)/ TI Food and Nutrition	
"Debating"	Wageningen (NL)	2006
"Networking"	Wageningen (NL)	2008
"Writing press releases"	Wageningen (NL)	2009
Talent day "Write it right" and "Creative thinking"	NWO Utrecht (NL)	2006
PhD Introduction week	Graduate School VLAG Ermelo (NL)	2006
PhD Competence Assessment	Wageningen Graduate Schools (WGS) Wageningen (NL)	2006
Course "Experimental Design"	WIAS Wageningen (NL)	2008
Course "Personal Efficacy"	Meijer & Meijaard Wageningen (NL)	2008
Course "Scientific Writing"	Language Centre, WUR Wageningen (NL)	2008
Course "Philosophy and Ethics of Food Science & Technology"	WGS/ Graduate School VLAG Wageningen (NL)	2009
Master class "Starting with the client: New approaches to effective health promotion"	Graduate School VLAG Wageningen (NL)	2009
European Nutrition Leadership Programme (ENLP)	ENLP Luxembourg (L)	2009
OPTIONAL COURSES AND ACTIVITIES		
Preparation research proposals	WUR, Wageningen (NL)	2006 - 2009
Organizing and participating PhD study tour USA	Division of Human Nutrition, WUR	2007
Literature group "Journal Club" and "Oldsmobiles"	Division of Human Nutrition, WUR	2006 - 2009
Research presentations	Division of Human Nutrition, WUR	2006 - 2010
Research presentations	Wageningen Centre for Food Sciences / TI Food and Nutrition, Wageningen (NL)	2005 - 2010

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