The role of oral exposure to taste on meal termination

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Abstract

Background and aim

The rise in obesity over the last decades is considered to be related to changes in the food environment. Our current diet exists of foods that facilitate fast intake of energy and minimal oral processing. Various studies showed that higher eating rate leads to higher food intake, and therefore promote energy overconsumption. When consuming at a high eating rate, the food spends less time in the oral cavity, resulting in less sensory exposure per gram food. The exposure to the taste of the food in the oral cavity is potentially important in controlling food intake. The studies in this thesis investigated the principle mechanisms through which orosensory exposure affects satiation. The factors that were studied were taste intensity, oral residence duration and bite size. The impact of these factors and their relative contributions to satiation will provide tools for designing new foods to prevent overconsumption.

Methods

We conducted five studies. The subjects that participated in the studies were healthy young normal weight adults. Satiation was measured by ad libitum intake and subjective ratings of hunger and fullness. Tomato soup was used as test product in all studies. We started by investigating the effect of taste intensity on ad libitum intake (n=48). Salt was used to vary the taste intensity in soup. We selected two salt concentrations for low-salt and high-salt soup that were similar in pleasantness on an individual basis. In the next study, salt taste intensity in soup was investigated again, but this time we changed the state of hunger (a preload was offered) and the meal composition (subjects were served a second course after the soup) (n=43). In the third study, the impact of taste intensity versus the duration of orosensory exposure (manipulated by changing the bite size) on satiation was investigated, by using peristaltic pumps to control the bites (n=55). The fourth study focussed on the underlying mechanisms of bite size on food intake (n=56). Therefore, separate effects of oral residence duration per gram food and number of bites per gram food on ad libitum intake were assessed. Finally, we investigated if bite size affects the perceived food intake. Subjects estimated the amount consumed after intake with small or large bites, in both focussed and a distracted states (n=53). In addition, effects of distraction on bite size were investigated.

Results

Taste intensity did not affect ad libitum intake when the soup was presented as single lunch-item in a hungry state. However, higher taste intensity reduced ad libitum intake by ~8%, when the soup was presented after a preload or as a starter followed by a second meal. Smaller bite sizes decreased ad libitum intake by ~25% and did not
interact with taste intensity. That smaller bites are more satiating than larger bites was confirmed by hunger and fullness ratings. Hunger decreased faster per consumed gram food when consuming with small bites compared to large bites. A similar effect was found for the increase in fullness. Ad libitum intake was separately reduced by longer oral residence duration and higher number of bites per gram food, there was no interaction between the two variables. Time-intensity measurements showed that both higher number of bites and longer oral residence duration increase the total magnitude of orosensory exposure to the taste of the food. Consumption with large bites resulted in underestimations of the amount consumed, whereas consumption with small bites did not. Distraction increased ad libitum intake. Distraction led to a higher number of bites over the meal but did not affect bite size.

Conclusions

This thesis demonstrates that consuming foods with smaller bite sizes, longer oral residence durations and higher taste intensities lowers food intake. These effects are possibly explained through their enhancement of the orosensory exposure to the taste of the foods. Consumption with large bites leads to underestimation of the amount that is consumed. An underestimation of the amount consumed is a risk factor for overconsumption. These results could be used by the food industry to enhance the satiating capacity of foods in order to prevent overconsumption and decrease the prevalence of obesity.
# Table of contents

**Chapter 1**
General introduction

**Chapter 2**
Effect of salt intensity on ad libitum intake of tomato soup similar in palatability and on salt preference after consumption  

**Chapter 3**
Effect of salt intensity in soup on ad libitum intake and on subsequent food choice  
*Appetite, 2012, 58: 48-55*

**Chapter 4**
Both longer oral sensory exposure to and higher intensity of saltiness decrease ad libitum food intake  
*Journal of Nutrition, 2012, 141: 2242-2248*

**Chapter 5**
Both higher number of bites and longer oral residence duration increase the oral sensory exposure to food and reduce ad libitum food intake  
*Submitted for publication*

**Chapter 6**
Consumption with large bite sizes increases food intake and leads to underestimation of the amount consumed  
*Submitted for publication in revised form*

**Chapter 7**
General discussion

**References**

**Samenvatting** (Summary in Dutch)

**Dankwoord** (Acknowledgements)

**About the author**
Chapter 1

General introduction
Food intake is critical for survival and health (1). The control of food intake is important for energy balance. A positive energy balance, thus higher energy intake than energy expenditure, may on the long-term result in overweight and obesity. Obesity has strong adverse effects on health, it increases risks of diabetes type II, cardiovascular disease and several types of cancer (2).

The prevalence of obesity has risen dramatically in the last decades (3). The rise in obesity is considered to be related to changes in the food environment (4, 5). The current 'obesogenic' environment is associated with a wide variety of high palatable, easy available, inexpensive, energy-dense foods and increased portion sizes. Moreover, our diet in general has become more energy dense. Dietary changes shifted to higher intake of fat, salt and sugar and lower intake of fibre (6). In addition, consumption of energy yielding beverages, like soda sweetened with sugar, energy drinks, fruit drinks and juices, has increased considerably over last decades (7, 8). Energy yielding beverages and energy dense foods that are low in fibre support a fast intake of energy and minimal oral processing (9, 10).

Higher eating rate (g/min) leads to higher food intake (9-14), and is therefore promoting energy overconsumption. Several studies have suggested a positive relationship between eating rate and body weight status (15-18). When consuming at a high eating rate, the food spends less time in the oral cavity, resulting in less sensory exposure per gram food. In other words, the taste perception per gram food decreases when food is eaten at a high eating rate compared to a low eating rate. The exposure to the taste of the food in the oral cavity is potentially important in controlling food intake. The research described in this thesis investigated the role of oral sensory (orosensory) exposure to taste in food intake.

Food intake regulation, satiation and satiety

Food is eaten in episodes, i.e., in meals and snacks. Food intake is initialized by a desire-to-eat. The desire-to-eat is the result of an integration of the internal state of hunger along with contextual aspects (e.g., time of the day, opportunity, habits, sight, and smell) (19, 20). Food intake is continued by reward signals from the brain generated from sensory signals from the food. These reward signals will finally be overruled by signals that bring the meal to an end (20, 21). The process that ends an eating episode is called satiation.

Immediately after an eating episode, there is low appetite for food. Food processing and nutrient absorption initiate neural responses and release of hormones from the gastrointestinal tract. These hormones and neural responses are translated in the brain to suppress hunger until the next eating episode (22, 23). The process that operates after
the meal that involves the suppression of hunger and inhibition of further eating is called satiety.

Satiation influences how much food is consumed in an eating episode, whereas satiety influences the frequency of eating episodes (19, 23). The research described in this thesis focuses on satiation.

**Factors that influence satiation**

Influences on satiation are divided into internal, sensory, cognitive and environmental factors, as shown in Figure 1. These factors are strongly related and mutually influence satiation and thereby meal size.

The internal signals, such as hormones from the gastrointestinal tract (e.g., ghrelin, leptin, glucose) are translated in the brain to reflect the state of hunger. The state of hunger prior to a meal influences the amount consumed (e.g., 24). During consumption, the degree of stomach distension and the release of hormones from the gastrointestinal tract (e.g., cholecystokinin and glucagon-like peptide 1) triggers brain signalling of satiation that brings a meal to an end (23, 25). Satiation is also regulated in the long-term by adiposity signals that signify body fat to control energy homeostasis (1, 19).

The environment determines the availability of the amount and types of food. It has been extensively shown that greater portion sizes lead to more food intake (26-33). The availability of a variety of different foods leads to more food intake than the presence of one or a few foods (e.g., 34); explained below by sensory specific satiety. The social setting also influences how much food will be consumed. The presence of family and friends leads to higher food intake because it distracts from consumption (e.g., 35). In addition, people tend to adjust their amount of food intake to that of their eating companions; they eat more when others eat more, and less when others eat less (36, 37). Other distracting activities, for example watching television or listening to music, increases the amount consumed (35, 38-41).

Sensory signals determine the hedonic value and the desire-to-eat the food. The pleasantness derived from food influences the amount consumed (42-47). During consumption, the repeated exposure to sensory signals leads to a decline in reward value of the eaten food, which contributes to meal termination (48). Sensory signals are considered of major importance in satiation due to their early onset during consumption (25). Sensory signals also encourage eating a variety of different foods, thereby providing different nutrients. When the desire-to-eat for the consumed food declines, the interest shifts to other foods with different sensory food properties, this phenomenon is called sensory specific satiety (e.g., 49).
Humans cognitive attitude towards food and eating influences meal size (50). From early childhood, humans learn to link sensory signals from food to post-ingestive consequences. Therefore, humans have associations about the satiating capacity of food. Beliefs regarding the satiating capacity of food influences how much will be consumed (50-53). This allows humans to make decisions on meal size before consumption (54). The cognitive control of food intake also occurs during a meal, humans monitor the amount they are consuming (55). Another important cognitive aspect that plays a role in meal size is restraint eating behaviour; the chronic tendency to limit food intake for controlling body weight (56). The research described in this thesis involves primarily effects of sensory signals on satiation (chapter 2-6). Cognitive effects combined with sensory signals were investigated in the last study (chapter 6) of this thesis.

**Figure 1.1** Meal size is determined by an integration of cognitive, sensory, internal and environmental factors.

**Sensory signals from food**

The perception of food starts when seeing, grasping and tasting the food. This evaluation is an integration of senses of taste, smell, touch (such as mouth feel, temperature, irritation), sight and hear. This multi-modal sensory integration is essential to encourage or discourage consumption.

Food aroma perception (smell) occurs via the nose from the external world (i.e., ortho-nasal) and via the mouth during food consumption (i.e., retro-nasal). The olfactory
system can recognize and discriminate a large number of different aroma qualities (57), whereas the taste system only distinguish five different qualities. Food texture involves structural and mechanical properties that are detected by the senses of touch, hear and sight (58). Examples of food texture parameters are viscosity, hardness, chewiness and stickiness. The research described in this thesis focused on effects of taste on satiation.

**Taste**

The current consensus is that human taste sensations can be divided into five qualities: sweet, salty, umami, sour, and bitter. Specific taste receptor cells organized in taste buds located within the gustatory papillae detect these tastants. The gustatory papillae are located on the tongue but also on other area’s in the oral cavity such as the palate, pharynx, the larynx and epiglottis (59).

The sense of taste is in charge of evaluating the nutritional value of a meal. Sweetness is an attractive taste. It is produced by sugar and a few other substances and is related to the carbohydrate content of foods. The function of sweetness is to identify energy rich foods. Saltiness is attractive in low concentrations. Saltiness primarily signals the presence of sodium. Sodium plays a fundamental role in regulating the volume of fluid compartments, nerve conductance, and muscle contraction (60). Almost all foods naturally contain sodium, meats and seafood more than plant-based foods. However, only 5-12% of our sodium intake directly originates from food, ~75-80% of the sodium intake originates from processed foods, and ~10-15% from table salt (61). Umami is an attractive taste described as a savoury, meaty taste. The taste of umami is mostly produced by monosodium glutamate (MSG). Foods rich in umami are: fish, meats, fermented foods, some vegetables and mushrooms. Bitterness evokes aversiveness. There are many different compounds that evokes a bitter taste, approximately 550 (62). A large number of bitter compounds are known to be toxic. Bitterness is therefore considered as a warning signal of toxins. Sourness is the taste that detects acidity. Fruits are the most common food group that naturally contain a sour taste component. Sourness in high concentrations evokes aversiveness and warns against spoiled foods or unripe fruits. The attraction towards sweet, umami, and low-salt, and the aversion towards bitter, high-sour, and high-salt are innate responses (63-65). The preference for taste can be modified during life. Individuals have differences in preferences and sensitivity for each taste quality. Preference for taste is also affected by experience, age, race and nutritional deficiencies (66, 67).
The role of food properties and sensory signalling in satiation

Sensory signals from food are learned to be associated with its energy and nutrient content (68, 69). These learned associations help to consume an appropriate amount of food in a meal that provides an adequate energy intake (68, 70, 71). Our current diet consists of many highly processed foods, including foods high in energy density, fat and sugar, and low in fibre (72). Sensory signals of highly processed foods have been shown to impair the identification of nutrients compared to raw or moderately processed foods (73). The impaired ability to link sensory signals from food to nutrient and energy intake may contribute to overconsumption and weight gain (74).

The inability to adjust food intake to its energy density have been demonstrated in studies that used variations in energy densities within the same foods. Intake was much more affected by the volume or weight of the food than the energy density (75-78). In one study (77), women were provided with meals for two days that varied in energy density. The diet lower in energy density contained approximately 30% less energy per gram than the diet higher in energy density. Participants ate a consistent amount of food (by weight) across conditions. The diet lower in energy density resulted in 31% less energy intake over two-days, without differences in hunger and fullness compared to the diet higher in energy density. In addition, also others have found that the consumed volume or weight has a greater impact on meal termination than its energy density (79-81). Foods high in energy density therefore promote overconsumption.

Influences of sensory modalities of taste, aroma and texture on satiation have been investigated separately. Food texture has been shown to greatly affect the amount consumed; ad libitum intake of liquid foods led to ~30% higher intake compared to intake of semi-solid foods equal in energy density and palatability (10, 80, 82). Differences in taste quality, sweet vs. savoury, did not affect ad libitum intake in foods that were similar in texture, palatability and energy density (83, 84). Differences in aroma quality, vanilla vs. lemon, did also not affect ad libitum intake in foods that were similar in texture and energy density (85). In line with these findings, Hogenkamp et al. (80, 86) showed that both food intake and expectations regarding the satiating capacity of food were mainly affected by texture and not by flavour quality.

In summary, satiation seems to be influenced by food texture rather than energy density and taste or flavour quality. Semi solids and solids are more satiating than liquids (10, 87, 88). The eating rates for solids (5 – 130 g/min) and semi solids (50-230 g/min) are much lower than for liquids (300-630 g/min) (11). Interestingly, ad libitum intake of a semi-solid and liquid food was not different in a study were the eating rate was kept constant (10). This suggests that the effect of texture on satiation is mediated via eating rate. The eating rate is negatively related to the orosensory exposure to taste, the latter is considered to be important in satiation.
The role of oral sensory exposure of taste in satiation

It has been repeatedly shown that higher rate of eating leads to higher food intake (9-13). This effect was found within the same foods (13, 89, 90). In addition, the eating rates of a wide range of different foods were positively correlated with their food intakes (in gram and energy) (11). Foods that promote high eating rate are liquid foods and foods low in fibre content (9-12). The effect of eating rate on food intake is suggested to be mediated via exposure to the taste of food in the oral cavity (orosensory exposure). The importance of orosensory exposure in meal termination was shown in an experiment where oral intake elicited much stronger responses on satiation compared to direct infusions of food into the stomach or duodenum (91). How eating rate influences orosensory exposure to taste and how this affects satiation is not exactly known. The eating rate is determined by the oral residence duration (i.e., residence time of food in the oral cavity), the bite size, and the bite frequency. Figure 1.2 illustrates a model that shows relationships between oral residence duration, bite size and eating rate and their potential effects on ad libitum intake.

Oral residence duration

Weijzen et al. (92) investigated the effect of oral residence duration on ad libitum food intake when eating rate (g/min) was constant. Longer oral residence duration decreased intake of lemonade (92). In addition, also Zijlstra et al. (93) found that longer oral residence duration decreased the ad libitum intake of a chocolate dairy product. These studies (92, 93) used sweet tasting foods. Humans associate sweetness with energy; longer oral residence duration of a sweet taste may therefore lead to an earlier onset of satiation. We do not know whether the effect of oral residence duration on satiation is a general effect or a taste specific effect. Saltines may not have strong association with energy compared to sweetness. The effect of oral residence duration to saltiness on satiation is one of the main research questions in this thesis (Figure 1.2).

Bite size

Consumption with large bite sizes increases the eating rate (g/min) (12, 94). A number of studies have found a positive relationship between bite size and food intake in laboratory settings and ‘real-life’ environments (13, 95-99). It is not clear why bite size affects satiation. In a normal eating situation, smaller bites are associated with longer oral duration per gram food (s/g) (100). The effect of bite size may therefore be explained by the oral duration per gram of food. Nevertheless, consuming with small bites rather than large bites involves a higher number of bites for consumption of the same amount of food. A relatively higher number of bites, for example three bites of 5 g instead of one bite of 15 g, mean a more pulsating exposure to the food. The pulsating exposure is possibly associated with more orosensory exposure per gram food, which
may lead to a faster satiation. The impact of oral residence duration (s/g) versus the number of bites (bites/g) on satiation has been studied in this thesis (Figure 1.2).

Bite size and cognition

Bite size may also cognitively affect food intake; humans may believe that intake is higher when taking relatively more small bites compared to fewer larger bites for consumption of the same amount of food. Humans’ beliefs of the amount consumed play an important role in satiation. For example, information about the calorie content (101-103), the serving size of the food (95, 97, 104-106), and the time of the day (107), were all shown to influence food intake and stress the importance of cognition on satiation. Cognitive associations of food intake may be disrupted when people are distracted during food consumption. A number of studies have shown that distraction by activities such as watching television or eating with friends usually leads to increased food intake (35, 38-41, 108). It is possible that distraction during consumption is associated with an impaired monitoring of the amount consumed through visual cues (35, 109). Other oral processing characteristics, such as bite size, meal duration, or number of bites may also be affected by distraction. In a distracted state, people may unconsciously take larger bites or increase their number of bites resulting in increased food intake (Figure 1.2).

Taste intensity

A stronger intensity of the taste of the food may also be associated with more orosensory exposure, which may result in an earlier onset of satiation. Warwick et al. (110) found that “tasty” foods were more satiating than “bland” foods equal in energy and macronutrient composition. This is in line with another study that found that ad libitum intake was lower for more intensely flavoured snacks compared to less intensely flavoured snacks (111). Studies that used a concentration range of sweetness in a food, one example is sugar in yoghurt, have shown that the optimal, most preferred sweetness in food led to highest intake (112-116). Two studies suggest that high-sweet foods decreased intake more than low-sweet foods (112, 113), one study suggests the opposite effect (116). Others found no differences between low-sweet and high-sweet foods (114, 115). Next to sweetness, humans have an optimal level of saltiness in food that is highest in pleasantness, less salt will be judged as “bland” and more salt will be judged as “too salty” (117-119). Yeomans et al. (120) have found highest intake of the pasta that was optimal in salt concentration, whereas lowest intake was found for high-salt pasta and the intake of low-salt pasta was in between. The taste intensity highly influences the pleasantness of the food (112-116, 120, 121), the latter is a strong predictor of the amount of food consumption (42-47). Pleasantness rather than the taste intensity may have affected the results of the studies described above. It is not clear
whether taste intensity *per se* affects satiation when pleasantness is similar between low-intense and high-intense tasting foods (Figure 1.2).

**Aim and thesis outline**

The research described in this thesis aims to investigate the role of orosensory exposure to taste in satiation. Clarification of principle mechanisms through which orosensory exposure affects satiation is important for the understanding of food intake regulation. The factors that are investigated are the taste intensity, oral residence duration and bite size. In addition, the effects of distraction on bite size and on number of bites during ad libitum intake were investigated (Figure 1.2). The impact of these factors and their relative contributions to ad libitum food intake will provide tools for designing new foods and advices to prevent overconsumption.

The aim of the first study was to investigate effects of taste intensity on ad libitum intake, independent of pleasantness (chapter 2). Salt was used to vary the taste intensity in soup. Concentration-intensity and concentration-pleasantness functions were conducted for each subject. We selected two salt concentrations for low-salt and high-salt soup that were similar in pleasantness on an individual basis. Subjects then consumed ad libitum from low-salt soup and high-salt soup. Whether sensory signals affect satiation may be dependent on the state of hunger and meal context. In the second study, we tested again the effect of saltiness on ad libitum intake, but this time within two different meal settings (chapter 3). In the first meal setting, subjects consumed the soup after a preload. In the second meal setting, subjects consumed the soup as a starter followed by a second course.

The aim of the third study was to investigate the effect of oral residence duration and bite size in combination with saltiness on ad libitum intake (chapter 4). Again, salt concentrations for the low-salt and high-salt soup were selected on an individual basis. In addition, we investigated the effect of saltiness on bite size determined by subjects themselves. In the fourth study, underlying mechanisms of bite size on food intake were studied. Therefore, separate effects of oral residence duration (s/g) and number of bites (bites/g) on ad libitum intake were assessed (chapter 5). In addition, effects of oral residence duration and number of bites on the orosensory exposure per gram food were determined. The fifth study was executed to investigate whether or not cognition plays a role in the effect of bite size on ad libitum intake (chapter 6). In addition, we investigated if the effect of bite size on intake was disturbed by distraction. Effects of distraction on bite size were also studied.

In the final chapter, the main results of all studies are discussed (chapter 7). Implications and suggestions for future research are presented.
Figure 1.2 Model that shows the potential effects of taste intensity, oral residence duration, and bite size on ad libitum intake that have been studied in this thesis (dotted lines). In addition, the effects of distraction on the bite size and on the effect of bite size on ad libitum intake were studied. The numbers indicate the chapters in which the results are described. The large arrows indicate the known effects of eating rate, pleasantness and distraction on ad libitum intake. The solid lines indicate known relationships between oral duration and bite size on eating rate, and between pleasantness and taste intensity.
Chapter 2

Effect of salt intensity on ad libitum intake of tomato soup similar in palatability and on salt preference after consumption

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Abstract

Sensory properties of food play an important role in satiation. Studies on the effect of taste intensity on satiation show conflicting results. This may be due to the notion that in these studies taste intensity and palatability were confounded. The objective of this study was to investigate the effect of salt intensity of tomato soup on ad libitum intake (satiation), while controlling for palatability on an individual basis. Forty-eight subjects consumed both a low-salt (LS) and high-salt (HS) soup ad libitum from a self-refilling bowl. The results showed no difference between LS and HS soup in ad libitum intake, eating rate, changes in appetite ratings and changes in hedonic ratings after intake. After intake of HS soup, LS soup was perceived as more bland than before intake of HS soup. After intake of LS soup, HS soup was perceived as more salt intense than before intake of LS soup. In conclusion, this study found no effect of salt intensity on satiation of tomato soups that were similar in palatability. During consumption, subjects adapted quickly to the exposed salt intensity, as contrasting salt intensities were rated further from the ideal salt intensity and therefore perceived as less pleasant after consumption.

Keywords: satiation, contrast effect, ad libitum intake, salt intensity
Introduction

Obesity is an increasing problem in Western society. Increased meal size is considered a major cause of weight gain (97, 122, 123). Insight in the meal termination (satiation) process may provide tools to prevent over-consumption during a meal. Satiation is regulated by sensory factors, physiological factors and psychological factors (23, 124). It is likely that sensory factors are of primary importance in satiation, due to their early onset during consumption (25, 48, 125).

Several sensory properties have been shown to influence satiation. For instance, a clear negative relationship was found between the viscosity of a food and the amount of ad libitum intake (10). The effect was attributed to the duration of sensory exposure in the oral cavity, as a prolonged sensory exposure per bite resulted in less ad libitum intake (12, 92, 93). Apart from sensory exposure time, the intensity of sensory exposure may also influence satiation, because a higher intensity also increases the amount of sensory exposure, in this case not in time but in strength.

A number of studies investigated the effect of taste intensity, mostly in sweet products, on ad libitum intake but the results are conflicting. Some studies indicated that yoghurts with high sweet intensity decrease ad libitum intake more than yoghurts with low sweet intensity (112, 113), while results from other studies found no clear differences (114, 115) or even an opposite effect (116). Moreover, a pasta sauce high in intensity, obtained by salt intensity (120) and oregano intensity (121), resulted in lower intake than the pasta sauces low in intensity. It is difficult to extrapolate from these studies whether intensity had an effect on ad libitum intake because pleasantness differs among intensities and this may have overruled the effect of intensity on intake. Palatability is considered to be a strong predictor of the amount consumed (43, 44, 120, 121, 126). Therefore, initial pleasantness should be kept constant to study the effect of taste intensity on satiation.

Exposure to a high or low intense taste may change the perception of intensity and preferred level of intensity. Helson’s theory of adaptation-level (127), originating from psychophysical experiments, suggests that judgments are made with respect to a frame of reference. People refer to the most recent experience in evaluating the sensory properties of a food. Studies that investigated contextual effects on perception of taste intensity showed a shift in perceived intensity when a product was tasted after exposure to a low or high intense product. The shift in intensity is the common result of a contrast effect, products are perceived more intense when exposed to low intense tastes and less intense when exposed to high intense tastes (128-130).

A change in perceived intensity may also affect palatability, because intensity is related to palatability (112-116, 120, 121, 131). By itself, palatability is also able to trigger a
contrast effect, for instance, a ‘neutral’ beverage was increased in palatability when subjects were previously exposed to an unpalatable beverage (132). The opposite of contrast is assimilation, meaning that the stimulus becomes similar to the preceding stimulus or expectation (133). Cardello and Sawyer (133) studied the effect of expectations on perception of foods and found mainly assimilation effects, for example, a higher sweetness expectation resulted in higher perceived sweetness.

The studies above (128-130, 132, 133) highlight the importance of contextual effects on perception of intensity and palatability. This indicates that consumption of one food can affect the perception of other foods, which is interesting because people consume different foods during one meal. In the experiments that showed contrast effects (128-130, 132), however, only small amounts were tasted. Whether these effects remain when a food is consumed until satiation is unclear. When consuming a food until satiation, pleasantness decreases specifically for the consumed food, while pleasantness of other foods does not decrease or decreases less, this phenomenon is called: “sensory specific satiety” (SSS) (49). When a food is eaten to satiation, its pleasantness decreases and people will switch to other foods that taste more pleasant, therefore SSS also encourages humans to consume a variety of different foods (134). When eating a food that is low in taste intensity, people may get tired of the bland taste and prefer foods higher in taste intensity afterwards and vice versa. Some studies showed a shift in preferred intensity towards lower concentrations, as observed in sweet intensity level (113, 115, 131) and in salt intensity level (135) after consumption of a food “optimal” in taste intensity.

The primary objective of the present study was to investigate the effect of taste intensity on satiation in foods similar in initial palatability. The effect of taste intensity on satiation when palatability is kept constant has not been studied before. For each subject individually, a low-salt (LS) and high-salt (HS) tomato soup were selected with similar initial pleasantness ratings. Subjects consumed ad libitum from the LS and HS tomato soup during lunchtime. The secondary objective was to assess changes in perception and preferences of salt intensity after ad libitum intake of LS versus HS soups.

Subjects and Methods

Experimental design

The study consisted of three different stages. In the first stage, analytical taste profiles of soups with varying salt concentrations were established. The aim of this stage was to verify whether salt intensity ratings increased linearly with geometric increasing salt.
concentrations (a factor 1.55 between adjacent salt concentrations) (118, 136) and to give insight in the perception of sweet and sour intensity when salt intensity increases.

In the second stage, subjects rated pleasantness and relative-to-ideal salt intensity ratings of soups with varying salt concentrations. This was performed to determine salt concentrations for LS, ideal-salt (IS) and HS soups per subject. An inverted U-shape describes the relationship between pleasantness and salt intensity with the most pleasant soup containing the ideal salt concentration on the top (118). One salt concentration below (LS) and one salt concentration above the ideal salt concentration (HS) were selected for each subject by linear interpolation based on equal initial pleasantness.

In the third stage, subjects visited the lab four times during lunch time and consumed LS soup and HS soup each two times. Subjects consumed the tomato soup from a self-refilling bowl as described by Wansink et al. (55); this was done to minimize self-monitoring of the amount consumed. Subjects were aware of the fact that the bowl was refilling.

Before and after ad libitum intake, small samples of LS, IS and HS soups were rated on several hedonic and analytical aspects (Table 2.1). Hedonic (pleasantness and relative-to-ideal salt intensity) and analytical aspects (salt intensity) were rated in separate lunches; therefore, both LS and HS soup were consumed twice. A distinction between hedonic and analytical aspects was made to measure salt intensity independent of hedonics. The aim was to get insight in changes in both salt intensity preference (pleasantness and relative-to-ideal salt intensity, measured in LS1 and HS1) and in salt intensity perception (salt intensity, measured in LS2 and HS2) after intake.

Table 2.1 Measurements during the four lunch sessions

<table>
<thead>
<tr>
<th>Ad libitum intake condition</th>
<th>Ratings</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS1 soup</td>
<td>pleasantness, desire-to-eat, relative-to-ideal salt intensity</td>
</tr>
<tr>
<td>LS2 soup</td>
<td>salt intensity, expected satiation</td>
</tr>
<tr>
<td>HS1 soup</td>
<td>pleasantness, desire-to-eat, relative-to-ideal salt intensity</td>
</tr>
<tr>
<td>HS2 soup</td>
<td>salt intensity, expected satiation</td>
</tr>
</tbody>
</table>

Subjects

Subjects were recruited from a database of people interested in taking part in trials from the Division of Human Nutrition at Wageningen University, Wageningen, The
Netherlands. Forty-eight subjects (24 females and 24 males) were selected; all were students from Wageningen University. Subjects were healthy, had a normal weight (Body Mass Index: BMI 18.5-25 kg/m\(^2\)), were aged between 18 and 27 year (mean ± SD = 20.8 ± 1.99) and liked creamy tomato soup (pleasantness score > 5 on a 9-point hedonic scale). Exclusion criteria were restrained eating (Dutch eating behaviour questionnaire (DEBQ) score men: >2.25, women: >2.79), having followed an energy-restricted diet during the last two months, gained or lost > 5 kg weight during the last year, having a lack of appetite, smoking, having gastrointestinal illness, having diabetes, having thyroid disease or any other endocrine disorder, having hypertension, suffering from kidney diseases and being pregnant or giving breast feeding. In addition, staff and students from the Division of Human Nutrition were excluded from participation. Subjects were unaware of the aim of the research. The study was approved by the Medical Ethical Committee of Wageningen University and all subjects signed an informed consent form.

**Test product: tomato soup**

Tomato soup with varying salt concentrations was used as test product in this study. One kilogram of soup was made from 600 g mashed tomato pieces (Heinz, Elst, The Netherlands), 80 g cream (kookroom, private label Albert Heijn, Zaandam, The Netherlands), 310 g water and 10 g sucrose. The mixture was heated to 80°C. The macronutrient composition was calculated at 0.8 g protein, 3.3 g carbohydrates, 1.6 g fat and 129 kJ (31 kcal) energy per 100 g soup. Eight sodium concentrations were used with equal geometric distances (factor 1.55): 63 (soup 1), 98 (soup 2), 151 (soup 3), 234 (soup 4), 363 (soup 5), 561 (soup 6), 870 (soup 7) and 1349 (soup 8) mg Na/100 g soup. The sodium concentration in soup 1, to which no salt was added, was calculated from the used ingredients. Soups were equal in viscosity; soup 1 and 7, the soups with lowest and highest salt concentration selected for ad libitum intake, had a viscosity of 0.246 Pa/s and 0.223 Pa/s, respectively, at a shear rate of 45 (1/s) at 55 °C.

**Analytical taste-profile**

Subjects rated all eight salt concentrations in soups on analytical attributes: salt intensity, sour intensity and sweet intensity. Subjects received 15 g of each soup in random order. The temperature of the soups was ± 55 °C. The salt intensity question was: “How strong is the salty taste of this soup?”; the scale was labelled “very weak” at the left end (0 mm) and “very strong” at the right end (100 mm) on a 100 mm visual analogue scale (VAS). Similar questions were asked for sweet and sour.
Selected LS, IS and HS soups and hedonic taste profile

To select LS, IS and HS soups on an individual basis, subjects rated 15 g of sampled soups with varying salt concentrations on relative-to-ideal salt intensity and pleasantness. The question that refers to relative-to-ideal salt intensity was: “How salty is the taste of this soup?”; the scale was labelled “not nearly salty enough” (-50 mm) at the left end, “just right” in the middle (0 mm) and “much too salty” at the right end (50 mm) of the scale. The pleasantness question was: “How pleasant is the taste of this soup?” the scale was labelled “very unpleasant” at the left end (0 mm) and “very pleasant” at the right end (100 mm). The soups were presented in an interactive procedure according to the method specified by Booth et al. (117). This procedure was developed as a quick method to find the individual ideal (i.e., most pleasant or optimal) salt concentration.

Soup 5, with a sodium concentration similar to that in commercially available tomato soups, was presented first. Depending on the rating of the first sample on relative-to-ideal, the second sample was chosen in a way to be rated on the other side of ideal from the first sample. For example, if the first sample was rated above ideal, then the second sample would be below ideal or vice versa. The procedure was continued until there were five ratings: two below ideal, one close to ideal (-10 < 0 < 10 mm) and two above ideal. After a 15 minute break, subjects received the same five soups in a different order, however, again alternating on each side of ideal (117).

For each subject, the means of duplicates were calculated and plotted against geometric sodium concentration. The IS soup was selected as the soup that was rated closest to the “just right” point (i.e., 0 mm on relative-to-ideal salt intensity ratings). The LS and HS concentrations were chosen at each side of ideal based on equal pleasantness (<10 mm difference on pleasantness ratings) as determined by linear interpolation. Each pair of LS and HS soups was selected in a way that the distance in geometric sodium concentration (i.e., the ratio) was the same between LS and HS soup. HS soup was for each individual 3.72 times higher that LS soup, which equals two soup numbers in between, Table 2.2.
Chapter 2 – Effect of salt intensity on ad libitum intake

Table 2.2 Distribution of the selected salt concentration for LS and HS soups.

<table>
<thead>
<tr>
<th>N</th>
<th>LS soup</th>
<th>HS soup</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg Na/100g</td>
<td>mg Na/100 g</td>
</tr>
<tr>
<td></td>
<td>soup no.</td>
<td>soup no.</td>
</tr>
<tr>
<td>3</td>
<td>63 1</td>
<td>234 4</td>
</tr>
<tr>
<td>4</td>
<td>98 2</td>
<td>363 5</td>
</tr>
<tr>
<td>2</td>
<td>121 2.5</td>
<td>503 5.5</td>
</tr>
<tr>
<td>17</td>
<td>151 3</td>
<td>561 6</td>
</tr>
<tr>
<td>2</td>
<td>168 3.25</td>
<td>626 6.25</td>
</tr>
<tr>
<td>12</td>
<td>188 3.5</td>
<td>698 6.5</td>
</tr>
<tr>
<td>1</td>
<td>210 3.75</td>
<td>779 6.75</td>
</tr>
<tr>
<td>7</td>
<td>234 4</td>
<td>870 7</td>
</tr>
</tbody>
</table>

Ad libitum intake

Over a period of four weeks, subjects visited the lab during lunchtime once a week to eat ad libitum from the selected LS soup and HS soup from a self-refilling bowl. LS1, LS2, HS1 and HS2 soups were presented in random order to the subjects. Subjects were instructed to consume the same breakfast and to abstain from eating and only allowed drinking water or weak tea three hours before the lunch started. Moreover, they were asked to refrain from drinking one hour before the test started. After each test lunch, subjects had to answer questions about what they consumed for breakfast and whether they consumed or drank between breakfast and test lunch. To make sure subjects would consume the soup until they were satiated, they were not allowed to eat one hour after the test.

The procedure of a test day was as follows: first, subjects started rating their feelings of hunger, fullness, prospective consumption (i.e., how much they thought they could eat) (137) and thirst on a 100 mm VAS. Thereafter, subjects tasted a small sample (15 g) of the individually selected LS, IS and HS soup at random and rated various aspects (Table 2.1). Following this, subjects were seated in front of a soup bowl covered by aluminium foil. A laptop was placed behind the bowl with instructions for the subjects. They were instructed to take off the aluminium foil and push a button when they started eating and when they finished eating, so that eating time was recorded. Subjects were instructed to terminate eating when they felt they had enough. The mean initial temperature of the ad
libitum selected soup was 55° C (SD = 5.3 °C). When they finished eating, they rated again their feelings of hunger, fullness, prospective consumption and thirst. Finally, they re-rated the soup samples on several aspects according to Table 2.1.

The ratings according to Table 2.1 were asked as follows. The question that refers to desire-to-eat was “How much would you like to eat this soup at this moment?” from “not at all” at the left end to “very much” at the right end. The question that refers to expected satiation was “How filling is this soup?” from “not at all” at the left end to “very much” at the right end. The remaining questions from Table 2.1 are previously described.

**Self-refilling bowl**

Subjects received the soup during the lunch in a self-refilling bowl as described by Wansink et al. (55). The self-refilling bowl can be visualized as follows. A bowl and a pan were placed on a table (82 cm distance); under the table, the bowl and pan were connected through a food-grade silicon tube. The bottom of the pan and bowl contained holes to be connected with the tube; however, subjects were not able to see the hole in the bowl, because the bowl was filled with soup. The soup was re-filled through a gravity-feed mechanism. During consumption, the level of the soup in the bowl decreased slowly, but was never empty.

**Data analyses**

Statistical analyses were performed using SAS version 9.1.4 (SAS Institute Inc., Cary, NC, USA). Data are presented as means ± standard deviation, P-values < 0.05 were considered significant.

During the taste tests, the effect of salt concentration on salt intensity, sour intensity, sweet intensity, pleasantness and relative-to-ideal salt intensity were analysed by a linear model that included the effect of subject.

One subject did not receive LS soup and was excluded from data analysis. Pearson correlations between intake of the same soup, HS or LS (duplicates) and between intakes of the different soups were calculated. Effects of salt intensity (LS vs. HS soup) on soup intake (mean of duplicates) were assessed with a linear model that included gender and subject nested within gender. Preliminary analyses revealed that gender only affected intake; therefore, gender was omitted from the other analyses (see below). Appetite ratings (hunger, fullness, prospective consumption and thirst), pleasantness, desire-to-eat, relative-to-ideal salt intensity, salt intensity and expected satiation were
Initial ratings of pleasantness, desire-to-eat, relative-to-ideal salt intensity, salt intensity and expected satiation were compared between LS, IS and HS sampled soups by a model that included the effect of subject. Delta ratings (post intake – pre intake) of pleasantness, desire-to-eat, relative-to-ideal salt intensity and salt intensity were compared between LS, IS and HS sampled soups by a linear split-plot model that included effects of salt intensity of the ad libitum soup (HS vs. LS); effects of salt intensity in ad libitum soup were tested against the effect of subject within ad libitum soup condition. Bonferroni adjustments were used for post hoc comparisons. The GLM procedure in SAS was used for all linear models.

Results

Analytical taste-profile

Salt intensity ratings increased with geometric salt concentrations, $F_{7, 321} = 174$, $P < 0.001$ (Figure 2.1). Sour intensity ratings did not change with increasing salt concentrations, $F_{7, 321} = 1.41$, $P = 0.20$. Sweet intensity decreased from 363 mg Na/100 g to higher salt concentrations $F_{7, 321} = 12.7$, $P < 0.001$.

![Figure 2.1 Mean ratings and SD of salt intensity (■), sour intensity (♦) and sweet intensity (○) as a function of salt concentration in tomato soup on 100 mm VAS.](image-url)
Selection of LS, IS and HS soups and hedonic taste profile

Relative-to-ideal salt intensity ratings showed an increase with increasing geometric salt concentrations, $F_{7, 178} = 169, P < 0.001$ (Figure 2.2). The ideal salt concentration is where the relative-to-ideal salt intensity curve crosses the x-axis (the just-about-right point), the mean was $363 \pm 56.8$ mg Na/100 g. The pleasantness curve showed an inverted U-shape against logarithmic salt concentration $F_{7, 178} = 30.6, P < 0.001$. The top of the inverted U-shape is defined as the ideal salt concentration. The pleasantness curve was asymmetrical; soups with salt concentrations above ideal decreased more in pleasantness than soups with salt concentrations below ideal. The relative-to-ideal salt intensity ratings did not reach the “not nearly salty enough” (-50 mm) end, while the “much too salty” end (50 mm) was almost reached; 43 mm.

For each individual, relative-to-ideal salt intensity and pleasantness curves were plotted individually. LS and HS concentrations were selected per individual by linear interpolation (Table 2.2). The mean salt concentration selected for LS was $165 \pm 52$ mg Na/100 g and the mean selected for HS was $613 \pm 194$ mg Na/100g. The distance in geometric salt concentration was equal between each selected LS and HS soup. The mean salt concentration selected for IS was $340 \pm 113$ mg Na/100g (range: 98 – 561 mg Na/100g).

Figure 2.3 illustrates individual differences in relative-to-ideal salt intensity ratings. It shows the difference in ideal salt concentration and the tolerance towards different salt concentrations (i.e., distance from ideal) between subjects. The slope expresses the tolerance for different salt concentrations in relative-from-ideal salt intensity; this varied from 22.8 mm/log mg Na per 100 g (most tolerant) to 132 mm/log mg Na per 100 g (least tolerant). The mean slope was $61.7 \pm 22.0$ mm/log mg Na per 100 g (mean $R^2 = 0.90 \pm 0.1$). There were no gender differences in relative-to-ideal salt intensity ratings and selection of LS and HS concentrations (data not shown).
Ad libitum intake

We found no differences between the ad libitum intakes of LS vs. HS soup, 375 ± 165 grams vs. 388 ± 147 grams, $F_{1,94} = 0.72, P = 0.39$ (Figure 2.4). In addition, eating rate did not differ between consumption of LS vs. HS soup, LS: 73.1 ± 3.6 g/min vs. HS: 76.4 ± 4.2 g/min; $F_{1,94} = 1.89, P = 0.18$. Ad libitum intake was highly correlated for duplicate measurements (LS soups: $r = 0.79$, HS soups $r = 0.85$, $P < 0.001$) and for different soups within subjects (LS versus HS soup, four different combinations: LS1 vs. HS1 $r = 0.68$, LS2 vs. HS1: 0.72, LS1 vs. HS2: 0.73 and LS2 vs. HS2: 0.76, $P < 0.001$).

Initial ratings of hunger, fullness, prospective consumption and thirst did not differ between LS vs. HS soup, which indicates that subjects were in the same hungry state before ad libitum intake of the soup (Table 2.3). After ad libitum intake of both LS and HS soup, ratings of hunger decreased (LS: $F_{1,93} = 431, P < 0.001$, HS: $F_{1,94} = 530, P < 0.001$) ratings of prospective consumption decreased (LS: $F_{1,93} = 340, P < 0.001$, HS: $F_{1,94} = 428, P < 0.001$) and ratings of fullness increased (LS: $F_{1,93} = 375, P < 0.001$, HS: $F_{1,94} = 668, P < 0.001$). Ratings of thirst decreased after intake of LS soup ($F_{1,93} = 4.38, P = 0.04$), but did not change after intake of HS soup ($F_{1,94} = 1.15, P = 0.29$). Changes in ratings of hunger, fullness, prospective consumption and thirst did not differ after intake of LS soup compared with HS soup (Table 2.3).

The mean sodium intake from LS soup was 593 ± 278 mg, the mean sodium intake from HS soup was 2356 ± 1173 mg. The mean sodium intake from the samples (LS, IS and HS before and after ad libitum intake) was 333 ± 49 mg.
Table 2.3 Mean ± SD of initial and delta (post intake – pre intake) ratings of hunger, fullness, prospective consumption and thirst for LS and HS soups.

<table>
<thead>
<tr>
<th></th>
<th>LS soup</th>
<th>HS soup</th>
<th>$F_{1.94}$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hunger</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>70.1 ± 11.8</td>
<td>71.1 ± 11.8</td>
<td>0.05</td>
<td>ns</td>
</tr>
<tr>
<td>$\Delta$</td>
<td>-51.2 ± 17.3*</td>
<td>-53.6 ± 15.9*</td>
<td>0.59</td>
<td>ns</td>
</tr>
<tr>
<td>Fullness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>23.6 ± 11.8</td>
<td>21.2 ± 10.4</td>
<td>0.97</td>
<td>ns</td>
</tr>
<tr>
<td>$\Delta$</td>
<td>50.9 ± 19.4*</td>
<td>54.7 ± 14.5*</td>
<td>2.37</td>
<td>ns</td>
</tr>
<tr>
<td>Prospective consumption</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>67.8 ± 10.4</td>
<td>68.8 ± 11.1</td>
<td>0.17</td>
<td>ns</td>
</tr>
<tr>
<td>$\Delta$</td>
<td>-43.8 ± 16.6*</td>
<td>-47.6 ± 15.9*</td>
<td>2.98</td>
<td>ns</td>
</tr>
<tr>
<td>Thirst</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>61.2 ± 13.9</td>
<td>65.6 ± 13.9</td>
<td>2.75</td>
<td>ns</td>
</tr>
<tr>
<td>$\Delta$</td>
<td>-6.72 ± 34.6*</td>
<td>-3.81 ± 22.2</td>
<td>0.88</td>
<td>ns</td>
</tr>
</tbody>
</table>

*Significance difference between pre- and post-intake ratings

Figure 2.4 Mean values + SD of ad libitum intake (g) of LS soup and HS soup

Changes in ratings for the consumed soup (LS after LS, and HS after HS)

The initial pleasantness and desire-to-eat did not differ between LS and HS soup (Table 2.4). HS soup was rated as higher intense according to both the relative-to-ideal salt intensity and the salt intensity ratings. Initial ratings of expected satiation (i.e., how filling they thought the soup was) was higher for the HS soup compared to the LS soup.
After ad libitum intake of both LS and HS soup, ratings of pleasantness decreased (LS: -9.34 ± 22.9, \( P = 0.006 \), HS: -13.8 ± 22.9, \( P < 0.001 \)) (Figure 2.5A), the degree of decrease did not differ between soups (\( F_{1, 46} = 1.19, P = 0.28 \)). In addition, the desire-to-eat after both soups decreased (LS: -30.2 ± 22.9, \( P < 0.001 \), HS: -29.6 ± 22.9, \( P < 0.001 \)) (Figure 2.5B), the degree of decrease did not differ between soups (\( F_{1, 46} = 0, P = 0.95 \)). After ad libitum intake of LS soup, relative-to-ideal salt intensity and salt intensity ratings did not change (Figure 2.5C, 5D). After ad libitum intake of HS, the relative-to-ideal salt intensity tended to be rated further to the “much too salty” end (4.2 ± 15.2, \( P = 0.08 \)), whereas salt intensity ratings did not change.

**Table 2.4** Mean ± SD of initial ratings of pleasantness, desire-to-eat, relative-to-ideal salt intensity, salt intensity and expected satiation for LS, IS and HS soups.

<table>
<thead>
<tr>
<th></th>
<th>LS soup</th>
<th>IS soup</th>
<th>HS soup</th>
<th>( F_{1, 94} )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pleasantness</strong></td>
<td>56.8 ± 2.19(^a)</td>
<td>66.1 ± 2.26(^b)</td>
<td>50.8 ± 2.79(^a)</td>
<td>12.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Desire-to-eat</strong></td>
<td>56.5 ± 2.49(^a)</td>
<td>65.4 ± 2.34(^b)</td>
<td>52.6 ± 2.90(^a)</td>
<td>10.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Relative-to-ideal salt intensity</strong></td>
<td>-12.9 ± 1.58(^a)</td>
<td>0.22 ± 1.26(^b)</td>
<td>14.5 ± 1.62(^c)</td>
<td>96.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Salt intensity</strong></td>
<td>31.7 ± 2.08(^a)</td>
<td>47.9 ± 1.88(^b)</td>
<td>71.0 ± 1.96(^c)</td>
<td>101</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Expected satiation</strong></td>
<td>45.0(^a) ± 1.85</td>
<td>53.9(^b) ± 1.57</td>
<td>57.8(^b) ± 2.07</td>
<td>12.6</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Mean ratings with different superscript letters (a, b, c) in the same row were significantly different.
Changes in ratings for soups with contrasting salt intensity (HS after LS and LS after HS)

Each sampled soup decreased in pleasantness after ad libitum intake of LS or HS soup ($P < 0.01$) (Figure 2.5A). After intake of LS soup, the decrease in pleasantness differed among samples ($F_{2, 138} = 4.14$, $P = 0.02$), HS soup decreased more in pleasantness compared to both IS and LS soup ($P < 0.05$). After intake of HS soup, the decrease in pleasantness did not differ significantly among the sampled soups ($F_{2, 141} = 1.18$, $P = 0.31$), however, LS soup numerically decreased the most in pleasantness. In addition, the decrease in desire-to-eat after LS and HS soup did not differ between the sampled soups (Figure 2.5B), however, drops in desire-to-eat showed similar patterns as the drops in pleasantness.

When comparing ratings from pre- to post-intake, after intake of LS soup, the sampled LS and IS soup did not differ in relative-to-ideal salt intensity, while the HS soup was rated more to the “much too salty” end ($P < 0.001$) (Figure 2.5C). Salt intensity ratings also showed that LS soup was not rated differently after intake of LS soup, whereas IS soup seemed be to rated somewhat more salt intense ($P = 0.14$) and HS soup was rated
as more salt intense \((P = 0.04)\) (Figure 2.5D). After intake of HS soup, HS and IS samples showed no change in relative-to-ideal salt intensity ratings, while LS soup was rated more to the “not nearly salty enough” end \((P = 0.004)\) (Figure 2.5C). In accordance, salt intensity ratings after intake of HS soup showed no change for HS and IS ratings, while LS soup was rated less salt intense \((P < 0.001)\) (Figure 2.5D). In general, total relative-to-ideal salt intensity ratings were lower after intake of HS soup compared to LS soup (HS: \(-1.25 ± 1.23\), LS: \(2.89 ± 1.51\); \(F_{1, 93} = 4.37, P = 0.04\)), as similar results were found for salt intensity ratings (HS: \(-2.64 ± 1.84\), LS: \(3.60 ± 1.72\); \(F_{1, 94} = 6.72, P = 0.01\)).

**Discussion**

The present study clearly shows that salt intensity does not affect satiation, which was measured as ad libitum intake. In accordance, neither did salt intensity affect the decrease in reward of the just consumed soup (i.e., subjective ratings of pleasantness and desire-to-eat) nor eating rate, hunger and fullness ratings after soup intake. The soups were only different in salt concentration and similar in initial pleasantness, energy density, temperature and viscosity. This is the first study that demonstrated that salt intensity does not affect satiation when controlling for palatability on an individual basis. After intake of HS soup, salt intensity ratings showed no difference for the consumed HS soup, however, LS soup was perceived as more bland. After intake of LS soup, salt intensity ratings showed no difference for the consumed LS soup, however, HS soup was perceived as higher in salt intensity.

Individuals vary largely in salt preference as shown by this and other studies (117, 118, 138, 139). Consequently, a certain salt concentration may be too salty for one person and just right or even not salty enough for another. Selecting two fixed concentrations for all subjects would give a great variability in perceived salt intensity and pleasantness and therefore ad libitum intake. To overcome these individual differences, we selected salt concentrations for LS and HS soups for each subject, as lower and higher in saltiness respectively, than their ideal salt concentration. Moreover, the salt concentrations were selected based on equal pleasantness for each subject. This allowed us to study the effect of salt intensity apart from hedonics. As stated in the introduction, satiation is not only determined by sensory factors, but also by certain physiological and psychological factors, which may disturb the effect of salt intensity on ad libitum intake. We tried to keep these factors as constant as possible. Visual cues, such as self-monitoring of the amount consumed and the natural tendency to finish the bowl have been shown to greatly influence the amount consumed (55, 140). Using a self-refilling bowl diminished these effects. This study attempted to keep the physiological
contribution constant by having the subjects arrived in the same metabolic state, as subjects were instructed not to eat three hours before and consume the same breakfast.

The perceived salt intensity did not change for the soup that was eaten ad libitum. In contrast, hedonic ratings decreased. This is consistent with previous findings showing that eating to satiation did not affect the perceived taste intensity, but resulted in a less pleasant taste (141, 142). Above findings are supported by several neurophysiological studies (142-147). In the brain, taste quality and intensity are processed in the primary taste cortex (i.e., the primate anterior insula and adjoining frontal operculum) whereas the secondary taste cortex (i.e., caudolateral orbitofrontal cortex) reflects the hedonic value and motivation to eat (142, 143). When eating to satiation, the response in the secondary taste cortex was shown to decrease in humans (142-146), while no decrease of response was seen in the primary taste cortex and in the nucleus of the solitary tract in non-human primates (143, 147). This is in line with a study that used a habituation paradigm, hedonic responses to repeated presentation of the same food habituated (i.e., decreased in response), while there was no clear habituation observed for the experienced intensity (148). Taken together, this underpins that taste intensity may not directly influence the motivational state of eating during intake and, therefore, may not cause an effect on ad libitum intake.

Another possible explanation why salt intensity does not influence ad libitum intake may be the lack of a physiological mechanism to adjust the amount of salt within a meal because it is not associated with energy. Sweet is considered associated with energy, as in sugar. Sweetness may affect meal size as a function of short-term energy regulation. Studies with animals illustrated that the amount intake was adjusted to carbohydrate concentration (68, 149, 150): this phenomenon is called “conditioned satiation”. This means that the orosensory stimuli derived from sweetness of carbohydrates could predict the post-ingestive energetic consequences and adjust the amount of intake (68, 151). It would be of interest to replicate the present study with sweetness.

Moreover, the experimental setting might have influenced effects of salt intensity on satiation. We assumed that sensory factors would be a major determinant of meal termination; however, this may not have been the case in the present study. Subjects were in a hungry state and soup was the only food available. It is possible that subjects consumed until their stomachs were filled and possible effects of salt intensity may have been overruled. Weight and volume are well-known controllers of short-term intake (78, 79, 152-154). De Castro (79) showed that the average weight of the nutrients and fluids estimated to be present in the stomach at the end of the meals was 400 g to 500 g. Intake in the present study was about the same (380 g plus in total six samples of 15 g for several ratings before and after consumption). Since people tend to eat a constant weight during a meal, sensory factors that contribute to satiation may be more important in circumstances when people are able to switch to other foods. In addition, a less hungry
state may reduce physiological contribution and enhance the sensory contribution of satiation. Whether salt intensity affects ad libitum intake when subjects, first, have more food choice and, second, are in a more satiated state will be investigated in the next study.

To get insight in changes of salt intensity preference and perception after ad libitum intake of either LS or HS soup, small samples of LS, IS and HS soup were tasted and evaluated. During ad libitum intake, the frame of reference in salt intensity became lower (in the case of LS) or higher (in the case of HS) than before consumption, which increases the difference in salt intensity when tasting the “opposite” salt intensity. Contrast effects were observed in both directions, LS was perceived as more bland after consumption of HS soup and HS soup was perceived as more salty after consumption of LS soup. The change in analytical salt intensity ratings suggests that subjects perceived the salt intensity differently after consumption, independent of hedonics. These contrasting effects observed in salt intensity ratings affected the hedonic value in a negative way. The “contrasting” salt intensities were rated further from the ideal-salt-intensity and therefore less palatable, which is confirmed by the pleasantness ratings as shown in the results. No contrast effect was observed for IS soup, as it was not rated differently in salt intensity after consumption. The difference between the consumed soup and the IS soup might be too small to produce a contrast effect. This suggests that a certain difference in salt intensity is needed to obtain a contrast effect.

The results of this study suggest that a substantial difference in salt intensity in a food decreases the palatability because people adapt to the exposed intensity. This adaptation towards lower salt intensity is in favour of the recommended salt intake, which is 5 g/day (WHO, 2006 (156)) and is much lower than the average consumption of 9-10 g/day (Dutch Health Council, 2000 (157)) in the Netherlands. The results showed that after consumption of LS soup, HS soup was rated as more salty and decreased more in pleasantness than the consumed LS soup. Therefore, it is unlikely that consumption of a low-salt soup will trigger higher salt intake from other foods afterwards. This is in accordance with the finding that subjects on a reduced salt diet did not compensate by increased table salt usage (158). However, there is a need to investigate to what extent the adaptation for low-salt intensity can be translated into other foods.

As far as we know, we showed for the first time that contrast effects remain after a food is consumed until satiation. Previously, contrast effects for intensity were shown after consuming a small amount of a liquid (128-130, 132). When a food is consumed to satiation, its pleasantness decreases and this decline is larger than the decline in pleasantness of uneaten foods (159-163). Therefore, people tend to choose foods that have different sensory properties compared to the consumed foods (162, 163). In this study, the used test foods (i.e., soup) that only differed in salt intensity. We were interested whether people would prefer a stronger taste after being exposed to a bland
soup and vice versa. In contradiction, this study showed a larger decrease in pleasantness for the “uneaten” soup (the soup with ‘contrasting’ salt concentration) compared to the eaten soup, caused by contrast effects. This indicates that the decrease in pleasantness is apparently not driven by taste intensity per se (bland or salty) and that exposure to a different salt concentration is perceived as less palatable.

IS soup (~363 mg Na/100 g) was most pleasant and is similar to the salt concentration in commercially available tomato soups (290 – 450 mg Na/100 g). The results illustrate, however, that there is a wide range in sodium concentration that is still acceptable (LS: ~151 mg Na/100 g - ~HS: 561 mg Na/100 g), which means pleasantness ratings of >50 mm on a 100 mm VAS scale. The results of this study suggest that when sodium is reduced by ~50% (mean IS compared to mean LS), the soup is still acceptable for consumption. Moreover, studies that expose subjects foods low in salt intensity for longer term, illustrated a preference shift towards lower salt intensities. Reduction of dietary salt for 3 months (155) or 5 months (164) showed a preference shift towards lower concentrations and a decreased preference for salty foods. In accordance, an increase of dietary salt for four weeks showed a preference shift to higher salt concentrations (165). In this study, we did not observe a preference shift in terms of a shift of the most preferred salt concentration (i.e., no shift of ideal and most pleasant salt concentration in soup) but we did observe a decreased preference of the contrasting salt concentrations in soup.

In conclusion, our study showed that salt intensity did not affect satiation in soups when they are similar in pleasantness. Subjects were shown to adapt to a low or high salt intensity during consumption. The contrasting salt intensities (LS after HS and HS after LS) were therefore perceived as less pleasant after consumption.

Acknowledgements

We thank all practical assistance in conduction of the study, especially Tijs Rovers, Matthijs Corbijn and Els Siebelink; and the technical department of Wageningen University for design and construction of the self-refilling bowl, especially André Sanders.
Chapter 3

Effect of salt intensity in soup on ad libitum intake and on subsequent food choice

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Abstract

The effect of salt intensity on ad libitum intake of tomato soup was investigated when soup was served as a first course and as a second course. In addition, the effect of salt intensity in soup on subsequent sweet vs. savoury choice of sandwich fillings was investigated. Forty-three healthy subjects consumed ad libitum a low-salt (LS), ideal-salt (IS) and high-salt (HS) tomato soup in both meal settings. The salt concentrations were selected on an individual basis, in a way that IS was most pleasant and LS and HS were similar in pleasantness. The ad libitum intake of IS soup was higher than that of LS and HS soup, and the ad libitum intake of LS soup was higher than that of HS soup. The meal setting, soup as a first or as a second course, did not affect ad libitum intake. Salt intensity in soup did not predict sweet vs. savoury choice of fillings in grams or energy, although most sodium from fillings was consumed after intake of HS soup. In conclusion, a higher salt intensity leads to lower ad libitum intake of soup similar in palatability (LS vs. HS). In addition, salt intensity in soup does not predict sweet vs. savoury food choice.

Keywords: salt intensity, sensory specific satiety, satiation, sweet, savoury, context
Introduction

Sensory food properties play an important role on meal termination (satiation) and food choice (166). For example, a palatable food will be chosen more frequently than a less palatable variant and will be consumed in larger amounts; both in laboratory settings (42-45, 112, 116, 120, 121) and in the natural everyday environment (46, 47). Another sensory food property that plays a role in satiation is texture, as a more viscous or solid product results in less ad libitum intake compared to liquids (9, 10, 12, 87, 88, 167). The effect has been attributed to the duration of sensory exposure in the oral cavity, because a prolonged sensory exposure per bite resulted in lower ad libitum intake (12, 92, 93). Taste and flavour intensity may also influence satiation, because increased taste intensity means an increased sensory exposure per bite and this may lead to faster satiation.

In literature, however, confounding results were found for the effect of taste and flavour intensity on satiation. Studies that used a concentration range of a tastant or flavour in a specific food, showed that the ideal, most preferred intensity in food resulted in highest intake (112-116, 120, 121). Most of these studies suggest that high-intense foods (i.e. over-ideal) decreased intake more than the low-intense foods (i.e. under-ideal). This was observed for salt intensity (120), oregano intensity (121), and sweetness intensity (112, 113). Other studies found, however, no differences in the effect of sweetness intensity (114, 115), and one study found even the opposite effect for sweetness intensity (116). In our previous study (chapter 2, 168), no effect of salt intensity on ad libitum intake of soup was found, when comparing a low-salt and high-salt soup similar in palatability.

The contribution of sensory factors on satiation may depend on the context in which the food is consumed. In daily life, the physiological states of hunger prior to consumption vary. In our previous study (chapter 2, 168) soup was the only food consumed during lunch. Subjects consumed the same amount of low-salt and high-salt soup. Prior to the lunch, subjects had not eaten for three hours, and consequently, subjects arrived in a hungry state. Therefore, the physiological signals to alleviate hunger may have overruled effects of sensory factors on satiation. Subjects may have consumed a certain amount of soup to fill their stomachs to alleviate hunger. It is well known that weight and volume were shown to be determinants of short-term intake (78, 79, 152-154, 169, 170).

In addition, the composition of the meal is a contextual aspect that may influence the contribution of sensory factors on satiation. Meals can be divided in single-item and multi-item meals. In multi-item meals, people may switch to other foods when the pleasantness is decreased for a specific food, whereas in a single-item meal people can only alleviate their hunger with that specific food. This may indicate that consumption
of a specific food in a multi-item lunch is less dependent on feelings of hunger or fullness.

After being satiated with one food, people tend to choose other foods that have different sensory properties compared to the consumed foods (162, 163). This can be explained by sensory specific satiation (SSS) which means that the reward (i.e., measured as ratings of pleasantness or desire-to-eat) decreases for the consumed food while the reward for other foods decreases less or remained unchanged (49). Foods that share the same sensory properties of the consumed foods also decrease in pleasantness together with the consumed foods (159-161, 171). Therefore, SSS encourages humans to eat a variety of foods (134). These effects were repeatedly observed for sweet vs. savoury foods. When a savoury food was consumed to satiation, also the pleasantness for other savoury foods decreased, while the pleasantness for sweet foods decreased less or remained unchanged (83, 159, 163, 172). The same effect was observed after consuming a sweet food to satiation (83, 159, 163, 172, 173). Moreover, Weenen et al. (174) even showed an increase in pleasantness for a sweet food (canned pears) after consumption of a savoury food (cheese biscuits) and vice versa. It is possible that when the intensity of a savoury food increases, for example by increasing the salt intensity, that after consumption, people prefer to choose sweet foods and less of other savoury foods. As far as we know, no studies have been performed that investigated the effect of salt intensity on subsequent food choice.

The first objective of the present study is to investigate whether salt intensity in soup affects ad libitum intake when soup is served both as a first course and as a second course. When soup is served as a first course, subjects are hungry but know that there will be more food afterwards. When soup is served as a second course, subjects are more satiated and know that the soup is the last meal-item. The second objective is to investigate if salt intensity in soup affects the choice of sweet vs. savoury sandwich fillings. Subjects consumed low-salt (LS), ideal-salt (IS) and high-salt (HS) tomato soup in both meal settings. The salt concentrations for the soups were selected on an individual basis, in a way that IS was most pleasant and LS and HS were similar in pleasantness.

**Subjects and methods**

**Experimental design**

For each individual, salt concentrations were selected for low-salt (LS), ideal-salt (IS) and high-salt (HS) soup. This was done in a taste test in which subjects tasted soups.
with various salt concentrations and rated pleasantness and relative-to-ideal salt intensity, as described below.

After the selection of individual salt concentrations, subjects visited the lab six times during lunchtime. Subjects consumed once the LS, IS and HS soup as a first course followed by a second course that consisted buns and fillings (“soup as first course” setting) and once LS, IS and HS soup after a preload (“soup as second course” setting). After ad libitum soup intake in the “soup as first course” setting, subjects consumed ad libitum from the second course that consisted buns presented with choice of several sweet and savoury fillings. In the “soup as second course” setting, subjects started with consumption of a fixed preload of raisin buns that was calculated as 50% of the energy needs during lunch.

The order of the six conditions was randomized between subjects. Subjects consumed the tomato soup from a self-refilling bowl as described by Wansink et al. (55), as in our previous study (chapter 2, 168). This was done to minimize the contribution of two psychological effects: first, the ability to self-monitor the amount consumed which influences portion size (55), and second, the tendency to finish the bowl, as subjects were aware of the fact that the bowl was re-filling.

**Subjects**

Forty-three subjects (13 males) participated in the study; all were students or employees from Wageningen University. Subjects were healthy, had a normal weight (BMI 18.5-25 kg/m², mean: 21.9 ± 1.8), were aged between 19 and 28 year (mean: 21.7 ± 2.2) and liked creamy tomato soup (pleasantness score > 5 on a 9-point hedonic scale). Exclusion criteria were restrained eating (Dutch eating behaviour questionnaire (DEBQ) score men: >2.25, women: >2.79), following an energy-restricted diet during the last two months, gained or lost >5 kg weight during the last year, having a lack of appetite, smoking, having gastrointestinal illness, having diabetes, having thyroid disease or any other endocrine disorder, having hypertension, suffering from kidney diseases and being pregnant or giving breast feeding. Subjects were unaware of the aim of the research. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the Medical Ethical Committee of Wageningen University. All subjects signed an informed consent form.

**Test foods**

Tomato soup with varying salt concentrations was used as test product in this study. One kilogram of soup was made from 600 g mashed tomato pieces (Heinz, Elst, The
Chapter 3 – Effect of salt intensity on ad libitum intake in different meal settings

Netherlands), 80 g cream (kookroom, private label Albert Hein Zaandam, The Netherlands), 310 g water and 10 g sucrose. The mixture was heated until 80 °C. The calculated macronutrient composition from the used ingredients was 0.8 g protein, 3.3 g carbohydrates, 1.6 g fat and 129 kJ (31 kcal) energy per 100 g soup. Eight sodium concentrations were used with equal geometric distances (factor 1.55): 63 (soup 1), 98 (soup 2), 151 (soup 3), 234 (soup 4), 363 (soup 5), 561 (soup 6), 870 (soup 7) and 1349 (soup 8) mg Na/100 g soup. The sodium concentration in soup 1, to which no salt was added, was calculated from the used ingredients. Soups were equal in viscosity; soup one and 7, the soups with lowest and highest salt concentration selected for ad libitum intake had a viscosity of 0.246 Pa/s and 0.223 Pa/s, respectively, at a shear rate of 45 (1/s) at 55 °C.

In both meal settings, subjects received a bottle of 500 ml of mineral water (Spa blauw, Brussels, Belgium). In the “soup as first course” setting, the following items were presented in the second course: buns (local bakery), margarine (VHC, Hendrik-Ido-Ambacht, the Netherlands), hazelnut paste (Nutella, Breda, the Netherlands), chocolate sprinkles (Chocoladehagel Puur, De Ruijter, Zeist, The Netherlands), strawberry jam (Geurts, Dodewaard, The Netherlands), cheese (Vergeer, Reeuwijk, The Netherlands), gammon (local butcher), and cervelat (local butcher). In the “soup as second course” setting, raisin buns (local bakery) were used as a preload. The nutritional compositions of the test foods are shown in Table 3.1 and are obtained from the Dutch Food Composition Database (NEVO, version 2009/1.0).

<table>
<thead>
<tr>
<th>Table 3.1 Nutrient compositions of tests foods in per 100 g.</th>
<th>Energy (kJ)</th>
<th>Protein (g)</th>
<th>Carbohydrate (g)</th>
<th>Fat (g)</th>
<th>Sodium (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raisin buns</td>
<td>1120</td>
<td>8</td>
<td>52</td>
<td>3</td>
<td>300</td>
</tr>
<tr>
<td>Buns</td>
<td>1013</td>
<td>10</td>
<td>45</td>
<td>2</td>
<td>571</td>
</tr>
<tr>
<td>Margarine</td>
<td>2956</td>
<td>0</td>
<td>0</td>
<td>80</td>
<td>331</td>
</tr>
<tr>
<td>Hazelnut paste</td>
<td>2215</td>
<td>7</td>
<td>56</td>
<td>31</td>
<td>30</td>
</tr>
<tr>
<td>Chocolate sprinkles</td>
<td>1725</td>
<td>5</td>
<td>73</td>
<td>14</td>
<td>27</td>
</tr>
<tr>
<td>Strawberry jam</td>
<td>1023</td>
<td>0.2</td>
<td>60</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Cheese</td>
<td>1561</td>
<td>24</td>
<td>0.3</td>
<td>31</td>
<td>932</td>
</tr>
<tr>
<td>Gammon</td>
<td>556</td>
<td>18</td>
<td>2</td>
<td>6</td>
<td>878</td>
</tr>
<tr>
<td>Cervelat</td>
<td>1622</td>
<td>19</td>
<td>0.8</td>
<td>35</td>
<td>1580</td>
</tr>
</tbody>
</table>
Selection of salt concentrations for LS, IS and HS soups

To select LS, IS and HS soups on an individual basis, subjects rated 15 g of sampled soups with varying salt concentrations on relative-to-ideal salt intensity and pleasantness. The question that refers to relative-to-ideal salt intensity was: “How salty is the taste of this soup?”; the scale was labelled “not nearly salty enough” (-50 mm) at the left end, “just right” in the middle (0 mm) and “much too salty” at the right end (50 mm) of the scale. The pleasantness question was: “How pleasant is the taste of this soup?” the scale was labelled “very unpleasant” at the left end (0 mm) and “very pleasant” at the right end (100 mm). The soups were presented in an interactive procedure according to the method specified by Booth et al. (117). This procedure was developed as a quick method to find the individual ideal (i.e., most pleasant or optimal) salt concentration.

Soup 5 (explained above in “Test foods”), with a sodium concentration similar to that in commercially available tomato soups, was presented first. Depending on the rating of the first sample on relative-to-ideal, the second sample was chosen in a way to be rated on the other side of ideal from the first sample. For example, if the first sample was rated above ideal, then the second sample would be below ideal or vice versa. The procedure was continued until there were five ratings: two below ideal, one close to ideal (-10 < 0 < 10 mm) and two above ideal. After a 15 minute break, subjects received the same five soups in a different order, however, again alternating on each side of ideal (117).

For each subject, the means of duplicates were calculated and plotted against geometric sodium concentration. The IS soup was selected as the soup that was rated closest to the “just right” point (i.e., 0 mm on relative-to-ideal salt intensity ratings). The LS and HS concentrations were chosen at each side of ideal based on equal pleasantness (<10 mm difference on pleasantness ratings) as determined by linear interpolation. Each pair of LS and HS soups was selected in a way that the distance in geometric sodium concentration (i.e., the ratio) was equal between LS and HS soup, which equals a factor 3.7.

General procedure

Subjects were instructed to consume the same breakfast and not to eat and only drink water or weak tea three hours before the lunch started. Moreover, they were asked to refrain from drinking one hour before the test started. After each test lunch, subjects had to answer questions about what they consumed for breakfast and whether they ate or drank between breakfast and test lunch. To make sure subjects would eat until they felt satiated; they were instructed not to eat one hour after the test.
Each subject was seated on a separate table with a soup bowl covered with aluminium foil, lunch-items (depended on condition: preload or buns and several fillings) and a laptop with instructions. During lunch, subjects had free access to water. Subjects rated their feelings of hunger, fullness, prospective consumption (how much they thought they could eat) (137) and thirst on a 100 mm VAS, before the lunch, in between the two courses (thus after preload or after soup), and at the end of the lunch. Before and after consumption of the soup, subjects were instructed to take a sip and to rate pleasantness and the desire-to-eat the soup, and after a second sip, subjects had to rate relative-to-ideal salt intensity and overall taste intensity on a 100 mm VAS.

Subjects had to push a button when they started and when they finished consumption of soup, so that eating time was recorded. They were instructed to terminate consumption when they felt they had enough. The mean initial temperature of the soup was 58.6 ± 3.8 ºC. From the start of soup consumption, subjects had to wait for at least ten minutes before consuming the second course. After ten minutes, an alert popped up on the laptop screen to inform subjects that the ten minutes were finished. From the start of consumption of the second course, subjects had to wait for at least twenty minutes; again, time was recorded by the laptop. These times were set to prevent subjects from leaving the research area for other reasons than being satiated with the presented foods.

All questions were presented on the laptop screen and answered by the use of a 100 mm VAS. The question that refers to desire-to-eat was “How much would you like to eat this soup at this moment?” from “not at all” at the left end to “very much” at the right end. The question that refers to overall taste intensity was “How strong is the taste of this soup?” from “very weak” at the left end to “very strong” at the right end. Pleasantness and relative-to-ideal salt intensity questions are described above.

“Soup as first course” setting

Subjects started with consumption of soup. After that, they were instructed to start consuming whatever they wanted from the second course. Buns and three types of sweet and three types of savoury fillings were supplied in excessive amounts, so that subjects could eat as much as they wanted. There were 15 buns, 100 g margarine, 200 g chocolate sprinkles, 200 g strawberry jam, 200 g hazelnut paste, ± 200 g cheese, ± 200 g gammon, ± 113 g cervelat (15 slices) per subject during one lunch. None of the items was finished during lunch over the whole period of the study. A 7-point scale questionnaire was used to get insight in both palatability and frequency of consumption of these fillings in daily life.

In addition to the general procedure, questions of desire-to-eat something sweet and desire-to-eat something savoury were added before lunch, after the soup consumption
and after the second course. These questions were rated on a 100 mm VAS, with at the left end “not at all” and at the right end “very much”.

“Soup as second course” setting

Subjects received an amount of small raisin buns as a preload. Each raisin bun weighed 22 g (246 kJ). The amount of buns was calculated for each subject at half of the energy provided by an average lunch in the Netherlands (175), that equals 11% energy of the daily energy needs. The daily energy needs for each subject were estimated by the Schofield I equation (176), taking into account: gender, age, weight and a physical activity level of 1.6. One subject received three buns, 18 subjects received four buns, 20 subjects received five buns and four subjects received six buns as a preload. Subjects were instructed to eat all the raisin buns that they were served. After finishing the preload, subjects had a pause of 30 minutes. This pause was chosen to diminish the possible interactions of the raisin buns on soup consumption, as a result of SSS (163), which showed largest effect immediately after consumption (177). After the pause, the self-refilling soup bowl was filled with soup, not visible for subjects, so that subjects could start soup consumption.

Data analyses

Statistical analyses were performed using SAS version 9.1.4 (SAS Institute Inc., Cary, NC, USA). Data are presented as means ± standard deviation (SD), p-values < 0.05 were considered significant.

Effects of salt intensity (LS, IS or HS), meal setting, and their interaction on soup intake were assessed with a mixed linear model that included the random effect of subject. Post hoc analyses of one-sided dunnett-tests were performed to compare intake of LS vs. IS vs. HS soup. One-sided tests were chosen because we had a priori an idea of the direction. We expected largest intake for the most palatable soup: IS soup. When comparing salt intensity in soups similar in palatability (LS vs. HS), we expected either no effect or less intake of the HS soup, in accordance to the results of most literature as stated in the introduction.

Effects of salt intensity, meal setting, and their interaction on eating rate and water consumption were assessed with a mixed linear model that included the random effect of subject. The LSD procedure was used for post hoc comparisons.

Ratings of pleasantness, desire-to-eat, relative-to-ideal salt intensity, overall taste intensity were compared between pre- and post-consumption of soup with paired t-tests.
Appetite ratings (hunger, fullness and prospective consumption) and ratings of thirst, were compared between pre- and post-consumption of the first course (either preload or soup) and compared between pre- and post-consumption of the second course (either soup or buns) with a paired t-tests. This was done for each salt intensity and each meal setting separately.

The effect of salt intensity in soup on initial ratings and changes in ratings (post-consumption ratings minus initial ratings) of appetite, hedonic and intensity ratings were compared by a mixed linear model that included the random effect of subject. This was done for both meal settings separately. The effect of meal course (first or second) on appetite ratings was assessed in a mixed linear model that included salt intensity and the random effect of subject.

In the “soup as first course” setting, the effect of salt intensity in soup on intake of foods in the second course was compared by a mixed linear model that included random effects of subjects. The LSD procedure was used for post hoc comparisons.

Pearson correlation coefficients were calculated between ratings of desire-to-eat sweet and desire-to-eat savoury after soup intake with actual intake in grams of sweet and savoury fillings in the second course.

Results

Individual selected salt concentrations for LS, IS and HS soups

The mean selected IS concentration was 320 ± 124 mg Na/100 g, the range between subjects was 98 - 561 mg Na/100 g. The mean LS concentration was 155 ± 47 mg Na/100 g, the range between subjects was 63 - 234 mg Na/100 g. The mean HS concentration was 575 ± 173 mg Na/100 g, the range between subjects was 234 - 870 mg Na/100 g.

Ad libitum intake of soup

Figure 3.1 shows the ad libitum intake of soup in both meal settings. Salt intensity in soup affected the ad libitum intake: F(2, 210) = 5.6, P = 0.004. Post hoc analyses showed that consumption of IS soup was higher than that of LS soup: P = 0.0497, and higher than the HS soup: P < 0.001. Consumption of LS soup was higher than that of HS soup: P = 0.045. There was no effect of meal setting on ad libitum intake of soup: F(1, 210) = 0, P = 0.99 and no interaction between salt intensity (LS, IS or HS soup) and meal setting on ad libitum intake of soup: F(2, 210) = 0.01, P = 1.0. In addition, the
eating rates of soup intake were 72 ± 28 g/min for LS; 71 ± 25 g/min for IS; and 67 ± 27 g/min for HS. Eating rate was not affected by salt intensity: F(2, 209) = 1.5, \( P = 0.23 \), or by meal setting: F(1, 209) = 0.7, \( P = 0.40 \). In addition, in the “soup as second course” setting, the amount of consumed raisin buns (described in “soup as second course” setting in the subjects and methods section) did not influence the amount of soup consumption: F(3, 125) = 1.6, \( P = 0.19 \).

**Figure 3.1:** Ad libitum intake (g) of LS, IS and HS soup (Mean ± SD), in “soup as first course” and “soup as second course” setting.

**Hedonic and intensity ratings**

Table 3.2 shows hedonic and intensity ratings of LS, IS and HS soups in both meal settings. Initial pleasantness and desire-to-eat of the IS soup was higher compared to both the LS and HS soups in both meal settings. Initial pleasantness and desire-to-eat was similar between the LS and HS soup in both meal settings. Both initial pleasantness and desire-to-eat ratings were higher when soup was consumed as a first course than when it was consumed as a second course: \( P < 0.001 \). The LS soup was rated below ideal towards the “not nearly salty enough” end (\(<0\)), the IS soup was rated around the ideal point (~0) and the HS soup was rated above ideal towards the “much too salty” end (\(>0\)). The overall taste intensity showed that the HS soup was rated as higher intense than the IS soup, and the IS soup was rated as higher intense than the LS soup.

After intake of soup, its pleasantness decreased in all cases. There were no differences in decrease in pleasantness between the three soups and between the two meal settings. In addition, desire-to-eat ratings decreased after soup consumption. The desire-to-eat after intake of the IS soup decreased more than the desire-to-eat after intake of both LS
and HS soups in both meal settings. Relative-to-ideal salt intensity ratings did not change after intake of soup. Ratings of overall taste intensity showed a small increase after intake of the IS soup.
Table 3.2: Initial and change in ratings (mean and SD) of pleasantness, desire-to-eat, relative-to-ideal salt intensity, and overall taste intensity for LS, IS and HS soups.

<table>
<thead>
<tr>
<th></th>
<th>Soup as first course</th>
<th></th>
<th>Soup as second course</th>
<th></th>
<th></th>
<th>P&lt;sup&gt;1&lt;/sup&gt;</th>
<th>P&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LS soup</td>
<td>Mean  SD</td>
<td>IS soup</td>
<td>Mean  SD</td>
<td>HS soup</td>
<td>Mean  SD</td>
<td>Mean  SD</td>
</tr>
<tr>
<td>Pleasantness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>60&lt;sup&gt;a&lt;/sup&gt; 15</td>
<td>69&lt;sup&gt;b&lt;/sup&gt; 12</td>
<td>63&lt;sup&gt;a&lt;/sup&gt; 19</td>
<td>55&lt;sup&gt;a&lt;/sup&gt; 17</td>
<td>65&lt;sup&gt;b&lt;/sup&gt; 16</td>
<td>53&lt;sup&gt;a&lt;/sup&gt; 18</td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>-8&lt;sup&gt;*&lt;/sup&gt; 16</td>
<td>-11&lt;sup&gt;*&lt;/sup&gt; 16</td>
<td>-13&lt;sup&gt;*&lt;/sup&gt; 20</td>
<td>-9&lt;sup&gt;*&lt;/sup&gt; 15</td>
<td>-11&lt;sup&gt;*&lt;/sup&gt; 18</td>
<td>-9&lt;sup&gt;*&lt;/sup&gt; 19</td>
<td></td>
</tr>
<tr>
<td>Desire-to-eat</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Initial</td>
<td>61&lt;sup&gt;a&lt;/sup&gt; 17</td>
<td>68&lt;sup&gt;b&lt;/sup&gt; 15</td>
<td>64&lt;sup&gt;a,b&lt;/sup&gt; 16</td>
<td>54&lt;sup&gt;a&lt;/sup&gt; 18</td>
<td>63&lt;sup&gt;b&lt;/sup&gt; 16</td>
<td>55&lt;sup&gt;a&lt;/sup&gt; 16</td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>-28&lt;sup&gt;a,b&lt;/sup&gt; 19</td>
<td>-36&lt;sup&gt;a,b&lt;/sup&gt; 19</td>
<td>-33&lt;sup&gt;a,b&lt;/sup&gt; 19</td>
<td>-25&lt;sup&gt;*&lt;/sup&gt; 16</td>
<td>-35&lt;sup&gt;b&lt;/sup&gt; 22</td>
<td>-28&lt;sup&gt;a&lt;/sup&gt; 18</td>
<td></td>
</tr>
<tr>
<td>Relative-to-ideal salt intensity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>-12&lt;sup&gt;a&lt;/sup&gt; 14</td>
<td>1&lt;sup&gt;b&lt;/sup&gt; 7</td>
<td>13&lt;sup&gt;c&lt;/sup&gt; 14</td>
<td>-12&lt;sup&gt;c&lt;/sup&gt; 13</td>
<td>0&lt;sup&gt;b&lt;/sup&gt; 11</td>
<td>15&lt;sup&gt;c&lt;/sup&gt; 17</td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>1 13</td>
<td>2 10</td>
<td>2 13</td>
<td>-1 12</td>
<td>1 9</td>
<td>3 18</td>
<td></td>
</tr>
<tr>
<td>Overall taste intensity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>47&lt;sup&gt;a&lt;/sup&gt; 15</td>
<td>59&lt;sup&gt;b&lt;/sup&gt; 12</td>
<td>63&lt;sup&gt;b&lt;/sup&gt; 15</td>
<td>43&lt;sup&gt;a&lt;/sup&gt; 15</td>
<td>56&lt;sup&gt;b&lt;/sup&gt; 11</td>
<td>64&lt;sup&gt;c&lt;/sup&gt; 14</td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>3 15</td>
<td>5&lt;sup&gt;*&lt;/sup&gt; 10</td>
<td>2 12</td>
<td>3 13</td>
<td>4&lt;sup&gt;*&lt;/sup&gt; 10</td>
<td>4 12</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup> Mean values within a row and within a meal setting with unlike superscript letter are significantly different (P < 0.05).

* Significant change: post-consumption minus pre-consumption ratings (P < 0.05)

<sup>1</sup> P-value of mixed model comparing initial and change in ratings of LS, IS and HS soup when soup is consumed as first course.

<sup>2</sup> P-value of mixed model comparing initial and change in ratings of LS, IS and HS soup when soup is consumed as second course.
**Appetite ratings and thirst**

Initial appetite ratings (i.e., hunger, fullness and prospective consumption) did not differ between soup conditions and meal settings (data not shown, all \( P \)-values > 0.53), which indicate that subjects were in the same hungry state before each lunch session.

After ad libitum intake of soup in the “soup as first course” setting, ratings of hunger and prospective consumption decreased and fullness increased. These changes in appetite ratings did not differ between soup conditions: \( P > 0.15 \). Ratings of thirst were affected by salt intensity in soup, thirst decreased -13 mm after LS soup; -7 mm after IS soup and -2 mm after HS soup: \( P = 0.012 \). After consumption of the second course (i.e., buns with fillings), ratings of hunger and prospective consumption decreased and fullness increased. Again, these changes in appetite ratings did not differ between soup conditions: \( P > 0.12 \).

After ad libitum intake of soup in the “soup as second course” setting, ratings of hunger and prospective consumption decreased and fullness increased, these changes in appetite ratings did not differ between soup conditions: \( P > 0.54 \). Ratings of thirst were affected by salt intensity in soup, thirst decreased -18 mm after LS soup; -8 mm after IS soup; and -2 mm after HS soup: \( P < 0.001 \).

Water consumption in the “soup as first course” setting was 284 ± 136 g in the LS soup condition; 297 ± 158 g in the IS soup condition; and 296 ± 158 g in the HS soup condition. Water consumption in the “soup as second course” setting was 223 ± 160 g in the LS soup condition; 221 ± 163 g in the IS soup condition; and 248 ± 165 g in the HS soup condition. Water consumption during lunch was not affected by the salt intensity in soup: \( F(2, 202) = 0.59, \quad P = 0.55 \), but was higher when soup was consumed as a first course than as a second course: \( F(1, 202) = 17.3, \quad P < 0.001 \).

**Differences in appetite ratings between meal settings**

Figure 3.2 shows the changes in hunger for both meal settings and for each course. In both meal settings, the first course (either preload or soup) led to smaller decreases in ratings of hunger and prospective consumption than the second course (either soup or buns and fillings): \( P < 0.001 \). This means that hunger after soup intake decreased more when it was consumed as a second course than as a first course: \( F(1, 210) = 33.8, \quad P < 0.001 \), despite the fact that the same amount of soup was consumed. In addition, the prospective consumption ratings led to larger decreases when soup was consumed as a second course (-34 ± 18) than as a first course (-20 ± 18): \( F(1, 210) = 54.5, \quad P < 0.001 \). The increase in fullness when soup was consumed as a second course (30 ± 19),...
however, did not differ from the increase when soup was consumed as a first course (27 ± 21): F(1, 210) = 1.3, P = 0.29.

Figure 3.2: Changes in hunger (Mean + SD) after intake of both soup and buns with fillings in “soup as first course” setting, and after intake of both preload and soup in “soup as second course” setting.

Food choice after ad libitum intake of soup in “soup as first course” setting

Table 3.3 shows intake in gram and energy of the second course after soup consumption. The total intake of the second course did not differ significantly in grams and energy between soup conditions, however the mean values show that intake in the second course partly compensates for soup consumption. Consequently, total lunch intake (soup plus second course) showed no differences in intake in grams and energy between soup conditions.

Savoury fillings in grams were consumed more than sweet fillings in grams after each soup: P < 0.001. There was no difference in sweet vs. savoury intake of fillings between soup conditions, neither in % of weight nor in % of energy. The absolute intake of total savoury fillings was largest after intake of the HS soup. However, intake of separate savoury fillings: cheese, gammon and cervelat, did not differ between soup conditions: P > 0.19. Total sweet fillings in grams and energy did not differ between soup conditions. Also intake of separate sweet fillings: chocolate sprinkles, jam and hazelnut paste, did not differ in intake between soup conditions: P > 0.09. Intake of buns was about 100 g and did also not differ between soup conditions: P = 0.65.

Salt intensity in soup affected sodium intake from fillings in the second course (Table 3.3); sodium intake was largest after consumption of the HS soup. Also the total intake of sodium was different between soup conditions, post hoc analyses showed that total
sodium intake (soup plus second course) was higher after consumption of HS soup than after consumption of IS and LS soup: \( P < 0.001 \), and sodium intake after IS soup was higher than after LS soup: \( P < 0.001 \).

To get insight in the palatability and frequency of consumption of the used sweet and savoury fillings in daily life, subjects rated these aspects on a 7-point scale questionnaire. Mean pleasantness ratings were around 5 for each filling (between 4.9 ± 1.3 and 5.6 ± 1.1), except for cervelat that was rated lower: 3.9 ± 1.8. Cheese was consumed most frequently: ± 2-3 days a week; followed by chocolate sprinkles: ± 1 day a week. Gammon, hazelnut paste and jam were consumed ± 2-3 days a month. Cervelat was consumed least frequently, less than one day a month.
<table>
<thead>
<tr>
<th></th>
<th>LS soup</th>
<th></th>
<th>IS soup</th>
<th></th>
<th>HS soup</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>$P^2$</td>
</tr>
<tr>
<td>Intake 2\textsuperscript{nd} course (g)</td>
<td>172</td>
<td>66</td>
<td>163</td>
<td>66</td>
<td>181</td>
<td>73</td>
<td>0.20</td>
</tr>
<tr>
<td>Intake 2\textsuperscript{nd} course (kJ)</td>
<td>2084</td>
<td>814</td>
<td>1996</td>
<td>761</td>
<td>2193</td>
<td>852</td>
<td>0.27</td>
</tr>
<tr>
<td>Total intake\textsuperscript{1} (g)</td>
<td>426</td>
<td>135</td>
<td>437</td>
<td>158</td>
<td>420</td>
<td>132</td>
<td>0.61</td>
</tr>
<tr>
<td>Total intake\textsuperscript{1} (kJ)</td>
<td>2411</td>
<td>844</td>
<td>2347</td>
<td>791</td>
<td>2501</td>
<td>879</td>
<td>0.50</td>
</tr>
<tr>
<td>Total savoury fillings (g)</td>
<td>45\textsuperscript{ab}</td>
<td>41</td>
<td>38\textsuperscript{a}</td>
<td>36</td>
<td>50\textsuperscript{b}</td>
<td>45</td>
<td>0.026</td>
</tr>
<tr>
<td>Total savoury fillings (kJ)</td>
<td>489</td>
<td>444</td>
<td>423</td>
<td>373</td>
<td>546</td>
<td>435</td>
<td>0.052</td>
</tr>
<tr>
<td>Total sweet fillings (g)</td>
<td>21</td>
<td>19</td>
<td>24</td>
<td>20</td>
<td>25</td>
<td>25</td>
<td>0.22</td>
</tr>
<tr>
<td>Total sweet fillings (kJ)</td>
<td>344</td>
<td>342</td>
<td>417</td>
<td>362</td>
<td>406</td>
<td>441</td>
<td>0.13</td>
</tr>
<tr>
<td>Weight% savoury\textsuperscript{3}</td>
<td>59</td>
<td>33</td>
<td>55</td>
<td>32</td>
<td>59</td>
<td>32</td>
<td>0.51</td>
</tr>
<tr>
<td>Energy% savoury\textsuperscript{3}</td>
<td>54</td>
<td>36</td>
<td>49</td>
<td>32</td>
<td>54</td>
<td>33</td>
<td>0.26</td>
</tr>
<tr>
<td>Na intake soup (mg)</td>
<td>394\textsuperscript{a}</td>
<td>220</td>
<td>890\textsuperscript{b}</td>
<td>648</td>
<td>1364\textsuperscript{c}</td>
<td>719</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Na intake from fillings (mg)</td>
<td>431\textsuperscript{ab}</td>
<td>376</td>
<td>368\textsuperscript{a}</td>
<td>328</td>
<td>487\textsuperscript{b}</td>
<td>408</td>
<td>0.017</td>
</tr>
<tr>
<td>Na intake 2\textsuperscript{nd} course (mg)</td>
<td>1020</td>
<td>507</td>
<td>926</td>
<td>447</td>
<td>1070</td>
<td>549</td>
<td>0.089</td>
</tr>
<tr>
<td>Total Na intake (mg)</td>
<td>1414\textsuperscript{a}</td>
<td>574</td>
<td>1816\textsuperscript{b}</td>
<td>827</td>
<td>2454\textsuperscript{c}</td>
<td>1018</td>
<td>$&lt;0.001$</td>
</tr>
</tbody>
</table>

\textsuperscript{a,b,c} Mean values within a row with unlike superscript letter are significantly different ($P < 0.05$).

\textsuperscript{1} Soup plus second course.

\textsuperscript{2} $P$-value of mixed models comparing intake of second course and sodium intake after consumption of LS, IS and HS soup.

\textsuperscript{3} Percentage savoury from total sweet plus total savoury filling.
Desire for sweet and savoury in “soup as first course” setting

Table 3.4 shows the initial and the change in ratings for desire-for-savoury and desire-for-sweet. Initial desire-for-savoury was higher than for sweet, and did not differ between soup conditions. After soup intake, the desire-for-savoury decreased; however, this decrease was not affected by salt intensity in soup. Desire-for-sweet remained unchanged after soup intake. The desire-for-sweet after soup intake was positively correlated with actual intake of total sweet fillings in gram: $r = 0.49, P < 0.001$, and negatively with actual intake of total savoury fillings in gram: $r = -0.37, P < 0.001$. The desire-for-savoury after soup intake was positively correlated with actual intake of total savoury fillings in gram: $r = 0.41, P < 0.001$, and negatively with actual intake of total sweet fillings in gram: $r = -0.37, P < 0.001$. After lunch, both desire-for-sweet and desire-for-savoury decreased (Table 3.4). The changes in desire-for-savoury did not differ between soup conditions after intake of the second course. However, the absolute value for desire-for-savoury was lower in the HS soup condition after the lunch (post-consumption ratings), this is in accordance with largest consumption of total savoury fillings (Table 3.3).

Table 3.4: Ratings (mean and SD) of desire-for-sweet and desire-for-savoury in the “soup as first course” setting.

<table>
<thead>
<tr>
<th></th>
<th>LS soup</th>
<th></th>
<th>IS soup</th>
<th></th>
<th>HS soup</th>
<th></th>
<th>$P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>Desire-for-savoury</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>72</td>
<td>15</td>
<td>71</td>
<td>14</td>
<td>72</td>
<td>16</td>
<td>0.97</td>
</tr>
<tr>
<td>Change after soup</td>
<td>-12*</td>
<td>18</td>
<td>-14*</td>
<td>19</td>
<td>-12*</td>
<td>18</td>
<td>0.65</td>
</tr>
<tr>
<td>Change after 2\textsuperscript{nd} course</td>
<td>-36*</td>
<td>24</td>
<td>-35*</td>
<td>19</td>
<td>-42*</td>
<td>23</td>
<td>0.11</td>
</tr>
<tr>
<td>Post lunch</td>
<td>24\textsuperscript{a}</td>
<td>20</td>
<td>22\textsuperscript{ab}</td>
<td>20</td>
<td>17\textsuperscript{b}</td>
<td>17</td>
<td>0.045</td>
</tr>
<tr>
<td>Desire-for-sweet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>56</td>
<td>20</td>
<td>51</td>
<td>23</td>
<td>54</td>
<td>22</td>
<td>0.24</td>
</tr>
<tr>
<td>Change after soup</td>
<td>1</td>
<td>19</td>
<td>3</td>
<td>24</td>
<td>-3</td>
<td>21</td>
<td>0.22</td>
</tr>
<tr>
<td>Change after 2\textsuperscript{nd} course</td>
<td>-32*</td>
<td>27</td>
<td>-29*</td>
<td>26</td>
<td>-29*</td>
<td>28</td>
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<tr>
<td>Post lunch</td>
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<td>20</td>
<td>25</td>
<td>21</td>
<td>23</td>
<td>19</td>
<td>0.65</td>
</tr>
</tbody>
</table>

\textsuperscript{a,b,c} Mean values within a row with unlike superscript letter are significantly different ($P < 0.05$)

$^*$P-value of mixed model comparing initial and change in ratings of LS, IS and HS soup conditions.
Discussion

The present study shows that salt intensity affected ad libitum intake. As expected, ad libitum intake of the most palatable IS soup was higher than both LS and HS soup. Moreover, salt intensity affected ad libitum intake even when palatability was kept constant, as intake of HS soup was about ~8% lower than LS soup. Ad libitum intake of soup was equal and also equally affected by palatability (IS vs. both LS and HS) and salt intensity (HS vs. LS) when either soup was consumed as a first or second course. In addition, salt intensity in soup did not predict sweet vs. savoury choice of sandwich fillings in grams or energy. Absolute intake of sodium from fillings, however, was highest after intake of HS soup compared to IS and LS soup.

Two other studies also suggested that higher salt intensity decreases intake (120, 135). Mashed potatoes were consumed in higher quantities when the salt concentration was low, whereas sensory evaluation tests showed preferences for higher salt intensities (135). In another study, pasta with tomato sauce that contained three different salt concentrations: low, ideal and high, showed the same intake pattern as in the present study: ideal>low>high (120). However, the low and high salt concentrations were not matched for equality in palatability. A higher salt intensity (i.e., higher than ideal) are often evaluated as less palatable than a lower salt intensity (i.e., lower than ideal) in soup. When pleasantness is plotted against salt concentration, the higher than ideal salt concentrations show a faster decline in pleasantness than lower than ideal salt concentrations (118, 119, 168). The present study shows that there even is an effect of salt intensity on ad libitum intake when the palatability is kept constant.

An explanation for the effect of salt intensity on ad libitum intake might be that an increased salt intensity results in an increased sensory exposure. The increased sensory exposure may lead to faster onset of satiation during consumption. Accordingly, Yeomans (20, 120) showed that an increased intensity of the taste of food led to faster decrease in hunger, and consequently lower intake. Increased taste intensity may also trigger to consume with smaller bite sizes. De Wijk et al. (178) has found that higher aroma intensities resulted in smaller bite sizes. Adjusting the bite size allows people to self-dose the taste intensity, and consequently the amount of nutrients. Smaller bite sizes are associated with lower intake (92, 93, 96). Whether salt intensity affects bite size and whether that leads to a decreased intake will be investigated in a next study.

In contrast to the present study, in our previous study, no effect of salt intensity was found on ad libitum intake of tomato soup when palatability was kept constant (LS vs. HS) (chapter 2, 168). The experimental design was almost equal to the design of the present study, except that tomato soup was presented as the only lunch-item. This suggests that the context in which the food is served determines effects of sensory signals on ad libitum intake. Also Vickers et al. (112, 116) showed when yoghurt was
consumed as only lunch-item, high-sweet yoghurt (higher than ideal) was consumed more than low-sweet yoghurt (lower than ideal) whereas the opposite result was found in a multi-item lunch. In our previous study, subjects were in a hungry state and soup was the only food to alleviate hunger (chapter 2, 168). In the present study, we assumed to create meal settings in which subjects terminate consumption based on sensory factors rather than on hunger. Subjects did not have to alleviate their hunger completely with soup because they were presented a second meal ("soup as first course" setting) or were less hungry prior to soup consumption when they consumed a preload before ("soup as second course" setting). The fact that we did not find an effect of salt intensity when soup was consumed as only lunch item, suggests that hunger may diminish effects of sensory signals on satiation. The contribution of sensory versus physiological or psychological factors on satiation in different contexts of food consumption needs to be studied further.

Ad libitum intake of soup was equal when soup was consumed either as a first or second course, while subjects were in a different state of hunger. Subjects were less hungry when soup was consumed as a second course than as a first course. The amount of consumed soup was around 250 g, which is an average serving size of soup. The portion size that people consume of a specific food is considered to be learned by previous experience (52, 170), but it is unsure how this exactly is regulated. Usually, visual cues play an important role in decisions on portion size (55). In this case, however, self-monitoring the amount consumed was not possible because a self-refilling bowl was used (55). Other processes that may have played a role might be the oral exposure time, the time course in which subjects usually consume soup, the number of bites or the degree of stomach filling.

Although ad libitum intake was equal in both meal settings, the change in hunger and prospective consumption ratings were much larger when soup was consumed as a second course. The changes in hunger and prospective consumption were about similar when the two first courses are compared (soup and preload) and when the two second courses are compared (buns with fillings and soup). This suggests a certain pattern of appetite during the meal. This was illustrated in a plot when hunger is plotted against intake (20, 120, 121), in the beginning of a meal, hunger remains constant or increases slightly, while during the meal hunger starts to decrease. As a result, hunger decreases faster at the end of the meal. This supports our result that hunger decreased more when the second course was consumed (buns with fillings or soup). We did not find differences in fullness when soup was consumed as a starter or after a preload. This is in accordance with findings that fullness ratings reflect the actual amount consumed (20, 121). Consuming the same amount of soup in both meal settings, despite different ratings of hunger, suggests an effect of habits or learned decisions on portion size.
There was no effect of salt intensity in soup on sweet vs. savoury choice of fillings in weight or energy percentages. Before soup intake, desire-for-savoury was higher than desire-for-sweet. This is in agreement with the finding that desire-for-savoury fluctuates and is highest before meals and that desire-for-sweet remains more constant during the day (172). After consumption of soup, desire-for-savoury decreased similarly between soup conditions, until a value that almost equals the desire-for-sweet. Subjects consumed more savoury fillings than sweet fillings in total, although the choice of sweet vs. savoury sandwich fillings did not differ between soup conditions. Vickers et al. (116) found a drop in liking for sweet products after consumption of a high-sweet yoghurt, compared to ideal-sweet and low-sweet yoghurts. We did not observe a larger drop in desire-for-savoury and consequently fewer intakes of savoury fillings after the HS soup. In contrast, savoury fillings even showed the largest consumption in grams after consumption of HS soup.

After consumption of HS soup, sodium intake from fillings was largest in the second course, this was due to the largest consumption of savoury fillings compared to the other soup conditions. All mean values of the separate savoury fillings were numerically highest in the HS soup condition (data of separate fillings not shown), so the increased sodium intake was not due to one specific filling. Subjects thus seemed to continue with high sodium intake after consuming HS soup. It might be that subjects preferred a salty taste after consuming the salty tasting HS soup, and found the sweet tasting fillings too “bland”. People may get used to the exposed salt intensity in a food, and may not prefer much different salt intensities at that moment, probably due to contrast effects (128, 129, 132). Accordingly, to our previous study (chapter 2, 168), in which we showed that after consumption of HS soup, LS soup was perceived as too “bland” and therefore less pleasant than just consumed HS soup. This means that sodium intake from soup is not compensated by sodium intake from a second course. A health beneficial consequence is that consumption of a low-salt soup does probably not lead to higher sodium intake from a second course.

In summary, the present study showed that salt intensity decreased ad libitum intake of tomato soup when palatability is kept constant. In addition, salt intensity in soup did not predict sweet vs. savoury food choice. Absolute intake of sodium from fillings, however, was highest after intake of HS soup compared to IS and LS soup. Ad libitum intake of soup was equal when soup was consumed as a first or second course. The latter indicates that habits or learned decisions are important in portion size.
Acknowledgements

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Chapter 4

Both longer oral sensory exposure to and higher intensity of saltiness decrease ad libitum food intake

Dieuwerke P. Bolhuis
Catriona M.M. Lakemond
Rene A. de Wijk
Pieternel A. Luning
Cees de Graaf

Abstract

Orosensory exposure to sweetness has been shown to be important in satiation, whereas the effect of exposure to a salty taste on satiation is not known. The primary objective was to investigate the effect of orosensory exposure time to and intensity of saltiness in soup on ad libitum intake. The orosensory exposure time was manipulated by changing the bite sizes. The secondary objective was to investigate the effect of intensity on bite size. Fifty-five healthy men consumed ad libitum from both a low-salt (LS) and a high-salt (HS) creamy tomato soup in two exposure time conditions, established by a small and large bite size condition (“small” and “large”) and a free bite size condition (“free”). Bites were administered and controlled via a pump. In the “small” condition, bites of 5 g were administered in 2 s at intervals of 5 s (oral exposure time: 40 s/100 g). In the “large” condition, bites of 15 g were administered in 3 s at intervals of 15 s (oral exposure time: 20 s/100 g). The eating rate was equal in the “small” and “large” conditions (60 g/min). In the “free” condition, participants adjusted their bite sizes at intervals of 15 s. The “large” condition resulted in ~34% higher ad libitum intake compared to the “small” condition ($P < 0.001$); there was no interaction with intensity. Ad libitum intake of HS soup was ~9% lower than LS soup ($P < 0.001$). The “free” condition showed that HS soup was consumed with smaller bite sizes during the first half of the intake period ($P < 0.05$). Longer orosensory exposure, established by smaller bites, and higher saltiness intensity both decreased food intake. Prolonging the orosensory exposure per gram food may be helpful to reduce food intake.

Keywords: orosensory exposure, bite size, intensity, salt, satiation, ad libitum intake
Introduction

Obesity is an increasing problem in the western society. Overconsumption during an eating episode is considered a major cause of overweight and obesity (97, 122, 123). Insight in food intake regulation is, therefore, of great concern. During a meal, consumption is driven by signals of reward in the brain (positive feedback). These reward signals will finally be overruled by signals of satiation (negative feedback) that result in meal termination (20, 121, 179). This interaction of feedback signals influences meal size. Exposure to food in the oral cavity (orosensory exposure) is essential for establishing feedback signals of satiation (20, 180). Accordingly, direct infusions of food into the stomach or duodenum elicit much weaker responses on satiation compared to oral intake of food (91, 180).

Eating rate has been shown to influence meal size; a faster consumption leads to higher intake (9-11). The most obvious distinction in eating rate is between liquids and solids. Liquids are consumed much faster than solids or semi-solids and show indeed higher ad libitum intakes (9-12, 181), even when they are equal in energy density (10, 12). Liquids are consumed with larger bite sizes than solids, which leads to a faster eating rate (12) and, consequently, a shorter orosensory exposure per gram food. Controlled experimental designs have shown a direct negative relation between orosensory exposure time and intake (92, 93). Until now, only sweet tasting foods have been used to investigate effects of texture (liquid vs. semi-solid or solid), eating rate or orosensory exposure time on satiation (10, 12, 87, 88, 92, 93).

Orosensory exposure to a sweet taste has been found to predict energy intake and is able to induce satiation (182-186). Other taste qualities may elicit different effects on satiation. Salt (i.e., sodium chloride) per se is a nutrient that does not contain energy and may therefore not be associated with energy. Sodium is needed to control the body’s fluid balance, but the intake of sodium is not regulated on the short-term, as is the intake of energy (187, 188). The contribution of orosensory exposure to salty, savoury tastes on satiation is not known.

Saltiness intensity has been shown to have a small effect on satiation (chapter 3, 189). Ad libitum intake of a high-salt soup was lower than that of a low-salt soup, while the soups were similar in palatability. The underlying mechanism of the effect of taste intensity on satiation is not known. One possible explanation is that the intensity affects intake via bite size. Recent data suggest that an increase in aroma intensity led to consumption with smaller bite sizes (178). In general, smaller bite sizes lead to lower food intake (92, 93, 96, 98). Higher intensity of taste, therefore, may drive people to consume with smaller bite sizes and this may result in lower intake.
The main objective was to investigate the effect of orosensory exposure time to and intensity of saltiness in soup on satiation. Orosensory exposure time was manipulated by changing the bite size. Satiation was measured as the amount of ad libitum intake of soup during lunch. The second objective was to investigate the effect of saltiness intensity in soup on bite size.

### Materials and Methods

#### Participants

Sixty-two male participants were recruited for participation. Fifty-five participants completed the study, five participants dropped out of the study before the start of the ad libitum intake sessions and two participants missed, respectively, two and three ad libitum intake sessions. Participants were healthy, had a normal weight (BMI 18.5-25 kg/m$^2$, mean ± SD: 22 ± 2 kg/m$^2$), were aged between 18 and 35 y (mean ± SD: 22 ± 3 y) and liked creamy tomato soup (pleasantness score > 5 on a 9-point hedonic scale). Exclusion criteria were restrained eating (Dutch eating behaviour questionnaire (DEBQ) score > 2.89 (56)), following an energy-restricted diet during the last two months, gained or lost > 5 kg weight during the last year, having a lack of appetite, smoking, suffering from gastrointestinal illness, diabetes, thyroid disease or any other endocrine disorder, hypertension and kidney diseases. Participants were informed that the aim of the research was to investigate the effect of individual taste sensitivity on taste perception of soup. All procedures in this study were approved by the Medical Ethical Committee of Wageningen University (NL31123.081.09). All participants signed an informed consent form before participation.

#### Test foods

Tomato soup with varying salt concentrations was used as test product in this study. One kilogram of soup was made from 600 g mashed tomato pieces (Heinz, Elst, The Netherlands), 40 g cream (kookroom, private label Albert Heijn, Zaandam, The Netherlands) and 360 g water. The mixture was heated until 60 °C. The calculated nutrient composition according to the labels of the used ingredients was: 1.0 g protein, 2.7 g carbohydrates, 0.9 g fat, 61 mg sodium and 99 kJ (24 kcal) energy per 100 g soup.

Raisin buns (local bakery) were used as a preload. The nutrient composition was: 8 g protein, 52 g carbohydrates, 3 g fat, 300 mg sodium and 1120 kJ (268 kcal) energy per 100 g, according to the Dutch Food Composition Database (NEVO, version 2009/1.0). The amount of raisin buns as preload was calculated for each participant at half of the
energy provided by an average lunch in the Netherlands (175), which is equal to 11% energy of the daily energy need. The daily energy need was estimated by the Schofield I equation (176), taking into account: gender, age, weight and a physical activity level of 1.6 x BMR.

Experimental design

Salt concentrations for the LS and HS soups were selected per subject during their first visit, as described below. After that, subjects visited the lab seven times during lunch, including one practice session, to consume either LS or HS soup in a “small”, “large”, or “free” bite size condition (2 x 3 cross-over design: Figure 4.1).

The six ad libitum conditions were presented in randomized order. Bites and intervals were administered and controlled via a pump. The eating rate was 60 g/min for both the “small” and “large” condition, which is somewhat lower than the mean eating rate of soup consumed in a “normal” manner with spoons (~72 g/min) found in two of our previous studies (chapter 2, 168 and chapter 3, 189). The time of exposure of soup in the oral cavity was 40 s/100 g in the “small” condition and 20 s/100 g in the “large” condition (Figure 4.1). In the “free” condition, participants were able to choose their own bite size. The “free” condition was used to investigate the effect of saltiness intensity on bite size.

**Figure 4.1** Bites and intervals in the “small”, “large” and “free” condition. In the “small” condition, participants received 5 g of soup each 5 s. One bite of 5 g was administered during 1 s and swallowed in the next second (arrow). In the “large” condition, participants received 15 g of soup each 15 s. One bite of 15 g was administered during 2 s and swallowed in the next second. Participants heard an auditory signal when the soup was administered and a double auditory signal when they had to swallow. In the “free” condition, participants received soup each 15 s and were allowed to stop the administration by themselves to adjust the bite size.
Selection of individual salt concentrations for LS and HS soups

We selected salt concentrations for LS and HS soups on an individual basis to be similar in palatability, because preference for saltiness intensity varies largely between individuals (117, 118, 138, 168). Additionally, we wanted to compare the ad libitum intakes of food that are similar in palatability. Palatability is a well-known determinant of the amount consumed (43-45, 112, 116, 120, 121).

The methodology for the individual selection is described in detail in Bolhuis et al. (168). In summary, the pleasantness ratings of soups with different salt concentrations were plotted against concentration on a logarithmic scale for each participant separately. A salt concentration below (LS) and a salt concentration above the most pleasant salt concentration (HS) were selected by linear interpolation based on similar pleasantness ratings, meaning < 10 mm difference in pleasantness ratings. The distance in geometric sodium concentration (i.e., the ratio) was equal between each selected pair of LS and HS soup, which was a factor 3.7.

Procedure of the ad libitum intake sessions

Participants were seated in sensory booths. They started with consumption of a preload of raisin buns. A preload was used so that participants would be less hungry before soup consumption. It is possible that feelings of hunger may overrule sensory factors to terminate consumption when participants are in a very hungry state (chapter 2, 168, chapter 3, 189). Participants were instructed to consume all served raisin buns and they were allowed to drink a maximum of 150 g water. After that, participants paused for 30 minutes, they were able to read or study. They were not allowed to leave the sensory room.

After the pause, participants received instructions and questions on a computer screen. After answering several appetite and hedonic questions, as described below, participants pushed a button on the screen to start soup consumption. They were instructed to terminate consumption when they felt they had enough. The mean (± SD) initial temperature of the soup was 52 ± 3 °C and the mean end temperature was 48 ± 2 °C. Participants were instructed to stay in the sensory booths for at least ten minutes after they started consuming the soup. After ten minutes, an alert popped up on the laptop screen to inform participants that the ten minutes had passed. This was to prevent participants from leaving the research area for other reasons than being satiated with the soup.

At the end of the last session, participants were asked what the most important reason was that they terminated consumption. They were able to choose between “full”,
“flavour was not pleasant anymore”, “the manner of consumption was not pleasant” and “other” (an open answer that could be filled in). This was only asked in the last sessions because we wanted to prevent participants from focusing too much on the termination of soup consumption.

Control of bites, intervals and swallowing in the ad libitum intake sessions

To control and measure the bite sizes and intervals, participants consumed the soup through a food-grade silicon tube that was connected to a peristaltic pump (Watson-Marlow, types 520 and 323Du, Watson-Marlow Bredel, Wilmington, MA, USA). The tube ended in a pan of soup that was placed on a balance (Kern, type 440-49A, KERN & Sohn GmbH, Balingen, Germany) to record the amount consumed. The pump, the pan and the balance were all located at the experimenters’ side of the sensory booths, thus participants did not see the experimental setup.

When the pump started, participants heard an auditory signal to prepare them that they would receive soup in their mouths. They heard a double auditory signal when they had to swallow. In the “small” condition, participants received 5 g in 1 s (pump speed was set at 300 g/min), and had to swallow in the next second (Figure 4.1). In the “large” condition participants received 15 g in 2 s (pump speed was set at 450 g/min), and had to swallow in the next second. In the “free” condition participants received soup at a rate of 2.5 g/s (pump speed was set at 150 g/min), starting at the first second in pulses of 15 s. They were able to push a button on a computer screen to stop the pump to enable their bite sizes. In this condition, participants were instructed to swallow as soon as they stopped administration.

Standardization of the satiety state in the ad libitum intake sessions

To standardize the satiety state, participants always started the lunch session at the same time. They were instructed to consume the same breakfast and not to eat and only drink water or weak tea 3 h before the lunch started. Moreover, they were asked to refrain from drinking 1 h before the test started. After each test lunch, participants had to answer questions about what they ate for breakfast and whether they ate or drank between breakfast and test lunch. To make sure participants would consume soup until they felt satiated; they were instructed not to eat until 1 h after the test.
Appetite and hedonic ratings in the ad libitum intake sessions

Just before soup intake, participants rated their feelings of hunger, fullness, prospective consumption (how much they thought they could eat) (137) and thirst on a 100 mm VAS. After that, participants were served a small sample of 10 g soup to rate pleasantness, desire-to-eat the soup and RTI saltiness intensity on a 100 mm VAS. In the “free” condition, participants rated saltiness intensity and overall taste intensity and not RTI-saltiness intensity on a 100 mm VAS. This distinction was made because it is considered difficult for participants to answer both RTI-saltiness intensity and saltiness intensity within the same session. The same questions were answered again at the end of the ad libitum intake.

In addition, in the “small” and “large” conditions, pleasantness, desire-to-eat, hunger, and fullness were rated after intake of every 75 g. These questions appeared on the computer screen in random order.

The scale that was used for the pleasantness ratings was labelled “very unpleasant” at the left end (0 mm) and “very pleasant” at the right end (100 mm). The scale that was used for the desire-to-eat was labelled “not at all” at the left end (0 mm) to “very much” at the right end (100 mm). The scale that was used for the RTI-saltiness intensity was labelled “not nearly salty enough” at the left end (-50 mm), “just right” in the middle (0 mm), and “much too salty” at the right end (+50 mm) (168). The scale that was used for the overall taste intensity and saltiness intensity was labelled “very weak” at the left end (0 mm) to “very strong” at the right end (100 mm).

Statistical analyses

Statistical analyses were performed using SAS version 9.1.4 (SAS Institute Inc., Cary, NC, USA). Data are presented as means ± SD.

Effects of saltiness intensity (LS vs. HS) on ratings of RTI-saltiness intensity, saltiness intensity, overall taste intensity and pleasantness before ad libitum intake were assessed in mixed linear models that included order and had participants as repeated factor.

The changes in hunger and fullness during ad libitum intake were fitted per participant in a linear model: y = a + b x intake. A linear model was chosen because this produced the best fit in most individual curves. The ‘b’ value (i.e., slope) indicates the change in appetite during ad libitum intake. The curves shown in the results section are calculated from the mean intercepts and mean slopes of the individual plots.

Effects of orosensory exposure time (“small” vs. “large” vs. “free”), saltiness intensity (LS vs. HS), and their interaction on ad libitum intake, appetite (ratings from before,
during and after ad libitum intake) and thirst ratings were assessed in mixed linear models that included order and pleasantness ratings and had participant as repeated factor. Tukey-Kramer adjustments were used for all post-hoc comparisons in this study.

Effects of saltiness intensity (LS vs. HS) on bite size and on the number of bites in the “free” condition were assessed in mixed linear models that included order, pleasantness ratings and had participant as repeated factor. Pearson correlations coefficients were calculated for ad libitum intake, saltiness intensity, hedonic and appetite ratings vs. bite size.

Results

Sensory characteristics of LS and HS soup

The selected sodium concentrations for LS soup ranged from: 61 to 258 mg Na/100 g (Table 4.1). The selected sodium concentrations for HS soup ranged from: 232 to 966 mg Na/100g. RTI-saltiness intensity ratings showed that HS soup was rated above ideal (>0) and LS soup was rated below ideal (<0). Moreover, the overall taste intensity and saltiness intensity of HS soup was higher than LS soup. On average, the individually selected HS soup was rated as more pleasant than the individually selected LS soup. However, this was a difference of only 6 mm on a 100 mm VAS, which is within the stated limit of a difference less than 10 mm in pleasantness (see “materials and methods” section). The sensory characteristics were measured before soup consumption at each lunch session and were averaged for both LS and HS soup.
Table 4.1 Sodium concentrations and sensory characteristics of LS and HS soups\textsuperscript{1,3}

<table>
<thead>
<tr>
<th></th>
<th>LS soup</th>
<th>HS soup</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na/100g soup, mg</td>
<td>146 ± 49</td>
<td>547 ± 183</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RTI-saltiness intensity\textsuperscript{3,4}, mm</td>
<td>-14 ± 15</td>
<td>10 ± 14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Saltiness intensity\textsuperscript{5}, mm</td>
<td>31 ± 17</td>
<td>60 ± 19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Overall taste intensity\textsuperscript{5}, mm</td>
<td>43 ± 19</td>
<td>63 ± 14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pleasantness\textsuperscript{4,5}, mm</td>
<td>53 ± 19</td>
<td>59 ± 20</td>
<td>0.009</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Values are means ± SD, \( n = 55 \)
\textsuperscript{2} The sensory characteristics were rated before ad libitum intake of soup on a 100 mm visual analogue scale (VAS)
\textsuperscript{3} LS = low-salt, HS = high-salt, RTI = relative-to-ideal
\textsuperscript{4} Rated in “small” and “large” conditions
\textsuperscript{5} Rated in “free” condition

Ad libitum intake

Ad libitum intake of soup was affected by effects of orosensory exposure time (\( P < 0.001 \)), and saltiness intensity (\( P < 0.001 \)) (Figure 4.2). There was no interaction between orosensory exposure time and saltiness intensity (\( P = 0.83 \)). Ad libitum intake of HS soup was 8-9% lower than LS soup in all three orosensory exposure time conditions. Ad libitum intake was ~34% higher in the “large” condition than in the “small” condition (\( P < 0.001 \)). Ad libitum intake in the “free” condition was ~13% lower than in the “large” condition (\( P = 0.005 \)) and ~17% higher than in the “small” condition (\( P = 0.037 \)).
Appetite and thirst ratings before and after ad libitum intake

The appetite ratings before ad libitum intake did not differ between conditions (Table 4.2), indicating that participants were in the same state of satiety before soup consumption. The change in appetite ratings after ad libitum intake was not affected by orosensory exposure time or saltiness intensity or by its interactions.

In the preload phase, participants were allowed to drink water (max. 150 g), this was on average: 142 ± 40 g, and did not differ between conditions ($P = 0.79$). Ratings of thirst increased after HS soup consumption and decreased after LS soup consumption (Table 4.2).

Appetite ratings during ad libitum intake in the “small” vs. “large” condition

Hunger decreased faster during soup consumption in the “small” condition compared to the “large” condition ($P = 0.014$) (Figure 4.3). The decrease in hunger was not affected by saltiness intensity ($P = 0.41$). Likewise, fullness increased faster in the “small” condition compared to the “large” condition ($P = 0.027$), but was not significantly affected by saltiness intensity ($P = 0.10$). There was no interaction effect (orosensory exposure time x salt intensity) on the decrease of hunger ($P = 0.91$) or the increase in fullness ($P = 0.38$). The dots visualize the mean ad libitum intakes in each condition (Figure 4.3). Interpolation from the mean ad libitum intakes to the appetite ratings visualizes that the appetite ratings after intake were not different in the “small” compared to the “large” condition. In summary, larger bites thus shorter orosensory exposure resulted in a slower change in hunger and fullness during consumption.
Chapter 4 – Effects of salt intensity and oral exposure on ad libitum intake

Figure 4.3 Linear functions of hunger (A) and fullness (B) ratings against soup intake in the “small” and “large” conditions, $n = 55$. The curves are calculated from the mean intercepts and mean slopes of the individual plots ($y = a + bx$ intake). The dots on the curves visualize the mean ad libitum intakes in each condition. LS = low-salt, HS = high-salt.

Bites sizes in the “free” condition

The mean bite sizes varied greatly between individuals, from 3.6 g to 25.2 g. The mean bite size for LS soup was $13.8 \pm 3.8$ g and the mean bite size for HS soup was $13.1 \pm 4.4$ g ($P = 0.053$). Bite size was mainly affected by saltiness intensity during the first quartile ($P = 0.048$), and second quartile ($P = 0.039$) of soup consumption (Figure 4.4). Bite size was negatively related to saltiness intensity, and positively to ratings of ad libitum intake, pleasantness, desire-to-eat, hunger and prospective consumption (Table 4.3). In addition, the mean number of bites of the ad libitum intake was higher when consuming LS soup: $24.1 \pm 12.4$, than when consuming HS soup: $22.4 \pm 12.4$ ($P = 0.008$).
Table 4.2 Initial appetite ratings and the change in ratings after ad libitum intake\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>Small</th>
<th></th>
<th>Large</th>
<th></th>
<th>Free</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LS(^3)</td>
<td>HS(^3)</td>
<td>LS</td>
<td>HS</td>
<td>LS soup</td>
<td>HS soup</td>
<td>Time</td>
<td>Salt</td>
<td>Time*salt</td>
</tr>
<tr>
<td>Hunger Initial</td>
<td>58 ± 19</td>
<td>58 ± 17</td>
<td>57 ± 19</td>
<td>60 ± 17</td>
<td>60 ± 20</td>
<td>58 ± 18</td>
<td>0.84</td>
<td>0.63</td>
<td>0.47</td>
</tr>
<tr>
<td>Hunger Change</td>
<td>-38 ± 24</td>
<td>-35 ± 24</td>
<td>-39 ± 22</td>
<td>-43 ± 22</td>
<td>-37 ± 22</td>
<td>-35 ± 24</td>
<td>0.07</td>
<td>0.55</td>
<td>0.13</td>
</tr>
<tr>
<td>Fullness Initial</td>
<td>37 ± 20</td>
<td>38 ± 16</td>
<td>41 ± 21</td>
<td>37 ± 19</td>
<td>36 ± 20</td>
<td>36 ± 18</td>
<td>0.51</td>
<td>0.53</td>
<td>0.60</td>
</tr>
<tr>
<td>Fullness Change</td>
<td>40 ± 23</td>
<td>36 ± 23</td>
<td>40 ± 27</td>
<td>42 ± 24</td>
<td>37 ± 26</td>
<td>38 ± 24</td>
<td>0.19</td>
<td>0.39</td>
<td>0.49</td>
</tr>
<tr>
<td>Prospective consumption Initial</td>
<td>57 ± 17</td>
<td>59 ± 14</td>
<td>57 ± 18</td>
<td>60 ± 17</td>
<td>61 ± 18</td>
<td>58 ± 16</td>
<td>0.67</td>
<td>0.85</td>
<td>0.11</td>
</tr>
<tr>
<td>Prospective consumption Change</td>
<td>-37 ± 23</td>
<td>-36 ± 22</td>
<td>-37 ± 23</td>
<td>-39 ± 23</td>
<td>-38 ± 20</td>
<td>-32 ± 23</td>
<td>0.35</td>
<td>0.09</td>
<td>0.13</td>
</tr>
<tr>
<td>Thirst Initial</td>
<td>53 ± 21</td>
<td>55 ± 19</td>
<td>53 ± 20</td>
<td>52 ± 20</td>
<td>53 ± 21</td>
<td>52 ± 20</td>
<td>0.68</td>
<td>0.83</td>
<td>0.69</td>
</tr>
<tr>
<td>Thirst Change</td>
<td>-7 ± 26(^a)</td>
<td>8 ± 26(^b)</td>
<td>-7 ± 22(^a)</td>
<td>9 ± 24(^b)</td>
<td>-7 ± 24(^a)</td>
<td>8 ± 22(^b)</td>
<td>0.83</td>
<td>&lt;0.001</td>
<td>0.98</td>
</tr>
</tbody>
</table>

1 Values are means ± SD, \(n = 55\). Mean values without a common letter differ, \(P < 0.05\)
2 The appetite and thirst ratings were rated on a 100 mm visual analogue scale (VAS)
3 LS = low-salt, HS = high-salt
4 \(P\)-value of interaction: orosensory exposure time*saltiness intensity
Figure 4.4 Bite sizes of each quartile during consumption in the “free” condition. Values are means + SD, n = 55. *Difference between LS and HS, P < 0.05. LS = low-salt, HS = high-salt.

Table 4.3 Correlations of bite size with intake and hedonic/appetite ratings in the “free” condition

<table>
<thead>
<tr>
<th></th>
<th>Bite size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad libitum intake</td>
<td>0.41***</td>
</tr>
<tr>
<td>Saltiness intensity</td>
<td>-0.23*</td>
</tr>
<tr>
<td>Pleasantness</td>
<td>0.29**</td>
</tr>
<tr>
<td>Desire-to-eat</td>
<td>0.28**</td>
</tr>
<tr>
<td>Hunger</td>
<td>0.24*</td>
</tr>
<tr>
<td>Prospective consumption</td>
<td>0.22*</td>
</tr>
</tbody>
</table>

† Values are Pearson correlation coefficients (r), n = 55
* P <0.05; ** P<0.01; ***P<0.001

Reasons to terminate consumption

Thirty-eight participants indicated that they had terminated consumption because they were full, twelve participants indicated that “the flavour of the soup was not pleasant anymore”, four participants indicated that they terminated because “the manner of consumption was not pleasant” and one participant indicated that it was a combination of the flavour and the manner of consumption.
Discussion

The present study shows that orosensory exposure time clearly affects satiation of a savoury, salty food. Larger bite sizes, thus shorter orosensory exposure per gram food, resulted in ~34% greater intake than smaller bite sizes, thus longer orosensory exposure. In accordance, a shorter orosensory exposure was associated with slower changes in ratings of hunger and fullness during intake. Both the increased intake and the slower changes in appetite ratings indicate that a shorter orosensory exposure to food delays signals of satiation. The effect of orosensory exposure time was not influenced by the saltiness intensity. In addition, higher saltiness intensity in soup led to lower ad libitum intake and to smaller bite sizes.

To our knowledge, this is the first time that the effect of orosensory exposure time of a savoury, salty food on ad libitum intake is measured. The results of the present study on ad libitum intake are in line with the results of studies that used sweet tasting foods (92, 93). Weijzen et al. (92) used a similar experimental setup as the present study: smaller sips were administered more frequently than larger sips and the exposure time per gram was twice as high in the small sip condition compared to the large sip condition. The results of that study (92) showed that intake with smaller sips, thus a longer orosensory exposure, led to ~29% lower intake for a regular energy orangeade and 16% lower intake for a no-energy containing orangeade. In addition, Zijlstra et al. (93) investigated the effect of bite size (5 g vs. 15 g) and oral exposure time (3 s vs. 9 s) separately on ad libitum intake. Both larger bite sizes and a shorter exposure time resulted in higher intakes of chocolate custard (effects of resp. ~31% and ~15%), which is again in line with the results of the present study.

Orosensory exposure time has a larger effect than the intensity to saltiness on ad libitum intake according to the present study. In addition, the effect of orosensory exposure time was independent of the saltiness intensity. This indicates that orosensory exposure time, apart from taste quality or taste intensity, is very important for food intake regulation. This is in accordance with a recent study (80) that showed that only the orosensory exposure, manipulated by the manner of consumption (spoon or straw), affected intake, whereas different flavours, even when combined with different energy densities, did not affect intake. Moreover, another study showed that manipulating the taste quality by either sweet or savoury, while all other aspects including palatability were held constant, did not influence intake (83). Together, the orosensory exposure time, which is affected by manner of consumption, may be more important in the process of satiation than taste quality or intensity when palatability is held constant.

The present study shows that a shorter orosensory exposure per gram food leads to more intake and delays feelings of hunger and fullness. A short orosensory exposure to food, therefore, may lead to insufficient sensing of nutrients in the oral cavity and this can
easily lead to overconsumption. In daily life, liquids are consumed with a much shorter orosensory exposure time than and non-liquid foods (9, 10, 181). The energy intake from liquids has been rising over last past decades along with the obesity epidemic (190, 191). Moreover, liquids have a low satiating capacity (87) and is only poorly compensated by subsequent food intake (88, 192).

In the present study, higher saltiness intensity results in a smaller bite size. Also de Wijk et al. (178) found that a higher aroma intensity resulted in smaller bite sizes. Adjusting the bite size allows people to self-dose the taste intensity and consequently the amount of nutrients. In literature, smaller bite sizes were found to be associated with lower intake (92, 93, 96, 98), although one study did not find an effect of bite sizes on intake (193). However, contrary to our hypothesis, the smaller bite sizes demonstrated for HS soup do not explain the lower intake for HS soup compared to LS soup. Fixed bite sizes (in “small” and “large” condition) led to the same difference in intake between LS and HS soup compared to the free bite sizes (in “free” condition), as the effect size was 8-9% in all three conditions. The effect of intensity on bite size in the “free” condition might have been too small to establish a larger decrease in intake of the HS soup. In addition, bite size is not only predicted by saltiness intensity in the present study, but also by palatability and hunger. The results of other studies already showed that bite size depends on palatability (194) and the state of hunger (13).

The results show that higher saltiness intensity leads to lower ad libitum intake. The salt concentration for the low and high saltiness intensity was selected to be similar in palatability on an individual basis. A similar effect size (~8%) was found in a previous study in which the palatability was also kept constant on an individual basis (chapter 3, 189). In that study, participants consumed soup in a “normal manner” with a spoon. A self-refilling bowl was used to diminish visual cues of the amount consumed. Also other studies found that a higher saltiness intensity led to lower ad libitum intake of pasta sauce (120) and mashed potatoes (135), however, palatability was not kept constant. It is not exactly clear why saltiness intensity affects satiation. As discussed above, the lower intake of HS soup is not only explained by smaller bite sizes. Higher taste intensity in general may be associated with a larger amount of nutrients. Therefore, people may feel satiated faster when consuming a food that is higher in taste intensity. In accordance, a previous study showed that the “expected satiation” value (measured by ratings of: “How satiating is this food?”) was indeed higher when the saltiness intensity increased (chapter 2, 168). Expectations of satiety/satiation have been shown to play a role in the amount consumed (52, 195). Participants may have perceived the HS soup as more satiating than the LS soup and therefore consumed less of the HS soup. Increasing the salt concentration in food, however, should not be used as a tool to lower energy intake. High levels of daily sodium intake are associated with hypertension (196) and cardiovascular disease (197, 198).
In the orosensory exposure time conditions chosen in the present study, we did not only vary the oral exposure time (40 s/100 g vs. 20 s/100 g), but also the bite sizes (5 g vs. 15 g) and the number of bites (12 bites/min vs. 4 bites/min). Also in a normal eating situation, a smaller bite size is associated with a lower eating rate (12, 199) and thereby with a relatively longer orosensory exposure per gram food, like in the present study. In addition, a smaller bite size automatically leads to a higher number of bites per gram food. Apart from the exposure time, also the number of bites may influence satiation. A possible role for the number of bites, apart from orosensory exposure time, will be investigated in a next study.

In conclusion, a shorter orosensory exposure per gram food, established by larger bite sizes, leads to higher intake and delays feelings of hunger and fullness in a savoury, salty food. Orosensory exposure time has more impact on satiation than saltiness intensity in the present study. Consumption of food with a longer orosensory exposure, for example with smaller bite sizes, will probably reduce intake within an eating episode. Likewise, designing foods that will be consumed with longer orosensory exposure may thereby contribute to the prevention of overweight and obesity.

Acknowledgements

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All authors contributed to the design of the study. D.P.B. wrote the study protocol, conducted the study, analysed the data and wrote the manuscript, all under supervision of C.M.M.L., R.A. de W., P.A.L and C. de G. All authors critically revised the manuscript and read and approved the final version.
Chapter 5

Both higher number of bites and longer oral residence duration increase the oral sensory exposure to food and reduce ad libitum food intake

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Submitted for publication
Abstract

Higher eating rate leads to higher food intake, considerably through shorter orosensory exposure to the food. Bite size largely affects eating rate. Smaller bites are associated with higher number of bites and longer oral residence duration per gram food. The separate role of these two aspects on satiation and on orosensory exposure needs further clarification. The objective was to investigate contributions of the number of bites (bites/g) and oral residence duration (s/g) on, first, ad libitum intake of soup, and second, on the orosensory exposure per gram food. In this 2 x 2 crossover study, 56 healthy male subjects consumed soup after a preload where number of bites and oral residence duration differed by a factor three, respectively: 6.7 bites/100g vs. 20 bites/100g, and 20 s/100g vs. 60 s/100g. All conditions had equal eating rate of 60 g/min. Effects on orosensory exposure of 30 g soup in all conditions were measured by time intensity functions by 22 different healthy subjects. Higher number of bites and longer oral residence duration reduced ad libitum intake by respectively ~22% and ~8% ($P < 0.007$), and both increased the orosensory exposure per gram food ($P < 0.001$). Hunger and fullness after intake did not differ between conditions. Higher number of bites and longer oral residence duration reduced food intake, possibly through the increased the orosensory exposure per gram food. Designing foods that will be consumed with small bites and long oral residence duration are both effective in reducing energy intake.

**Keywords:** orosensory exposure, oral residence time, satiation, bite size
Introduction

The current food supply consists of a majority of highly processed foods that support fast intake of energy and minimal oral processing, like energy-yielding beverages and foods low in fibre content (72, 200, 201). Foods that can be consumed quickly (i.e., fast eating rate, g/min) can facilitate over-consumption. A number of studies have shown that higher eating rate leads to higher energy intake (9-12, 199, 202, 203). Several studies suggest a positive association between eating rate and and body weight status (15-18, 204).

It is considered that the positive relationship between eating rate and energy intake is mediated through sensory exposure to food in the oral cavity (205). There is growing evidence that oral sensory (i.e., orosensory) exposure to food is essential for establishing feedback signals of satiation (71, 92, 93, 182). Direct infusions of food into the stomach or duodenum, thus bypassing the orosensory exposure, give much weaker responses of satiation compared to oral intake (91, 180, 183, 206).

In theory, there are three ways to slow down the eating rate, thus the amount of food consumed in time (g/min). The first is to prolong the duration of food in the oral cavity (i.e., oral residence duration), the second is to use smaller bite sizes, and the third is to lower the bite frequency. It is not clear how these factors that influence eating rate affect food intake. As far as we know, only one study investigated the effect of oral residence duration when all other variables, like bite size and eating rate were held constant. In this study, longer oral residence duration per gram food resulted in lower food intake (93). Prolonging the pauses between bites, thus lower the bite frequency, has been shown to reduce food intake but only in people that consumed large amounts (14), or have no effect on food intake (207), or even led to greater intake (208).

A number of studies have shown a link between bite size and food intake; larger bite sizes result in greater food intake (13, 92, 93, 95-99, 209). We do not exactly know why bite size affects satiation. In a normal eating situation, smaller bite sizes lead to relatively longer oral residence duration per gram food (100, 210). Some studies explain the effect of bite size on food intake by its effect on oral residence duration or orosensory exposure time (92, 209). Nevertheless, by definition, bite size also increases the number of bites per gram food. A higher number of bites per gram food, for example three bites of 5 g instead of one bite of 15 g, means a more pulsating exposure to food, thus may result in relatively more orosensory exposure, and thereby influencing satiation.

More insight into contributions of number of bites and oral residence duration per gram food on food intake will be helpful to a better understanding of the process of satiation. The primary objective was to investigate the separate effects of number of bites and oral
residence duration per gram food on ad libitum intake and on changes in hunger and fullness (study 1). Effects of number of bites and oral residence duration are possibly explained by their influence on the orosensory exposure. The secondary objective was to assess the influences of number of bites and oral residence duration on the orosensory exposure per gram food, by executing time intensity measurements (study 2).

**Subjects and Methods**

*Study 1*

**Subjects**

Fifty-nine male subjects were recruited for participation. Fifty-six subjects completed the study, two subjects dropped out before the start of the study and one subject missed three ad libitum intake sessions. Subjects were healthy, had a normal weight (BMI 18.5-25 kg/m\(^2\), mean ± SD: 22 ± 2 kg/m\(^2\)), were aged between 18 and 35 y (mean ± SD: 22 ± 3 y) and liked creamy tomato soup (pleasantness score > 5 on a 9-point hedonic scale). Exclusion criteria were restrained eating behaviour (Dutch eating behaviour questionnaire (DEBQ) score > 2.89), following an energy-restricted diet during the last two months, gained or lost > 5 kg weight during the last year, having a lack of appetite, smoking, suffering from gastrointestinal illness, diabetes, thyroid disease or any other endocrine disorder, hypertension and kidney diseases. Subjects were informed that the aim of the research was to investigate the effect of bite size on flavour perception of soup. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Medical Ethical Committee of Wageningen University. All subjects signed an informed consent form before participation. This study was registered with the Dutch trial registration at [http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=2601](http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=2601) as NTR2601.

**Test foods**

Tomato soup was used as test product in this study. One kilogram of soup was made from 333 g sieved tomatoes (Heinz, Elst, The Netherlands), 662.7 g water, and 4.7 g salt (NaCl). The mixture was heated until 60 °C. The calculated nutrient composition from the used ingredients was: 0.57 g protein, 1.6 g carbohydrates, 0.03 g fat, 253 mg sodium and 38 kJ (9.1 kcal) energy per 100 g soup.

Raisin buns (local bakery) were used as preload. The nutrient composition was: 8 g protein, 52 g carbohydrates, 3 g fat, 300 mg sodium and 1120 kJ (268 kcal) energy per
100 g, according to the Dutch Food Composition Database (NEVO, version 2009/1.0). Each raisin bun weighed 22 g (246 kJ). The number of raisin buns as preload was calculated for each subject at half of the energy provided by an average lunch in the Netherlands (175), that is equal to 11% energy of the daily energy need. The daily energy need for each subject was estimated by the Schofield I equation (176), taking into account: gender, age, weight and a physical activity level of 1.6. Thirty subjects received 5 buns and 27 subjects received 6 buns. Subjects were instructed to eat all the raisin buns that they were served.

**Experimental design**

The experimental design is summarized in Figure 5.1 and Table 5.1. The study consisted of a 2 x 2 crossover design. Subjects came five times to the lab, including a “practice session” (first session), to consume soup in each of the four conditions. The eating rate was equal in each condition (Table 5.1). The oral residence duration was three times longer in the “long duration, low number of bites (Long-LB)” and “long duration, high number of bites (Long-HB)” conditions compared to the “short duration, low number of bites (Short-LB)” and “short duration, high number of bites (Short-HB)” conditions. The bite frequency was three times higher in the “Short-HB” and “Long-HB” conditions compared to the “Short-LB” and “Long-LB” conditions, to keep the eating rate constant between conditions.

![Figure 5.1 Bites and intervals in the four experimental conditions.](image)

<table>
<thead>
<tr>
<th>Time (s)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short-LB:</td>
<td>15g</td>
<td></td>
<td></td>
<td>15g</td>
</tr>
<tr>
<td>Long-LB:</td>
<td>15g</td>
<td></td>
<td></td>
<td>15g</td>
</tr>
<tr>
<td>Short-HB:</td>
<td>5g</td>
<td>5g</td>
<td>5g</td>
<td>5g</td>
</tr>
<tr>
<td>Long-HB:</td>
<td>5g</td>
<td>5g</td>
<td>5g</td>
<td>5g</td>
</tr>
</tbody>
</table>

Subjects heard an auditory signal when they received the soup and a double auditory signal when they had to swallow.
Table 5.1 Eating rate, oral residence duration and the number of bites of the four conditions\(^1\).

<table>
<thead>
<tr>
<th></th>
<th>Eating rate (g/min)</th>
<th>Oral residence duration (s/100g)</th>
<th>Number of bites (bites/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short-LB(^1)</td>
<td>60</td>
<td>20</td>
<td>6.7</td>
</tr>
<tr>
<td>Long-LB</td>
<td>60</td>
<td>60</td>
<td>6.7</td>
</tr>
<tr>
<td>Short-HB</td>
<td>60</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Long-HB</td>
<td>60</td>
<td>60</td>
<td>20</td>
</tr>
</tbody>
</table>

\(^1\)Short-LB = “Short duration, low number of bites” condition. Long-LB = “Long duration, low number of bites” condition. Short-HB = “Short duration, high number of bites” condition. Long-HB = “Long duration, high number of bites” condition.

Control of bite sizes, intervals and swallowing

To control the bites and intervals, subjects consumed the soup through a food-grade silicon tube that was connected to a peristaltic pump (Watson-Marlow, type 323Du, Watson-Marlow Bredel, Wilmington, MA, USA). The tube ended in a pan of soup that was placed on a balance (Kern, type 440-49A, KERN & Sohn GmbH, Balingen, Germany) to record the amount consumed. The pump, the pan and the balance were all located at the experimenters’ side of the sensory booths, thus subjects did not see the experimental setup.

At the moment that the pump started driving, subjects heard an auditory signal to prepare them that they would receive soup in their mouths. They heard a double auditory signal at the moment they had to swallow. The instruction to swallow was given 0.4 s after termination of the administration of the bite. Pilot studies revealed that a short pause in between the termination of administration and the moment of swallowing was more pleasant than without a pause. Before the start of each session, subjects were instructed that it was very important to swallow at the double auditory signal.

The “Short-LB” and “Long-LB” conditions consisted of intervals of 15 s (Figure 5.1). In the “Short-LB” condition, subjects received 15 g in the first 2.6 s of each interval (pump rate was set at 346 g/min) and swallowed after 3 s. In the “Long-LB” condition, subjects received 15 g in the first 8.6 s of each interval (pump rate was set at 105 g/min) and swallowed after 9 s. The “Short-HB” and “Long-HB” conditions consisted of intervals of 5 s. In the “Short-HB” condition, subjects received 5 g in the first 0.6 s of each interval (pump rate was set at 500 g/min) and swallowed after 1 s. In the “Long-HB” condition, subjects received 5 g in the first 2.6 s of each interval (pump rate was set at 115 g/min) and swallowed after 3 s.
First session

Subjects familiarized with the experimental procedures during their first visit. Subjects were seated in sensory booths. They received instructions and questions via a computer screen. Subjects received 45 g soup in each of the four conditions. The order of conditions between subjects was randomized. After consumption of soup in each condition, subjects had to rate several sensory characteristics of the soup.

Sensory characteristics

The sensory characteristics that were rated in the first session were overall taste intensity, saltiness, after taste intensity, thickness and “expected satiation”. This was done to get insight whether the number of bites or oral residence duration affected sensory characteristics.

All aspects were rated by using a 100 mm visual analogue scale (VAS). The question that refers to overall taste intensity, saltiness and after taste intensity was “How strong is the overall taste/salty taste/after taste of this soup?” from “very weak” at the left end to “very strong” at the right end. The question that refers to thickness was “How thick is the texture of this soup?” from “very thin” at the left end to “very thick” at the right end. The question that refers to “expected satiation” was “How filling is this soup?” from “very little filling” to “very much filling”.

Ad libitum intake sessions

Subjects came four times during lunch for the ad libitum intake of soup, with one week in between sessions. The four conditions were presented in randomized order. Subjects started with consumption of the preload that consisted of raisin buns. A preload was used so that participants would be in a less hungry state prior to soup consumption. It is possible that feelings of hunger will overrule sensory factors to terminate consumption (chapter 3, 189). They were instructed to consume all served raisin buns and they were allowed to drink water. After consumption of preload and water, subjects paused for 30 minutes. In the pause, subjects were allowed to study or read, but they were not allowed to leave the sensory room.

After the pause, subjects received a tube from which they had to consume soup. Subjects received instructions and questions via a computer screen. After answering several appetite and hedonic questions, as described below, subjects pushed a button on the screen to start soup consumption. They were instructed to terminate consumption when they had enough. The mean (± SD) initial temperature of the soup was 56 ± 5 °C.
and the mean end temperature was 51 ± 3 ºC. Subjects were instructed to stay in the sensory booths for at least ten minutes after they started consuming the soup. After ten minutes, a visual warning signal popped up on the laptop screen to inform subjects the ten minutes had passed. This was done to prevent subjects from leaving the research area for other reasons than being satiated with the soup.

**Appetite, hedonic ratings and questionnaires**

Just before soup consumption, subjects rated their feelings of hunger, fullness, prospective consumption and thirst. After that, subjects were served a small sample of 10 g soup and rated pleasantness and desire-to-eat the soup. The same questions were answered again at the end of the ad libitum intake. In addition, the same questions, except for thirst, were rated at random after consumption of every 75 g soup. All questions were answered by using a 100 mm VAS, on a scale from “not at all” at the left end to “very much” at the right end.

At the end of the session, subjects had to indicate the reasons of termination of soup consumption. The subjects were asked to what extent they agreed with the propositions: “I terminated consumption because I was full”; “I terminated consumption because the flavour of the soup was not pleasant anymore”; and “I terminated consumption because I did not like the manner of consumption”. The propositions were answered on a 5-point scale from “totally disagree” (1) to “completely agree” (5).

**Standardization of the satiety state**

To standardize the satiety state, subjects always started the lunch session at the same time. They were instructed to consume the same breakfast and not to eat and only drink water before the lunch started. Moreover, they were asked to refrain from drinking one hour before the test started. After each test lunch, subjects answered questions about what they ate for breakfast and whether they ate or drank between breakfast and test lunch. To make sure subjects would consume the soup until they felt satiated; they were instructed not to eat until one hour after the test.
Study 2

Subjects

Twenty-two different subjects (12 male, BMI 18.5-25 kg/m², mean ± SD: 22 ± 2 kg/m²), aged between 18 and 35 y (mean ± SD: 22 ± 2 y) participated in the second part of the study. The same exclusion and inclusion criteria as in the first study were used to recruit subjects, except for gender.

Time intensity measurements

Time intensity (TI) measurements were used to measure the total orosensory exposure of 30 g of soup (53 ± 2 ºC) in each of the four different conditions used in the first study. Subjects were used to consume via a tube and peristaltic pump with controlled bite sizes and intervals, because they were participating in another study that used a similar experimental set up. They had one practice session to train the procedure of the TI measurements.

Subjects were instructed to rate their perceived taste intensity constantly for one minute on a VAS from 0 to 100 mm. All four conditions were presented in randomized order. The area under the curve (AUC) is the sum of the total perceived taste, which represents the orosensory exposure. Subjects were instructed not to rate the first bite to have a short time to adapt to the procedure, therefore, the AUC was calculated between t=30 s and t=60 s.

Statistical analyses

Statistical analyses were performed using SAS version 9.1.4 (SAS Institute Inc., Cary, NC, USA). Data are presented as means ± SDs, P-values < 0.05 were considered significant.

Effects of number of bites, oral residence duration, and their interaction on sensory characteristics, initial appetite and initial hedonic ratings, were assessed in a mixed linear model that included order and had subject as repeated factor. Changes in appetite and hedonic ratings between before (initial) vs. after (post) ad libitum intake were assessed by paired t-tests.

Effects of number of bites, oral residence duration, and their interaction on ad libitum intake, appetite and hedonic ratings from after and during ad libitum intake, were assessed in a mixed linear model that included order, ratings of initial hunger and
ratings of “I terminated consumption because I did not like the manner of consumption” and had subject as repeated factor.

The changes in appetite and hedonic ratings during ad libitum intake were fitted per subject in a linear model: \( y = a + b \times \text{intake} \). A linear model was chosen because this was the best fit in most individual curves. The curves shown in the results section were calculated from the mean intercepts and mean slopes of the individual plots.

Effects of number of bites, oral residence duration, and their interaction on the reason to terminate consumption were assessed in a mixed linear model that included order and initial hunger ratings and had subject as repeated factor. Effects of number of bites, oral residence duration, and their interaction on the AUC of the taste intensity were assessed in a generalized linear model that included subject. Fisher’s LSD procedure was used for all post hoc comparisons in the present study.

**Results**

**Study 1**

**Sensory characteristics**

Table 5.2 shows the sensory characteristics of the soup as rated in each of the four conditions. Overall taste was not affected by number of bites \((P = 0.37)\) and not by oral residence duration \((P = 0.30)\), although there was an interaction effect \((P = 0.019)\). Saltiness was affected by bite size \((P = 0.024)\), but not by oral residence duration \((P = 0.91)\), and there was no interaction effect \((P = 0.92)\). After taste intensity, thickness and “expected satiation” were not affected by number of bites, oral residence duration, or by their interaction (all \(P\)-values > 0.19).
Table 5.2 Sensory characteristics of the soup as measured in the four conditions  

<table>
<thead>
<tr>
<th></th>
<th>Short-LB³</th>
<th>Long-LB</th>
<th>Short-HB</th>
<th>Long-HB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall taste intensity⁴</td>
<td>53 ± 18ᵃ</td>
<td>58 ± 17ᵇ</td>
<td>59 ± 16ᵇ</td>
<td>56 ± 20ᵇ</td>
</tr>
<tr>
<td>Saltiness⁵</td>
<td>48 ± 17</td>
<td>48 ± 19</td>
<td>53 ± 20</td>
<td>53 ± 21</td>
</tr>
<tr>
<td>After taste intensity</td>
<td>51 ± 18</td>
<td>53 ± 18</td>
<td>54 ± 20</td>
<td>53 ± 19</td>
</tr>
<tr>
<td>Thickness</td>
<td>44 ± 19</td>
<td>47 ± 18</td>
<td>42 ± 18</td>
<td>44 ± 17</td>
</tr>
<tr>
<td>Expected satiation</td>
<td>48 ± 18</td>
<td>49 ± 19</td>
<td>43 ± 20</td>
<td>47 ± 22</td>
</tr>
</tbody>
</table>

¹ Values are means ± SDs. Values in a row with different superscript letters are significantly different (P < 0.05)  
² Rated on a 100 mm VAS after consumption of 45 g soup.  
³ Short-LB = “Short duration, low number of bites” condition. Long-LB = “Long duration, low number of bites” condition. Short-HB = “Short duration, high number of bites” condition. Long-HB = “Long duration, high number of bites” condition.  
⁴ Significant interaction effect of the number of bites and orosensory exposure time: P = 0.019  
⁵ Significant main effect of the number of bites: P = 0.024, post hoc comparisons (LSD procedure) showed no significant differences.

Ad libitum intake of soup

The ad libitum intake was 453 ± 173 g in the “Short-LB” condition, 421 ± 190 g in the “Long-LB” condition, 358 ± 171 g in the “Short-HB” condition, and 330 ± 156 g in the “Long-HB” condition (Figure 5.2). The number of bites (P < 0.001) affected ad libitum intake; it was 21% lower in the “Short-HB” compared to the “Short-LB” condition and 22% lower in the “Long-HB” compared to the “Long-LB” condition. Also oral residence duration affected ad libitum intake (P = 0.006); it was 7% lower in the “Long-LB” compared to the “Short-LB” condition and 8% lower in the “Long-HB” compared to the “Short-HB” condition. There was no interaction effect between number of bites and oral residence duration on ad libitum intake (P = 0.94).
Figure 5.2 Means (+ SD) of ad libitum intakes of soup. A higher number of bites: \( P < 0.001 \), and longer oral residence duration: \( P < 0.006 \), reduced ad libitum intake (mixed linear model). Mean values with different letters are significantly different between conditions (LSD procedure). The difference in ad libitum intake of the short vs. the long exposure in the LB conditions showed a trend: \( P = 0.06 \). LB = “low number of bites”, HB = “high number of bites”.

Appetite and hedonic ratings before and after ad libitum intake

Initial appetite ratings (i.e., hunger, fullness and prospective consumption) and hedonic ratings (i.e., pleasantness and desire-to-eat the soup) did not differ between conditions (all \( P \)-values > 0.59) (Table 5.3). Initial thirst did also not differ between conditions (\( P = 0.17 \)). In the preload phase, subjects drank: 146 ± 38 g and this were not different between conditions (\( P = 0.20 \)).

After ad libitum intake, ratings of hunger, prospective consumption, pleasantness and desire-to-eat the soup decreased and fullness increased in each condition compared to the initial ratings (all \( P \)-values < 0.001). Ratings of thirst were not significantly different after intake compared to the initial ratings (all \( P \)-values > 0.10). Appetite, thirst and hedonic ratings after intake were not affected by oral residence duration (all \( P \)-values > 0.44), or by number of bites (all \( P \)-values > 0.21), and there were no interaction effects (all \( P \)-values > 0.26).
Table 5.3 Appetite and hedonic ratings from before and after ad libitum intake of soup in the four conditions

<table>
<thead>
<tr>
<th></th>
<th>Short-LB</th>
<th>Long-LB</th>
<th>Short-HB</th>
<th>Long-HB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hunger</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>60 ± 20</td>
<td>61 ± 21</td>
<td>62 ± 19</td>
<td>62 ± 19</td>
</tr>
<tr>
<td>Post&lt;sup&gt;4&lt;/sup&gt;</td>
<td>18 ± 18</td>
<td>19 ± 19</td>
<td>21 ± 19</td>
<td>21 ± 19</td>
</tr>
<tr>
<td><strong>Fullness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>33 ± 21</td>
<td>32 ± 19</td>
<td>30 ± 18</td>
<td>31 ± 19</td>
</tr>
<tr>
<td>Post&lt;sup&gt;4&lt;/sup&gt;</td>
<td>75 ± 20</td>
<td>73 ± 18</td>
<td>73 ± 20</td>
<td>76 ± 17</td>
</tr>
<tr>
<td><strong>Prospective consumption</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>62 ± 24</td>
<td>64 ± 20</td>
<td>65 ± 17</td>
<td>64 ± 19</td>
</tr>
<tr>
<td>Post&lt;sup&gt;4&lt;/sup&gt;</td>
<td>24 ± 21</td>
<td>25 ± 23</td>
<td>29 ± 22</td>
<td>27 ± 18</td>
</tr>
<tr>
<td><strong>Thirst</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>59 ± 21</td>
<td>54 ± 21</td>
<td>55 ± 21</td>
<td>57 ± 19</td>
</tr>
<tr>
<td>Post&lt;sup&gt;5&lt;/sup&gt;</td>
<td>54 ± 27</td>
<td>54 ± 25</td>
<td>54 ± 23</td>
<td>57 ± 24</td>
</tr>
<tr>
<td><strong>Pleasantness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>64 ± 20</td>
<td>65 ± 18</td>
<td>64 ± 18</td>
<td>66 ± 18</td>
</tr>
<tr>
<td>Post&lt;sup&gt;4&lt;/sup&gt;</td>
<td>51 ± 23</td>
<td>51 ± 18</td>
<td>50 ± 22</td>
<td>52 ± 22</td>
</tr>
<tr>
<td>** Desire-to-eat**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>65 ± 19</td>
<td>64 ± 18</td>
<td>65 ± 19</td>
<td>67 ± 19</td>
</tr>
<tr>
<td>Post&lt;sup&gt;4&lt;/sup&gt;</td>
<td>29 ± 21</td>
<td>28 ± 21</td>
<td>31 ± 20</td>
<td>32 ± 22</td>
</tr>
</tbody>
</table>

<sup>1</sup> Values are means ± SDs.
<sup>2</sup> Rated on a 100 mm VAS.
<sup>3</sup> Short-LB = “Short duration, low number of bites” condition. Long-LB = “Long duration, low number of bites” condition. Short-HB = “Short duration, high number of bites” condition. Long-HB = “Long duration, high number of bites” condition.
<sup>4</sup> Significant differences between initial and post appetite and hedonic ratings in all conditions: all *P*-values < 0.001.
<sup>5</sup> No significant difference between initial and post thirst ratings.

**Appetite and hedonic ratings during ad libitum intake**

Figure 5.3 shows the linear curves of the changes in rated hunger (A) and fullness (B) as a function of intake. Higher number of bites led to faster decrease in hunger (*P* = 0.003); faster increase in fullness (*P* < 0.001); faster decrease in ratings of prospective consumption (*P* = 0.009, data not shown); and faster decrease in desire-to-eat (*P* =
Chapter 5 – Effects of number of bites and oral residence duration on ad libitum intake

0.004, data not shown). Oral residence duration did not significantly affect the decrease in hunger ($P = 0.36$); the increase in fullness ($P = 0.16$); and the decrease in the desire-to-eat ($P = 0.16$). However, a trend was observed for ratings of prospective consumption, longer oral residence duration resulted in faster decrease in prospective consumption ($P = 0.07$). Pleasantness (data not shown), however, was neither affected by number of bites ($P = 0.36$) nor oral residence duration ($P = 0.27$). None of changes in appetite and hedonic ratings showed an interaction effect between number of bites and oral residence duration (all $P$-values $> 0.30$).

![Figure 5.3](image)

**Figure 5.3** Linear functions of rated hunger (A) and fullness (B) against intake in the four conditions. A higher number of bites led to a faster decrease in hunger ($P = 0.003$), and a faster increase in fullness ($P < 0.001$) per consumed gram food. Longer oral residence duration did not significantly affect the appetite ratings. The dots visualize the mean ad libitum intake in each condition. Short-LB = “Short duration, low number of bites” condition. Long-LB = “Long duration, low number of bites” condition. Short-HB = “Short duration, high number of bites” condition. Long-HB = “Long duration, high number of bites” condition.

**Reasons to terminate consumption**

“I terminated consumption because I was full” was the most important reason in all conditions to terminate consumption (Table 5.4). The reasons to terminate consumption: “I was full” and “flavour not pleasant anymore” were both not affected by number of bites or oral residence duration (all $P$-values $> 0.38$). Ratings of “I terminated consumption because I did not like the manner of consumption” were higher in high number of bites conditions compared to low number of bites conditions ($P = 0.002$), but were not affected by oral residence duration ($P = 0.19$).
Table 5.4 The reasons to terminate soup consumption in the four conditions

<table>
<thead>
<tr>
<th>Reason</th>
<th>Short-LB</th>
<th>Long-LB</th>
<th>Short-HB</th>
<th>Long-HB</th>
</tr>
</thead>
<tbody>
<tr>
<td>“I was full”</td>
<td>4.0 ± 1.1</td>
<td>4.0 ± 1.1</td>
<td>4.0 ± 1.1</td>
<td>3.9 ± 1.0</td>
</tr>
<tr>
<td>“Flavour not pleasant anymore”</td>
<td>2.6 ± 1.2</td>
<td>2.6 ± 1.1</td>
<td>2.6 ± 1.1</td>
<td>2.5 ± 1.3</td>
</tr>
<tr>
<td>“Manner of consumption”</td>
<td>2.4 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.6 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.9 ± 1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.0 ± 1.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> Values are means ± SDs.

<sup>2</sup> The propositions were answered on a 5-point scale from “totally disagree” (1) to “completely agree” (5).

<sup>3</sup> Short-LB = “Short duration, low number of bites” condition. Long-LB = “Long duration, low number of bites” condition. Short-HB = “Short duration, high number of bites” condition. Long-HB = “Long duration, high number of bites” condition.

Study 2

Area under the curve of perceived taste intensity of 30 g soup

Figure 5.4 shows the mean perceived taste during 30 seconds, which is equal to consumption of 30 g in each condition. The mean AUC of each condition is shown in Figure 5.5. Both higher number of bites (<i>P</i> < 0.001) and longer oral residence duration (<i>P</i> < 0.001) led to a greater AUC, which means an increase in perceived taste, thus higher orosensory exposure per consumed gram food.

The taste of the soup is highest in intensity directly after swallowing, shown by the top of the peaks (Figure 5.4). The mean heights of the peak were 71 ± 19 mm in the “Short-LB” condition; 71 ± 17 mm in the “Long-LB” condition; 63 ± 22 mm in the “Short-HB” condition; and 65 ± 22 mm in the “Long-HB” condition. Higher number of bites resulted in lower heights of the peaks (<i>P</i> < 0.001). Longer oral residence duration did not affect the mean height of the peaks (<i>P</i> = 0.47).
Figure 5.4 Mean rated taste intensity during 30 s, thus 30 g, of soup in each of the four conditions. The grey areas (AUC) represent the total perceived taste and thereby the total magnitude of orosensory exposure. In the low number of bites conditions, two bites of 15 g were administered in 30s, at t=30 and t=45, each bite was swallowed after 3 s in the “Short-LB” condition and after 9 s in the “Long-LB” condition. In the high number of bites conditions, 6 bites were administered in 30s (in pulses of 5 s), each bite was swallowed after 1 s in the “Short-HB” condition and after 3 s in the “Long-HB” condition. The maxima of the peaks were reached directly after swallowing. Short-LB = “Short duration, low number of bites” condition. Long-LB = “Long duration, low number of bites” condition. Short-HB = “Short duration, high number of bites” condition. Long-HB = “Long duration, high number of bites” condition.
Figure 5.5 Means (+ SD) of AUC that represents total magnitude of orosensory exposure to the taste of 30 g of soup. Higher number of bites: $P < 0.001$, and longer oral residence duration: $P < 0.001$, resulted in an increased AUC (generalized linear model). Mean values with different letters are significantly different between conditions (LSD procedure). LB = “low number of bites”, HB = “high number of bites”.

Discussion

The primary objective of the study was to determine separate effects of the number of bites and the oral residence duration on ad libitum intake. The results showed that both higher number of bites per gram food, thus smaller bites, and longer oral residence duration per gram food, independently, reduced food intake by 22% and 8%, respectively (Figure 5.2). The ratings for hunger and fullness did not differ after consumption (Table 5.3). This can be explained by a faster decrease in hunger and faster increase in fullness per consumed gram food in the high number of bites conditions (Figure 5.3). Both higher number of bites and longer oral residence duration per gram food led to an increase in orosensory exposure per gram food (Figure 5.4, 5.5). The increased orosensory exposure per consumed gram because of smaller bites and longer oral residence duration food may explain the reduction in food intake.

To measure effects of oral residence duration and number of bites on the orosensory exposure per gram food, time intensity functions (211) were conducted. Prolonged duration of food in the oral cavity is associated with more time to sense the taste, which explains the increased orosensory exposure per gram food (greater AUC, Figure 5.5). Interestingly, higher number of bites, thus smaller bites, also results in more orosensory exposure per gram food. One bite of 5 g almost reached the same taste intensity as one bite of 15 g (top of the peaks, Figure 5.4). Three bites of 5 g results in greater taste perception than one bite of 15 g (greater AUC, figure 5.4, 5.5). Three bites of 5 g
possibly lead to more contact of the tastants with the taste receptors in the oral cavity compared one bite of 15 g. An increased orosensory exposure per gram food may be associated with relatively more sensing of the nutrients, this may lead to a faster onset of internal signals of satiation (71, 182).

We learned from previous studies that higher eating rate leads to higher food intake in a natural way of eating (10, 13, 212). In this study, underlying mechanisms were investigated that gives more insight in how the factors that influence eating rate (bite size, oral residence duration) influence satiation. Investigation of these underlying mechanisms required a controlled experimental design in which the eating rate was kept constant. The results of this study suggest that both bite size and oral residence duration influence food intake. We investigated effects of the number of bites per gram food, which is related to bite size. However, in a natural setting, bite size also influences the oral residence duration; smaller bites are associated with longer oral residence duration per gram food (100, 210). This means that reducing the bite size is probably more effective in reducing food intake than only prolong the oral residence duration, because in a natural setting bite size affects both the number of bites and oral residence duration.

In theory, eating rate is also affected by bite frequency; the latter may also influence satiation. To keep the eating rate constant in this study, the bite frequency was three times higher for 5 g bites (HB conditions) than for 15 g bites (LB conditions). Zijlstra et al. (93) investigated effects of bite size when both the bites of 5 g and 15 g were administered at the same bite frequency, thus eating rate was three times higher for the 15 g bites. The size of the effect of bite size on ad libitum intake shown by Zijlstra et al. (93) was similar compared to the results of this study. This indicates ad libitum intake was primarily affected by bite size and not by bite frequency. In accordance, studies that lower the eating rate by lowering bite frequency fail to find a reduction in food intake (213, 214) or fail to find effects on postprandial hormonal secretion (215). However a recent study (14) has demonstrated a reduction in food intake when bite frequency was lowered, but only for individuals who consumed large amounts of food. Overall, decreasing the bite frequency might be less effective in reducing the energy intake. This is possibly explained by the fact that bite frequency does not influence the orosensory exposure per gram food, and thereby not influencing feedback signals of satiation (71, 182).

Strengths of this study are the within subjects design and the tight controlled experimental design which allowed us to investigate underlying mechanisms of eating rate on food intake. However, this tight controlled design is also a limitation of the study. Controlling the bite size and frequency, and the unnatural way of soup consumption may have influenced the results to some extent. Nevertheless, “fullness” was the most important reason to terminate consumption in all conditions, which indicates that subjects consumed till they were satiated (Table 5.4). Another limitation
is that the study population consisted of mostly young healthy adults. We do not know whether oral residence duration and number of bites affect food intake to the same extent in the obese/overweight population. In addition, preloads were used to prevent subjects from being in a very hungry state. The state of hunger may influence the impact of sensory signals on food intake. This requires further investigation.

As far as we know, the effect of number of bites per gram food on intake was not studied before. The impact of the number of bites on food intake was greater than that of the oral residence duration, where both factors differed a factor three. This indicates that the number of bites per gram food is an important explanation of the effect of bite size on food intake. Consuming smaller bites is more satiating than consuming fewer large bites of the same amount of food.

However, consumption with relatively higher number of bites led to higher ratings for “I terminated consumption because I did not like the manner of consumption” (Table 5.4). This may be due to the increased effort that is associated with smaller bites. The “manner of consumption” may partly influence the size of the effect of number of bites on intake. Nevertheless, relatively higher number of bites (smaller bites) is more satiating, because it led to faster decrease in hunger and faster increase in fullness per consumed gram food (Figure 5.3). It seems that subjects in the present study consumed till a certain state of satiety, as the mean “end-point” was for hunger ~20 mm and for fullness ~75 mm on a 100 mm VAS, (Figure 5.3, Table 5.3). This certain state of satiety was faster established when subjects took relatively more bites, which may consequently have led to a lower food intake.

Bite size or the number of bites per gram food may also cognitively affect food intake. It is possible that a higher number of small bites are associated to be more satiating than fewer larger bites. It has been repeatedly shown that feelings of satiation are typically influenced by the amount of food people believe they have consumed (50, 55, 216, 217). In a normal situation, taking more bites leads to higher intake, considering that bite size is quite constant for a specific food matrix by a specific person (89, 218-221). In the present study, the rated expected satiation value (i.e., rated as: how filling do you think this soup is?) after 45 g in each condition was not influenced by the number of bites (Table 5.2). It is possible that consumption of 45 g is not enough to find differences in expected satiation, or the interpretation of the question focuses on the sensory attributes of the soup per se, and does not address effects of number of bites on expected satiation.

We do not know whether lower food intake as a result of smaller bites or longer oral residence duration will be compensated during the rest of the day or over more days. It seems unlikely that people accurately compensate their energy intake as a result of changes in energy intake from a meal or a day (101, 222, 223). Two studies (98, 224),
that used an oral device to decrease the bite sizes, showed that the device led to a reduction in meal size without changes on rated satiety between meals compared to normal intake. Moreover, consumption of liquids is associated with larger bite sizes and shorter oral residence duration (10-12), and energy intake from liquids was shown to be poorly compensated (225, 226). More research, however, is needed to investigate effects of bite size or orosensory exposure on long-term energy intake.

In conclusion, higher number of bites and longer oral residence duration per gram food resulted in reduced food intake, where the number of bites showed the greatest effect. In addition, a higher number of smaller bites led to a faster decrease in hunger and increase in fullness per consumed per gram food. The increased orosensory exposure to the food and the cognitive aspects of taking relative more bites per gram food may explain the effect of bite size on satiation. Advices to consume with smaller bites and prolong the oral residence duration, by for example increase chewing, may be helpful in body weight management. Moreover, designing foods that will be consumed with small bites and long oral residence duration may also be an effective tool to reduce food intake. Food properties, such as hardness, viscosity, dryness, stickiness, tenderness, affect oral residence duration, bite size, and other oral processes like chewing behaviour and salivation. More insight in associations between food properties, oral processes and food intake will contribute to the understanding of the satiating effects of different foods.

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Chapter 6

Consumption with large bite sizes increases food intake and leads to underestimation of the amount consumed

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Submitted for publication in revised form
Abstract

A number of studies have shown that bite size influences the amount of food intake. Consuming small rather than large bites involves relatively more bites for consumption of the same amount of food. People may believe that their intake is higher which leads to a faster satiation. However, this cognitive effect may be disturbed when people are distracted. The objective of the study is to assess the effects of bite size in a focused state and a distracted state on ad libitum intake and on the estimated amount consumed. In this 3 x 2 cross-over design, 53 healthy subjects consumed ad libitum soup with small bites (5 g, 60 g/min), large bites (15 g, 60 g/min), and free bites (where bite size was determined by subjects themselves), in both a distracted and focused state. Bites were administered via a pump. There were no visual cues toward consumption. Subjects then estimated their amount consumed by filling soup in soup bowls. Intake in the small-bites condition was ~30% lower than in both the large-bites and free-bites conditions ($P < 0.001$). In addition, subjects underestimated their amount consumed in the large-bites and free-bites conditions ($P < 0.029$). Distraction led to a general increase in food intake ($P = 0.008$), independent of bite size. Distraction did not influence bite size or estimations. Consumption with large bites led to higher food intake and underestimation of the amount consumed. This implies that consuming with large bites impairs the control of food intake. Reducing bite sizes may successfully lower food intake, even in a distracted state.

Keywords: bite size, number of bites, cognition, distraction, satiation
Introduction

Obesity is an increasing problem in Western society. Overweight and obesity are the result of a long-term positive energy balance in which energy intake is higher than energy expenditure. There is growing evidence that oral processing is important in the regulation of food intake (9, 10, 12, 91-93, 180, 206, 209, 227). Foods that are consumed quickly and require minimal oral processing, such as beverages and foods low in fibre content, lead to higher ad libitum intake (9-12), and therefore promote over-consumption.

Eating rate (g/min) is influenced by bite size (12, 94). A number of studies have demonstrated a positive relationship between bite size and the amount of food intake (13, 92, 93, 95-99, 209). Controlled experimental studies with fixed bite sizes showed that 5 g bites led to a reduction in food intake of 15 to 30% compared to 15 g and 20 g bites, even when eating rate was constant (92, 93, 209, 227).

Consuming small bites rather than large bites involves more bites for consumption of the same amount of food. Smaller bites may affect peoples’ assumption that intake is higher compared to relatively fewer larger bites, and therefore lead to lower food intake. Beliefs about the amount consumed play an important role in satiation (50, 55, 216, 217). For example, when people were not able to monitor their amount eaten by consuming soup from self-refilling bowls, intake was 70% higher compared to consumption from a normal soup bowl (55). In addition, information about calorie content (101-103), the serving size (95, 97, 104-106), and time of the day (107), all influenced amount of food intake. These findings stress the importance of cognitive aspects on satiation.

Cognitive aspects of food intake may be disrupted when people are distracted during food consumption. Cognitive restraint eating behaviour (i.e., chronic tendency to limit food intake to control body weight), was offset by distraction; food intake increased when listening to a detective story (228). A number of studies have shown that distraction through activities such as watching television or eating with friends usually led to higher food intake (35, 38-41, 108). It is possible that distraction during consumption is associated with impaired monitoring of the amount consumed by visual cues (35, 109). Other regulators of food intake, such as number of bites, bite size, eating rate, or meal duration may also be affected by distraction. In a distracted state, people may unconsciously increase their number of bites that leads to higher food intake. Consumption with smaller bites in a distracted state may, therefore, be less effective in reducing food intake.

The objective of this study is to assess effects of bite size in both focused and distracted states on ad libitum intake. Subjects estimated the amount consumed after intake to
determine if bite size affects perceived food intake. We hypothesize that consumption with larger bites results in higher intake and underestimation of the amount consumed. We then hypothesize that the effect of bite size on food intake is diminished in a distracted state, and that subjects, generally, underestimate the amount consumed when they are distracted.

Subjects and Methods

Subjects

Fifty-seven subjects were recruited for participation, 53 of whom (33 males, 20 females) completed the study. Three subjects dropped out before the start of the study and one subject missed four sessions. Subjects were healthy, had normal weight (BMI 18.5 to 25 kg/m$^2$, mean ± SD: 22 ± 2 kg/m$^2$), were aged between 18 and 35 y (mean ± SD: 22 ± 3 y) and liked creamy tomato soup (pleasantness score > 5 on a 9-point hedonic scale). Exclusion criteria were: restrained eating behaviour (Dutch Eating Behaviour Questionnaire (DEBQ) score men: > 2.89, women: > 3.39); an energy-restricted diet during the last two months; gained or lost > 5 kg weight during the last year; lack of appetite; smoking; gastrointestinal illness; diabetes; thyroid disease, or any other endocrine disorder; or being pregnant or breast feeding. Subjects were informed that the research aimed to investigate the effect of distraction on flavour perception of soup. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Medical Ethical Committee of Wageningen University. All subjects signed an informed-consent form before participation. This study was registered (NTR: 3091) with the Dutch trial registration at: www.trialregister.nl/trialreg/admin/rctview.asp?-TC=3091.

Test foods

Tomato soup was used for this study. One kg of soup was made from 333 g sieved tomatoes (Heinz, Elst, The Netherlands), 662.7 g water, and 4.7 g salt (NaCl). The mixture was heated to 60 °C. The calculated nutrient composition from the ingredients was: 0.57 g protein, 1.6 g carbohydrates, 0.03 g fat, 253 mg sodium and 38 kJ (9.1 kcal) energy per 100 g soup.

Raisin buns (local bakery) were used as preload. The nutrient composition was: 8 g protein, 52 g carbohydrates, 3 g fat, 300 mg sodium and 1120 kJ (268 kcal) energy per 100 g, according to the Dutch Food Composition Database (NEVO, version 2009/1.0). Each raisin bun weighed 22 g (246 kJ). The number of raisin buns was calculated at half
of the energy provided by an average lunch in the Netherlands (175), equal to 11% energy of the daily energy need. The daily energy need for each subject was estimated by the Schofield I equation (176), taking into account: gender, age, weight and a physical activity level of 1.6. Sixteen subjects received 4 buns, 25 subjects received 5 buns, 12 subjects received 6 buns. Subjects were instructed to eat all the raisin buns they were served.

**Experimental design**

The experimental design is summarized in Figure 6.1. The study consisted of a 3 x 2 cross-over design. Subjects came to the lab seven times, including a first practice session. There were six different ad libitum intake conditions: small-bites, large-bites, and free-bites, presented in both a focused and a distracted state. The bite frequency was three times higher in the small-bites condition than in the large-bites condition, to keep the eating rate (g/min) equal. The eating rate was set at 60 g/min for both the small-bites and large-bites conditions. The oral residence duration (i.e., duration of food in the oral cavity) was 40 s/100g for both small-bites and large-bites conditions. Subjects regulated the administration of the soup by themselves in the free-bites condition. They could start and stop the pump by themselves to determine bite sizes and bite frequencies.

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**Figure 6.1** Bites and intervals in the three bite size conditions.

= administration of soup, = instruction to swallow, = pauses between bites, = regulation of bites and pauses by subjects themselves.

All three conditions were presented in a focused and distracted state, resulting in six conditions. In the small-bites condition, bites of 5 g were exposed in 2 s (from the start of soup administration until swallowing) in pulses of 5 s. In the large-bites condition, bites of 15 g were exposed in 6 s in pulses of 15 s. In the free-bites condition, subjects were free to start and stop the pump, thereby determining bite sizes and frequency by themselves. In the small-bites and large-bites conditions, subjects heard an auditory signal when they received the soup and a double auditory signal when they had to swallow.
Chapter 6 – Effect of bite size on ad libitum intake and on the estimated amount consumed

Control of bite sizes, intervals and swallowing

Subjects consumed soup through a food-grade tube (Saint-Gobain, Norprene, A-60-F, Charny, France) connected to a peristaltic pump (Watson-Marlow, type 323Du, Watson-Marlow Bredel, Wilmington, MA, USA) to control bites and intervals. The tube ended in a pan of soup that was placed on a balance (Kern, type 440-49A, KERN & Sohn GmbH, Balingen, Germany) to record the amount consumed.

Subjects heard an auditory signal to inform them that the pump started working and they would receive soup in their mouths. They heard a double auditory signal when they had to swallow. The instruction to swallow was given 0.5 s after termination of bite administration. Subjects were instructed that it was very important to swallow at the double auditory signal before the start of each session.

The large-bites condition consisted of 15 s intervals (Figure 6.1). Subjects received 15 g during the first 5.5 s of each interval and swallowed after 6 s. The small-bites condition consisted of 5 s intervals. Subjects received 5 g during the first 1.5 s of each interval and swallowed after 2 s. In the free-bites condition, subjects could start and stop the pump by themselves. The pump rate was set at 2.5 g/s. This meant that, for example, a 4 s administration resulted in a 10 g bite. Subjects in the free-bites condition were instructed to swallow as soon as they stopped administration.

First session

Subjects were familiarized with the experimental procedures during their first visit. They were seated in sensory booths. They received instructions and questions via a computer screen. Subjects received 45 g soup in both the small-bites and large-bites conditions, in randomized order. Subjects rated several sensory aspects after consumption of soup in both conditions to determine if bite size influences sensory characteristics.

Sensory characteristics

The sensory characteristics rated in the first session were overall taste intensity, saltiness, thickness, after-taste intensity, and “expected satiation”. All aspects were rated on a 100 mm visual analogue scale (VAS). The question that referred to overall taste intensity, saltiness intensity and after-taste intensity was “How strong is the taste/saltiness/after-taste of this soup?” from “very weak” at the left end to “very strong” at the right end. The question that referred to thickness was “How thick is the texture of this soup?” from “very thin” at the left end to “very thick” at the right end.
The question that referred to “expected satiation” was “How filling is this soup?” from “hardly filling” to “very much filling”.

Ad libitum intake sessions

There were six lunch sessions for ad libitum intake of soup, with one week between sessions. The six conditions were presented in randomized order. Subjects started by consuming the preload of raisin buns. A preload was used so that participants would be less hungry prior to soup consumption (chapter 3, 189). It is possible that feelings of hunger would overrule sensory factors to terminate consumption. Subjects were instructed to consume all raisin buns and were allowed to drink water. Subjects then paused for 20 minutes. During that time, subjects were allowed to study or read, but were not allowed to leave the sensory room.

After the pause, subjects received instructions and questions via a computer screen. Before ad libitum intake, subjects first rated appetite and hedonic aspects, as described below. Subjects could push a button on the computer screen to start soup consumption. The pan and balance were placed on the experimenters’ side of the sensory booth, so there were no visual cues of the amount consumed. Subjects were instructed to terminate consumption any time when they felt they had enough. The mean (± SD) initial temperature of the soup was 55 ± 3 °C and the mean end temperature was 48 ± 3 °C.

Subjects were instructed to stay in the sensory booths for at least 15 minutes in both the focused and the distracted states. A visual warning signal popped up on the laptop screen to inform subjects that the 15 minutes had passed. This prevented subjects from leaving the research area other for than being satiated with the soup.

Focus versus distraction

Subjects in the focused state were instructed to focus on the taste and flavour of the soup. Subjects in the distracted state were told they would see a short (~15 min) animation film (“Pat and Mat”, in Dutch: “Buurman en Buurman”) during consumption and would answer questions about the film afterwards. This was done to ensure they focused on the film. There were six different films randomized between conditions and subjects. Subjects answered between 8 to 11 questions about the film. The film was started once subjects started consuming soup.
Estimated amount consumed

At the end of each session, subjects estimated the amount they had consumed. They were given a jug containing 2 kg soup and six soup bowls (250 g). Subjects filled the bowls with the amount of soup they thought they had consumed. The estimated amount consumed was calculated by weighing the jug before and after estimation.

Appetite, hedonic ratings and questionnaires

Subjects rated feelings of hunger, fullness, and thirst on a 9-point scale from “not at all” (0) to “very much” (9). This was rated before and directly after intake, and 1 hour, 2 hours and 3 hours after ad libitum intake.

Before and after intake, subjects were served a small sample of 10 g soup and rated pleasantness and desire-to-eat the soup on a 100 mm visual analogue scale (VAS) that was scaled from “not at all” (0) to “very much” (100).

At the end of the session, subjects indicated reasons for terminating soup consumption. Subjects were asked to what extent they agreed with the propositions: “I terminated consumption because I was full”, “I terminated consumption because the flavour of the soup was not pleasant anymore”, and “I terminated consumption because I did not like the manner of consumption”. The propositions were answered on a 5-point scale from “totally disagree” (1) to “completely agree” (5).

Standardization of satiety

To standardize the satiety state, subjects always started the lunch session at the same time. They were instructed to consume the same breakfast and only drink water before lunch started. Moreover, they were asked to refrain from drinking one hour before lunch. After each lunch, subjects answered questions about what they ate for breakfast and if they ate or drank between breakfast and lunch. Subjects were instructed not to eat until three hours after the lunch to rate subjective satiety.

Statistical analyses

Statistical analyses were performed using SAS version 9.1.4 (SAS Institute Inc., Cary, NC, USA). Data were presented as means ± SDs. P-values of < 0.05 were considered significant.
Effects of bite size (small-bites vs. large-bites) on sensory characteristics were assessed in within-subjects ANOVA (PROC GLM, SAS). Effects of bite size (small-bites vs. large-bites vs. free-bites) and distraction on ad libitum intake, estimated amount consumed, appetite ratings, and reasons to terminate consumption, were assessed in a two-way within-subjects ANOVA (PROC GLM, SAS). The difference between the ad libitum intake and the estimated amount consumed was assessed per condition in within-subjects ANOVA (PROC GLM, SAS). The accuracy of the estimations was assessed by the absolute difference between the ad libitum intake and the estimated amount consumed in percentiles. Effects of distraction on meal duration, bite size, number of bites, and bite frequency were assessed in a within-subjects ANOVA (PROC GLM, SAS). Gender and order of presentation affected most parameters and were added as covariates in the ANOVA models. The rating of “I terminated consumption because I did not like the manner of consumption” affected the ad libitum intake, so was added in the ANOVA model when ad libitum intake was assessed. Parameters not normally distributed were log-transformed before assessment. Fisher’s LSD procedure was used for all post hoc comparisons.

Results

Sensory characteristics

Table 6.1 shows the sensory characteristics of the soup as rated in the small-bites and large-bites conditions. The bite size did not affect sensory characteristics and the pleasantness of the soup. In addition, the “expected satiation” value was not affected by the bite size.

<table>
<thead>
<tr>
<th>Table 6.1 Sensory characteristics of the soup consumed in the small-bites and large-bites conditions12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small-bites</td>
</tr>
<tr>
<td>Pleasantness</td>
</tr>
<tr>
<td>Overall taste intensity</td>
</tr>
<tr>
<td>Saltiness</td>
</tr>
<tr>
<td>Thickness</td>
</tr>
<tr>
<td>After-taste intensity</td>
</tr>
<tr>
<td>Expected satiation</td>
</tr>
</tbody>
</table>

1 Values are means ± SDs, n = 54.
2 Scores were rated on a 100 mm VAS after 45 g of soup in the practice session (first session).
Ad libitum intake of soup

The ad libitum intake in the small-bites condition was ~30% lower than in the large-bites and free-bites conditions ($F(2, 248) = 41, P < 0.001$) in both the focused and distracted states (Figure 6.2). The ad libitum intake in the large-bites and free-bites conditions did not differ ($P = 0.15$). The rating of “I terminated consumption because I did not like the manner of consumption” was added as covariate in the ANOVA model, because it also affected ad libitum intake ($F(1, 248) = 9.5, P = 0.002$). This means that with correction of the manner of consumption, intake in the small-bites condition remained significantly lower compared to the large- and free-bites conditions. The ad libitum intake was 5 to 11% higher when subjects were distracted than when they were focused ($F(1,248) = 7.1, P = 0.008$). There was no interaction between bite-size conditions and distraction on ad libitum intake ($P = 0.74$). In the distracted state, subjects correctly answered 85 ± 12% of the questions (min – max: 50 - 100%). This outcome was not different between the different bite-size conditions ($P = 0.39$).

![Figure 6.2](image-url)

**Figure 6.2** Mean + SD of ad libitum intakes. Ad libitum intake was higher in the large-bites and free-bites conditions compared to the small-bites condition ($P < 0.001$), and was higher in the distracted state than in the focused state ($P = 0.008$). Values on columns with different superscript letters are significantly different ($P < 0.05$). + = trend between the focused and distracted state in the small-bites condition: $P = 0.057$.

Estimated amount consumed

The direction of the estimations, negative (underestimation) or positive (overestimation), was affected by bite-size ($P < 0.001$), but not by distraction ($P = 0.72$) (Figure 6.3). There was no interaction effect ($P = 0.34$). Taking into account that distraction did not influence the estimations, subjects significantly underestimated their amount consumed in the large-bites condition (both focused and distracted state) and
the free-bites condition \((P < 0.029)\). Estimations in the small-bites condition did not significantly differ from ad libitum intake \((P = 0.16)\).

The mean values of the estimations in the small-bites condition was \(332 \pm 190 \text{ g}\) in the focused state, which is 11\% more than ad libitum intake (difference between ad libitum intake and estimation: \(P = 0.09\)), and \(342 \pm 175 \text{ g}\) in the distracted state, which is 4\% more than ad libitum intake \((P = 0.66)\). The estimations in the large-bites condition was \(386 \pm 206 \text{ g}\) in the focused state, which is 13\% less than ad libitum intake \((P = 0.04)\), and \(441 \pm 208 \text{ g}\) in the distracted state, which is 6\% less than ad libitum intake \((P = 0.33)\). The estimations in the free-bites condition was \(397 \pm 227 \text{ g}\) in the focused state, which is 10\% less than ad libitum intake \((P = 0.07)\) and \(419 \pm 202 \text{ g}\) in the distracted state, which is also 10\% less than ad libitum intake \((P = 0.12)\).

The mean difference in absolute values between the estimated the amount consumed minus the ad libitum intake over all conditions was \(134 \pm 131 \text{ g}\) (min-max: 0.1 - 808 g). Estimation accuracy (i.e., the absolute difference in percentiles between estimated amount consumed minus ad libitum intake) did not differ between the bite-size conditions \((P = 0.36)\) and did not differ between the distracted and focused states \((P = 0.88)\). There was a significant gender effect \((P = 0.018)\); women were 5\% more accurate in their estimations than men.

![Figure 6.3](image)

**Figure 6.3** Mean + SD of the difference between the estimated the amount consumed and the ad libitum intake. The difference (estimated intake minus intake) was affected by bite size \((P < 0.001)\), but not by distraction \((P = 0.72)\), and there was no interaction \((P = 0.34)\). * = significant difference between estimated intake and intake \((P < 0.05)\), + = trend between estimated intake and intake \((P < 0.10)\). Values on columns with different superscript letters are significantly different \((P < 0.05)\).
Effect of distraction on bite size and total number of bites

In the free-bites condition, subjects determined their bite sizes and bite frequency by themselves. The bite size was not affected by distraction (Table 6.2). The total number of bites was 11% higher in the distracted state than in the focused state. In the distracted state, the total duration of ad libitum intake was longer, and the eating rate and bite frequency were lower.

Table 6.2 Duration, bite size, number of bites, and bite frequency in the free-bites condition

<table>
<thead>
<tr>
<th></th>
<th>Free-bites</th>
<th>Distraction</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total duration (min)</td>
<td>6.2 ± 3.7</td>
<td>8.1 ± 4.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Eating rate (g/min)</td>
<td>72.0 ± 19.1</td>
<td>60.3 ± 23.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bite size (g)</td>
<td>14.3 ± 5.8</td>
<td>13.5 ± 4.8</td>
<td>0.13</td>
</tr>
<tr>
<td>Total number of bites</td>
<td>32.3 ± 18.6</td>
<td>36.0 ± 18.9</td>
<td>0.02</td>
</tr>
<tr>
<td>Bite frequency (bites/min)</td>
<td>5.6 ± 2.1</td>
<td>4.8 ± 1.9</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± SDs, n = 53.

Appetite and hedonic ratings

Initial ratings of hunger, fullness and prospective consumption did not differ between conditions (all P-values > 0.75), indicating that subjects were in the same state of satiety before ad libitum intake in each condition (Figure 6.4).

After ad libitum intake (t=30 min), hunger (Figure 6.4A) was affected by the bite size (P = 0.004), but not by distraction (P = 1.0). Hunger ratings were higher after the small-bites, compared to both the large-bites and free-bites (P < 0.020). Hunger was not affected by bite size after 1, 2, and 3 hours (P > 0.32). Likewise, ratings for fullness (Figure 6.4B) were affected by bite size after ad libitum intake (t=30 min) (P < 0.001), but not by distraction (P = 0.31). The ratings for fullness were lower after the small-bites compared to both the large-bites and free-bites (P < 0.003). Fullness was not significantly affected by bite size after 1 hour (P = 0.07), 2 hours (P = 0.11), and 3 hours (P = 0.70).

Decrease in pleasantness and desire-to-eat the soup after ad libitum intake (data not shown) was not affected by bite size (P > 0.33), or distraction (P > 0.52).
Figure 6.4 Means of hunger (A) and fullness (B) ratings over time (9-point scale).*After ad libitum intake (t = 0.5 h), hunger and fullness were affected by bite size ($P < 0.004$), but not by distraction ($P > 0.31$). *The ratings for hunger (A) were higher after the small-bites condition compared to both the large-bites and the free-bites conditions ($P < 0.02$). The ratings for fullness (B) were lower after the small-bites condition compared to both the large-bites and the free-bites conditions ($P < 0.003$).

Reasons to terminate consumption

“I terminated consumption because I was full” was the most important reason in all conditions to terminate consumption (Table 6.3). All three reasons to terminate consumption were affected by bite size ($P < 0.012$), but not significantly by distraction ($P > 0.07$). The importance of the reason “I terminated consumption because I did not like the manner of consumption” differed between all three bite size conditions ($P < 0.015$): small-bites > large-bites > free-bites. The reasons “I was full” and “Flavour was not pleasant anymore” were more important in the large-bites and free-bites conditions compared to the small-bites condition ($P < 0.036$).
Table 6.3 The reasons to terminate soup consumption

<table>
<thead>
<tr>
<th>Reason</th>
<th>Small-bites</th>
<th>Large-bites</th>
<th>Free-bites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Focus</td>
<td>Distraction</td>
<td>Focus</td>
</tr>
<tr>
<td>“I was full”</td>
<td>3.7 ± 1.1</td>
<td>4.0± 1.0</td>
<td>4.0bc ± 1.0</td>
</tr>
<tr>
<td>“Flavour not pleasant”</td>
<td>2.6 ± 1.1</td>
<td>2.8ab ± 1.3</td>
<td>3.0bc ± 1.2</td>
</tr>
<tr>
<td>“Manner of consumption”</td>
<td>3.2 ± 1.3</td>
<td>3.2a ± 1.2</td>
<td>2.7b ± 1.2</td>
</tr>
</tbody>
</table>

1 Values are means ± SDs. Values in rows with different superscript letters are significantly different (P < 0.05).
2 The propositions were answered on a 5-point scale from “totally disagree” (1) to “completely agree” (5).
3 Significant main effects of bite size: P < 0.012.

Discussion

Effect of bite size on food intake and on the estimated amount consumed

We hypothesized that ad libitum intake is higher when subjects consume larger bites and that they would underestimate their amount consumed. The results show, indeed, that ad libitum intake was higher when consuming large bites, in agreement with previous studies (13, 92, 93, 95-99, 209). Consuming large bites led to underestimation, whereas small-bites, led numerically, but not significantly, to overestimation of the amount consumed. This indicates that bite size affects beliefs about food intake. Larger bites are by definition associated with fewer bites per gram food. The fact that fewer bites are taken when people consume with large bites may explain the underestimation of food intake. This underestimation during consumption may delay satiation, because food intake is highly influenced by cognitive processes (50).

Interestingly, when subjects determined their bite size and frequency by themselves (free-bites condition), ad libitum intake was similar to large-bites condition. Subjects also underestimated their amount consumed in the free-bites condition. Moreover, subjects consumed soup in the free-bites condition with almost similar bite size to the large-bites condition (~14 g and 15 g, respectively). The results of the free-bites condition indicates that underestimation of consumption also occurs when people take relatively large bites by themselves.

The mean bite size in the free-bites condition of ~14 g is larger than the bites that are taken when the soup is consumed with spoons: 7 - 9 g (229). The bite size was probably influenced by the manner of consumption, which was through a tube. It has been shown that consuming with a straw instead of a spoon increased eating rate, possibly through
relatively large bites facilitated by straws (80). The tube may therefore facilitate large bites compared to spoons.

The reason “I terminated consumption because I did not like the manner of consumption” was more important in the small-bites condition compared to the large-bites and free-bites conditions. It probably contributes to the 30% lower intake in the small-bites condition. The lower intake in the small-bites condition may also explain why subjects felt less full directly after consumption. However, when the statistical model on ad libitum intake was corrected for “manner of consumption”, there is still a strong significant effect of bite size on ad libitum intake (see results). Smaller bites are always associated with more effort per gram food. Increased effort has been related to lower food intake (26). Our previous study showed that hunger decreased faster per consumed gram food (rated after each consumed 75 g) with small bites compared to large bites (chapter 5). This supports the theory that smaller bites lead to faster satiation and is not just the result of a more uncomfortable manner of consumption.

Subjects felt less full after consumption in the small-bites condition compared to the large-bites and free-bites conditions. However, these differences in hunger and fullness ratings diminished at one to three hours after consumption (Figure 6.4). No differences in hunger after three hours may indicate that the reduced food intake in the small-bites condition will not be compensated. Two studies (98, 224), that used an oral device to decrease the bite sizes, have shown that the device led to a reduction in meal size without changes on rated satiety between meals. Small bites may therefore lead to a reduction in food intake on longer term.

Bite size did not influence sensory characteristics of the soup (Table 6.1). In addition, the initial pleasantness and the decrease in pleasantness after ad libitum intake were not affected by bite size. Therefore, the effect of bite size on ad libitum intake was not mediated via differences in flavour perception or pleasantness of the food.

**Effects of distraction on food intake, estimated amount consumed and bite size**

Distraction led to greater intake (5-11%), in agreement with a number of studies (35, 38-41, 108). Other studies have found an increase in energy intake of ~14% when watching TV (35, 38). This is somewhat greater than the effect found in the present study. Others have suggested that the increased food intake in distracted states is explained by impaired ability to monitor visually the amount consumed (35, 50, 109). This study differed from others because subjects were not able to monitor visually the amount consumed. Therefore, it is possible that impaired visual cues play a role, but there must be other mechanisms that explain higher food intake during distraction.
Distraction led to lower bite frequency and longer meal duration in the free-bites condition. In addition, distraction was associated with higher number of total bites, whereas bite size was not affected. Bite size may be an individual behavioural characteristic that is not influenced by distraction. This is in agreement with the finding that bite size is constant within individuals for specific types of food (218, 221).

Another study (41) also showed prolonged meal duration and increased food intake when people were distracted by listening to music. The present study also showed that the distracted state slowed down eating rate but prolonged meal duration that eventually resulted in higher food intake. Longer meal duration, thus more opportunity to eat, may explain increased food intake in distracted states. It is also possible that the sensory exposure per gram food is less in the distracted state. Watching the film distracted attention away from oral food processing. Oral sensory exposure to food is important for termination of food consumption (e.g., 134, 183).

To ensure subjects were distracted, they watched an animation film during consumption and were instructed to answer questions afterwards. The distraction was successful because these questions were well answered. The minimum score was 50% correct (out of 8 to 11 questions). These questions could not be answered if no attention was paid to the film.

We hypothesized that the effect of bite size on food intake is diminished in a distracted state. Distraction did not influence the effect size of bite size on food intake; there was no interaction effect. This means that the effect of reducing intake by consuming small bites is not overruled by increasing the number of bites in a distracted state. Therefore, smaller bite sizes are effective in reducing food intake even when people are distracted.

We hypothesized that subjects would underestimate their amount consumed when they were distracted. The results showed that both the direction and the accuracy of the estimated amount consumed were not affected by distraction. This contradicts a recent study that showed distraction resulted in impaired memory for the consumed foods (108). In that study, subjects had to recall the different lunch items they ate after 30 minutes, which is different from estimating the amount consumed directly after intake. The results of the present study suggest that in a distracted state without visual cues, people somehow know how much they approximately consumed. Probably, their attention to the film did not completely diminish attention towards food consumption.
Conclusion

Consumption with large bites, thus relatively fewer bites per gram food, led to much higher food intake and led to an underestimation of the amount consumed. When subjects were able to determine bite sizes by themselves, they took relatively large bites and also underestimated the amount consumed. Underestimating the amount consumed is a possible risk factor for overconsumption. This implies that consuming with large bites impairs the control of food intake. Distraction led to a general increase in food intake, independent of bite size. In addition, subjects did not adjust their bite sizes when they were distracted. This implies that small bite sizes may successfully reduce food intake, even in a distracted state. Designing foods with properties that involves consumption of small bites/sips may prevent overconsumption and decrease the prevalence of obesity.

Acknowledgements

We thank all who assisted in conducting the study, especially Henri Raatjes, Fabian Griens and Marijke Hummel, and the technical department of Wageningen University for the software used in this study.

D.P.B. designed and carried out the study, interpreted the results and wrote the manuscript, all under supervision of C.M.M.L., R.A. de W., P.A.L and C. de G. None of the authors had a conflict of interest.
Chapter 7

General discussion
Figure 7.1 Model that shows the main findings of this thesis, namely the effects of taste intensity, oral residence duration, bite size and distraction on ad libitum intake. Negative effects are illustrated with – and positive effects with +. Ad libitum intake is negatively affected by taste intensity (chapters 3, 4) and oral residence duration (chapter 5). Ad libitum intake is positively affected by bite size (chapters 4-6), pleasantness (chapter 3) and distraction (chapter 6). Eating rate is negatively affected by oral residence duration and positively by bite size and bite frequency. Bite size is positively affected by pleasantness and negatively by taste intensity (chapter 4). Larger bites lead to shorter oral residence duration per gram food, as was found in other studies (100, 210). Distraction decreases the bite frequency that leads to a lower eating rate (chapter 6).

Table 7.1 Effect sizes of factors that reduced ad libitum intake investigated in this thesis.

<table>
<thead>
<tr>
<th>Intake was reduced by</th>
<th>Variable</th>
<th>Factor</th>
<th>Reduction intake (%)</th>
<th>Chapter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Higher taste intensity</td>
<td>Salt concentration(^1)</td>
<td>3.7</td>
<td>~7.5 – 9</td>
<td>3,4</td>
</tr>
<tr>
<td>Smaller bite size</td>
<td>5 vs. 15 g</td>
<td>3</td>
<td>~22 – 30</td>
<td>4-6</td>
</tr>
<tr>
<td>Longer oral residence duration</td>
<td>20 vs. 60 s/100g</td>
<td>3</td>
<td>~8</td>
<td>5</td>
</tr>
</tbody>
</table>

\(^1\) The mean selected salt concentration for the low-salt soup was ~150 mg Na/100g and the mean selected salt concentration for the high-salt soup was ~575 mg Na/100g (chapters 2-4). The low-salt soup was rated at ~30 mm and the high-salt soup was rated at ~65 mm on a 100 mm VAS for saltiness (from very weak to very strong).
The studies described in this thesis focused on the role of orosensory exposure of taste on satiation. Clarification of principle mechanisms through which orosensory exposure affects satiation is important for the understanding of the regulation of food intake. Effects of taste intensity, oral residence duration and bite size on ad libitum intake have been investigated. This discussion starts with an overview of the main findings of this thesis, followed by a number of methodological considerations. After that, the interpretation of the results is discussed, followed by theoretical explanatory mechanisms. Lastly, implications and suggestions for future research are given.

Main findings

Figure 7.1 summarizes the main findings of this thesis and the interrelationships between variables. Table 7.1 quantifies the main effects that are described in this thesis. The effect of taste intensity on satiation was investigated by varying the salt concentrations in soup. When the soup was presented as single lunch-item in a hungry state, there were no differences in intake between low-salt and high-salt soup similar in pleasantness (chapter 2). However, intake of high-salt soup was ~8% lower than that of low-salt soup, either when the soup was presented after a preload or as a starter followed by a second meal (chapters 3, 4).

Smaller bite sizes decreased ad libitum intake when the eating rate was kept constant (chapters 4-6). This effect was independent of saltiness, because the size of the effect (~25%) was similar for the low-salt and high-salt soup (chapter 4). Hunger decreased faster per consumed gram food when consuming with small bites compared to large bites (chapter 4, 5). Higher saltiness led to smaller bite sizes when subjects were able to determine the bite size by themselves (chapter 4).

To investigate the underlying mechanisms of bite size on satiation, we separated effects of oral residence duration (s/g) from the number of bites (bites/g) in a design were the eating rate was kept constant (chapter 5). Both longer oral residence duration and higher number of bites per gram food decreased ad libitum intake separately. In addition, both longer oral residence duration and higher number of bites increased the total magnitude of orosensory exposure to the taste per consumed gram of food.

Consumption with large bites increased ad libitum intake and resulted in underestimations of the amount consumed, whereas consumption with small bites did not (chapter 6). Distraction increased ad libitum intake, this effect was independent of bite size. Distraction led to a higher total number of bites but did not affect bite size.
Test foods

Tomato soup was chosen as test product in all studies because of a number of reasons. Tomato soup is a common food to consume at lunch in the Netherlands (175, 230). Tomatoes contain four different taste qualities: sweet (glucose/fructose), sour (aspartic acid), salty (sodium) and savoury (glutamate). Soup from tomatoes is associated as a savoury tasting food. It is more appropriate to serve a savoury tasting food than a sweet tasting food during lunch, and there are not so many savoury liquid products besides soup. A liquid product allowed us to vary the bite sizes and oral residence duration by using peristaltic pumps (chapters 4-6). Soup is a liquid product that is usually consumed with a spoon, and is therefore associated with “food” instead of “beverage”. Blended tomatoes are a suitable basis to vary the salt concentration, due to its naturally low sodium content (chapters 2-4). In summary, tomato soup is an appropriate food for lunch and was considered suitable to vary effects of orosensory exposure to taste on satiation.

Preloads were used in the studies of chapters 3-6 to prevent subjects from being in a very hungry state before ad libitum intake. In the study described in chapter 2, we found no effect of saltiness on ad libitum intake, whereas we did find effects of saltiness when subjects received preloads before ad libitum intake in the studies described in chapters 3 and 4. The state of hunger in chapter 2 may have overruled effects of sensory signals on satiation. This illustrates that experimental designs have to be chosen carefully to demonstrate effects of sensory signals (231). Raisin buns were used as preloads. Raisin buns are appropriate to consume during lunch in the Netherlands (175). The energy provided from the preload was calculated as half of the energy provided by an average lunch in the Netherlands (175). This was calculated per individual, taking into account: gender, age and weight (Schofield I equation). During the preload phase, subjects were able to drink water to ensure that they were not thirsty before ad libitum intake. Using preloads prevented subjects from being very hungry and allowed us to investigate effects of orosensory exposure to taste on satiation.

Palatability strongly affects the amount consumed (42-47), and may therefore be a confounder when investigating sensory signals on satiation. First, all included subjects liked tomato soup; they scored at least 6 on a 9-point scale (from very unpleasant (1) to very pleasant (9)). In chapters 2-4, the salt concentrations for low-salt and high-salt soup were individually selected to be similar in pleasantness (< 10 mm on a 100 mm VAS). As far as we know, this individual selection to correct for pleasantness was not used before in other studies. In chapters 5, 6, bite size and oral residence duration did not have an influence on the pleasantness of the soup. This means that palatability is not a confounding factor in the studies described in this thesis.
**Internal, environmental and cognitive factors**

As stated in the introduction (Figure 1.1), also internal, environmental and cognitive factors play a role in satiation. These factors were standardized or excluded as much as possible to investigate effects of sensory exposure on satiation. To standardize the internal state of satiety, subjects always started the lunch session at the same time. In addition, they were instructed to consume the same breakfast and only drink water before the test lunch started. Ratings of hunger and fullness before ad libitum intake of soup did not differ between conditions in all studies of this thesis (chapters 2-6). This suggests that standardization of the satiety state before ad libitum intake was successful.

Visual cues toward food have been demonstrated to influence the amount consumed (26, 55). In the studies described in this thesis, visual cues toward the amount consumed were diminished (chapters 2, 3) or excluded (chapters 4-6). In the first two studies (chapters 2, 3) subjects consumed soup from a soup bowl with a spoon. The soup bowl was “self-refilling” as described by Wansink et al. (55). Subjects were aware of the fact that the bowl was refilling, to exclude the cognitive effect of the natural tendency to finish the bowl (“clean the plate” (232)). In the last three studies (chapters 4-6), subjects consumed soup through a tube that was connected with a pump. The pump and the pan that contained soup were invisible to the subjects. There were no visual cues toward soup consumption. Nevertheless, subjects were familiar with the taste and sight of the soup, because they had one test session before the start of the ad libitum intake sessions, and always tasted a small sample of soup in a transparent small cup before ad libitum intake. The designs used in this thesis diminished effects of visual cues in order to focus primarily on effects of orosensory exposure to taste on satiation.

Other environmental and cognitive factors that influence satiation were standardized as much as possible. During the ad libitum intake, subjects were not able to see others, thus intake was not influenced by social settings. Potential subjects that scored high on restrained eating behaviour in the Dutch eating behaviour questionnaire (DEBQ) were excluded from participation. Tomato soup was always available in excessive amounts, so that subjects could eat as much they wanted.

**Experimental design**

Effects of eating rate and taste intensity on food intake were already known from previous studies that used ‘real life’ environments (10, 13, 212). In this thesis, underlying mechanisms were studied that give more insight in how these factors influence satiation. Investigation of these underlying mechanisms required tightly controlled experimental designs. These designs were setup to enable variation of the parameters of interests while keeping all other parameters that potentially influence ad libitum intake as constant as possible. In chapters 4-6, eating rate, bite sizes and bite
frequencies were fixed, by administrating soup via a tube using a peristaltic pump. This unnatural way and tight control of eating may have influenced the results on intake to some extent. Nevertheless, subjects consumed until they were satiated because the reason “I was full” was more important than “I did not like the manner of consumption” or “I did not like the flavour of the soup anymore” to terminate consumption (chapter 4-6). Moreover, chapters 4 and 6 had “free” conditions that allowed subjects to determine the bite size and bite frequency by themselves. This did not result in much larger or much lower ad libitum intakes, the intakes were comparable to those in fixed conditions. Thus, more ‘freedom’ in consumption did not lead to obvious differences in ad libitum intake.

External validity

The studies consisted of within-subject designs in which 43 to 56 subjects participated. This means that there was sufficient statistical power to demonstrate effects of at least ~10% (power calculation for within-subject designs). Taste intensity and oral residence duration affected intake by 8%, resulting in significant main effects (chapters 4, 5), but did not always reached significance in post hoc comparisons within different conditions. However, we found similar effects of taste intensity on satiation in two different studies (chapters 3, 4). Taste intensity affected satiation in different meal compositions (chapter 3) and when consuming with different bite sizes (chapter 4). Similar reduction due to longer oral residence duration was found in both the low- and high number of bites conditions in chapter 5. In addition, the effect of bite size on satiation was repeatedly demonstrated (chapters 4-6). The fact that similar effects were found between different conditions and over more studies suggests that the results in this thesis are robust and accurate.

The results in chapter 6 showed that consumption with large bites led to underestimations of the amount consumed. This was not only found when the bites were fixed, but also when subjects determine their bites by themselves. This suggests that taking large bites in a natural way of eating also lead to underestimations of the amount consumed, which is a risk factor for overconsumption.

All subjects participated in the studies of this thesis were healthy, young (18-35 y), normal weight (BMI 18.5-25 kg/m²), non-restrained adults. We assume that, in general, humans without taste and smell impairments, including obese and overweight people, will be responsive to effects of orosensory exposure to food on satiation. Obese people were shown in other studies to reduce food intake at a decreased eating rate (233), thus by increasing the orosensory exposure per gram food. Moreover, the positive relationships that were found between eating rate, bite size and body weight status (15-18, 234, 235) suggest a causal relationship. This suggests that obese people are also sensitive to effects of orosensory exposure to taste on satiation.
Eating rate, which is related to orosensory exposure, has been demonstrated to affect intake over a wide range of foods (9-13). Orosensory exposure to food has been shown to be important for feedback signals of satiation in several human and animal studies (71, 180, 182). Tomato soup was used to clarify the principle mechanisms through which orosensory exposure affects satiation. Hence, we believe that the mechanisms found in this thesis for tomato soup are also valid for other foods.

**Discussion and interpretation of the results**

Smaller bite sizes, longer oral residence duration and higher taste intensity led to lower food intake (Table 7.1, Figure 7.1). These three factors separately affect food intake, because no interaction effects were found between taste intensity, bite size and oral residence duration (chapters 4, 5). Table 7.1 shows that reducing the bite or sip sizes would be the most efficient way to lower food intake.

**Bite size, oral residence duration**

As stated in the introduction, eating rate (g/min) affects the amount of food intake, and is affected by oral residence duration and bite size (Figure 7.1.). We showed that both bite size and oral residence duration affected food intake when the eating rate was fixed (chapters 4-6). Therefore, Figure 7.1 does not show a direct arrow from eating rate to ad libitum intake, which is different from the model in the introduction (Figure 1.2). Eating rate does affect intake in a natural way of eating, but this is considered as an indirect effect. Bite size and oral residence duration may be the explaining factors of the effect of eating rate on satiation.

In a natural way of eating, bite size and oral residence duration are related to each other; smaller bites are associated with longer oral residence duration per gram food (100, 210). We showed that smaller bites are not only more satiating because of the longer oral residence duration, but also because of the higher the number of bites per gram food as such (chapter 5). This explains the large effect that was found for bite size on food intake compared to oral residence duration *per se* (Table 7.1). That smaller bites are more satiating is confirmed by the faster decrease in hunger and faster increase in fullness compared to large bites per consumed 75 g (chapter 4, 5). Smaller bites reduce ad libitum intake not only in a food-focused state but also in a distracted state (chapter 6). Similar size of effect of bite size on ad libitum intake has been found for sweet tasting products (92, 93). These studies also compared bites of 5 g with bites of 15 g (93) or 20 g (92).

Consuming with either large or small bites affected believes about the amount consumed (chapter 6). Consumption with large bites led to an underestimation of the
amount consumed, whereas consumption with small bites led numerically, although not significantly, to an overestimation of the amount consumed. Consuming with large bites means that fewer bites need to be consumed compared to smaller bites for intake of the same amount of food. The number of bites may be related to beliefs regarding the amount consumed. Relatively fewer bites, due to large bites, may explain the underestimations of the amount consumed. Beliefs toward the amount consumed play an important role in how much food is actually consumed (e.g., 50). Underestimating the amount that is consumed during a meal, may lead to higher food intake within that meal. It is possible that the association of bite size with food intake depends on the effort; large bites means less effort compared to small bites per gram food. Effort is related to the ease with which a food can be consumed and has a great impact on the amount consumed (236). Increased effort was shown to decrease consumption (26).

The reduction in intake of ~8% due to longer oral residence duration is smaller than found by Zijlstra et al. (93), who found a difference of 15% in intake of a sweet tasting product when the oral residence duration was varied by a factor three, similar to our design (Table 7.1). It is possible that oral residence duration to sweetness has a stronger effect on reduction in food intake than saltiness. Sweetness signals energy and saltiness signals sodium. Sodium is essential for many physiological processes, but the intake of sodium is probably not regulated on the short-term, as is the intake of energy (187, 188).

The effect sizes found in this thesis strongly depend on the experimental designs that were chosen. This thesis showed that reducing the bite size is probably more efficient than only increasing the oral residence duration, when they would be manipulated to same degree. The fixed bite sizes that were chosen, 5 g and 15 g (chapters 4-6), are within the range of natural bite sizes of common foods. Bite sizes for solid foods are between 2 to 10 g (89, 100), and between ~10 to 20 g for liquids and semi-solid foods (12, 89, 221). Bite size is also determined by individual characteristics and manner of consumption. In chapter 3, mean bite sizes differed from 4.6 to 13.0 g when subjects consumed soup with a spoon. In chapters 4 and 6, mean bite sizes differed from 3.6 to 32.0 g when subjects consumed soup via a tube.

For the oral residence duration, we chose 20 s/100g vs. 60 s/100g food, which is shorter than found for solid foods. The oral residence duration is between ~200 to 700 s/100g for hard solid foods, and between ~60 to 300 s/100g for more softly textured solid foods (100). As far as we know, there are no data of oral residence duration of liquid foods, but this is has to be much lower than for solid foods because liquids need minimal oral processing and are swallowed quickly. We found a reduction in intake of 8% when the oral duration was three times extended. The variation in oral residence duration used in this thesis is much smaller than found in common foods. Therefore, oral residence
duration may play a more important role in satiation in a natural ways of eating than found in this thesis.

Eating rate (g/min) is also influenced by bite frequency in addition to oral residence duration and bite size (Figure 7.1.). Effects of bite frequency on ad libitum intake were not directly investigated in this thesis, because bite frequency was considered less important in food intake. The contributions of bite size and bite frequency on ad libitum intake of chapter 3 (where subjects consumed soup with a spoon) were analysed by Bayesian modelling (237). Predicting intake by 10% increases of bite size and bite frequency shows that bite size has much more impact on ad libitum intake than bite frequency (Figure 7.2). In addition, Zijlstra et al. (93) investigated effects of bite size when both the bites of 5 g and 15 g were administered at equal bite frequency. In this thesis, bites of 5 g were administrated at a three times higher bite frequency to keep the eating rate equal (chapters 4-6). The effects of bite size on ad libitum intake found by Zijlstra et al. (93) was of similar effect size than the results in this thesis. This indicates ad libitum intake was primarily affected by bite size and not by bite frequency. Bite frequency may therefore not affect satiation in such an extent as bite size and oral residence duration. This is possibly explained by less impact on the orosensory exposure to the food compared to bite size and oral residence duration. This may explain why some studies that prolong the pauses between bites, thus lower the bite frequency, fail to find a reduction in food intake (213, 214) or fail to find effects on postprandial hormonal secretion (215).

Figure 7.2 Comparing contribution of bite size and bite frequency on intake by Bayesian modelling, using data of chapter 3. The mean and standard deviation of intake were predicted at every 10% increase of bite size (A) and bite frequency (B). The 10%-increase step of each variable was calculated to be one-tenth of the range between the mean ± 3 x SD (covering more than 99.7% observations). This step was equal to 0.93 g for bite size and 2.25 bites/min for bite frequency. Adopted with permission from (237).
Taste intensity

The studies described in this thesis were the first that showed that taste intensity directly affects ad libitum intake, and is not necessarily mediated via pleasantness (Figure 7.1) (chapters 3, 4). We showed that higher saltiness decreased ad libitum intake when the pleasantness was kept constant. The effect of taste intensity on food intake found in this thesis may explain why humans usually eat higher amounts of neutral tasting or staple foods like potatoes or rice than high-intense tasting foods, such as olives or sweets.

In a real life situation, taste intensity or saltiness affects the pleasantness of a food, and pleasantness is an important determinant of food intake (42-47). We showed that the most pleasant level of saltiness in soup (i.e., the ideal-salt soup, salt concentration in between low-salt and high-salt soup) resulted in highest intake (chapter 3). This is in agreement with other studies that have shown that the food that has the most pleasant taste intensity led to highest intake (112-116, 120). Pleasantness overruled the effect of taste intensity on satiation, because the ideal-salt soup was higher in taste intensity than the low-salt soup, but led to higher intake (chapter 3).

Pleasantness positively and taste intensity negatively affected bite size (chapter 4, Figure 7.1). Adjusting bite sizes according to taste intensity allows humans to self-dose the amount of nutrients. Similarly, de Wijk et al. (178) found that a higher aroma intensity resulted in smaller bite sizes. However, the effect of taste intensity on bite size does not explain the effect of taste intensity on intake in this thesis, because fixed bite sizes led to the same size of effects on ad libitum intake (chapter 4).

Although we found an effect of saltiness on satiation, it is a relatively small effect. A large difference in salt concentration in soup (~150 mg Na/100g (low-salt) vs. ~575 mg Na/100g (high-salt)) led to a small difference in food intake (chapters 3, 4 and Table 7.1). The low-salt soup was rated at ~30 mm and the high-salt soup was rated at ~65 mm on a 100 mm VAS for saltiness (from very weak to very strong). It is possible that saltiness is not associated as a satiating nutrient because is it not associated with energy. Taste intensity manipulated by sweetness or umami (i.e., savouriness) may gave different effects on satiation due to their associations with energy and protein, respectively. However, humans may not always distinguish an umami taste from a salty taste (238). As far as we know, the effect of taste intensity of sweetness or umami have not been investigated in designs were pleasantness was kept constant.

With regard to taste intensity, nowadays many processed foods contain flavour enhancers or artificial sweeteners. These ingredients increase the intensity of the taste but do not contribute to the nutrient density of the foods. The taste of highly processed foods have been shown to impair the prediction the nutrient content (73). The association between taste intensity and nutrient density may have weakened along with
the introduction of processed foods. For example, humans in the industrialized world may have a mixed diet of foods that contain energy rich sweeteners (sugar) and foods that contain sweeteners without energy (artificial sweeteners). In rats, ingestion of sweet foods that contained artificial sweeteners disrupted the association between energy density and sweetness. This resulted in increased body weight when the rats were exposed to sweet foods that contained energy (151). In addition to artificial sweeteners, also flavour enhancers may impair the prediction between taste and nutrient content, especially taste intensity and nutrient density. This may explain the relative small effect of taste intensity on satiation found in this thesis (chapters 3, 4). The poor prediction of the taste and its nutrient content may interfere with fundamental physiological processes that may lead to positive energy balances.

No effect of saltiness on ad libitum intake was found when subjects only consumed the soup for lunch and did not receive a preload (chapter 2). Probably, feelings of hunger may have overruled effects of sensory signals on satiation. Humans in a very hungry state may be less sensitive to sensory processes and this may result in higher consumption once they have access to food. Hunger is positively related to bite size and eating rate (chapter 4) (12, 15), which facilitates overconsumption. In contradiction, when subjects consumed soup as a starter while they knew they would be served a second course, they did show effects of taste intensity on soup intake (one treatment of chapter 3). In this treatment, subjects were in the same hungry state before soup intake because they did not receive a preload as in study of chapter 2. The difference is that in chapter 3, subjects knew that they would be served a second meal, whereas soup was the only lunch-item in chapter 2. People eat more of a food when they know that they have no access to other foods for a certain time (44). Possibly, in a meal that consists of multiple items, the intake each food item is primarily regulated by sensory processes, whereas in a single-item meal, internal signals of hunger/fullness play a more important role in satiation.

The role of orosensory exposure to taste on satiation: Explanatory mechanisms

This thesis demonstrates the importance of taste in satiation. The sense of taste was previously known to be important in food choice and meal initiation (20, 49, 71). The sense of taste does not only inform the brain what kind of nutrients are ingested but also how much nutrients are ingested. More contact of tastants with taste receptors leads to an increase in orosensory exposure to taste (i.e., an increased taste perception). We showed that changing the orosensory exposure to the taste of food influences satiation.

The intensity and oral residence duration of taste are considered to affect the orosensory exposure to taste in strength and duration, respectively (Figure 7.3). In theory, foods
higher in taste intensity contain a higher number of tastants. More binding of tastants with taste receptor leads to an increase in orosensory exposure. Longer oral residence duration is associated with more time for tastants to bind taste-receptors before swallowing. This also increases the orosensory exposure. The effect of oral residence duration on orosensory exposure is not only based on theory but has been demonstrated in chapter 5. Execution of time-intensity measurements showed that longer oral residence duration increases the total magnitude of orosensory exposure to the food (Figure 5.4 and 5.5). Bite size also affected orosensory exposure, smaller bites led to an increased total magnitude of orosensory exposure to the taste of the food (chapter 5, Figure 5.4 and 5.5). Consuming with smaller bites rather than larger bites probably leads to relative more tastants-receptor binding per gram food. This means in practice, that relatively more per gram food is tasted when consuming with small bites compared to large bites.

![Figure 7.3](image_url)

**Figure 7.3** Theoretical model of taste intensity, oral residence duration and bite size on the total magnitude of orosensory exposure. The straight line (-----) illustrates one bite of 15 g. The area under the curve (grey area) is the [taste intensity x oral residence duration] which represents the total orosensory exposure to the taste of the bite. The dotted line (-----), illustrates a bite that is higher in taste intensity. The dashed line (-----), illustrates a bite longer in oral residence duration. Both the taste intensity and the oral residence duration increase the area under the curve and thereby the orosensory exposure per gram food. The thin line (-----) illustrates one bite of 5 g. Three bites of 5 g lead to larger area under the curve than one bite of 15 g. This is measured and demonstrated in Figures 5.4 and 5.5 in chapter 5. Thus, smaller bites lead to more orosensory exposure to the taste per gram food compared to larger bites.

In a natural way of eating, oral residence duration strongly depends on other oral processes like chewing and other mouth movements that are needed before swallowing. Mouth movements and chewing diminish taste adaptation and increase the number of taste receptors that are stimulated (239). This probably also leads to more orosensory exposure to the taste and may contribute to satiation. Li et al. (240) showed that increasing the number of chews led to lower ad libitum intake and higher hormonal
satiety responses. Oral processes as mouth movements and chewing may also contribute to satiation in addition to the effect of oral residence duration and bite size.

We showed that orosensory exposure per gram food is negatively influenced by shorter oral residence duration and larger bite sizes (chapter 5, Figures 5.3, 5.4, Figure 7.3). Liquid foods are consumed with large sips and minimal oral residence duration (12). Energy-yielding liquids were repeatedly found to have low satiating capacity. This was demonstrated by high intakes and low hormonal satiety responses compared to liquids/semi-solids higher in viscosity (10, 12, 88, 241). Moreover, energy intake from liquids was shown to be compensated poorly (225, 226). The minimal orosensory exposure to taste obtained from energy-yielding liquids may explain the low satiating capacity.

Orosensory exposure to taste may be the key explanation why volume or weight is a more important determinant of food intake than energy density (28, 78, 79). Humans were shown to consume a constant weight or volume over time (77, 79, 80). The volume of food that is consumed is related linearly to the orosensory exposure to food in a natural way of eating. The controlled experimental studies in this thesis showed that variations in orosensory exposure to the food affect the volume/weight of the food that was consumed (chapters 3-6). Therefore, we assume that orosensory exposure to the food may be the controlling factor of food intake rather than the volume. This is supported by the results of Hogenkamp et al. (80), who showed that intake was primarily affected by eating rate. Consumption with straws increased eating rate, thus less orosensory exposure, and led to a constant higher intake over 10 days compared to consumption with spoon. Moreover, consumption of liquid foods results in greater intakes compared to solids and semi-solid foods, due to less orosensory exposure (9-12, 181). When the orosensory exposure (the bites and bite intervals) was held constant between the liquid and semi-solids, the intakes were similar (10, 12).

In addition to sensory signals, also cognition may have played an important role in the results that were found in this thesis. Besides sensory processes, cognitive processes highly influence satiation (25, 50). Humans may associate foods higher in taste intensity, thus stronger tastes, as foods that are higher in nutrient density. Less food needs to be consumed for an appropriate ingestion of nutrients of a nutrient dense food. Therefore, foods higher in taste intensity may lead to earlier satiation. Foods that require long oral residence duration and small bites are mostly solid foods. Solids are more energy dense than liquids in general. Humans, therefore, may have an association between oral residence duration, bite size and the energy density of the foods. This association may affect food intake. We showed that consuming with large bites led to an underestimation of the amount consumed (chapter 6). Consumption with larger bites is associated with relatively fewer bites for the same amount of food to be consumed. The fact that relatively fewer bites are taken when consuming with larger bites may explain
the underestimation. This underestimation during consumption may have led to higher food intake compared to consumption with small bites.

Consumption in a food-focused state led to lower intake compared to consumption in a distracted state, as shown by the results in chapter 6 and many other studies (35, 38-41). Awareness of eating, or ‘mindful’ eating, increases the sensitivity of the senses toward eating, like the taste, smell, sight and (mouth)feel. Mindful eating has been shown to be efficient in body weight management (242-244). Distraction impairs the sensory experience among eating, which may delay satiation. Some studies suggest that increased food intake in distracted states are caused by the decrease of visual cues toward food (35, 50, 109). We showed that distraction still leads to higher food intake also when visual cues were excluded. This suggests an important role for the attention to the taste of the food in satiation.

**Implications and future research**

With regard to the prevalence of obesity, more attention should be paid to the satiating capacity of foods. The satiating capacity should be added as a quality attribute in designing and marketing of new foods. This thesis learned that orosensory exposure is an important factor in the satiating capacity of foods. The orosensory exposure is influenced by bite size, oral residence duration and probably other oral processing like chewing. In this thesis, the focus was on orosensory exposure per gram because we used the same test food throughout all studies. In order to reduce energy intake, it is important to change the focus to orosensory exposure per calorie. The orosensory exposure per calorie can be influenced by the physical and extrinsic (that influence manner of consumption) properties of food, and by the energy density.

Physical food properties influence the bite size, oral residence duration, chewing and other mouth movements (94, 219). Viscosity is negatively related to bite size and positively to oral residence duration (12). The roles of food properties like coatings, particles, hardness, tenderness, stickiness, and chewiness, on orosensory exposure have to be investigated. Also food unit size, height and width, (chips, French fries, chocolate) influences the orosensory exposure and may contribute to the satiating capacity. That smaller food unit size lead to considerably lower intake without differences in fullness compared to larger unit sizes was recently illustrated in a study that used different sizes of rice balls (245). Large portion sizes were also associated with higher eating rate and bite size compared to normal portion sizes (97, 246). Besides physical food properties, the manner of consumption also determines the orosensory exposure to food. Drinking from a cup lead to larger sip sizes than drinking with a straw (221), and straws, lead to larger sip/bite sizes than consumption with spoons (80). In addition, packaging can
influence the manner of consumption. Decreasing the diameter of the neck of a bottle may reduce sip size. Smaller spoons may be helpful to reduce bite size. In conclusion, the results in this thesis give insight in how to change the satiating capacity of foods. The oral residence duration and especially the bite size seems to be very efficient in this respect (chapters 4-6). The influence of other oral processes, for example chewing, on satiation need to be further investigated. Moreover, the relations between of physical and extrinsic food properties on the satiating capacity should be investigated in order to prevent overconsumption.

Opposite approaches can be used to decrease the satiating capacity of foods, for example for underweight elderly or infants. Fast eating rate, thus short orosensory exposure, diminishes the satiating capacity of foods and may increase energy intake. Some studies attempted to increase energy intake in elderly by offering flavour enhanced foods (247-249). Taste and smell is impaired with age, therefore flavour enhanced foods were offered to elderly to increase the palatability. Flavour enhanced foods did not increase food intake in these studies (247-249). This thesis learned that taste intensity directly negatively affects satiation and not necessarily has to be mediated via pleasantness (Figure 7.1). Increasing the taste or flavour may therefore not be a helpful way to delay satiation. Facilitate food intake by increasing the eating rate may be more effective in increasing food intake than change the taste of the food.

We do not know if reductions in food intake due to an increased orosensory exposure per gram food are compensated later on the day of over a few days. The orosensory exposure to the taste of food is considered to not only influence satiation but also satiety. Slower eating rate, that increases the orosensory exposure to the food, led to higher responses of satiety hormones (241, 250, 251). Reducing bite sizes by an oral device, thus increasing the orosensory exposure, led to reductions in meal size, whereas the changes in hunger and fullness did not differ from normal intake (98, 224). Moreover, some studies suggest that humans do not compensate well to moderate changes in energy intake over multiple days (223, 252, 253). Levitsky et al. (253) showed no energy compensation when lunch was replaced a by lunch lower in energy content over a period of 10 days. These results encourage the idea that achieving earlier satiation may lead to decreases in energy intake on the long term. The final challenge will be to investigate if increased orosensory exposure the taste of food, established by physical food properties or manner of consumption, leads to sustainable reductions in energy intake.

The eating rate, bite size and oral processing are determined by food properties (10-12, 94, 218, 219), but also by the individual (219, 221). Several studies have suggested a positive relationship between eating rate and body weight status (15-18). Laboratory studies found that obese people take larger bites, eat more quickly, and this has been associated with greater food intake (15, 234, 235). Spiegel (94), however, found no
differences in eating rate and bite size between lean and obese subjects. Zijlstra et al. (89) found that obese subjects consumed with larger bites from one food but not from another food, whereas eating rates did not differ compared to lean subjects. Slow eating rate interventions resulted in different outcomes regarding food intake. It has led to decreased food intake (13, 233), decreased food intake only in men but not in women (254), decreased food intake only in subjects who consumed large amounts of foods (14), no change in food intake (255), or even higher food intake (213). Together, these interventions do not clearly link slower eating rate to reductions in energy intake and thereby not supporting slow-eating training as a component of behaviour treatments for obesity. This may due to the way that slow eating rate was established. The results of this thesis demonstrate that lowering eating rate by smaller bite size and longer oral residence duration would be more effective than by lowering the bite frequency (Figure 7.2). Slowing down the bite frequency or increase the pauses during consumption may not be an effective way to reduce food intake, as confirmed by the outcome of some of these studies (14, 213, 255).

Training obese subjects to slow down eating rate is possibly efficient to increase the sensitivity to signals of satiation (251). A sustained lower eating rate is not easy to maintain. Slow down eating rate by a mandometer (i.e., a computerised device that provides real time feedback to participants during meals to slow down eating rate) has been shown to improve weight loss during 12 months in addition to standard dietary and activity counselling (233). However, the results weakened within six months after a treatment of one year with the mandometer. Moreover, consuming a food high in energy density at a slow eating rate still involves a relative high caloric eating rate (energy/time). Choosing foods that involve low caloric eating rate (i.e., “slow foods” 212), thus more orosensory exposure per calorie, is probably easier and therefore more effective in reducing energy intake than slow down a person’s eating rate. More attention to the satiating capacity of foods by the food industry will make it easier to choose for satiating foods. This will not only help the obese population but will also be effective in decreasing the prevalence of obesity.

Food intake regulation in infants starts with drinking milk from mother’s breast or bottle. In the Netherlands, 81% of the infants are breastfed after birth (0 months), the number is reduced to 48% at the age of 1 month and reduced to 13% at 6 months (256). There is evidence that breastfeeding reduces the risk of obesity by 10-30% through adulthood (257-259). A possible explanation is that breastfeeding involves self-regulation of energy intake, as it is primarily regulated by the infants’ responses on satiation. Bottle-feeding involves much more maternal control of energy intake than breastfeeding. In addition, bottle-feeding is associated with a lower frequency of the feeds and probably alters the eating rate and duration. A recent retrospective study (260) showed that children aged 3 to 6 years who were breastfed in the first three months of
life had higher responsiveness to satiety than children who were bottle-fed with human milk (measured by the Child Eating Behaviour Questionnaire (261)). Breastfeeding may be important for the fundamental control of food intake throughout life. Effects of breastfeeding vs. bottle-feeding on food intake regulation in later life needs to be further investigated.

**Main conclusions**

This thesis demonstrates that consuming foods with smaller bite sizes, longer oral residence duration and higher taste intensity lowers food intake. These effects are possibly explained by influencing the total magnitude of orosensory exposure to the taste of the foods. More orosensory exposure to the taste led to faster satiation. However, taste intensity did not influence satiation when food was presented as single lunch-item in a hungry state. Sensory processes of satiation may be dependent on state of hunger and meal composition.

The largest impact on satiation was shown by changing the bite size. Consumption with large bites led to underestimations of the amount consumed. This implies that consumption with large bite sizes may impair the control of food intake, which is a risk factor for overconsumption.

With regard to the high prevalence of obesity, more attention is needed for the satiating capacity of foods. The latter is influenced by the total magnitude of orosensory exposure. Increasing the orosensory exposure can be achieved by changing physical and extrinsic food properties. More research is needed to link these food properties to factors that influence orosensory exposure, as bite size, oral residence duration, chewing and other oral processes.
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Samenvatting

(Summary in Dutch)
Samenvatting

Achtergrond

De prevalentie van obesitas en overgewicht is de laatste decennia fors toegenomen. Obesitas en overgewicht zijn een gevolg van een langdurige positieve energiebalans, waarbij de energie inname uit voedsel hoger is dan het energie verbruik. De toename van obesitas en overgewicht in onze samenleving is waarschijnlijk gerelateerd aan de veranderingen in het voedselaanbod. Ons huidig voedselaanbod wordt gekenmerkt door producten die met weinig moeite snel gegeten kunnen worden. Dit leidt tot een relatief snelle inname van energie. Voeding die geassocieerd is met een snelle inname van energie zijn bijvoorbeeld energie houdende frisdranken en producten met weinig vezels. Onderzoek heeft uitgewezen dat een snelle inname van energie gemakkelijk leidt tot inname van meer energie.

Een snelle inname van voedsel leidt tot minder orale sensorische blootstelling aan dit voedsel. Orale sensorische blootstelling is de waarneming van smaak, geur en textuur in de mond, in andere woorden, het proeven van voedsel. De orale sensorische blootstelling aan voedsel is belangrijk voor de regulatie van de voedselinname. De smaak van voedsel bepaalt de voedselkeuze. Naast het belang van smaak voor het beginnen van een eetmoment, is het ook belangrijk voor de beëindiging van een eetmoment, dus voor verzadiging. Eerdere onderzoeken hebben uitgewezen dat wanneer voedsel direct in de maag wordt toegediend, mensen zich veel minder verzadigd voelen dan wanneer ze hetzelfde voedsel gewoon via de mond gegeten hadden. Het proeven van voedsel is dus belangrijk voor het verzadigingsgevoel en het uiteindelijk beëindigen van een eetmoment.

Doel van dit proefschrift

Het doel van dit proefschrift is om de belangrijkste mechanismes te onderzoeken waarmee de orale blootstelling aan smaak van voedsel de verzadiging beïnvloedt. We hebben onderzocht of de sterkte van de smaak, de duur van blootstelling aan de smaak en de hapgrootte van invloed zijn op verzadiging. De resultaten leiden tot meer inzichten in het verzadigingsproces en bieden mogelijkheden om overconsumptie (het consumeren van te veel energie) tegen te gaan.

Onderzoeken

In vijf experimenten onderzochten we of de smaakintensiteit, de duur van blootstelling aan smaak in de mond, en de hapgrootte en van invloed zijn op verzadiging. Om verzadiging te meten hebben we gekeken naar de hoeveelheid voedsel die mensen aten en naar subjectieve scores van honger en verzadiging. Als testproduct werd in alle experimenten tomatensoep gebruikt die onbeperkt kon worden geconsumeerd tijdens de lunch. In alle experimenten deden rond de 50 gezonde, jongvolwassen (18-35 jaar) deelnemers mee die allen een normaal gewicht hadden.
In de eerste drie experimenten (hoofdstukken 2-4) at elke deelnemer twee soepen, één met een lage en één met een hoge smaakintensiteit, op twee verschillende dagen. De smaakintensiteit was aangepast door het zoutgehalte te variëren. Er werden twee zoutgehaltes geselecteerd (een lage en een hoge) die ongeveer even lekker waren. Het eerste onderzoek wees uit dat er geen effect was van smaakintensiteit op de hoeveelheid soep inname (hoofdstuk 2). In dit onderzoek waren de deelnemers hongerig, na het ontbijt hadden ze niets meer gegeten en kregen ze enkel de soep aangeboden als lunch. Wanneer de deelnemers in een matig verzadigde staat waren voor consumptie van de soep, werd 8-9% minder van de soep met de hoge smaakintensiteit gegeten vergeleken met de soep met de lage smaakintensiteit (hoofdstukken 3 en 4). Dit effect werd ook gevonden als de deelnemers hongerig waren (dus na het ontbijt niets meer gegeten hadden) maar wisten dat ze na de soep nog verder mochten eten van een broodmaaltijd (hoofdstuk 3). Deelnemers gaven zich even vol te voelen na het eten van de soep met lage als hoge smaakintensiteit. Een hogere smaakintensiteit leidt dus tot een snellere verzadiging en minder voedsel inname. Dit effect is echter afhankelijk van de context, zoals de staat van honger en uit hoeveel verschillende componenten de maaltijd bestaat.

In hoofdstuk 5 keken we naar het effect van de duur van blootstelling aan het voedsel in de mond op verzadiging. Dit hebben we gedaan door deelnemers happen aan te bieden via een slang die verbonden was met een pomp. De pomp reguleerde de happen. De duur van het voedsel (per hap) in de mond werd gereguleerd door middel van geluiden (piepjjes) die aangaven wanneer er een hap aankwam en wanneer deze werd geacht doorgeslikt. Een verlenging de duur van een hap in de mond met factor drie (een hap 3 seconden tegenover 9 seconden in de mond, en 1 seconde tegenover 3 seconden) leidde tot 8% minder soep inname, terwijl de deelnemers zich even vol voelden. Een langere blootstelling aan de smaak van voeding in de mond leidt tot een snellere verzadiging en minder voedsel inname.

In de experimenten beschreven in hoofdstukken 4-6 blijkt dat hapgrootte een belangrijke rol speelt in verzadiging. Happen van 5 gram leidden tot ongeveer 25% minder inname dan wanneer er met happen van 15 gram werd gegeten. In deze experimenten werden de happen eveneens gereguleerd door middel van een pomp. Deelnemers beoordeelden gevoel van honger en verzadiging tijdens het eten, namelijk na consumptie van elke 75 gram soep. Kleinere happen zorgden voor een snellere daling van het hongergevoel per gegeten gram voedsel (hoofdstukken 4 en 5). Kleinere happen bleken tot een grotere blootstelling aan de smaak per gram voedsel te leiden (hoofdstuk 5). Deze grotere blootstelling aan de smaak zou kunnen verklaren waarom kleine happen meer verzadigend zijn dan grote happen.

Eten met kleine happen betekent dat je meer happen moet nemen voor het consumeren van dezelfde hoeveelheid voedsel. Eten met grote happen kost minder moeite dan eten
Samenvatting

met kleine happen. Doordat grotere happen geassocieerd zijn met relatief minder happen, zou het kunnen zijn dat mensen hun werkelijke inname onderschatten. Dit zou kunnen leiden tot een hogere voedselinname. In hoofdstuk 6 onderzochten we het cognitieve effect van hapgrootte op verzadiging. Deelnemers aten onbeperkt met zowel grote als kleine happen (aangestuurd via de pomp). Daarna kregen ze de instructie om zelf soepkommen te vullen met de hoeveelheid soep die ze dachten gegeten te hebben. De deelnemers bleken hun soepconsumptie te onderschatten als ze met grote happen hadden gegeten. Dit was eveneens het geval als de hapgrootte zelf bepaald werd door de deelnemer. Eten met kleinere happen leidde niet tot een onderschatting van de gegeten hoeveelheid voedsel, maar tot een niet-significante overschatting.

Conclusies

De experimenten beschreven in dit proefschrift laten zien dat het consumeren van voedsel met kleinere happen, met een langere verblijfsduur in de mond en met een hogere smaakintensiteit leiden tot een lagere voedselinname. Deze effecten worden mogelijk verklaard door de verhoogde sensorische blootstelling aan de smaak van het voedsel in de mond. Consumptie met grotere happen leidt tot een onderschatting van de gegeten hoeveelheid, dit kan leiden tot overconsumptie. De voedingsindustrie zou deze resultaten kunnen gebruiken om de verzadigingscapaciteit van voedsel te verhogen om de prevalentie van obesitas te verlagen.
Dankwoord

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About the author
Curriculum Vitae

Dieuwerke Bolhuis was born on January 11\textsuperscript{th}, 1982 in Groningen, the Netherlands. After completing secondary school at ‘Het Hogeland College’, she started the Bachelor’s programme of ‘Pharmacy’ at the University of Groningen. After having received her Bachelor’s degree she enrolled in the research Master’s programme ‘Medical Pharmaceutical Sciences’. Dieuwerke’s first thesis focused on the relationship between glucose tolerance during pregnancy and the status of long-chain polyunsaturated fatty acids and essential fatty acids in the fetus. Research for this thesis was carried out at the Sint Elisabeth Hospital in Willemstad, Curaçao, Netherlands Antilles and at the Clinical Chemistry Group of the University of Groningen. The second thesis was in the field of Medical Genetics, this was executed at the Center for Medical Genetics at the Miller School of medicine at the University of Miami, USA. She graduated in November 2007 for her Master’s degree. She was appointed as PhD-student at the Wageningen University at the Product Design and Quality Management Group in December 2007. The topic of this PhD-project was the role of taste and texture on sensory specific satiation. Dieuwerke joined the educational programme of the Graduate School VLAG and she was involved in teaching and supervision of Master and Bachelor students. Dieuwerke attended several (international) conferences and courses. Furthermore, she was a member of the organizing committee of the PhD study tour from the Product Design and Quality Management Group in 2010. This was a two-week tour to visit several companies and universities in Melbourne and Sydney in Australia. Besides her PhD-project, Dieuwerke was involved in a large intervention study on salt-reduction in bread, which was in collaboration with scientists of the group of “Consumer Science & Intelligent Systems” of Wageningen University and Research. This study is published in the Journal of Nutrition (see List of Publications) and has showed up in the media several times. Currently, Dieuwerke is working as a postdoctoral researcher at the Division of Human Nutrition of Wageningen University, where her research focusses on salt perception and salt preference.
List of Publications

Publications in peer-reviewed journals


Bolhuis DP, Lakemond CMM, de Wijk RA, Luning PA, de Graaf C. Longer oral sensory exposure time and higher intensity of saltiness both lead to lower ad libitum food intake. Journal of Nutrition. 2011;141(12):2242-8.


Submitted papers

Bolhuis DP, Lakemond CMM, de Wijk RA, Luning PA, de Graaf C. Both higher number of bites and longer oral residence duration increase the oral sensory exposure to food and reduce ad libitum food intake.

Bolhuis DP, Lakemond CMM, de Wijk RA, Luning PA, de Graaf C. Consumption with large bite sizes increases food intake and leads to underestimation of the amount consumed.


Abstracts

Bolhuis DP, Lakemond CMM, de Wijk RA, Luning PA, de Graaf C. Both the number of bites and the oral residence duration increase the oral sensory exposure to food and reduce ad libitum food intake. Appetite. 2012;59(S1):e8 (abstract for Society for the Study of Ingestive Behavior (SSIB), annual meeting, 2012, Zurich, Switzerland, poster presentation)
About the author

Bolhuis DP, Lakemond CMM, de Wijk RA, Luning PA, de Graaf C. Effect of salt intensity on ad libitum intake of tomato soup and on sweet vs. savoury food choice afterwards. Appetite. 2011;57(2):542 (abstract for the British Feeding and Drinking Group, annual meeting 2010, Maastricht, The Netherlands, oral presentation)


Overview of completed training activities

**Discipline specific courses**

Course “New directions of sensory food research”, 2009 (ABS Finnish Graduate School, Helsinki, Finland).

Course “Smell and Taste”, 2008 (University of Dresden, Germany).

Course “Regulation of food intake”, 2008 (graduate school VLAG, Maastricht, The Netherlands).

Workshop “The influence of sensory and normative cues on human food intake”, 2008 (Radboud University, Nijmegen, The Netherlands).

Course “Food perception and preference”, 2007 (VLAG Wageningen, The Netherlands).

**Conferences and meetings**

Conference: VoedingNederland, 2012 (Nieuwegein, the Netherlands, oral presentation, winner of own session).

Annual meeting (20th) of the Society for the Study of Ingestive Behavior (SSIB), 2012 (Zurich, Switzerland, poster presentation).

Pangborn Sensory Science Symposium (8th and 9th), 2009 and 2011 (Florence, Italy and Toronto, Canada, poster presentations).


Annual meetings (33rd and 34th) of British Feeding and Drinking Group, 2009 and 2010 (Swansea, UK, and Maastricht, The Netherlands, oral presentations).


Weurman Flavor Research Symposium (12th), 2008 (Interlaken, Switzerland, poster presentation).

**General courses**

Course “A practical and theoretical introduction into fMRI”, 2011 (University of Utrecht, Utrecht, the Netherlands).

Statistical courses “Linear models and Mixed linear models”, 2010 (Graduate school PE&RC, Wageningen, the Netherlands).
About the author

Educational course “Teaching and supervision thesis students” 2010 (Wageningen, The Netherlands).

Training day “Funding” 2010 (NWO (Dutch organization for scientific research) Den Haag, The Netherlands).

Workshop “How to write a world class paper” 2010 (Wageningen Graduate Schools (WGS) Wageningen, The Netherlands)

Course “Effective behavior in your professional surrounding” 2009 (WGS, Wageningen, The Netherlands).


Course “Advanced guide to scientific artwork” 2009 (WGS, Wageningen, The Netherlands)

Course “Interpersonal communication skills for PhD-students” 2009 (WGS, Wageningen, The Netherlands).

“PhD Introduction course” 2008 (VLAG, Bilthoven, The Netherlands).

Course “Introduction Literacy, including introduction to Endnote” 2008 (WGS, Wageningen, The Netherlands).

“PhD-competence assessment” 2008 (WGS, Wageningen, The Netherlands)

Optional courses and activities

Organizing and participating in PhD study tour to Australia, 2012

Preparation research proposals, 2008-2012

Research presentations, 2008-2012
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