# THE ROLE OF SWEET AND SAVOURY TASTE IN FOOD INTAKE AND FOOD PREFERENCES

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# The role of sweet and savoury taste in food intake and food preferences

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Thesis
submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus
Prof. dr. M.J. Kropff,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Friday 20 January 2012
at 1:30 p.m. in the Aula

# Abstract

**Background and aim**

The sensory attributes of food play a key role in the selection and termination of meals and their rewarding properties. The majority of our foods are either sweet or savoury tasting. In addition, within our food range, savoury-tasting foods contain in general higher levels of protein. The effect of specific taste modalities on human food intake, however, requires further clarification. The primary aim of this thesis was to investigate the role of sweet and savoury taste in food intake and food preferences. The secondary aim was to provide more insight into the processes of explicit and implicit liking and wanting, to be able to identify underlying reward mechanisms involved in food intake behaviour.

**Methods**

We conducted series of experiments where healthy young adults participated. We started by investigating the difference between a sweet and savoury taste on satiation, independent of palatability, texture, energy density, and macronutrient composition (n=64). Next, the effect of sweet and savoury taste of a single meal on subsequent satiety and food preferences was investigated (n=61). To further explore the effect of taste in the context of a complete diet on satiety and food preferences, the effect of three 24-h diets that differed only in taste (predominantly sweet tasting, predominantly savoury tasting, or a mixture of sweet and savoury tasting) were compared (n=39). Next, we separated the influence of taste from within-meal protein content on satiety and food preferences, by comparing the effect of sweet and savoury high and low protein single meals (n=60). Finally, the effect of long-term protein status on satiety and food preferences was investigated by comparing the effect of two 14-d diets that differed in protein content (a low protein diet vs. a high protein diet) (n=37).

**Results**

Sweet and savoury taste, independent of palatability, texture, energy density, and macronutrient composition, did not differ in their effect on satiation and satiety in terms of subsequent *ad libitum* intake. Sweet and savoury taste did differ in their effect on subsequent food preferences. In general, after eating a food with a certain taste, appetite for foods with a similar taste was lower than for foods with a dissimilar taste, hence, a clear transfer effect of sensory specific satiety was demonstrated. This transfer effect was not equipotent for sweet and savoury taste; after eating a sweet single meal or sweet 24-h diet, preferences for sweet and savoury foods did not differ. Eating a savoury single meal or savoury 24-h diet, however, led to a clear preference for sweet foods. Neither sweet or savoury tasting single meals nor sweet or savoury 24-h diets shifted food preferences towards high or low protein foods. It was shown that protein content of a meal, inde-

pendent of taste, did not have an effect on satiety and food preference. We did observe, however, an effect of protein status: after a 14-d low protein diet, there was an increase in *ad libitum* protein intake, compared with after a 14-d high protein diet, while total energy intake was not different. In addition, food preference for savoury high protein foods was increased.

Regarding the different components of food reward it was demonstrated that in all studies both explicit and implicit measures correlated with several aspects of eating. It appeared that in a controlled setting, i.e. in the sensory booths, explicit processes played a stronger determining role in satiation (meal size) than implicit processes. Food choices appeared to be made on a more unconscious level. In a setting where subjects could behave more naturally (i.e. self-selection and serving of foods in a relaxed environment where subjects could sit and eat together), implicit, unconscious processes seemed to explain food intake behaviour more than explicit processes. When subjects experienced protein shortage, after the 14-d low protein diet, it appeared that implicit processes of wanting played a stronger determining role in decisions about what to eat.
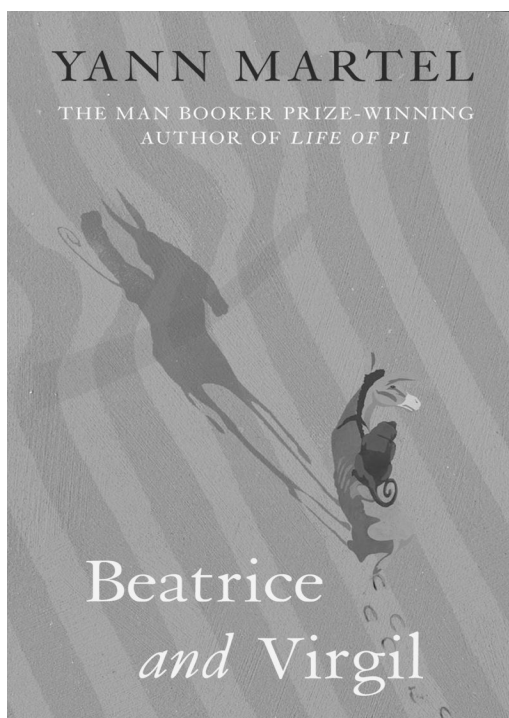
### Conclusion

Sweet and savoury taste do not differ in their effect on satiation or satiety in terms of subsequent *ad libitum* intake. The taste of a meal or diet does have a large effect on subsequent food preferences, thereby showing a clear transfer effect which is not equipotent for sweet and savoury taste. Savoury taste exerts a stronger modulating effect on subsequent food preferences than sweet taste. Sweet and savoury taste of a single meal or 24-h diet do not differ in their effect on food preferences for high or low protein foods. In addition, within-meal protein content seems not to influence satiety or food preferences. A low protein status, however, through selective reduction of dietary protein intake, elicits compensatory changes in food intake and food preferences to restore adequate protein status. It appears that both conscious (explicit) and unconscious (implicit) processes are involved in satiation and food choice. The role implicit motivational processes play in driving food choice is not static, but appears to vary. This is especially the case when homeostasis is challenged (by depleting macronutrient stores), where implicit processes of wanting appear to play a stronger determining role in decisions about what to eat.

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# Appetizer



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# Chapter one

## —General introduction

Food intake is critical for survival (1). It provides the body with all the necessary macronutrients (carbohydrates, fats, and proteins) and most micronutrients (minerals and vitamins). In addition, food intake is a fundamental determinant of energy balance (2, 3), contributing to maintenance of stable body weight (3).

The initiation and termination of an eating episode is a complex behaviour that involves many regulated parameters (4). In our modern society, humans have the opportunity to select foods from a wide array of alternatives with varying hedonic attributes, nutrient compositions, and energy contents. The result is that the average number and size of meals per day varies widely among and within individuals. Neither the timing nor the size of meals are fixed, and individuals are able to adjust to a wide array of schedules (5). From an evolutionary point of view, the ability to be flexible and adaptable with regard to meal patterns provides a great advantage, as it allows organisms to adjust to a broad range of different environments (6). In addition to this flexibility, however, physiological controllers involved in food intake and food preferences must exist, in order to keep daily energy and macronutrient intake somewhat constant.

The research described in this thesis investigated the role of sweet and savoury taste in food intake and food preferences. To explore underlying reward mechanisms involved in food intake behaviour, different components of food reward were investigated. This introduction starts with a brief overview of the main contributors to food intake, with a focus on the sensory processes involved. This will be followed by an overview of the different components of food reward and the proposed ways of measuring these. Lastly, the aim and thesis outline are described.

## Food intake regulation

Controlling meal size is an effective strategy for maintaining stable body weight (7). Many studies have shown the existence of meal-generated 'satiety signals' that arise during eating and contribute to meal termination (or meal size) (1).

When food is ingested, the food interacts with receptors on the tongue, the oropharynx, the stomach, and the intestine, as well as the liver and other organs. The detection, processing, and absorption of food generate hormonal and neural signals from the gastrointestinal tract that signals to the brain which in turn modulates feelings of satiety. Stretch receptors in the stomach and various hormones, like cholecystokinin, glucagon-like peptide 1, and insulin, but also nutrients like glucose, fatty acids, and amino acids, are involved in this process. After eating the meal, the duration of satiety depends on the amount and the composition of the foods ingested. As well as regulation of food intake

in the short-term, energy balance is also regulated in the long-term, and involves signals generated from adipose and lean tissue mass (1, 7, 8).

Over 20 years ago the mediating process involved in the initiation and termination of an eating episode were conceptualized by Blundell, Rogers and Hill (9) in the 'satiety cascade' (Figure 1.1). Within this framework, two separate processes are identified: satiation and satiety. Satiation is used to describe the processes that bring an eating episode to an end, incorporating all events that operate during the course of the meal. Satiety is used to describe the processes that operate after a meal has been eaten, involving the suppression of hunger and inhibition of further eating. Together, satiation and satiety are major determinants of the size and frequency of meals in the pattern of eating.
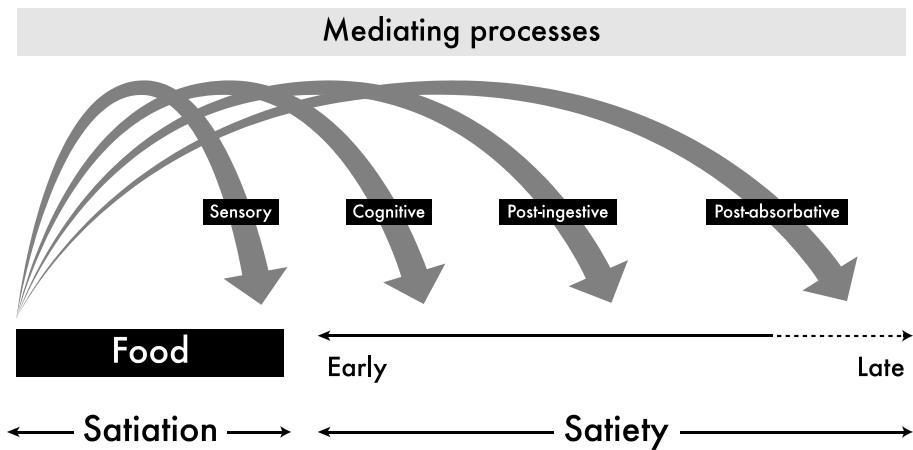


**Figure 1.1** *The satiety cascade of Blundell, Rogers and Hill, 1987 (9).*

# Sensory processes

Determining when and how much to eat presents a crucial daily challenge for an organism. Living in a stable environment with predictable access to food allows establishing regular eating patterns. It is believed that in this kind of environment, sensory attributes of food, like taste, texture and smell, can become reliable predictors of food quality and energy content. These sensory signals can hereby obtain the ability to guide food intake behaviour and how much to eat of a particular food (7).

**Conditioned satiety**
It is conceived that through repeated consumption of food during our lifetime we learn

to associate the sensory attributes of food with their physiological effects and consequently learn to estimate their metabolic effects (Figure 1.2).

The phenomenon of linking sensory signals to metabolic consequences is called conditioned satiety, and was first demonstrated by Le Magnen (10) and Booth (11). They showed that by repeated ingestion of a food, an unconscious learning process is induced, in which the central nervous system associates the sensory attributes of this food with its post-ingestive metabolic effects and its energy content (12). For example, in hungry rats where protein was infused in the stomach while they drank a distinctively flavoured non-nutritive fluid, a conditioned preference for this particular flavour was seen when they experienced hunger again. However, when protein was infused during the deprivation period before the test, no flavour preference was observed (13). Once the taste of an ingested food is associated with an appetitive or aversive signal, the individual reacts to subsequent exposure by increasing or decreasing ingestion of this food. This associative learning influences selection of food and food intake and has been suggested to play a central role in the development of specific appetites (13, 14).
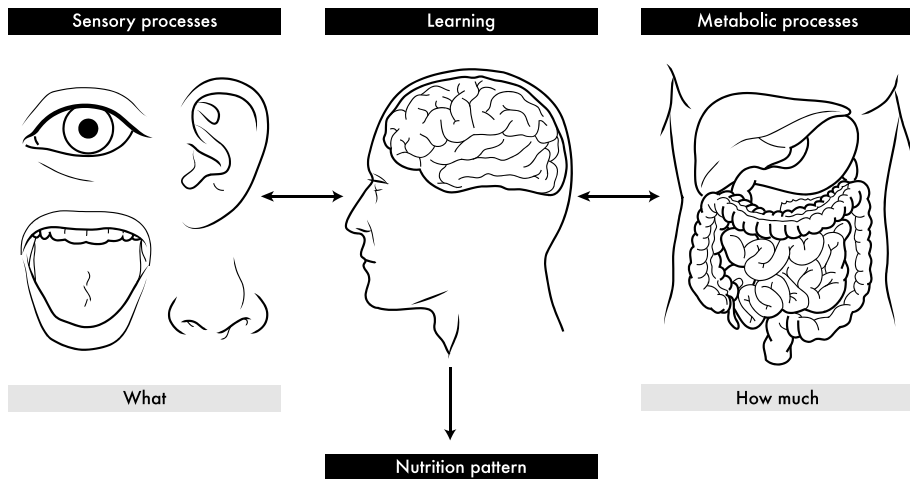


*Figure 1.2* Sensory signals during eating are linked to the metabolic consequences. These learning processes shape our eating patterns [adapted from de Graaf and Kok (15)].

### Sensory specific satiety/satiation

As absorption of nutrients occurs mostly after a meal is ingested, the use of sensory signals arising before and during contact with food may also be involved in the control of food intake (7). And indeed, it has been established that satiation is to a large extent mediated by sensory processes, generated from the sensory qualities of food. Decreas-

ing pleasure from prolonged exposure to the sensory qualities is a key factor believed to contribute to the termination of a meal (16, 17).

The first statement linking the sensory attributes of food to the processes of satiety was made by Katz in 1934, who observed in chickens that satiety occurred very soon when only one type of food was offered. However, by enriching the food with one or two other types, satiety could be delayed. In 1940, Young showed that pre-feeding rats in a controlled manner could reverse food preferences. Normally, rats prefer sugar to wheat. But if rats were pre-fed sugar before a choice task, this preference reversed (18). In 1956, Le Magnen showed in rats that changing the odour of offered food within one meal produced a substantial increase in intake, in comparison to when the odour was kept constant (10).

The first demonstration of 'sensory specific satiety/satiation' (SSS) in humans was made by Rolls, Rolls, Rowe, and Sweeney (19), who showed that the pleasantness of a particular food was decreased after it was eaten as a first course in a meal. Moreover, the observed decrease in pleasantness was associated with a lower intake of that particular food when presented in a second course. As SSS has been demonstrated from as early as two minutes following meal initiation (20), before digestion and absorption of nutrients can occur, it is likely to be specific for the sensory attributes of the eaten food. SSS have been demonstrated for several attributes of food, including taste, smell, texture, and appearance (16, 21-26).

SSS is also conceived as the drive for variety-seeking behaviour: "Variety in a meal enhances food intake in man" (27, 28). When a large variety of different foods are available, the surest way for an organism to receive adequate nutrition would be to ingest a wide selection of foods. It appears that when more than one food is available there is a natural tendency to switch between foods rather than just consume the most preferred food (18).

## Taste

The sensory system is responsible for generating an internal representation of the outside world, which includes its chemical (taste and smell) and physical (tactile, sound, sight and temperature) features (29). The sense of taste is involved in evaluating the nutritional content of food and preventing the ingestion of toxic substances. Among the senses, taste is unique in its innate association with mechanisms of reward and aversion (30) in addition to its recognition of quality (31).

The sense of taste detects and discriminates between sweet, bitter, sour, salty, and savoury stimuli (with savoury referring to non-sweet, 'umami taste', which can also be

described as 'meaty' or 'broth-like') (32). It has been posed that sweet taste acts as a signal for energy-rich nutrients, that savoury taste allows the recognition of amino acids, salt taste has been linked to dietary electrolyte balance, and sour and bitter tastes are posed to warn against the intake of potentially toxic substances (29). Recently, evidence has accumulated to support the existence of a taste component for the perception of fatty acids (33, 34). The taste system is capable of distinguishing between these various taste modalities and can generate innate behavioural responses. For instance, animals have a strong innate aversion to bitter-tasting compounds, but are attracted to sweet and savoury stimuli (35, 36). In humans, sweet and savoury are the main attractive taste modalities (37).

### Sweet and savoury taste

An important distinction can be made between sweet and savoury taste, which includes almost 90% of the food we eat (38). Over the course of a day, profiles of appetite for something savoury and appetite for something sweet show different patterns; it appears that appetite for something savoury oscillates more in line with the pattern of meals, i.e. more related to feelings of hunger, whereas appetite for something sweet is more stable during the day (39). It is unclear, however, whether sweet and savoury differ in their influence on satiation and satiety. In addition, their role in food intake regulation beyond a single eating occasion needs further clarification.

Interestingly, within our food range, savoury-tasting foods contain in general higher levels of protein, while sweet-tasting foods contain more carbohydrates (40, 41). Protein is an indispensable component within the human diet. It provides the body with nitrogen and amino acids that are of crucial importance in preserving and maintaining bodily functions (42). In humans, the range of protein intake has remained relatively constant over time and across the population, both as a percentage of energy in the diet (~10-25%) and in terms of absolute amount eaten (~40-100 g) (43-45). It is therefore argued that protein intake is tightly regulated, and prioritised over the intake of carbohydrate and fat (44, 45). The role sensory attributes of food play in protein intake regulation, however, is far from clear and requires further clarification.

# Components of food reward

As food intake is critical for survival, it follows that eating is a highly rewarding behaviour (46). As previously stated, taste is involved in evaluating the nutritional content of food and prevents the ingestion of toxic substances. Taste, however, has the additional attribute of contributing to the overall pleasure and enjoyment of a meal (29). While food intake is clearly regulated by short and long term energy homeostasis, it also appears

to be regulated by hedonic brain systems (46-53). A food that is pleasant when hungry may be deemed unpleasant when satiated (54). Moreover, enhancing the palatability of a food often results in an increased intake (even when satiated). Hedonic responses to foods are closely associated with food choice, and mere exposure to a preferred food has been shown to stimulate appetite and craving (e.g. references 16, 55-59). It is thought that both homeostatic and hedonic sensory mechanisms contribute to making food intake such a rewarding experience (46).

### Food liking and food wanting

In 1996, Berridge proposed that when examining the role of food reward in food intake behaviour, one should make the distinction between food 'liking' and food 'wanting', with liking corresponding closely to the concept of palatability and pleasure (hedonic feelings), and wanting corresponding more closely to appetite or craving (the motivation to engage in eating) (60). Research in animals has shown that these components can be manipulated and measured separately, and have separable neural substrates. Liking is associated with opioid activation in specific limbic forebrain structures, or 'hedonic hotspots' (61), while wanting involves mesotelencephalic dopamine systems. In addition, Berridge argued that objective liking and wanting responses reflect 'core' processes that can operate without conscious awareness (for further reading e.g. references 62, 63-65).

| Major categories | Liking (pleasure) | | Wanting (motivation) | |
|---|---|---|---|---|
| **Psychological Components** | Conscious – Pleasure<br><br>Explicit hedonic feelings | Unconscious – Hedonic impact<br><br>Objective affective reactions<br><br>Implicit affect | Conscious – Cognitive incentives<br><br>Goal-directed plans<br><br>Explicit desires | Unconscious – Incentive salience<br><br>Conditioned stimuli (motivational magnet)<br><br>Cue-triggered 'wanting' |
| **Measured Behaviours** | Direct – Explicit<br><br>Subjective ratings of liking or pleasure | Indirect – Implicit<br><br>Facial affective expressions<br><br>Unconscious 'liking' reactions | Direct – Explicit<br><br>Subjective ratings of desire<br><br>Cognitive goals | Indirect – Implicit<br><br>Conditioned approach (reaction time tasks)<br><br>Pavlovian-instrumental responses<br><br>Intake |

*Figure 1.3* Components of liking and wanting, and how each has been measured in experiments. The reward components have different psychological components, both explicit (conscious) and implicit (unconscious), which can be measured in different ways [adapted from Berridge (63)].

**Measuring food reward**

Various tasks and tests have been developed to study these components of food reward separately (Figure 1.3). There are several direct 'explicit' measurements, e.g. subjective ratings (e.g. references 21, 66), which typically tap into conscious attitudes. Most often, however, individuals do not analyse their attitudes towards stimuli consciously. Rather, behaviour seems to be guided by spontaneous interactions with the environment. Indirect 'implicit' measures are proposed to better capture these spontaneous (automatic) reactions to stimuli. Hence, implicit measures could be particularly suited to predict spontaneous, unmonitored behaviour (67, 68).

The definition of an implicit measure is that "the outcome of a measurement procedure is causally produced by psychological attributes in an automatic manner" (68). Many of the implicit measures that have been introduced during the past two years are based on reaction time tasks (68-71). Another procedure which has been suggested to measure implicit wanting processes is the assessment of a subject's willingness to work (i.e. instrumental responses) to get access to a food (72-75). In food research actual intake has always been the ultimate measure of (sensory) satiety, and recently it has been proposed that this is also a likely marker of implicit wanting processes (63).

In terms of fully understanding the processes involved in food intake, it is important that we know how these different components of food reward, operating at explicit (conscious) and implicit (unconscious) levels, relate to eating behaviour (76).

# Aim and thesis outline

The sensory attributes of food play a key role in the selection and termination of meals and their rewarding properties. However, the wider significance of the effect of specific taste modalities on human food intake requires further clarification. The primary aim of this thesis was to investigate the role of sweet and savoury taste in food intake and food preferences. The secondary aim was to provide more insight into the processes of explicit and implicit liking and wanting, to be able to identify underlying reward mechanisms involved in food intake behaviour.

In the first study we investigated the difference between sweet and savoury taste on satiation, independent of palatability, texture, energy density, and macronutrient composition. We assessed this by comparing the intake of homogeneous meals with a sweet and savoury taste (**chapter 2**). In the second study, we investigated the effect of sweet and savoury taste on satiety and food preferences. We assessed this by comparing the effect of sweet and savoury single meals on subsequent *ad libitum* intake and choice of food prod-

ucts that differed in taste and fat content (**chapter 3**). In our third study, we separated the influence of taste from within-meal protein content on satiety and food preferences. We assessed this by comparing the effect of sweet and savoury high and low protein single meals on subsequent *ad libitum* intake and choice of food products that differed in taste and protein content (**chapter 4**). These first three studies were all performed in a controlled setting (sensory booths) and focused on a better understanding of short-term effects, i.e. measurements within one eating episode.

The study described in **chapter 5** investigated the effect of taste in the context of a complete diet on satiety and food preferences. The approach consisted of comparing the effect of three 24-h diets that differed in taste only (predominantly sweet tasting, predominantly savoury tasting, or a mixture of sweet and savoury tasting) on subsequent *ad libitum* intake and choice of food products that differed in taste and protein content. The study described in **chapter 6** investigated the effect of long-term protein status on satiety and food preferences. We assessed this by comparing the effect of two 14-d diets that differed in protein content (a low protein diet vs. a high protein diet) on subsequent *ad libitum* intake and choice of a large array of food products in the 2.5 d that followed. These last two studies were situated in a more naturalistic setting and focussed on revealing long-term effects, i.e. behaviour over several eating episodes.

In all studies described in this thesis one or more advanced psychological tools were included in the design to explore underlying reward mechanisms involved in the displayed eating behaviour.

In the final chapter of this thesis (**chapter 7**) the main findings of the studies are summarized and discussed. Implications and suggestions for further research are given.

**2**

# Chapter two

—Satiation due to equally palatable sweet and savoury meals does not differ in normal weight young adults

Sanne Griffioen–Roose, Monica Mars, Graham Finlayson, John E. Blundell, Cees de Graaf

# Abstract

*Sensory properties are greatly involved in the process of satiation. Regarding the nature of sensory signals, an important distinction can be made between sweet and savoury taste. It is unclear, however, whether sweet and savoury differ in their influence on satiation. Our objective was to investigate the difference between a sweet and savoury taste on satiation, independent of palatability, texture, energy density, and macronutrient composition. A crossover design was used, consisting of 3 test conditions in which 2 tastes (sweet and savoury) were compared. Sixty-four healthy, non-smoking, unrestrained participants (18 males and 46 females), with a mean age of 22.3±2.4 y and a mean BMI of 21.6±1.7 kg/m², enrolled. Rice was used as a test meal served in either a sweet or savoury version. The meals were similar in palatability, texture, energy density, and macronutrient composition. Ad libitum intake, eating rate, and changes of pleasantness and appetite during the meals were measured. Ad libitum intake did not differ between the 2 meals; participants ate a mean of 314±144 g of the sweet meal and 333±159 g of the savoury meal. Eating rate (sweet: 38±14 g/min; savoury: 37±14 g/min) and changes in pleasantness and appetite during the meals were similar. Homogeneous meals with a sweet or savoury taste, similar in palatability, texture, energy density, and macronutrient composition, do not differ in their influence on satiation in normal weight young adults.*

# Introduction

Satiation is defined as the process that develops during eating and brings an eating episode to an end (9). In terms of preventing overconsumption, it is important to identify properties of foods that influence this process. Regarding energy balance, no strong relationship has been found between eating frequency and body weight (77, 78). Because weight gain is characterized by excess energy intake (79-83), meal size might be an important factor contributing to obesity.

Numerous studies have shown that palatability plays a key role in satiation (for review, see reference 16). Enhancing palatability results in an increased intake, observed both inside and outside the laboratory (55, 56, 84). Other properties of food shown to influence satiation are weight/volume (21, 85, 86), texture (87) and macronutrient composition (88, 89). Energy density might play a role as well but appears to involve learning processes (90, 91). Most of above-mentioned factors are thought to play a role in satiation through the sensory properties of the food (9, 16, 17). When a food is eaten to satiety, the pleasantness of that food is decreased in comparison to foods that have not been eaten (19). This is called sensory specific satiety (SSS). SSS can be detected within 2 minutes after consumption has started, before digestion and absorption can occur, and therefore specific for the sensory properties of the eaten food. Because SSS can be

an important factor for meal termination (9, 92), we might better speak of sensory specific satiation. This refers to the decline in reward (60) during consumption of a food, i.e. due to repeated exposure to a particular sensory signal. Regarding sensory signals, an important distinction can be made between sweet and savoury taste, which includes almost 90% of the food we eat (38). It is unclear, however, whether sweet and savoury differ in their influence on satiation.

Our objective was to investigate the difference between a sweet and savoury taste on satiation, independent of palatability, texture, energy density, and macronutrient composition. We assessed this by comparing homogeneous rice meals with a sweet and savoury taste. It is our hypothesis that sweet taste suppresses hunger less, and stimulates appetite more, compared with a savoury taste, resulting in a lower intake of the savoury meal. A second part of our study was focused on the effect of taste on satiety processes, however these results are outside the scope of this paper.
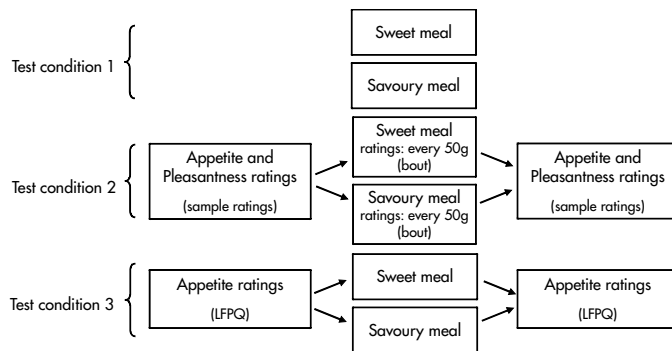


**Figure 2.1** *Overview of study design.*

# Methods

## Participants

Healthy, normal weight participants, aged 18-35 y, were recruited from Wageningen and the surroundings. Exclusion criteria were restrained eating (Dutch Eating Behaviour Questionnaire (DEBQ): men score >2.25; women score >2.80) (93), lack of appetite, an energy restricted diet during the last 2 months, change in body weight >5 kg during the last 2 months, stomach or bowel diseases, diabetes, thyroid disease or any other endocrine disorder, having difficulties with swallowing/eating, hypersensitivity for food products under study, smoking, being a vegetarian and for women being pregnant or lactating. Body weight and height were measured. In total, 64 participants (18 males and

46 females) aged 22.3±2.4 y, with a mean BMI of 21.6±1.7 kg/m² enrolled in the study. All participants completed the study and received financial compensation. Participants were unaware to the exact aim of the study and were informed we were interested in comparing several methods of assessing palatability of rice products. The study was approved by the Medical Ethical Committee of Wageningen University and all participants signed an informed consent.

## Design

We used a randomized crossover design consisting of 3 test conditions in which 2 tastes (sweet and savoury) were compared, resulting in 6 experimental conditions (Figure 2.1). The wash-out period between the experimental conditions was at least 3 d. Preceding the experiment there was one practice day to accommodate participants to the test conditions.

## Test food

Two versions of a rice meal were used as test products, a sweet and a savoury version. Similarity on palatability and texture  was established with a pilot study (Figure 2.2). By comparing the sweet meal with 5 different sucrose concentrations (0, 0.125, 0.25, 0.5 and 1.0 mol/L) and the savoury meal with 5 different NaCl concentrations (0, 0.125, 0.25, 0.5 and 1.0 mol/L) perceived intensity of both meals could be expressed in physical units (94). It was shown that the sweet meal had a perceived intensity that was comparable to 0.38 mol/L sucrose in water and perceived intensity of the savoury meal was comparable to 0.22 mol/L NaCl in water. Prior to the study, energy and macronutrient contents were calculated using the Dutch nutrient database (95). Afterwards, macronutrient content was determined by chemical analysis of samples taken from a homogenous mixture of samples which were collected every testing day (Table 2.1). The core component of both meals was risotto rice (Lassie, Wormer, The Netherlands) (78.0%). The sweet version was made with semi skimmed milk (17.0%), butter (2.2%), cinnamon (0.08%), vanilla sugar (0.5%) and aspartame (3.0%). The savoury version
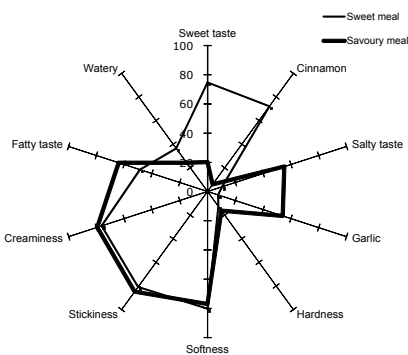


**Figure 2.2** *Results of a pilot study showing the sensory profiles of the sweet and savoury meal. In this study, 12 healthy, non-smoking participants (mean age 22.6±2.2 y) rated the meals on sensory aspects. Ratings were performed on a 100-mm VAS. These participants did not participate in the main study.*

was made with semi skimmed milk (12.0%), crème fraîche (8.0%), bouillon (0.3%), garlic powder (0.02%), and NaCl (0.8%). A standardized protocol was used to make fresh meals every morning prior to the test and they were kept warm with a mean temperature of 75ºC (range 65ºC – 85ºC). The meals were served in large bowls containing 800 g and were consumed with a tablespoon.

### Procedure and data collection

On test days, participants were instructed to eat a normal, standardized breakfast and standardize their morning physical activities. They were not allowed to eat or drink anything except for non-energy-containing beverages 3 h before the start of a test session and not to consume anything in the previous hour before the start. Furthermore, they were instructed not to eat anything until 1 h after the test to make sure they consumed the test product until they were satiated. Tests were scheduled during lunchtime and performed in isolated tasting booths throughout the experiment. There were 3 time shifts: 11:30-12:15, 12:30-13:15 and 13:30-14:15. All experimental measurement of 1 participant took place in the same time shift. When participants arrived at the laboratory they were seated and given specific instructions depending on test condition (described below) shown on a computer screen. In all test conditions, participants were offered an *ad libitum* meal with instructions 'to eat as much as they liked, until comfortably satiated'. Food intake was monitored using a hidden scale (model KERN 440, ATP-Messtechnik) that was connected to a computer. The computer was programmed (VISUAL BASIC) to record weight with a 2-s interval throughout the meal with a precision of 0.1 g. When weight of the meal fell below 100 g, a researcher was alerted by the computer and the bowl was replaced with a new one (during the experiments, this happened in total 8 times for 2 male participants; 4 times each). To reduce errors in weighting,

**Table 2.1** Nutritional composition (energy content and macronutrient composition) per 100 g of the sweet and savoury test meal[1]

|  | Sweet meal | Savoury meal |
| --- | --- | --- |
| **Energy content, kcal (kJ)** | 112 (470) | 98 (411) |
| **Protein, g (% energy)** | 1.8 ( 6%) | 2.0 (8%) |
| **Carbohydrate, g (% energy)** | 20.4 (73%) | 17.4 (71%) |
| **Fat, g (% energy)** | 2.6 (21%) | 2.3 (21%) |
| **Fibre, g** | 0.26 | 0.25 |

[1]*Values determined by chemical analysis of samples taken from homogenous mixture of samples collected every testing day. Nitrogen was determined by the Kjeldahl method (96, method 920.87), and the amount of protein was calculated using a conversion factor of 6.25; fat by the acid hydrolysis method (96, method 14.019); available carbohydrate was calculated by subtracting moisture, ash, protein, dietary fibre and fat from total weight. Energy content was calculated from the macronutrient composition by using the following energy conversion factors: protein, 16.7 kJ/g; fat, 37.7 kJ/g; carbohydrate, 15.7 kJ/g.*

participants were given instructions to avoid contact with the bowl, to only take food onto their spoon until ready to ingest it, and to leave the spoon next to the food bowl when completing ratings or finished eating. *Ad libitum* intake and total eating time were recorded for all experimental conditions. The used appetite questionnaire consisted of 5 dimensions: hunger, fullness, prospective consumption, desire to eat something sweet, and desire to eat something savoury. For pleasantness ratings a distinction was made between 'pleasantness of the taste' and 'desire to eat the food'. All ratings were performed on a computer on a 100-unit visual analogue scale (VAS), anchored with 'not at all' to 'extremely'.

During test sessions a cup of 200 mL tap water was available. If needed, the experimenter refilled the cup. A*d libitum* intake of water was measured afterwards by weighting the residues in the glasses. Intake of water did not significantly differ between the 2 meals in any of the test conditions (test condition 1: sweet meal 149±81 g vs. savoury meal 159±73 g; test condition 2: sweet meal 295±123 g vs. savoury meal 295±119 g test condition 3: sweet meal 164±95 g vs. savoury meal 168±98 g). In test condition 2, however, overall intake of water was higher due to obliged intake during sample rating ($p<0.001$).

Procedures of the different test conditions were as follows. In test condition 1, once seated, participants received a bowl of rice. After finishing eating, they left. In test condition 2, participants first filled out the appetite questionnaire. Then they received a plate containing 6 samples of rice (each weighting ~5 g), varying in taste and intensity. Participants were instructed to neutralize their mouth with a sip of water between tasting and rating the different samples. Afterwards, participants received a bowl of rice. They had to taste a bite and rate the pleasantness, after which they could start eating. When 50 g (1 bout) was consumed, the computer gave a buzz, accompanied with on-screen instructions to stop eating and perform pleasantness and appetite ratings, after which eating was continued until the next bout was consumed. This cycle continued until participants indicated they were satiated. They were then asked to repeat pleasantness and appetite ratings. Lastly, participants received a new plate of 6 rice samples and re-rated them. In test condition 3, participants rated their appetite similarly as in test condition 2. Afterwards, explicit and implicit aspects of food choice were assessed by means of a computerized food preference questionnaire (LFPQ). Next, participants received a bowl of rice. When participants indicated they were satiated, they had to re-rate their appetite and the LFPQ was re-run.

Please note that results of 'desire to eat something sweet/savoury' ratings and rice sample ratings of test condition 2 and the LFPQ of test condition 3 are not discussed in this current paper but are only mentioned to give an overview of the experimental setting.

## Statistical analyses

Data presented are means±SD. Per test condition, *ad libitum* intake (g) were compared between the 2 meals using a paired *t* test. Differences in overall intake between the 3 test conditions were compared with a 1-way ANOVA (Proc GLM) with participant and test condition as independent variables. For test condition 1, eating rate (total intake divided by total consumption time) was compared between the 2 meals with a paired *t* test. Interaction between time and intake was compared between the 2 meals by means of a mixed-model ANOVA (Proc mixed with fixed factors time, product and time x product and random factor participants). For test condition 2 and 3, initial ratings were compared and analysed by means of a paired *t* test. Change scores were calculated by subtracting ratings before the meal from ratings after the meal. Differences in change scores between the sweet and savoury meal were analysed using a paired *t* test. Mean ratings of pleasantness and appetite during the meals, measured in test condition 2, were calculated per bout and compared between tastes by means of a *t* test. Initial pleasantness and appetite ratings were tested for correlation to intake (Pearson correlation coefficient) and 95% CI were calculated. To take into account individual preferences for sweet or savoury foods, a secondary analysis was included. Based on initial pleasantness of the meals measured in test condition 2, participants were divided into 3 groups; high-sweet likers, high-savoury likers and a group that was indifferent. When initial liking of the 2 meals was >20 units apart (ratings were performed on a 100-unit VAS scale) a participant was either identified as a high-sweet liker or a high-savoury liker. Per group, *ad libitum* intake (g) in test condition 2 was compared between the 2 meals by means of a paired *t* test. Data were analysed using SAS 9.1 for Windows. Results were considered significantly different at a *p*-value of <0.05.

# Results

### *Ad libitum* intake

*Ad libitum* intakes of the sweet and savoury meals per test condition did not differ (Figure 2.3). Overall intake in test condition 2 was lower compared with intake in test condition 1 and 3 ($p<0.0001$). In test condition 1, total eating time comprised 8.7±3.4 min for the sweet and 9.2±4.1 min for the savoury meal and overall eating rate was similar (38±14 g/min for the sweet meal, and 37±14 g/min for the savoury meal). There was a main effect of time for both meals ($p<0.0001$). However, there was no effect of meal or time x meal interaction. In the other 2 test conditions, total eating time of the sweet and savoury meals did not significantly differ. In test condition 2, total eating time comprised 8.9±4.2 min for the sweet and 9.6±4.5 min for the savoury meal. In test condition 3, total eating time comprised 8.2±3.2 min for the sweet and 8.7±4.5 min for the savoury meal. Of the 64 participants, 16 were categorized as high-sweet likers (pleasantness
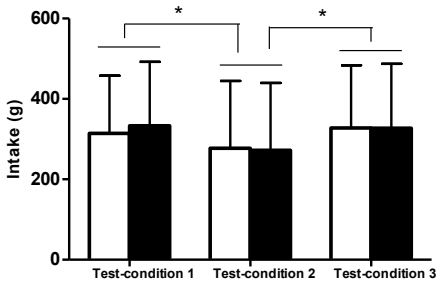
*Figure 2.3 Ad libitum intake by normal weight young adults of sweet (-□-) and savoury (-■-) meals in test condition 1, 2 and 3. Values are means±SD (n=64).*

sweet meal, 75±10 vs. savoury meal, 37±20), 14 were categorized as high-savoury likers (pleasantness sweet meal, 29±17 vs. savoury meal, 74±12), and 34 were indifferent (pleasantness sweet meal, 68±15 vs. savoury meal, 68±14). The indifferent group ate similar amounts of both meals; 319±168 g of the sweet meal and 328±173 g of the savoury meal. High-sweet likers ate more of the sweet meal (295±168 g) than of the savoury meal (203±151 g) ($p<0.01$). High-savoury likers ate more of the savoury meal (216±127 g) than of the sweet meal (155±104 g) ($p<0.001$).

### Pleasantness and appetite ratings

Initial ratings were similar between the 2 experimental conditions (Table 2.2). Changes in ratings were significant, but did not differ between the 2 meals. The number of bouts consumed varied among participants (mean 5.1±3.2). Pleasantness and appetite ratings did not differ between the 2 meals for any of the bouts (Figure 2.4).

### Correlations

Initial pleasantness ratings, measured in test condition 2, were correlated with intake (Table 2.3). Only initial prospective consumption, measured in test condition 2, was correlated to intake of the savoury meal. In test condition 3, both initial hunger and initial prospective consumption were correlated with intake of the savoury meal. None of the appetite ratings were correlated with intake of the sweet meal.

# Discussion

Our objective was to investigate the difference between a sweet and savoury taste on satiation, independent of palatability, texture, energy density and macronutrient composition. Intake did not differ between the 2 meals. Progress of the meals and eating rates were similar. Both the sweet and savoury meals suppressed hunger and prospective consumption and increased fullness equally during the eating episode.

**Table 2.2** Initial and changes pleasantness and appetite ratings for the sweet and savoury meal for test condition 2 and 3[1]

| | Sweet meal | | Savoury meal | |
|---|---|---|---|---|
| **Test condition 2** | **Initial** | **Change** | **Initial** | **Change** |
| **Pleasantness ratings** | | | | |
| Pleasantness of taste | 61±22 | -21±20* | 61±21 | -22±23* |
| Desire to eat the food | 60±24 | -34±21* | 61±22 | -38±23* |
| **Appetite ratings** | | | | |
| Hunger | 71±15 | -52±20* | 70±14 | -49±21* |
| Fullness | 23±16 | 50±21* | 24±17 | 48±23* |
| Prospective consumption | 68±14 | -47±18* | 66±14 | -44±18* |
| **Test condition 3** | | | | |
| **Appetite ratings** | | | | |
| Hunger | 72±16 | -56±20* | 70±18 | -54±20* |
| Fullness | 22±16 | 54±20* | 23±17 | 53±22* |
| Prospective consumption | 68±15 | -47±20* | 68±15 | -49±18* |

[1]*Ratings performed on a 100-unit VAS. Values are means±SD (n=64). *Different from initial ratings, p<0.001. Changes in ratings did not differ between the meals.*

These findings were not consistent with our hypothesis. The circadian rhythm of appetite for something sweet and appetite for something savoury show different patterns during the day; appetite for something savoury is more meal/hunger related, whereas appetite for something sweet is more stable (39, 97). In addition, it has been observed that appetite for something sweet is less suppressed by a meal than appetite for something savoury (98, 99). And studies have shown that sweetness might have a stimulatory effect on appetite, and is therefore less satiating than savouriness (16, 98-100). Several studies showed that the intake of sweet products was higher than of savoury products (99, 101, 102). The products used in these experiments, however, differed greatly, not only on energy density but also in sensory properties. Components other than taste could have been responsible for differences in satiation.

It might be that the stimulatory effect of sweetness on appetite is only valid for a certain population. Laeng *et al.* (103) reported that both gender and degree of individual "sweetness liking" influenced the experience of sweet tastes. Appleton *et al.* (98, 104) showed that low consumers of artificially sweetened beverages demonstrated an increase in appetite in response to sweet taste, whereas high consumers did not. Our high-sweet likers ate more of the sweet meal than of the savoury meal. Whether this was due, however, to a stimulatory effect of sweetness or to liking of the product (high-savoury likers ate more of the savoury meal) is unclear. Due to small number of participants in these groups, no elaborate analysis could be performed.

## A. Hunger



## B. Fullness



## C. Prospective consumption



## D. Pleasantness of taste
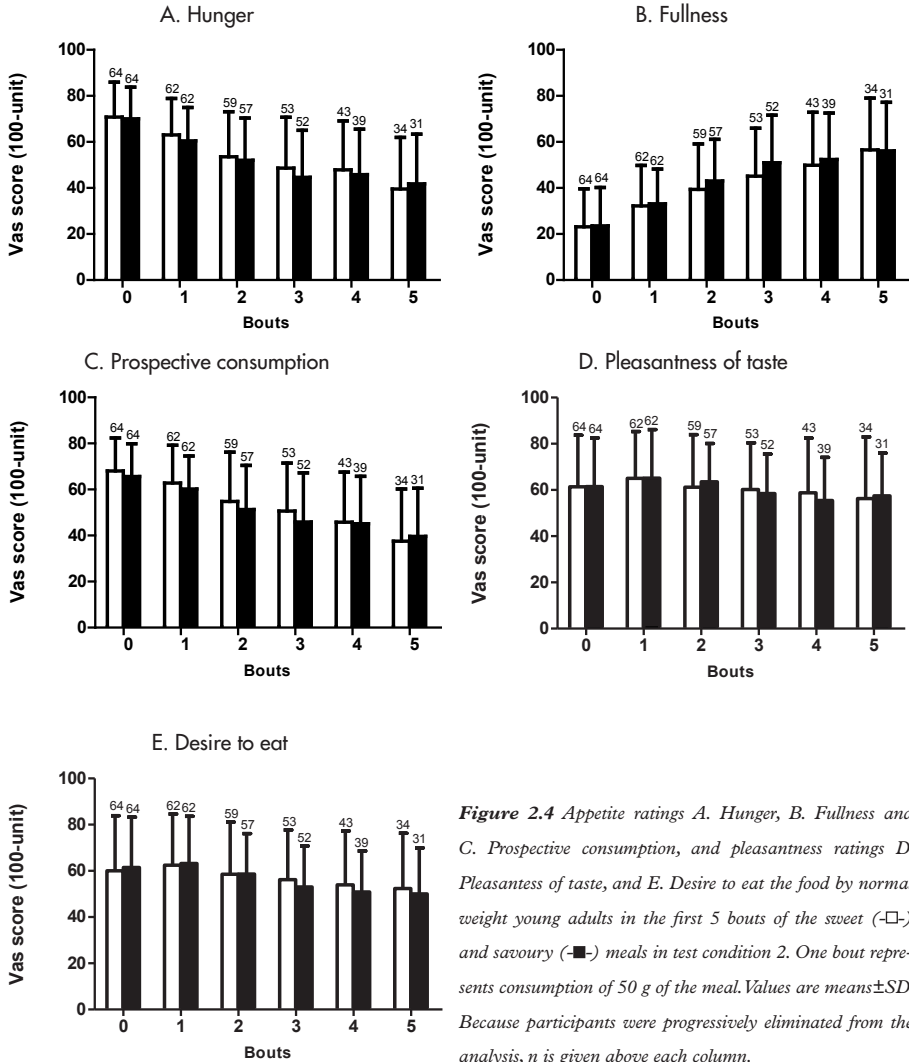


## E. Desire to eat



*Figure 2.4* Appetite ratings A. Hunger, B. Fullness and C. Prospective consumption, and pleasantness ratings D. Pleasantess of taste, and E. Desire to eat the food by normal weight young adults in the first 5 bouts of the sweet (-□-) and savoury (-■-) meals in test condition 2. One bout represents consumption of 50 g of the meal. Values are means±SD. Because participants were progressively eliminated from the analysis, n is given above each column.

We focused primarily on sensory specific satiation. Alongside sensory properties, however, environmental/contextual factors and cognitive factors appear to play a role (9). Although environmental factors were controlled for (all sessions were conducted under similar circumstances), cognitive factors might have contributed to our outcome. Through consumption of foods during our lifetime we learn to estimate their satiating effects. This plays an important and independent role in decisions about portion size (105, 106). Although we did not assess beliefs about our meals, the appearance, texture and core product (rice) were very similar, which might explain equal intake of both

meals. Another factor that might have contributed to equal intake is visual cues, which have been shown to greatly influence portion size (86). Although we tried to avoid this by serving the meals in large quantities, participants might still have been able to determine their intake by using visual cues, also because the appearance of both meals was so similar.

In our study we let participants ingest the meals. Therefore we cannot with certainty claim that post-ingestive satiety mechanisms were not involved. In all test conditions, however, eating time was <10 min. In addition, by controlling the composition of both meals, we do not think post-ingestive satiety mechanisms influenced our outcome.

Results of all 3 test conditions showed that satiation did not differ between the sweet and savoury meal. In test condition 2, however, an overall lower intake was observed. This could have been due to the exposure of rice samples and water before the meal; the rice samples in total were ~30 g and participants knew they would receive them again after the meal. Interruptions during the meals could have also caused lower intake. It led to a slower eating rate, which has been linked to smaller intake (107). And when pauses are introduced, participants are cognitively more aware that they can stop eating (108), although Yeomans *et al.* (109) reported that interruptions increased intake. Due to our design, we cannot distinguish these processes and their effects, but we do know that they influenced the 2 meals equally.

**Table 2.3** Pearson correlations (*r*) between initial pleasantness and appetite ratings with *ad libitum* intake in normal weight young adults

| | Intake sweet meal | | Intake savoury meal | |
|---|---|---|---|---|
| **Test condition 2** | *r* | [C.I. 95%] | *r* | [C.I. 95%] |
| **Pleasantness ratings** | | | | |
| Pleasantness of taste | 0.50*** | [0.29-0.66] | 0.34* | [0.10-0.54] |
| Desire to eat the food | 0.52*** | [0.31-0.68] | 0.44** | [0.21-0.62] |
| **Appetite ratings** | | | | |
| Hunger | 0.09 | [-0.16-0.32] | 0.19 | [-0.06-0.41] |
| Fullness | -0.04 | [-0.28-0.21] | -0.14 | [-0.37-0.11] |
| Prospective consumption | 0.17 | [-0.08-0.40] | 0.37* | [0.13-0.56] |
| **Test condition 3** | | | | |
| **Appetite ratings** | | | | |
| Hunger | -0.00 | [-0.25-0.25] | 0.38* | [0.14-0.57] |
| Fullness | -0.06 | [-0.30-0.19] | -0.14 | [-0.37-0.11] |
| Prospective consumption | 0.13 | [-0.12-0.36] | 0.36* | [0.12-0.56] |

*$p<0.01$ **$p<0.001$ ***$p<0.0001$ (n = 64)*

It can be argued whether our products were similar in energy density. The compositions of both meals were calculated using the Dutch nutrient database (95). Chemical analysis afterwards showed that the similarity of energy density was less than had been established. Numerous studies have shown, however, that within a meal, participants do not compensate for energy intake (90, 110). And if energy density does play a role, it probably involves learning processes (91). When examining the order effect of both meals (each was given 3 times to participants), no effect of time was evident (data not shown). We are therefore confident that the difference in energy density did not affect our outcome.

The change in pleasantness ratings for both meals were significantly decreased after finishing the meal, which is indicator for SSS (Table 2.2). When inspecting the ratings in the first bouts, however, it appeared that these ratings were not decreased (Figure 2.4). This may be due to the fact that intake of the meals were *ad libitum* and therefore participants were progressively eliminated from the analysis. When comparing the correlation of the sweet and savoury meals, initial pleasantness ratings were strongly correlated with intake. Appetite ratings for 'hunger' and 'prospective consumption' were correlated to intake of the savoury meal and not to the sweet meal (Table 2.3). This seems to be consistent with observations that appetite for something savoury is more related to meal time/hunger feelings than appetite for something sweet (39, 97). Because the correlation of intake and hunger was not replicated in test condition 2, however, more research is needed to clarify this finding.

Our results are in concordance with a study performed by Rolls *et al.* (111). They investigated the effect of serving a sweet or savoury food, equal in energy density and palatability, as either a first or last course in a 3-course meal. Intake of the sweet and savoury products before the main course was similar. After the savoury main course, however, intake of the sweet product appeared to be higher than of the savoury product (not main outcome). This is an example of a many studies showing that sweet and savoury taste do differ on several aspects. SSS research has shown that eating either taste (sweet or savoury) to satiety invariably leads to a decrease in the pleasantness of foods with similar taste (22, 98, 99, 112), leading to adjusted food choice and intake (65, 76, 111).

To quantify the role of sensory properties on food intake, research with single foods is most sensitive and provide clear results. In everyday life, however, we ingest varied meals, which have a more complex taste, in a less controlled environment. It is therefore difficult to extrapolate these findings to everyday life. There are still many eating occasions, however, in which people eat homogeneous or single foods.

In conclusion, we have shown that homogeneous meals with a sweet or savoury taste, similar in palatability, texture, energy density and macronutrient composition, do not differ in their influence on satiation in normal weight young adults. We therefore postulate that, when considering that the sweet-savoury domain is an important dimension from taste perspective, taste seems not to have a large influence on satiation in equal palatable foods. However, more research, including testing other foods, is needed to strengthen this claim.

# Acknowledgements

# Chapter three

## —Measuring food reward and the transfer effect of sensory specific satiety

Sanne Griffioen–Roose, Graham Finlayson, Monica Mars, John E. Blundell, Cees de Graaf

# Abstract

*The main objectives of our study were (1) to compare several direct and indirect measures of liking and wanting for food and thereby (2) investigating the transfer effect of sensory specific satiety (SSS) for sweet and savoury taste to other foods. We used a cross-over design whereby 61 healthy, unrestrained subjects (19M/42F), with a mean age of 21.9±3.1 y and a mean BMI of 21.7±1.5 kg/m² were offered a standardized amount of rice meal with either a sweet or savoury taste. Afterwards, liking and wanting for 16 snack products, varying in taste (sweet/ savoury) and fat (high/low), were assessed. Method 1 assessed ad libitum intake, method 2 the willingness to work for access, and method 3 explicit and implicit responses to photographic food stimuli. All the methods used showed a similar pattern of results; after eating a preload with a certain taste, the liking and wanting of snacks with a similar taste were less than for snacks with a dissimilar taste. This transfer effect was not equipotent for sweet and savoury tastes. It appears that in young, healthy adults, savoury taste has a stronger modulating effect on subsequent food choice than sweet.*

# Introduction

Sensory properties play an important role in food selection and intake (9, 16). When a food is eaten to satiety, the pleasantness of the sensory properties of that food decreases more than of foods that have not been eaten. This is sensory specific satiety (SSS) and was first demonstrated in humans by Rolls *et al.* (19).

Not only eaten foods, but also foods that share sensory characteristics of the eaten foods decline in pleasantness relative to foods that do not share these properties (e.g. reference 112). This has implications for the operation of SSS in a natural setting where availability of foods is unconstrained and varied. In addition, the strength of this transfer effect for different tastes is far from clear. For example, an important distinction regarding taste can be made between sweet and savoury, which includes almost 90% of the food we eat (38). However, the profiles of appetite for something sweet and appetite for something savoury show different patterns over the course of a day. It appears that appetite for something savoury is more in line with the pattern of meals (more hunger/ meal related), whereas appetite for something sweet is more stable during the day (39). It is unclear why this occurs.

It has been suggested that SSS is not only represented by a decrease in pleasantness (e.g. 'liking': hedonic sensation), but also in 'wanting', which refers to the motivation to engage in eating (60, 113). In addition, it is believed that both measures of liking and wanting reflect 'core' processes that can operate without conscious awareness (for

further reading e.g. references 62-65). It is important that we know how these different components of food reward, operating at explicit (conscious) and implicit (unconscious) levels, relate to behaviour, to fully understand processes involved in food selection and intake (76).

To measure these processes of food reward separately, however, is challenging, as many manipulations alter these processes together (60). There are several direct measurements, e.g. subjective ratings (e.g. references 21, 66), which typically tap into conscious attitudes. But most often people do not analyse their attitudes towards stimuli consciously. Rather, their behaviour is guided by a spontaneous interaction with the environment. Implicit measures are proposed to measure these spontaneous (automatic) reaction to a stimuli. Hence, indirect, implicit measures could be particularly suited to predict spontaneous, uncontrolled behaviour (67, 68).

The definition of an implicit measure is that "the outcome of a measurement procedure is causally produced by psychological attributes in an automatic manner" (68). Many of the implicit measures that have been introduced during the past 2 years are based on reaction time tasks (68). Recently a novel computer-based procedure has been developed, the Leeds Food Preference Questionnaire (LFPQ). Aside from subjective ratings of photographic food stimuli it includes a "forced choice" behavioural measure, whereby the speed with which one stimulus is chosen in preference to its alternative is the indirect measure, proposed to assess implicit wanting (71, 76). Another procedure which has been suggested to measure implicit wanting processes is the assessment of a subject's willingness to work (i.e. instrumental responses) to get access to a food (72-75). In food research actual intake has always been the ultimate measure of (sensory) satiety, and recently it has been proposed that this is also a measurement of implicit wanting processes (63). The outcomes of above mentioned measures might be tapping more into unconscious processes which are involved in food intake[1].

The main objectives of our study were (1) to compare several direct and indirect measures of liking and wanting for food and thereby (2) investigating the transfer effect of SSS for sweet and savoury taste to other foods. We assessed this by measuring, after eating a sweet or savoury preload, the *ad libitum* intake (method 1), the willingness to work (i.e. instrumental responding) for access (method 2), and explicit and implicit responses to photographic food stimuli using the LFPQ (method 3), of several snack products which varied in taste and fat content. By comparing the outcomes of these measures we advance the understanding of the relationship between hedonic and motivational aspects of eating.

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[1]For further reading on different approaches measuring explicit and implicit processes see Figure 5 in Berridge, 2009 (63)

# Methods

## Subjects

We recruited subjects aged 18-35 years, with a normal weight. Exclusion criteria were restrained eating (Dutch Eating Behaviour Questionnaire (DEBQ), men: score >2.25; women: score >2.80) (93), lack of appetite, an energy restricted diet during the last 2 months, change in body weight >5 kg during the last 2 months, stomach or bowel diseases, diabetes, thyroid disease or any other endocrine disorder, having difficulties with swallowing/eating, hypersensitivity for the food products under study, smoking, being a vegetarian, and for women: being pregnant or lactating. Sixty-one subjects (19 males and 42 females) with a mean age of 21.9±3.1 y and a mean BMI of 21.7±1.5 kg/m² completed the study and received a financial compensation. Subjects were informed they were participating in a study designed to compare several methods to assess palatability of different rice products. The study was approved by the Medical Ethical Committee of Wageningen University and all subjects signed an informed consent.
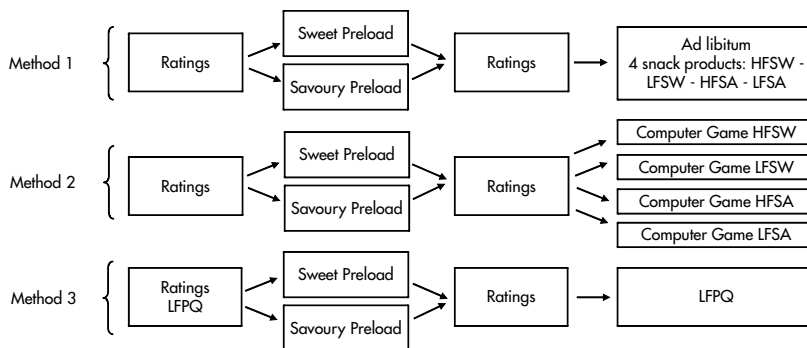


*Figure 3.1* Overview of study design. LFPQ: Leeds Food Preference Questionnaire, HFSW: high fat sweet snack category, LFSW: low fat sweet snack category, HFSA: high fat savoury snack category, LFSA: low fat savoury snack category.

## Design

We used a randomized cross-over design, consisting of 3 methods in which the transfer effect of 2 tastes (sweet and savoury) were compared, resulting in 6 test sessions (Figure 3.1). The order of sessions was randomized per subject according a generalized Latin square design. Each session took place on a separate testing day, with a minimum wash-out period of 3 days. After a standardized amount of either a sweet or savoury preload, liking and wanting for 16 snack products, varying in taste (sweet/savoury) and fat (high/low), were assessed. Preceding the experiment there was one practice day to accommodate subjects to isolated tasting booths and usage of computers without consumption of test foods.

**Table 3.1** Palatability ratings and nutritional composition (energy content and macronutrient composition) of the sweet and savoury preload

|  | Sweet preload | Savoury preload |
|---|---|---|
| **Palatability ratings[1]** | | |
| Liking | 72±2 | 69±2 |
| Wanting | 72±2 | 71±2 |
| **Composition[2]** | | |
| Energy content, kcal (kJ) | 101 (423) | 91 (381) |
| Protein, g (% energy) | 1.9 (7) | 1.9 (8) |
| Carbohydrate, g (% energy) | 19.4 (77) | 16.5 (73) |
| Fat, g (% energy) | 2.2 (16) | 2.5 (19) |
| Fibre, g | 0.4 | 0.4 |

[1]Values are means±SE after the first bite, measured on a 100-unit VAS, averaged for the 3 methods (n=61). [2]Shown per 100 g preload: Nitrogen was determined by the Kjeldahl method (96, method 920.87), and the amount of protein was calculated using a conversion factor of 6.25; fat was determined by the acid hydrolysis method (96, method 14.019); available carbohydrate was calculated by subtracting moisture, ash, protein, dietary fibre and fat from total weight. Energy content was calculated from the macronutrient composition by using the following energy conversion factors: protein, 16.7 kJ/g; fat, 37.7 kJ/g; carbohydrate, 15.7 kJ/g.

### Test foods

*Preload.* Rice was used as a preload, served in either a sweet or savoury variant. To ensure equal sensory exposure in all experimental conditions the amount (g) of rice meal was fixed. Per individual, the amount of rice was determined by individual energy needs, estimated by means of the Schofield I equation (114), taking into account age, weight, gender and a physical activity level of 1.6. About 10% of energy of daily estimated energy needs was provided by the preload, which is about half the amount of energy provided by lunch in the Netherlands (115). The calculated amounts were categorized per 25 g: 4 subjects received 200 g (192 kcal; averaged for taste), 24 subjects received 225 g (216 kcal), 14 subjects received 250 g (240 kcal), 7 subjects received 275 g (264 kcal), 7 subjects received 300 g (288 kcal) and 5 subjects received 325 g (312 kcal) (=an average intake of 252 g - 242 kcal).

Palatability and composition of both preloads are given in Table 3.1. Energy content and macronutrient composition were calculated prior to the study using the Dutch nutrient database (95). In addition, macronutrient content was determined afterwards by chemical analysis of samples taken from a homogenous mixture of samples which were collected every testing day. The core component of both preloads was risotto rice (Lassie, Wormer, The Netherlands) (78.5%). The sweet version was made with semi-skimmed milk (16.1%), butter (2.4%), cinnamon (0.08%), and aspartame (3.1%). The savoury version was made with semi-skimmed milk (11.8%), crème fraîche (8.6%), bouillon

(0.3%), garlic powder (0.02%), and salt (0.8%). We used a standardized protocol to make fresh preloads every morning prior to the test and they were kept warm with a mean temperature of 73°C (range 65–80°C). The preloads were served in bowls and were consumed with a tablespoon. Subjects were instructed to finish their bowl.

*Snack products.* Sixteen snack products were used to assess the transfer effect of SSS. These snack products were selected based on their taste (sweet/savoury) and fat content (high/low) and are shown in Table 3.2. Prior to the experiment the general liking of these 16 foods was assessed with a food questionnaire containing pictures of these foods. Ratings were performed on a 9-point hedonic scale. For each individual we selected snack products rated as 5 or higher and matching on liking as closely as possible to be used in

**Table 3.2** Energy content and macronutrient composition of the food products used in all methods (per 100 g)[1]

|  | Kcal | Protein, g (% energy) | Carbohydrates, g (% energy) | Fat, g (% energy) |
|---|---|---|---|---|
| **Low Fat Sweet** | | | | |
| Marshmallows | 328 | 2 (2) | 80 (98) | 0 (0) |
| Gingerbread | 305 | 3 (4) | 70 (92) | 1 (4) |
| Low fat cake ('eikerkoek') | 299 | 7 (10) | 59 (79) | 4 (11) |
| Jelly sweets (candies) | 204 | 1 (2) | 50 (98) | 0 (0) |
| **Average** | **284** | **3 (5)** | **65 (92)** | **1 (4)** |
| **High Fat Sweet** | | | | |
| Chocolate | 538 | 7 (5) | 55 (41) | 33 (55) |
| Sweet pastry | 286 | 4 (5) | 37 (51) | 14 (44) |
| Chocolate cake | 447 | 5 (4) | 52 (46) | 24 (49) |
| Donuts | 413 | 6 (6) | 35 (34) | 28 (61) |
| **Average** | **421** | **5 (5)** | **44 (43)** | **25 (52)** |
| **Low Fat Savoury** | | | | |
| Pretzels | 373 | 12 (13) | 70 (75) | 5 (13) |
| Rice crackers | 409 | 6 (5) | 86 (84) | 5 (11) |
| Ham rolls | 132 | 18 (54) | 2 (6) | 6 (40) |
| Salty snacks ('Japanse mix') | 386 | 9 (9) | 86 (89) | 1 (2) |
| **Average** | **325** | **11 (20)** | **61 (63)** | **4 (16)** |
| **High Fat Savoury** | | | | |
| Salted peanuts | 625 | 28 (18) | 11 (7) | 52 (75) |
| Salted crisps | 536 | 5 (4) | 51 (38) | 35 (58) |
| Sausages ('bifi') | 392 | 19 (19) | 1 (1) | 35 (80) |
| Cheese 48+ | 377 | 24 (25) | 0 (0) | 31 (74) |
| **Average** | **483** | **19 (17)** | **16 (12)** | **38 (72)** |

[1]*Values derived from the Dutch nutrient database 2006 (95).*

methods 1 and 2. In method 1, subjects received one high fat sweet product (HFSW), one low fat sweet product (LFSW), one high fat savoury product (HFSA), and one low fat savoury product (LFSA). The mean liking scores for these four snack categories, based on the prior assessment on the 9-point hedonic scale, were: HFSW 7.5±0.1, LFSW 7.0±0.1, HFSA 7.2±0.1, and LFSA 7.2±0.1. In method 2, subjects had to work for access to one of the snack products by playing a computer game. Subjects were divided into four groups (between-subjects): one group worked for a HFSW product (n=16), one group worked for a LFSW product (n=15), one group worked for a HFSA product (n=15) and one group worked for a LFSA product (n=15). Subjects worked for the same snack product under sweet and savoury preload conditions. The individually selected snack product was determined by their initial liking as described above, and was the same snack product as the one received in method 1. Mean liking scores in method 2 for the different snack categories were: HFSW 7.4±0.1, LFSW 7.3±0.2, HFSA 7.4±0.1, and LFSA 7.3±0.2. In method 3, subjects assessed and responded to all 16 snack products using the LFPQ, which included photographic images of each snack product.

## Procedure and data collection

On test days subjects were asked to standardize their breakfast and morning physical activity. They had to refrain from eating and drinking energy-containing beverages 3 h prior to the start of the test session and to refrain from any drinks 1 h prior to session. Furthermore, subjects were asked not to eat anything until 1 h after the test session, to encourage compliance with the experimental protocol. Subjects were tested either at: 11:30-12:15, 12:30-13:15, or 13:30-14:15. All experimental measurements of one individual took place at the same time of day in an isolated booth. When subjects arrived at the laboratory they were seated and given specific instructions shown on a computer screen. All test sessions started with subjects filling out an appetite questionnaire, consisting of 5 dimensions: hunger, fullness, prospective consumption, appetite for something sweet and appetite for something savoury. The 100-unit visual analogue scale (VAS) was anchored with 'not at all' to 'extremely'. Next, subjects were presented with a preload. Before starting eating, they had to taste a bite and rate their liking ('how pleasant do you find the taste of this food right now?') and wanting ('how much do you want to eat this food right now?'). Then, subjects were instructed to start their meal. After indicating they had finished their bowl they were asked to re-rate the preload on liking and wanting and to repeat appetite ratings.

In method 1, after post meal ratings, subjects were offered a tray with 4 bowls (as previously described): each containing 100 g of one snack category. Subjects were instructed to eat until they were comfortably satiated. They could ask for extra bowls if necessary. *Ad libitum* intake of snack products was measured by weighing remaining food in the bowls.

In method 2, after post meal ratings, a computerized procedure, developed by Havermans *et al.*, was performed. This procedure has been extensively described elsewhere (72, 73). In brief, subjects had to play a computer game comprising a series of choices between gaining points to get access to a snack product (as described earlier) or stop playing. A picture of the snack product and a picture of a 'stop sign' were displayed at the left and right centre of a computer screen. When selecting the snack product, subjects received immediate feedback whether a point was earned. For the first 5 points, subjects had to choose the snack product 4 times to earn a single point; a fixed ratio reinforcement schedule of 4 (FR 4). For each subsequent 5 point increment, the response requirement was doubled; progressing through FR 8, 16, 32 and 64. Subjects could earn a maximum of 25 points (100 g of snack product). When subjects decided to stop playing, the total number of points obtained was displayed on screen. They then received the amount of snack product they earned, which had to be immediately consumed (this was communicated prior to the task). Motivation of a subject to obtain a certain snack product was expressed as number of mouse clicks.

In method 3, before and after eating the preload, the LFPQ was run, which is a validated tool developed and extensively described by Finlayson *et al.* (71, 76). The program was translated to Dutch and included photographs of the 16 snack products shown in Table 3.2. For explicit measures, a single presentation of a snack product was shown and people had to rate their liking ('how pleasant would you find the taste of this food right now?') and their wanting ('how much do you want to eat this food right now?') on a 100-unit VAS. In addition, a paired presentation of snack products was shown where subjects had to select their most wanted food ('select the food which you most want to eat right now') as quickly and accurately as possible. During this last procedure both frequency of preferred choice (relative food preference) and reaction time were measured. Reaction times (RT) were transformed to a standardized 'd-score' (D-RT) using a validated algorithm (70): the smaller the D-RT, the greater the implicit wanting for that food category relative to other categories in the task.

During consumption of the preload a hidden scale (model KERN 440, ATP-Messtechnik, Balingen, Germany), connected to a computer, recorded food intake (precision 0.1 g), enabling calculation of eating rate and total eating time of the preloads. Throughout the test sessions, water was freely available and served in cups of 200 ml. *Ad libitum* intake of water was measured by weighing remaining water in the glasses. Intake of water was not significantly different between the 2 preloads in any of the conditions; mean intake of water during the sweet preload conditions was 112±6 g and during the savoury preload conditions 117±6 g.

### Statistical analyses

Data are presented as means with standard error unless otherwise specified. An ANOVA was used to compare eating time and eating rate (total intake divided by total consumption time) between the preloads (SAS Proc GLM with taste of preload (sweet and savoury) and method (1, 2, and 3) as independent variables). Pre and post appetite and pleasantness ratings for the preloads were analysed using ANOVA (SAS Proc GLM with taste of preload (sweet and savoury), time of rating (pre and post preload) and method (1, 2 and 3) as independent variables). The outcome measures of method 1 (intake in g and kcal), method 2 (number of mouse clicks) and method 3 (explicit liking, explicit wanting, relative food preference and implicit wanting) were analysed using ANOVA (SAS Proc GLM with taste of preload (sweet and savoury), taste of snack product (sweet and savoury) and fat content of snack product (high fat and low fat) as independent variables). A similar secondary analyses was run, however, the taste of the snack products was not defined as sweet/savoury, but as similar/dissimilar with the preload taste. In all analyses both main effects and interactions between the independent variables were analysed. In addition, except for the analysis of method 2, participants were included in the model (within-subject design). For method 3 one subject was excluded from the analysis due to missing data.

Post appetite ratings were tested for correlation to intake in method 1 (Pearson's correlation coefficient). To compare the outcomes of each method on their agreement, the mean results of all methods, per preload, were tested for correlation (Pearson's correlation coefficient). In addition, the correlations for the individual scores for the different outcomes were correlated per preload. Post-hoc analyses were made using Tukey's correction. Results were considered significantly different at a $p$-value of <0.05.

# Results

### Preload

As analyses showed that eating time, eating rate, appetite ratings, and pleasantness ratings did not differ between the 3 methods, these were averaged per preload. Sweet and savoury preloads were eaten within a similar duration (sweet preload: 4.9±0.2 min, savoury preload: 5.0±0.3 min, F(1, 300)=0.30, $p$=0.58), and at a similar pace (sweet preload: 58±2 g/min, savoury preload: 58±3 g/min, F(1, 300)=0.51, $p$=0.48).

Eating a fixed amount of preload irrespective of taste decreased hunger, prospective consumption and appetite for something savoury and increased fullness (all $p$-values <0.0001, Table 3.3). Appetite for something sweet was only decreased after eating the sweet preload, ($p$<0.0001), but not after the savoury preload ($p$=0.31). In addition, ap-

**Table 3.3** Pre and post appetite ratings per preload averaged for the 3 methods[1]

|  | Sweet preload | | Savoury preload | | |
|---|---|---|---|---|---|
|  | **Pre** | **Post** | **Pre** | **Post** | $p_{post}$[2] |
| **Hunger** | 74±2 | 34±2* | 73±2 | 36±2* | 0.18 |
| **Fullness** | 21±2 | 63±2* | 22±1 | 60±2* | 0.13 |
| **Prospective consumption** | 72±1 | 39±2* | 71±1 | 40±2* | 0.67 |
| **Appetite for sweet** | 58±2 | 33±2* | 58±2 | 55±2 | <0.0001 |
| **Appetite for savoury** | 71±2 | 55±2* | 72±2 | 44±2* | <0.0001 |

[1]*Ratings performed on a 100-unit VAS. Values are means±SE (n=61).* [2]*p-value of differences in post meal ratings between sweet and savoury preload averaged for 3 methods.* \**Significant difference between pre and post ratings per preload.*

petite for something sweet was decreased more after eating the sweet preload than after the savoury preload, ($p<0.0001$). Appetite for something savoury was decreased more after eating the savoury preload than after the sweet preload ($p<0.0001$).

### Method 1: *Ad libitum* intake after sweet and savoury preload

Total intake (g) of the snacks after eating the sweet preload and savoury preload is shown in Figure 3.2. Intake of the total snacks after the sweet preload was 94±10 g, and after the savoury preload 100±8 g, which was not significantly different [$F_{(1,420)}=0.50$, $p=0.48$], as were the energy intakes (intake after sweet preload 337±33 kcal; intake after savoury preload 352±28 kcal [$F_{(1,420)}=0.24$, $p=0.62$)].

In general, intake of the sweet snacks was higher than of the savoury snacks [$F_{(1,420)}=19.12$, $p<0.0001$]. The taste of preload interacted with snack intake [$F_{(1,420)}=5.96$, $p<0.05$]: after eating the sweet preload, similar amounts of sweet snacks (51±7 g) and savoury snacks (43±5 g) were eaten ($p=0.52$). After eating the savoury preload, however, intake of the savoury snacks (36±5 g) was lower than of the sweet snacks (65±6 g) ($p<0.0001$).

The intake of the high fat snacks was higher than of the low fat snacks [$F_{(1,420)}=21.30$, $p<0.0001$], and specifically, the intake of the high fat sweet snacks (37 g) was 85% higher in comparison to the other snack categories (20 g) [$F_{(1,420)}=9.27$, $p<0.0001$]. But this was irrespective of taste of preload [$F_{(1,420)}=0.04$, $p=0.85$]. Figure 3.3A shows the distribution of the intake (g) over the 4 different snack categories after eating the sweet preload and the savoury preload. Within the separate snack categories, the left bar of a pair represent the intake of snacks with a similar taste with the preload whereas the right bar represent the intake of snacks with a dissimilar taste. Overall, the intake of snacks with a dissimilar taste (27±2 g) was higher than the intake of snacks with a similar taste (22±2 g) [$F_{(1,420)}=5.96$, $p<0.05$].

## Method 2: Willingness to work for access to snack product after sweet and savoury preload

Willingness to work for access (number of mouse clicks) for the 4 different snack categories after eating the sweet preload and the savoury preload is shown in Figure 3.3B. In general, the number of mouse clicks for the sweet snacks was higher than for the savoury snacks [$F_{(1, 114)}$=4.38, $p<0.05$]. This was irrespective of taste of preload [$F_{(1, 114)}$=1.62, $p=0.21$]. Similar as in Figure 3.3, the left bar of a pair represent the responding for snacks with a similar taste with the preload, while the right bar represents the responding for snacks with a dissimilar taste. Subjects responded more for snacks with a dissimilar taste (32±5 mouse clicks) in comparison for snacks which had a similar taste (25±3 mouse clicks), however this difference was not statistically significant ($p=0.21$).

## Method 3: Results of the LFPQ after the sweet and savoury preload

Prior to consumption of the preloads, there were no differences on explicit liking, explicit wanting, relative food preference and implicit wanting between the sweet and savoury preload conditions. Figure 3.4 shows the explicit liking, explicit wanting, relative food preference, and implicit wanting for the 4 different snack categories after eating the sweet and savoury preload. In general, all measurements showed a preference for the sweet snacks in comparison to the savoury snacks (explicit liking, $F_{(1,420)}$=11.85, $p<0.001$; explicit wanting, $F_{(1,420)}$=10.75, $p<0.01$; relative food preference, $F_{(1,420)}$=22.29, $p<0.001$, and implicit wanting, $F_{(1,413)}$=8.16, $p<0.01$). The taste of the preload, however, interacted with this preference: after eating the sweet preload no preference was evident for either snack taste (explicit liking, $p=0.15$; explicit wanting, $p=0.09$; relative food preference, $p=0.28$, and implicit wanting, $p=0.18$). After eating the savoury preload, a preference was seen for the sweet snacks in comparison to the savoury snacks (difference on explicit liking 30%, $p<0.0001$; explicit wanting 31%, $p<0.0001$; relative food preference 59%, $p<0.001$, implicit wanting Δ0.35 D-RT, $p<0.0001$). There was a preference evident for the high fat snacks in comparison to the low fat snacks, and specifically for the high fat sweet snacks in comparison to the other snack categories (explicit liking 29%, $F_{(3, 419)}$=29.36, $p<0.001$; explicit wanting of 31%, $F_{(3, 419)}$=28.98, $p<0.001$; relative food preference of 52%, $F_{(3, 419)}$=37.99, $p<0.001$, implicit wanting
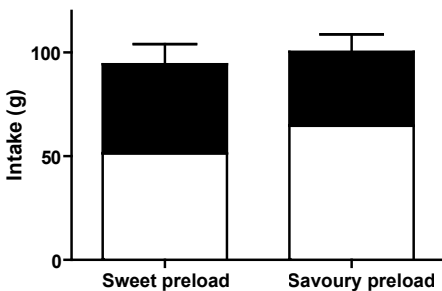


***Figure 3.2** Method 1: Total intake (g) of the sweet snack products (-□-) and savoury snack products (-■-) after eating the sweet preload and the savoury preload. Values are means±SE (n=61).*
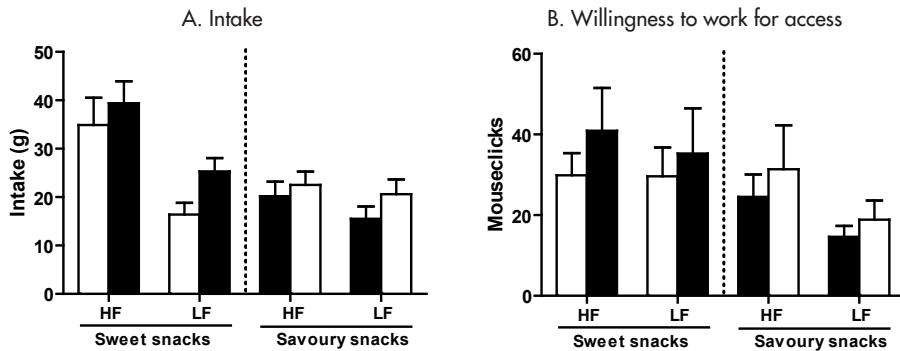
*Figure 3.3 A. Method 1: Intake (g) of the high fat (HF) and low fat (LF) sweet and savoury snacks after eating the sweet preload (-□-) or savoury preload (-■-). Within the separate snack categories, the left bar of a pair represent the intake of snacks with a similar taste with the preload whereas the right bar represent the intake of snacks with a dissimilar taste. Values are means±SE (n=61) B. Method 2: Willingness to work for access (number of mouse clicks) for the high fat (HF) and low fat (LF) sweet and savoury snacks after eating the sweet preload (-□-) or savoury preload (-■-). Within the separate snack categories, the left bar of a pair represent the intake of snacks with a similar taste with the preload whereas the right bar represent the intake of snacks with a dissimilar taste. Values are means±SE [n: high fat sweet (n=16), low fat sweet (n=15), high fat savoury (n=15), low fat savoury (n=15)].*

$\Delta 0.18$ D-RT, $F(3, 412)=4.94$, $p<0.01$). But for all measures this was irrespective of taste of preload. Overall, there was a preference for snacks with a dissimilar taste in comparison to snacks with a similar taste (difference on explicit liking 19%, $p<0.0001$; explicit wanting 20%, $p<0.0001$; relative food preference 32%, $p<0.0001$; and for reaction time $\Delta 0.24$ D-RT, $p<0.0001$).

### Comparison of the different methods

Post appetite ratings were significantly correlated with total intake (g) in method 1 (hunger $r_{122}=0.44$, $p<0.0001$; fullness $r_{122}=-0.40$, $p<0.0001$; prospective consumption $r_{122}=0.51$, $p<0.0001$). Appetite for something sweet was only significantly correlated with intake (g) of the sweet snack products ($r_{122}=0.42$, $p<0.0001$) but not with the intake (g) of the savoury snack products ($p=0.48$). Appetite for something savoury was only significantly correlated with intake (g) of the savoury snack products ($r_{122}=0.33$, $p<0.001$), but not with the intake (g) of the sweet snack products ($p=0.44$).

Mean intake (g) in method 1 and mean number of mouse clicks in method 2 were significantly correlated ($r_8=0.72$, $p<0.05$), as was intake with all outcomes of method 3 (mean explicit liking $r_8=0.92$, $p<0.01$; mean explicit wanting $r_8=0.91$, $p<0.01$; mean food preference $r_8=0.92$, $p<0.01$; mean implicit wanting $r_8=-0.77$, $p<0.05$). The correlations between the mean number of mouse clicks (method 2) and all outcome means of method 3

were mostly trends (mean explicit liking $r_s$=0.68, $p$=0.07; mean explicit wanting $r_s$=0.65, $p$=0.08; mean food preference $r_s$=0.68, $p$=0.07; mean reaction time $r_s$=-0.75, $p$<0.05).

Pearson's correlation analyses of all outcomes per preload on individual level are shown in Table 3.4. *Ad libitum* intake (method 1) was correlated with the number of mouse clicks (method 2) and all outcomes of method 3, except for implicit wanting after the sweet preload. The number of mouse clicks (method 2) was not significantly correlated with the outcomes of method 3, except with explicit wanting after the sweet preload. Within the outcomes of method 3, for both preloads, explicit liking and explicit wanting were highly positively correlated, and in turn correlated with food preference. All ratings and food preference were negatively correlated with implicit wanting, indicating than an increase in explicit liking and wanting was associated with a faster response time.
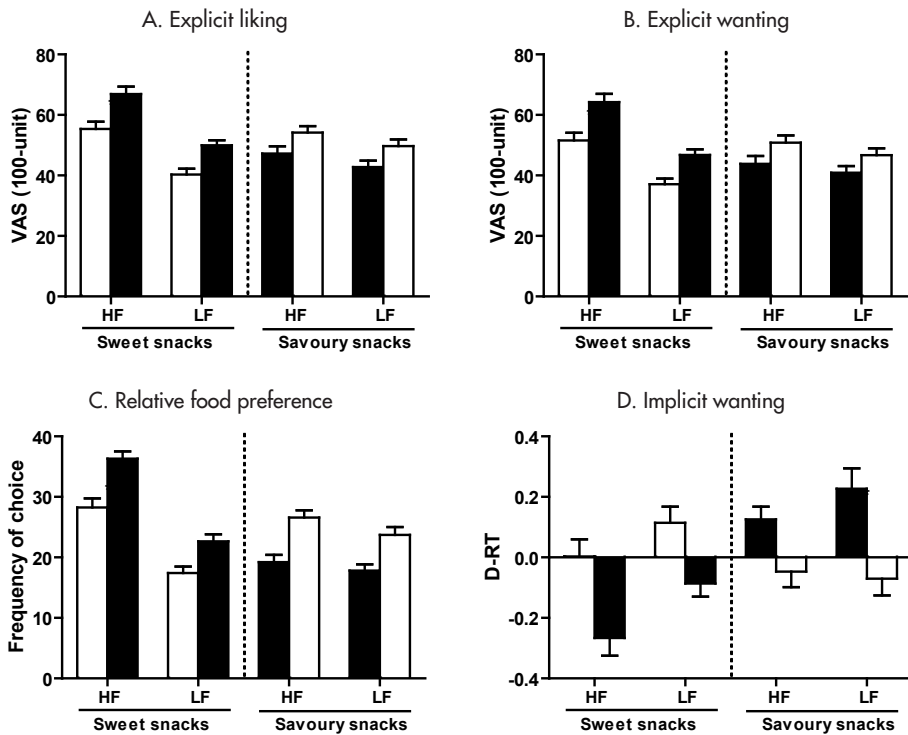


*Figure 3.4 Method 3: A. Explicit liking rating, B. Explicit wanting rating, C. Relative food preference, and D. Implicit wanting for the high fat (HF) and low fat (LF) sweet and savoury snacks after eating the sweet preload (-□-) or savoury preload (-■-). Within the separate snack categories, the left bar of a pair represent the response for snacks with a similar taste with the preload whereas the right bar represent the response for snacks with a dissimilar taste. Values are means±SE (n=61).*

**Table 3.4** Pearson's correlation analysis ($r$) of the outcomes of method 1, 2 and 3 after the sweet and savoury preload

| | Sweet preload | | | | | Savoury preload | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Method 2 | Method 3 | | | | Method 2 | Method 3 | | | |
| | Work | EL | EW | FP | IW | Work | EL | EW | FP | IW |
| **Method 1** | | | | | | | | | | |
| Intake | $r_{61}$=.45$^c$ | $r_{244}$=.27$^d$ | $r_{244}$=.30$^d$ | $r_{244}$=.18$^c$ | $r_{240}$=-.09 | $r_{61}$=.54$^d$ | $r_{244}$=.34$^d$ | $r_{244}$=.35$^d$ | $r_{244}$=.31$^d$ | $r_{240}$=-.24$^c$ |
| **Method 2** | | | | | | | | | | |
| Work | | $r_{61}$=.23$^a$ | $r_{61}$=.26$^b$ | $r_{61}$=.04 | $r_{60}$=.00 | | $r_{61}$=.18 | $r_{61}$=.21 | $r_{61}$=.20 | $r_{60}$=-.12 |
| **Method 3** | | | | | | | | | | |
| EL | | | $r_{244}$=.92$^d$ | $r_{244}$=.67$^d$ | $r_{240}$=-.42$^d$ | | | $r_{244}$=.93$^d$ | $r_{244}$=.67$^d$ | $r_{240}$=-.45$^d$ |
| EW | | | | $r_{244}$=.66$^d$ | $r_{240}$=-.38$^d$ | | | | $r_{244}$=.62$^d$ | $r_{240}$=-.42$^d$ |
| FP | | | | | $r_{240}$=-.56$^d$ | | | | | $r_{240}$=-.64$^d$ |

$^a$p=0.07 $^b$p<0.05 $^c$p<0.001 $^d$p<0.0001. EL: explicit liking, EW: explicit wanting, FP: food preference, IW: implicit wanting.

# Discussion

The main objectives of or our study were (1) to compare several direct and indirect measures of liking and wanting for food and thereby (2) investigating the transfer effect of SSS for sweet and savoury taste to other foods. In general all methods showed a similar pattern of results; after eating a preload with a certain taste, the liking and wanting of snacks with a similar taste were less than for snacks with a dissimilar taste.

As mentioned in the introduction, it is important that we know how the different component of food reward relate to behaviour, to fully understand processes involved in food selection and intake (76). In our experiment we measured the transfer effect of SSS by *ad libitum* intake (method 1), by willingness to work for access (method 2), and by explicit and implicit responses to photographic stimuli (method 3). When investigating the correlation between the mean outcomes of all the approaches, it is clear that all methods show a similar pattern of response, suggesting both that subjects behave very consistently in the laboratory, and all methods are likely measuring the same underlying processes. On the other hand, due to these correlations between the liking and wanting measures, we cannot draw conclusions regarding SSS being a dual process phenomenon as we cannot separate the two processes. It might be that the dissociation of these processes is difficult to measure within our study population: healthy, young individuals. In other groups (e.g. obese, restrained eaters, eating disorders) this might be different (63). As stated in the introduction, it has been proposed that food intake and working tasks could be measurements of implicit processes (63). However, *ad libitum* intake (method

1) and mouse clicks (methods 2) correlated less well with the implicit measure of the LFPQ (method 3) compared with explicit ratings of liking and wanting under these conditions. Indeed, it could be argued that providing subjects with snack foods in an isolated booth, and instructing them to eat "as much as they want" is more likely to raise the importance of explicit than implicit processes. The same argument has been raised for working tasks where subjects are required to continuously monitor whether the amount of effort invested is in balance with the reward at stake (72). Previous research suggests that when subjects are more distracted (66), are less aware they are being measured, or are in a more naturalistic environment (116), results could favour implicit processes and indirect measures.

From the perspective of feasibility, the motivational task (method 2) seems less attractive. The method required food to be assessed on separate occasions which made a full within-subjects design impractical. And although the difference in number of mouse clicks for snacks which had a similar taste to the preload with the number of mouse clicks for snacks which had a dissimilar taste was quite substantial (28%), this difference did not reach significance. In addition, the variation within and between subjects was larger than expected, e.g. a posteriori sample size calculations showed that with this difference of 28%, given an alpha of 0.05 and a power of 0.8, we would have needed 137 subjects for a significant effect. A posteriori power calculations for method 1 (intake) showed that we had just a sufficient number of participants, as our power was 0.82 (with an alpha of 0.05 and a difference of 23% between snack with a similar taste to the preload in comparison to the intake of snacks with dissimilar taste). A posteriori power calculations for the outcomes of method 3 showed that the power reached almost 1.

The method of Finlayson *et al.* was feasible to use in this context. As the results of the snack intake and the results of this method were very similar, it appears that photographic stimuli can be used in SSS research. Berridge *et al.* (117) stated that a "vivid imagery of reward cues may suffice, especially in humans, to trigger incentive salience" (page 68). In addition, it was shown recently using neuroimaging that the brain rapidly tracks the energetic content of food images (118). By using photographic stimuli the dimensions of the categories can be simply adapted and it permits dissociation between explicit and implicit responses.

A limitation of our study is that we did not include a measurement of implicit liking processes. The implicit component of liking relates to unconscious (objective) affective reactions (e.g. unintentional smiling after eating something tasty), which has been made most clear in animals and human infants (119). In adults, there is one elegant study performed where subjects were presented with subliminally photographs of happy facial expression. This presentation failed to produce any conscious report of affect or emotion

or shift in hedonic feeling at all, yet it did increase the subject's subsequent behavioural consumption of a fruit drink and subjective affective rating of it later (120). To incorporate these kind of procedures into SSS research, however, is complicated and needs further investigation.

The second objective of our study was to investigate the transfer effect of SSS for sweet and savoury taste to other food. Firstly, when looking at the total intake of snacks (method 1), this was not different after the sweet and savoury preload (both around 100 g). This is in concurrence with the appetite ratings hunger, fullness and prospective consumption, which also did not differ between the 2 preloads (Table 3.2). These results support our earlier finding that sweet and savoury taste do not differ in their influence on satiation (121).

As mentioned earlier, there was a clear transfer effect shown for both preloads, i.e. after eating a preload with a certain taste, the preference for snacks with a similar taste was less than for snacks with a dissimilar taste. Interestingly, this transfer effect was not equipotent for the sweet and savoury preload; there was a clear interaction between the taste of the preload and the liking and wanting for the snack categories. After eating the sweet preload, the intake (method 1), subjective ratings (method 3), relative food preference (method 3), and reaction time (method 3) for the sweet and savoury snacks did not differ. After eating the savoury preload, however, there was a clear preference for the sweet snack category. The subjective ratings of appetite for something sweet and appetite for something savoury (Table 3.3) point in the same direction; after eating a sweet preload, the decreases for appetite for sweet and appetite for savoury are quite close (post rating minus pre rating: sweet: -25; savoury: -16), whereas after eating a savoury preload the decreases differ largely (post rating minus pre rating: sweet: -3; savoury: -28). In conclusion, it appears that savoury taste has a stronger modulating effect on subsequent food choice than sweet taste.

Although the transfer effect of SSS has been shown in earlier studies (39, 98, 99, 112, 122), to our knowledge it has never been investigated in a fully controlled and balanced scientific design. In our view, the asymmetry of SSS transfer between sweet and savoury taste might be a psychobiological phenomenon. Firstly, the hedonic properties of sweetness embody strong reward potential with the capacity to reinforce its own consumption and behaviour associated with consumption. For this reason it can be expected that sweetness will have a positive and distinctive effects on eating behaviour, food selection and other aspects of appetite control (123). In contrast, savoury taste is generally associated with foods high in protein, and it has been shown that in a satiated state, aversion for high-protein (savoury tasting) foods develops (124). Therefore, when a savoury meal is eaten, an aversion for other products containing protein could emerge. Interestingly,

this idea corresponds with the 'protein-leverage' hypothesis, where it is posed that protein intake is tightly regulated in the human body (45). Future research could explore the role of protein content as a factor in sensory satiety transfer.

In all outcomes it was clear that the high fat (especially sweet) snacks were preferred to the low fat snacks. Although this was unexpected based on the prior assessment of the products (see method section), it could be that when we had used a larger preload, results would be different. The amount of preload, which is about half the amount of energy provided by lunch in the Netherlands (115), was chosen in order to get subjects (sensory) satiated (which, based on the appetite ratings, was achieved), but not too overfed, in order for subjects to still be motivated to eat and work for snack products.

In conclusion, all the methods used in this study show similar patterns of results and indicate that SSS transfers to snacks with a similar taste. The specificity of this effect, however, is not equipotent for sweet and savoury taste. It appears that in young, healthy adults, savoury taste has a stronger modulating effect on subsequent food choice than sweet.

# Acknowledgements

# Chapter four

## —The effect of within-meal protein content and taste on subsequent food choice and satiety

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# Abstract

*It is posed that protein intake is tightly regulated by the human body. The role of sensory qualities in the satiating effects of protein, however, requires further clarification. Our objective was to determine the effect of within-meal protein content and taste on subsequent food choice and satiety. We used a cross-over design whereby sixty healthy, unrestrained subjects (twenty-three males and thirty-seven females) with a mean age of 20.8±2.1 years and a mean BMI of 21.5±1.6 kg/ m² were offered one of four iso-energetic preloads (rice meal) for lunch: two low in protein (about 7% energy derived from protein) and two high in protein (about 25% energy derived from protein Both had a sweet and savoury version. At 30 min after preload consumption, subjects were offered an ad libitum buffet, consisting of food products differing in protein content (low/high) and taste (sweet/savoury). In addition, the computerized Leeds Food Preference Questionnaire (LFPQ) was run to assess several components of food reward. The results showed no effect of protein content of the preloads on subsequent food choice. There was an effect of taste; after eating the savoury preloads, choice and intake of sweet products were higher than of savoury products. No such preference was seen after the sweet preloads. No differences in satiety were observed. To conclude, within one eating episode, within-meal protein content in these quantities seems not to have an effect on subsequent food choice. This appears to be mostly determined by taste, whereby savoury taste exerts the strongest modulating effect. The results of the LFPQ provided insight into underlying processes.*

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# Introduction

Within our food range, products with a savoury taste are in general higher in protein levels, while food products with a sweet taste are more related to carbohydrate content (40, 41) [i.e. savoury taste refers to non-sweet, salty taste, closely linked to the 'umami taste', and is also described as 'brothy' or 'meaty'(32)]. It has been shown that hungry subjects show a marked preference for high-protein foods, while after being satiated, an aversion for high-protein foods emerges (124, 125). In addition, a high-protein meal produces a significantly greater reduction in liking for high-protein foods than high-carbohydrate foods (124, 125). These findings are in concurrence with the 'protein-leverage' hypothesis, which poses that protein intake is tightly regulated in the human body, and prioritised over the intakes of carbohydrate and fat (44, 45). This might also explain why protein has been found to be the more satiating macronutrient. However, the role of sensory qualities in the satiating effects of protein requires further clarification.

It has been shown that sensory properties of food play an important role in food selection and intake (9, 16, 17). When a food is eaten to satiety, the hedonic value of the sensory properties of that food decreases more than of foods that have not been eaten

(19). And not only eaten foods, but also foods that share sensory characteristics of the eaten foods decline in pleasantness relative to foods that do not share these properties (112). In addition, the strength of this transfer effect for different tastes is not equal. It appears that savoury taste has a stronger modulating effect on subsequent food choice than sweet taste (126).

The objective of the present study was to determine separately the effect of within-meal protein content and taste on subsequent food choice and satiety. Our approach consisted of measuring the effect of four different preload (rice meals), varying in protein content (low and high) and taste (sweet and savoury) on subsequent food choice and intake of sixteen food products differing in protein content and taste.

We postulated that the high-protein preloads would be more satiating than the low-protein preloads, and that this effect would be most evident when this high protein content was linked with the appropriate savoury taste. In addition, we hypothesised that after the low-protein preloads the intake of products with a high protein content at the buffet would be higher than after the high-protein preloads, but that this effect would be most evident when the low-protein preload was linked with sweet taste.

# Methods

### Subjects

Healthy, normal weight subjects, aged 18-35 years, were recruited. Exclusion criteria were restrained eating (Dutch Eating Behaviour Questionnaire (DEBQ), men: score >2.25; women: score >2.80) (93), lack of appetite, an energy restricted diet during the last 2 months, change in body weight >5 kg during the last 2 months, stomach or bowel diseases, diabetes, thyroid disease or any other endocrine disorder, having difficulties with swallowing/eating, hypersensitivity for the food products under study, smoking, being a vegetarian, and, for women, being pregnant or lactating. Body weight and height were measured. In total, sixty subjects (twenty-three males and thirty-seven females) aged 20.8±2.1 years, with a mean BMI of 21.5±1.6 kg/m$^2$ completed the study.

Subjects were unaware of the exact aim of the study and were informed that we were interested in comparing several methods of assessing palatability of rice products. The present 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. This trial has been registered with the Dutch Trial register (NTR) (registration no. NTR 2162). Written informed consent was obtained from all subjects.

## Design

We used a randomized cross-over design with four conditions (Figure 4.1). Subjects were offered one of four iso-energetic preloads for lunch: a low-protein sweet, a high-protein sweet, a low-protein savoury, or a high-protein savoury. This was followed by an *ad libitum* lunch buffet. In addition, after the preload and before the buffet, several components of food reward were measured using the Leeds Food Preference Questionnaire (LFPQ) (procedure explained in 'procedure and data collection'). The four sessions were scheduled in four subsequent weeks with a minimal wash-out of 5 d (preferably subjects came to the laboratory on the same day of the week, but this was not always possible). The order of the sessions was randomized for each subject according a generalised Latin square design. Preceding the experiment there was one practice day to familiarise participants to the test conditions without consumption of the test foods.
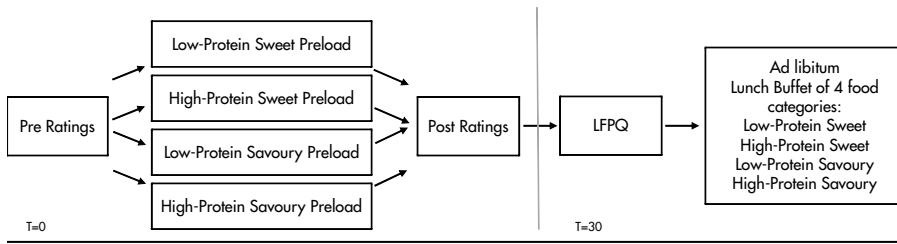


**Figure 4.1** *Overview of study design. LFPQ: Leeds Food Preference Questionnaire.*

## Test foods

*Preload.* A rice meal was used as preload. For each individual the amount of rice was determined by individual energy needs estimated by means of the Schofield I equation (114), taking into account age, weight, sex and a physical activity level of 1.6. About 10% of energy of daily estimated energy needs was provided by the preload, which is about half the amount of energy provided by lunch in the Netherlands (115). The calculated amounts were categorized per 25 g: five subjects received 200 g, twenty-two subjects received 225 g, twelve subjects received 250 g, nine subjects received 275 g, nine subjects received 300 g, and three subjects received 325 g (equal to an average intake of 252±35 g). Palatability and composition of all preloads are given in Table 4.1. The low-protein preloads derived about 7% of their energy from protein, the high-protein versions about 25%. Energy content and macronutrient composition were calculated before the study using the Dutch nutrient database (95). In addition, macronutrient content was determined afterwards by chemical analysis of a homogenous mixture of samples that were collected every testing day. The core component of all preloads was risotto rice (Lassie, Wormer, The Netherlands) (65%). The sweet versions were made with semi-skimmed milk (22%), butter (4%), water (4%), cinnamon (0.08%) and sucralose (0.05%). The savoury version was made with semi-skimmed milk (17%), crème fraîche (11%), bouil-

**Table 4.1** Palatability ratings and nutritional composition (energy content and macronutrient composition) of the sweet and savoury low- and high-protein preloads

|  | Sweet preloads | | Savoury preloads | |
|---|---|---|---|---|
|  | Low protein | High protein | Low protein | High protein |
| **Palatability ratings[1]** | | | | |
| Liking | 73±19 | 75±20 | 58±23*** | 54±24*** |
| Wanting | 68±22 | 70±22 | 56±27** | 55±24** |
| **Composition - per 252 g serving[2]** | | | | |
| Energy content, kJ (kcal) | 1149 (275) | 1170 (280) | 1162 (278) | 1176 (281) |
| Protein, g (% energy) | 4 (6) | 18 (26) | 5 (7) | 18 (25) |
| Carbohydrate, g (% energy) | 51 (70) | 37 (49) | 51 (69) | 39 (52) |
| Fat, g (% energy) | 7 (24) | 8 (25) | 7 (24) | 7 (23) |
| Fibre (g) | 0.5 | 1.1 | 0.6 | 0.8 |

[1]*Ratings after the first bite, measured on a 100-unit VAS. Values are means±SD (n=60). Mean value was significantly lower than for the sweet preloads: **p<0.01, ***p<0.001. No differences existed between the low- and high-protein preloads.* [2]*Shown per average serving of 252 g preload. N was determined by the Kjeldahl method (96, method 920.87), and the amount of protein was calculated using a conversion factor of 6.25. Fat was determined by the acid hydrolysis method (96, method 14.019); available carbohydrate was calculated by subtracting moisture, ash, protein, dietary fibre and fat from total weight. Energy content was calculated from the macronutrient composition by using the following energy conversion factors: protein, 16.7 kJ/g; fat, 37.7 kJ/g; carbohydrate, 15.7 kJ/g.*

lon (0.3%), garlic powder (0.02%), and salt (0.8%). The low-protein versions contained 6% maltodextrine (Fantomalt, Nutricia, The Netherlands), the high-protein versions contained 6% whey protein (EWP, The Netherlands). We used a standardised protocol to make fresh preloads every morning before the test and they were kept warm with an average temperature of 66°C (range 61°C – 75°C). The preloads were served in bowls and were consumed with a tablespoon. Subjects were instructed to finish their bowl.

*Food products.* The *ad libitum* buffet consisted of sixteen food products which were selected on the basis of their protein content (low/high) and taste (sweet/savoury). Energy content and macronutrient composition of the selected products are shown in Table 4.2. The food products were offered in portions of 40 g (there were four exceptions, as multiplying the weight of a single piece did not add up to an exact 40 g. Therefore all-butter biscuits and Dutch cookies were served in quantity of three (adding up to servings of 35 g), and little frankfurters and Dutch tiny pancakes in quantity of five (little frankfurters 35 g; Dutch tiny pancakes 42 g). The LFPQ, which was run before the buffet, included photographic images of all the food products present at the lunch buffet. Before the experiment the general liking of these sixteen foods was assessed with a food questionnaire containing pictures of these foods. Ratings were performed on a nine-point hedonic scale. For inclusion, at least one product of a category should be scored 5 or higher.

## Procedure and data collection

Subjects were asked to refrain from eating and drinking energy-containing beverages from 23.00 hours on the day before each test day, and were instructed to standardise both their morning activity and breakfast. Before the experiment subjects received a list of high-protein products that were not allowed to be consumed during breakfast, including the following products: cheese, peanut butter, curd, egg products, meat products and

**Table 4.2** Energy content and macronutrient composition of the food products offered during the *ad libitum* lunch buffet and shown in the Leeds Food Preference Questionnaire (per 100 g)[1]

| | Energy, kJ (kcal) | Protein, g (% energy) | Carbohydrates, g (% energy) | Fat, g (% energy) |
|---|---|---|---|---|
| **Low Protein Sweet** | | | | |
| Chocolate mousse | 1052 (252) | 3 (5) | 26 (41) | 15 (54) |
| M&Ms chocolate | 2044 (487) | 5 (4) | 70 (57) | 21 (39) |
| Gingerbread | 1295 (305) | 3 (4) | 70 (92) | 1 (3) |
| All-butter biscuits | 2171 (519) | 6 (5) | 62 (48) | 27 (47) |
| **Average** | **1641 (391)** | **4 (4)** | **57★(60)** | **16 (36)** |
| **High Protein Sweet** | | | | |
| Dutch cookies[2] | 1947 (464) | 16 (14) | 55 (47) | 20 (39) |
| Dutch tiny pancakes[3] | 821 (195) | 8 (16) | 29 (59) | 5 (23) |
| Sugared peanuts | 1423 (340) | 14 (16) | 33 (39) | 17 (45) |
| Curd with fruit taste | 488 (116) | 7 (24) | 15 (52) | 4 (31) |
| **Average** | **1170 (279)** | **11★ (18)** | **33 (49)** | **12 (34)** |
| **Low Protein Savoury** | | | | |
| Potato salad | 753 (180) | 2 (4) | 16 (36) | 12 (60) |
| Crisps | 2235 (536) | 5 (4) | 51 (38) | 35 (59) |
| Rice crackers | 1711 (409) | 6 (6) | 86 (84) | 5 (11) |
| Prawn crackers | 2137 (510) | 4 (3) | 69 (54) | 24 (42) |
| **Average** | **1709 (409)** | **4 (4)** | **56★ (53)** | **19 (43)** |
| **High Protein Savoury** | | | | |
| Russian salad | 642 (154) | 6 (16) | 14 (36) | 9 (53) |
| Cheese 48+ | 1561 (377) | 24 (25) | 0 (0) | 31 (74) |
| Dry roasted peanuts | 2334 (563) | 24 (17) | 11 (8) | 47 (75) |
| Little frankfurters | 833 (200) | 13 (26) | 6 (12) | 14 (63) |
| **Average** | **1343 (324)** | **17★ (21)** | **8 (14)** | **25 (66)** |

[1]*Values derived from the Dutch nutrient database 2006 (95). *[2]*Bokkepootjes *[3]*Poffertjes ★An analysis was run to investigate whether the energy content and macronutrient composition of the food products offered during the ad libitum lunch buffet (per 100 g) differed between the food categories using ANOVA (SAS Proc GLM with protein content of product (low and high) and taste of product (sweet and savoury) as independent variables). It was shown that the high-protein categories indeed contained more protein (g) [F(1, 12)=14.77, p<0.01] and less carbohydrates (g) [F(1, 12)=12.47, p<0.01].*

fish products. Subjects had to consume their breakfast at least 3 h before the start of the test and report the time of breakfast and products consumed in a diary. Energy-free beverages were allowed up to 1h before the test session. Furthermore, subjects were asked not to eat anything until 1h after the test session. Food diaries were used to monitor compliance with these procedures. Assessment of the food diaries showed that subjects indeed consumed a standardized breakfast at least 3h before the test and that the main taste of the breakfast that all subjects ate was predominantly sweet (for example bread with jam, or milk with muesli, etc.). Subjects were tested either at 11:30-12:30, 12:30-13:30 or 13:30-14:30. All experimental measurements of one individual took place at the same time of the day. When subjects arrived at the laboratory they were seated in an isolated sensory booth and given specific instructions shown on a computer screen. All test sessions started with subjects filling out an appetite questionnaire, consisting of five dimensions: hunger, fullness, prospective consumption, desire to eat something sweet, and desire to eat something savoury. The 100-unit visual analogue scale (VAS) was anchored with 'not at all' to 'extremely'. Next, subjects were presented with a preload. Before starting to eat, they had to taste a bite and rate their liking ('how pleasant do you find the taste of this food right now?') and wanting ('how much do you want to eat this food right now?'). Then, subjects were instructed to start their meal. After finishing their bowl, they were asked to re-rate the preload on liking and wanting and to repeat appetite ratings. During consumption of the preload, a hidden scale (model KERN 440; ATP-Messtechnik, Balingen, Germany) connected to a computer, recorded food intake every 2 s (precision 0.1 g), enabling calculation of eating rate and total eating time of all preloads.

At 30 min after eating the preload, different components of food reward were assessed by the LFPQ, which is a validated tool developed and extensively described by Finlayson *et al.* (71, 76). The program was translated to Dutch and included photographs of the sixteen food products shown in Table 4.2. For explicit measures, a single presentation of a food product was shown and individuals had to rate their liking ('how pleasant would you find the taste of this food right now?') and their wanting ('how much do you want to eat this food right now?') on a 100-unit VAS. In addition, a paired presentation of food products was shown where subjects had to select their most wanted food ('select the food which you most want to eat right now') as quickly and accurately as possible. During this last procedure both frequency of preferred choice (relative food preference) and reaction time were measured. As participants were not informed about the measurement of their reaction time for each choice, this measure provided an indication of non-verbal, implicit processes of motivation (implicit wanting). Reaction times (RT) were transformed to a standardized 'd-score' (D-RT) using a validated algorithm (70): the smaller the D-RT, the greater the implicit wanting for that food category relative to other categories in the task.

After finishing the LFPQ, subjects were escorted to an adjacent room where an *ad libitum* lunch buffet was present and where separate individual eating areas had been created. At the buffet, subjects were allowed to choose as many products as they wanted, and they could re-visit the buffet as many times as they liked. Subjects were not obliged to finish their plate. The buffet area only allowed one subject at the time. The buffet was continuously refilled, so twelve portions of every product were displayed at all times. *Ad libitum* intake of the food products was measured by weighing the remaining amount in the food packages.

Throughout the test sessions, both during the preload and at the buffet, water was freely available and served in cups of 200 ml. *Ad libitum* intake of water was measured by weighing remaining water in the glasses. Intake of water was not significantly different during the four sessions; mean intake of water during the low-protein sweet preload session was 320±107 g, during the high-protein sweet session 337±119 g, during the low-protein savoury session 329±89 g, and during the high-protein savoury session 352±126 g. Between finishing the preload and starting the LFPQ subjects remained in the isolated sensory booths but were allowed to read or play a computer game.

**Statistical analyses**

Data are presented as mean values with standard deviation unless otherwise specified. An ANOVA was used to compare eating time and eating rate (total intake divided by total eating time) between the four preloads (SAS Proc GLM with protein content of preload (low and high) and taste of preload (sweet and savoury) as independent variables). The cumulative food intake was fitted for each person for each preload to a quadratic equation: $y=a+bt+ct^2$, where b is the constant slope of the curve over time, i.e. initial eating rate, and c is the change in the slope of the curve over time, i.e. rate of deceleration (127, 128). To investigate whether cumulative food intake differed between the four preloads the a's, b's, and c's were analysed using an ANOVA. Due to measurement errors, there were in total eight missing values on eating time.

Pre- and post-appetite ratings for the preload were analysed using ANOVA (SAS Proc GLM with protein content of preload (low and high), taste of preload (sweet and savoury), and time of rating (pre- and post-preload) as independent variables). Differences between the four preloads on total intake (kJ) of the food products at the *ad libitum* buffet and the choice of food products from the different categories at the buffet were analysed using ANOVA (SAS Proc GLM with protein content of preload (low and high), taste of preload (sweet and savoury), protein content of product at the buffet (low and high), and taste of product at the buffet (sweet and savoury) as independent variables). The choice of food products from the different categories at the buffet is expressed in percentage of the total, irrespective of amount. For example if a subject

chose sweet low- and high-protein products, both categories represent 50% of the total, even if of one category more products were chosen and eaten than of the other category. As one subject refrained from eating at the buffet during two out of four sessions, no percentages could be calculated for the four categories for these sessions (missing data). In addition, macronutrient intake (g) at the *ad libitum* buffet, irrespective of food categories, was calculated per preload and analysed for each macronutrient separately (protein, carbohydrates, and fat) using ANOVA (SAS Proc GLM with protein content of preload (low and high) and taste of preload (sweet and savoury) as independent variables). In all analyses, both main effects and interactions between the independent variables were analysed. In addition, participants were included in the model (within-subject design).

The *ad libitum* intake (kJ) and food choice (%) were tested for correlation (Pearson's correlation coefficient) with the measures of the LFPQ. In addition, the predictive values of the measures of the LFPQ on intake (kJ) and food choice (%) were investigated using a multiple linear regression analyses with backward elimination. As one subject refrained from choosing one particular food category during one session of the four sessions, no implicit wanting could be calculated for the four categories for this session (missing data). Post hoc analyses were made using Tukey's correction. Results were considered significantly different at a *p* value of <0.05. Analyses were conducted using SAS, 9.1 (SAS Institute Inc., Cary, NC, USA).

# Results

## Preload
The four preloads were eaten within a similar time period of 4.4±2.1 min and at a similar pace of 64±29 g/min; neither protein content nor taste of the preloads had a significant effect on eating time (protein content, $F_{(1, 170)}=0.79$, $p=0.38$; taste, $F_{(1, 170)}=0.19$, $p=0.66$) or eating rate (protein content, $F_{(1, 170)}=1.02$, $p=0.31$; taste, $F_{(1, 170)}=0.22$, $p=0.64$). In addition, there were no differences between the four preloads regarding cumulative food intake.

Pre-preload appetite ratings (hunger, fullness, prospective consumption, appetite for sweet and appetite for savoury) were similar across conditions and therefore averaged (Table 4.3). Eating a fixed amount of preload irrespective of protein content and taste decreased hunger, prospective consumption and increased fullness (all *p*-values <0.05). Appetite for something sweet was only decreased after eating the sweet preloads, but not after the savoury preloads. In addition, post-preload rating appetite for something sweet was lower after eating the sweet preloads (both low- and high-protein preloads combined) than after the savoury preloads (*p*<0.0001). Post-preload rating appetite for

**Table 4.3** Pre-preload and post-preload appetite ratings according to preload[1]

| | **Pre-ratings** | **Sweet preloads** | | **Savoury preloads** | | $p_{post}$[2] | | |
|---|---|---|---|---|---|---|---|---|
| | Averaged over all preloads (n=240) | Low protein | High protein | Low protein | High protein | main effect taste | main effect protein | taste x protein |
| **Hunger** | 71±15 | 42±23* | 43±22* | 44±22* | 45±22* | 0.23 | 0.85 | 0.90 |
| **Fullness** | 24±16 | 57±21* | 56±20* | 52±20* | 52±22* | 0.01 | 0.69 | 0.55 |
| **Pros cons** | 68±14 | 45±22* | 48±18* | 49±18* | 48±19* | 0.18 | 0.50 | 0.26 |
| **App for sw** | 58±21 | 37±26* | 44±24* | 60±24 | 61±22 | <0.0001 | 0.14 | 0.25 |
| **App for sav** | 69±17 | 62±20 | 60±20* | 52±20* | 48±21* | <0.0001 | 0.19 | 0.71 |

[1]*Ratings are performed on a 100-unit VAS. Values are means±SD (n=60).* [2]*p-values of differences in post-meal ratings between the four preloads.* \**Mean value was significantly different from that for the pre-preload rating (p<0.05). Pros cons: prospective consumption, App for sw: Appetite for sweet, App for sav: Appeite for savoury.*

something savoury was lower after the savoury preloads than after the sweet preloads ($p<0.0001$). No main effect of protein content of the preloads was seen in either of the appetite ratings.

### Effect of protein content of the preloads on food choice and satiety

No effect of protein content of the preloads was seen on total intake (kJ) at the *ad libitum* buffet [$F_{(1, 885)}=0.01$, $p=0.93$] (Figure 4.2A). When investigating the intake of the different food categories, no differences were seen in intake of the different food categories after the low-protein preloads (both sweet and savoury preloads combined) in comparison to intake after the high-protein preloads [$F_{(1, 885)}=0.16$, $p=0.69$]. When investigating choice of food products from the different categories at the buffet (Figure 4.2B), also no differences were seen in preferences for the different food categories after the low-protein preloads in comparison to preferences after the high-protein preloads [$F_{(1, 877)}=0.87$, $p=0.35$]. The overall macronutrient intake (g) after the different preloads at the *ad libitum* buffet, irrespective of the four different food categories, is shown in Table 4.4. No effect of protein content of the preloads was seen on intake of the different macronutrients (g) (protein, $F_{(1, 177)}=0.05$, $p=0.82$; carbohydrates, $F_{(1, 177)}=0.70$, $p=0.41$; fat, $F_{(1, 177)}=0.16$, $p=0.69$).

### Effect of taste of the preloads on satiety and food choice

No effect of taste of the preloads was seen on total intake (kJ) at the *ad libitum* buffet [$F_{(1, 885)}=0.02$, $p=0.89$] (Figure 4.2A). When investigating the intake of the different categories, it was shown that the taste of the preload significantly interacted with the taste of the food products at the buffet [$F_{(1, 885)}=51.92$, $p<0.0001$]; after eating the sweet preloads (both low- and high-protein preloads combined) no difference was seen

in intake between the sweet foods (1148 kJ) and savoury foods (1211 kJ) at the buffet ($p$=0.90). After the savoury preloads, however (both low- and high-protein preloads combined), the intake of the sweet foods (1624 kJ) was higher than of the savoury foods (751 kJ) ($p<0.0001$). No interaction was evident between taste of the preload and intake of food products differing in protein content [$F_{(1, 885)}$=0.67, $p$=0.41]. When investigating choice of food products at the buffet (Figure 4.2B), it was shown that the taste of the preload significantly interacted with the taste of the food products chosen at the buffet [$F_{(1, 877)}$=71.15, $p<0.0001$]; after eating the sweet preloads (both low- and high-protein preloads combined) no preference for a certain category existed ($p$=0.93). After eating the savour preloads, however, a large preference for the sweet foods appeared (68%) in comparison to the savoury foods (32%) ($p<0.0001$). There was no difference in preferences for low- or high-protein foods after any of the preloads. No effect of taste of the preload was seen on intake of protein (g) [$F_{(1, 177)}$=1.34, $p$=0.25] and fat (g) [$F_{(1, 177)}$=2.42, $p$=0.12] at the *ad libitum* buffet (Table 4.4). The intake of carbohydrates (g), however, was slightly higher after the savoury preloads (63 g) in comparison to after the sweet preloads (58 g) [$F_{(1, 177)}$=6.45, $p<0.05$].

### LFPQ

Pearson's correlation analyses of the *ad libitum* intake (kJ) and food choice (%) with the measures of the LFPQ are shown in Table 4.5. All measures of the LFPQ were significantly correlated with *ad libitum* intake (kJ) and food choice (%). The multiple linear regression analyses showed that the intake (kJ) of the food products at the *ad libitum* buffet was predicted only by the explicit wanting and relative food preference measures of the LFPQ ($R^2$ 0.33). Food choice (%) at the buffet was predicted by the relative food preference and implicit wanting measures of the LFPQ ($R^2$ 0.38).
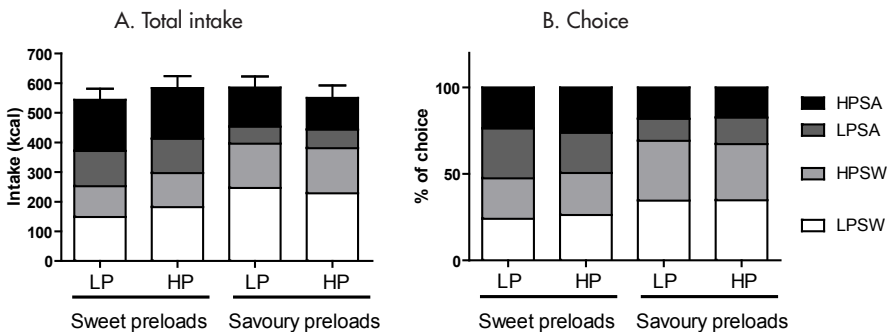


***Figure 4.2*** *A. Total intake (kJ) of the high-protein savoury (HPSA), low-protein savoury (LPSA), high-protein sweet (HPSW), and low-protein sweet (LPSW) products at the ad libitum buffet after eating the sweet and savoury low-protein (LP) and high-protein (HP) preloads. Values are means±SE (n=60). B. Choice (%) of the food products at the ad libitum buffet after eating the sweet and savoury LP and HP preloads. Values are means±SE (n=59).*

**Table 4.4** Total energy intake and macronutrient intake at the *ad libitum* lunch buffet after the sweet and savoury low- and high-protein preloads

| Preloads | Total intake, kJ (kcal) | Protein, g (% energy) | Carbohydrates, g (% energy) | Fat, g (% energy) |
|---|---|---|---|---|
| **Sweet Low protein** | 2276 (544) | 18 (13) | 55 (41) | 28 (47) |
| **Sweet High protein** | 2243 (584) | 19 (13) | 60 (41) | 30 (47) |
| | | | | |
| **Savoury Low protein** | 2448 (585) | 18 (12) | 64* (44) | 29 (45) |
| **Savoury High protein** | 2301 (550) | 17 (12) | 63* (45) | 26 (43) |

*Mean value was significantly higher than after the sweet preloads (p<0.05).*

# Discussion

With the present study we investigated the effect of within-meal protein content and taste on subsequent food choice and satiety. Results showed that food choice at the *ad libitum* buffet differed between the preloads but seemed to be mainly determined by taste and not by protein content. That we did not find an effect of protein content on subsequent food choice and intake was actually unexpected. Hill and Blundell (124) investigated the effects of consuming a high-protein or high-carbohydrate meal on subjective feelings of appetite. They showed that the high-protein meal produced a significantly greater reduction in liking for high-protein than high-carbohydrate foods, but that the converse was not true for the carbohydrate meal. In addition, it was shown that hungry subjects showed a marked preference for high-protein foods, and that after eating, when satiety was high and hunger low, a relative aversion for high-protein foods and a preference for carbohydrate foods were displayed. Barkeling *et al.* (125) replicated these results and showed that in a satiated state, a relative aversion for high-protein foods was present, and that this aversion was greater after having eaten a high-protein lunch meal than after a high-carbohydrate lunch meal. Recently, Chung Chun Lam *et al.* (129) showed that after a protein preload, but not after a carbohydrate preload, subjects choose subsequent foods higher in carbohydrate and lower in protein.

The strength of the present study, however, is that we varied *both* taste and protein content, in order to separate the course of action of these two components. To our knowledge this is the first study using this approach. As the majority of earlier studies did not do this, this might have affected their results (124, 125, 129). If you consider that in general savoury products contain higher protein levels than sweet products, the finding that the savoury preloads had a stronger modulating effect on subsequent food choice than sweet does seem to be in concordance with the protein-leverage hypothesis, which poses that protein intake is tightly regulated in the human body (44, 45). After the

savoury meal, a strong preference for sweet products was shown, while after the sweet meal, no preference was evident.

Through consumption of foods during our lifetime, we learn to estimate their satiating effects (106), and it has been suggested that this also plays a central role in the development of specific macronutrient appetites (13, 14). It might be that the 'learned' link between taste and macronutrient composition is quite strong and cannot be affected within one eating episode. It would be interesting to investigate whether this link between taste and macronutrient composition could be learned over a longer period of time.

Results showed that both protein content and taste had no effect on satiety. The preloads were eaten at a similar pace, no differences were seen in cumulative intake and no effects were seen on the appetite ratings. In addition, at 30 min after the preloads there was no difference seen on total intake at the *ad libitum* buffet. That we did not find a difference between the sweet and savoury preloads was no surprise and concurred with our previous research where we showed that taste had no effect on satiety parameters (121).

The lack of effect of protein content of the preloads, however, was not what we expected. Several studies have shown that protein seems to be more satiating than the iso-energetic ingestion of carbohydrate or fat [for a review see Halton and Hu (130)]. However, although it appears that the relationship between satiety and protein is clear, it is actually still quite ambiguous. For example, De Graaf *et al.* (131) did not find any effect of macronutrient composition of a preload on energy and macronutrient intake during the remainder of the day. Akhavan *et al.* (132) reported a suppressive effect of whey protein on appetite, but when inspecting the cumulative intake of the total day, no effect on intake was seen. And when investigating the results of Vozzo *et al.* (133) Hursel *et al.* (134), Leidy *et al.* (135), Chung Chun Lam *et al.* (129), and a recent study of Potier *et al.* (136), no effect of protein on short-term appetite is evident. In addition, the European Food Safety Authority (EFSA) concluded recently that a cause-and-effect relationship

**Table 4.5** Pearson's correlation analysis (*r*) of the intake (kJ) and food choice (%) at the *ad libitum* buffet with the measures of the Leeds Food Preference Questionnaire (LFPQ)

| | *Ad libitum* **buffet** | |
|---|---|---|
| **LFPQ** | Intake (kJ) | Food choice (%) |
| Explicit liking | $r_{960}=0.50\star$ | $r_{952}=0.47\star$ |
| Explicit wanting | $r_{960}=0.53\star$ | $r_{952}=0.46\star$ |
| Relative food preference | $r_{960}=0.53\star$ | $r_{952}=0.61\star$ |
| Implicit wanting | $r_{956}=-0.37\star$ | $r_{948}=-0.48\star$ |

*\*p<0.0001*

has not been established between the dietary intake of protein and a sustained increase in satiety leading to a reduction in energy intake (137). The cause of the heterogeneous finding regarding the satiating effect of protein might lie with some methodological issues concerning this type of research. Satiety appears to be influenced by a wide variety of factors, including palatability, food mass, energy density, fibre, and glycaemic index. It is very hard to control for all of these influences at the same time while still delivering different amounts of protein (130). In addition, many study designs do actually not allow us to draw conclusions on whether the effects observed can be attributed to dietary protein or to the concomitant modification of carbohydrate and/or fat as it is impossible to vary dietary protein, carbohydrate and fat content independently of one another using a single control preload (137).

There are some limitations to our study that could have influenced our findings. It could be that our intervention was too subtle; although the relative energy percentage derived from protein differed largely between the low- and high-protein preloads, the absolute difference in protein intake averaged about 13 g (with a preload intake of 250 g). However, when considering that the average daily intake of protein in the Netherlands is about 80 g (115), which is similar as reported in other parts of the world (138), a difference of 13 g within one lunch meal is still quite substantial. But it might be that subjects need to be more deprived of protein to shift their choice to high-protein foods, or be more protein satiated to shift their choice to low-protein foods. In the literature, many studies use larger amounts of protein to investigate the satiating effect [for example more than 40% of energy derived of protein (130)], and most often in liquid preloads. We chose a more naturalistic approach, with ranges of protein that were still within the normal (Dutch) range (139). In addition, we chose a solid product to ensure adequate sensory exposure, as this has been shown to be very important for satiety (140). Solids do bring the difficulty, however, of being less flexible in terms of manipulating macronutrient composition. Although there are some studies showing effects of protein on satiety with only minor differences in protein content [for example Bertenshaw *et al.* (141)], as mentioned before, the relationship between satiety and protein is still ambiguous.

It has also been suggested that a mild protein deficiency is needed to be sensitive to protein manipulations (14). Although we instructed subjects to have an overnight fast and avoid high-protein food products at breakfast, we cannot claim with certainty that our subjects were mildly protein deficient. Moreover, it might be that an interval of 30 min between the preloads and *ad libitum* buffet is too short to see an effect of protein, although Bertenshaw *et al.* (142) showed an effect of protein with this small interval. Also our preloads were not similar in palatability. Although in the past it has been shown that palatability has no effect on satiety (55), we ran an extra analysis on a sub-group of subjects which rated the preloads <20 units apart on a 100-unit VAS (n=37). This analy-

sis yielded similar results as the results for the group in total, strengthening our view that this issue did not influence our findings.

Another issue which needs to be touched upon is the macronutrient content of the different food categories that were offered at the *ad libitum* buffet. As shown in Table 4.3, it appears that the savoury categories contain more fat (g) than the sweet categories (although not significantly). Recently there have been more and more indications that humans might have a fat receptor in the oral cavity that might influence food choice and intake (33, 143). This might have interfered with the present results. To gain more insights into the intake of the participants an analysis was run on their macronutrient intake, irrespective of the different food categories. As shown in Table 4.4, no effect of protein content or taste of the preload had an effect on fat intake. The intake of carbohydrates was slightly higher after the savoury preloads. This result strengthens us in our view that the fat content of the food products did not affect the intake and food preferences differently after the preloads.

In previous research we have shown that the LFPQ can be an appropriate tool to investigate food reward (126). In the present study, the results of the LFPQ provided interesting insights regarding human eating behaviour. All measures of the LFPQ correlated with both intake and food choice, implying that both conscious (explicit) and subconscious (implicit) processes, measured by the LFPQ, are involved in self-determination of meal sizes and in the self-selection of foods within a meal; it is not just conscious decisions that determine what we eat or how much. Moreover, the regression analyses show that the amount of food that individuals ingested was significantly predicted by explicit wanting and food choice. The choice for a specific food category, however, was more predicted by the implicit measurement. This suggests that the choice of food to ingest might be made on a more subconscious level, whereas the amount of intake is a more a conscious event. Of course, the laboratory setting is not the optimum environment to investigate human eating behaviour (116), but by creating a buffet with free choice in a more relaxed eating environment in comparison with the sensory booths, we strived to create a more natural situation. In our opinion the LFPQ, or other psychological tools that can assess underlying processes [for example Calitri *et al.* (144)], is a valuable tool to use in human eating studies, not to replace measuring actual eating behaviour, but to use to unravel underlying mechanisms involved in human eating behaviour.

To summarize, the present results show that within one eating episode within-meal protein content in these quantities seems not to have an effect on subsequent food choice. This appears to be mostly determined by taste, whereby savoury taste exerts the strongest modulating effect.

# Acknowledgements

# Chapter five

## —Taste of a 24-h diet and its effect on food preferences and satiety

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# Abstract

*Sensory attributes of food play an important role in food selection and food intake. An important distinction regarding sensory signals can be made between sweet and savoury taste. The role of sweet and savoury taste in food intake regulation over a day, however, needs further clarification. The objective of this study was to investigate the effect of taste of a 24-h diet on food preferences and satiety. We used a cross-over design, consisting of a 24-h fully controlled dietary intervention, where 39 healthy subjects consumed diets that were predominantly sweet tasting, savoury tasting, or a mixture. The diets were similar in energy content, macronutrient composition, and variety, i.e. comprised equal amounts of different products. Following the intervention an ad libitum lunch buffet was offered, consisting of food items differing in taste (sweet/savoury) and protein content (high/low) and intake was measured. The results showed that taste of the diet had a large effect on food preferences (p<0.0001); after the savoury diet, intake of sweet foods was higher than of savoury foods. After the sweet diet, savoury foods tended to be preferred (p=0.07). No effect was found on preferences for high or low protein foods (p=0.67). No differences in total intake (kJ) at the ad libitum lunch buffet were observed (p=0.58). It appears that in healthy subjects, taste of a 24-h diet largely affects food preferences in terms of sensory appetite, whereby savoury taste exerts the strongest modulating effect. Taste of a 24-h diet has no effect on macronutrient appetite.*

# Introduction

Sensory attributes of food play an important role in food selection and food intake (9, 16, 17). When a food is eaten to satiety, the pleasantness of that food is decreased in comparison to foods that have not been eaten. This is termed sensory specific satiety (SSS) and was first demonstrated in humans by Rolls *et al.* (19). SSS has been demonstrated for several attributes of food, including, taste, smell, texture, and appearance (e.g. references 16, 21-26). In addition, not only eaten foods, but also foods that share sensory characteristics of the eaten foods decline in pleasantness relative to food that do not share these properties, a so-called transfer effect (112, 126). SSS is conceived as the drive for variety-seeking behaviour (27, 28, 145). When a large variety of different foods is available, the safest way for an organism to ensure adequate nutrition would be to ingest a wide selection of foods. It appears that when more than one food is available there is a natural tendency to switch between foods rather than just consume the most preferred food (18). Until now SSS has mainly been studied as a within-meal phenomenon. As far as we are aware, there are no data on SSS across a whole day.

Through repeated consumption of food during our lifetime we learn to associate the sensory attributes of food with their physiological effect and thereby learn to estimate

their satiating effects (11, 12). This has been suggested to play a central role in the development of specific appetites (13, 14). An important distinction regarding sensory signals can be made between sweet and savoury taste, which includes almost 90% of the food we eat (38). Over the course of a day, profiles of appetite for something savoury and appetite for something sweet show different patterns; it appears that appetite for something savoury oscillates more in line with the pattern of meals, i.e. more hunger related, whereas appetite for something sweet is more stable during the day (97). In addition, savoury-tasting foods contain in general higher levels of protein, while sweet-tasting foods contain more carbohydrates (40). In earlier studies we showed that sweet and savoury taste of a single meal differ in their effect on subsequent food preference, i.e. that savoury taste exerts a stronger modulating effect. The role of sensory signals in food intake regulation over a day, however, needs further clarification. Therefore the objective of this study was to investigate the effect of taste of a 24-h diet on food preferences and satiety.

The approach consisted of measuring the effect of three different 24-h diets that varied in taste only (predominantly sweet tasting, predominantly savoury tasting, or a mixture of sweet and savoury tasting). Afterwards food intake was measured during an *ad libitum* lunch buffet where a large array of food items was available differing in taste (sweet/savoury) and protein content (high/low). We hypothesized that through sensory specific satiety effects, the taste of the diet would modulate food preferences to foods with a dissimilar taste. In addition, it was hypothesized that this modulation would occur in relation to the protein content of the offered food products, i.e. that a savoury diet would modulate food preferences to sweet low protein foods.

# Methods

## Subjects

Thirty-nine subjects (11 males, 28 females) aged 21±2 years, with a mean BMI of 21.3±1.7 kg/m$^2$ completed the study. Healthy, normal weight subjects, aged 18-35 years were recruited. Exclusion criteria were the following: restrained eating (Dutch Eating Behaviour Questionnaire (DEBQ), men: score >2.25, women: score >2.8) (93), lack of appetite, an energy restricted diet during the last two months, change in body weight >5 kg during the last two months, stomach or bowel diseases, diabetes, thyroid disease or any other endocrine disorder, use of daily medication other than birth control pills, having difficulties with swallowing/eating, hypersensitivity for the foods used in the study, smoking, being a vegetarian, and for women, being pregnant or lactating. Potential participants filled out an inclusion questionnaire including a medical history questionnaire. In addition, the general liking of the products offered during the *ad libitum* lunch was

assessed with a food questionnaire containing pictures of these foods and for inclusion, at least one product of each category (see 'ad libitum lunch buffet') should be scored average or higher. There was a screening session where weight and height were measured and where subjects were familiarized with the computer tasks. Subjects were unaware to the exact outcome measurements of the study (food intake) and were informed we were interested in the effect of taste on food habits and specifically how to measure this. The present 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. This trial has been registered with the Dutch Trial Register (NTR) (registration no. NTR 2875). Written informed consent was obtained from all subjects.
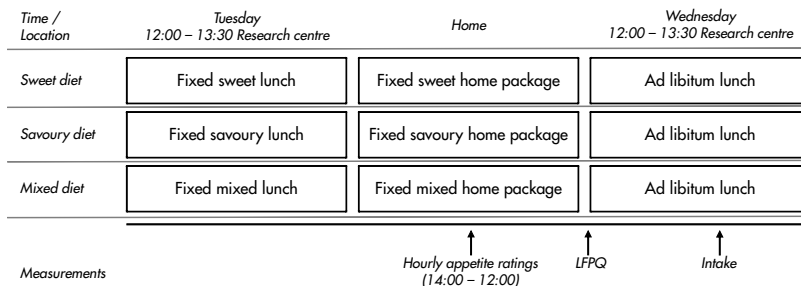


**Figure 5.1** *Overview of study design. LFPQ: Leeds Food Preference Questionnaire. Order of diets was randomized.*

## Design

The study consisted of a 24-h fully controlled dietary intervention, where subjects consumed three diets that were either predominantly sweet tasting, predominantly savoury tasting or a mixture of sweet and savoury tasting, in a cross-over design (Figure 5.1). The diets were similar in energy content, macronutrient composition, and variety, i.e. comprised equal amounts of different products. The three 24-h diets were scheduled in three subsequent weeks, always starting with a lunch on Tuesday. The order of the diets was randomized for each subject according a generalized Latin square design. Following the intervention, an *ad libitum* lunch buffet was offered on Wednesday, consisting of food items differing in taste (sweet/savoury) and protein level (high/low) and food intake was measured. In addition, during the intervention, feelings of appetite were assessed every waking hour. To identify underlying mechanisms involved in eating behaviour two advanced psychological tools were included: the Leeds Food Preference Questionnaire (LFPQ) (71, 126) and the Sorting Paired Features Task (SPF) (69), which were completed before and after the *ad libitum* lunch buffet. Please note that the results of SPF and the measurements after the *ad libitum* lunch buffet are not reported in this current paper.

**Table 5.1a** Palatability and nutritional composition (energy content and macronutrient composition) of the sweet diet, the savoury diet, and the two mixed diets.

| | Sweet diet | Savoury diet | Mixed diets | |
| --- | --- | --- | --- | --- |
| | | | #1 | #2 |
| **Palatability[1]** | | | | |
| Lunch | 5±2 | 3±2 | 5±2 | 3±2 |
| Home package | 6±2 | 6±2 | 6±2 | 7±2 |
| **Composition[2]** | | | | |
| Energy content, MJ (kcal) | 11.0 (2623) | 10.9 (2613) | 11.0 (2637) | 11.1 (2650) |
| Protein, g (% energy) | 72 (11) | 83 (13) | 69 (11) | 75 (11) |
| Carbohydrate, g (% energy) | 321 (50) | 309 (48) | 312 (48) | 320 (49) |
| Fat, g (% energy) | 116 (39) | 116 (39) | 123 (41) | 118 (40) |

[1]*Ratings after consumption using a 10-point Likert scale. Values are means±SD (n=39).* [2]*Composition is shown for the 11 MJ group. The proportion of macronutrients was similar for the 8 MJ and 13 MJ groups. The composition of the diets was calculated using food composition tables (95).*

## Test foods

*Diets.* Each subject's total energy requirement was estimated by means of the Schofield I equation (114), taking into account age, weight, sex, and a physical activity level of 1.6. Based on this calculation, subjects were assigned to either an 8 MJ diet (n=15), an 11 MJ diet (n=21), or a 13 MJ diet (n=3). The composition of the three diets is shown in Table 5.1a and comprised of products that had sweet and savoury versions (Table 5.1b). Foods were provided during a fixed lunch and in a fixed home package. The fixed lunch comprised of a rice meal with a tomato salad and a shake. The fixed home package contained two bread meals and afternoon, evening, and morning snacks. Macronutrient composition of all diets was matched for each separate eating occasion. The mixed diet comprised all products that were used in the sweet and savoury diets and products with a bland taste. To avoid a higher variety (i.e. more different products) in the mixed diet, two mixed diets were compiled. All the products of the sweet and savoury diets were divided over these two diets (Table 5.1b). In terms of palatability and composition, mixed diet #1 was slightly more coherent with the sweet diet, and mixed diet #2 slightly more coherent with the savoury diet (Table 5.1a). The subjects were randomly divided over the two mixed diets (Mixed diet #1, n=20; Mixed diet #2, n=19).

On test days, subjects were instructed to standardize their breakfast and physical activity. They had to refrain from eating and drinking energy-containing beverages 3 h prior to the start of the intervention. The intervention started Tuesday with a warm fixed lunch that was served between 12:00 and 13:30 at the research centre. Afterwards they received their fixed home package. Subjects were instructed to eat all foods that were provided and were only allowed to drink water, coffee and tea without milk or sugar.

They recorded the time of consumption of their meals and snacks in a diary and were instructed to standardize these times over the three diets.

*Ad libitum lunch buffet.* On Wednesday, the *ad libitum* lunch buffet was served between 12:00 and 13:30 at the research centre. The buffet consisted of sixteen food products

**Table 5.1b** Foods provided during the sweet diet, the savoury diet, and the two mixed diets[1]

| | Sweet diet (n=39) | Savoury diet (n=39) | Mixed diets | |
| --- | --- | --- | --- | --- |
| | | | #1 (n=20) | #2 (n=19) |
| **Fixed lunch** | | | | |
| | Rice with sweet taste[2] | Rice with savoury taste[2] | Rice with bland taste | Rice with bland taste |
| | Sliced tomatoes with sweetener[3] | Sliced tomatoes with salt | Sliced tomatoes with salt | Sliced tomatoes with sweetener[3] |
| | Vanilla shake[4] | Spicy chicken shake[5] | Vanilla shake[4] | Spicy chicken shake[5] |
| **Fixed home package** | | | | |
| Bread meals and Toppings | Sweetened bread[6] | Salted bread[7] | Bland (normal) bread | Bland (normal) bread |
| | Chocolate sprinkles | Cheese spread | Chocolate sprinkles | Chocolate paste |
| | Jam | Sausages | Jam | Apple syrup |
| | Chocolate paste | Cheese | Cheese spread | Cheese |
| | Apple syrup | Ham | Sausages | Ham |
| Afternoon and Morning snack (biscuits) | Wholegrain Natural | Barbeque bacon Mexican spices | Wholegrain Barbeque bacon | Natural Mexican spices |
| Evening snack (nuts) | Chocolate peanuts Sugared peanuts | Dry roasted peanuts Beer nuts | Chocolate peanuts Dry roasted peanuts | Sugared peanuts Beer nuts |

[1]All subjects (n=39) received the sweet diet and the savoury diet. To avoid a higher variety (i.e. more different products) in the mixed diet, two mixed diets were compiled. All the products of the sweet and savoury diets were divided over these two mixed diets and the subjects were randomly divided. [2]These rice meals have been used in previous experiments and were matched on macronutrient composition, energy density, texture, and intensity (121, 126, 146). [3]The used sweetener contained aspartame (3%) with bulking agent maltodextrin (97%) (Albert Heijn, Zaandam, The Netherlands). [4]The Vanilla shake was made by mixing vanilla custard with water and vegetable oil. [5]The Spicy chicken shake was made by blending spicy chicken soup (Unox, Unilever Nederland, Rotterdam, The Netherlands) with Fantomalt (Nutricia Nederland BV, Zoetermeer, The Netherlands). [6]The sweetened bread was made by adding sugar and Protifar (Nutricia Nederland BV, Zoetermeer, The Netherlands) during the baking process. [7]The salted bread was made by adding salt and Fantomalt (Nutricia Nederland BV) during the baking process.

that were selected on the basis of their taste (sweet/savoury) and protein content (high/low). The resulting four categories were matched on energy density, fat content, and type of food (each category contained one meal item, one salad, one sandwich, and one semi-solid product). Energy content and macronutrient composition of the selected products are shown in Table 5.2. The food products were offered in large quantities, and if they consisted of single pieces these were around 40 g (pancakes 34 g, apple turnovers 43 g, small pizzas 30 g, rösti rounds 43 g). The buns were 22 g with 22 g of topping. During the *ad libitum* lunch buffet, subjects could select foods and serve themselves and eat until comfortably satiated. Individual food intake was measured by weighing the remainders of food on the plate and number of different products eaten was counted. *Ad libitum* energy and macronutrient intake was calculated using food composition tables (95).

### Measurements

During the intervention, each subject completed an appetite questionnaire hourly during waking hours over a 24-h period using a Personal Digital Assistant (HP IPAQ with software of EyeQuestion Version 3.8.3., Logic8 BV, 2010, Elst, The Netherlands) starting after the fixed lunch on Tuesday from 14:00 until 12:00 the next morning. The questionnaire consisted of seven dimensions: hunger, fullness, prospective consumption, desire to eat, appetite for something sweet, appetite for something savoury, and thirst. The 10-point Likert scale was anchored with 'not at all' to 'extremely'. This appetite questionnaire was also run prior to and immediately after the consumption of the fixed lunch and the *ad libitum* lunch buffet.

Before the *ad libitum* lunch buffet, the LFPQ was completed. The LFPQ is a computerized hedonic analysis platform that measures explicit and implicit components of food reward and included photographs of the 16 foods shown in Table 5.2. For explicit measures, each food was shown and subjects had to rate their liking ('how pleasant would you find the taste of this food right now?') and their wanting ('how much do you want to eat this food right now?') on a 100-unit visual analogue scale (VAS). In addition, foods were presented in randomized pairs where subjects had to select their most wanted food ('select the food which you most want to eat right now') as quickly and accurately as possible. During the latter procedure both frequency of preferred choice (relative food preference) and reaction time were measured. As participants were not informed about the measurement of their reaction time for each choice and unable to monitor their responses, this measure provided a non-verbal, implicit assay of their motivation (implicit wanting). Reaction times (RT) were transformed to a standardized 'd-score' using a validated algorithm (70): the smaller the d-score, the greater the implicit wanting for that food category relative to other categories in the task.

**Table 5.2** Energy content and macronutrient composition of the food products offered during the *ad libitum* lunch buffet and shown in the Leeds Food Preference Questionnaire (per 100 g)[1]

| | Energy, kJ (kcal) | Protein, g (% energy) | Carbohydrates, g (% energy) | Fat, g (% energy) |
|---|---|---|---|---|
| **Low Protein Sweet** | | | | |
| Apple turnover | 1690 (405) | 4 (4) | 39 (39) | 26 (57) |
| Salad with pineapple and raisins with raspberry dressing | 311 (73) | 1 (7) | 17 (93) | 0 (1) |
| Fruity sprinkles sandwich | 1342 (316) | 5 (6) | 72 (91) | 1 (3) |
| Chocolate mousse | 1052 (252) | 3 (4) | 26 (43) | 15 (53) |
| **Average** | **1099 (262)** | **3 (5)** | **38 (66)** | **11 (29)** |
| **High Protein Sweet** | | | | |
| Pancakes | 821 (195) | 8 (17) | 29 (60) | 5 (22) |
| Salad with nuts and raisins with raspberry dressing | 717 (172) | 5 (11) | 16 (37) | 10 (52) |
| Peanut butter honey sandwich | 1508 (359) | 11 (12) | 45 (51) | 15 (36) |
| Curd with fruit taste | 562 (133) | 9 (26) | 17 (51) | 3 (23) |
| **Average** | **902 (215)** | **8 (17)** | **27 (50)** | **8 (33)** |
| **Low Protein Savoury** | | | | |
| Rösti rounds | 608 (145) | 2 (5) | 23 (64) | 5 (31) |
| Salad with croutons and fried onions with herbs dressing | 557 (133) | 2 (7) | 15 (46) | 7 (47) |
| Savoury spread sandwich | 979 (233) | 5 (9) | 30 (51) | 10 (39) |
| Potato salad | 715 (172) | 2 (5) | 14 (34) | 12 (62) |
| **Average** | **715 (171)** | **3 (7)** | **20 (49)** | **9 (45)** |
| **High Protein Savoury** | | | | |
| Small pizzas | 848 (202) | 9 (17) | 25 (51) | 7 (32) |
| Salad with nuts and cheese with herbs dressing | 849 (205) | 7 (14) | 3 (7) | 18 (79) |
| Egg sandwich | 801 (190) | 11 (24) | 21 (45) | 7 (31) |
| Russian salad | 642 (154) | 6 (15) | 14 (36) | 9 (49) |
| **Average** | **785 (188)** | **8 (18)** | **16 (35)** | **10 (48)** |

[1] *Values derived from the Dutch nutrient database 2006 (95).*

## Statistical analyses

Data are presented as mean values with standard deviation unless otherwise specified. Comparisons of the 24-h appetite ratings between the three diets were made by calculating the areas under the curve (AUC) for all ratings (trapezoidal method) and these were compared by means of ANOVA (mixed model procedure) with type of diet (sweet, savoury, mixed) as independent variable. Pre and post appetite ratings for the lunches

were analysed by means of ANOVA (mixed model procedure) with type of diet and type of lunch (fixed, *ad libitum*) as independent variables. Intake during the *ad libitum* lunch buffet was compared by means of ANOVA (mixed model procedure) with diet as independent variable. Analyses were performed on total energy intake (kJ), amount of different products (variety), and intake of each macronutrient (g) separately (protein, carbohydrates and fat). In all analyses, both main effects and interactions were analysed. In addition, participants were included in all models as random factor. Post-hoc comparisons were made using Tukey's correction. The *ad libitum* intake (kJ) was tested for correlation (Pearson's correlation coefficient) with the measures of the LFPQ. In addition, the predictive values of the measures of the LFPQ on intake (kJ) were investigated using a multiple linear regression analyses with backward elimination. Analyses were conducted using SAS, 9.1 (SAS Institute, Inc., Cary, NC, USA).

# Results

**Appetite ratings during the sweet diet, the savoury diet, and the mixed diet**
Analyses on the 24-h ratings showed that during the savoury diet, subjects reported significantly less hunger than during the sweet diet and mixed diet [$F_{(2, 76)}=12.6$, $p<0.0001$] (Figure 5.2A). These results were similar for fullness ($p<0.01$), prospective consumption ($p<0.0001$) and desire to eat ($p<0.0001$). During the sweet diet, subject reported significantly less appetite for sweet than during the savoury diet and the mixed diet [$F_{(2, 76)}=22.6$, $p<0.0001$] (Figure 5.2B). During the savoury diet, subjects reported significantly less appetite for savoury than during the sweet diet and mixed diet [$F_{(2, 76)}=26.9$, $p<0.0001$] (Figure 5.2C). During the savoury diet, subjects reported significantly more thirst than during the sweet diet [$F_{(2, 76)}=4.1$, $p<0.05$].

**Intake at *ad libitum* lunch buffet after the sweet diet, the savoury diet, and the mixed diet**
Total energy intake (kJ) during the *ad libitum* lunch buffet did not differ between the sweet diet (4598±1354 kJ), the savoury diet (4517±1745 kJ) and the mixed diet (4682±1592 kJ) [$F_{(2, 76)}=0.5$, $p=0.58$] (Figure 5.3). Nor did the average number of different products the subjects chose differ between the sweet diet (6.5±1.5 products), the savoury diet (6.1±1.5 products), and the mixed diet (6.4±1.0 products) [$F_{(2, 76)}=1.4$, $p=0.25$]. Total protein intake (g) at the *ad libitum* lunch buffet did not differ between the sweet diet (31±12 g), the savoury diet (31±15 g) and the mixed diet (31±14 g) [$F_{(2, 76)}=0.06$, $p=0.94$]. The intake of carbohydrates tended to be slightly higher after the savoury diet (124±49 g) in comparison to after the sweet diet (114±38 g) [$F_{(2, 76)}=2.9$, $p=0.06$]. Intake of fat was lower after the savoury diet (51±22 g) than after the sweet diet (58±19 g) and mixed diet (57±22 g) [$F_{(2, 76)}=4.3$, $p<0.05$].

## A. Hunger

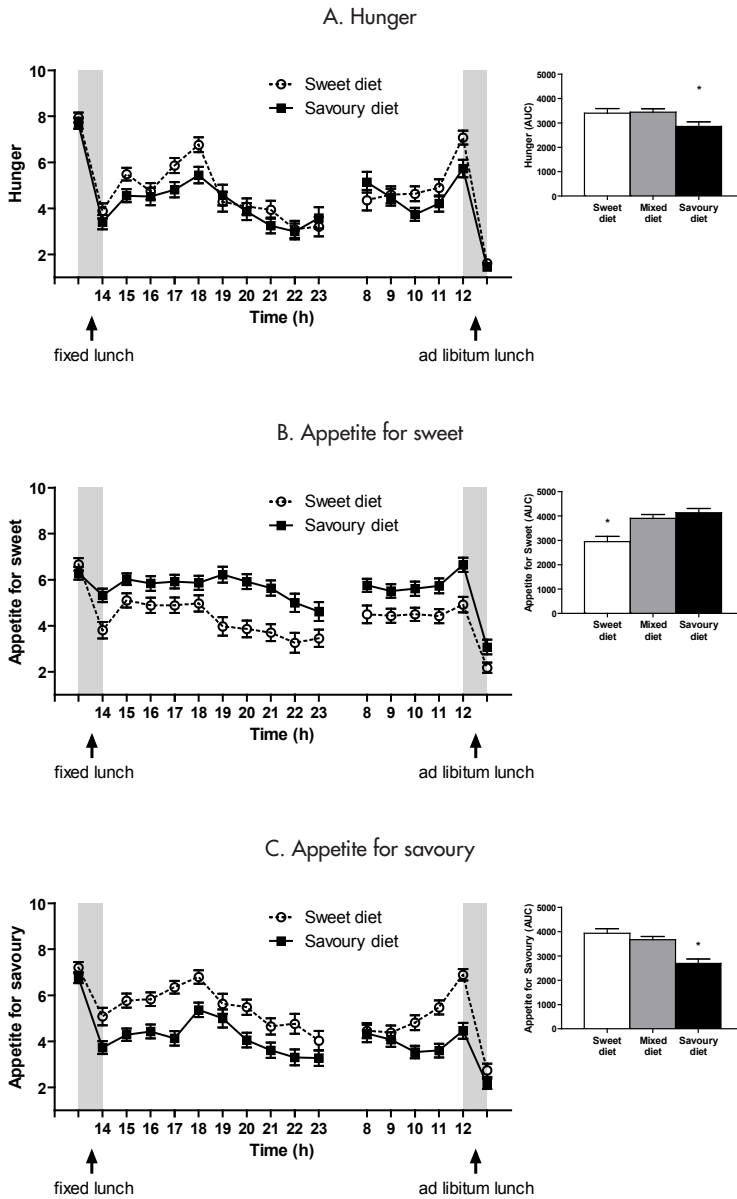

## B. Appetite for sweet



## C. Appetite for savoury



**Figure 5.2** *The hourly rated feelings of A. Hunger, B. Appetite for something sweet, and C. Appetite for something savoury during waking hours from 14:00 till 12:00 the next morning during the sweet diet and savoury diet assessed on a 10-point Likert scale (for readability, mixed diet is not included). AUCs are shown for sweet diet, mixed diet, and savoury diet. Values are means±SE.*
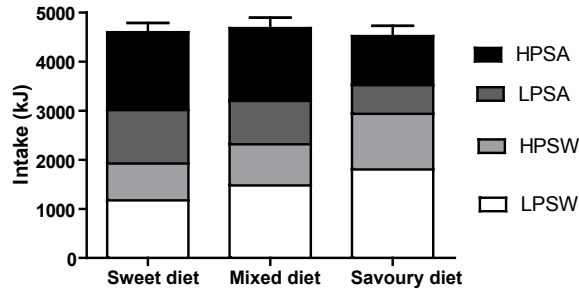
**Figure 5.3** *Total intake (kJ) of the high-protein savoury (HPSA), low-protein savoury (LPSA), high protein sweet (HPSW), and low-protein sweet (LPSW) products at the ad libitum lunch buffet after the sweet diet, the mixed diet, and the savoury diet. Values are means±SE (n=39).*

The energy intake (kJ) of the different food categories at the lunch buffet revealed that the taste of the diet significantly altered preference for foods according to their taste properties; [F (2, 76)=16.0, *p*<0.0001]; after the savoury diet, the intake of the savoury foods (1582±1312 kJ) was lower that of the sweet foods (2935±1270 kJ) (*p*<0.0001). After the sweet diet, the intake of sweet foods (1924±1035 kJ) tended to be lower than of the savoury foods (2674±991 kJ) (*p*=0.07). After the mixed diet, intake of sweet foods (2317±1020 kJ) and savoury foods (2365±1159 kJ) did not differ (*p*=1.00) (Figure 5.3). No interaction was seen between the taste of the diet and food preference according to their protein content [F(2, 76)=0.4, *p*=0.67].

**Correlation of intake at the *ad libitum* lunch buffet with results of the LFPQ**
The results of the LFPQ concurred with the *ad libitum* food intake at the lunch buffet. All measures of the LFPQ were significantly correlated with the *ad libitum* intake at the lunch buffet (kJ); explicit liking $r_{468}$=0.36, *p*<0.0001; explicit wanting $r_{468}$=0.38, *p*<0.0001; relative food preference $r_{468}$=0.53, *p*<0.0001; implicit wanting $r_{468}$=-0.39, *p*<0.0001 (a negative correlation indicates that an increase in intake was associated with a faster response time). The multiple linear regression analyses showed that the *ad libitum* intake at the lunch buffet (kJ) was predicted mostly by relative food preference and implicit wanting measures of the LFPQ ($R^2$ 0.30).

# Discussion

With this study the effect of taste of a 24-h diet on food preferences and satiety was investigated. Results showed, concurrent with the hypothesis, that through sensory specific satiety effects, taste of the diet modulated food preferences towards foods with a

dissimilar taste. We did, however, not find any effect of taste of the diets on preferences for high and low protein foods. In addition, no differences in total intake at the *ad libitum* lunch buffet were observed.

The intake at the *ad libitum* lunch buffet after the three diets showed a clear transfer effect of sensory specific satiety, i.e. after consuming a diet with a certain taste, the intake of foods with a similar taste was less than of food with a dissimilar taste, while there was no effect on total intake. These results concur with earlier short-term studies investigating the effect of taste of a single meal on subsequent food preferences and satiety (e.g. references 98, 99, 126, 146).

The transfer effect of sensory specific satiety was not equipotent for the sweet and savoury diets however: after the savoury diet a large preference was seen for sweet foods. After the sweet diet subjects seemed to favour savoury foods, but this preference was less pronounced. These findings are in coherence with previous finding where it was shown that savoury taste of a meal has a stronger modulating effect on subsequent food choice than sweet (126, 146). We believe this asymmetry of SSS transfer between sweet and savoury taste might come about via the association with their physiological effect. Within the food range, savoury-tasting foods contain in general higher levels of protein, while sweet-tasting foods contain more carbohydrates (40). Several studies have shown a positive association between feelings of hunger and appetite for high-protein (savoury) foods (124, 125, 147). These data align with the 'protein leverage hypothesis' which poses that protein intake is tightly regulated (43-45). This makes a sensory-nutrient interaction plausible; that regulation of macronutrient intake may come about via the sensory signals from our food. As savoury taste would signal for a source of protein, it could be hypothesized that after a savoury diet, the intake of protein-rich foods would be lower than after the sweet diet. The data, however, showed that intake of high and low protein foods was similar after the three diets – and intake of protein at the *ad libitum* lunch buffet was exactly 31 g after all three diets, indicating that taste of the diet did not have an effect on food preference in terms of protein content.

It has been shown that energy and macronutrient balance are regulated over time (5). It can be assumed that the participating healthy subjects were not deficient in any macronutrient and therefore, from a physiological point of view, there was no need to adjust protein intake. The results favour the hypothesis that sensory signals of a diet do not change macronutrient appetites, but do change sensory appetites. From an evolutionary point of view, rapid adaptive changes in sensory appetites might serve to ensure a diet with lots of variety (27). In an earlier study it was established that when subjects experienced macronutrient imbalance through selective reduction of dietary protein, food preferences shifted to high protein savoury foods (148). Therefore we propose that

sensory signals of food play an important role in guiding food intake to maintain macronutrient balance. Only when homeostasis is challenged, e.g. when facing prolonged macronutrient deficit, will macronutrient driven appetite engage to restore macronutrient balance. There are several recent animal studies supporting a role for taste in maintaining macronutrient balance (149), but also for taste-independent sensors when homeostasis is threatened (150).

Interestingly, feelings of appetite during the 24-h savoury diet were reduced compared with the sweet and mixed diets. It could be hypothesized this effect is due to the association that exists between savoury taste and protein content. Protein has been implicated to be more satiating than carbohydrates and fat (130), and this might be partly facilitated by the savoury taste. In addition, it has been suggested that sweet taste has an enhancing effect on appetite - the hedonic properties of sweetness embody strong reward potential with the capacity to reinforce its own consumption (123). The observed reduction in appetite during the savoury diet, however, was not reflected in the total food consumed at the *ad libitum* lunch buffet. The objective of this study was to investigate the effect of taste of three different diets on satiety and food preference. To examine the latter, a large variety of food products at the *ad libitum* lunch buffet was offered. This might have interfered with the subjects' intake. If the average intake at the lunch buffet (4.6 MJ) is compared with the Dutch average daily energy intake within this age group during lunch (2.2 MJ) or dinner (3.1 MJ) (115), it is apparent that the subjects ingested more than average after all three diets. It has long been know that variety increases intake (27), and it has been shown in earlier studies that buffet style dinners facilitate overeating (108). It might be that the presence of multiple items in the buffet nullified differences in satiety state brought about by the intervention.

As mentioned, in general, savoury-tasting food contain higher levels of protein, while sweet-tasting foods contain more carbohydrates (40). It is challenging to change the taste of a diet independently of macronutrient composition, while preserving the use of relatively normal foods. Consequently, some compromises were made regarding control over macronutrient content and palatability (Table 5.1a). As the savoury diet contained slightly more protein and was less palatable than the sweet diet, the effects of these differences were further investigated by comparing the two variants of the mixed diet. Mixed diet #1 had a protein level and a palatability rating more coherent with the sweet diet, and mixed diet #2 had a protein level and palatability rating more coherent with the savoury diet (Table 5.1a). It might be expected that mixed diet #2 would have higher satiety effects as it was higher in protein (130) and lower in palatability (55) than mixed diet #1. The opposite, however, was found: mixed diet #2 appeared to be lower in satiety (Hunger AUC 3576±985) than mixed diet #1 (Hunger AUC 3299±807). This strengthened us in our view that this minor issue did not affect the results.

To identify underlying mechanisms involved in eating behaviour the LFPQ was included in the study to assess different components of food reward. All measures of the LFPQ correlated with the intake at the lunch buffet, implying that both conscious (explicit) and unconscious (implicit) processes were involved in the selection and ingestion of food. The regression analyses, however, suggests that food intake was mostly determined by implicit wanting and relative food preference. We aimed to create an environment where subjects could behave as naturally as possible (self-selection and serving of foods in a relaxed environment where subjects could sit and eat together) and it is interesting to see that in this setting implicit, unconscious processes were more strongly determining food intake behaviour. This is unlike the more controlled setting we have used in the past, where explicit ratings were more predictive of food intake (126, 146).

In conclusion, the results of this study show that in healthy subjects, taste of a 24-h diet largely affects food preferences in terms of sensory appetite, whereby savoury taste exerts the strongest modulating effect. The taste of a 24-h diet has no effect on macronutrient appetite. More long term studies are warranted to further investigate the relationship between sensory signals of food and the role they play in food intake regulation.

# Acknowledgements

# Chapter six

## —Protein status elicits compensatory changes in food intake and food preferences

Sanne Griffioen–Roose, Monica Mars, Els Siebelink, Graham Finlayson, Daniel Tomé, Cees de Graaf

# Abstract

*Background: Protein is an indispensable component within the human diet. It is unclear, however, whether behavioural strategies exist to avoid shortages.*

*Objective: The objective was investigate the effect of a low protein status compared with a high protein status on food intake and food preferences.*

*Design: We used a randomized crossover design that consisted of a 14-d fully controlled dietary intervention involving 37 subjects (age: 21±2 y; BMI: 21.9±1.5 kg/m²) who consumed individualized, isoenergetic diets that were either low in protein (0.5 g protein/kg BW/day) or high in protein (2.0 g protein/kg BW/day). The diets were followed by an ad libitum-phase of 2.5 d, during which a large array of food items was available, and protein and energy intake were measured.*

*Results: We showed that in the ad libitum-phase protein intake was 13% higher after the low protein diet than after the high protein diet (253±70 g compared with 225±63 g, p<0.001), whereas total energy intake was not different. The higher intake of protein was evident throughout the ad libitum-phase of 2.5 d. In addition, after the low protein diet, food preferences for savoury high protein foods were enhanced.*

*Conclusion: After a protein deficit, food intake and food preferences show adaptive changes that suggest that compensatory mechanisms are induced to restore adequate protein status. This indicates that there are human behavioural strategies present to avoid protein shortage, and that these involve selection of savoury high protein foods.*

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# Introduction

Protein is an indispensable component within the human diet. It provides the body with nitrogen and amino acids, including the nine amino acids classified as indispensable that are of crucial importance in preserving and maintaining bodily functions and life (42). Both in animals (151) and in humans (5, 45, 152) it has been shown that energy and macronutrient balance are regulated over time, and it has been posed that specifically protein intake is tightly regulated (43-45, 153). Accordingly, animal studies have shown that rodents have several behavioural strategies for regulating the ingestion of indispensable amino acids, including meal termination, altered food choice, foraging for foods that will complement or correct for deficiency, development of learned aversion to a deficient or imbalanced food to avoid that food in the future, and memory for the taste, smell, or place associated with protein-containing food (154-158). In humans, the range of protein intake has remained relatively constant over time and across populations, both as a percentage of energy in the diet (~10-25%) and in terms of absolute amount eaten (~40-100 g), but it is less clear whether behavioural strategies exist to avoid shortages (43-45). Several studies have shown that hungry subjects show a preference for high-

protein foods (e.g. references 124, 125, 147), but the causal short-term relationship between protein content, food choice, and satiety remains unclear (137), because there are many contradictory findings (e.g. references 130, 131, 159). The objective of this study was to investigate the effect of a low protein status compared with a high protein status on food intake and food preferences.

Our approach consisted of measuring the effect of two different diets that varied in protein content (a low protein diet compared with a high protein diet). To achieve differences in protein status, the dietary intervention lasted for 14 d. Afterwards *ad libitum* food intake was measured for 2.5 d. We hypothesized that when protein status is low, after 14 d of consuming a low protein diet, food preferences will shift to high protein foods, resulting in a higher protein intake than after consuming a high protein diet.

# Methods

### Subjects

Thirty-seven subjects (12 men, 25 women) with a mean±SD age of 21±2 y and a mean BMI of 21.9±1.5 kg/m$^2$ completed the study. Of the 41 participants enrolled in the study, four participants dropped out during the first week (two of each treatment). We recruited healthy, normal-weight subjects, aged 18-35 y. Exclusion criteria were as follows: restrained eating (Dutch Eating Behaviour Questionnaire (DEBQ), men: score >2.25, women: score >2.80) (93), lack of appetite, an energy restricted diet during the past two months, change in body weight >5 kg during the past two months, stomach or bowel diseases, diabetes, thyroid disease or any other endocrine disorder, prevalent cardiovascular disease, use of daily medication other than birth control pills, having difficulties with swallowing/eating, hypersensitivity for the foods used in the study, being a vegetarian, and for women, being pregnant or lactating. Potential participants filled out an inclusion questionnaire including a medical history questionnaire. Then they attended a screening session, which included measurement of weight and height. In addition, the procedures were explained and a Food Frequency Questionnaire (FFQ) was filled out. Results of the FFQ showed that the mean±SD daily energy intake reported by the subjects was 11.3±3.8 MJ, and the protein intake 93±31 g. Subjects were unaware of the exact aim of the study and were informed we were investigating the effect of specific diets, which varied in macronutrient content, on food preferences. Subjects were naïve to the fact that we specifically varied the protein and carbohydrate content of the diets.

The study was approved by the Medical Ethical Committee of Wageningen University. This trial has been registered with the Dutch Trial register (registration no. NTR 2491). Written informed consent was obtained from all subjects.

## Design

The study consisted of a 14-d fully controlled dietary intervention involving subjects who consumed isoenergetic diets that were either low in protein (containing 0.5 g protein/kg BW/d; ~5% of energy derived from protein) or high in protein (containing 2.0 g protein/kg BW/d; ~21% of energy) in a randomized, crossover design (Figure 6.1). The amount of protein in the low protein diet was below the average daily recommendation and was considered to be inadequate (160).
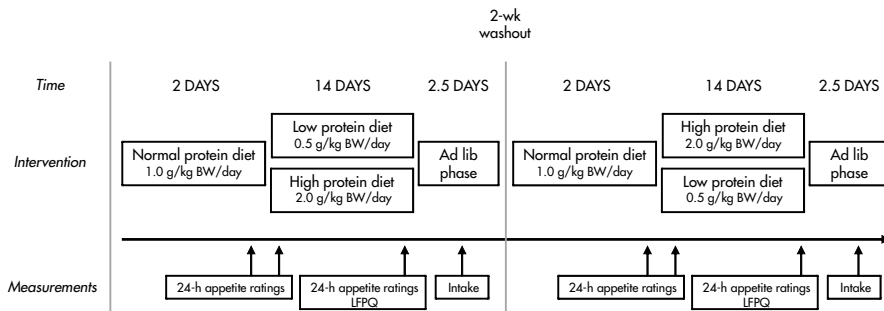


***Figure 6.1*** *Overview of study design. LFPQ: Leeds Food Preference Questionnaire.*

Both diets were preceded by 2 d during which subjects ate a normal protein diet containing 1.0 g protein/kg BW/d (~11% of energy), which is the average consumption of the Dutch population within this age group (115). These two d were used to adapt subjects to the procedure and to ensure energy balance. To assess the effect of protein status on food intake and food preferences, the intervention was followed by an *ad libitum*-phase of 2.5 d during which a large array of food items was available. During the dietary intervention, appetite was assessed during three single 24-h periods: on day 2 of the normal protein diet (baseline rating) and on days 1 and 14 of the low and high protein diets. In addition, the Leeds Food Preference Questionnaire (LFPQ) (71, 126, 146) was completed on day 14 of each dietary condition, before the first *ad libitum* lunch. The two dietary conditions were separated by a 2-wk washout period, and during this time subjects were instructed to consume their habitual diet.

Protein in the diets was exchanged for carbohydrate, and the amount of fat was kept similar (Table 6.1). Protein manipulation was achieved by varying commercially available foods in the diets and by changing protein contents within foods (e.g. low protein bread). In addition, whey protein isolate powder (Nectar, pink grapefruit, Syntrax, Scott City, MO, USA) was added to drinks, desserts, or both, which were consumed during the hot meal to enable the variations in required individual protein amounts.

## Procedure

During the dietary intervention we provided the subjects with foods and beverages, except for water, coffee, and tea (*ad libitum* intake without milk and sugar), which covered ~90% of their estimated daily energy requirement. Subjects chose the remaining 10% of energy from a list of choice items that included virtually protein-free and fat-free foods (common procedure within our division, see reference 161). Their choice was recorded in a diary. Each subject's total energy requirement was estimated by using the results of the FFQ, which was filled out during screening, and by means of the Schofield equation taking into account age, weight, height, sex, and a physical activity level of 1.6 (114).

During weekdays at lunch, the participants visited the division and consumed their hot meal. All other foods were supplied daily as a meal package and consumed at home. The home meal package contained two bread meals with toppings for dinner and breakfast, and beverages, fruits, and snacks. On Fridays, subjects received a home meal package with foods and beverages for the entire weekend plus instructions for the preparation of these foods. Subjects were instructed to eat all foods that were provided. They were allowed to use seasoning and table salt.

During weekdays, palatability of the hot meals was rated by using a 10-point Likert scale (mean±SD results: low protein diet 8±2; high protein diet 7±2). Palatability of the home meal packages was measured three times for both dietary conditions (day 1, day 8, day 14, mean±SD results: low protein diet 7±1; high protein diet 8±1).

**Table 6.1** Nutritional composition (energy content and macronutrient composition) of the daily low and high protein diets for a participant with an energy intake of 11 MJ/d and a body weight of 68 kg[1]

|  | Low protein diet | High protein diet |
|---|---|---|
| **Energy, MJ** | 10.7 | 11.4 |
| **Protein, g/kg BW** | 0.5 | 1.9 |
| **Protein, g (% energy)** | 31 (5) | 127 (19) |
| **Carbohydrates, g (% energy)** | 353 (56) | 303 (45) |
| **Fat, g (% energy)** | 108 (37) | 106 (34) |
| **Alcohol, g (% energy)** | 5 (1) | 6 (2) |
| **Fibre, g** | 30 | 31 |

[1]*Duplicate portions of the provided diets were collected every day for an imaginary participant, stored at -20°C, and analysed for energy and macronutrient composition after the experiment. Nitrogen was determined by the Kjeldahl method (96, method 920.87), and the amount of protein was calculated by using a conversion factor of 6.25; fat by the acid hydrolysis method (96, method 14.019); available carbohydrate was calculated by subtracting moisture, ash, protein, dietary fibre and fat from total weight. Energy content was calculated from the macronutrient composition by using the following energy conversion factors: protein, 17 kJ/g; fat, 37 kJ/g; carbohydrate, 17 kJ/g; alcohol, 29 kJ/g. The average of the calculated composition of the free-choice items (10%), which were recorded in a diary by all participants (n=37), was added.*

The *ad libitum*-phase of 2.5 d that followed the dietary intervention started with a hot lunch; participants could select foods themselves and eat until comfortably satiated. Large meal packages were provided for consumption at home and contained >200% of the estimated energy requirements. The home meal packages consisted of foods that were not available during the intervention and included buns with toppings for dinner and breakfast and beverages, fruits, and snacks. The foods were provided in unusual portion sizes to prevent habitual intake. In addition, many foods were offered in both a low and high protein version to enable selective protein intake (Table 6.2). In total, the *ad libitum*-phase comprised three lunches and two home meal packages. Individual food intake was measured by weighing the remainder of food on the plate (during lunch) and the home meal packages the next day. *Ad libitum* energy intake and macronutrient selection were calculated by using Dutch food composition tables (95).

### Measurements

During the dietary intervention, appetite was assessed during three single 24-h periods: on day 2 of the normal protein diet (baseline rating) and on day 1 and 14 of the low and high protein diets. Each subject completed an appetite questionnaire hourly during waking hours over the 24-h period using a Personal Digital Assistant (HP IPAQ with EyeQuestion Version 3.8.3 software, Logic8 BV, 2010, Elst, The Netherlands) starting after lunch from 14:00 until 12:00 the next day. The questionnaire consisted of seven dimensions: hunger, fullness, prospective consumption, desire to eat, appetite for something sweet, appetite for something savoury, and thirst. The 10-point Likert scale was anchored with 'not at all' to 'extremely'.

The LFPQ was completed on day 14 of each dietary condition, before the first *ad libitum* lunch. The LFPQ is a computerized hedonic analysis platform that measures explicit and implicit components of food reward and included photographs of 16 foods varying in two dimensions – protein (low and high) and taste (sweet and savoury). These four categories were matched on energy density, fat content, and type of food (each category contained one sandwich, one snack, one cookie, and one meal item). For explicit measures, each food was shown and subjects had to rate their liking ('how pleasant would you find the taste of this food right now?') and their wanting ('how much do you want to eat this food right now?') on a 100-mm visual analogue scale (VAS). In addition, foods were presented in randomized pairs, and subjects had to select their most wanted food ('select the food which you most want to eat right now') as quickly and accurately as possible. During the latter procedure, both frequency of preferred choice (relative food preference) and reaction time were measured. Because participants were not informed about the measurement of their reaction time for each choice and were unable to monitor their responses, this measure provided a nonverbal, implicit assay of their motivation (implicit wanting). Reaction times were transformed to a standardized 'd-score' by using

a validated algorithm (70): the smaller the d-score, the greater the implicit wanting for that food category relative to other categories in the task.

### Body weight, urine nitrogen excretion, and analytical methods

Body weight was measured twice a week before subjects ate their hot meal while subjects were wearing no shoes or heavy clothing. If a subjects' weight fluctuated >0.2 kg from baseline, the research dietician decided whether energy intake needed to be adjusted for weight maintenance.

As an independent, objective marker of dietary compliance, total urine nitrogen excretion was measured from two 24-h urine collections made during day 14 of each dietary condition. Results showed that total urine nitrogen excretion decreased with low dietary protein intake (low protein diet: 84±17 mg/kg BW/day, high protein diet: 248±38 mg/kg BW/day). These data confirm that the low protein diet was inadequate and contained protein levels below the average daily recommendation (equivalent to 105 mg nitrogen/kg BW/day) (160). Completeness of the two 24-h urine samples was verified by recovery of three 80 mg doses of paraaminobenzoic acid given with the meals (162). Analyses showed an average recovery rate of 96.5%. Nitrogen in urine was determined colorimetrically according to the Kjeldahl method (96, method 920.87) on a Vitros 250 Chemistry System (Ortho-clinical Diagnostics, Raritan, NJ, USA).

Although we relied primarily on the total nitrogen excretion data as an independent, objective marker of dietary compliance, we also used other means to promote compliance. These included instructing participants to keep a diary to record any deviations from the diet, illness, and use of drugs. Subjects were urged not to change their smoking habits and their physical activities. The latter was also monitored by assessing the number of steps taken each weekday with pedometers (Yamax Digi-walker, SW-200, Tokyo, Japan)

### Statistical analyses

Comparisons of appetite ratings between the low and high protein diet were made by calculating the areas under the curve (AUC) for all ratings (trapezoidal method), and these were compared by means of ANOVA (mixed model procedure). Because baseline ratings did not differ between the two diets, these were not incorporated in the analyses. Intake during the *ad libitum*-phase were compared by means of ANOVA (mixed model procedure). Analyses were performed on protein intake (g) and total intake (MJ) for the total 2.5 d, and separate analyses were performed with days (3 d), lunches (3 lunches) and home meal packages (2 packages) as factors in the model. Intake of the different food categories were compared by means of a paired *t* test. The results of the LFPQ were analysed by using ANOVA (GLM procedure). In all analyses, both main effects and interactions were analysed. In addition, participants were included in all models as
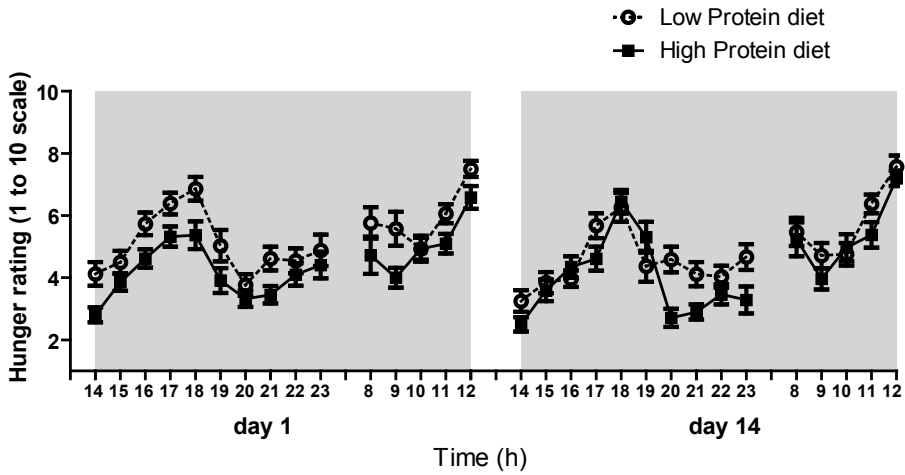
**Figure 6.2** *The hourly rated feelings of hunger during waking hours from 14:00 untill 12:00 the next day on days 1 and day 14 during the low protein diet and the high protein diet assessed on a 10-point Likert scale. Values are means±SE.*

random factor. Post-hoc comparisons were made by using Tukey's correction. Analyses were conducted by using SAS, 9.1 (SAS Institute, Inc., Cary, NC, USA). Data are presented as mean values with standard deviation unless otherwise specified.

# Results

### Appetite ratings during the low and high protein diets

During the low protein diet subjects reported significantly more hunger than during the high protein diet ($p<0.0001$) on both day 1 and 14 (Figure 6.2); the magnitude of this difference did not change (diet x day interaction: $p=0.52$). These results were similar for fullness ($p<0.001$), prospective consumption ($p<0.0001$), desire to eat ($p<0.0001$), and appetite for something savoury ($p<0.0001$). On day 1 during the low protein diet subjects experienced more appetite for something sweet than during the high protein diet ($p<0.05$). On day 14, however, the difference was no longer evident ($p=0.85$). The diets had no differential effects on ratings of thirst.

### Intake during the *ad libitum*-phase after the low and high protein diets

Total protein intake (g) during the *ad libitum*-phase was 13% higher after the low protein diet (253±70 g) than after the high protein diet (225±63 g) ($p<0.001$). This difference in protein intake was evident on all three days ($p<0.01$): day 1, 105±30 g (1.6 g/kg BW/day) compared with 92±25 g (1.4 g/kg BW/day); day 2, 101±41 g (1.5 g/kg BW/day) compared with 94±28 g (1.4 g/kg BW/day); day 3, 46±20 g compared with 39±17 g;

and was evident both during the hot lunches ($p<0.01$) and during consumption at home ($p<0.05$) (Figure 6.3A). The proportion of energy derived from protein during the *ad libitum*-phase was also significantly higher after the low protein diet (12.9%) than after the high protein diet (11.8%) ($p<0.001$). The proportions of energy derived from carbohydrates and fat after the low and high protein diets, respectively, were 52.1% compared with 54.0% ($p<0.01$) and 34.1% compared with 32.8% ($p<0.05$). Total energy intake (MJ) during the *ad libitum*-phase after the low protein diet (33.5±9.3 MJ) did not differ from the intake after the high protein diet (32.1±7.0 MJ) ($p=0.20$). On all three d and both during consumption of the hot lunches ($p=0.68$) and during consumption at home ($p=0.12$) there were no differences (Figure 6.3B) (day 1, 14.9±4.3 MJ compared with 14.3±3.5 MJ; day 2, 15.0±5.2 MJ compared with 14.4±3.3 MJ; day 3, 3.6±1.2 MJ compared with 3.3±1.6 MJ - $p=0.20$). With comparison of the intake of foods during the *ad libitum*-phase according to sensory and protein composition (i.e. high or low protein foods with neutral, savoury or sweet taste), it was shown that subjects selectively consumed certain foods in response to the dietary intervention (Table 6.2). Specifically, after the low protein diet subjects had a higher intake of savoury high protein foods than after the high protein diet ($p<0.01$).

### Results of the LFPQ after the low and high protein diets

The results from the LFPQ showed that the dietary condition significantly altered preference for foods according to their taste properties; after the low protein diet, there was an enhanced preference for the savoury foods compared with the sweet foods. No such preference was seen after the high protein diet. This finding was observed in all four outputs: explicit liking, $p<0.0001$; explicit wanting, $p<0.001$, (Figure 6.4A); relative food
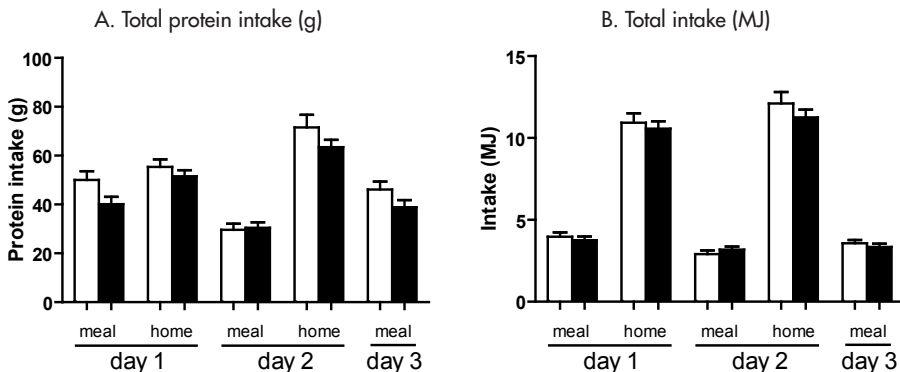


**Figure 6.3** *A. Total protein intake (g) of the lunch meals (meal) and home meal packages (home) during the three d in the ad libitum-phase after the low protein diet (-□-) and high protein diet (-■-). B. Total intake (MJ) of the lunch meals and home packages on the three d in the ad libitum-phase after low protein diet (-□-) and the high protein diet (-■-). Values are means±SE (n=37).*

preference, $p<0.01$; implicit wanting, $p<0.05$, (Figure 6.4B). In terms of effects on food preference according to the protein composition of the images, the dietary condition significantly interacted with implicit wanting according to protein content of the foods. After the low protein diet, greater implicit wanting was observed for high protein foods than for low protein foods ($p<0.05$). No such preference was seen after the high protein diet. This specific interaction was not evident in the other outputs (explicit liking, $p=0.20$; explicit wanting, $p=0.31$; relative food preference, $p=0.42$).

# Discussion

This study investigated the effect of a low protein status with a high protein status on food intake and food preferences. The present results show that there was a spontaneous 13% higher intake of protein after a low protein diet compared with after a high protein diet, whereas total energy intake was not different. In addition, after a low protein diet preferences for savoury high protein food were enhanced. These results indicate that after a protein deficit, food intake and food preferences change to restore adequate protein status.

In animal studies it has long been determined that protein balance is achieved by behavioural strategies (154-158), whereas in humans it is less clear whether behavioural strategies exist to avoid protein shortages. (43-45). Several studies have shown that hungry subjects show a preference for high-protein foods (e.g. references 124, 125, 147). However, the role of the sensory qualities in the influence of protein on food intake
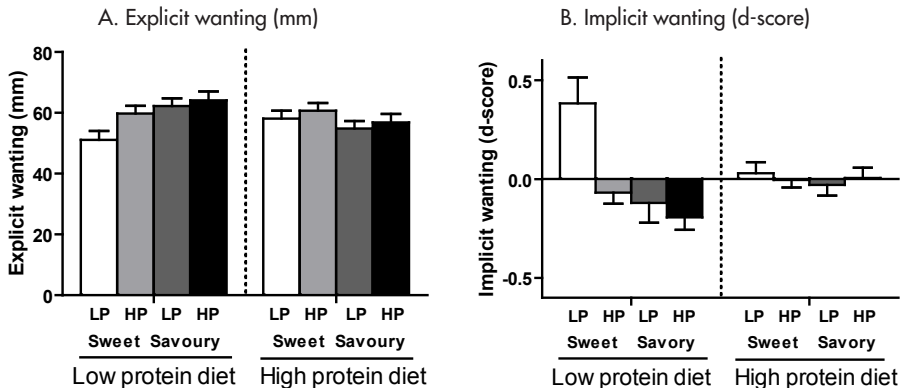


**Figure 6.4** *A. Explicit wanting for the low protein (LP) and high protein (HP) sweet and savoury products after the low protein diet and high protein diet. B. Implicit wanting for the low protein (LP) and high protein (HP) sweet and savoury products after the low protein diet and high protein diet. Values are means±SE (n=37).*

and food choice requires further clarification. Within our food range, foods containing high amount of protein are in general more savoury-tasting, whereas foods containing carbohydrates are generally sweeter (40) ['savoury taste' refers to non-sweet taste, closely linked to the 'umami taste', which is also described as 'broth-like' or 'meaty' (32)]. Through consumption of food during our lifetime we learn to estimate their satiating effects (11, 12), and it has been suggested that this plays a central role in the development of specific macronutrient appetites (13, 14). The intake of different foods observed during the *ad libitum*-phase of our study indicates that sensory attributes play a role in selecting food for macronutrient balance. Indeed, after the low protein diet, food choice was directed toward savoury high protein foods in comparison with after the high protein diet. These findings were reinforced by the results of the LFPQ which showed that after the low protein diet, food preferences were enhanced and oriented towards savoury foods, whereas after the high protein diet preferences remained stable.

As shown in our data, the preference for high protein foods was still present after three days (Figure 6.3). It appears, therefore, that protein appetite induced through two wk of selective reduction of dietary protein is not extinguished after three days of *ad libitum* intake. It might be that a longer period of time is needed to recover from the protein shortage that has been imposed. More research is needed to quantify the time needed for an organism to regain macronutrient balance.

To be able to create a large difference in protein amounts between the two diets, some compromises were made with regard to the control for the sensory differences between the diets. To obtain more insight, we calculated a taste ratio of the low and high protein diets by classifying the offered foods as sweet tasting, savoury tasting, or neutral tasting. Subsequently, the total amount of food (g) per taste was divided by the total amount of food (g) provided by the diet. The ratio of sweet:savoury:neutral for the low protein diet was 53:9:39 and for the high protein diet 54:15:31, indicating that the low protein diet contained slightly fewer savoury tasting foods, and more neutral tasting foods. This might have affected the choice behaviour during the *ad libitum*-phase, because long-term sensory specific satiety has been shown to affect food choice and intake (19). The intake of the different foods during the *ad libitum*-phase, however, indicates that after the low protein diet a specific selection for high protein foods was present, and not just for savoury foods in general (see Table 6.2). Because we offered foods during the *ad libitum*-phase that were not offered during the intervention, we believe that this specific selection for savoury high protein foods is a result of compensatory mechanisms that are induced to restore adequate protein status. In future research, however, it would be preferable during the preparation phase of such a study to perform sensory tests on the foods that are included. This would enable a more specific characterization of the diets on a sensory level, facilitating an even better match.

**Table 6.2** Mean total intake of foods and beverages during the 2.5-d *ad libitum*-phase

| Foods | After low protein diet | | After high protein diet | | Difference in intake[1] |
|---|---|---|---|---|---|
| | kJ | (g) | kJ | (g) | |
| **Lunch items** | | | | | |
| **Neutral taste** | **2667±972[2]** | **(1010)[3]** | **2679±1090** | **(1029)** | **0%** |
| Starch - 2 kinds | 1874±864 | (425) | 1777±735 | (399) | 5% |
| Vegetables - 2 kinds | 645±291 | (500) | 709±424 | (521) | -9% |
| Salad and dressing | 148±169 | (85) | 193±205 | (109) | -24% |
| **Savoury taste** | **4050±1497** | **(600)** | **3728±1917** | **(555)** | **9%** |
| Sauce - 2 kinds | 1682±944 | (302) | 1588±1095 | (286) | 6% |
| Meat - High protein version | 1248±655 | (161) | 948±829 | (119) | 32% |
| Meat - Low protein version | 1120±762 | (136) | 1192±802 | (151) | -6% |
| **Sweet taste** | **3835±1769** | **(534)** | **4181±1746** | **(558)** | **–8%** |
| Dessert - High protein version | 385±581 | (108) | 254±528 | (73) | 51% |
| Dessert - Low protein version | 3450±1642 | (427) | 3926±1789 | (485) | -12% |
| **Home package items** | | | | | |
| **Neutral taste** | **5105±1620** | **(421)** | **4736±1635** | **(401)** | **8%** |
| Buns | 3829±1448 | (378) | 3709±1498 | (366) | 3% |
| Margarine | 1276±683 | (43) | 1028±775 | (35) | 24% |
| **Savoury taste - High protein** | **2791±1677** | **(203)** | **2087±1156** | **(149)** | **34%\*** |
| Egg | 548±417 | (89) | 382±365 | (62) | 44% |
| Sandwich fillings | 1175±1042 | (68) | 825±779 | (49) | 42% |
| Snacks | 1068±1150 | (46) | 880±994 | (38) | 21% |
| **Savoury taste - Low protein** | **2256±1281** | **(158)** | **2200±1210** | **(155)** | **3%** |
| Sandwich fillings | 569±477 | (82) | 558±479 | (81) | 2% |
| Snack | 1687±1204 | (75) | 1643±1183 | (73) | 3% |
| **Sweet taste - High protein** | **6329±2908** | **(1018)** | **6237±2621** | **(949)** | **1%** |
| Sandwich fillings | 1114±906 | (59) | 1365±1158 | (71) | -18% |
| Snack | 791±977 | (37) | 622±722 | (29) | 27% |
| Cookie | 1660±1740 | (94) | 1772±1565 | (101) | -6% |
| Fruit drinks | 2763±1452 | (828) | 2478±1368 | (748) | 11% |
| **Sweet taste - Low protein** | **5659±2197** | **(1610)** | **6039±2406** | **(1713)** | **–6%** |
| Sandwich fillings | 221±490 | (22) | 181±220 | (18) | 22% |
| Sweet snack | 1696±1159 | (83) | 1852±1146 | (91) | -8% |
| Cookie | 746±719 | (58) | 816±763 | (63) | -9% |
| Fruit drinks | 1865±853 | (997) | 1837±983 | (982) | 2% |
| Fruit | 1131±696 | (451) | 1354±830 | (559) | -16% |

[1]*Intake (kJ) after low protein diet divided by intake after high protein diet, multiplied by 100%, minus 100%.* [2]*Mean±SD (all such values).* [3]*Mean intake (all such values) (n=37)* **\****p<0.01.*

The results from the LFPQ indicated that the changes in food preferences appear to involve both conscious (explicit) and subconscious (implicit) processes. It is recognized that both explicit and implicit processes are involved in human eating behaviour (e.g. references 117, 163); the degree to which implicit processes are involved, however, is not clear. The results of the present study suggest that the role of implicit motivational processes in driving food choice is not static, but can vary. When the human body is in balance (e.g. macronutrient balance) it appears that explicit and implicit hedonic responses to foods are similar (e.g. after the high protein diet explicit and implicit outcomes showed similar results). However, when homeostasis is challenged (e.g. prolonged macronutrient imbalance), implicit processes appear to play a stronger determining role in decisions about what to eat (e.g. after the low protein diet subjects implicitly, but not explicitly, preferring high protein foods). Results from intake in the *ad libitum*-phase showed that subjects indeed ingested selectively more high protein foods, even among savoury foods. These data advocate the use of these kinds of advanced psychological tools in behavioural food research to help identify underlying mechanisms involved in human eating behaviour.

In conclusion, after a protein deficit, food intake and food preferences show adaptive changes that suggest compensatory mechanisms that are induced to restore adequate protein status. Hence, it appears that human protein intake can be controlled in a very specific manner when allowed by the composition of the food available. This indicates that there are human behavioural strategies present to avoid protein shortage and these involve selection of savoury high protein foods, made either consciously or unconsciously.

# Acknowledgements

# Chapter seven

## —General discussion

**Table 7.1** Overview of the main findings

| Topic | Chapter | Results and Conclusion |
|---|---|---|
| **Taste** | | |
| Effect of taste of a single meal on satiation | 2, 3, 4 | *Ad libitum intake*, eating rate, and changes in pleasantness and appetite were similar during consumption of a sweet or savoury meal. *Sweet and savoury taste of a single meal do not differ in their effect on satiation* |
| Effect of taste of a single meal or 24-h diet on satiety (subsequent *ad lib* intake) | 3, 4, 5 | *Ad libitum* intake after a sweet or savoury single meal or a sweet or savoury 24-h diet was similar. *Sweet and savoury taste of a single meal or 24-h diet do not differ in their effect on satiety* |
| Effect of taste of a single meal or 24-h diet on food preferences (subsequent food choice and intake of specific food categories) | 3, 4, 5 | Intake, pleasantness, and food choice did not differ for sweet and savoury foods after eating a sweet single meal or sweet 24-h diet. After eating a savoury single meal or savoury 24-h diet, food choices were directed to sweet foods. Food choice for low and high protein foods were similar after a sweet or savoury single meal and sweet or savoury 24-h diet. *Savoury taste has a stronger modulating effect on subsequent food preferences than sweet taste. Sweet and savoury taste of a single meal or 24-h diet do not differ in their effect on food preferences for high or low protein foods.* |
| Effect of within-meal protein content on satiety (subsequent *ad lib* intake) and food preferences (subsequent food choice and intake of specific food categories) | 4 | *Ad libitum* intake and food choice for sweet or savoury low or high protein foods was similar after a high protein or low protein single meal. *Within-meal protein content seems not to have an effect on satiety and food preferences.* |
| Effect of protein status on satiety (subsequent *ad lib* intake) and food preferences (subsequent food choice and intake of specific food categories) | 6 | *Ad libitum* intake after a 14-d low or high protein diet was similar. After a low protein diet, food choices were directed to savoury high protein foods. Protein intake was significantly higher after a low protein diet compared to after a high protein diet. *Protein status elicits compensatory changes in food intake and food preferences to restore adequate protein status.* |
| **Components of food reward** | | |
| Involved in a controlled setting - short term effects | 3, 4 | In a sensory booth, intake and motivation to get access to foods correlated best with explicit measures. Regression analyses suggested that food intake was mostly predicted by results of the explicit measures. The choice for a specific food was mostly explained by results of the implicit measures. *In a controlled setting, explicit processes appear to play a more important role in decisions about portion size than implicit processes. The choice which food to ingest might be made on a more unconscious level.* |
| Involved in a naturalistic setting - long term effects | 5, 6 | Regression analysis suggested that after 24-h of a specific diet, food intake was mostly explained by results of the implicit measures. After a 14-d low protein diet subjects were implicitly, not explicitly, preferring high protein foods. After the high protein diet explicit and implicit measures showed similar results. *In a more naturalistic setting, it appears that implicit, unconscious processes are mostly explaining food intake behaviour. In addition, when homeostasis is challenged, implicit processes of wanting appear to play a stronger determining role in decisions about what to eat.* |

The primary aim of this thesis was to investigate the role of sweet and savoury taste in food intake and food preferences. The secondary aim was to provide more insight into the processes of explicit and implicit liking and wanting, to be able to identify underlying reward mechanisms involved in food intake behaviour. This final chapter starts with an overview of the main finding of this thesis. This is followed by a discussion on the results regarding the role of sweet and savoury taste in food intake and food preferences and its relation to protein intake regulation. Next the findings on the different components of food reward are discussed. Lastly the implications and suggestions for future research are given.

# Main findings

The main findings described in this thesis are presented in Table 7.1. It was shown that sweet and savoury taste, independent of palatability, texture, energy density, and macro-nutrient composition, did not differ in their effect on satiation and satiety in terms of subsequent *ad libitum* food intake (**chapter 2-5**).

With respect to the effect of taste on subsequent food preferences, it was shown that sweet and savoury taste did differ in their effect. In general, after eating a food with a certain taste, appetite for foods with a similar taste was lower than for foods with a dissimilar taste, hence, a clear transfer effect of sensory specific satiety (**chapter 3-5**). This transfer effect was not equipotent for sweet and savoury taste; after eating a sweet single meal or sweet 24-h diet, no strong preference arose for either sweet or savoury foods. Eating a savoury single meal or savoury 24-h diet, however, led to a clear preference for sweet foods. Neither sweet or savoury tasting single meals nor sweet or savoury 24-h diets shifted food preferences towards high or low protein foods **(chapter 3-5)**.

It was shown that protein content of a meal, independent of its taste, did not have an effect on satiety and food preferences (**chapter 4**). We did observe, however, an effect of protein status on satiety and food preferences (**chapter 6**). After a 14-d low protein diet, there was an increase in protein intake, compared with after a 14-d high protein diet, while total energy intake was not different. After the low protein diet, food preference for savoury high protein foods was increased.

By incorporating several psychological tools in the studies, we were able to explore underlying mechanisms that were involved in food intake behaviour. In all studies it was demonstrated that both explicit and implicit measures correlated with several aspects of eating (**chapter 3-6**). It appeared that in a controlled setting, i.e. in the sensory booths, explicit processes played a stronger determining role in satiation (meal size) than implic-

it processes (**chapter 3, 4**). Food choices appeared to be made on a more unconscious level as they were more strongly associated with implicit processes (**chapter 4**). In a setting where subjects could behave more naturally (i.e. self-selection and serving of foods in a relaxed environment where subjects could sit and eat together), implicit, unconscious processes seemed to explain food intake behaviour more than explicit processes (**chapter 5**). When subjects experienced protein shortage, after the 14-d low protein diet, it appeared that implicit processes of wanting played a stronger determining role in decisions about what to eat (**chapter 6**).

# The role of sweet and savoury taste in food intake and food preferences

The first aim of this thesis was to investigate the role of sweet and savoury taste in food intake and food preferences. Before discussing and interpreting the results it is important to consider a number of methodological issues.

## Methodological considerations

*Test foods.* In all our studies we made a distinction between sweet tasting foods and savoury tasting foods. To investigate the effect of these tastes on the processes of satiation and satiety, savoury and sweet tasting versions of identical rice-based meals were developed. These test foods were specifically designed and varied only on the taste dimension. Aspects like texture, energy density, and palatability, that have all been found to affect the processes of satiation and/or satiety (e.g. references 55, 87, 90), were kept similar. In addition, it was possible to vary the protein contents in these rice meals without altering sensory aspects, thereby allowing us to specifically investigate the effect of taste and protein content on food intake independently, in order to separate the course of action of these two dimensions.

Savoury taste has less specific properties compared with the 'pure' tastes sweet, sour, salt, and bitter. Strictly speaking, the fifth basic taste is 'umami'. This is a term that identifies the taste of amino acids such as monosodium glutamate (32), which associates this taste with protein containing foods. As there is no English word synonymous for umami, the closely related term 'savoury' is used, describing 'broth-like', 'meaty' and non-sweet flavours (32). This might be the reason that savoury taste is also very closely related to the experience of salty taste (164). In our studies, we have chosen to use commonly savoury Dutch products or ingredients (termed 'hartig'), without strictly determining the presence of certain amino acids. As a consequence our results on savoury taste might represent effects of savoury and salty tastes combined.

The strength of several studies described in this thesis is that we aimed to investigate the effect of taste and protein content on food intake independently. The buffets used in these studies consisted of both sweet and savoury foods with low and high protein contents (=four distinguishable categories). We carefully selected food products for each category to match energy density, fat content, and type of food (snacks, meal items, etc.) between the categories. The consequence was, however, that it narrowed the range of foods we could include in the studies. As in general savoury foods contain more protein, and sweet foods more carbohydrates (40, 41), very typical high protein savoury foods and low protein sweet foods could not be included. This might have restricted the subjects' food intake behaviour, as differences between the categories on macronutrient content were limited. It is important to note that as these two dimensions of sensory attributes and macronutrient content are so intertwined in the regular food supply it is challenging to investigate these independently in one study. We believe, however, that our approach is most appropriate in order to determine the independent effects. In addition, by using a large variety of common food products, the results remain ecological relevant.

*Study population.* The subjects that participated in the studies described in this thesis were all healthy young adults. We screened their medical history and only non-restrained subjects with a BMI between 18.5 and 25 kg/m$^2$ were included. This has been shown an appropriate population to investigate the relation between sensory signals and food intake behaviour in (165, 166). In the short-term studies, we investigated the effect of taste and/or within-meal protein content on subsequent food preferences. No effect of taste of a meal on specific macronutrient appetites was observed (i.e. preferences for high or low protein foods). It can be assumed that in the short-term studies, the participating healthy subjects were in energy balance and not deficient in any macronutrient store. Therefore, from a physiological point of view, there was actually no need for these subjects to adjust their protein intake. In the long-term study on protein status, it was observed that when subjects experienced protein shortage, food intake and food preferences showed adaptive changes to restore adequate protein status. These results suggest that when macronutrient specific appetites are investigated, it is important to include some kind of macronutrient depletion into the study design.

*Environmental and cognitive factors.* Alongside sensory attributes of food, environmental and cognitive factors have been shown to play a role in food intake behaviour (9). It has been shown in earlier studies that appetite for something savoury is more related to feelings of hunger, whereas appetite for something sweet is more stable during the day (97), implicating that savoury meals might be viewed as more appropriate that sweet meals. To avoid 'appropriateness' influencing the results, we chose to perform our single meal studies during lunchtime. In the Netherlands, it is quite common to have a sweet

lunch, i.e. use sweet bread toppings (115). In addition, it has been shown that at this time of the day, appetite for savoury and appetite for sweet do not diverge largely in the Netherlands (97).

We demonstrated that the transfer effect of sweet and savoury taste was not equipotent; after eating a sweet meal no strong preference arose for either sweet or savoury foods. Eating a savoury meal however, led to a clear preference for sweet foods. We cannot with certainty dismiss the notion that this asymmetric transfer effect of sweet and savoury taste is a culture specific phenomenon (43). In Western society it is commonly accepted that sweet desserts usually follow savoury entrees. It might be that the increased preference for sweet after a savoury meal is an anticipated response. In future research it would be interesting to compare these findings with those drawn from non-Western populations, where sweet and savoury taste may be viewed differently.

Through repeated consumption of food during our lifetime we learn to associate the sensory attributes of food to their physiological effects and consequently learn to estimate their metabolic effects (11, 12). This leads to expectations about the level of satiety that is likely to develop after consuming particular foods. These expectations have been shown to play an important and independent role in food intake behaviour (106). In our studies we did not assess beliefs about the foods we used. As previously stated, within the regular food supply savoury-tasting foods contain in general higher levels of protein, while sweet-tasting foods contain more carbohydrates. This learned association might have interfered with the results, as participants could have adjusted their intake based on their believes rather than on the actual feelings of satiety (40, 41). In future studies it might be worthwhile to incorporate this aspect into the study design.

Large *ad libitum* buffets were always included in the study designs. At these buffets subjects could select foods and consume as much as they wanted, in order to quantify effects on food intake and food preferences. In the two long-term studies we encountered a discrepancy between the feelings of appetite during the diets and subsequent food intake at the buffets. Over the course of a day, a savoury 24-h diet seemed to reduce feelings of appetite more, compared with a sweet tasting diet (chapter 5). In addition, a 14-d high protein diet seemed to reduce feelings of appetite more, compared with a low protein diet (chapter 6). During the *ad libitum* phases of these studies, however, total intake was not different between the conditions. It was observed that in general, subjects ate more than was expected, based on the known habitual intakes for that specific time of the day (115). It is known that variety increases intake (27), and it has been shown in earlier studies that buffet style dinners facilitate overeating (108). Thus it might be that the large variety of items in our buffets nullified differences in satiety state brought about by the interventions.

### Discussion and interpretation of the results

*Sensory-nutrient interaction.* As previously stated, through repeated consumption of food during our lifetime we learn to associate the sensory attributes of food to their physiological effects and consequently learn to estimate their metabolic effects (11, 12). This has been suggested to play a central role in the development of specific appetites (13, 14). Over the course of a day, profiles of appetite for something savoury and appetite for something sweet show different patterns; it appears that appetite for something savoury oscillates more in line with the pattern of meals, whereas appetite for something sweet is more stable during the day (39). In general within our food supply, savoury-tasting food contain higher levels of protein, while sweet-tasting foods contain more carbohydrates (40, 41).

Protein is an indispensable component within the human diet. It provides the body with nitrogen and amino acids that are crucial in preserving and maintaining bodily functions (42). Interestingly, in humans, the range of protein intake is relatively constant over time and across populations, both as a percentage of energy in the diet (10-25%) and in terms of absolute amount eaten (40-100 g). Simpson *et al.* explained this observation with the 'protein-leverage hypothesis' which poses that protein intake is tightly regulated and prioritised over intake of carbohydrates and fat (44, 45).

These observations make a sensory-nutrient interaction plausible; in other words, that regulation of macronutrient intake may come about via the sensory signals from our food. As savoury taste would signal a source of protein, and sweet taste would signal carbohydrates, different effects of these tastes on food intake behaviour might be expected.

It was originally the hypothesis that sweet taste would suppress hunger less and stimulate appetite more compared with a savoury taste, resulting in a lower satiating capacity of a sweet meal. Evidence suggests that sweet tasting foods generally have a positive effect on the expression of appetite that can lead to the facilitation of eating, and that appetite for something sweet is less suppressed by a meal than appetite for something savoury (98-100). Many studies have indeed confirmed that when comparing, *ad libitum* intake of sweet products is higher than intake of savoury products (99, 101, 102).

It was also hypothesized that if savoury taste would signal for a source of protein, this would help to explain why savoury taste tends to relate to feelings of hunger and satiety. Several studies have shown that hungry subjects show a marked preference for high-protein (savoury) foods, while after being satiated, an aversion for high-protein foods emerges. In addition, a high-protein meal has been shown to produce a significantly greater reduction in liking for high-protein foods than high-carbohydrate foods (124, 125).

The results of our first studies show that within one eating episode, sweet and savoury taste do not differ in their effect on satiation and satiety in terms of subsequent *ad libitum* intake. Considering that the sweet-savoury domain is important from a sensory perspective, it appears that within a single meal aspects other than taste determine meal size. We showed that the taste of a single meal or 24-h diet does have a large effect on subsequent food preferences. In general, after eating a food with a given taste, appetite for foods with a similar taste was lower than for foods with a dissimilar taste. This is in accordance with other studies showing that sensory-specific satiety effects are not only specific for the eaten food, but also for foods that share sensory characteristics with the eaten food (18, 112). Interestingly, this transfer effect was not equipotent for sweet and savoury taste; after eating a single sweet meal or sweet 24-h diet, no strong preference arose for either sweet or savoury foods. Eating a savoury single meal or savoury 24-h diet however, led to a clear preference for sweet foods. Hence, savoury taste had a stronger modulating effect on subsequent food choice than sweet taste. This observation supports the hypothesis that intake of savoury tasting foods would signal for protein intake and therefore decreases appetite for other (protein containing) savoury tasting foods, as posed by the protein-leverage hypothesis (45).

When we incorporated the dimension of protein content into our studies, it could not be established that taste of a single meal or 24-h diet shifted food preferences towards high or low protein foods. In addition, within-meal protein content had no effect on subsequent food preferences or satiety. It can be assumed, however, that the participants (healthy subjects) were not deficient in any macronutrient. Therefore there was no physiological stimulus to adjust protein intake. It appears, when a person is in protein balance, taste has no macronutrient specific effects on appetite.

These results favour the hypothesis that the sensory signals of a meal or diet do not change macronutrient appetite, but do change sensory appetite. From an evolutionary point of view, rapid adaptive changes in sensory appetites may serve to ensure a diet with a lot of variety (27). When subjects experienced a protein imbalance over a longer period of time, food preferences did shift to high protein savoury food. It appears that only when homeostasis is challenged, e.g. when facing a prolonged protein deficit, will macronutrient driven appetite engage to restore protein balance.

*The protein leverage hypothesis.* The results showed that both within-meal protein content and protein status had no effect on satiety in terms of subsequent energy intake. Interestingly, protein has been described to be the more satiating macronutrient [reviewed by Halton and Hu (130)], although the causal short-term relationship between protein content and satiety remains unclear (137), as there are many contradictory findings (130, 131, 159). Simpson *et al.* posed that the higher satiating capacity of protein can be

explained by the earlier mentioned protein leverage hypothesis. This hypothesis states that protein intake is tightly regulated, and prioritised over the intake of carbohydrate and fat and suggests a form of protein intake regulation relative to carbohydrate and fat (a regulated 'target' ratio) (44, 45). So when the diet contains a lower ratio of protein than the target ratio, the response is to maintain the amount of protein eaten, potentially leading to increased consumption of carbohydrate and fat as an unintended consequence. If, on the other hand, the protein ratio is higher than the target ratio, the extent to which carbohydrate and fat are consumed to maintain adequate intake of protein is reduced. Eating foods high in protein would thereby indirectly lead to a reduction in overall energy intake.

Our results however, do not confirm this hypothesis. Both after a high protein single meal and a 14-d high protein diet, total energy intake was similar compared with the total energy intake after the low protein versions. After depriving the subjects of protein in such a way that a protein stores were depleted, there were adaptive changes in food intake and food preferences to restore adequate protein status (160). It might be, that when given the opportunity, organisms regulate their macronutrient intake (i.e. maintain a target ratio) by selecting foods with different macronutrient contents, instead of decreasing or increasing their total energy intake.

This may not be the case in an environment where only a limited variety of food is available (e.g. only high in protein, or only low in protein). In this situation, as Simpson *et al.* (44, 45) suggests, macronutrient balance (i.e. achieving the target ratio) would be achieved by adjusting the total amount eaten. This kind of adaptive food intake behaviour has been observed in both animals (167, 168) and humans (44). Our results favour the hypothesis that individuals can control their macronutrient intake in a very specific manner when permitted by the composition of the available food. Whether there exists an upper limit of protein intake in humans needs to be established.

In conclusion, we propose that sweet and savoury taste play an important role in guiding food intake to maintain macronutrient balance, i.e. via rapid adaptive changes in sensory appetites, thereby ensuring a diet with a large variety in nutrients. Only when homeostasis is challenged, e.g. when facing prolonged macronutrient deficit, macronutrient driven appetite will engage to restore macronutrient balance, hence directing food preferences towards savoury high protein foods when protein status is low.

*Underlying mechanisms.* The sense of taste uses G-protein coupled receptors (GPCRs) for transduction of environmental signals. Taste sensation is mediated through two families of GPCRs known as T1Rs and T2Rs (29). Sweet taste and savoury (umami) taste are both dependent on T1R-receptors. Sweet taste sensation is initiated through het-

erodimeric receptor complex formed by two GPCRs, T1R2 and T1R3. Savoury taste sensation is initiated through a heterodimer of T1R1 and T1R3 (37).

Taste signals provide the system with information regarding the food that is being ingested. It has been shown that the responses to sensory cues, such as taste, includes a cascade of pre-absorptive physiological reactions: the so-called cephalic phase responses (CPRs). CPRs are thought to prepare the gastrointestinal tract for optimal digestion and absorption of nutrients. The purpose of initial CPRs is to help maintain homeostasis, i.e. to minimize disturbances of the internal milieu resulting from food intake (169).

In 1996, Höfer *et al.* were the first to show that the taste cell specific receptors were also present in the gut of rodents (170). Subsequent studies showed that this was also true for humans and established the presence of T1R sweet and savoury taste receptors and T2R bitter taste receptors in the gut. These taste receptors in the gut are linked to the secretion of peptides that have a function in metabolism and satiety (171, 172). It has been shown that for example the gut tastes sugars in a similar way as the tongue and by using many of the same signalling elements. This has been posed to explain the observation that orally ingested glucose is more effective than intravenous glucose in raising plasma insulin concentrations (173). So taste signals may have an important physiologic role in the gut and modulate responses to luminal nutrients.

There are several recent animal studies supporting a role for taste in maintaining energy and macronutrient balance (149, 174). In rats, the taste system has been demonstrated to alter in sensitivity; guiding food choice by aligning the physiological needs with gustatory-driven hedonics. In addition, there are also reports indicating the existence of taste-independent sensors when homeostasis is threatened (150). Both in flies and in mice it has been shown that post-ingestive cues can drive feeding behaviour (150, 175). In addition, this metabolic sensing has been shown to be dependent on the internal milieu (150).

Protein intake provides the organisms with amino acids, including the nine amino acids which are classified as essential; they are neither synthesized nor stored in animals and are rapidly depleted when not provided by the diet. It is therefore suggested that protein intake is tightly regulated and involves peripheral and central signalling processes (42, 176). Theories on how this regulation operates are mainly based on animal research. To maintain homeostasis, organisms must sense the deficiency of an essential amino acid and then respond to it (174). It has been posed that the sensory system is involved in these processes to detect the essential amino acid depletion and activate an appropriate neural circuitry to mobilise behaviour. Based on animal research, it has been proposed that the chemosensor for this depletion lies in the anterior piriform cortex of the brain,

that activates when a deficiency is detected (158). When protein is ingested, it is believed that there are many complex pathways involved in protein and amino acid signalling to the brain. These signals originate from visceral and metabolic processes and involve both direct (via the blood stream) and indirect pathways (vagus mediated). Amino acids are also probably directly involved in signalling the vagus pathway in the hepatoportal area and the arcuate nucleus in the hypothalamus (167).

# Components of food reward

The second aim of this thesis was to provide more insight into the processes of explicit and implicit liking and wanting, to be able to identify underlying reward mechanisms involved in food intake behaviour. Before discussing and interpreting the results it is important to consider a number of methodological issues.

### Methodological considerations

In 1996, Berridge proposed that when examining the role of food reward in food intake behaviour, one should make the distinction between food liking and food wanting, with liking corresponding closely to the concept of palatability and pleasure (hedonic feelings), and wanting corresponding more closely to appetite or craving (the motivation to engage in eating) (60). In addition, Berridge argued that objective liking and wanting responses reflect 'core' processes that can operate without conscious awareness. Measuring these processes of food reward separately, however, has been found challenging, as many manipulations seem to alter both. It is even debated whether these processes can be addressed separately in humans (177). Before one can independently measure food liking and food wanting, a theory is needed to motivate why they should diverge in the first place. It has been hypothesized that when ingesting food, the sensory signals mainly influence the desire to eat that specific food (wanting), as distinct from a change in the actual (sensory) pleasantness derived from eating it (liking) (113). In our studies, however, explicit ratings of food liking and food wanting were always highly correlated (~r=0.9, table 3.4, page 52[1]). This could be either interpreted as food intake affecting both food liking and food wanting, and that these processes are intertwined and cannot be addressed separately. It could also be that explicit ratings of food liking and food wanting are not specific enough to detect small differences between the effect on both separate processes.

Our study population consisted of healthy young individuals. It has been suggested that a dissociation of food liking and food wanting is involved in deviations from normal eat-

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[1]Note that in all studies where we incorporated psychological tools these correlations showed similar results. These are however only reported in chapter 3.

ing behaviour (62, 75). For example, it has been shown that susceptible individuals at risk of weight gain, and with a high tendency to binge eat, showed an increased liking for all food types that were presented but an increase in implicit wanting only for high-fat sweet foods. These foods could be defined as highly palatable, and the implicit wanting scores correlated highly with the amounts of these foods freely selected and consumed. Subjects who did not show any tendency for binge eating did not display an implicit wanting for any type of food (178). Epstein *et al.* showed on many occasions that the willingness to work for food (measured with instrumental responding) is higher in obese than in non-obese individuals (80, 179). These individual differences in food reinforcement are suggested to be linked to differences in genotypes (80, 180). It appears that in these groups of subjects (e.g. obese subjects, restrained eaters, subjects with eating disorders) processes of liking and wanting have independent and separable roles. We believe that food liking and food wanting also have independent and separable roles in healthy young individuals. However, in our studies we were not able to dissociate them on an *explicit level*.

In our studies we also dissociated explicit and implicit measurements. As an explicit measurement subjective ratings were used. As implicit measurements, a reaction time task, an instrumental responding task, and food intake were used. The results showed that in a controlled environment the outcomes of the instrumental responding task and food intake had a lower correlation with the implicit reaction time task (~r=0.1, table 3.4, page 52) than with the explicit subjective ratings (~r=0.3). The definition of an implicit measure is that "the outcome of a measurement procedure is causally produced by psychological attributes in an automatic manner" (68). It should be noted that in general, reaction time tasks are mostly used and viewed as implicit measures (68-71). It can be argued that performing an instrumental responding task or ingesting food in a controlled environment is more likely to raise the importance of explicit processes rather than implicit processes. These results implicate that individuals might behave differently in a controlled setting than in a more natural setting. This has been shown in many prior studies (e.g. reference 116) and needs to be considered when interpreting the results.

In most of our studies, explicit and implicit measures correlated well (~r=0.4). In our last study, however, when subjects faced a prolonged protein deficit through selective reduction of dietary protein, dissociation between explicit and implicit processes was observed. Here the implicit measurement (reaction time) was a better predictor of actual food intake behaviour than explicit subjective ratings. Our results indicate that under certain circumstances (e.g. protein imbalance), explicit and implicit processes dissociate and can be measured independently. We believe that tasks that rely on reaction time measures of motivation can provide a non-verbal, implicit assay of incentive salience.

In our studies we have not included measurements of implicit liking processes. The implicit component of liking relates to unconscious (objective) affective reactions (e.g. unintentional smiling after eating something tasty), which has been made most clear in animals and human infants (119). In adults, there is one elegant study performed where subjects were presented with subliminally photographs of happy facial expressions. This presentation failed to produce any conscious report of affect or emotion or shift in hedonic feeling at all, yet it did increase the subject's subsequent behavioural consumption of a fruit drink and subjective affective rating of it later (120). This study, however, *changed* the implicit liking, which was then reflected in explicit ratings and behaviour. Whether it is possible to really measure implicit liking in human adults is debatable.

The psychological tools we used to measure different components of food reward incorporated photographs of food items. It has been established that vivid imagery of reward cues may suffice, especially in humans, to trigger reward processes (117). In addition, it was shown using neuroimaging that the brain rapidly tracks the energetic content of food images (118). By using photographic stimuli, the dimensions of the categories could be simply adapted and it permitted in our case dissociation between explicit and implicit responses. However, a picture of a food only stimulates the visual system; there are no accompanying odours and/or tactile stimulations. This might limit the generalization of the results. As we always incorporated an actual food intake measurement in our studies, however, we were able to show that the results of the psychological tools always highly correlated with the actual eating behaviours. We are therefore confident that the use of photographs was a valid approach in our research on food reward.

### Discussion and interpretation of the results

When Berridge proposed that food reward was not a unitary process, but could be distinguished into the two components food liking and food wanting, this changed the research field on food intake behaviour. It has provided investigators of human appetite behaviour with a useful framework for the interpretation of their data (e.g. references 65, 75, 181-183). It is the general view that to be able to fully understand the processes involved in food intake, it is important to know how these different components of food reward, operating at explicit (conscious) and implicit (unconscious) levels, relate to eating behaviour (76). The difficulty with these concepts, however, is that they rely on animal research. Berridge showed that food liking and food wanting have different neural substrates and could be manipulated and measured separately (60). He showed that liking involves neurotransmitter systems such as opioid and GABA/benzodiazepine systems, and anatomical structures such as the ventral pallidum and the brainstem. Wanting involves mesoteleenchephalic dopamine systems, and divisions of nucleus accumbens and amygdala. He stated that both liking and wanting arise from vastly distributed neural systems, and that they are separable. For example, it has been shown than when rats are

depleted of dopamine (by 6-OHDA lesions) this leaves them in an aphagic state. Their hedonic and aversive reaction to sweet and bitter solutions, however, remains normal. So in animal research, food liking and food wanting can be studied separately by imposing specific brain lesions or other kinds of manipulations. The difficulty in identifying and dissecting these food reward components in human food intake behaviour, however, lies in the obvious fact that we cannot perform lesion studies in humans.

In the last one and a half decade researchers have been trying to clarify important issues regarding food liking and food wanting in humans, including whether these concepts of food liking and food wanting can be operationalised for use in human appetite research. If these concepts can be translated into observable components that reflect the underlying neural mechanisms. Whether these concepts operate independently to produce significant changes in behaviour. And whether they can be truly separated or if an expression of one inevitably contains elements of the other (65) (many of which we also encountered during the interpretations of the results).

Until this day there are no straightforward answers to these questions and there is much debate (65, 177). This does have implications on how to interpret the results described in this thesis. The results show that both explicit and implicit measures were shown to correlate with several aspects of food intake behaviour, implying that both conscious (explicit) and unconscious (implicit) processes were involved in satiation and food choice. These results seem to indicate that in this healthy young study population under normal conditions, explicit food liking and food wanting did not operate independently.

Interestingly, the role implicit wanting plays in driving food choice seemed not static, but appeared to vary. When macronutrient stores were in balance, it appeared that explicit and implicit responses to foods were similar, e.g. after the 14-d high protein diet explicit and implicit outcomes showed similar results. However, when homeostasis was challenged (e.g. by prolonged macronutrient imbalance), implicit processes appeared to play a stronger determining role in decisions about what to eat, e.g. after the 14-d low protein diet subjects were implicitly, but not explicitly, preferring high protein foods. This suggests that in this healthy young population, when homeostasis is threatened, explicit and implicit processes dissociate, whereby the implicit drives seem to be influenced by internal physiological needs. Our results indicate that in a healthy young population, explicit and implicit processes might serve different roles, especially in guiding intake behaviour when homeostasis is challenged.

To conclude, it appears that by incorporating psychological tools in behavioural food research, underlying reward mechanisms involved can to some extent be identified. This provides further insights into the observed behaviour and serves to better explain behav-

ioural patterns. We do want to emphasize that we do not think that these kinds of tools can replace actual behavioural research.

# Implications and suggestions for future research

Through repeated consumption of food during our lifetime we learn to associate the sensory attributes of food to their physiological effects and consequently learn to estimate their metabolic effects (11, 12). The results described in this thesis indicate that a sensory-nutrient interaction might be plausible; that regulation of macronutrient intake may come about via the sensory signals from our food. This has implications for the current food environment, where up to 60% of all consumed foods is highly processed (184). Processing foods is applied for instance to enhance palatability by adding flavours and aromas, or to reduce energy content by using fat replacers or non-nutritive sweeteners. The discrepancy between sensory signals and nutrient content that may occur because of these technological processes could thereby undermine the predictive power of the sensory signals, hence affecting food intake regulation. It has been shown that in highly processed foods, the association between taste and nutrient content is less pronounced than in raw or moderately processed foods (164). In addition, in both animal and human research it has been shown that exposure to sweeteners or fat-replacers disrupts food intake regulation processes (104, 185, 186). This interfering of the predictive relationship between sensory properties of foods and nutrient content has been posed to contribute to dysregulation of energy balance, overweight and obesity and should therefore be considered an important research area.

As mentioned several times in this thesis, savoury-tasting foods contain in general higher levels of protein, while sweet-tasting foods contain more carbohydrates (40, 41). The latter is mainly due to sweet foods containing sugars (i.e. sweet-carbohydrates). Aside from sweet-carbohydrates, however, there is also a large food group containing non-sweet, neutral tasting carbohydrates. This group comprises mainly starch products like bread, pasta, or potatoes. There has been a debate whether sweet and non-sweet carbohydrate products differ in their effect on satiation and satiety (99). Non-sweet carbohydrates account for large part of our daily food intake (187), and these foods are generally conceived as staple food. Interestingly, staple foods have been found to be most resistant to boredom-associated decreases in rated acceptability (113, 188). It would be interesting to further investigate the role of non-sweet carbohydrates in food intake regulation.

In our studies we have made a distinction between sweet tasting foods and savoury tasting foods. Foods that were broth-like, meaty, and non-sweet were included, without strictly determining the presence of certain amino acids (the 'umami taste'). There are

some studies known that specifically investigated the effect of umami taste on aspects of appetite (e.g. reference 189). It appears that monosodium glutamate enhances 'depth' of savoury taste when added to soups, which has been linked to a shorter suppression of hunger compared with soup without additional monosodium glutamate (190). In addition, enhancing the general savoury taste with mono sodium glutamate may also facilitate flavour acceptance and subsequent intake of soup (191). It would be worthwhile to further investigate specifically the effect of the fifth basic taste 'umami', and its relation to the more general savoury food category.

As stated, Simpson *et al.* poses that protein intake is tightly regulated, and prioritised over the intake of carbohydrate and fat and he suggests that organisms balance protein intake against carbohydrate and fat (a regulated 'target' ratio) (44, 45). Eating foods high in protein would thereby lead to a reduced overall intake. This would also explain the finding that protein is the more satiating macronutrient [reviewed by Halton and Hu (130)]. Our results, however, did not confirm this hypothesis but favour the hypothesis that individuals can control their macronutrient intake in a very specific manner when allowed to do so by the composition of available foods. Only after depriving subjects of protein in such a way that a protein stores were depleted (160), were adaptive changes in food intake and food preferences evident to restore adequate protein status. It would be interesting to further explore this observation in groups that are expected to endure protein deprivation from time to time, for example in individuals that perform top sports (e.g. reference 192) or individuals that follow extreme vegan diets (193). In addition, the hypothesis on protein regulation is based on the organisms' need for the essential amino acids. As essential amino acids are neither synthesized nor stored, the maintenance of a full complement of the amino acids is solely dependent on food intake (158, 174). In future studies it would be interesting to incorporate specifically the role of these essential amino acids in food intake and food preferences. In addition, further studies are warranted to investigate whether an upper limit of protein intake occurs in humans.

Our studies show that both explicit and implicit liking and wanting measures of food reward correlated with several aspects of food intake, implying that both conscious (explicit) and unconscious (implicit) processes are involved in satiation and food choice; it is not just conscious decisions that determine what we eat or how much. It has indeed been posed that most of our eating is conducted mindlessly (194). As stated before, when an individual encounters an object, an evaluation can occur without effort, quickly and without intention. Such associative and automatic evaluations have been termed implicit attitudes and they have been shown to correlate with a range of health behaviours (163). In our studies we have incorporated implicit measures to assess implicit wanting for food categories *after* being exposed to a certain treatment (a single meal or a diet). It would be interesting to further explore the implicit attitudes subjects already have

towards certain products, how stable these attitudes are, and how they relate to actual eating decisions [a method that could be used is for example 'the sorting paired feature task' (69)].

In the last one and a half decade a new exciting research field has emerged. With the arrival of functional neuroimaging techniques such as electroencephalography (EEG), magneto encephalography (MEG), positron emission tomography (PET) en most recently, functional magnetic resonance imaging (fMRI), it has become possible to reveal functional brain activity (195). Experiments using these techniques have located brain areas involved in food reward and in turn helped to understand how the brain operates. It has for example been shown that after the sensory information is carried from the periphery to the primary sensory cortices (where the stimulus is identified and decoded), the orbitofrontal cortex is involved in mediating the hedonic experience. Also other brain regions are identified that are thought to be part of the hedonic networks in the human brain using these techniques, such as the anterior cingulate, the insular cortex and ventral striatum (47). More specifically, Spetter *et al.* have for example used fMRI to locate brain regions where taste activation covaries with sweet and salty taste intensity (196). Aside from locating functional brain areas, however, these techniques can also be used to identify differences between individuals regarding food reward. For example, Stice *et al.* showed, by using fMRI, that individuals who show greater activation in the gustatory cortex and somatosensory regions in response to anticipation and consumption of food, but who show weaker activation in the striatum during food intake, may be at risk for overeating and consequent weight gain (197). Leidy *et al.* investigated in a recent fMRI pilot study the effect of breakfast, with additional protein or not, on neural activity in overweight 'breakfast skipping' adolescent girls. They showed that eating breakfast led to alterations in brain activation in regions previously associated with food motivation and reward, with additional alterations following a higher-protein breakfast. This study suggested that increased dietary protein at breakfast might be a beneficial strategy to reduce reward-driven eating behaviour in overweight young girls (198). In our case, it would for example be very interesting to incorporate these techniques to investigate further the role of reward in protein intake regulation.

It is becoming clear that environmental factors play an important role in food intake regulation. Already in 1968, Schachter introduced the idea that external cues are involved in human food intake (199). It has been posed that humans are to a great part genetically still adapted to the diet of the hunter-gatherer ancestors. In an environment where periods of food abundance and food shortage alternated, periodical overeating when food was available enabled normal reproduction. The current environment where food is continuously abundant (i.e. an 'obesogenic environment') might therefore stimulate people to continuously overeat, resulting in a positive energy balance, overweight

and obesity (200). There have been studies to identify which external factors contribute to overeating, for example portion sizes, plate shapes, package sizes, etc. (108). Interestingly, as there is still a large part of the population able to maintain a healthy body weight despite the environment they are living in, it appears there are important individual differences in responses to the obesogenic environment (201, 202). To fully understand human food intake, more research should be performed on the effect of external cues, thereby taking into account these individual differences.

# Main conclusions

Sweet and savoury taste do not differ in their effect on satiation or satiety in terms of subsequent *ad libitum* intake. When considering that the sweet-savoury domain is important from a sensory perspective, taste in general seems not to have a strong influence on satiation and satiety. The taste of a meal or diet does have a large effect on subsequent food preferences. In general, after eating a food with a certain taste, appetite for foods with a similar taste is less than for foods with a dissimilar taste. This transfer effect is not equipotent for sweet and savoury tastes: savoury taste exerts a stronger modulating effect on subsequent food preferences than sweet taste.

Sweet and savoury taste of a single meal or 24-h diet do not differ in their effect on food preferences for high or low protein foods. In addition, within-meal protein content seems not to influence satiety or food preferences. A low protein status however, through selective reduction of dietary protein intake, elicits compensatory changes in food intake and food preferences to restore adequate protein status. This indicates the presence of behavioural strategies in humans to avoid protein shortage, and these involve specific selection of foods.

It appears that both conscious (explicit) and unconscious (implicit) processes are involved in satiation and food choice; this suggests it is not just conscious decisions that determine what we eat or how much. The role implicit motivational processes play in driving food choice is not static, but appears to vary. This is especially the case when homeostasis is challenged (by depleting macronutrient stores), where implicit processes of wanting appear to play a stronger determining role in decisions about what to eat.

# References

1. Woods, S.C., R.J. Seeley, D. Porte, and M.W. Schwartz, *Signals that regulate food intake and energy homeostasis.* Science, 1998. **280**(5368): p. 1378-1383.

2. Cannon, W.B., *The wisdom of the body.* Vol. xv, 1 1. 1932: W.W. Norton and Company.

3. Hervey, G.R., *Physiological mechanisms for the regulation of energy balance.* Proc Nutr Soc, 1971. **30**(02): p. 109-116.

4. Stubbs, R.J., *Appetite, feeding behaviour and energy balance in human subjects.* Proc Nutr Soc, 1998. **57**(03): p. 341-356.

5. de Castro, J.M., *Prior day's intake has macronutrient-specific delayed negative feedback effects on the spontaneous food intake of free-living humans.* J Nutr, 1998. **128**(1): p. 61-67.

6. Strubbe, J.H. and S.C. Woods, *The timing of meals.* Psychol Rev, 2004. **111**(1): p. 128-141.

7. Woods, S.C., *The control of food intake: behavioral versus molecular perspectives.* Cell Metab, 2009. **9**(6): p. 489-498.

8. de Graaf, C., W.A.M. Blom, P.A.M. Smeets, A. Stafleu, and H.F.J. Hendriks, *Biomarkers of satiation and satiety.* Am J Clin Nutr, 2004. **79**(6): p. 946-961.

9. Blundell, J.E., P.J. Rogers, and A.J. Hill, *Evaluating the satiating power of foods: implications for acceptance and consumption*, in *Food Acceptance and Nutrition*, J. Solms, et al., Editors. 1987, Academic Press: London. p. 205–219.

10. Le Magnen, J., *Increased food intake induced in rats by changes in the satiating sensory input from food (first published in French in 1956).* Appetite, 1999, reprinted from 1956. **33**(1): p. 33-35.

11. Booth, D.A., *Conditioned satiety in the rat.* J Comp Physiol Psychol, 1972. **81**(3): p. 457-471.

12. Booth, D.A., *Food-conditioned eating preferences and aversions with interoceptive elements - conditioned appetites and satieties.* Ann NY Acad Sci, 1985. **443**(JUN): p. 22-41.

13. Baker, B.J., D.A. Booth, J.P. Duggan, and E.L. Gibson, *Protein appetite demonstrated: learned specificity of protein-cue preference to protein need in adult rats.* Nutr Res, 1987. **7**(5): p. 481-487.

14. Gibson, E.L., C.J. Wainwright, and D.A. Booth, *Disguised protein in lunch after low-protein breakfast conditions food-flavor preferences dependent on recent lack of protein intake.* Physiol Behav, 1995. **58**(2): p. 363-371.

15. de Graaf, C. and F.J. Kok, *Slow food, fast food and the control of food intake.* 2010. **6**(5): p. 290-293.

16. Sørensen, L.B., P. Møller, A. Flint, M. Martens, and A. Raben, *Effect of sensory perception of foods on appetite and food intake: a review of studies on humans.* Int J Obes, 2003. **27**(10): p. 1152-66.

17. Swithers, S.E. and W.G. Hall, *Does oral experience terminate ingestion?* Appetite, 1994. **23**(2): p. 113-38.

18. Rolls, B.J., *Sensory-specific satiety.* Nutr Rev, 1986. **44**(3): p. 93-101.

19. Rolls, B.J., E.T. Rolls, E.A. Rowe, and K. Sweeney, *Sensory specific satiety in man.* Physiol Behav, 1981. **27**(1): p. 137-42.

20. Hetherington, M., B.J. Rolls, and V.J. Burley, *The time course of sensory-specific satiety.* Appetite, 1989. **12**(1): p. 57-68.

21. Weijzen, P.L.G., D.G. Liem, E.H. Zandstra, and C. de Graaf, *Sensory specific satiety and intake: The difference between nibble- and bar-size snacks.* Appetite, 2008. **50**(2-3): p. 435-442.

22. Guinard, J.X. and P. Brun, *Sensory-specific satiety: comparison of taste and texture effects.* Appetite, 1998. **31**(2): p. 141-57.

23. Rolls, B.J., E.A. Rowe, and E.T. Rolls, *How flavour and appearance affect human feeding.* Proc Nutr Soc, 1982.

**41**(2): p. 109-17.

24. Romer, M., J. Lehrner, V. Van Wymelbeke, T. Jiang, L. Deecke, and L. Brondel, *Does modification of olfacto-gustatory stimulation diminish sensory-specific satiety in humans?* Physiol Behav, 2006. **87**(3): p. 469-477.

25. Vickers, Z., E. Holton, and J. Wang, *Effect of yoghurt sweetness on sensory specific satiety.* J Sens Stud, 1998. **13**: p. 377-388.

26. Rolls, B.J., E.A. Rowe, and E.T. Rolls, *How sensory properties of foods affect human feeding behavior.* Physiol Behav, 1982. **29**(3): p. 409-417.

27. Rolls, B.J., E.A. Rowe, E.T. Rolls, B. Kingston, A. Megson, and R. Gunary, *Variety in a meal enhances food intake in man.* Physiol Behav, 1981. **26**(2): p. 215-221.

28. Raynor, H.A. and L.H. Epstein, *Dietary variety, energy regulation, and obesity.* Psychol Bull, 2001. **127**(3): p. 325-341.

29. Chandrashekar, J., M.A. Hoon, N.J.P. Ryba, and C.S. Zuker, *The receptors and cells for mammalian taste.* 2006. **444**(7117): p. 288-294.

30. Steiner, J.E., *Innate, discriminative human facial expressions to taste and smell stimulation.* Ann N Y Acad Sci, 1974. **237**(1): p. 229-233.

31. Yamamoto, T., *Central mechanisms of roles of taste in reward and eating.* Acta Physiol Hung, 2008. **95**(2): p. 165-186.

32. Yamaguchi, S. and K. Ninomiya, *Umami and food palatability.* J Nutr, 2000. **130**(4): p. 921-926.

33. Mattes, R.D., *Fat taste and lipid metabolism in humans.* Physiol Behav, 2005. **86**(5): p. 691-697.

34. Stewart, J.E., C. Feinle-Bisset, and R.S.J. Keast, *Fatty acid detection during food consumption and digestion: Associations with ingestive behavior and obesity.* Prog Lipid Res, 2011. **50**(3): p. 225-233.

35. Grill, H.J. and R. Norgren, *The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats.* Brain Res, 1978. **143**(2): p. 263-279.

36. Ginane, C., R. Baumont, and A. Favreau-Peigné, *Perception and hedonic value of basic tastes in domestic ruminants.* Physiol Behav, 2011. **104**(5): p. 666-674.

37. Zhao, G.Q., Y. Zhang, M.A. Hoon, J. Chandrashekar, I. Erlenbach, N.J.P. Ryba, and C.S. Zuker, *The receptors for mammalian sweet and umami taste.* Cell, 2003. **115**(3): p. 255-266.

38. Mattes, R.D., *Gustation as a determinant of ingestion: methodological issues.* Am J Clin Nutr, 1985. **41**(4): p. 672-683.

39. de Graaf, C., P. Jas, K. Van der Kooy, and R. Leenen, *Circadian rhythms of appetite at different stages of a weight loss programme.* Int J Obes, 1993. **17**(9): p. 521-526.

40. Luscombe-Marsh, N.D., A.J.P.G. Smeets, and M.S. Westerterp-Plantenga, *Taste sensitivity for monosodium glutamate and an increased liking of dietary protein.* Br J Nutr, 2008. **99**(04): p. 904-908.

41. Blundell, J.E., S. Green, and V. Burley, *Carbohydrates and human appetite.* Am J Clin Nutr, 1994. **59**(3): p. 728S-734.

42. Tome, D., *Protein, amino acids and the control of food intake.* Br J Nutr, 2004. **92**(SupplementS1): p. S27-S30.

43. Metcalf, P.A., R.R.K. Scragg, D. Schaaf, L. Dyall, P.N. Black, and R. Jackson, *Dietary intakes of European, Maori, Pacific and Asian adults living in Auckland: the Diabetes, Heart and Health Study.* Aust N Z J Public Health, 2008. **32**(5): p. 454-460.

44. Simpson, S.J., R. Batley, and D. Raubenheimer, *Geometric analysis of macronutrient intake in humans: the power*

*of protein?* Appetite, 2003. **41**(2): p. 123-140.

45. Simpson, S.J. and D. Raubenheimer, *Obesity: the protein leverage hypothesis.* Obes Rev, 2005. **6**: p. 133-142.

46. Saper, C.B., T.C. Chou, and J.K. Elmquist, *The need to feed: homeostatic and hedonic control of eating.* Neuron, 2002. **36**(2): p. 199-211.

47. Kringelbach, M.L., *Food for thought: hedonic experience beyond homeostasis in the human brain.* Neuroscience, 2004. **126**(4): p. 807-819.

48. Lowe, M.R. and M.L. Butryn, *Hedonic hunger: A new dimension of appetite?* Physiol Behav, 2007. **91**(4): p. 432-439.

49. Zheng, H., N.R. Lenard, A.C. Shin, and H.R. Berthoud, *Appetite control and energy balance regulation in the modern world: reward-driven brain overrides repletion signals.* 2009. **33**(S2): p. S8-S13.

50. Fulton, S., *Appetite and reward.* Front Neuroendocrinol, 2010. **31**(1): p. 85-103.

51. Rolls, E.T., *Sensory processing in the brain related to the control of food intake.* Proc Nutr Soc, 2007. **66**(1): p. 96-112.

52. Appelhans, B.M., *Neurobehavioral inhibition of reward-driven feeding: implications for dieting and obesity.* Obesity, 2009. **17**(4): p. 640-647.

53. Levitsky, D.A., *The non-regulation of food intake in humans: hope for reversing the epidemic of obesity.* Physiol Behav, 2005. **86**(5): p. 623-632.

54. Cabanac, M., *Physiological role of pleasure.* Science, 1971. **173**(4002): p. 1103-1107.

55. de Graaf, C., L.S. de Jong, and A.C. Lambers, *Palatability affects satiation but not satiety.* Physiol Behav, 1999. **66**(4): p. 681-688.

56. de Graaf, C., F.M. Kramer, H.L. Meiselman, L.L. Lesher, C. Baker-Fulco, E.S. Hirsch, and J. Warber, *Food acceptability in field studies with US army men and women: relationship with food intake and food choice after repeated exposures.* Appetite, 2005. **44**(1): p. 23-31.

57. Yeomans, M.R., J.E. Blundell, and M. Leshem, *Palatability: response to nutritional need or need-free stimulation of appetite?* Br J Nutr, 2004. **92 Suppl 1**: p. S3-14.

58. Jansen, A., A. Vanreyten, T. van Balveren, A. Roefs, C. Nederkoorn, and R. Havermans, *Negative affect and cue-induced overeating in non-eating disordered obesity.* Appetite, 2008. **51**(3): p. 556-562.

59. Mustonen, S., I. Hissa, A. Huotilainen, S.-M. Miettinen, and H. Tuorila, *Hedonic responses as predictors of food choice: flexibility and self-prediction.* Appetite, 2007. **49**(1): p. 159-168.

60. Berridge, K.C., *Food reward: brain substrates of wanting and liking.* Neurosci Biobehav Rev, 1996. **20**(1): p. 1-25.

61. Pecina, S., K.S. Smith, and K.C. Berridge, *Hedonic hot spots in the brain.* Neuroscientist, 2006. **12**(6): p. 500-511.

62. Berridge, K.C., *Motivation concepts in behavioral neuroscience.* Physiol Behav, 2004. **81**(2): p. 179-209.

63. Berridge, K.C., *`Liking' and `wanting' food rewards: brain substrates and roles in eating disorders.* Physiol Behav, 2009. **97**(5): p. 537-550.

64. Berridge, K.C. and T.E. Robinson, *Parsing reward.* Trends Neurosci, 2003. **26**(9): p. 507-513.

65. Finlayson, G., N. King, and J.E. Blundell, *Liking vs. wanting food: importance for human appetite control and weight regulation.* Neurosci Biobehav Rev, 2007. **31**(7): p. 987-1002.

66. Brunstrom, J.M. and G.L. Mitchell, *Effects of distraction on the development of satiety.* Br J Nutr, 2006. **96**(4): p. 761-9.

67. De Houwer, J., *Comparing measures of attitutes at the functional and procedural level: Analysis and implications*, in *Attitudes: Insights from the new implicit measures*, R.E. Petty, R.H. Fazio, and P. Brinol, Editors. 2009, Taylor & Francis Group, LLC: New York. p. 361-390.

68. De Houwer, J. and A. Moors, *Implicit measures: similarities and differences*, in *Handbook of implicit social cognition*, B. Gawronski and B.K. Payne Editors. 2010, Guilford Press: New York.

69. Bar-Anan, Y., B.A. Nosek, and M. Vianello, *The sorting paired features task: a measure of association strengths*. Exp Psychol, 2009. **56**(5): p. 329-343.

70. Greenwald, A.G., B.A. Nosek, and M.R. Banaji, *Understanding and using the Implicit Association Test: I. An improved scoring algorithm.* J Pers Soc Psychol, 2003. **85**(2): p. 197-216.

71. Finlayson, G., N. King, and J.E. Blundell, *Is it possible to dissociate 'liking' and 'wanting' for foods in humans? A novel experimental procedure.* Physiol Behav, 2007. **90**(1): p. 36-42.

72. Havermans, R.C., T. Janssen, J.C.A.H. Giesen, A. Roefs, and A. Jansen, *Food liking, food wanting, and sensory-specific satiety.* Appetite, 2009. **52**(1): p. 222-225.

73. Epstein, L.H., R. Truesdale, A. Wojcik, R.A. Paluch, and H.A. Raynor, *Effects of deprivation on hedonics and reinforcing value of food.* Physiol Behav, 2003. **78**(2): p. 221-227.

74. Lemmens, S.G.T., P.F.M. Schoffelen, L. Wouters, J.M. Born, M.J.I. Martens, F. Rutters, and M.S. Wester terp-Plantenga, *Eating what you like induces a stronger decrease of `wanting' to eat.* Physiol Behav, 2009. **98**(3): p. 318-325.

75. Mela, D.J., *Eating for pleasure or just wanting to eat? Reconsidering sensory hedonic responses as a driver of obesity.* Appetite, 2006. **47**(1): p. 10-17.

76. Finlayson, G., N. King, and J. Blundell, *The role of implicit wanting in relation to explicit liking and wanting for food: Implications for appetite control.* Appetite, 2008. **50**(1): p. 120-127.

77. Bellisle, F., R. McDevitt, and A.M. Prentice, *Meal frequency and energy balance.* Br J Nutr, 1997. **77**: p. 57-70.

78. Whybrow, S., C. Mayer, T.R. Kirk, N. Mazlan, and R.J. Stubbs, *Effects of two weeks mandatory snack consumption on energy intake and energy balance.* Obesity, 2007. **15**(3): p. 673-685.

79. Blundell, J.E., R.J. Stubbs, C. Golding, F. Croden, R. Alam, S. Whybrow, J. Le Noury, and C.L. Lawton, *Resistance and susceptibility to weight gain: Individual variability in response to a high-fat diet.* Physiol Behav, 2005. **86**(5): p. 614-622.

80. Epstein, L.H., J.L. Temple, B.J. Neaderhiser, R.J. Salis, R.W. Erbe, and J.J. Leddy, *Food reinforcement, the dopamine D2 receptor genotype, and energy intake in obese and nonobese humans.* Behav Neurosci, 2007. **121**(5): p. 877-886.

81. Laessle, R.G., S. Lehrke, and S. Dückers, *Laboratory eating behavior in obesity.* Appetite, 2007. **49**(2): p. 399-404.

82. Epstein, L.H., J.L. Robinson, J.L. Temple, J.N. Roemmich, A.L. Marusewski, and R.L. Nadbrzuch, *Variety influences habituation of motivated behavior for food and energy intake in children.* Am J Clin Nutr, 2009. **89**(3): p. 746-754.

83. Swinburn, B.A., G. Sacks, S.K. Lo, K.R. Westerterp, E.C. Rush, M. Rosenbaum, A. Luke, D.A. Schoeller, J.P. DeLany, N.F. Butte, and E. Ravussin, *Estimating the changes in energy flux that characterize the rise in obesity prevalence.* Am J Clin Nutr, 2009. **89**(6): p. 1723-1728.

84. Yeomans, M.R., *Palatability and the micro-structure of feeding in humans: the appetizer effect.* Appetite, 1996.

**27**(2): p. 119-133.

85. Rolls, B.J., E.A. Bell, and B.A. Waugh, *Increasing the volume of a food by incorporating air affects satiety in men.* Am J Clin Nutr, 2000. **72**(2): p. 361-368.

86. Wansink, B., J.E. Painter, and J. North, *Bottomless bowls: why visual cues of portion size may influence intake.* Obes Res, 2005. **13**(1): p. 93-100.

87. Zijlstra, N., M. Mars, R.A. de Wijk, M.S. Westerterp-Plantenga, and C. de Graaf, *The effect of viscosity on ad libitum food intake.* Int J Obes, 2008. **32**(4): p. 676-683.

88. Porrini, M., A. Santangelo, R. Crovetti, P. Riso, G. Testolin, and J.E. Blundell, *Weight, protein, fat, and timing of preloads affect food intake.* Physiol Behav, 1997. **62**(3): p. 563-570.

89. Westerterp-Plantenga, M.S., *Protein intake and energy balance.* Regul Pept, 2008. **149**(1-3): p. 67-69.

90. Rolls, B., *The relationship between dietary energy density and energy intake.* Physiol Behav, 2009. **97**: p. 609 - 615.

91. Yeomans, M.R., L. Weinberg, and S. James, *Effects of palatability and learned satiety on energy density influences on breakfast intake in humans.* Physiol Behav, 2005. **86**(4): p. 487-499.

92. Hetherington, M.M., *Sensory-specific satiety and its importance in meal termination.* Neurosci Biobehav Rev, 1996. **20**(1): p. 113-117.

93. Van Strien, T., *Dutch Eating Behavior Questionnaire manual (Nederlandse Vragenlijst voor eetgedrag Handleiding).* 2005, Amsterdam: Boom Test Publishers.

94. de Graaf, C. and J.E.R. Frijters, *Interrelationships among sweetness, saltiness and total taste intensity of sucrose, NaCl and sucrose/NaCl mixtures.* Chem Senses, 1989. **14**(1): p. 81-102.

95. NEVO, *Tabel Dutch Nutrient Database 2006.* 2006, Voedingscentrum: Den Haag, The Netherlands.

96. AOAC-International, *Official methods of analysis of AOAC international* 16th ed. 1997, revised 1999, Gaithers burg, MD, USA.

97. de Graaf, C., *The validity of appetite ratings.* Appetite, 1993. **21**(2): p. 156-60.

98. Appleton, K.M., P.J. Rogers, and J.E. Blundell, *Effects of a sweet and a nonsweet lunch on short-term appetite: differences in female high and low consumers of sweet/low-energy beverages.* J Hum Nutr Diet, 2004. **17**(5): p. 425-434.

99. de Graaf, C., A. Schreurs, and Y.H. Blauw, *Short-term effects of different amounts of sweet and nonsweet carbohy-drates on satiety and energy intake.* Physiol Behav, 1993. **54**(5): p. 833-843.

100. Rogers, P.J. and J.E. Blundell, *Separating the actions of sweetness and calories: effects of saccharin and carbohy-drates on hunger and food intake in human subjects.* Physiol Behav, 1989. **45**(6): p. 1093-1099.

101. Kringelbach, M.L., J. O'Doherty, E.T. Rolls, and C. Andrews, *Activation of the human orbitofrontal cortex to a liquid food stimulus is correlated with its subjective pleasantness.* Cereb Cortex, 2003. **13**(10): p. 1064-1071.

102. Weenen, H., A. Stafleu, and C. de Graaf, *Dynamic aspects of liking: post-prandial persistence of sensory specific satiety.* Food Qual Prefer, 2005. **16**(6): p. 528-535.

103. Laeng, B., K.C. Berridge, and C.M. Butter, *Pleasantness of a sweet taste during hunger and satiety: effects of gender and "sweet tooth".* Appetite, 1993. **21**(3): p. 247-254.

104. Appleton, K.M. and J.E. Blundell, *Habitual high and low consumers of artificially-sweetened beverages: Effects of sweet taste and energy on short-term appetite.* Physiol Behav, 2007. **92**(3): p. 479-486.

105. Brunstrom, J.M. and N.G. Shakeshaft, *Measuring affective (liking) and non-affective (expected satiety) determi-*

*nants of portion size and food reward.* Appetite, 2009. **52**(1): p. 108-114.

106. Brunstrom, J.M., N.G. Shakeshaft, and N.E. Scott-Samuel, *Measuring `expected satiety' in a range of common foods using a method of constant stimuli.* Appetite, 2008. **51**(3): p. 604-614.

107. Andrade, A.M., G.W. Greene, and K.J. Melanson, *Eating slowly led to decreases in energy intake within meals in healthy women.* J Am Diet Assoc, 2008. **108**(7): p. 1186-1191.

108. Wansink, B., *Environmental factors that increase the food intake and consumption volume of unknowing consumers.* Annu Rev Nutr, 2004. **24**: p. 455-79.

109. Yeomans, M.R., R.W. Gray, C.J. Mitchell, and S. True, *Independent effects of palatability and within-meal pauses on intake and appetite ratings in human volunteers.* Appetite, 1997. **29**(1): p. 61-76.

110. Viskaal-van Dongen, M., C. de Graaf, E. Siebelink, and F.J. Kok, *Hidden fat facilitates passive overconsumption.* J Nutr, 2009. **139**(2): p. 394-399.

111. Rolls, B.J., L.J. Laster, and A. Summerfelt, *Meal order reversal: effects of eating a sweet course first or last.* Appetite, 1991. **16**(2): p. 141-148.

112. Rolls, B.J., P.M. Vanduijvenvoorde, and E.T. Rolls, *Pleasantness changes and food-intake in a varied 4-course meal.* Appetite, 1984. **5**(4): p. 337-348.

113. Mela, D.J., *Why do we like what we like?* J Sci Food Agr, 2001. **81**(1): p. 10-16.

114. WHO, *Principles for the estimation of energy requirements. Energy and protein requirements. Report of a joint FAO/WHO/UNU expert consultation.* 1985, World Health Organization: Geneva. p. 34–52.

115. Hulshof, K., M. Ocké, C. Van Rossum, E. Buurma-Rethans, H. Brants, and J. Drijvers, *Results of the national food consumption survey 2003.* 2003, RIVM, National Institute for Public Health and the environment.

116. Meiselman, H.L., *Methodology and theory in human eating research.* Appetite, 1992. **19**(1): p. 49-55.

117. Berridge, K.C., T.E. Robinson, and J.W. Aldridge, *Dissecting components of reward: `liking', `wanting', and learning.* Curr Opin Pharmacol, 2009. **9**(1): p. 65-73.

118. Toepel, U., J.-F. Knebel, J. Hudry, J. le Coutre, and M.M. Murray, *The brain tracks the energetic value in food images.* NeuroImage, 2009. **44**(3): p. 967-974.

119. Steiner, J.E., D. Glaser, M.E. Hawilo, and K.C. Berridge, *Comparative expression of hedonic impact: affective reactions to taste by human infants and other primates.* Neurosci Biobehav Rev, 2001. **25**(1): p. 53-74.

120. Winkielman, P., K.C. Berridge, and J.L. Wilbarger, *Unconscious affective reactions to masked happy versus angry faces influence consumption behavior and judgments of value.* Pers Soc Psychol Bull, 2005. **31**(1): p. 121-135.

121. Griffioen-Roose, S., M. Mars, G. Finlayson, J.E. Blundell, and C. de Graaf, *Satiation due to equally palatable sweet and savoury meals does not differ in normal weight young adults.* J Nutr, 2009. **139**(11): p. 2093-2098.

122. Jiang, T., R. Soussignan, D. Rigaud, S. Martin, J.-P. Royet, L. Brondel, and B. Schaal, *Alliesthesia to food cues: Heterogeneity across stimuli and sensory modalities.* Physiol Behav, 2008. **95**(3): p. 464-470.

123. Blundell, J.E. and G. Finlayson, *Role of sweetness in appetite control (Le rôle du sucré dans le contrôle de l'appétit).* Cahiers de Nutrition et de Dietetique, 2008. **43**(S1): p. 242-246.

124. Hill, A.J. and J.E. Blundell, *Macronutrient and satiety: the effects of a high-protein or high-carbohydrate meal on subjective motivation to eat and food preferences.* Nutr Behav, 1986. **3**: p. 133-144.

125. Barkeling, B., S. Rossner, and H. Bjorvell, *Effect of a high-protein meal (meat) and a high-carbohydrate meal*

(vegetarian) on satiety measured by automated computerized monitoring of subsequent food intake, motivation to eat and food preferences. Int J Obes, 1990. **14**(9): p. 743-751.

126. Griffioen-Roose, S., G. Finlayson, M. Mars, J.E. Blundell, and C. de Graaf, *Measuring food reward and the transfer effect of sensory specific satiety.* Appetite, 2010. **55**(3): p. 648-655.

127. Dovey, T.M., D. Clark-Carter, E.J. Boyland, and J.C.G. Halford, *A guide to analysing Universal Eating Monitor data: assessing the impact of different analysis techniques.* Physiol Behav, 2009. **96**(1): p. 78-84.

128. Kissileff, H.R., J. Thornton, and E. Becker, *A quadratic equation adequately describes the cumulative food intake curve in man.* Appetite, 1982. **3**(3): p. 255-272.

129. Chung Chun Lam, S.M.S., P.J. Moughan, A. Awati, and H.R. Morton, *The influence of whey protein and glycomacropeptide on satiety in adult humans.* Physiol Behav, 2009. **96**(1): p. 162-168.

130. Halton, T.L. and F.B. Hu, *The effects of high protein diets on thermogenesis, satiety and weight loss: a critical review.* J Am Coll Nutr, 2004. **23**(5): p. 373-385.

131. de Graaf, C., T. Hulshof, J.A. Weststrate, and P. Jas, *Short-term effects of different amounts of protein, fats, and carbohydrates on satiety.* Am J Clin Nutr, 1992. **55**(1): p. 33-38.

132. Akhavan, T., B.L. Luhovyy, P.H. Brown, C.E. Cho, and G.H. Anderson, *Effect of premeal consumption of whey protein and its hydrolysate on food intake and postmeal glycemia and insulin responses in young adults.* Am J Clin Nutr, 2010. **91**(4): p. 966-975.

133. Vozzo, R., G. Wittert, C. Cocchiaro, W.C. Tan, J. Mudge, R. Fraser, and I. Chapman, *Similar effects of foods high in protein, carbohydrate and fat on subsequent spontaneous food intake in healthy individuals.* Appetite, 2003. **40**(2): p. 101-107.

134. Hursel, R., L. van der Zee, and M.S. Westerterp-Plantenga, *Effects of a breakfast yoghurt, with additional total whey protein or caseinomacropeptide-depleted -lactalbumin- enriched whey protein, on diet-induced thermogenesis and appetite suppression.* Br J Nutr, 2010. **103**(05): p. 775-780.

135. Leidy, H.J., C.L.H. Armstrong, M. Tang, R.D. Mattes, and W.W. Campbell, *The influence of higher protein intake and greater eating frequency on appetite control in overweight and obese men.* Obesity, 2010. **18**(9): p. 1725-1732.

136. Potier, M., G. Fromentin, A. Lesdema, R. Benamouzig, D. Tomé, and A. Marsset-Baglieri, *The satiety effect of disguised liquid preloads administered acutely and differing only in their nutrient content tended to be weaker for lipids but did not differ between proteins and carbohydrates in human subjects.* Br J Nutr, 2010. **104**(09): p. 1406-1414.

137. EFSA Panel on Dietetic Products, N.a.A.N., *Scientific opinion on the substantiation of health claims related to protein and increase in satiety leading to a reduction in energy intake (ID 414, 616, 730), contribution to the maintenance or achievement of a normal body weight (ID 414, 616, 730), maintenance of normal bone (ID 416) and growth or maintenance of muscle mass (ID 415, 417, 593, 594, 595, 715) pursuant to article 13(1) of regulation (EC) No 1924/2006.* EFSA J, 2010. **8**(10): p. 1811.

138. FAOSTAT. *FAOSTAT database.* 2007; Available from: http://faostat.fao.org/faostat/collections.

139. Gezondheidsraad, *Voedingsnormen energie, eiwitten, vetten en verteerbare koolhydraten publicatie nr 2001/19 - Den Haag.* 2001.

140. Zijlstra, N., M. Mars, R.A. de Wijk, M.S. Westerterp-Plantenga, J.J. Holst, and C. de Graaf, *Effect of viscosity on appetite and gastro-intestinal hormones.* Physiol Behav, 2009. **97**(1): p. 68-75.

141. Bertenshaw, E.J., A. Lluch, and M.R. Yeomans, *Dose-dependent effects of beverage protein content upon short-term intake.* Appetite, 2009. **52**(3): p. 580-587.

142. Bertenshaw, E.J., A. Lluch, and M.R. Yeomans, *Satiating effects of protein but not carbohydrate consumed in a between-meal beverage context.* Physiol Behav, 2008. **93**(3): p. 427-436.

143. Simons, P.J., J.A. Kummer, J.J.F.P. Luiken, and L. Boon, *Apical CD36 immunolocalization in human and porcine taste buds from circumvallate and foliate papillae.* Acta Histochem, 2011. **113**(8): p. 839-843.

144. Calitri, R., E.M. Pothos, K. Tapper, J.M. Brunstrom, and P.J. Rogers, *Cognitive biases to healthy and unhealthy food words predict change in BMI.* Obesity, 2010. **18**(12): p. 2282-2287.

145. Hetherington, M.M., R. Foster, T. Newman, A.S. Anderson, and G.N.M. Norton, *Understanding variety: tasting different foods delays satiation.* Physiol Behav, 2006. **87**(2): p. 263-71.

146. Griffioen-Roose, S., M. Mars, G. Finlayson, J.E. Blundell, and C. de Graaf, *The effect of within-meal protein content and taste on subsequent food choice and satiety.* Br J Nutr, 2011. **106**(05): p. 779-788.

147. Vazquez, M., P.B. Pearson, and G.K. Beauchamp, *Flavor preferences in malnourished mexican infants.* Physiol Behav, 1982. **28**(3): p. 513-519.

148. Griffioen-Roose, S., M. Mars, E. Siebelink, G. Finlayson, D. Tomé, and C. De Graaf, *Protein status elicits compensatory changes in food intake and food preferences.* Am J Clin Nutr, in press.

149. Scott, T.R., *Taste as a basis for body wisdom.* Physiol Behav, 2011. **104**(1): p. 57-63.

150. Dus, M., S. Min, A.C. Keene, G.Y. Lee, and G.S.B. Suh, *Taste-independent detection of the caloric content of sugar in Drosophila.* Proc Natl Acad Sci U S A, 2011. **108**(28): p. 11644-11649.

151. Bensaïd, A., D. Tomé, D. Gietzen, P. Even, C. Morens, N. Gausseres, and G. Fromentin, *Protein is more potent than carbohydrate for reducing appetite in rats.* Physiol Behav, 2002. **75**(4): p. 577-582.

152. Apolzan, J.W., N.S. Carnell, R.D. Mattes, and W.W. Campbell, *Inadequate dietary protein increases hunger and desire to eat in younger and older men.* J Nutr, 2007. **137**(6): p. 1478-1482.

153. Long, S.J., A.R. Jeffcoat, and D.J. Millward, *Effect of habitual dietary-protein intake on appetite and satiety.* Appetite, 2000. **35**(1): p. 79-88.

154. Booth, D.A. and P.C. Simson, *Food preferences acquired by association with variations in amino acid nutrition.* Q J Exp Psychol (Colchester), 1971. **23**(1): p. 135-145.

155. Booth, D.A., *Food intake compensation for increase or decrease in the protein content of the diet.* Behav Biol, 1974. **12**(1): p. 31-40.

156. Gibson, E.L. and D.A. Booth, *Acquired protein appetite in rats: dependence on a protein-specific need state.* Experientia, 1986. **42**(9): p. 1003-1004.

157. Simpson, S.J. and P.R. White, *Associative learning and locust feeding: evidence for a `learned hunger' for protein.* Anim Behav, 1990. **40**(3): p. 506-513.

158. Gietzen, D.W., S. Hao, and T.G. Anthony, *Mechanisms of food intake repression in indispensable amino acid deficiency.* Annu Rev Nutr, 2007. **27**(1): p. 63-78.

159. Blatt, A.D., L.S. Roe, and B.J. Rolls, *Increasing the protein content of meals and its effect on daily energy intake.* J Am Diet Assoc, 2011. **111**(2): p. 290-294.

160. WHO, *Protein and amino acid requirements in human nutrition. Report of a Joint WHO/FAO/UNU Expert Consultation.* 2007, World Health Organization: Geneva.

161. Siebelink, E., A. Geelen, and J.H.M. de Vries, *Self-reported energy intake by FFQ compared with actual energy*

*intake to maintain body weight in 516 adults.* Br J Nutr, 2011. **106**(02): p. 274-281.

162. Bingham, S. and J.H. Cummings, *The use of 4-aminobenzoic acid as a marker to validate the completeness of 24 h urine collections in man.* Clin Sci (Lond), 1983. **64**(6): p. 629-35.

163. Prestwich, A., R. Hurling, and S. Baker, *Implicit shopping: attitudinal determinants of the purchasing of healthy and unhealthy foods.* Psychol Health, 2011. **26**(7): p. 875-885.

164. Viskaal-van Dongen, M., M.C. van den Berg, N. Vink, F.J. Kok, and C. de Graaf, *Taste – nutrient relationships in commonly consumed foods.* Br J Nutr, 2011. **FirstView Article : pp 1-8**.

165. Essed, N.H., W.A. van Staveren, F.J. Kok, W. Ormel, G. Zeinstra, and C. de Graaf, *The effect of repeated exposure to fruit drinks on intake, pleasantness and boredom in young and elderly adults.* Physiol Behav, 2006. **89**(3): p. 335-341.

166. Rolls, B.J. and T.M. McDermott, *Effects of age on sensory-specific satiety.* Am J Clin Nutr, 1991. **54**(6): p. 988-996.

167. Tome, D., J. Schwarz, N. Darcel, and G. Fromentin, *Protein, amino acids, vagus nerve signaling, and the brain.* Am J Clin Nutr, 2009. **90**(3): p. 838-843.

168. Sorensen, A., D. Mayntz, D. Raubenheimer, and S.J. Simpson, *Protein-leverage in mice: the geometry of macronutrient balancing and consequences for fat deposition.* Obesity, 2008. **16**(3): p. 566-571.

169. Smeets, P.A.M., A. Erkner, and C. De Graaf, *Cephalic phase responses and appetite.* Nutr Rev, 2010. **68**(11): p. 643-655.

170. Höfer, D., B. Püschel, and D. Drenckhahn, *Taste receptor-like cells in the rat gut identified by expression of alpha-gustducin.* Proc Natl Acad Sci U S A, 1996. **93**(13): p. 6631-6634.

171. Beauchamp, G.K., *Sensory and receptor responses to umami: an overview of pioneering work.* Am J Clin Nutr, 2009. **90**(3): p. 723S-727S.

172. Sclafani, A., *Sweet taste signaling in the gut.* Proc Natl Acad Sci U S A, 2007. **104**(38): p. 14887-14888.

173. Egan, J.M. and R.F. Margolskee, *Taste cells of the gut and gastrointestinal chemosensation.* Mol Interv, 2008. **8**(2): p. 78-81.

174. Gietzen, D.W. and Q.R. Rogers, *Nutritional homeostasis and indispensable amino acid sensing: a new solution to an old puzzle.* Trends Neurosci, 2006. **29**(2): p. 91-99.

175. Sclafani, A., *Post-ingestive positive controls of ingestive behavior.* Appetite, 2001. **36**(1): p. 79-83.

176. Mellinkoff, S.M., M. Frankland, D. Boyle, and M. Greipel, *Relationship between serum amino acid concentration and fluctuations in appetite.* J Appl Physiol, 1956. **8**(5): p. 535-538.

177. Remco C, H., *"You say it's liking, I say it's wanting …". On the difficulty of disentangling food reward in man.* Appetite, 2011. **57**(1): p. 286-294.

178. Finlayson, G., A. Arlotti, M. Dalton, N. King, and J.E. Blundell, *Implicit wanting and explicit liking are markers for trait binge eating. A susceptible phenotype for overeating.* Appetite, 2011. **57**(3): p. 722-728.

179. Temple, J.L., A.M. Giacomelli, J.N. Roemmich, and L.H. Epstein, *Overweight children habituate slower than non-overweight children to food.* Physiol Behav, 2007. **91**(2-3): p. 250-254.

180. Wardle, J., C. Llewellyn, S. Sanderson, and R. Plomin, *The FTO gene and measured food intake in children.* 2008. **33**(1): p. 42-45.

181. Zandstra, E.H., C. de Graaf, D.J. Mela, and W.A. Van Staveren, *Short- and long-term effects of changes in pleasantness on food intake.* Appetite, 2000. **34**(3): p. 253-60.

182. Hetherington, M.M., L.M. Pirie, and S. Nabb, *Stimulus satiation: effects of repeated exposure to foods on pleasantness and intake.* Appetite, 2002. **38**(1): p. 19-28.

183. Morewedge, C.K., Y.E. Huh, and J. Vosgerau, *Thought for food: imagined consumption reduces actual consumption.* Science, 2010. **330**(6010): p. 1530-1533.

184. Slimani, N., G. Deharveng, D.A.T. Southgate, C. Biessy, V. Chajes, M.M.E. van Bakel, M.C. Boutron-Ruault, A. McTaggart, S. Grioni, J. Verkaik-Kloosterman, I. Huybrechts, P. Amiano, M. Jenab, J. Vignat, K. Bouckaert, C. Casagrande, P. Ferrari, P. Zourna, A. Trichopoulou, E. Wirfalt, G. Johansson, S. Rohrmann, A.K. Illner, A. Barricarte, L. Rodriguez, M. Touvier, M. Niravong, A. Mulligan, F. Crowe, M.C. Ocke, Y.T. van der Schouw, B. Bendinelli, C. Lauria, M. Brustad, A. Hjartaker, A. Tjonneland, A.M. Jensen, E. Riboli, and S. Bingham, *Contribution of highly industrially processed foods to the nutrient intakes and patterns of middle-aged populations in the European Prospective Investigation into Cancer and Nutrition study.* Eur J Clin Nutr, 2010. **63**(S4): p. S206-S225.

185. Swithers, S.E. and T.L. Davidson, *A role for sweet taste: calorie predictive relations in energy regulation by rats.* Behav Neurosci, 2008 **122**(1): p. 161-173.

186. Swithers, S.E., S.B. Ogden, and T.L. Davidson, *Fat substitutes promote weight gain in rats consuming high-fat diets.* Behav Neurosci, 2011. **125**(4): p. 512-518.

187. Cust, A.E., M.R. Skilton, M.M.E. van Bakel, J. Halkjar, A. Olsen, C. Agnoli, T. Psaltopoulou, E. Buurma, E. Sonestedt, M.D. Chirlaque, S. Rinaldi, A. Tjonneland, M.K. Jensen, F. Clavel-Chapelon, M.C. Boutron-Ruault, R. Kaaks, U. Nothlings, Y. Chloptsios, D. Zylis, A. Mattiello, S. Caini, M.C. Ocke, Y.T. van der Schouw, G. Skeie, C.L. Parr, E. Molina-Montes, J. Manjer, I. Johansson, A. McTaggart, T.J. Key, S. Bingham, E. Riboli, and N. Slimani, *Total dietary carbohydrate, sugar, starch and fibre intakes in the European Prospective Investigation into Cancer and Nutrition.* Eur J Clin Nutr, 2009. **63**(S4): p. S37-S60.

188. Schutz, H.G. and F.J. Pilgrim, *A field study of food monotony.* Psychol Rep, 1958. **4**(7): p. 559-565.

189. Uematsu, A., T. Tsurugizawa, A. Kitamura, R. Ichikawa, K. Iwatsuki, H. Uneyama, and K. Torii, *Evaluation of the `liking' and `wanting' properties of umami compound in rats.* Physiol Behav, 2011. **102**(5): p. 553-558.

190. Rogers, P.J. and J.E. Blundell, *Umami and appetite: effects of monosodium glutamate on hunger and food intake in human subjects.* Physiol Behav, 1990. **48**(6): p. 801-804.

191. Yeomans, M.R., N.J. Gould, S. Mobini, and J. Prescott, *Acquired flavor acceptance and intake facilitated by monosodium glutamate in humans.* Physiol Behav, 2008. **93**(4-5): p. 958-966.

192. García-Rovés, P.M., N. Terrados, S. Fernández, and A.M. Patterson, *Comparison of dietary intake and eating behavior of professional road cyclists during training and competition.* Int J Sport Nutr, 2000. **10**(1): p. 82-98.

193. Abdulla, M., I. Andersson, N. Asp, K. Berthelsen, D. Birkhed, I. Dencker, C. Johansson, M. Jagerstad, K. Kolar, B. Nair, P. Nilsson-Ehle, A. Norden, S. Rassner, B. Akesson, and P. Ockerman, *Nutrient intake and health status of vegans - chemical analyses of diets using the duplicate portion sampling technique.* Am J Clin Nutr, 1981. **34**(11): p. 2464-2477.

194. Wansink, B., *From mindless eating to mindlessly eating better.* Physiol Behav, 2010. **100**(5): p. 454-463.

195. Neary, M.T. and R.L. Batterham, *Gaining new insights into food reward with functional neuroimaging*, in

*Frontiers in Eating and Weight Regulation*. 2010. p. 152-163.

196. Spetter, M.S., P.A.M. Smeets, C. de Graaf, and M.A. Viergever, *Representation of sweet and salty taste intensity in the brain.* Chem Senses, 2010. **35**(9): p. 831-840.

197. Stice, E., S. Spoor, C. Bohon, M. Veldhuizen, and D. Small, *Relation of reward from food intake and anticipated food intake to obesity: a functional magnetic resonance imaging study.* J Abnorm Psychol, 2008. **117**(4): p. 924-935.

198. Leidy, H.J., R.J. Lepping, C.R. Savage, and C.T. Harris, *Neural responses to visual food stimuli after a normal vs. higher protein breakfast in breakfast-skipping teens: a pilot fMRI study.* 2011. **19**(10): p. 2019-2025.

199. Schachter, S., *Obesity and eating.* Science, 1968. **161**(3843): p. 751-756.

200. King, D.A. and S.M. Thomas, *Big lessons for a healthy future.* 2007. **449**(7164): p. 791-792.

201. Herman, C.P. and J. Polivy, *External cues in the control of food intake in humans: The sensory-normative distinction.* Physiol Behav, 2008. **94**(5): p. 722-728.

202. Wardle, J. and S. Carnell, *Appetite is a heritable phenotype associated with adiposity.* Ann Behav Med, 2009. **38**(0): p. 25-30.

# Et cetera

—Summary in Dutch, acknowledgements, and more about the author

# Samenvatting

Sensorische eigenschappen van voedsel, zoals de smaak, geur, textuur, en hoe een product eruit ziet, spelen een belangrijke rol in ons eetgedrag. Er wordt vaak aan de hand van deze eigenschappen bepaald of een product wel of niet gegeten gaat worden, en zo ja: hoeveel. Heel veel producten die we eten zijn over het algemeen óf zoet, óf hartig. De rol die zoet en hartig spelen in ons voedingspatroon en keuzes die we gedurende een dag maken was tot dusver niet duidelijk. Interessant is dat de hartige producten over het algemeen vaak meer eiwit bevatten en zoete producten meer koolhydraten. Het wordt verondersteld dat eiwit en koolhydraten een verschillende verzadigende werking hebben. Welke rol zoet en hartig hierbij bij spelen is vooralsnog ook niet duidelijk.

Daarnaast zijn er veel psychologische processen betrokken bij de keuzes die we maken. We vinden producten lekker en daarom willen we ze eten. Het kan ook voor komen dat we een product lekker vinden, maar het op een bepaald moment niet zouden willen eten (denk bijvoorbeeld aan bitterballen als ontbijt). Veel keuzes worden natuurlijk ook onbewust gemaakt.

Het doel van dit proefschrift is tweeledig:
1. De rol die zoete en hartige smaak spelen in voedselinname en voedselkeuze onderzoeken;
2. Meer inzicht verkrijgen in bewuste en onbewuste psychologische processen betrokken bij voedselinname en voedselkeuze om zo de onderliggende mechanismen beter te begrijpen.

In het eerste onderzoek (hoofdstuk 2) hebben we het verschil tussen zoete en hartige smaak in het proces van verzadiging onderzocht. Dit hebben we gedaan door 64 deelnemers een zoete of een hartige rijstmaaltijd te geven en te meten hoeveel de deelnemers ervan aten. De maaltijden hadden dezelfde textuur, ze bevatten dezelfde hoeveelheid energie en nutriënten, en de deelnemers vonden ze ook even lekker. Op deze manier konden we dus specifiek het effect van de smaak onderzoeken. De deelnemers consumeerden deze maaltijden in een sensorisch hokje[1]. Zo werden ze niet afgeleid en konden zich concentreren op de maaltijd. De resultaten lieten zien dat de deelnemers evenveel aten van beide rijstmaaltijden. Ook werden beide maaltijden gegeten met dezelfde snelheid en voelden de deelnemers zich even verzadigd na het stoppen met eten. Hieruit kunnen we concluderen dat zoet en hartig niet verschillen in hun effect op het proces van verzadiging.

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[1]Sensorische hokjes zijn individuele smaakhokjes die worden ingericht om sensorische evaluaties uit te voeren onder gecontroleerde en gestandaardiseerde omstandigheden. In zo'n hokje is er minimale afleiding voor de proefpersonen.

In het tweede onderzoek (hoofdstuk 3) hebben we gekeken naar het effect van zoete en hartige smaak op opvolgende voedselkeuzes. Dit hebben we gedaan door 61 deelnemers eerst een gelijke hoeveelheid zoete of hartige rijst te laten eten. Vervolgens hebben gekeken naar voorkeuren en inname van verschillende producten die varieerden in smaak. Ook dit onderzoek werd uitgevoerd in sensorische hokjes. De resultaten lieten zien dat de deelnemers na het eten van de zoete rijst geen voorkeur hadden voor zoet of hartig; ze aten evenveel zoete als hartige producten. Na het eten van de hartige rijst zagen we wat anders: deelnemers hadden een grote voorkeur voor zoete producten en aten dus meer zoete dan hartige producten. De totale hoeveelheid die de deelnemers aten was niet verschillend.

Dit effect hebben we verder onderzocht in de context van een compleet dieet (hoofdstuk 5). In totaal hebben we 39 deelnemers drie verschillende 24-uurs diëten gegeven bestaand uit drie maaltijden (lunch, diner en ontbijt) en tussendoortjes die óf alleen maar zoet waren, óf alleen maar hartig waren, óf een mix van zoet en hartig waren. Na het 24-uurs dieet werd er een lunchbuffet aangeboden waar de deelnemers zelf mochten kiezen wat en hoeveel ze aten. De inname van het buffet hebben we gemeten. Dit onderzoek werd uitgevoerd in een iets normalere omgeving: de deelnemers mochten zelf hun bord opscheppen en de maaltijd werd genuttigd in een gezamenlijke ruimte. De resultaten waren hetzelfde als na een enkele maaltijd: na het eten van het zoete dieet (en het gemixte dieet) hadden de deelnemers geen uitgesproken voorkeur voor zoete of hartige producten. Na het eten van het hartige dieet hadden de deelnemers een grote voorkeur voor zoet, en aten dus meer zoete dan hartige producten. Ook in deze studie was de totale hoeveelheid die de deelnemers aten niet verschillend. Uit deze twee studies kunnen we concluderen dat de smaak van een gegeten product effect heeft op de keuze voor een volgend te eten product; de voorkeur verandert dus. Maar het lijkt erop dat hartig een groter effect heeft op opvolgende voedselkeuzes (voorkeur) dan zoet.

Hartige producten bevatten over het algemeen meer eiwit en zoete producten vaak meer koolhydraten. Het is daarom soms moeilijk te onderscheiden of voedselkeuze gebasseerd is op alleen de smaak van een product, of dat de 'de inhoud' hierbij ook een rol speelt. Wij hebben daarom een onderzoek uitgevoerd (hoofdstuk 4) waarbij we het effect van smaak en het effect van eiwit op voedselkeuze en verzadiging los van elkaar hebben onderzocht. Dit hebben we gedaan door 60 deelnemers een vaste hoeveelheid van vier verschillende rijstmaaltijden te laten eten; een zoete rijstmaaltijd met veel eiwit, een zoete rijstmaaltijd met weinig eiwit, een hartige rijstmaaltijd met veel eiwit en een hartige rijstmaaltijd met weinig eiwit. Na deze maaltijd werd er een buffet aangeboden waar de deelnemers zelf mochten kiezen wat en hoeveel ze aten. De inname van het buffet hebben we gemeten. Dit onderzoek werd weer uitgevoerd in de sensorische hokjes. Het buffet bevatte producten die speciaal geselecteerd waren op hun smaak (zoet of

hartig) en eiwitgehalte (hoog of laag). De resultaten van dit onderzoek lieten zien dat de hoeveelheid eiwit in de rijstmaaltijd geen effect had op opvolgend keuzegedrag en inname van de deelnemers. Ook in dit onderzoek zagen we alleen een effect van smaak: na de zoete maaltijd hadden de deelnemers geen voorkeur voor zoet of hartig, maar na de hartige maaltijden was er een grote voorkeur voor zoete producten.

In de laatste studie (hoofdstuk 6) hebben we gekeken naar de lange termijn effecten van eiwit op voedselkeuze en verzadiging. Dit hebben we gedaan door 37 deelnemers 14 dagen een dieet te laten volgen dat of heel weinig eiwit bevatte of juist heel veel eiwit. Na deze 14 dagen mochten de deelnemers 2,5 dag zelf weten wat en hoeveel ze aten van producten die wij ze gedurende deze dagen aanboden. Deze inname hebben we gemeten. De producten die we aanboden hadden grote variëteit in smaak en eiwitgehalte. Dit onderzoek werd weer uitgevoerd in een iets normalere omgeving. De resultaten van dit onderzoek lieten zien dat na het lage eiwit dieet deelnemers spontaan meer eiwit innamen dan na het hoog eiwit dieet. Maar in totaal aten ze wel dezelfde hoeveelheid producten. Deze verhoogde inname is dus het gevolg van een verhoogde inname van eiwitrijke producten. En inderdaad, na het lage eiwitdieet hadden de deelnemers een grote voorkeur voor hartige eiwitrijke producten ontwikkeld.

In alle studies die hierboven zijn beschreven hebben we ook een of meerdere psychologische testen uitgevoerd. We hebben een aantal keer de 'Leeds Voedsel Voorkeur Vragenlijst' gebruikt waarbij deelnemers aan de hand van foto's van producten konden aangeven hoe lekker ze dat product zouden vinden en hoe graag ze dat product zouden willen eten. Ook werd er een reeks foto's laten zien waar de deelnemers steeds keuzes moesten maken welk product ze op dat moment het liefst wilde eten. Bij deze laatste meting werd ook de deelnemers' reactietijd gemeten. Dit hebben we gedaan omdat de snelheid waarmee een persoon op een voedselproduct reageert iets zegt over de hoe graag de deelnemer dat product wil eten. Hier zijn de deelnemers zich niet van bewust, hierdoor is dit een meting waarmee we onbewuste processen kunnen meten. Naast dit fotoprogramma hebben we tevens een methode gebruikt waar deelnemers moesten 'werken' om toegang te krijgen tot een bepaald voedselproduct. Door middel van een soort spelletje konden deelnemers punten verzamelen: hoe meer punten, hoe meer product. Ook dit is een methode waarmee we kunnen meten hoe graag deelnemers het voedingsproduct willen eten.

Door het gebruik van deze verschillende methoden hebben we kunnen laten zien dat zowel bewuste als onbewuste processen een rol spelen bij verschillende aspecten van eetgedrag. Het lijkt erop dat in een gecontroleerde omgeving (zoals in een sensorisch hokje), bewuste processen een grotere rol spelen bij keuzes over hoeveel er wordt gegeten. De keuze welk voedselproduct wordt gegeten lijkt op een onbewuster niveau

te worden gemaakt. In een wat meer natuurlijke omgeving waar de deelnemers zelf hun eten opschepten en de maaltijd genuttigd werd in een gezamenlijke ruimte lijken onbewuste processen het eetgedrag beter te verklaren. Wanneer deelnemers een eiwittekort ervoeren (na 14 dagen op een laag eiwit dieet) hadden ze onbewust een voorkeur voor hartige eiwitrijke producten; ze waren zich daar niet bewust van. Het lijkt erop dat als het lichaam tekorten ervaart, dit onbewust de voedselvoorkeuren verandert en het eetgedrag beïnvloedt.

In deze slotalinea wil ik graag terugkomen op de doelen van dit proefschrift, namelijk 1. de rol die zoete en hartige smaak spelen in voedselinname en voedselkeuze onderzoeken en 2. meer inzicht verkrijgen in bewuste en onbewuste psychologische processen betrokken bij voedselinname en voedselkeuze om zo de onderliggende mechanismen beter te begrijpen. De resultaten van de studies laten zien dat:

- Zoet en hartig niet verschillen in hun effect op het proces van verzadiging.
- De smaak van een maaltijd of dieet wel een groot effect heeft op voedselkeuzes die erna worden gemaakt. En dit effect is niet gelijk voor zoet en hartig. Hartig lijkt een groter modulerend effect te hebben op opvolgende voedselvoorkeuren dan zoet.
- Het eiwitgehalte van een maaltijd geen effect lijkt te hebben op voedselkeuze en verzading.
- Een eiwittekort (na 14 dagen op een laag eiwit dieet) wel veranderingen brengt in voedselinname en voedselkeuze om zo de eiwitbalans weer te herstellen.
- Zowel bewuste als onbewuste processen betrokken zijn bij verzadiging en voedselkeuze, maar de bijdrage van beide niet altijd hetzelfde lijkt. Dit wordt vooral duidelijk wanneer er tekorten dreigen te ontstaan; dan lijkt het dat onbewuste processen een grote rol gaan spelen in de keuzes die worden gemaakt over wat en hoeveel te gaan eten.

# Dankwoord

En dan is het gewoon af! Vier jaar voorbij gevlogen en wat is het leuk geweest! Ik wil graag een aantal mensen bedanken die hebben bijgedragen aan dit proefschrift (en/of aan de leuke tijd ;) Ten eerste, mijn promotor Kees. Kees, bedankt dat je mij de gelegenheid hebt gegeven om aan dit project te gaan werken. Ik heb onze gesprekken over onderzoek, of dat nu in je werkkamer was, of in een vliegtuig, altijd heel erg gewaardeerd. Ik heb bewondering voor je visie en ben heel blij dat ik komend jaar nog bij je groep kan blijven werken. Monica, als co-promotor ben je heel dicht betrokken geweest bij mijn project. Bedankt voor al je hulp en adviezen. Heerlijk dat ik altijd bij je binnen kon lopen! Graham, thank you for wanting to be my co-supervisor. I really appreciated all your input and thoughts on the subject. I loved working with your method and hope we can continue this collaboration. John Blundell, during these years we have met on several occasions to discuss my research. Thank you so much for your hospitality and your time. Our meetings were always very inspiring and I hope I will continue to meet you in the future. Daniel Tomé, once my research started to move towards the topic protein I was lucky that you could get involved. I was very happy that you were also willing to be part of my thesis committee. Thank you for all your valuable input and I'm looking forward to our future collaboration! Graag wil ik hierbij ook de leden van de begeleidingscommissie van STW bedanken. Jos, Liesbeth, Hugo, David, Jan, Henk, Annette, Harry, Henry en Pascalle. Bedankt voor alle meetings waar we resultaten en plannen hebben bediscussieerd. Jullie zorgden er altijd voor dat ik scherp bleef, dank voor jullie input! Pascalle, hierbij wil ik je ook bedanken voor het feit dat je deelneemt aan mijn thesis committee. En ik vind het ontzettend leuk dat ik komend jaar samen met je kan gaan werken in Pidon! Hereby I would also like to thank the two other members of my thesis committee: Henk Wouters, and Suzanne Higgs. Suzanne, thank you for flying all the way to the Netherlands to be part of this. I hope I will continue seeing you on meetings and conferences.

Om mijn onderzoeken te laten slagen heb ik ontzettend veel hulp gehad van een heleboel mensen. Els, jij bent fantastisch! Dank voor al je hulp en inzet en bemoediging als ik het weer eens niet zag zitten. Dit was gewoon allemaal niet gelukt zonder jou. Karin, dank voor je expertise en je rust. En alle andere diëtisten die zo hard hebben gewerkt en gerekend aan mijn onderzoeken: Ingrid, Merel, Dorthe, Corine, Pauline en Emmy, bedankt! Jullie waren echt te gek! Tijdens mijn onderzoeken hebben er ook een aantal studenten en studentassistenten meegewerkt. Iris, jij was mijn eerste student. En daarna heb je ook nog als studentassistent tijdens mijn tweede studie meegewerkt. Man, wat hebben wij een lol gehad. Dank voor je inzet en enthousiasme. Ik vind het ook heel leuk dat je nu zelf aan het promoveren bent. Maaike, jij hebt helemaal solo je eigen onderzoek gerund. Heel knap! Fabian, wat was het leuk om met jou samen te werken! Bedankt voor

je inzet en ik wens je heel veel succes in de toekomst. Inge, Nika, Eva, Elles en Evren: jullie hebben heel hard meegewerkt aan het succes van ProTime. Daar ben ik jullie heel erg dankbaar voor! Studentassistenten Emmy, Martine, Claudy, Rianne en Cecile: zonder jullie waren mijn onderzoeken nooit zo smooth verlopen. Bedankt! Wieke, tijdens ProTime hebben we close samengewerkt. Dank voor al je hulp! Hierbij wil ik ook de medewerkers van het lab bedanken, en in het speciaal: Anita, Tineke, Betty, Nhien en Paul. Zonder jullie geen chemische analyses. Dank voor al jullie werk! Jan, dank dat ik altijd weer bij je terecht kon voor foto's voor mijn onderzoeken. Heel fijn dat we het zo hebben kunnen professionaliseren. Dione, dankzij jou heb ik altijd weer genoeg deelnemers voor mijn studies kunnen vinden en daarvoor ben ik je heel erg dankbaar! Hans en Reinoud, dank voor al jullie technische ondersteuning, vooral in het begin van mijn project! Cornelia, Gabrielle, Gea en Karen. Met al mijn vragen kon ik altijd bij jullie terecht. Dank dat jullie er zijn. Gabrielle, ik ga je missen volgend jaar! Lidwien, bedankt voor alles wat je voor mijn project hebt geregeld. Eric en Riekie, jullie bedankt voor het zorgdragen van alle financiën!

Ik wil ook graag nog een aantal andere collega's bedanken. Ten eerste, de (ex-)leden van de Eetclub. Deze is de afgelopen jaren behoorlijk gegroeid! Ik wil met name Gerry, Marcus, Sanne, Gertrude, Nicolien, Anne, Anne, Iris en Svetlana bedanken voor de fijne samenwerking van de afgelopen jaren. Paul, ik kijk uit naar onze toekomstige samenwerking! Victoire, bedankt voor je energie, interesse en enthousiasme. Dat er nog maar veel gezellig lunches mogen volgen! Mirre, ik heb onze samenwerking altijd als heel prettig ervaren. Knap hoe jij alles in je leven altijd zo goed weet te combineren! Ik wens je heel veel succes met de laatste kleine loodjes van je proefschrift! Coraline, tijdens onze buitenlandse reisjes ben ik je iets beter leren kennen. Ik waardeer je rust en interesse. Ook jij heel veel succes met het afronden van je (monster)project! Ik wil hier ook mijn STW-collega's noemen: Van-Anh, Maartje en Marielle. Bedankt voor de prettige samenwerking van de afgelopen jaren, en ik wens jullie heel veel succes met het afronden van jullie proefschrift. En ik wil speciaal mijn twee paranimfen bedanken. Dieuwerke, ik vond het ontzettend leuk met jou binnen het STW project te werken en je daardoor beter te leren kennen. Ik heb waardering voor je manier van onderzoek en hoop komend jaar nog veel met je samen te werken! Pleunie, wat hebben wij een te gekke tijd achter de rug: 13 gezamenlijke 'business trips' naar binnen- en buitenland (die vaak gepaard gingen met leuke feestjes), de 4-uur-bier-uren, het 'half-half-en' met eten, ons verblijf in de prime minister suite, het laatste gezamenlijke onderzoek. Deze tijd was niet hetzelfde geweest zonder jou en ik ben je enorm dankbaar. Door onze samenwerking heb ik ook heel veel over mezelf geleerd. Ik ga je missen volgend jaar maar wens je heel veel succes en plezier in Zweden samen met Wouter!

Ook zijn er nog een aantal andere (ex-)collega's van de afdeling die ik speciaal even wil noemen. Michel, bedankt voor al je energie! Ik heb zo enorm met je gelachen. Je was echt mijn maatje en ik hoop dat we ons contact behouden (ook al zit je 4 maanden per jaar in Harvard!). Akke, wat ben jij leuk. De afgelopen jaren ben je een vriendin van me geworden en ik ga je missen hier in Wageningen! Janneke, Michel, Michael, Anne, Sandra, Maria-Elisa and Pleunie, I will never forget our trip to Saint-Petersburg, sweet memories! Rianne, Cecile and Sandra, together with Michel we organized the PhD trip in 2009. I'm still very proud of what we achieved. Elise, Esmée, Linda, Janette, Simone en Antonie: het was gezellig!

Lieve vrienden, familie en schoonfamilie. De afgelopen jaren zijn jullie altijd geïnteres-seerd geweest in wat ik aan het doen was in Wageningen. Hester, Yvonne, Sarianne en Anke, of het nu van dichtbij of ver weg is, jullie zijn er alle vier op een hele verschillende manier altijd voor mij en dat is zo waardevol. Dank voor alle gezellige avondjes/weekend-jes die het werk altijd weer even relativeerde! Mireille, Wouter, Arnout (bedankt voor de hulp met InDesign!), Annelies, Guido, Karin en Wendy, als broers (zwager) en (schoon) zussen staan jullie heel dichtbij. Ik ben zo trots dat ik jullie zusje ben! Dank dat jullie er zijn. Pa en ma, ik ben jullie zo ontzettend dankbaar voor alle mogelijkheden die jullie mij hebben gegeven. Jullie zijn mijn rotsen en dit werk was niet geworden wat het is zonder jullie vertrouwen in mij. Ik hou van jullie!

En dan lieve Alex. Ik ben je heel erg dankbaar wat je de afgelopen jaren allemaal voor me hebt gedaan; van het ontwerpen van logo's voor mijn onderzoeken, tot het toasten met bubbels als er weer een paper was geaccepteerd, of me opbeuren als er juist één was afgewezen. Ik kan altijd met al mijn verhalen bij je terecht! En daarnaast heb je ook nog eens deze fantastische cover ontworpen die zo ík is. Alex, ik kijk uit naar alle avonturen die we samen nog gaan beleven. Love you!

— Sanne

# Curriculum Vitae

Sanne Griffioen-Roose was born on May 12th, 1982 in Amersfoort, the Netherlands. After completing secondary school at the 'Farel College' in Amersfoort, she started the Bachelor's programme 'Biomedical Sciences' at the University Utrecht. After having received her Bachelors degree in 2004, she enrolled in the Master programme 'Neuroscience and Cognition'. During her Masters she did two internships: one on social play behaviour in juvenile rats, and one play behaviour in children with autism. Sanne wrote her Master's thesis on early environmental influences on the development of the stress response. After having received her Masters degree in 2006, she was appointed as a junior researcher at TNO (a Dutch acronym for applied scientific knowledge) Quality of Life in Zeist. Within one year Sanne decided she would like to pursue a PhD. In October 2007, Sanne was appointed as a PhD candidate at the Division of Human Nutrition at the Wageningen University. Human behaviour has always been her main topic of interest, and this project offered her an opportunity to investigate this in relation to food. Her research specifically focussed on the role of sweet and savoury taste in food intake and food preferences. During her PhD project, Sanne attended several (international) conferences and courses, and was involved in teaching. Furthermore, she was a member of the organising committee of the PhD study tour to Denmark, Sweden and Finland in 2009. In 2011 she was selected for the European Nutrition Leadership Programme (ENLP). Currently, Sanne is working as a postdoctoral fellow at Wageningen where her research is focussed on food and brain reward.

# List of publications

### Publications in peer-reviewed journals

**Griffioen-Roose, S.**, M. Mars, E. Siebelink, G. Finlayson, D. Tomé, and C. De Graaf, *Protein status elicits compensatory changes in food intake and food preferences.* Am J Clin Nutr, in press.

**Griffioen-Roose, S.**, M. Mars, G. Finlayson, J.E. Blundell, and C. de Graaf, *The effect of within-meal protein content and taste on subsequent food choice and satiety.* Br J Nutr, 2011. **106**(05): p. 779-788.

**Griffioen-Roose, S.**, G. Finlayson, M. Mars, J.E. Blundell, and C. de Graaf, *Measuring food reward and the transfer effect of sensory specific satiety.* Appetite, 2010. **55**(3): p. 648-655.

**Griffioen-Roose, S.**, M. Mars, G. Finlayson, J.E. Blundell, and C. de Graaf, *Satiation due to equally palatable sweet and savoury meals does not differ in normal weight young adults.* J Nutr, 2009. **139**(11): p. 2093-2098.

Vanderschuren, L.J.M.J., V. Trezza, **S. Griffioen-Roose**, O.J.G. Schiepers, N. Van Leeuwen, T.J. De Vries, and A.N.M. Schoffelmeer, *Methylphenidate disrupts social play behavior in adolescent rats.* Neuropsychopharmacology, 2008. **33**(12): p. 2946-2956.

#### Submitted papers
**Griffioen-Roose, S.**, P.S. Hogenkamp, M. Mars, G. Finlayson, and C. de Graaf, *Taste of a 24-h diet and its effect on food preferences and satiety.* submitted.

#### Abstracts
**Griffioen-Roose S.**, M. Mars, E. Siebelink, D. Tomé, C. de Graaf, *Protein status modulates food intake and preferences.* Appetite, 2011. **57**: p. 559 (abstract for the British Feeding and Drinking group meeting, 2011, Belfast, Ireland, oral presentation)

**Griffioen-Roose S.**, G. Finlayson, M. Mars, J. Blundell, C de Graaf, *Measuring food reward and the transfer effect of sensory specific satiety.* Appetite, 2011. **57**: p. 541 (abstract for the British Feeding and Drinking group meeting, 2010, Maastricht, The Netherlands, oral presentation)

**Griffioen-Roose S.**, M. Mars, G. Finlayson, J. Blundell, C. de Graaf, *The effect of protein content and taste on satiety and food choice.* Appetite, 2010. **54**: p. 648 (abstract for the 18th Annual meeting of the Society of the Study of Ingestive Behavior, 2010, Pittsburgh, USA, poster presentation)

**Griffioen-Roose S.**, G. Finlayson, M. Mars, J. Blundell, C. de Graaf, *Measuring food reward and the transfer effect of sensory specific satiety.* Appetite, 2010. **54**: p. 648 (abstract for the 18th Annual meeting of the Society of the Study of Ingestive Behavior, 2010, Pittsburgh, USA, poster presentation)

**Griffioen-Roose S.**, M. Mars, G. Finlayson, J. Blundell, C. de Graaf, *No difference in satiation between equally palatable meals with a sweet or savoury taste.* Obesity Facts, 2009. **2**(Suppl.2): p. 142 (abstract for the 17th European congress on Obesity, 2009, Amsterdam, The Netherlands, poster presentation)

**Griffioen-Roose S.**, M. Mars, G. Finlayson, J. Blundell, C. de Graaf, *No difference in satiation between equally palatable meals with a sweet or savoury taste.* Eur J Clin Nutr, 2009. **63** (Suppl.3): p. 10 (abstract for Wageningen Nutritional Science Forum, 2009, Arnhem, The Netherlands, poster presentation)

# Overview of completed training activities

### Discipline specific courses and activities

- 9th Pangborn Sensory Science Conference, 2011 (Toronto, Canada)
- 11th Benjamin Franklin La Fayette Seminar, 2011 (Fréjus, France)
- 9th Dutch Endo-Neuro-Psycho Meeting Lunteren, 2011 (Lunteren, The Netherlands)
- 35th British Feeding and Drinking group meeting, 2011 (Belfast, UK)
- 18th Annual meeting of the Society of the Study of Ingestive Behavior, 2010 (Pittsburgh, USA)
- 34th British Feeding and Drinking group meeting, 2010 (Maastricht, The Netherlands)
- 3rd Annual Frontiers meeting on Ingestive Behavior, 2009 (Leeds, UK)
- 8th Pangborn Sensory Science Conference, 2009 (Florence, Italy)
- 17th European congress on Obesity, 2009 (Amsterdam, The Netherlands)
- Wageningen Nutritional Science Forum, 2009 (Arnhem, The Netherlands)
- 16th Annual meeting of the Society of the Study for ingestive Behavior, 2008 (Paris, France)
- Course 'Epigenesis and Epigenetics - Physiological consequences of perinatal programming', 2008 (Wageningen, The Netherlands)
- Course 'Regulation of Food Intake', 2008 (Maastricht, The Netherlands)
- Course 'Food Perception and Preference', 2007 (Wageningen, The Netherlands)

### General courses and activities

- Course 'A practical and theoretical introduction into fMRI', 2011 (Utrecht, The Netherlands)
- 17th European Nutrition Leadership Program, 2011 (Luxembourg, Luxembourg)
- Annual PCDI postdoc retreat, 2011 (Heeze, The Netherlands)
- Master class 'Linear and Logistic Regression', 2010 (Wageningen, The Netherlands)
- Course 'Linear models and Mixed Linear models', 2010 (Wageningen, The Netherlands)
- Educational course 'Teaching and supervising thesis students', 2010 (Wageningen, The Netherlands)

- Course 'Philosophy and Ethics of Food Science and Technology', 2010 (Wageningen, The Netherlands)
- NWO training day 'Presentation skills' and 'Project planning', 2009 (Utrecht, The Netherlands)
- Course 'Techniques for Writing and Presenting a Scientific Paper', 2009 (Wageningen, The Netherlands)
- PhD Introduction Course, 2008 (Eindhoven, The Netherlands)
- Educational course 'How to be a good tutor', 2007 (Wageningen, The Netherlands)

### Optional courses and activities

- Organizing and participating in PhD study tour to Denmark, Sweden, and Finland, 2009
- Preparation research proposals and research presentations, 2008-2011
- Literature group 'Journal Club' and 'Oldsmobiles', 2007-2011

# Colophon

**Cover design and layout**
Oscar Alexander Griffioen and Sanne Griffioen–Roose

**Printing**
Printed by Grafisch Service Centrum, Wageningen