Effects of hunger state on food-related brain responses across the lifespan

Lisette Charbonnier

Colophon

Cover: A child confronted with a high versus low calorie food choice. Its attention completely goes to the high calorie food, while the low calorie food is completely out of sight (back cover).

Effects of hunger state on food-related brain responses across the lifespan
PhD thesis, Utrecht University, The Netherlands

Cover photo & lay-out: Lisette Charbonnier
Cover lay-out: Onno P.C. Steman
Child on cover photo: Daan M. Steman
ISBN: 978-90-393-6644-8
Printing: PrintSupport4u

Effects of hunger state on food-related brain responses across the lifespan

Effecten van honger status op voedselgerelateerde breinresponsen over de levensduur

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op donderdag 13 oktober 2016 des middags te 12.45 uur

door

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The work described in this thesis is part of the Full4Health project (www.full4health.eu), funded by the European Union Seventh Framework Program (FP7/2007-2013) under grant agreement nr. 266408.

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CHAPTER 1
GENERAL INTRODUCTION

1.1 Background

As the prevalence of overweight and obesity continues to rise (McLellan, 2002), research on food evaluation and choice has become of increased interest. Normal human physiology is innately geared towards obtaining food, which is a powerful reinforcer (Epstein and Leddy, 2006). Easy availability of tasty foods has caused a shift from eating for survival to eating for the pleasure obtained from food reward, even when not actually hungry (Mela, 2006; Peters et al., 2002; Saper et al., 2002). Consequently, human eating behavior is no longer controlled by metabolic need (Berthoud, 2006; Mela, 2006).

1.2 Brain development

The human brain does not fully mature until early adulthood and gradually deteriorates with age. Especially frontal regions important in response inhibition are known to mature the latest. Consequently, children and adolescents preferably choose an immediate reward over a delayed one (Killgore and Yurgelun-Todd, 2005). In contrast to children, elderly experience a gradual decline in response inhibition and working memory with age (for a review see Luna and Sweeney, 2006). This is most likely due to degeneration of grey and white matter volumes in especially frontal and hippocampal regions. To date it is still unknown to what extent developmental changes in brain structure and function influence food-related brain responses. This is important to be able to finetune possible interventions aimed at healthier eating decisions to different age groups.

1.3 Measuring food related brain responses

In the past two decades, functional magnetic resonance imaging (fMRI) has become an established method for investigating food-related brain responses (Smeets et al., 2012). The most commonly used technique is blood-oxygen level-dependent (BOLD) fMRI. This technique provides an indirect measurement of neuronal activation. It is based on regional changes in blood oxygenation in brain areas that become increasingly active due to for example sensory stimulation or a cognitive task. Different magnetic properties of oxy- and deoxyhaemoglobin, which vary with blood oxygenation level, result in a small increase in the BOLD signal (~1%), which can be measured with fMRI.

1.4 Neural correlates of food cue exposure

We are continuously exposed to food and during the day we make many choices regarding food consumption. Especially brain responses to the presentation of food pictures have been widely investigated with fMRI (Coletta et al., 2009; Frank et al., 2010a; Killgore et al., 2003; LaBar et al., 2001; Siep et al., 2009; Simmons et al., 2005; Smeets et al., 2013; Stoeckel et al., 2008; Toepel et al., 2009, 2010). Food versus non-food viewing paradigms are most common (Beaver et al., 2006; Cornier et al., 2007; Führer et al., 2008; Killgore et al., 2003; LaBar et al., 2001; Malik et al., 2008; Miller et al., 2007; Rothemund et al., 2007; Simmons et al., 2005), although high versus low calorie (or energy) food viewing comparisons are increasingly popular especially in light of the current obesity epidemic (Beaver et al., 2006; Cornier et al., 2007; Goldstone et al., 2009; Killgore et al., 2003; Passamonti et al., 2009; Rothemund et al., 2007). Several meta-analyses have been conducted to give insight in the consistency of the findings in these paradigms across studies, which turns out to be rather low due to differences in stimuli and tasks used and the variable hunger states examined (Brooks et al., 2013; Pursey et al., 2014; Van der Laan et al., 2012; van Meer et al., 2014).

The vast majority of the food viewing studies has been conducted in adults. Activation in response to food compared to non-food image viewing in healthy normal-weight (predominantly) adults, was found most concurrently across studies in the posterior fusiform gyrus, the lateral orbitofrontal cortex and the left middle insula (van der Laan et al., 2011). During high versus low calorie food viewing the hypothalamus and ventral striatum were most consistently found. Nevertheless, the overall concurrency across studies is low with at best 43% of the studies contributing to concurrent clusters and the majority of the reported activation foci not overlapping at all. An important factor that is known to modulate food related brain responses is hunger state. Hunger has been found to increase brain responses to food image viewing in healthy adults and is thought to be altered in obesity. The variability in this motivational state could therefore very well contribute to the low overlap across studies. In children and adolescents similar regions to those found in adults, seem to activate in response to food cues (van Meer et al., 2014). In addition, children have been found to be more responsive to food images when hungry (Killgore and Yurgelun-Todd, 2005). However, the low number of studies makes it hard to draw firm conclusions. Furthermore, to date, no food evaluation studies in elderly have been conducted.

In summary, there is a fair body of literature on food viewing, however, meta-analyses show that results are not very consistent. In particular, studies directly comparing brain responses to food cue exposure between age groups and hunger states are lacking.

1.5 Neural correlates of food decision making

Another paradigm of interest is actual (food) decision making. On a daily base we make many choices between different options of which the best option might depend on all kinds of secondary variables on a given day in a given choice context. In order to make optimal decisions, valuation of the available choice options is required. This includes consideration of short versus long-term consequences and goals, and incorporation of the goal relevance (Samanez-Larkin et al., 2013). Subsequent choice outcome evaluation and learning are essential for the optimization of future choices. This so-called adaptive decision making requires fine-tuning of executive functions and emotional regulation, functions that gradually develop and change with age. The executive functions underlying cognitive control such as attention, response inhibition, learning and memory, are regulated by complex brain networks. In general, choice values are thought to converge in the ventromedial prefrontal cortex (vmPFC). Different values relevant for the choice at hand are compared by a network including the dorsolateral prefrontal cortex (dlPFC), pre-supplementary motor area and bilateral inferior parietal sulcus, which influences action e.g. by modulation of activation in the motor cortex (Basten et al., 2010; Hare et al., 2011b).

Compared to the overall decision making literature, relatively few studies have examined food choice, all in adults (Hare et al., 2009; Hare et al., 2011a; van der Laan et al., 2014a; Van der Laan et al., 2012; Van Der Laan et al., 2014b)). As previously mentioned, food choices are of great importance, since they determine energy intake and therefore play a crucial role in weight management and overall healthiness across the lifespan. On an average day we make more than 200 food related decisions (Wansink and Sobal, 2007) the majority of which is made based on visual appearance. Through learned associations, this elicits expectations about taste and metabolic consequences. Food choice paradigms greatly vary from single and dual food choices (Grabenhorst et al., 2013; Hare et al., 2009; Hare et al., 2011a; Lim et al., 2011; van der Laan et al., 2014a; Van der Laan et al., 2012; Van Der Laan et al., 2014b), to willingness to pay for a particular food (Plassmann et al., 2007) and auction paradigms (Linder et al., 2010).

Compared to the food viewing fMRI literature, the literature on food choice is limited. Also, the choice tasks employed vary greatly. Overall valuation of choice options (e.g. the degree of tastiness and healthiness) involves the ventromedial prefrontal cortex (vmPFC), which can be modulated by the dorsolateral prefrontal cortex (dlPFC) a region involved in inhibitory control. Hunger state is expected to influence food choice related brain activation, however, to date studies comparing different hunger states are lacking. In children, only one food choice study has been conducted (Lim et al., 2016). It found that children predominantly

choose foods based on their tastiness and that this coincided with activation in the vmPFC, similar to adults. Again, literature on elderly is lacking.
In summary, there is limited literature on the neural correlates of food decision making. Especially studies examining effects of hunger state and differences in food decision-making across the lifespan are lacking.

1.6 Neural correlates of monetary reward

From previous studies we know that food reward processing is altered in (DelParigi et al., 2005; Ng et al., 2011; Stice et al., 2008a; Stice et al., 2008b; Stice et al., 2010; Stice et al., 2011). However, how this relates to alterations in reward processing in general is unknown. A task that has been extensively used to study reward processing in healthy volunteers and in a broad range of disorders, is the monetary incentive delay task (de Leeuw et al., 2015; Figee et al., 2011; Knutson et al., 2001a; van Hell et al., 2010; Vink et al., 2015). The anticipation of monetary reward has consistently been linked to increased activation in the ventral striatum (Knutson et al., 2001a), while reward receipt activates the orbitofrontal cortex/ventromedial prefrontal cortex (OFC/vmPFC) in healthy participants (Knutson et al., 2001a; Sescousse et al., 2013). In addition, studies comparing the valuation of food and money, report overlapping brain regions for the processing of different reward types, including the striatum (FitzGerald et al., 2009) and vmPFC (Chib et al., 2009; Kim et al., 2011). To date, only few monetary reward studies have been conducted in obesity. They report alterations in monetary reward anticipation and/or receipt (Balodis et al., 2013; Stice et al., 2011). However, due to the very limited number of studies available and differences in study designs comparison is problematic.

In summary, the literature on monetary reward processing in overweight and obesity is limited. Especially studies examining the effects of weight-status on monetary reward are missing.

1.7 Thesis aims

The studies conducted in this thesis were part of the Full4Health project. The aims of the Full4Health project were to assess the differences in the brain responses to food presentation and food choice and how these responses are modulated by hunger and gut signals in lean and obese subjects across the lifespan.

In this thesis we focus on the effect of hunger state on food-related brain responses across the lifespan. In addition, to be able to better examine alterations

in reward processing we aimed to establish the effects of hunger state and weight-status on monetary reward processing.

1.8 Thesis overview

In **Chapter 2** we present a standardized food image photographing protocol and image database tested and validated across countries which can be used in food cue research. In **Chapter 3** we utilize these images in a sophisticated fMRI food choice design in which food choices are matched on individual preference but differ in caloric content. A group of healthy normal-weight adults performed this choice task in de absence of hunger. In **Chapter 4** the standardized food images are used in a food viewing fMR task. With this task, the effect of hunger state on the brain responses to low and high calorie food cues across the lifespan was investigated. In **Chapter 5** we examine the effect of hunger state on the brain responses to food choice across the lifespan. In **Chapter 6** we investigate the effect of hunger state and weight status on monetary reward processing. We finish this thesis with a general discussion of the results in **Chapter 7**.

CHAPTER 2

STANDARDIZED FOOD IMAGES: A PHOTOGRAPHING PROTOCOL AND IMAGE DATABASE

Based on: Charbonnier, L., van Meer, F., van der Laan, L.N., Viergever, M.A., Smeets, P.A. (2016) Standardized food images: A photographing protocol and image database. Appetite 96, 166-173.

Abstract

The regulation of food intake has gained much research interest because of the current obesity epidemic. For research purposes, food images are a good and convenient alternative for real food because many dietary decisions are made based on the sight of foods. Food pictures are assumed to elicit anticipatory responses similar to real foods because of learned associations between visual food characteristics and post-ingestive consequences. In contemporary food science, a wide variety of images are used which introduces between-study variability and hampers comparison and meta-analysis of results. Therefore, we created an easy-to-use photographing protocol which enables researchers to generate high resolution food images appropriate for their study objective and population. In addition, we provide a high quality standardized picture set which was characterized in seven European countries.

With the use of this photographing protocol a large number of food images were created. Of these images, 80 were selected based on their recognizability in Scotland, Greece and The Netherlands. We collected image characteristics such as liking, perceived calories and/or perceived healthiness ratings from 449 adults and 191 children.

The majority of the foods were recognized and liked at all sites. The differences in liking ratings, perceived calories and perceived healthiness between sites were minimal. Furthermore, perceived caloric content and healthiness ratings correlated strongly ($r \geq 0.8$) with actual caloric content in both adults and children. The photographing protocol as well as the images and the data are freely available for research on http://nutritionalneuroscience.eu/. By providing the research community with standardized images and the tools to create their own, comparability between studies will be improved and a head-start is made for a world-wide standardized food image database.

2.1 Introduction

The regulation of food intake has gained much research interest because of the current obesity epidemic. Accordingly, a broad range of studies from several fields of research has investigated the determinants of (un)healthy eating behavior. This includes studies in psychiatry (Cowdrey et al., 2013), psychology (Junghans et al., 2013; Veling et al., 2013), food-related neuroscience studies (Kringelbach and Rolls, 2004; Simmons et al., 2005; Tiggemann and Kemps, 2005a; van der Laan et al., 2011; Van Der Laan et al., 2014b; van Meer et al., 2014) consumer research (van der Laan et al., 2015), nutritional sciences and sensory sciences. Many of these studies use food images as a substitute for real foods to investigate the determinants of eating behavior caused by visual food cue exposure. Visual food cues are omnipresent in our environment and many of our dietary decisions are based on the sight of food due to learned associations between visual food characteristics and their post-ingestive effects (Lappalainen, 1992; Wardle, 1990). Therefore, food images are a good and easy-to-use alternative to real food to study responses to visual food exposure.

A wide variety of food images has been used. For example, in neuroimaging studies alone, images vary from single food items to filled plates or even complete pots with food (Brooks et al., 2012; Frank et al., 2010; Führer et al., 2008; Malik et al., 2008; Mehta et al., 2012a; Siep et al., 2009; Simmons et al., 2005). Examples of food stimuli are shown in Figure 2.1. Some studies used images made by the researchers themselves, often poor in lighting; other studies used images from commercial stock photograph websites (Killgore et al., 2003; Killgore and Yurgelun-Todd, 2005; Rothemund et al., 2007; Siep et al., 2009), which are of high photographic quality but highly diverse in portion size and presentation. Commonly, studies only report a brief description of the images used, which complicates replication (Beaver et al., 2006; Cornier et al., 2007; Davids et al., 2009; Holsen et al., 2006; Holsen et al., 2005; LaBar et al., 2001; Miller et al., 2007; Santel et al., 2006; Schienle et al., 2009). In an attempt to stimulate replication studies, a few groups have started to share their image sets online (e.g. (Blechert et al., 2014)). These images are often collected from the internet, pasted on a plain background (white, grey or black) and adjusted in brightness and contrast. The downsides of this are the lack of shadows (which gives the food the appearance of hanging in the air), the different angles at which foods are depicted, different magnifications (e.g., a sandwich shown the same size as a tomato), the lack of a visual reference item such as a plate, and image deterioration owing to the adjustments in brightness and contrasts, which can affect the attractiveness of the food items (e.g. (Knebel et al., 2008)). Until now,

only a few image sets have been used in multiple studies, usually limited to use in the same research lab (e.g. (Holsen et al., 2006; Holsen et al., 2005; LaBar et al., 2001) and (Killgore et al., 2003; Killgore and Yurgelun-Todd, 2005; Rothemund et al., 2007)). In conclusion, the images used in food research are highly variable and only seldom re-used. This is unfortunate, because it introduces within- and between-study variability and hampers comparison and meta-analysis of results (van der Laan et al., 2011). Thus, there is a clear need for a high quality food image set, standardized in luminance and presentation, which researchers can use for multiple research purposes.

For across-nation comparison of results, an important point to consider is that each country has its own food specialties and availability of fruits and vegetables. This complicates the (re)use of one set of images in experiments conducted in different countries. Because results are often compared between countries and the number of international multicenter studies is increasing, there is a need for a standardized food image set suitable for use across nations. Furthermore, with the increasing interest in age differences in eating behavior, it is important that images are suitable for research in children as well as adults.

The most important characteristic of food images is the recognizability of the portrayed food. Additional image characteristics that can be of interest are for example liking (e.g. to ensure equal attractiveness across food categories), the perceived and actual caloric content, and perceived healthiness (e.g. especially important when a high versus low calorie paradigm is used). Besides these characteristics, the ability of an image to exert a similar response as real food (e.g. do liking ratings of the images increase with hunger) is also of importance.

To address the need for high quality standardized food images we here present our easy to use standardized photographing protocol which enables researchers with a broad range of research objectives to generate additional study- or country-specific high resolution food images. In addition, we used the protocol to create images for a standardized food viewing fMRI task that is being used in several European countries. For these images, data was collected from adults and children to facilitate image selection on both visual appearance and food characteristics (e.g. caloric content, recognizability ratings, liking ratings etc.). We thus make important first steps in image standardization and validation across countries.

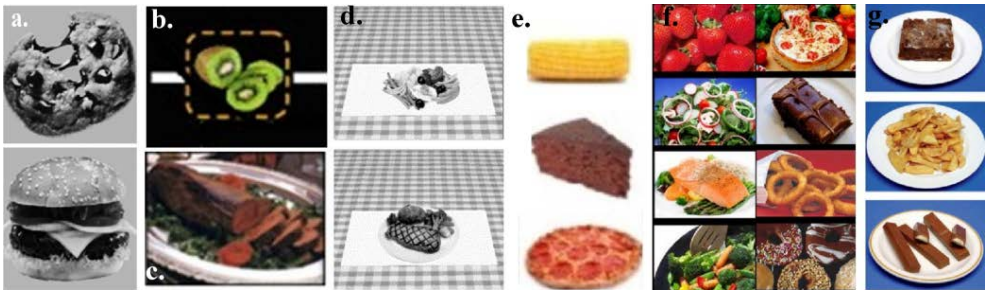


Figure 2.1 Food stimuli used in several neuroimaging studies
a: (Simmons et al., 2005); b: (Siep et al., 2009); c: (Malik et al., 2008); d: (Führer et al., 2008); e: (Frank et al., 2010b); f: (Mehta et al., 2012); g:(Brooks et al., 2012)

2.2 Method

2.2.1 Standardized photographing protocol

The images were created with the use of a high resolution digital single lens reflex camera which was mounted on a tripod. The focal length used was 32.0 mm. In order to depict each food as attractive as possible, shutter speed and aperture were automatically adjusted for each food. The pictures were taken in a 60 x 60 x 60 cm photo studio (i.e., a cubic photo tent made from snow white fabric which softens and reflects light). The tent was completely closed. An incision was made in the front to accommodate the camera objective. The tent could be opened from the top to change the plates. In addition, two daylight lamps (E27/55W) were used to create optimal lighting conditions. The lens angle was approximately 45◦, the distance from center plate to center tripod was 39.5 cm and the height of the center of the camera on the tripod was 38 cm to resemble the viewing of a plate of food on a table during a meal time. Figure 2.2 shows the protocol set-up on scale (when 2.2a is printed on A0 it can be used as a blue print), which is freely available online at http://nutritionalneuroscience.eu. Each food was presented on a white plate (FÄRGRIK, Ikea) with a diameter of 17.0 cm. Plates were covered with food, to ensure a similar visual appearance. In addition, a light grey background was chosen to ensure sufficient contrast between plate and background (a white background makes the plate disappear, while a black or dark blue background results in a very strong unnatural contrast between plate and background). Owing to the optimization of the food appearance on the plates, differences in background brightness occurred. To standardize the background, MeVisLab (MeVis Medical Solutions AG, Bremen, Germany) and the open-source registration software Elastix (http://elastix.isi.uu.nl/) were used (Klein et al.,

2010). Each plate was segmented, registered on a standardized background which was taken from one image, and smoothened on the plate edges.

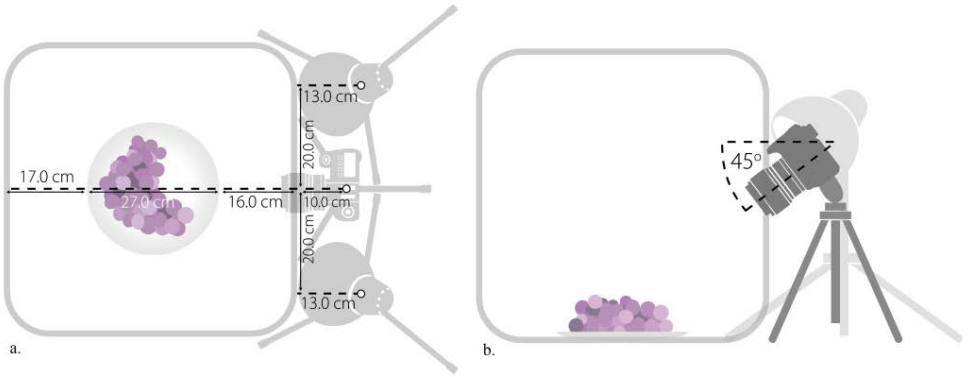


Figure 2.2 Standardized photographing protocol set-up
a. side view; b. top view

2.2.2 Image subset selection

The image subset selection was performed to develop a standardized food viewing fMRI task that could be used in Scotland, The Netherlands and Greece as part of the Full4Health project (www.full4health.eu)**.** For this purpose, 286 images (including different preparations and country specific foods) were created with the photographing protocol. The vast majority of the images were taken in The Netherlands (186). Only the images that were found to be recognizable by nutritional scientists of each country were subsequently tested in the general population: 223 images were rated in The Netherlands, 202 in the UK (i.e., Scotland and England) and 214 in Greece. For these images data was collected from adults on recognizability, liking, healthiness and perceived number of calories. From these images a subset of 80 was selected based on recognizability ratings of Scotland, The Netherlands and Greece. Each image in this subset had to be recognized at least 85% of the time at all three sites (i.e. 85% “yes” answers to the question: ”Do you recognize this product”). The subset includes images of 40 high calorie foods and 40 low calorie foods and comprises both sweet and savory foods (i.e., 16 high calorie savory, 24 high calorie sweet, 21 low calorie savory, and 19 low calorie sweet). Figure 2.3 shows examples of the images including available non-foods which are not discussed in this paper as ratings are not yet available. The complete list of images can be found on http://nutritionalneuroscience.eu/. Later on, additional data was collected from Dutch children. Furthermore, as part of another European project (I.Family, www.ifamilystudy.eu) additional images

were created for Germany, Sweden and Hungary. For this project, data was collected in children from these countries. Because different criteria applied for the image selection, for these countries data was available from 57 images of the previously described subset.

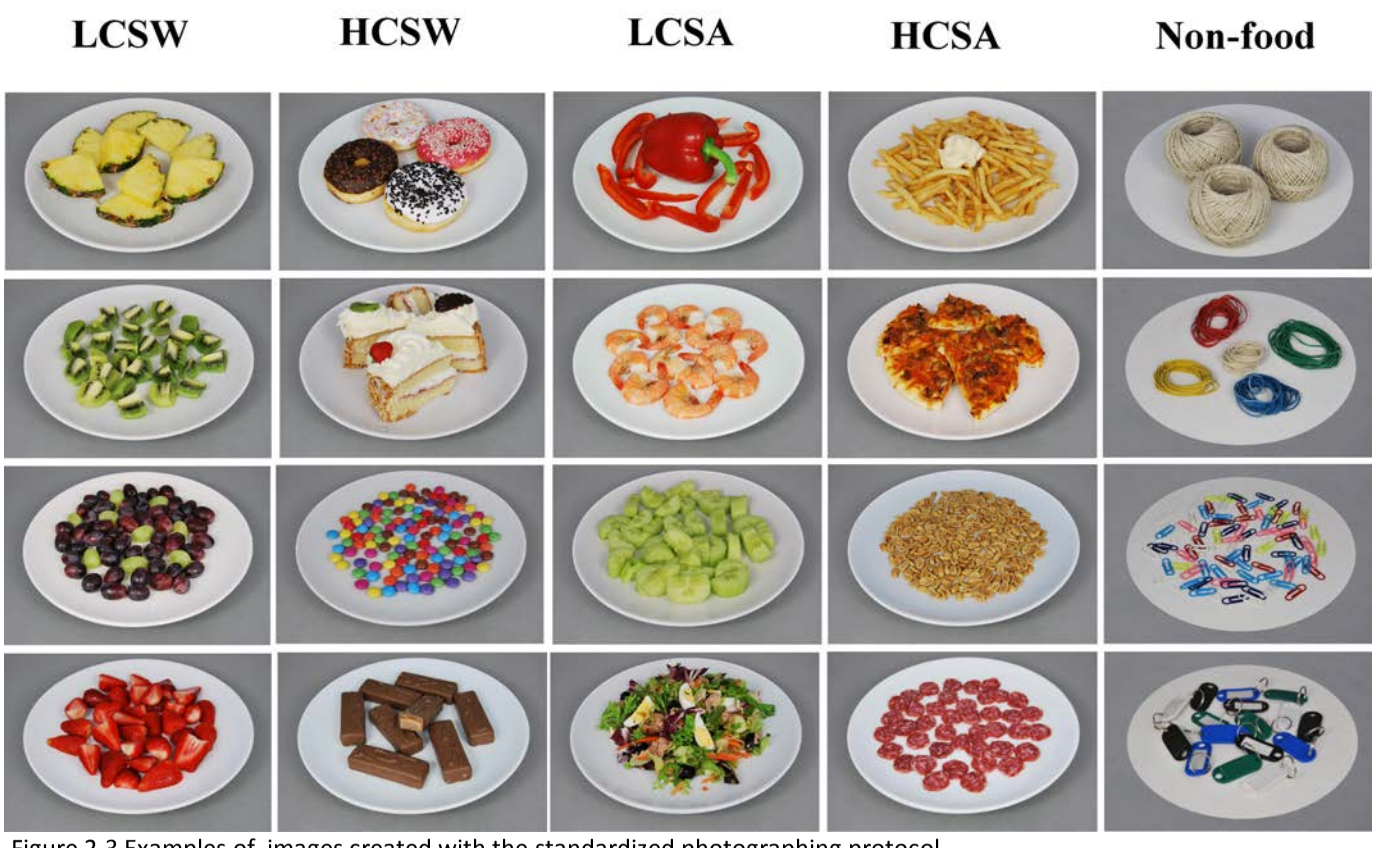


Figure 2.3 Examples of images created with the standardized photographing protocol
LCSW: Low Calorie Sweet; HCSW: High Calorie Sweet; LCSA: Low Calorie Savory; HCSA: High Calorie Savory.

2.2.3 Data collection

In adults, data was collected with an online web application in Scotland, England, The Netherlands and Greece. An invitation to participate was sent out to university employees and students, colleagues, friends etc. Participants were asked to provide their personal characteristics such as gender, age, weight, height and educational level (see Table 2.1 for the complete list). Subsequently, they were asked to rate a subset of approximately 40-60 images (10-15 high calorie savory, 10-15 high calorie sweet, 10-15 low calorie savory and 10-15 low calorie sweet) randomly selected from a larger set of images. Only ratings of the 80 previously selected images were included in this study. The mean number of ratings per picture (only ratings from recognized images included) was 72 (SD = 13) for the high calorie category an 77 (SD = 10) for the low calorie food category. The first question they were asked was: "Do you recognize the product?". If the answer was no, the image was skipped and the next image was shown. If the answer was yes, the question was followed by: "How much do you like the product (1, not at all – 9, very much)?", "How many calories do you think the product consists of (1, very few calories – 9, many calories)?" and "How healthy do you think the product is (1, not healthy at all – 9, very healthy)?". The answers were provided on a 9-point Likert scale with numbers. They were instructed to answer the question as quickly as possible, and were told that there were no correct or incorrect answers and that we were interested in their opinion. This task was part of a longer procedure.

In children, data were acquired with a computer-based questionnaire. In The Netherlands the data was collected at after school daycares and at scouting clubs, while in Germany, Sweden and Hungary, data was collected at schools. We started data collection in Dutch children and subsequently expanded data collection to other sites. Children were asked to provide us with personal characteristics such as age, gender, height and weight (see Table 2.2 for the complete list). After that they rated a subset of 60-80 images (15-20 high calorie savory, 15-20 high calorie sweet, 15-20 low calorie savory and 15-20 low calorie sweet) randomly selected from a larger set of images. Only ratings of the subset of 80 previously selected were included in the present study. The mean number of ratings per picture was 44 (SD = 31) for the high calorie images and 59 (SD = 29) for the low calorie images (again only ratings from recognized images included). Participants were first asked: "Do you recognize the product?". If the answer was no, the image was skipped and the next image was shown. If the answer was yes, the question was followed by: "How much do you like the product (1, not at all – 5, very much)?", and "How healthy do you think the product is (1, not healthy at all – 5, very healthy)?". The answers were provided on and 5-point Likert scale

with numbers and smiley faces, instead of the 9-point Likert scale used for the adults. The scale was simplified for children as the original 9-point scale was too difficult for young children. Except for the first question, the task instructions were identical to the adults. The first question was “Have you ever eaten this product?” instead of “Do you recognize this product?” because we noticed, during the test in the Dutch children, that some children recognized certain foods but subsequently did not know what it tasted like because they had never eaten it before.

2.2.4 Participants

In Table 2.1 the characteristics of the adult participants are shown. For this study 449 adults (70% females) from Scotland, England, The Netherlands and Greece participated. A Mann-Whitney U test showed that the average BMI of the Scottish participants was significantly higher than that of the other sites. There were no significant differences in BMI between the other sites. The mean age differed significantly between all sites, except for Greece and the Netherlands. The majority of the total adult sample had a normal weight (68.4%).
191 children (55% girls) from The Netherlands, Germany, Hungary and Sweden participated. The demographics of the children are shown in Table 2.2. Mean age was significantly different for all sites, except Hungary and Sweden. In line with the adults, the majority of the children had a normal weight, however, 34% of the children had an unknown BMI because especially the younger children did not know their weight.

Table 2.1 Subject characteristics adults.

	Sites				
	SCT N=121	**ENG** N=90	**NL** N=136	**GR** N=102	**Total sample** N=449
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Gender (%)	67.8F	83.3F [2]	70.2F [3]	64.7F	70.2F [5]
Age (y)	40.6 ± 10.8 [a]	20.0 ± 1.9 [b]	36.5 ± 16.0 [c]	33.9 ± 6 [c]	33.7 ± 13.1
BMI (kg/m^2)	25.7 ± 4.8 [a,1]	22.8 ± 3.7 [b]	23.4 ± 4.6 [b]	23.8 ± 5 [b]	24.0 ± 4.7 [6]
Normal-weight (%)[*]	54.5	74.4	80.1	63.7	68.4
Overweight (%)[**]	42.1	20.0	16.2	28.4	26.7
Underweight (%)[***]	2.5	5.6	3.7	7.8	4.7
Education [****]	5.2 ± 0.7 [a]	4.2 ± 0.4 [b]	5.0 ± 1.0 [a]	5.3 ± 0.7 [a]	5.0 ± 0.9
Hunger[8]	3.4 ± 2.2 [a,b]	3.8 ± 2.2 [a]	3.3 ± 2.2 [a,b]	2.8 ± 2.2 [b]	3.3 ± 2.3
Fullness[8]	5.1 ± 2.2 [a]	5.1 ± 2.2 [a]	4.7 ± 2.2 [a]	6.3 ± 2.5 [b]	5.3 ± 2.3
Thirstiness[8]	4.8 ± 2.2 [a]	5.8 ± 1.9 [b]	4.9 ± 2.1 [a]	4.6 ± 2.2 [a]	5.0 ± 2.2
Food allergies (%)	9.9	7.8	8.1	5.9	8.0
Diet (%)	11.6	11.1	2.9	12.7	9.1
Overweight in family (%)	23.1	10.0	27.2	29.4	23.3
Diabetes (%)	2.5	0	2.5 [4]	0	1.4 [7]
Eating disorder (%)	0	3.3	1.6 [4]	1.0	1.4 [7]
Smoking (%)	7.4	20.0	8.1	42.2	18.0
Time since last food consumption (%)					
<1 h ago	41.1	40	51.1	45.1	44.5
1h – 2 h	27.6	28.8	25.9	20.6	26
≥ 2h ago	30.9	31.2	23.1	34.3	29.4

SCT = Scotland; ENG = England; NL = The Netherlands; GR: Greece; [*] = BMI 18.5-25 kg/m^2; [**] = BMI > 25 kg/m^2; [***] BMI < 18.5 kg/m^2; [****] Education level 5 equals higher education ; SCT site: [1] = 0.8% unknown; ENG site: [2] = 26.7% unknown; NL site: [3] = 3.7% unknown; [4] = 10.3% unknown; Total: [5] = 6.5% unknown; [6] = 0.2% unknown [7] = 3.1% unknown; Unequal letters, differences between the sites were significant at $p \leq 0.001$; [8] 9-point Likert scale.

Table 2.2 Subject characteristics children.

	Sites				Total sample N=191
	NL N=30	**DE** N=50	**HU** N=57	**SE** N=54	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Gender (%)	70 F [1]	40 F	61.4 F	52 F	55 F [6]
Age (y)	8.7 ± 1.4 [a, 1]	13.8 ± 1.4 [b]	13.1 ± 0.9 [c]	12.7 ± 1.7 [c]	12.5 ± 2.2 [6]
Normal-weight (%)*	100 [2]	100 [3]	72.1 [4]	84.1 [5]	85.7 [7]
Overweight (%)**	-	-	23.3	9.1	11.9
Underweight (%)***	-	-	4.7	6.8	4.8
Hunger [8]	2.5 ± 1.0 [a]	1.9 ± 1.0 [a]	2.3 ± 1.4 [a]	2.4 ± 0.9 [a]	2.2 ± 1.1 [6]
Fullness[8]	2.5 ± 1.2 [a,b]	2.3 ± 1.1 [b]	3.0 ± 1.4 [a]	3.1 ± 1.1 [a]	2.8 ± 1.2 [6]
Thirstiness[8]	3 ± 1.4 [a]	2.6 ± 1.2 [a]	3.1 ± 1.1 [a]	2.9 ± 1.2 [a]	2.9 ± 1.2 [6]
Last food consumption (%)					
<1 h ago	60 [1]	48	63.2	27.8	48.7 [7]
1h – 2 h	26.7	20	15.8	42.6	26.2
≥ 2h ago	13.3	32	21.1	29.6	25.2

NL = The Netherlands; DE = Germany; HU = Hungary; SE = Sweden; Weight categorization based on growth reference data for 5-19 year olds of the World Health Organization, * = BMI between +1SD and -2SD; ** = BMI >+1SD; *** = <-2SD; NL site: [1] = 6% unknown; [2] = 59% unknown; DE site: [3] = 44% unknown; HU site: [4] = 24.6: SE site: [5] = 18.5% unknown; Total sample: [6] = 1% unknown ; [7] = 34 % unkown; Unequal letters = differences between the sites were significant at $p < 0.008$; [8] 5-point Likert scale.

2.2.5 Data analyses

Data were analyzed with SPSS statistics 19. The demographics (i.e., age, BMI, education), self-report ratings (i.e., hunger, fullness, thirstiness, liking, perceived caloric content and perceived healthiness), and actual caloric content per 100 gram and per plate were not normally distributed. Therefore, differences between countries in age, BMI, education, hunger, fullness and thirstiness, and differences between image categories in liking, perceived caloric content, actual caloric content (per 100 gram and per plate) and perceived healthiness, were

examined with the non-parametric Independent Samples Mann-Whitney U Test. Alpha was subsequently Bonferroni-corrected for multiple testing. To ensure equal contribution of each image to the calculated means of the liking, perceived calorie and perceived healthiness ratings (due to unequal number of ratings per image), first mean ratings per image were calculates and subsequently mean liking, perceived calories and healthiness were calculated per country and food category. In addition, Cohen's d was calculated using an online application (http://www.uccs.edu/~lbecker/). It calculates Cohen's d by using the means and the standard deviations of the two categories (i.e. high and low caloric images). Furthermore, Spearman rank correlations were calculated between the mean high calorie food liking per subject and hunger, perceived and actual caloric content and perceived healthiness and actual caloric content.

2.3 Results

2.3.1 Standardized food images

Figure 2.4 shows examples of the images created on the different sites. Although different types of food were photographed, this figure clearly shows the similarity of the images produced at different sites that can be achieved.

2.3.2 Food image recognizability

As previously mentioned, the 80 images presented in this study were selected on recognizability of adults in Scotland, The Netherlands and Greece (see section 2.2). The mean ± SD percentages of recognized ratings per image for these sites were: SCT = 98.9 ± 2.4; NL = 98.3 ± 2.7; GR = 98.1 ± 5.5.
The mean percentages of recognized ratings per image for the other sites were for adults (mean ± SD): ENG = 96.1 ± 6.5, and for children (mean ± SD): NL = 86 ± 16%; DE = 87 ± 13%; HU = 85 ± 12% and SE = 77 ± 17%.
In total, the mean percentage of recognized images was 98.0 % (SD = 2.3) in adults and 81% (SD = 14) in children.

2.3.3 Food characteristics:liking, perceived caloric content & perceived healthiness

High versus low calorie food images

In Figure 2.5 the mean liking, perceived caloric content and perceived healthiness ratings of the food pictures are shown per category (high versus low calorie). In adults, the mean perceived caloric content was significanly higher for the high calorie food images ($p<0.001$ and Cohen's $d = 2.52$). The mean healthiness ratings in both children and adults were significantly lower for the high calorie food images ($p<0.001$ and Cohen's d children = -1.82 and adults d=-2.67). In both children and adults liking ratings did not differ significantly between the high and low calorie categories (children $p=0.028$, Cohen's $d= 0.12$ and adults $p= 0.118$ and Cohen's $d= -0.11$). The differences between sites were minimal.

Correlation between perceived and actual caloric content

In Figure 2.6 the correlations between perceived caloric content, perceived healthiness and actual caloric content are shown. Perceived caloric content and actual caloric content were strongly positively correlated in adults (Spearman's rho: 0.801; $p < 0.001$) while perceived healthiness and actual caloric content were negatively correlated in both adults and children (Spearman's rho adults: -0.798; $p < 0.001$; children: -0.806; $p = < 0.001$). Note that perceived caloric content was not measured in children.

Correlation between liking and hunger ratings

Hunger ratings were positively correlated with mean perceived liking for the high calorie foods: Hunger ratings (9-point Likert scale): mean ± SD = 3.3 ± 2.2; HC perceived liking ratings (9-point Likert scale): mean ± SD = 6.4 ± 1.4; Spearman's rho = 0.153, $p = 0.001$. The low Spearman's rho shows that there was a small but significant correlation between liking and hunger. This correlation difference was not found in children.

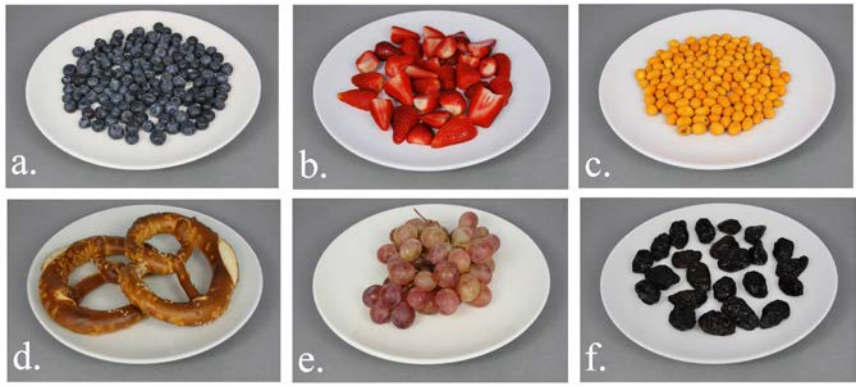


Figure 2.4

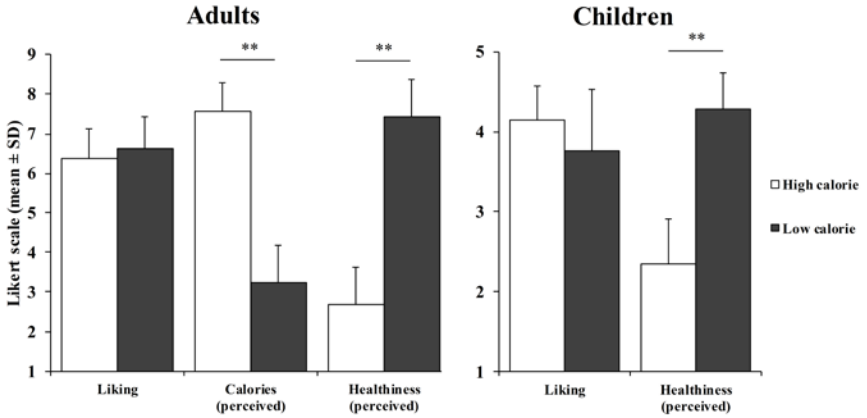


Figure 2.5

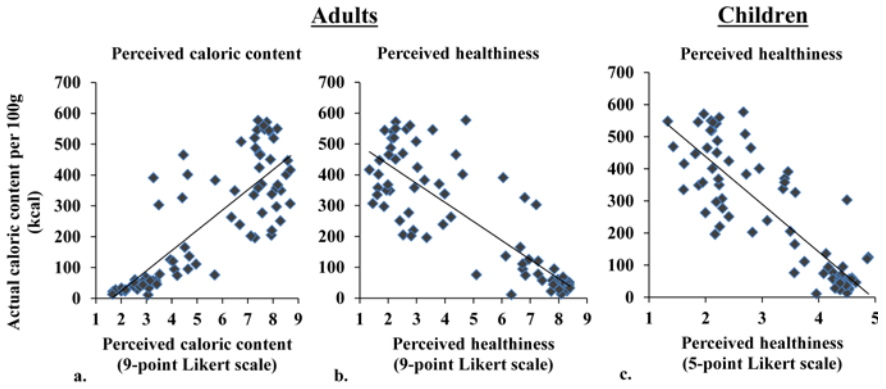


Figure 2.6

2.4 Discussion

We made a photographing protocol suitable for creating high-quality standardized food images. Using this protocol we created a food image set and tested a subset of 80 images in several European countries in both adults and children. Currently, the total set comprises 370 images (including country specific images and different preparations) of 260 different food items and the number of images is still growing.

A large proportion of the images was recognized by the sites not included in the image selection procedure. Furthermore, differences between linking, perceived calories and healthiness ratings across sites were minimal. The images were scored high in liking and there were high associations between perceived caloric content, perceived healthiness and actual caloric content. Furthermore, we found that liking ratings were influenced by hunger.

The vast majority of the images was recognized by both adults and children in all countries involved. Although this was not surprising for the sites that were included in the image selection procedure based on recognizability (i.e ratings from adults from: The Netherlands, Scotland and Greece), the mean percentage of recognized ratings per image from the five remaining sites (i.e ratings from adults from England, and children from The Netherlands, Germany, Sweden and Hungary) was also high. The mean percentage of recognized ratings per image was somewhat lower in children than in adults, but still high (81% in children versus 98% in adults). Moreover, there was more variation in the number of foods recognized by children (SD in children 14% versus 2.4% in adults). Overall, all images from the high and low calorie categories were highly liked, which makes them useful to study food reward since it would allow one to match low and high calorie foods on liking, see e.g. Wardle (1990).

Furthermore, liking ratings of adults were slightly influenced by hunger ratings. For real foods it has been shown that they are more rewarding in a hungry state (Rozin and Vollmecke, 1986) and it has been found in neuroimaging studies that hunger increases the brain response to viewing food images in reward areas e.g. van der Laan et al. (2011). It should be noted that we did not instruct participants to refrain from eating for a certain period prior to rating the images. Therefore, we expect this finding to be even more pronounced in studies in which measurements are collected in more controlled hunger or satiety states.

The perceived caloric content ratings were significantly higher for high compared to low calorie images. Thus, on average perceived caloric content matches well with actual caloric content. Furthermore, high caloric images were perceived as significantly less healthy than low caloric images by both adults and children. This

makes the images suitable for investigating effects of (perceived) caloric content. Although the recognizability of the image set was high, the differences between sites were low and the perceived caloric content was congruent with the actual caloric content, we advise to pretest all images under study before use. Especially because there may be differences in how foods are perceived e.g. depending on SES (Hoogendam et al., 2013), gender (O'Doherty et al., 2002; Pelchat et al., 2004) or other factors like health-mindedness. This is very hard to control for as this would require a substantial amount of data on all the images.
In conclusion, we presented a standardized photographing protocol for making high-quality food images. Data collected on a subset of 80 images show that the images are suitable for use in food research across the investigated countries both in adults and children. However, because there are factor that may influence one's perception of food, pretesting the images for each study population is necessary. With this first set of images we provide a head-start for a world-wide standardized food image database that will ultimately foster international collaboration and increase comparability of research findings. The total image set is freely available on http://nutritionalneuroscience.eu/.

CHAPTER 3

FUNCTIONAL MRI OF CHALLENGING FOOD CHOICES: FORCED CHOICE BETWEEN EQUALLY LIKED HIGH- AND LOW- CALORIE FOODS IN THE ABSENCE OF HUNGER

Based on: Charbonnier, L., van der Laan, L.N., Viergever, M.A., Smeets, P.A. (2015) Functional MRI of Challenging Food Choices: Forced Choice between Equally Liked High-and Low-Calorie Foods in the Absence of Hunger. PLoS ONE 10, e0131727.

Abstract

We are continuously exposed to food and during the day we make many food choices. These choices play an important role in the regulation of food intake and thereby in weight management. Therefore, it is important to obtain more insight into the mechanisms that underlie these choices. While several food choice functional MRI (fMRI) studies have been conducted, the effect of energy content on neural responses during food choice has, to our knowledge, not been investigated before. Our objective was to examine brain responses during food choices between equally liked high- and low-calorie foods in the absence of hunger. During a 10-min fMRI scan 19 normal weight volunteers performed a forced-choice task. Food pairs were matched on individual liking but differed in perceived and actual caloric content (high-low). Food choice compared with non-food choice elicited stronger unilateral activation in the left insula, superior temporal sulcus, posterior cingulate gyrus and (pre) cuneus. This suggests that the food stimuli were more salient despite subject's low motivation to eat. The right superior temporal sulcus (STS) was the only region that exhibited greater activation for high versus low calorie food choices between foods matched on liking. Together with previous studies, this suggests that STS activation during food evaluation and choice may reflect the food's biological relevance independent of food preference. This novel finding warrants further research into the effects of hunger state and weight status on STS, which may provide a marker of biological relevance.

3.1 Introduction

We are continuously exposed to food and during the day we make many choices regarding food consumption. As the prevalence of overweight and obesity continues to rise (McLellan, 2002), research on food choice is becoming of increased interest because food choices play an important role in determining energy intake. Normal human physiology is innately geared towards obtaining food, which is a powerful reinforcer (Epstein and Leddy, 2006). Easy availability of tasty foods has caused a shift from eating for survival to eating for the pleasure obtained from food reward (hedonic eating) (Mela, 2006; Peters et al., 2002; Saper et al., 2002). Consequently, metabolic need no longer governs human eating behavior (Berthoud, 2006; Mela, 2006).

In the past two decades, functional magnetic resonance imaging (fMRI) has become an established method for investigating food-related brain responses (Smeets et al., 2012). Especially brain responses to the presentation of food pictures have been widely investigated with fMRI (Coletta et al., 2009; Frank et al., 2010a; Killgore et al., 2003; LaBar et al., 2001; Siep et al., 2009; Simmons et al., 2005; Smeets et al., 2013; Stoeckel et al., 2008; Toepel et al., 2009, 2010). Several of these studies have investigated neural responses to pictures of high and low calorie foods (Coletta et al., 2009; Frank et al., 2010a; Killgore et al., 2003; Rothemund et al., 2007; Siep et al., 2009; Stoeckel et al., 2008; van der Laan et al., 2011). They have shown that high calorie foods are more rewarding than low calorie foods. However, these studies were limited by studying high versus low calorie food viewing contrast in the absence of a choice context. Furthermore, the food stimuli were not matched on liking, which might explain the difference in reward. In addition, participants were usually in a hungry condition, which increases food reward (van der Laan et al., 2011).

The neuroimaging literature on decision making including the investigation of food choices is growing (Born et al., 2011; Frank et al., 2010a; Grabenhorst and Rolls, 2011; Grabenhorst et al., 2013; Hare et al., 2011a; Levy and Glimcher, 2011; Linder et al., 2010; Piech et al., 2010; Plassmann et al., 2010; Uher et al., 2006; Van der Laan et al., 2012). In these studies various manipulations were used to examine different aspects of food choice-related processing in the brain including the effects of taste (Levy and Glimcher, 2011) and willingness to pay for different foods types (Plassmann et al., 2010). However, studies investigating food choice between foods differing in caloric content have, to our knowledge, not been described in the literature. The absence of literature might be explained by the complexity of the topic because of the many factors that may influence the choice between foods differing in caloric content. These factors include the food's

palatability, personality traits and motivational state (Finlayson et al., 2008; Finlayson et al., 2007; Griffioen-Roose et al., 2010). Hunger increases the rated pleasantness of foods and brain regions involved in reward processing are stronger activated when people are viewing pictures of foods in a hungry state (van der Laan et al., 2011). In line with these findings it is often assumed that there would be minor differences in rewarding properties between high and low calorie food in a sated condition. Yet, studies investigating this are lacking. This is important because it has been shown that many people eat in the absence of hunger. In an environment where food is scarce this is an adaptive characteristic because energy can be stored for later in adipose tissue. However, in our Western society this eventually contributes to overweight (Fisher and Birch, 2002; Shomaker et al., 2010). To our knowledge, it is unknown which neural mechanisms subserve this phenomenon.

Therefore, the aim of the present study is to investigate the neural mechanisms underlying the choice between equally liked high calorie and low calorie foods in the absence of hunger. We predict minimal differences between brain responses during high versus low calorie food choices, as the subjects are sated and the choices are matched on liking. Because the majority of the food evaluation studies examine the food versus non-food contrasts, we additionally aim to investigate the neural mechanisms underlying food choice versus non-food choice in the absence of hunger. We hypothesize increased activation during food choice in brain regions predominately involved in attention as foods are thought to be more salient than office utensils (i.e. the non-foods used in this study) (Castellanos et al., 2009; Nijs et al., 2010) (Smeets et al., 2013; van der Laan et al., 2011).

3.2 Materials and methods

3.2.1 Participants

Participants were recruited by distributing flyers and posters in the University Medical Center Utrecht and at the university campus. Forty-two participants enrolled in the study. We included healthy participants with a normal weight (i.e., BMI 18-25 kg/m^2), between 20-40 years old, right-handed, non-smoking, with a stable weight (did not gain or lose > 5 kg in the past 6 months), no use of medication (except aspirin/paracetamol and oral contraceptives) and no current alcohol consumption of > 28 units per week. We excluded participants who scored above average on restraint eating (restraint eating subscale score of the DEBQ could not exceed 2.89 for males and 3.39 for females)(Van Strien et al., 1986),

since this characteristic is known to influence food relationships (Fedoroff et al., 2003). Furthermore, common fMRI exclusion criteria (e.g. claustrophobia, pregnancy and metal implants in the body) and criteria that might influence response to food cues (e.g. food allergies, special diets, eating disorders, gastrointestinal disorders or metabolic or endocrine disease) were used. In addition, runs with any single movement greater than 4 mm translation or 4 degrees rotation were excluded. From the original sample (N=42), subjects meeting one of the following criteria were excluded for the current analysis: nausea (self-report >5 on a 9-point Likert scale) after test meal consumption (N=8), too much hunger (self-report >5 on a 9-point Likert scale) after test meal consumption (N=1) or prior to the scan (N=4) and <10 high and low calorie choices during the forced choice fMRI task (N=10). No subjects had to be excluded for excessive movement. The 19 remaining participants (9 males, 10 females; age (Mean, SD) = 25.4 ± 5.1; BMI (Mean, SD) = 22 ± 1.6; DEBQ dietary restraint (Mean ± SD): males = 1.82 ±0.66; females = 2.41 ± 0.49) were examined in this study.

3.2.2 Experimental design

3.2.2.1 Study procedures

The study consisted of one MRI scan session conducted in the morning. Subjects were scanned after an overnight fast (≥ 10h) after consumption of an ad libitum test meal (a commercially available drink called Nutridrink from Nutricia). They provided hunger and fullness ratings before and after test meal consumption. These served to ensure that their hunger decreased after test meal consumption. The amount of protein shake consumed ranged from 117-631 ml (Mean, SD = 449.7 ± 170.9 ml). Hunger ratings decreased and fullness ratings increased significantly after protein shake consumption (9-point Likert scale measurements: pre-meal hunger (Mean, SD) = 6.1±1.8; after meal hunger (Mean, SD) = 2.1 ± 1.1; and pre-meal fullness (Mean, SD) = 2.5±1.2; after meal fullness (Mean, SD) = 7.2 ± 1.5).

Before the scan, participants conducted a computerized food picture rating task (based on (Finlayson et al., 2007)). Subsequently, the participants underwent a 30-min MRI scan session. The first functional run consisted of a food and non-food viewing task, the second consisted of a forced choice task. In this paper we report the results of the forced choice task (see Fig. 3.1).

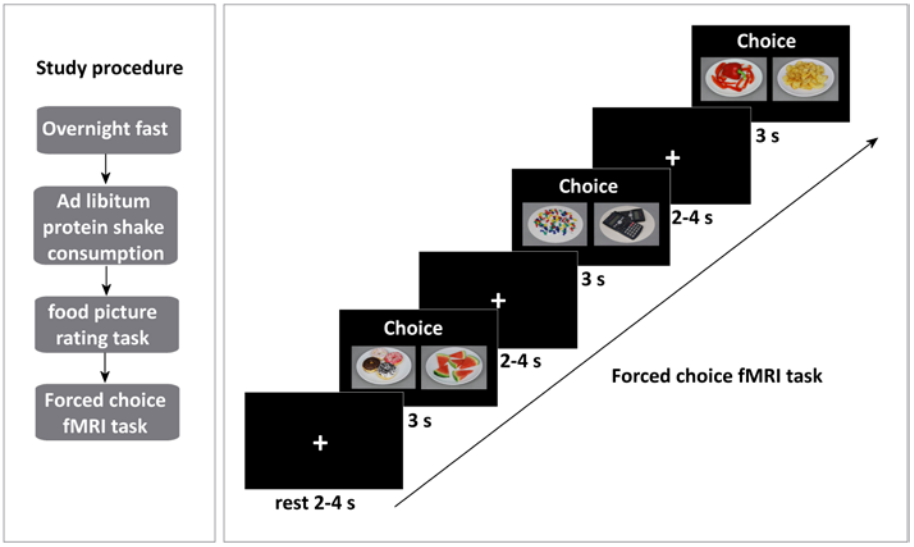


Figure 3.1

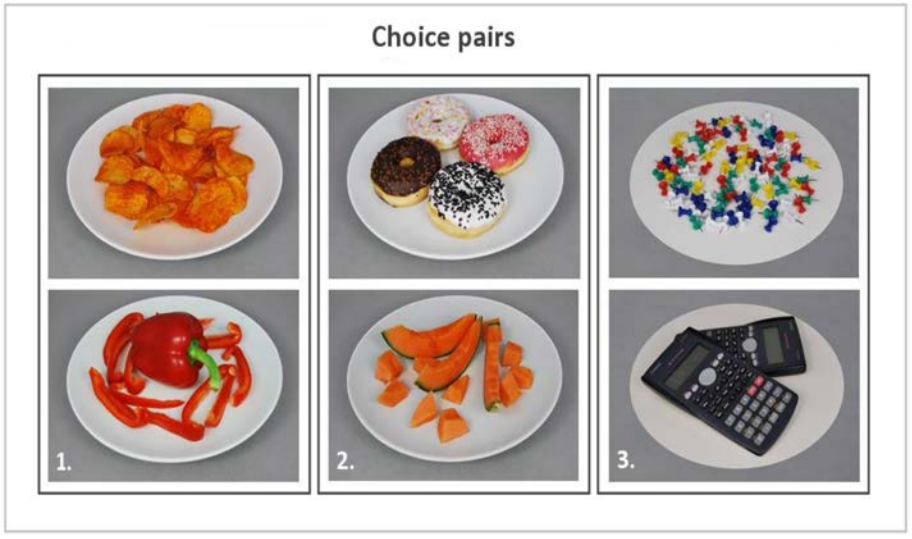


Figure 3.2

3.2.2.2 Stimuli

The stimuli used in this study were 96 food and 51 non-food images custom-made for this study. The food image set contained mostly snacks, ready for consumption, without package and brand information. The foods can be divided into two categories: high calorie and low calorie images (including both sweet and savoury items). Each food was presented on a plate, filled with the food. The plates were shown on a standardized background. To ensure the background was identical for every picture, each plate was registered to a standardized background with the use of MeVisLab (MeVis Medical Solutions AG, Bremen, Germany) and Elastix (Klein et al., 2010). The non-foods were office utensils, depicted in a similar way as the food items, on a white round piece of paper instead of a plate.

3.2.2.3 Food picture rating task

Shortly before the MRI scan the participants executed a food picture rating task which was based on the Leeds Food Preference Questionnaire (LFPQ) (Finlayson et al., 2007). During this task participants rated 96 food pictures on liking, caloric content and healthiness on a 9-point Likert scale. The food pictures were divided in high calorie and low calorie categories (including both sweet and savoury items). All images are freely available on request, see S3 PDF for an overview of all images used in this study. Each food picture was shown for 3 seconds (which was similar to the time the participants had to choose during the forced choice fMRI task). After that the following questions were asked: 'How much do you like the product?' (1 not at all – 9 very much), 'How many calories do you think this product consists of?' (1 very few calories– 9 many calories) and 'How healthy do you think this product is?' (1 not healthy at all- 9 very healthy). The participants received the following instruction: 'Try to answer the questions as quickly as possible. There are no correct or incorrect answers, it's about your opinion. Don't think too long about an answer, the first answer that occurs to you is usually the best one'.

3.2.2.4 Forced choice fMRI task

Based on the ratings collected during the food picture rating task, food pairs were created for each subject. Food pairs were matched on liking (i.e. equal ratings or plus/minus 1 on a 9-point scale) and taste (i.e. sweet or savoury), to make the pairs as equal as possible, but differed in caloric content (i.e. a minimum of 2 points difference on a 9-point scale) (see Fig. 3.2). Each pair was unique although a picture could appear in several food pairs (repetition (means, SD) = 1.17 ± 0.08; range = 1-2). To check whether our manipulations were successful, mean actual

caloric content (kcal), perceived caloric content (9-point Likert scale), healthiness (9-point Likert scale) and mean liking (9-point Likert scale) were calculated. As expected, all variables except liking, differed significantly between the choice options within a food choice pair (Table 3.1). Hence, the study manipulations were effective. The participants were verbally giving the following instructions: "choose the product of which you most want to eat at this moment", whenever a food pair appeared, and "choose one of the products", when a non-food pair appeared (without giving any direction or further instructions). In addition to the verbal instruction, each question was shown above every choice pair. Subjects had 3 seconds to indicate their choice. Whenever a subject failed to make a choice within the restricted time, the event was labelled as a missed choice. The choice pairs were projected on a screen with a projector. The subjects viewed the images via a mirror attached to the headcoil. The stimuli were presented in the scanner by using the PRESENTATION software (Neurobehavioral Systems Inc., Albany, CA).The mean ± SD scan duration of the forced choice task was 508 ± 30s. The length of this scan varied between participants due to the variable number of food pairs (Mean ± SD = 40.7 ± 4.8; range = 28 -49 food pairs) that could be created per individual. Furthermore, food choices were alternated with non-food choices (i.e., choices between office utensils) to serve as a control condition and to avoid adaptation to the food stimuli. After each choice a fixation cross of variable length (2-4 s), was shown.

Table 3.1

	High calorie pictures		**Low calorie pictures**	
	mean±SD	range	mean±SD	range
Actual cal. [1]	376.2 ± 13	358.4 - 412	151 ± 26.1**	99.9 - 193.5
Perceived cal.[2]	7.5 ± 0.6	6.3 - 8.4	3.1 ± 0.6**	2.1 - 4.6
Liking[2]	6.7 ± 0.7	5.3 - 7.5	6.7 ± 0.6 [ns]	5.4 - 7.5
Health[2]	3.0 ± 0.7	2 - 4.6	7.1 ± 0.4 **	6.3 - 7.9

** Differences between high& low calorie pictures were significant $p < 0.001$; [ns] Differences between high& low calorie pictures were not significant; [1]Actual caloric content kcal per 100 grams; [2] 9-point Likert scale.

3.2.3 Image acquisition

Scans were performed with a 3 Tesla Philips Achieva MRI scanner (Philips Healthcare, Best, The Netherlands) using an 8-channel SENSE head coil. A high resolution anatomical image (T^1-weighted scan) was acquired at 1 x 1 x 1 mm resolution (TR = 8.4 ms, total scan duration = 473 s). Functional scans were acquired with a T^2*-weighted gradient-echo 2D-EPI sequence (TR/TE = 1400/23 ms, flip angle = 72.5°, voxel size = 4 x 4 x 4 mm, FOV = 208 × 119.6 x 256 mm, dynamic scan duration = 1400 ms). Six dummy volumes were automatically discarded. The total number of volumes collected varied between participants due to the different number of food choice pairs that could be generated (range: 295-400 volumes).

3.2.4 Data analyses

3.2.4.1 Behavioral analyses

The behavioral data were analyzed with SPSS statistics 19. The self-report ratings on a 9-point Likert scale (i.e. liking, perceived caloric content and perceived healthiness), actual caloric content, the number of high and low calorie choices made and reaction times (RTs) were normally distributed. Differences in liking, perceived caloric content, actual caloric content and healthiness between the high and low calorie choice options, the choices made and RTs were analyzed by using paired t-tests. In addition, the percentage of high and low calorie choices made was examined by using a one-sample t-test.

3.2.4.2 Image preprocessing

Data processing was performed with the SPM8 software package (Wellcome Department of Imaging Neuroscience, London, United Kingdom, (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) run with MATLAB R2012a (The MathworksInc, Natick, MA). The functional images were realigned to the first image. Subsequently, the functional images and the anatomical images were coregistered and normalized to MNI space (Montreal Neurological Institute – International Consortium for Brain Mapping). In addition, the functional images were smoothed with a Gaussian kernel of 8 mm full width at half maximum (FWHM). The mean functional images were visually inspected for artefacts. Furthermore, the realignment parameters of all subjects were also examined."

3.2.4.3 fMRI analyses

The following five conditions were modeled: high calorie food choice, low calorie food choice, non-food choice, spare choices and missed choices. Because

participants were free to choose, the number of high and low calorie choices was unequal for most participants (range number high calorie choices = 11-26; range number low calorie choices= 11-38). To control for this bias, balanced designs were created by selecting equal number of choices per condition of interest per subject. In addition, the choices used for this analysis were selected based on a minimum of 2 points difference in the individual caloric content ratings on a 9 point Likert scale (to ensure each food pair differed in perceived caloric content). The choices that did not meet this criterion, in addition to spare choices (i.e. rest choices due to equal number of choice selection) and missed choices, were modeled as separate conditions.

High calorie vs low calorie choices

For the high (HCC) versus low calorie choice (LCC) analysis equal numbers of high calorie, low calorie and non-food choices were selected (range 11-21 choices per category). On first level (single subject analysis) the high calorie choice and low calorie choice versus baseline and high versus low calorie choice contrasts (i.e. conditions of interest) were created. On second level (group analysis) one sample t-tests were performed to examine the significant brain activation of the group during the contrasts mentioned above. The statistical parametric map generated of the HCC-LCC contrast, was thresholded at $p < 0.001$ uncorrected for multiple comparisons with a cluster-extent k=20 (Lieberman and Cunningham, 2009). The statistical parametric maps calculated for the single conditions (i.e. HCC and LCC) were thresholded more conservatively ($p < 0.05$ FWE corrected for multiple comparisons at whole brain level, k=10) since these conditions were contrasted against rest.

Food vs non-food choices

For the food choice versus non-food choice analysis, equal numbers of food choices (containing both high & low calorie choices) and non-food choices were selected (range 19-26 choices per category). On first level (single subject analysis) the food choice versus non-food choice contrast was created. On second level (group analysis) a one sample t-test was performed to establish the brain regions that are differentially activated by food and nonfood choices. The generated statistical parametric map was thresholded at $p < 0.001$ uncorrected for multiple comparisons, k=20 (Lieberman and Cunningham, 2009).

3.3 Results

3.3.1 Behavioral data

Overall, the participants chose significantly more low calorie than high calorie foods (LCC percentage (Mean, SD) = 57% ± 11.3%; t = 2.75; p = 0.013). Because all liking ratings were included in the creation of the food choice pairs, liked, neutral but also disliked pairs could be present. To check whether the choices made per category did not differ significantly in liking a paired sample t-tests was conducted. We found no significant difference in liking ratings between high and low calorie choices (see Table 2). See for more detailed ratings per subject, S4 Table. Furthermore, the RT's of the high calorie choices were significantly larger than the RT's of the low calorie choices (RT HCC (Mean, SD)= 1.6 s ± 0.4 s; RT LCC (Mean, SD)= 1.5 ± 0.3; t = 2.45; p = 0.025).

Table 3.2

	High calorie choices		**Low calorie choices**	
	mean±SD	range	mean±SD	range
N	17.3 ± 4.2	11–26	23.4 ± 6.1*	11–38
Actual cal. [1]	366.4 ± 34.3	310.1–416.6	155 ± 36.3**	74.6–226
Perceived cal.[2]	7.4 ± 0.5	6.3–8.3	3.1 ± 0.7**	2.2–4.5
Liking[2]	6.7 ± 0.7	5.6–7.8	6.7 ± 0.7 ns	5.3–7.8
Health[2]	3.1 ± 0.7	1.9–4.5	7.1 ± 0.4**	6.5–7.9

* Differences between high& low calorie choices were significant p = 0.011; ** p< 0.001; [ns] Differences between high& low calorie choices were not significant; [1]Actual caloric content kcal per 100 grams; [2] 9-point Likert scale.

3.3.2 fMRI data

3.3.2.1 High calorie & low calorie choices vs baseline

Fig. 3.3 shows the results of the single food contrasts (i.e. high calorie choice and low calorie choice versus baseline) (p < 0.05 FWE corrected, k = 10) are shown. This figure clearly shows the similarity of the brain activation pattern during high and low calorie choice. Regions that were stronger activated compared with rest

in both high and low calorie choice include the midbrain, insula, supplemental motor area, middle cingulate gyrus and several visual areas.

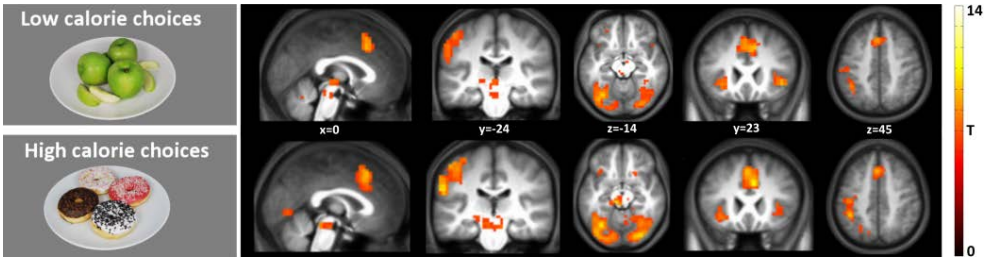


Figure 3.3 Brain regions with stronger activation in response to HCC and LCC vs baseline. Shown is a T-map thresholded at P<0.05 (FWE-corrected; T>6.25), superimposed on the mean anatomical image of all subjects (MNI-space).

3.3.2.2 High calorie vs low calorie choices

Few differences were found between high versus low calorie food choices (p<0.001 uncorrected, cluster extent threshold k= 20). Significantly stronger activation was found in the posterior part of the right superior temporal sulcus (See Fig. 4; MNI peak coordinate (62, -36, 22; T = 4.32; Z = 3.53; k=20) for high versus low calorie choice. This activation did not correlate (pearson r = 0.098, p=0.691) with the differences in RT's for the high and low calorie choices (reported in the Behavioural data section). No differences were found in the low compared with high calorie food choice contrast (p<0.001 uncorrected, cluster extent threshold k=20).

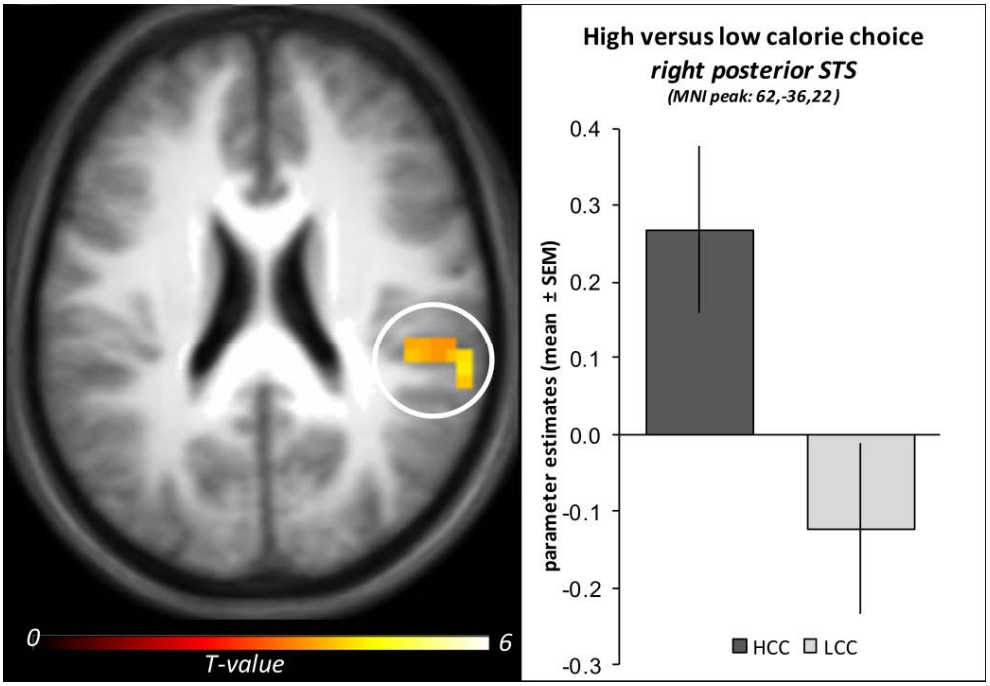


Figure 3.4 Mean parameter estimates, peak coordinate (62. -36. 22) of the brain region with stronger activation in response to HCC versus LCC. Shown is a T-map for visualization thresholded at T = 3.5 p<0.001 uncorrected for multiple comparisons, superimposed on the mean anatomical image of all subjects (MNI-space).

3.3.2.3 Food vs non-food choices

In addition, differences between food and non-food choices in the absence of hunger ($p < 0.001$ uncorrected, cluster extent threshold $k = 20$), were investigated. The results are depicted in Figure 3.5 and the peak coordinates are given in Table 3.3. Several brain regions, including the insula, posterior cingulate gyrus, cuneus, precuneus and superior temporal gyrus, were stronger activated during food choice ($p<0.001$ uncorrected, $k=20$). In addition the overlap between the food versus non-food choices and the individual contrasts high calorie food choices vs rest and low calorie-food choices vs rest were examined. The left insula was active in all three contrasts.

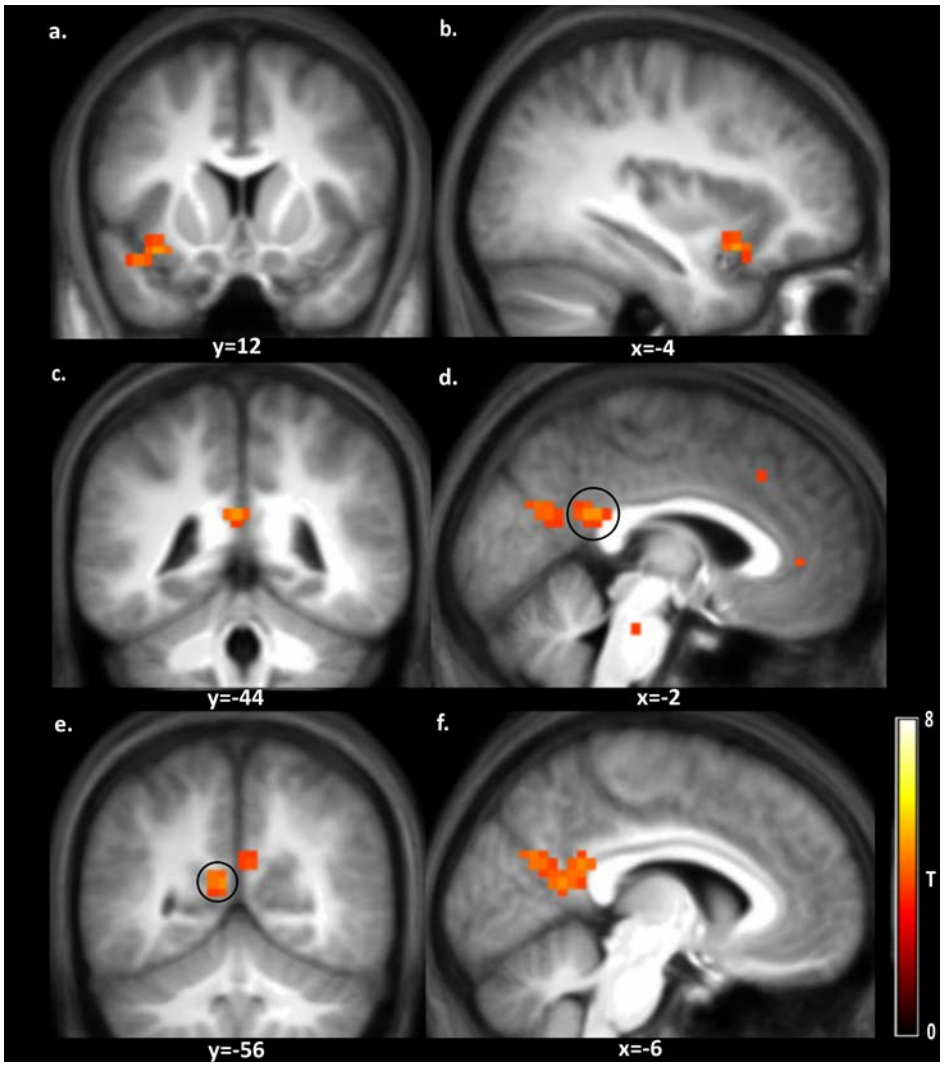


Figure 3.5 Brain regions with stronger activation in response to food choice versus non-food choice. Shown is a T-map thresholded for visualization purposes at $p < 0.001$ uncorrected for multiple comparisons ($T > 3.6$), superimposed on the mean anatomical image of all subjects. a: L, insula and L, superior temporal sulcus; b: L, superior temporal sulcus; c & d: L, posterior cingulate gyrus; e: L, precuneus; f: L, precuneus & L, cuneus; See corresponding peak coordinates in Table 3.

Table 3.3 Brain regions with stronger activation in response to food choices versus non-food choices

		Peak MNI-coordinates (mm)				
Region	**k**	**x**	**y**	**z**	**T**	**Z**
L, insula (a)	21	-34	12	-14	4.77	3.79
L, superior temporal sulcus (a,b)		-42	12	-18	4.31	3.53
L, posterior cingulate gyrus (c,d)	67	-2	-44	22	4.70	3.75
L, precuneus (e,f)		-6	-56	14	4.64	3.72
L, cuneus (f)		-6	-68	26	4.44	3.60

Peaks are reported for all clusters ≥ 20 voxels at $p<0.001$ uncorrected for multiple comparisons; L = left and R= right hemisphere; The regions a-f are depicted in Figure 3.5.

3.4 Discussion

We investigated brain responses during food choices between foods matched on liking but differing in caloric content in the absence of hunger. Although the participants were not above average dietary restraint, not dieting and had a stable weight, they made more low compared to high calorie food choices. In addition, the RTs were higher for the high calorie choices compared to the low calorie choices. We speculate that the subjects in this study choose more low calorie foods because they were in a fed state and were presented with equally liked foods. In this scenario, the low calorie option was, physiologically, the best option to choose to maintain a stable weight.

Brain regions which elicited stronger activation during high and low calorie choice compared to rest include the midbrain, insula, supplemental motor area, middle cingulate gyrus and several regions involved in visual processing. While, the posterior part of the right superior temporal sulcus (STS), was the only region found to be more active during high compared with low calorie choice matched on liking in healthy sated normal-weight volunteers. In addition, this activation did not correlate with the differences in RTs between the high and low calorie choices.

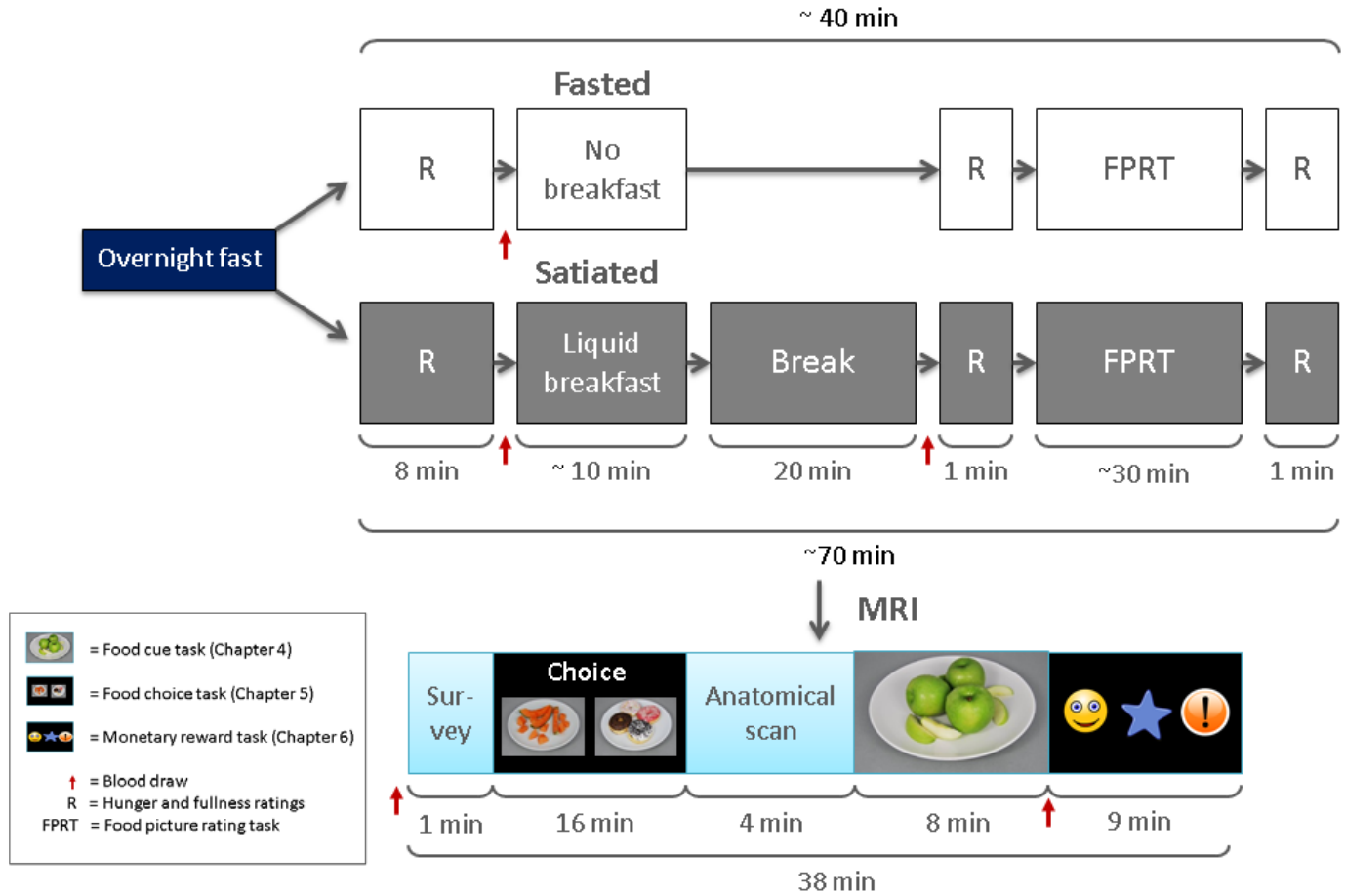
The superior temporal sulcus is thought to be a multifunctional region. The literature on this region is characterized by a large variety of cognitive studies in different fields, ranging from facial recognition to social cognition and theory of mind (Hein and Knight, 2008). Studies investigating face processing have compared brain responses to faces with abstract images having similar contours e.g., Narumoto et al. (2001). In these studies, the right STS is more activated during emotional face expressions (as fear, sadness and happiness). Effects of attention on STS activation have also been reported (Pessoa et al., 2002). Finally, the right posterior STS has been found to be more active during viewing of highly palatable foods versus moderate palatable foods in unrestraint volunteers of normal weight in a fasted state (Coletta et al., 2009). Although the region that has been reported in Colletta et al., is a different part of the STS than we found (most likely due to the different nature of the task used), it is interesting and suggests that the right posterior STS is not only involved in the processing of faces and emotion but also in other biological relevant processes such as high calorie food evaluation and choice. High calorie food choice is an especially biologically relevant process as these foods are highly energy-dense. Interestingly, the difference in right posterior STS activation seems to be independent of state and palatability since its activation was found in both the hungry state (Coletta et al., 2009) and in our fed state controlled for liking. This suggests that the right posterior STS activation may reflect a food's biological relevance, irrespective of satiety and independent of food preference. However, to obtain more insight in the exact function of the STS in food choice and how this may be modulated by hunger and satiety, more research is needed.

Furthermore, we examined the differences between food and non-food choices. We found increased activation in the left insula, left superior temporal sulcus, left posterior cingulate gyrus and left (pre)cuneus, in response to food compared with non-food choices.
The insula is known for its involvement in value-based decision making. It integrates internal state and sensory signals and is important during response selection. In addition, it integrates information about the salience and relative value of stimuli (Paulus and Stein, 2006). Previous studies found significantly stronger activation in both the left insula and left posterior cingulate gyrus during food viewing tasks (van der Laan et al., 2011). Both the insula (FitzGerald et al., 2009; Kang et al., 2011; Kim et al., 2011; Knutson et al., 2007) and the left posterior cingulate gyrus (Ballard and Knutson, 2009; Knutson et al., 2001a; Knutson et al., 2003; Plassmann et al., 2007), were also found to be active during choice valuation tasks. Furthermore, activation in the posterior cingulate gyrus has been found to correlate with monetary reward magnitude (Ballard and

Knutson, 2009; Knutson et al., 2001a; Knutson et al., 2003) and willingness to pay for primary rewards (e.g. food) (Plassmann et al., 2007). Although in our study the foods were most likely devaluated due to satiety, insula activation and posterior cingulate gyrus activation found in food viewing studies and choice valuation studies suggest that, despite a low motivation to eat, food items were more salient than non-food items. Other studies support this view and showed that non-food stimuli attract less attention than food images in both eye tracking (Castellanos et al., 2009; Nijs et al., 2010) and neuroimaging studies using visual food cues (Smeets et al., 2013; van der Laan et al., 2011).
During food choice, activation in the (pre)-cuneus and the left superior temporal sulcus was also increased. The precuneus is especially known for its involvement in attention (Cavanna and Trimble, 2006; Stoll et al., 2008). This suggests that the increased activation in the precuneus reflects increased attention for the food pairs compared with the non-food pairs. The left STS is involved in simple moral decisions versus semantic decisions (Heekeren et al., 2003). Increased activation in this region during food choice compared with non-food choice might reflect the different nature of the choices made, namely simple decisions between two office utensils versus more complex decisions between which food one would most want to eat.

In conclusion, we observed increased insula, posterior cingulate gyrus and precuneus activation during food choice versus non-food choice. This suggests that the food stimuli were more salient than the non-food stimuli despite the low motivation to eat. In addition, in line with our hypothesis, we did not find major differences between high versus low calorie choices between equally-liked food items in the absence of hunger. The right superior temporal sulcus was the only region found to be stronger activated during high calorie compared with low calorie choice independent of liking. Together with previous studies, this may suggest that right STS activation during food evaluation and choice reflects the food's biological relevance independent of food preference. This novel finding warrants further research into the effects of hunger state and weight status on right STS, which may provide a marker of biological relevance.

STUDY PROCEDURE


~ 40 min
Fasted
R
No breakfast
R
FPRT
R
Overnight fast
Satiated
R
Liquid breakfast
Break
R
FPRT
R
8 min
~ 10 min
20 min
1 min
~30 min
1 min
~70 min
MRI
= Food cue task (Chapter 4)
= Food choice task (Chapter 5)
= Monetary reward task (Chapter 6)
= Blood draw
R = Hunger and fullness ratings
FPRT = Food picture rating task
Sur-vey
Choice
Anatomical scan
1 min
16 min
4 min
8 min
9 min
38 min

CHAPTER 4

EFFECTS OF HUNGER STATE ON THE BRAIN RESPONSES TO FOOD CUES ACROSS THE LIFESPAN

Based on: Charbonnier, L., van Meer, F., Johnstone, A.M., Crabtree, D., Buosi, W., Manios, Y., Androutsos, O., Giannopoulou, A., Viergever, M.A., Smeets, P.A.M. Effects of hunger state on the brain responses to food cues across the lifespan. *(submitted for publication)*

Abstract

The abundant exposure to food cues in our environment is one of the drivers of overconsumption. Food evaluation is important for the regulation of food intake by the brain and interacts with hunger state. Children are especially susceptible to food cues. Understanding the mechanisms behind this regulation in healthy individuals across the lifespan can help to elucidate the mechanisms underlying overconsumption and aid the development of future obesity prevention strategies. Few functional neuroimaging studies have been done in children and elderly. Furthermore, it is unknown what effect hunger state has, since this has not been examined consistently.

We examined the effects of hunger state and age on the brain responses to low- and high calorie foods. On two mornings, 122 subjects (17 children; 38 teens; 36 adults; 31 elderly) performed a food viewing task while being scanned using fMRI, either fasted or sated.

Hunger induced greater activation during high vs low calorie food viewing than satiety in the dorsomedial, ventromedial and dorsolateral prefrontal cortex across age groups. In addition, children showed the highest activation in visual areas, while the lowest activation was apparent in teens.

The greater activation of the dlPFC across age groups during high calorie food viewing in a fasted state might reflect increased inhibitory control in response to these foods. This may underlie the ability to resist overconsumption of high calorie foods. Furthermore, increased medial prefrontal cortex activation during hunger might reflect increased reward value of high calorie foods, which declines with satiation. Further studies are needed to better understand these results. Notably, overweight individuals should be included to examine whether these responses are altered by weight status across age.

4.1 Introduction

The abundant exposure to food cues in our environment is one of the drivers of overconsumption. Food evaluation is important for the regulation of food intake and therefore weight management and general health. It plays a crucial role in food selection throughout the lifespan. However, relatively little is known about the impact of developmental changes on the brain responses to food. Understanding the mechanisms behind the regulation of food evaluation in normal-weight individuals across the lifespan is an important step in elucidating the mechanisms driving obesity and the development of future obesity prevention strategies.

During the day we are continuously exposed to food cues. Food cue exposure can influence the motivation to eat (Ferriday and Brunstrom, 2011). It has been shown that brief exposure to the sight or smell of food can increase perceived hunger (Fedoroff et al., 2003; Ferriday and Brunstrom, 2008; Oakes and Slotterback, 2000) and initiates responses similar to those of actual food consumption, e.g. insulin secretion, salivation, heart rate changes, gastric activity and altered blood pressure (Nederkoorn et al., 2004; Nederkoorn et al., 2000; Overduin et al., 1997). Children are especially sensitive to food cues. They are easily influenced by images of tasty but, most often, unhealthy food (Ferguson, Munoz et al 2011). This could promote unhealthy eating habits like excess energy intake, which are major predictors of overweight and obesity later in life (Perry CL 1997).
Relatively little is known about the effect of age on food cue-related brain responses. In most studies, food images are commonly utilised as substitute for real foods (Kringelbach and Rolls, 2004; Simmons et al., 2005; Tiggemann and Kemps, 2005b). This resembles food cue exposure in the environment like we encounter in our daily life. It has been suggested that these types of tasks, without an active choice component, nevertheless elicit activation in certain brain regions similar to that seen during making actual choices (Levy et al., 2011).
In healthy normal-weight (predominantly) adults, the posterior fusiform gyrus, the lateral orbitofrontal cortex (OFC; also known as ventrolateral prefrontal cortex; vlPFC) and the left middle insula were found as most concurrent brain regions (35-41% concurrency across studies) activated in response to food compared to non-food image viewing (van der Laan et al., 2011). Activation in the lateral OFC is thought reflect the anticipated pleasantness of the food, especially since it has also been reported during palatable taste exposure and anticipation of consumption (O'Doherty et al., 2002).

During high compared to low calorie food viewing the hypothalamus and ventral striatum were most consistently found in adults (43% contributing studies), although part of the middle frontal gyrus/dorsolateral prefrontal gyrus (dlPFC), cerebellum, middle occipital gyrus and inferior temporal gyrus were also reported across studies (29% contributing) (van der Laan et al., 2011). Overall the congruency across studies is rather low and the vast majority of foci do not overlap.

There are many factors that could cause this poor reproducibility, ranging from large variety in study designs and tasks used to highly variable hunger states examined. Especially hunger state seems to be of importance as it is known to modulate food-related brain responses. Activation in the hippocampal gyrus and amygdala has been found to increase during hunger across several studies (60% contributing, van der Laan et al, 2011). Similarly, lateral OFC (vlPFC) activation was found to increase in response to food images in a hungry compared to a sated state (40%). This effect was found to be more pronounced for high compared to low calorie food image viewing (Siep et al., 2009).

In children and adolescents only few studies have been conducted. The brain regions that were most consistently found to activate in response to food cues in children and adolescents were the inferior frontal gyrus, fusiform gyrus and right superior parietal lobule (van Meer et al., 2014). These findings are similar to those found in adults (van Meer et al., 2014). However, given the low number of studies conducted in children it is hard to draw firm conclusions. Due to developmental brain changes in especially frontal areas, differences between children, adolescents and adults may be expected. Furthermore, similar to adults, children have been found to be more responsive to food images when hungry (Bruce et al., 2010; Holsen et al., 2005; Killgore and Yurgelun-Todd, 2005), but also here the number of studies is low, age ranges of the children investigated are rather broad and hunger state was not consistently varied across studies.

In elderly, no food evaluation studies have been conducted to date. Therefore, it is unknown whether the effects seen in adults persist later in life. Similar to children, alterations may be expected since frontal regions in particular degenerate with ageing (Kaup et al., 2011; Raz, 2000), which affects cognitive functions like decision making.

In summary, there is a fair body of literature on food viewing, however, meta-analyses show that results are not very consistent (van der Laan et al., 2011; van Meer et al., 2014), due to differences in study designs, analyses approaches and multiple influencing factors like hunger state and BMI. In particular, studies directly comparing brain responses to food cue exposure between age groups and hunger states are lacking. Therefore, the primary objective of this study was to compare brain responses to food cues across the lifespan and to examine to what

extent these are modulated by hunger state. We expected lower activation in frontal areas in children, teens and elderly compared to adults. In addition, we hypothesized that this effect would be modulated by hunger.

4.2 Materials and methods

4.2.1 Participants

We included healthy children, teens, adults and elderly with a normal weight (i.e., BMI 20–25 kg/m^2 equivalent) in four age groups (between 8-10, 13-17, 25–45 and 65-75 years of age) and three countries (The Netherlands, Scotland and Greece). These groups were chosen to ensure that the vast majority of the children were pre-pubertal, teens pubertal, adults post-pubertal and most likely pre-menopausal and elderly post-menopausal. Additionally, the gap between the age groups maximized the change of finding differences between the groups. Additional criteria: right-handed, non-smoking, with a stable weight (did not gain or lose > 5 kg in the past 6 months), no use of medication (except aspirin/paracetamol and oral contraceptives and anticoagulants and cholesterol medication in elderly) and no current alcohol consumption of > 28 units per week. Furthermore, common fMRI exclusion criteria (e.g. claustrophobia, pregnancy and metal implants in the body) and criteria that might influence response to food cues (e.g. food allergies, special diets, eating disorders, gastrointestinal disorders or metabolic or endocrine disease, highly restraint eating scores on The Dutch Eating Behavior Questionnaire (Van Strien et al., 1986)) were used. In addition, runs with excessive movement were excluded from the analyses. 145 eligible participants enrolled in the study, 10 participants (4 adults, 3 elderly, 2 children, 1 teen) were excluded because they did not have a successful viewing task run for each condition, 8 participants (6 children and 2 teens) were excluded due to excessive movement, one child had a neurological disorder, the remaining two children from Scotland were excluded because adding an extra variable to the already low number of children was not preferable, one adult was excluded because he fell asleep and one additional child was excluded due to average signal during food versus non-food viewing was >2 SD different from the group mean and since its movement was on the upper boundaries of our threshold it was excluded from the analysis. The resulting sample included in the analyses consisted of n=122 subjects (17 children, 38 teens, 36 adults, 31 elderly).

Table 4.1. Participant characteristics

	Children n =17	Teens n =38	Adults n =36	Elderly n =31
Country ratio (NL:SCT:GR)	17:0:0	26:5:7	19:8:9	22:6:3
Gender (M:F)	6:11	21:17	18:18	14:17
Age (y)	9.6±0.9	15.5±1.7	32.6±5.8	69.8±3.2
BMI (kg/m^2)	n/a	n/a	23.0±1.8	21.3±7.4
SDS BMI	-0.33±0.8	0.34±0.8	n/a	n/a
Interscan interval (days)	9.6±2.9	9.7±6.1	8.5±4.2	9.3±4.1
First visit (S:H)	8:8	19:19	14:22	13:18
Amount liquid breakfast consumed (mL)	277±108	445±51	510±80	492±124

4.2.2 Experimental design

4.2.2.1 Study procedures

The study consisted of two morning MRI scan sessions. On both days, the participants came in after an overnight fast of at least ten hours. During the sated condition session participants were scanned after the consumption of a fixed amount of liquid breakfast (a commercially available vanilla (vanilla or strawberry flavour in children and teens) whey protein shake from XXL Nutrition, The Netherlands prepared with whole milk), 1.4 x basic metabolic rate (BMR), calculated with the Schofield equation. With this equation an individual's BMR can be estimated by using age, gender and weight (Schofield, 1985; Schofield, 1984). During the hungry condition session participants were scanned after an overnight fast of ≥10 h. The time between the two scan sessions was 1-2 weeks. The conditions (i.e. hungry or sated state) were counterbalanced. Upon arrival on a study morning, subjects filled out several questionnaires and executed a computerized food picture rating task. During this task, 133 images were rated on liking, perceived caloric content and perceived healthiness (9 point Likert scales; 5 point Likert scales in children; caloric content question not applicable in children). On the sated morning session, the computerized food picture rating task was executed 20 minutes after liquid breakfast consumption. Participants entered the

scanner approximately 1 hour after liquid breakfast consumption. For hormone analyses (not part of the current analysis) blood was collected through a cannula on several time points during both morning sessions. Subsequently, they underwent a 38-min MRI scan session consisting of four functional MRI runs during which they performed a food viewing task, a food choice task (two parts) and a monetary reward task. The results of the food viewing task are the focus of this paper. . See for more details the study procedure on page 50.

4.2.2.3 Food viewing fMRI task

In the food viewing task, participants watched 18 blocks of 7 images each (12 blocks with foods, i.e. 6 blocks with high and 6 blocks with low calorie food images; and 6 blocks with non-foods). Each block was followed by an inter-block interval (i.e. black screen with crosshair) with a randomized duration between 8 and 16 s. In total, participants viewed 126 images over 454 seconds (~8 min). They were given the following task instruction: “In the next task you will see food and non-food products. Please look at the images and pay close attention, since at the end of the MRI session you will be asked a couple of question regarding the images shown during this task.” After the MRI session, participants were shown 10 images for which they had to indicate whether they had seen them during the task.

4.2.3 Image acquisition

Imaging was performed on a Philips Achieva 3.0 T MRI scanner (Philips Healthcare, Best, NL). Functional images were obtained with an 8-channel SENSE head-coil using a 2-D echo planar imaging (EPI) sequence with the following parameters: voxel size 4 mm isotropic; repetition time (TR) = 1600 ms; echo time (TE) = 23 ms; flip angle = 72.5°; 30 axial slices; SENSE-factor *R* = 2.4 (anterior-posterior). A total of 316 functional images were acquired. A high resolution anatomical image (T_1-weighted scan) was acquired at 1 × 1 × 1 mm resolution (TR/TE = 8.4/3.8 ms, total scan duration = 454 s).

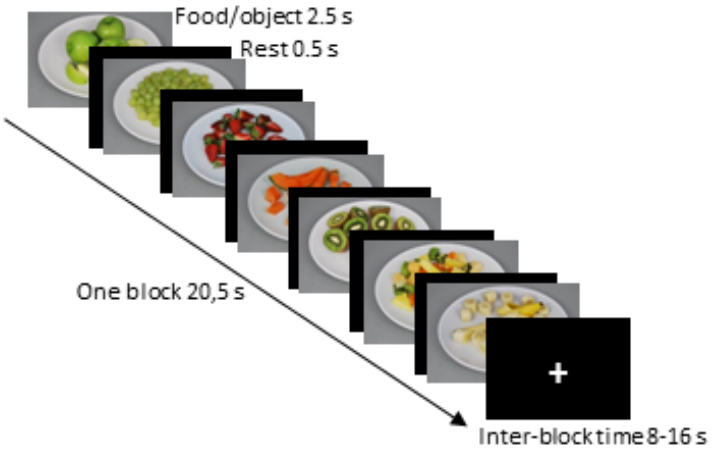


Figure 4.3 Structure of the food viewing task. Depicted is one low calorie block. The task included both low and high calorie food blocks and non-food blocks (showing images of office utensils).

4.2.4 Data analyses

4.2.4.1 Behavioral analyses

Behavioral data were analyzed with the use of SPSS Statistics 23. Hunger and fullness ratings were analyzed by using four paired sample t-test per age group. Additionally the significance threshold was Bonferroni corrected (p=0.0125 (0.05/4)). The 5-point liking ratings of children were linearly transformed to the 9-point scale of the other groups to facilitate group comparisons. Subsequent differences in liking ratings were analyzed by performing a repeated measures ANOVA and Bonferroni post-hoc tests.

4.2.4.2 Image preprocessing

Image preprocessing and analyses were carried out with the SPM12 software (http://www.fil.ion.ucl.ac.uk/spm). After slice timing correction and realignment, the structural scan was coregistered to the mean functional scan. Next, the structural scan was segmented using unified segmentation, and normalization parameters were estimated. A study-specific anatomical template was created using DARTEL, and after coregistration DARTEL was used to normalize this template and the functional scans to MNI space (Montreal Neurological Institute–International Consortium for Brain Mapping). The data were then smoothed with

an 8 mm full width at half maximum isotropic Gaussian kernel. The Volume Artefact tool from ArtRepair (http://cibsr.stanford.edu/tools/ArtRepair/ArtRepair.htm) was used to detect and repair anomalously noisy volumes. Volumes that were moved more than 1mm/TR were repaired. Based on this detection three children had to be excluded from analysis because of too many volumes (>30%) that had to be repaired.

4.2.4.3 Individual & group analyses

The following conditions were modelled: high calorie food viewing, low calorie food viewing and non-food viewing. For the first analysis, the average brain activation during food versus non-food viewing and high versus low calorie food viewing across conditions (mean hungry and satiated) and between conditions (hungry – satiated) were calculated for each subject. These contrast images were then submitted to two 2x4 ANOVAs to investigate age-group and condition differences in activation during high versus low calorie food viewing. In addition, scan order and country were added as covariates. The statistical parametric maps generated were masked with an average grey matter mask of the group and thresholded at a threshold equivalent to $p<0.05$ corrected for multiple comparisons across the analysis mask. This threshold was derived using Monte Carlo simulations (10,000 iterations) of random noise distribution in the whole brain mask using the 3dClustSim in AFNI. This approach combines an individual voxel probability threshold with a minimum cluster size to estimate the probability of a false positive. The resulting threshold was $p<0.001$ with a cluster extent $k\geq29$ for the food versus non-food viewing analysis and $k\geq30$ for the high versus low calorie food viewing analysis.

4.3 Results

4.3.1 Behavior

Baseline hunger and fullness ratings, except for adult fullness, did not differ significantly between the study days (hungry-sated: hunger ratings: (children: $t(16) =-0.251$, $p=0.805$; teens: $t(37) =1.260$, $p=0.216$; adults: $t(33) =2.149$, $p=0.039$; elderly: $t(28)=1.386$, $p=0.177$; hungry-sated: fullness ratings: children: $t(16) =-1.595$, $p=0.130$; teens: $t(37) =-1.503$, $p=0.141$; adults: $t(33) =-3.043$, $p=0.005$; elderly: $t(28)=0.660$, $p=0.515$). For all age groups hunger ratings prior to the scan were significantly lower on the sated day (hungry-sated: hunger ratings: (children: $t(14) =4.365$, $p=0.001$; teens: $t(33) =7.970$, $p<0.001$; adults: $t(27) =10.842$, $p<0.001$; elderly: $t(27)=4.877$, $p<0.001$), while fullness ratings were significantly higher prior to the scan on the sated day (hungry-sated: fullness ratings: children:

t(14) =-3.287, p=0.005; teens: *t*(33) = -9.949, *p*<0.001; adults: *t*(27) =-11.301, *p*<0.001; elderly: *t*(27)=-3.156, *p*=0.004). See Table 4.2 for a complete overview.

Table 4.2 Hunger and fullness ratings in all age groups[9]

	Children	Teens	Adults	Elderly
Hungry condition				
Hunger ratings				
Baseline	3.1±0.8	5.9±2.5	6.7±1.7[1]	4.7±2.1[7]
20 min after drink	n/a	n/a	n/a	n/a
Prior to scan	4.1±0.8[4]	6.8±2.0[5]	7.4±1.2[2]	5.1±2.1[7]
Fullness ratings				
Baseline	1.6±0.6	2.7±1.8	2.2±1.4[1]	3.5±1.7[7]
20 min after drink	n/a	n/a	n/a	n/a
Prior to scan	1.7±0.8[4]	2.7±1.6[5]	1.9±1.4[2]	3.0±1.6[7]
Sated condition				
Hunger ratings				
Baseline	3.3±1.1	4.4±2.1	6.1±1.6[1]	5.4±2.4[7]
20 min after drink	2.1±0.8	2.8±2.1	2.7±1.7[1]	2.4±1.6[7]
Prior to scan	2.9±1.2[4]	3.6±2.0[6]	3.1±1.8[3]	3.7±1.9[6]
Fullness ratings				
Baseline	2.1±0.8	3.3±1.8	2.9±1.4[1]	3.2±1.7[7]
20 min after drink	3.6±0.8	5.7±1.9	6.8±1.7[1]	7.1±1.6[7]
Prior to scan	2.6±1.1[4]	4.4±2.0[6]	7.0±1.8[3]	6.5±1.8[8]

[1]N=35 [2]N=30 [3]N=28 [4]N=17 [5]N=37 [6]N=34 [7]N=30 [8]N=28[9] ;In teens, adults and elderly 9-point Likert scales were used, while in children 5-point Likert scales were utilized.

High-low calorie food image liking ratings differed between age groups (Figure 4.4, main effect group: $F(3,117) = 16.879$, $p<0.001$). Children and teen's high-low calorie food liking ratings were significantly greater than those from adults and elderly ($p<0.001$; children-teens: $p=0.917$; children-adults: $p=0.001$; children-elderly: $p<0.001$; teens-adults: $p=0.008$; teens-elderly: $p<0.001$; adults-elderly: $p=0.082$).

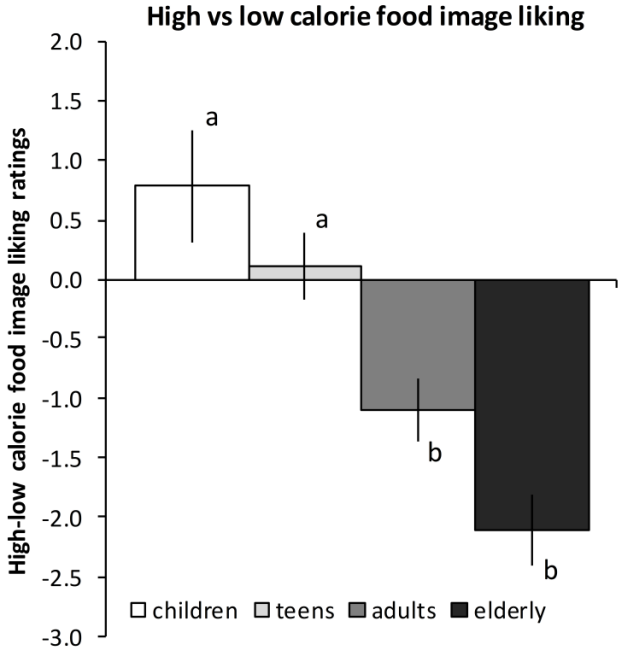


Figure 4.4 High-low calorie food image liking ratings. In the graph, different letters indicate significant group differences (for example teens (a) do not differ from children (a) but do differ from adults (b) and elderly (b)).

4.3.2 Food versus non -food viewing

There was no significant main effect of hunger state on food vs. non-food viewing related-brain activation. There was also no significant main effect of age group on food vs. non-food viewing activation. However, a cluster on the borders of the vermis, precuneus and lingual gyrus showed a trend for a main effect of age (Figure S4.1, MNI peak coordinate: 4, -48, 8; $F = 9.18$; $Z = 4.14$; k = 28). In this region, the activation in children was lower compared to adults ($p=0.001$) and

elderly (p<0.001). Activation between children and teens, and between teens, adults and elderly did not differ significantly (children-teens: p=0.135; teens-adults: p=0.358; teens-elderly: p=0.061; adults-elderly: p=1.000).

4.3.3 High versus low calorie food viewing

During high vs. low calorie food viewing there was a significant main effect of hunger state in two clusters (Table 4.3). These clusters cover parts of the bilateral dorsomedial prefrontal cortex (dmPFC, Figure 4.5), left ventromedial prefrontal cortex (vmPFC) and the right dlPFC (Figure 4.6). In all these clusters, activation during high compared to low calorie food viewing was higher in the hungry compared to the sated state. When controlling for differences in food liking between high and low calorie foods and between hunger states by including average ratings, only the left dmPFC/vmPFC cluster became 3 voxels smaller (k=35). Thus, the observed differences between the hunger states are not driven by differences in food image liking.

Table 4.3 Brain regions showing a main effect of hunger-state during high vs. low calorie food viewing

		Peak MNI-coordinate (mm)				
Region	*k*	x	y	z	*F*	*Z*
dmPFC, L (medial superior frontal gyrus)	38[L]	-4	56	24	19.61	4.09
dmPFC, L (medial superior frontal gyrus)		-4	64	8	16.94	3.80
vmPFC, L (medial orbitofrontal cortex)		-4	56	-8	15.08	3.58
dlPFC, R (superior frontal gyrus)	31	20	52	24	18.85	4.01
dmPFC, R (Superior medial frontal gyrus)		4	52	36	16.75	3.78
dmPFC, R (Superior medial frontal gyrus)		8	48	44	16.57	3.75

Peaks are reported for all clusters ≥30 voxels at p<0.001 uncorrected for multiple comparisons; L = left and R= right hemisphere. [L]k=35 when controlling for differences in liking.

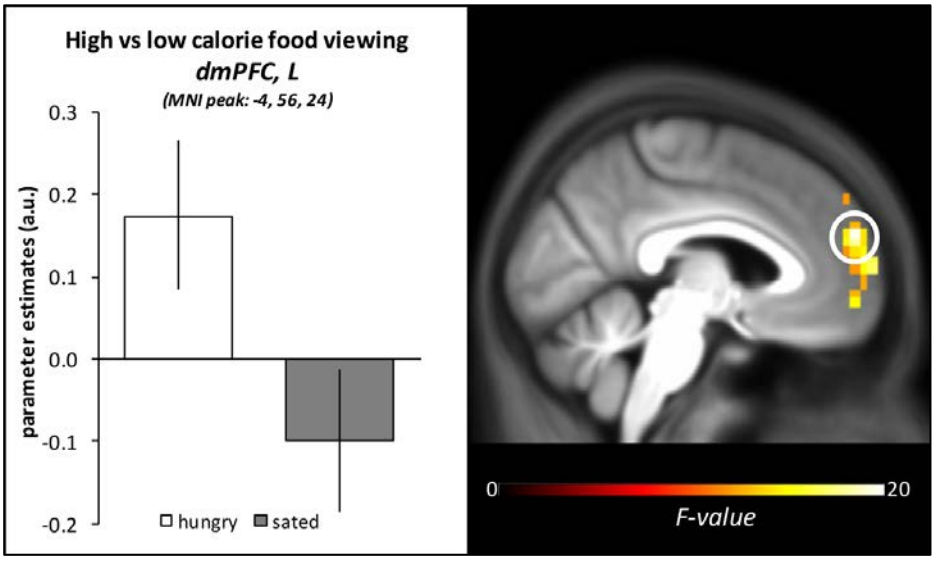


Figure 4.5 Average dmPFC cluster parameter estimates (mean ± s.e.m) during high vs. low calorie food viewing in the hungry and sated state (main effect hunger state). Shown is a *F*-map thresholded for visualization at F=11.0, p<0.001 uncorrected for multiple comparisons and superimposed on the mean anatomical image of all subjects.

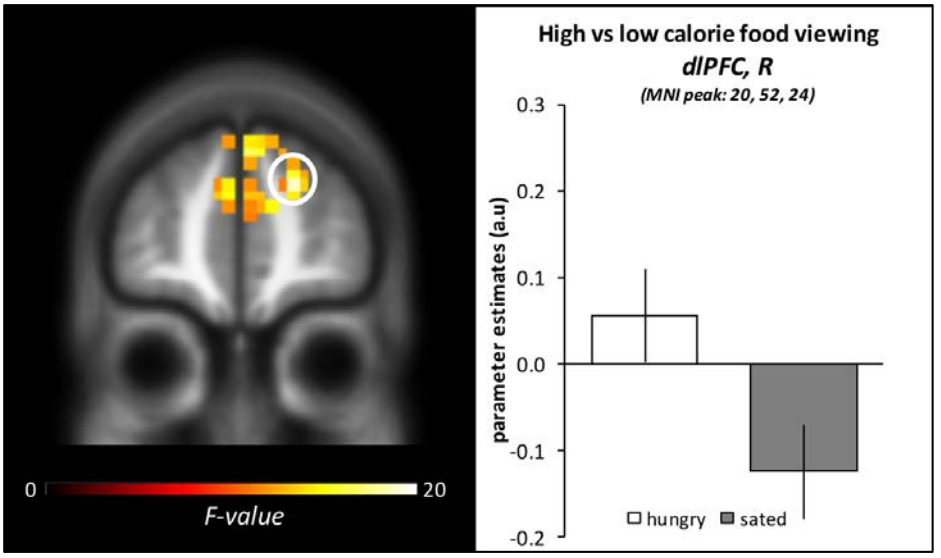


Figure 4.6 Average dlPFC cluster parameter estimates (mean ± s.e.m) during high vs. low calorie food viewing in the hungry and sated state (main effect hunger state). Shown is a *F*-map thresholded for visualization at F=11.0, p<0.001 uncorrected for multiple comparisons and superimposed on the mean anatomical image of all subjects.

4.3.3.2 Effects of age

There was a significant main effect of age on high vs. low calorie food viewing in the right superior and bilateral middle occipital gyrus, left middle temporal gyrus and left superior parietal gyrus (Table 4.4). In the middle occipital gyrus clusters, children showed greater activation compared to teens and elderly during high compared to low calorie food viewing (Figure 4.7). When controlling for liking, the left middle occipital gyrus cluster (including the middle temporal gyrus) became 3 voxels smaller (k=129) and the right middle occipital gyrus became 1 voxel smaller (k=105). Hence, only a very small part of the differences between age groups is driven by differences in liking of the food images.

In the middle occipital gyrus cluster, which extends into the superior parietal gyrus, activation in children was greater than that in elderly (Figure 4.8). When controlling for liking, this cluster became 35 voxels smaller (k=4), which is below our cluster extent threshold. Thus, activation differences between the age groups in the middle occipital gyrus are strongly driven by differences in liking of the food images.

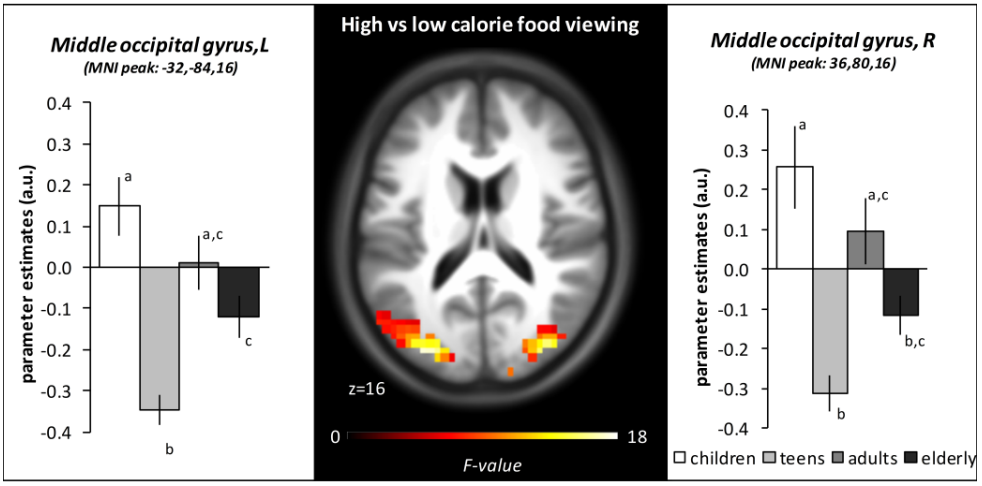


Figure 4.7 Average middle occipital gyrus cluster parameter estimates (mean ± s.e.m) during high versus low calorie food viewing in the different age groups across hunger states (main effect age group). Shown is a *F*-map thresholded for visualization at F=6.0, p<0.001 uncorrected for multiple comparisons and superimposed on the mean anatomical image of all subjects. In the graph, different letters indicate significant group differences (for example children (a) do not differ from adults (a) but do differ from teens (b) and elderly (c)).

Table 4.4 Brain regions showing a main effect of group during high versus low calorie food viewing.

		Peak MNI-coordinate (mm)				
Region	k	x	y	z	F	Z
Middle occipital gyrus, L[a]	132[L1]	-32	-84	16	17.33	5.85
Middle temporal gyrus, L		-48	-64	0	12.25	4.88
Middle occipital gyrus, R[b]	106[L2]	36	-80	16	15.53	5.53
Superior occipital gyrus, R		24	-84	40	12.81	5.00
Middle occipital gyrus, L[c]	39[L3]	-28	-64	40	7.92	3.79
Superior parietal gyrus, L		-20	-68	52	6.32	3.27

Peaks are reported for all clusters ≥ 27voxels at p<0.001 uncorrected for multiple comparisons; L = left and R= right hemisphere. [a] *children-teens: p<0.001; children-adults: p=0.730; children-elderly: p=0.021 ; teens-adults: p<0.001 ; teens-elderly: p=0.014 ; adults-elderly: p=0.454;* [b] *children-teens: p<0.001; children-adults: p=0.864; children-elderly: p=0.008 ; teens-adults: p<0.001 ; teens-elderly: p=0.187 ; adults-elderly: p= 0.138;* [c] *children-teens: p= 0.203; children-adults: p=0.627; children-elderly: p<0.001 ; teens-adults: p=1.000; teens-elderly: p=0.071 ; adults-elderly: p=0.014;*[L1]*Cluster was slightly influenced by differences in food image liking ratings, when controlling for liking, k=129;* [L2]*Cluster was slightly influenced by differences in food image liking ratings, when controlling for liking, k=105;* [L3]*Cluster was highly influenced by differences in food image liking ratings, when controlling for liking k=4.*

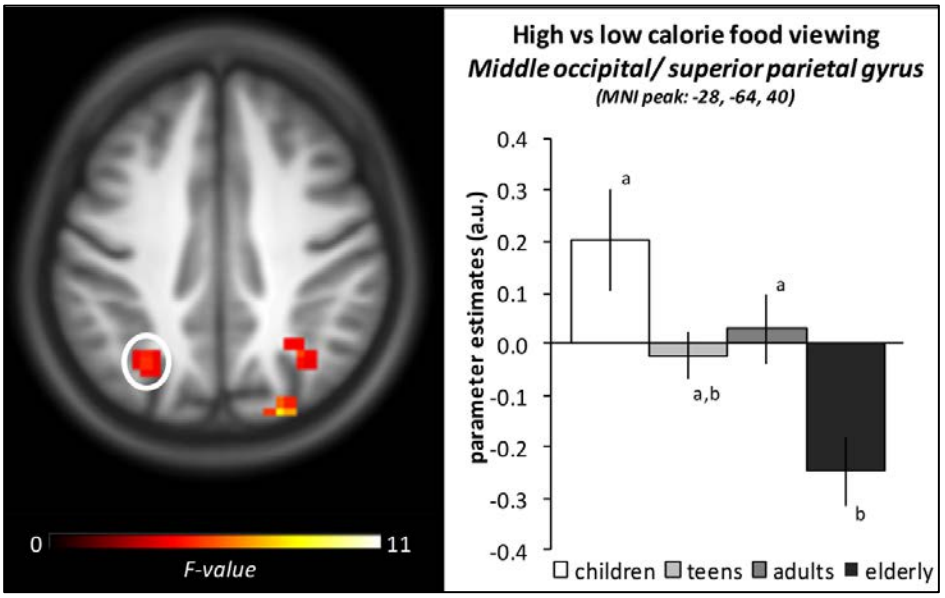


Figure 4.8 Average Middle occipital gyrus/superior parietal gyrus cluster (parameter estimates (mean ± s.e.m) during high vs. low calorie food viewing in the different age groups across hunger states (main effect age group). Shown is a *F*-map thresholded for visualization at F=6.0, p<0.001 uncorrected for multiple comparisons and superimposed on the mean anatomical image of all subjects. In the graph, different letters indicate significant group differences (for example children (a) do not differ from adults and teens (a) but do differ from elderly (b)).

4.4 Discussion

To our knowledge, we were the first to examine food-related brain responses across the lifespan during both hunger and satiety. We found increased dmPFC, vmPFC and dlPFC activation during high compared to low calorie food viewing in a hungry compared to a sated state across age groups. In addition, activation in several visual processing areas including the bilateral middle occipital and superior occipital gyri was affected by age during high compared to low calorie food viewing irrespective of hunger state. We found no such differences during food versus non-food viewing.

Food vs. non-food viewing

During food versus non-food viewing there was only a trend for an effect of age group in the vermis on the border of the lingual gyrus and precuneus. Interestingly, a meta-analysis on food viewing brain activation comparing adults and children/adolescents found that across several studies the lingual gyrus

showed lower activation in children/adolescents (9-18 y) compared to adults (19-45 y) (van Meer et al., 2014). This region is implicated in attention. Thus, our finding suggests that children may have had a higher attention for the non-food items (office utensils). This may be because they do not come across these items on a daily base and may be unfamiliar with them.

Contrary to our expectations, there was no significant effect of hunger state on food vs. non-food viewing across age groups. Unlike other studies, our food stimuli blocks consisted of 50% high and 50% low calorie images. Most studies used high calorie palatable foods in their food vs. non-food comparison, which means that this differs substantially from the comparison we examined (Beaver et al., 2006; Cascio et al., 2012; Cornier et al., 2009; Davids et al., 2009; Malik et al., 2011; Murdaugh et al., 2012; Rubinstein et al., 2011; Schienle et al., 2009; Simmons et al., 2005; Smeets et al., 2013). Additionally, the non-foods used in these studies were highly variable ranging from animals to blurred images, landscapes, non-edible food related utensils, cars, locations and buildings, office utensils and a combination of different types of non-foods (for a complete overview see (van Meer et al., 2014)). The vast majority of these non-foods are very different from the non-foods we utilized (office utensils). This, along with a more rigorous statistical threshold, could account for the fact that we observed no effect of hunger state on food cue activation. This suggests that the degree of hunger we employed (overnight fast) does not increase the salience of food in general across age groups. Rather, hunger effects appear to be specific for high-calorie foods, which is in line with previous studies and also reflected in what we found for high vs. low calorie foods (discussed below).

High vs. low calorie food viewing

We did find an effect of hunger state across age groups for high versus low calorie food viewing; the right dlPFC, bilateral dmPFC and left vmPFC showed increased activation during the fasted compared with the sated state. Only a minor part of the activation differences found in the left dmPFC and left vmPFC could be attributed to differences in food liking between the hunger states. The vmPFC encodes the subjective value of foods but also other goods (Chib et al., 2009). Thus, our findings show that hunger specifically increases the subjective value of high calorie foods irrespective of age, and that this effect is not coupled to changes in liking.

Activation in the dlPFC during high versus low calorie food viewing was increased during hunger as well. The dlPFC is implicated in exercising inhibitory control. In line with our results, increased activation in the medial and lateral PFC was reported during a hungry (overnight fast) compared with a sated state during high compared to low calorie food viewing in normal-weight adults (Goldstone et al.,

2009), while the left dlPFC was found in adults during high compared to low calorie food viewing (Killgore et al., 2003). Although the participants in this study did not eat for 90 min prior to the scan, hunger state was not systematically controlled for. Similar to these results, our results could reflect increased engagement of inhibitory control for high calorie foods during hunger, which we show is irrespective of age.
Furthermore, several predominantly visual areas showed a main effect of age. In these areas, children showed the highest activation and teens the lowest in response to high compared to low calorie foods. Previous studies showed increased activation in visual areas including the middle occipital gyrus and middle temporal gyrus during high compared to low calorie food viewing in adults (Killgore et al., 2003). However, to the contrary, activation in this region in (predominantly) adolescents was decreased during high compared to low calorie food viewing (Killgore and Yurgelun-Todd, 2005), similar to our results in teens. Teens seem to have an attentional bias for low calorie foods although they like high calorie foods more. This might indicate that they consider low calorie options more carefully which may reflect the development of weight-concern. However, more research is needed to further examine this. Children, on the contrary, seem to have an attentional bias for high calorie foods, which is further supported by their higher overall high calorie food liking.

Conclusion
Overall, we can conclude that the effect of hunger state on the brain response to food cue exposure is similar across all age groups. Increased activation in the dmPFC and vmPFC during hunger could reflect the increased reward value of high calorie foods across age groups due to the overnight fast. Additionally, increased dlPFC activation during hunger might reflect a higher inhibitory response to high calorie foods. This may underlie the ability to resist overconsumption of such foods. Age differences were most prominent in visual areas. In these areas, bilateral middle occipital activation was highest in children and lowest in teens, irrespective of food liking. This may reflect an overall higher attention of young children (8-10y) for high calorie foods and a higher attention for low calorie foods in teens. Future studies should further investigate these findings and include overweight and obese individuals of all ages.

4.5 Supplementary material

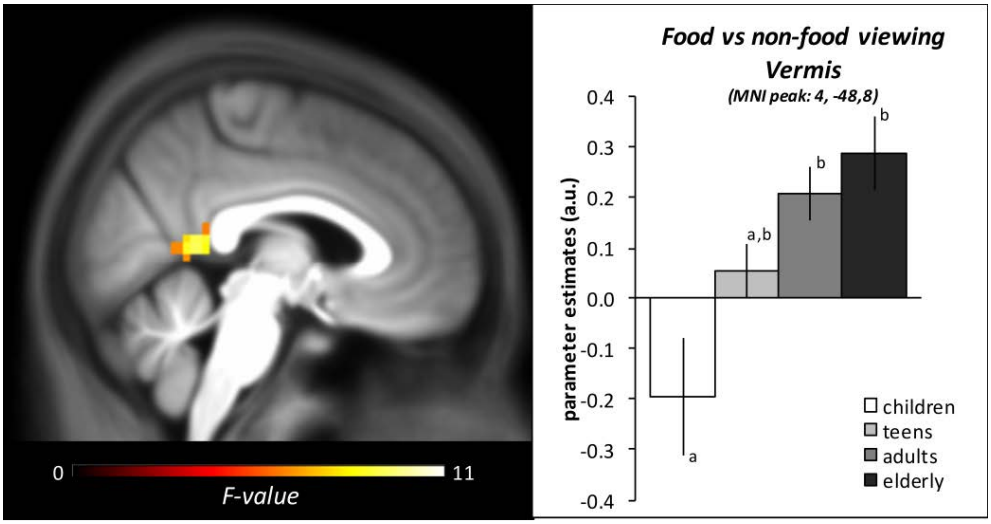


Figure S4.1. Average vermis cluster parameter estimates (mean ± s.e.m) during food vs. non-food viewing in the different age groups, across hunger states (main effect age group). Shown is a *F*-map thresholded for visualization at F=6.0, p<0.001 uncorrected for multiple comparisons and superimposed on the mean anatomical image of all subjects. In the graph, different letters indicate significant group differences (for example children (a) do not differ from teens (a) but do differ from adults (b) and elderly (b)).

Chapter 5

EFFECTS OF HUNGER STATE ON THE NEURAL CORRELATES OF FOOD CHOICE ACROSS THE LIFESPAN

Based on: Charbonnier, L., van Meer, F., Johnstone, A.M., Crabtree, D., Buosi, W., Manios, Y., Androutsos, O., Giannopoulou, A., Viergever, M.A., Smeets, P.A.M. Effects of hunger state on the neural correlates of food choice across the lifespan *(submitted for publication)*

Abstract

In our surroundings food is everywhere. It is easily available and we are continuously tempted to consume food even when we are not actually hungry. Food choices are of great importance, since they determine energy intake and therefore play a crucial role in weight management and overall healthiness across the lifespan. It is unknown how developmental changes in brain structure and function influence food decision making. Thus, it is important to elucidate the mechanisms behind food decisions during childhood, adolescence and adulthood, and how these change with ageing.

In the present study we examined how food choices and associated brain activation are modulated by hunger state and age. In addition we examined to what extent caloric content modulates these brain responses. On two mornings, 95 subjects (18 children, 25 teens, 27 adults, 25 elderly) performed a food choice task while being scanned using fMRI, either fasted or sated.

Children and teens had lower dlPFC activation and lower activation in visual processing areas compared to adults and elderly during food choice. Additionally these made more high calorie food choices. Furthermore, activation between children and teens and between adults and elderly did not differ. In addition, caloric content of the food choices negatively modulated activation in the calcarine sulcus during hunger, while the opposite was true after satiation.

In conclusion, it may be harder for children and teens to resist palatable foods because of their reduced inhibitory capacities, which is reflected in their lower overall dlPFC activation. In addition, hunger appears to lessen attention during high calorie food choice across all ages, which makes people more likely to choose high calorie foods when hungry. The overall higher percentage high calorie choices during hunger further supports this. Children and teens seem to be especially vulnerable during hunger, having the highest percentage of high calorie choices compared to adults and elderly, despite the equal liking of the high and low calorie food choice options. Identifying these vulnerabilities and corroborating them with neuroimaging data may provide a target for interventions to prevent overconsumption of unhealthy foods.

5.1 Introduction

In our surroundings food is everywhere. It is easily available and we are continuously tempted to consume food, even when we are not actually hungry. Food choices are of great importance, since they determine energy intake and therefore play a crucial role in weight management and overall healthiness across the lifespan. The human brain does not fully mature until early adulthood and gradually deteriorates with age. It is unknown how developmental changes in brain structure and function influence food decision making. Thus, it is important to elucidate the mechanisms behind food decisions during childhood, adolescence and adulthood, and how these change with aging. This is especially relevant in the context of the current overweight and obesity epidemic with a high prevalence in all ages.

Every day we make many food choices, which requires valuation of the available choice options. In general, choice values are thought to converge in the ventromedial prefrontal cortex (vmPFC). Different values relevant for the choice at hand are compared by a network including the dorsolateral prefrontal cortex (dlPFC), pre-supplementary motor area and bilateral inferior parietal sulcus, which influences action e.g. by modulation of activation in the motor cortex (Basten et al., 2010; Hare et al., 2011b). Compared to the overall decision making literature, only few studies have examined food choice (Hare et al., 2009; Hare et al., 2011a; van der Laan et al., 2014a). The majority of food choices are made based on visual appearance which, through learned associations, elicits expectations about taste and metabolic consequences. In light of the high prevalence of overconsumption and the intention to eat healthier, it is of interest how perceived healthiness of foods influences the food decision making process. Healthiness of a choice option has been found to increase the value signal formed in the vmPFC when dieters make choices, and this signal is modulated by the dlPFC when self-control is needed, e.g. for rejection of an unhealthy but tasty food (Hare et al., 2009; Hare et al., 2011a). Similar results have been found in non-dieting adults when they considered healthiness during food choices (Hare et al., 2011a). In addition, when weight concerned women fail to choose a healthy food over a highly liked unhealthy food, they show reduced activation of the anterior cingulate cortex (ACC), putatively reflecting a lack of conflict between tastiness (eating enjoyment) and their health goal (Van Der Laan et al., 2014b).

An important factor that can influence food decision making and neural responses to food, is the degree of hunger (e.g. (Hinton et al., 2004; Killgore and Yurgelun-Todd, 2006; Kringelbach and Rolls, 2004; Small et al., 2001). To our knowledge, we were the only ones to examine food choice in a sated state, in normal-weight

adults. We found increased superior temporal sulcus (STS) activation in response to high compared to low calorie food choices with choice options matched on tastiness (Charbonnier et al., 2015). However, the question remains how hunger would affect such food choice activation, as this would make high calorie foods more salient.

The literature on food-related brain responses and food choice in children is very limited. To our knowledge, only one study examined food choice in children and showed that they predominantly base their food choices on tastiness, which correlated with vmPFC activation (Lim et al., 2016). This is similar to what has been found for food valuation signals in adults. However, when the children were instructed to make the choice their mother would make (i.e., healthier choices), their choices correlated with dlPFC activation (Lim et al., 2016). This is in line with the dlPFC activation found in adults during healthy food decision making (Hare et al., 2011a). However, food decision making-related brain responses have not been directly compared between children and adults. Differences can be expected, especially since inhibition areas play an important role in food decision making and these areas in particular change across the lifespan. Children and adolescents are known to have more difficulties with response inhibition, choosing an immediate reward over a delayed one (Killgore and Yurgelun-Todd, 2005). During food choice, this might translate into an increased number of highly palatable high caloric food choices, which may coincide with reduced activation in inhibitory areas such as the dlPFC. Furthermore, hunger state might modulate food choice related brain responses differentially in different age groups. Moreover, it is unknown how these results extend to food choice.

In contrast to children, in which response inhibition and working memory improve with age, elderly experience a gradual decline in these functions (for a review see Luna and Sweeney, 2006). This is most likely caused by reductions in both grey and white matter volumes in especially frontal and hippocampal regions (Kaup et al., 2011; Raz, 2000). To date, no food choice studies in elderly have been conducted. However, since their capacity to inhibit responses declines with age (Sweeney et al., 2001), they might experience difficulty with withstanding highly palatable high calorie foods, similar to children.

When comparing age groups, it is especially of interest to examine choices differing in caloric content (or healthiness) matched on liking, to control for within and between group differences in food preferences, similar to our previous food choice study in adults (Charbonnier et al., 2015).Therefore, the primary aim of the present study was to examine how food choices and associated brain activation are modulated by hunger state and age. The secondary aim was to establish to what extent caloric content modulates these brain responses. Additionally, we aimed to investigate whether STS activation, as found in our previous food choice

study (Charbonnier et al., 2015), is modulated by age and hunger state. We expected overall lower activation in the dlPFC in children and elderly compared to adults during food choice, and additionally expected this lower activation to be more pronounced when hungry, since more inhibition is required in this condition. In addition, we hypothesized higher dlPFC activation during choices in a hungry state. Furthermore, this activation was thought to be modulated by the caloric content of the choices made. In addition, STS activation was expected to be positively correlated with caloric content of choices and this effect was thought to be more pronounced in a hungry state since high calorie foods are more salient then.

5.2 Materials and methods

5.2.1 Participants

We included healthy normal weight (i.e., BMI 20–25 kg/m2 or equivalent) and overweight (i.e., BMI >27.5 kg/m2 or equivalent) adults and children in four age groups (between 8-10, 13-17, 25–45 and 65-75 years). These groups were chosen to ensure that the majority of the children were pre-pubertal, teens pubertal, adults post-pubertal and most likely pre-menopausal and elderly post-menopausal. Additionally, the gaps between the age groups maximize the chance of finding differences between groups. Additional criteria were: being right-handed, non-smoking, having a stable weight (did not gain or lose > 5 kg in the past 6 months), no use of medication (except aspirin/paracetamol and oral contraceptives, and blood thinners and cholesterol medication in elderly) and no current alcohol consumption of > 28 units per week. Furthermore, common fMRI exclusion criteria (e.g. claustrophobia, pregnancy and metal implants in the body) and criteria that might influence response to food cues (e.g. food allergies, special diets, eating disorders, gastrointestinal disorders or metabolic or endocrine disease, highly restraint eating scores on the Dutch Eating Behavior Questionnaire) were used. In addition, fMRI runs with excessive head movement were excluded from the analyses. 147 participants enrolled in the study. However, data from 44 participants (11 children; 4 teens; 12 adults; 17 elderly) were excluded because. These participants did not enough food choice pairs could be generated during one or both visits (see design below): not enough food choice pairs during the hungry condition (n=5: 2 teens; 3 elderly), not enough pairs during the sated condition (n=28: 7 children; 2 teens; 10 adults; 9 elderly), not enough pairs during both conditions (n=11: 2 adults; 4 children; 5 adults). This is further explained in paragraph 5.2.2.2. Additionally, one adult was excluded

because of missing food picture rating task data, one adult because of medication use, one adult did not complete both study days, one child had a neurological disorder, one child had an incomplete dataset and 3 children had excessive head movement. The final sample included in the analyses consisted of 95 subjects (18 children, 25 teens, 27 adults, 25 elderly).

Table 5.1. Participant characteristics

	Children[1] n =18	Teens[2] n =25	Adults[3] n =27	Elderly[4] n =25
Gender (M:F)	5:13	10:15	15:12	14:11
Age (yr)	9.8±0.9	15.1±1.4	34.7±7.2	68.9±3.0
BMI (kg/m^2)	n/a	n/a	27.2±4.1	26.6±3.9
BMI-SDS	0.5±1.2	0.4±1.1	n/a	n/a
Ratio NW:OW	13:5	22:3	12:15	14:11
Interscan interval (days)	9.7±3.1	10.3±3.7	8.6±2.6	9.2±2.9
First visit (S:H)	9:9	13:12	11:16	12:13
Amount liquid breakfast consumed (ml)	324±142	458±140	579±95	478±59

Participant overlap with Chapter 4: [1]children: n= 15; [2]teens: n=21; [3]adults: n=10; [4]elderly: n=14

5.2.2 Experimental design

5.2.2.1 Study procedures

The study consisted of two morning MRI scan sessions. On both days, participants came in after an overnight fast of at least ten hours. During one session the participants were scanned after the consumption of a fixed amount of a liquid breakfast (a commercially available vanilla whey protein shake from XXL Nutrition prepared with whole milk), 1.4 x basic metabolic rate (BMR), calculated with the Schofield equation. With this equation an individual's BMR can be estimated by using age, gender and weight (Schofield, 1985; Schofield, 1984). During the other session participants were scanned in after an overnight fast (≥10h). The time between the two scan sessions was 1-2 weeks. The order of conditions (i.e. hungry or sated state) was counterbalanced. Upon arrival on the study day, participants filled out several questionnaires and executed a computerized food picture rating task. During this task, 133 images were rated on liking, perceived caloric content and perceived healthiness (caloric content question omitted in children). These ratings were used to create food choice pairs during the food choice fMRI task (see for more details the picture rating task paragraph). On the sated day, the food picture rating task was executed 20 minutes after liquid breakfast consumption. Participants entered the scanner approximately 1 hour after liquid breakfast consumption. For hormone analyses (not part of the current analysis) blood was collected in adults through a cannula placed in an antecubital vein at several time points on both mornings. Subsequently, participants underwent a 38-min MRI scan session consisting of four functional MRI runs during which they performed a food viewing task, a food choice task and a monetary reward task. The results of the food choice task are the focus of this paper. See for more details the study procedure on page 50.

5.2.2.2 Food picture rating task

Before each MRI session participants performed a food picture rating task (FPRT), during which they rated 133 food images. Teens, adults and elderly rated the images on liking (How much do you like this product at this moment? 1, not at all - 9, very much), perceived caloric content (How many calories do you think the product has? 1, very few calories – 9, no calories at all) and healthiness (How healthy do you think the product is? 1, not healthy at all – 9, very healthy) on 9-point Likert scales. For children, 5-point Likert scales were used and the calorie question was omitted. Each food image was shown for 3 seconds after which the liking question appeared. When answered, the question disappeared and the calorie question appeared, similarly followed by the health question. There was no time limit to answer a given question, although participants were instructed to

answer each question as quickly as possible (see the supplement S5.1 for the exact instructions). A given answer could not be changed. When an image was missed or not recognized, the participants were instructed to answer all the questions about that image with "1". These ratings were excluded from the matching process. Since the vast majority of the images was expected to be recognized, based on ratings collected from a separate group of adults in a pre-test, this method was preferred over adding an extra question on recognizability for each image. Based on the ratings a list of image pairs (matched images), similar in liking (±1 point on the 9- point Likert scale) but different in caloric content (calorie ratings >5 were matched with ratings <5) was created. Images with liking ratings ≤4 were categorized as disliked and were not used. The liking matching was done to ensure that the choice options in each choice pair only differed in caloric content. This is important since food liking is thought to differ across age (Steiner, a977; Anliker, 1991; Zandstra, 1998; Griep, 1996) and may be affected by hunger state.
In children the FPRT included a question on the recognizability of each image. When an image was not recognized, the additional questions were skipped and the next image was shown. For all recognized images a question on liking and healthiness was asked. The question on perceived calories was expected to be too difficult and was omitted. Here, a 5-point Likert scale with smiley faces was used. Similar to the other age groups, for children a list of choice pairs similar in liking (±1 point on the 5 point Likert scale) but differing in caloric content (calorie ratings >3 were matched with ratings <3) was created. Images with liking ratings ≤2 were categorized as disliked and were not used.

5.2.2.3 Food choice fMRI task

The food choice fMRI task (Figure 5.1) consisted of two parts (runs). Each part started with two practice choices, followed by 50 binary food choices alternated with 23 binary non-food choices. Participants were verbally instructed to choose the product of which they most wanted to eat at that particular moment when they were presented with a food pair, and to choose the largest product whenever they were presented with a non-food pair (see the supplement S5.1 for the exact instructions). As a reminder a brief instruction was shown above every choice pair (for food pairs: choose the product you most want to eat at this moment; for non-food pairs: choose the largest product). Participants were told that they would receive one of the chosen foods after the scan session.

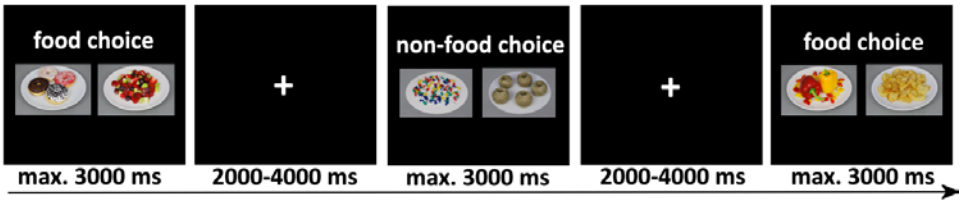


Figure 5.1. Structure of the food choice task.

5.2.3 Image acquisition

All imaging was performed on a Philips 3.0T Achieva whole-body MRI scanner (Philips Healthcare, Best, NL). Functional images were obtained using a 2-D echo planar imaging-sensitivity encoding (EPI-SENSE) sequence with the following parameters: voxel size 4 mm isotropic; repetition time (TR) = 1400 ms; echo time (TE) = 23 ms; flip angle = 72.5°; FOV = 208 x 119.6 x 256 mm; 30 axial slices; SENSE-factor *R* = 2.4 (anterior-posterior); scan duration ~473 s). A high resolution anatomical image (T_1-weighted scan) was acquired at 1 × 1 × 1 mm resolution (TR = 8.4 ms; TE = 3.8 ms; flip angle 8°; FOV = 288 mm × 288 mm × 175 mm; 175 sagittal slices; total scan duration =284 s).

5.2.4 Data analyses

5.2.4.1 Behavioral analyses

Behavioral data were analyzed with the use of SPSS statistics 23. Hunger and fullness ratings were analyzed by using four paired sample t-tests per age group. The significance threshold was Bonferroni corrected (p=0.05/4 = 0.0125). Differences in high calorie food choice percentages and reaction times were analyzed by using a repeated measures ANOVA and subsequent Bonferroni-corrected post-hoc tests.

5.2.4.2 fMRI image preprocessing

Image preprocessing and analyses were carried out with the SPM12 software (http://www.fil.ion.ucl.ac.uk/spm). After slice timing correction and realignment, the structural scan was coregistered to the mean functional scan. Next, the structural scan was segmented using unified segmentation, and normalization parameters were estimated. A study-specific anatomical template was created using DARTEL, and after coregistration DARTEL was used to normalize this template and the functional scans to MNI (Montreal Neurological Institute) standard space. The functional data were then smoothed with an 8 mm full width

at half maximum isotropic Gaussian kernel. The Volume Artefact tool from ArtRepair (http://cibsr.stanford.edu/tools/ArtRepair/ArtRepair.htm) was used to detect and repair anomalously noisy volumes. Volumes that moved more than 1 mm/TR were repaired. Three children were excluded from analysis because of too many volumes (>30%) that had to be repaired.

5.2.4.3 fMRI Individual & group analyses

For each subject the following conditions were modeled: food choice, non-food choice, spare choices and missed choices. For the first analysis, the average brain activation during food choice across conditions (mean hungry and sated) and between conditions (hungry – sated) were calculated for each subject. These contrast images were then submitted to two 2x4 ANOVAs to test for effects of hunger and age-group on food choice activation. The resulting statistical parametric maps were masked with an average grey matter mask of the group (grey matter probability >0.1) and thresholded at p=0.001 with a cluster extent k≥23, which corresponds to p<0.05 corrected for multiple comparisons across the whole analysis mask. This threshold was derived using Monte Carlo simulations (10,000 iterations) of random noise distributions in the brain mask using the 3dClustSim routine in AFNI. This approach combines an individual voxel probability threshold with a minimum cluster size to estimate the probability of a false positive.

For the second analysis, perceived healthiness ratings for every chosen food were added as a parametric modulator on first level for each subject during food choice versus rest. Subsequently, the average level of food choice modulation by perceived caloric content across conditions (mean hungry and sated) and between conditions (hungry – sated) were calculated for each subject. These contrast images were then submitted to two 2x4 ANOVAs to investigate age-group and hunger condition differences in the modulation of food choice activation by perceived caloric content of the chosen food. Similar to the first analysis, the statistical parametric maps generated were masked with an average grey matter mask of the group (grey matter probability >0.1). The threshold used was p<0.001 with a cluster extent k≥26, which corresponds to p<0.05 corrected for multiple comparisons across the analysis mask.

In addition, for each analysis described above, an ROI analysis was conducted using a mask containing the OFC, amygdala and STS thresholded at p<0.001 corrected for mask volume.

5.3 Results

5.3.1 Behavioral analyses

5.3.1.1 Hunger and fullness ratings

Baseline hunger and fullness ratings did not differ significantly between the study days (Table 5.2) (hungry: (children: $t(17) = -0.615$, $p=0.547$; teens: $t(24) = 2.487$, $p=0.020$; adults: $t(26) = 1.521$, $p=0.140$; elderly: $t(24)= -0.084$, $p=0.933$; fullness: children: $t(17) = -2.500$, $p=0.023$; teens: $t(24) = 2.324$, $p=0.029$; adults: $t(26) =-0.284$, $p=0.779$; elderly: $t(24)=-0.109$, $p=0.914$). For all age groups hunger ratings prior to the scan were significantly lower on the sated day (children: $t(17)=5.17$, $p<0.001$; teens: $t(23)=5.24$, $p<0.001$; adults: $t(17) = 7.75$, $p<0.001$; elderly: $t(24)=5.06$, $p<0.001$).

Table 5.2 Hunger and fullness ratings[1]

	Children	Teens	Adults	Elderly
Hunger condition				
Hunger ratings				
Baseline	3.1±0.9	5.5±2.3	6.2±1.8	4.6±2.1
20 min after drink	n/a	n/a	n/a	n/a
Prior to scan	4.2±0.9	6.8±1.7	7.0±1.3	5.5±1.8
Fullness ratings				
Baseline	1.6±0.6	3.0±1.7	2.9±1.7	3.4±1.9
20 min after drink	n/a	n/a	n/a	n/a
Prior to scan	1.5±0.7	3.1±1.7	2.6±1.3	3.5±1.5
Sated condition				
Hunger ratings				
Baseline	3.3±1.1	5.6±2.0	5.6±2.0	4.0±2.3
20 min after drink	2.1±0.9	2.4±1.6	2.7±1.5	3.1±2.4
Prior to scan	2.9±1.1	4.4±1.9	3.6±1.8[6]	3.8±2.2
Fullness ratings				
Baseline	2.2±0.7	3.0±1.3	3.0±1.5	2.8±1.7
20 min after drink	3.5±0.6	6.8±1.6	6.4±1.9	5.6±1.5
Prior to scan	2.6±1.1	6.5±1.6	5.7±2.3	4.2±1.7

[1] *In teens, adults and elderly 9-point Likert scales were used, while in children 5-point Likert scales were utilized.*

Similarly, fullness ratings, except for elderly, were significantly higher on sated days (children: $t(17)=-0.52$, $p=0.001$; teens: $t(23)=-7.07$, $p<0.001$; adults: $t(17) = -5.53$, $p<0.001$; elderly: $t(24)=-1.74$, $p=0.094$).

5.3.1.2 Food choice outcome

There was a main effect of hunger state across age groups for the percentage high calorie choices ($F(1,91)=6.930$, $p=0.010$); all groups chose less high calorie foods when sated (Figure 5.2). Additionally, there was a main effect of age group ($F(3,91)= 11.067$, $p<0.001$); Elderly chose significantly more low calorie foods compared to the other age groups (elderly-children: $p<0.001$; elderly-teens: $p<0.001$; elderly-adults: $p=0.038$) while the other groups did not differ significantly from each other (children-teens: $p=1.000$; children-adults: $p=0.109$; teen-adults: $p=0.178$).

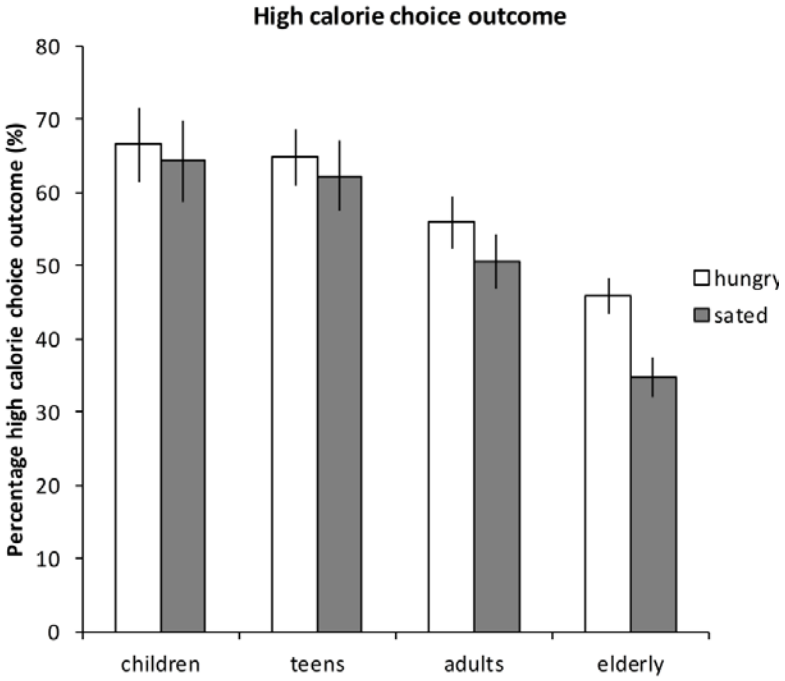


Figure 5.2 High calorie choice outcome per age group and hunger state. Main effect of hunger state: F(1,91)=6.930, p=0.010; Main effect of age group: F(3,91)= 11.067, p<0.001 (children-teens: p=1.000; children-adults: p=0.109; children-elderly: p<0.001; teen-adults: p=0.178; teens-elderly: p<0.001; adults-elderly: p=0.038).

5.3.1.3 Food choice reaction times

There was a main effect of age on reaction times ($F(1,85)=70.414$, $p<0.001$); both children and teens had lower reaction times (i.e., chose quicker) than adults and elderly (Figure 5.3). All groups differed significantly from one another except for children and teens (children-teens: $p=1.000$; children-adults: $p=<0.001$; children-elderly: $p<0.001$; teens-adults: $p<0.001$; teens-elderly: $p<0.001$; adults-elderly: $p<0.001$). Elderly had the longest reaction times relative to the other groups. In addition, there was a significant effect of age group by caloric content of the choice outcome ($F(1,85)=3.516$, $p=0.019$). Children and teens had lower reaction times during high calorie choices, while adults and elderly did not (elderly even showed an opposite pattern; higher reaction time during high calorie choice).

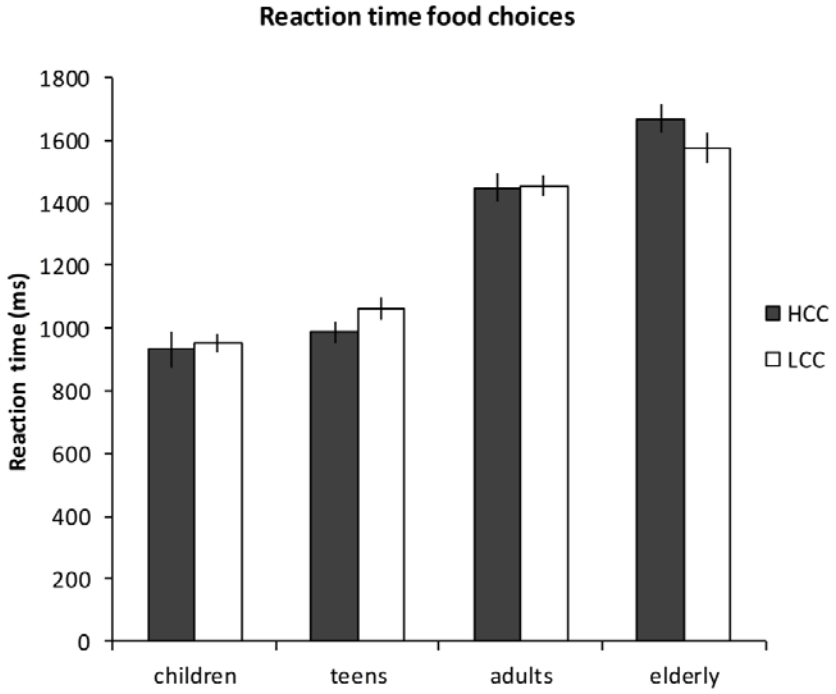


Figure 5.3 Reaction time food choices
Reaction time (mean ± s.e.m) high calorie choices (HCC) and low calorie choices (LCC).

5.3.2 Food choice brain activation

There was no significant effect of hunger state on food choice related-brain activation across the age groups.
There were significant main effects of age in several regions including the dorsolateral prefrontal cortex (dlPFC, Figure 5.4), postcentral gyrus, middle cingulum, middle and superior occipital gyrus (Table 5.4). In the middle occipital gyrus cluster, activation in children and teens was lower compared to that in adults and elderly. In the other clusters, activation in children and teens tended to be lower compared to adults, however only activation in teens differed significantly from adults and elderly. In all clusters, children and teens, and adults and elderly did not differ significantly. See for more details Figure S5.1.
There was no significant interaction between hunger state and age group.

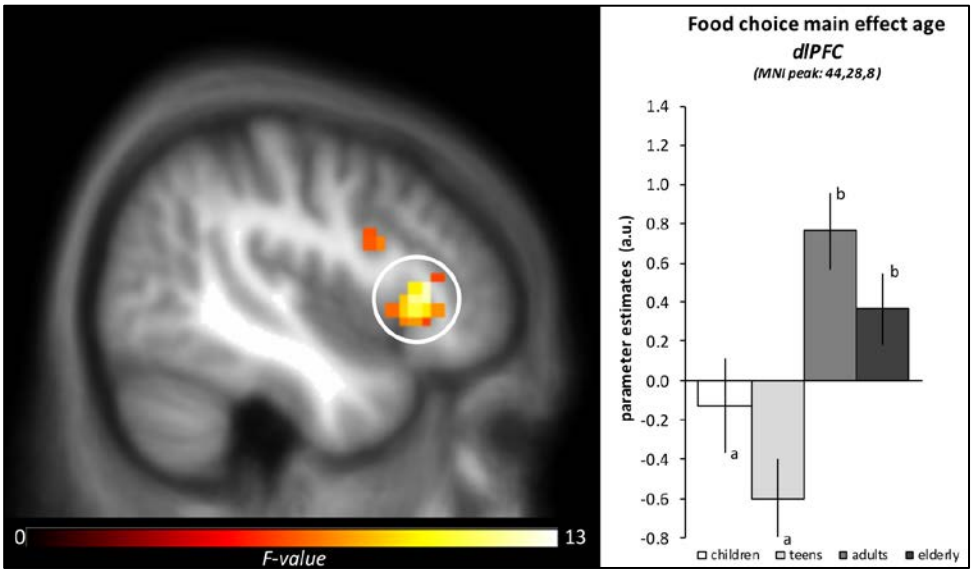


Figure 5.4 Average dlPFC cluster parameter estimates (mean ± s.e.m.) during food choice. Shown is an F-map, thresholded for visualization at F=6.0 p<0.001 uncorrected for multiple comparisons and superimposed on the mean anatomical image of all subjects. In the graph, different letters indicate significant group differences (for example teens (a) do not differ from children (a) but do differ from adults (b) and elderly (b)); Children-teens: p=0.757; children-adults: p=0.023; children-elderly: p=0.680; teens-adults: p<0.001; teens-elderly: p=0.005; adults-elderly: p=0.877.

Table 5.4 Brain regions showing a main effect of age on food choice activation[1]

		Peak MNI-coordinate (mm)				
Region	*k*	x	y	z	*F*	*Z*
Middle occipital gyrus, R[2]	70	28	-84	8	12.58	4.84
Middle occipital gyrus, R		28	-68	32	7.73	3.67
dlPFC (Inferior frontal gyrus, triangular part, R)[3*]	66	44	28	8	12.14	4.75
Insula, R		36	20	-8	9.44	4.13
Superior occipital gyrus, L[4]	227	-20	-68	24	11.88	4.70
Calcarine sulcus, L		-16	-68	8	10.68	4.43
Middle occipital gyrus, L		-28	-72	20	10.07	4.29
Postcentral gyrus, L[5]	119	-48	-20	44	11.29	4.57
Inferior parietal gyrus, L		-56	-36	40	7.50	3.60
Middle cingulum, R[6]	69	8	16	40	9.80	4.22
Supplemental motor area, R		4	8	52	9.39	4.12

*[1] Whole brain analysis. Peaks are reported for all clusters ≥23voxels at p<0.001 uncorrected for multiple comparisons; L = left and R= right hemisphere. *in the ROI mask the same area was significant. [2]Middle occipital gyrus: children-teens: p= 1.000; children-adults: p=0.008; children-elderly: p=0.002 ; teens-adults: p<0.001; teens-elderly: p<0.001; adults-elderly: p=1.000. [3] dlPFC: children-teens: p=0.757; children-adults: p=0.023; children-elderly: p= 0.680; teens-adults: p<0.001; teens-elderly: p=0.005; adults-elderly: p=0.877. [4]Superior occipital gyrus: children-teens: p=0.919; children-adults: p=0.076; children-elderly: p=0.001 ; teens-adults: p<0.001; teens-elderly: p<0.001; adults-elderly: p=0.709. [5]Postcentral gyrus: children-teens: p=0.446; children-adults: p=0.334; children-elderly: p=0.018 ; teens-adults: p<0.001; teens-elderly: p<0.001; adults-elderly: p=1.000; [6]Middle cingulum: children-teens: p=1.000; children-adults: p=0.007; children-elderly: p=0.061 ; teens-adults: p=0.001; teens-elderly: p=0.013; adults-elderly: p=1.000.*

5.3.3 Modulation of food choice activation by perceived caloric content

There was a main effect of hunger state in the calcarine sulcus and precuneus (Table 5.5); Calcarine sulcus and precuneus activation were positively modulated by perceived caloric content in the sated state. Thus, food choices higher in perceived calories were associated with greater calcarine sulcus and precuneus activation. In the hungry state, the contrary pattern was apparent (Figure 5.5).
There was no significant effect of age on the modulation of food choice activation by perceived caloric content and no interaction effect.

Table 5.5 Brain regions showing a main effect of hunger state on the modulation of food choice activation by perceived caloric content[1].

		Peak MNI-coordinate (mm)				
Region	*k*	x	y	z	*F*	*Z*
Calcarine sulcus, L	38	-4	-52	8	19.08	3.98
Precuneus, R		20	-52	16	14.20	3.43
Calcarine, R		16	-48	8	12.50	3.21

[1] *Whole brain analysis. Results of an ANOVA main effect hunger state; Peaks are reported for all clusters ≥ 26 voxels at p<0.001 uncorrected for multiple comparisons; L = left and R= right hemisphere.*

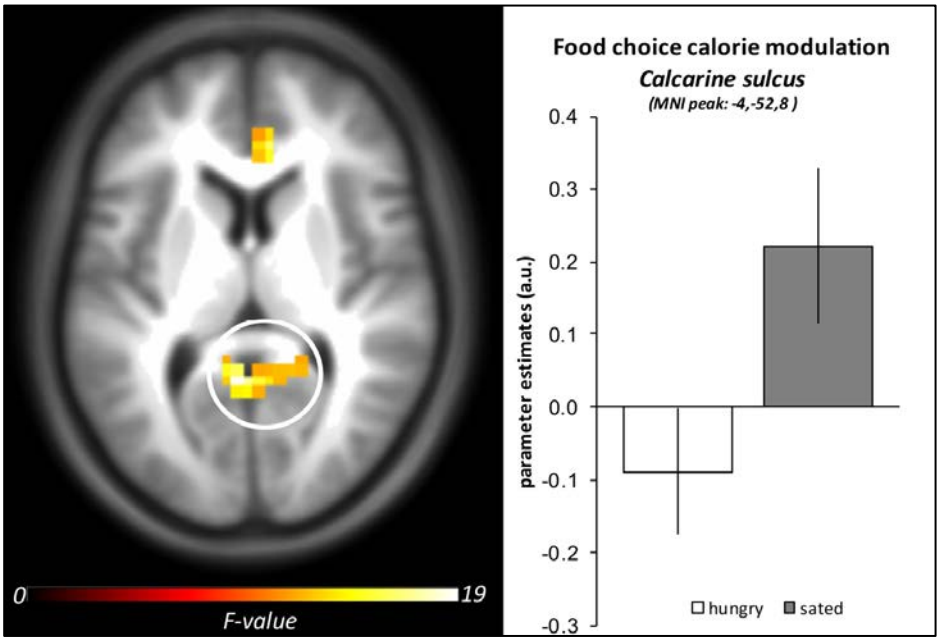


Figure 5.5 Average calcarine sulcus cluster parameter estimates (mean ± s.e.m.) of the modulation of food choice activation by caloric content of the choice outcome in the hungry and sated state (main effect hunger state). Shown is a *F*-map, thresholded for visualization at F=11.0, p<0.001 uncorrected for multiple comparisons, superimposed on the mean anatomical image of all subjects.

5.4 Discussion

We examined how hunger state and age affect brain activation while choosing between low and high calorie foods, matched on preference. Hunger state had no overall effect on choice activation, however, there were several differences between age groups. Children and teens showed lower choice activation compared to adults and elderly in the dlPFC, visual processing areas such as the middle occipital gyrus, postcentral gyrus and middle cingulum. There was no difference in choice activation between children and teens, and between adults and elderly. In addition, calcarine activation was negatively modulated by caloric content of the chosen food in the hungry state, while there was a positive modulation in the sated state.

dlPFC

Children and teens showed lower (right) dlPFC activation than adults during food decision-making, irrespective of hunger state. In addition, dlPFC activation in elderly was higher than that in teens, but did not differ from that in children and adults, although there was a trend towards lower activation in children and higher activation in elderly. The dlPFC has been implicated in response inhibition (Aron et al., 2004) and plays a crucial role in self-control (Hare et al., 2009; Hare et al., 2011a). Thus, our results suggest that children and teens exert less inhibition during making food choices between equally preferred choice options differing in caloric content. This may explain their higher overall percentage of high caloric choices made, compared to adults and elderly and their lower reaction times during a high compared to low calorie choices. dlPFC choice activation did not differ between adults and elderly, although elderly tended to have lower dlPFC activation. Despite this trend, elderly made more low calorie choices than adults. Thus, contrary to our hypothesis, aging is not associated with diminished inhibitory control during food decision-making, at least not when matching choice options on preference.

Visual processing areas

Similar to the dlPFC activation pattern, the superior and middle occipital gyrus showed decreased activation during food choice in children and teens, while increases were apparent in adults and elderly. In all these regions, choice activation did not differ between children and teens, or between adults and elderly. The superior and middle occipital gyri are involved in visual processing. More specifically, the middle occipital gyrus has been found to activate while choosing packaged foods in adults: activation was increased during presentation of subsequently chosen compared to not chosen packages (Van der Laan et al., 2012). Additionally, in a meta-analysis, the middle occipital gyri were found to be most consistently activated across several studies during highly palatable versus neutral food viewing in adults (van der Laan et al., 2011). Similarly, activation in this region was increased during viewing of high compared to low calorie food pictures, matched on visual properties (Frank et al., 2010b). This suggests that in our study, with carefully standardized food images (Charbonnier et al., 2016)and additional matching on liking, activation differences in the middle occipital gyri are related to the caloric content contrast. In addition, activation in this region can be influenced by attention and emotional valence by increasing activation in related visual processing areas (Gerdes et al., 2010; Lane et al., 1999; Novemsky and Ratner, 2003). Thus, in our study greater middle frontal gyrus activation may reflect heightened visual attention during the decision making process. Adults and elderly might have experienced the choices as more challenging than children due

to the matching on liking and their greater knowledge and awareness of caloric content and healthiness of foods. This effort may have resulted in heightened visual attention during food decision making, resulting in increased middle frontal gyrus activation. Conversely, children may be more inclined to just choose one of the options, with less consideration, reflected in less visual attention and thus less middle frontal gyrus activation. This interpretation is supported by the longer reaction times of adults and elderly. However, reaction time differences may also reflect general age effects; children and teens are known for their quicker reaction times.

Modulation of food choice activation by caloric content

Calcarine sulcus

In the calcarine sulcus, which contains the primary visual cortex, activation was negatively modulated by caloric content during hunger while there was positive modulation of choice activation in the sated condition. A recent food viewing meta-analysis found that the calcarine sulcus is among the most consistently activated regions during food compared to non-food viewing in both children/adolescents and adults (van Meer et al., 2014). More specifically, several studies showed increased activation in this region in response to high calorie food images in adults during hunger (Kullmann et al., 2012) and in adolescent non-smokers compared to smokers (hunger state not reported) (Rubinstein et al., 2011). Additionally, a food choice study reported increased calcarine sulcus activation during high compared to low calorie choices during mild hunger (2-3 h fast) in weight-concerned women (Van Der Laan et al., 2014b). These results are thought to reflect increased visual attention for high calorie foods, especially when hungry (Kullmann et al., 2012; Smeets et al., 2013; Van Der Laan et al., 2014b). In contrast, we found less calcarine sulcus activation when choosing foods higher in calories when hungry. This suggests that in a hungry state, less visual attention is engaged for choices higher in calories. This is putatively because they are more biologically salient and thus have a higher choice value, which may facilitate the decision-making process, requiring less attentional resources. Reversely, when sated higher calorie food choices engage more attention. These findings align well with the finding that the reward value of familiar foods depends on 'implicit knowledge' of their caloric content(Tang et al., 2014) and additionally demonstrate the importance of hunger state as a powerful modulator of food-related decision-making.

Superior temporal sulcus (STS)
In our previous study in sated normal-weight adults we found increased STS activation during high versus low calorie choices (Charbonnier et al., 2015). In the present study we did not replicate this finding, although we hypothesized that STS activation would be modulated by perceived caloric content. This might be due to several differences in the design: in the present study, disliked images were not included in choice pairs. Furthermore, the participant group was more heterogeneous including both normal-weight and overweight children, teens, adults and elderly. In addition, the ad libitum intake was replaced by a personalized load, based on the Schofield equation. All participants were instructed to completely consume their drink and were most likely more sated that the group in our previous study.
Secondly, we did not compare average activation during low- and high calorie choices but rather used perceived caloric content as a parametric modulator of choice activation.

Conclusion
In conclusion, it may be harder for children and teens to resist palatable foods because of their reduced inhibitory capacities, which is reflected in their lower dlPFC activation, greater number of high calorie food choices and quicker response during high calorie choices. Hunger appears to lessen attention during higher calorie food choice across all ages, which may make it more likely that people choose high calorie foods when hungry. This is supported by the higher percentage of high calorie food choices made when hungry across all age groups. However, children and teens seem to be especially vulnerable when hungry, choosing the highest number of high caloric foods, even though the low calorie options were similar in liking. Identifying these vulnerabilities and corroborating the underlying choice mechanisms with neuroimaging data may provide better targets for interventions aimed at the prevention of overconsumption of unhealthy foods.

5.5 Supplementary material

S5.1 Instruction given before the food choice fMRI task:
During this task you will have to make choices between two options (i.e. two non-food options, a non-food pair and two food options, a food pair). During the non-food pairs (i.e. office utensils) you'll have to choose the largest object (the left or the right object). During the food pairs, you'll have to choose the product of which you most want to eat now. It's important that you keep in mind that you can eat as much or as little of the food as you like and that you will receive one of the foods you chose after the task. You'll have a limited time to choose so choose as quickly as possible.

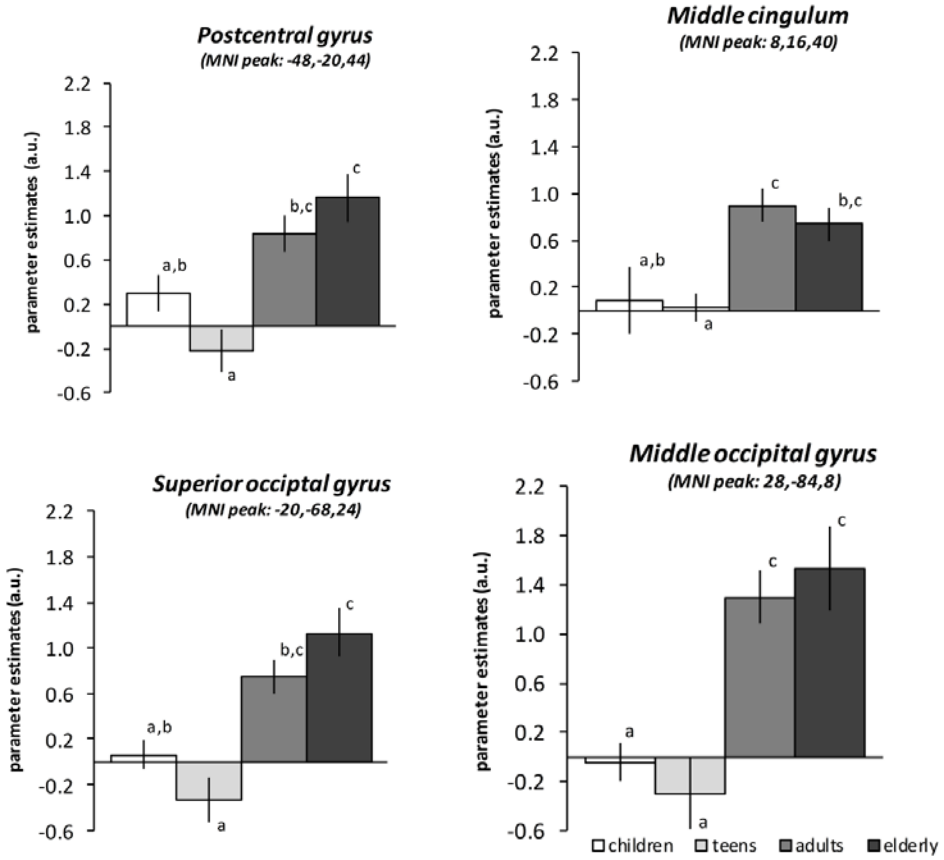


Figure S5.1 Average parameter estimates activation (mean ± s.e.m.) for each significant cluster during food choice effected by age irrespective of hunger state. In the graphs, significant differences are indicated with different letters. Postcentral gyrus: children-teens: p= 0.446; children-adults: p=0.334; children-elderly: p=0.018 ; teens-adults: p<0.001; teens-elderly: p<0.001 ; adults-elderly: p =1.000. Middle cingulum: children-teens: p=1.000; children-adults: p=0.007; children-elderly: p=0.061 ; teens-adults: p=0.001; teens-elderly: p=0.013; adults-elderly: p = 1.00. Superior occipital gyrus: children-teens: p=0.919; children-adults: p=0.076; children-elderly: p=0.001; teens-adults: p<0.001; teens-elderly: p<0.001 ; adults-elderly: p = 0.709. Middle occipital gyrus: children-teens: p=1.000; children-adults: p=0.008; children-elderly: p=0.002; teens-adults: p<0.001; teens-elderly: p<0.001; adults-elderly: p =1.000.

Chapter 6

HUNGER FOR MORE: LACK OF STRIATAL DOWNREGULATION AFTER SATIATION DURING MONETARY REWARD ANTICIPATION IN OVERWEIGHT INDIVIDUALS

Based on: Charbonnier, L., Vink, M., van Meer, F., Viergever, M.A., Smeets, P.A.M. Hunger for more: Lack of striatal downregulation after satiation during monetary reward anticipation in overweight individuals. *(submitted for publication)*

Abstract

Overweight and obesity are persistent conditions with major health implications. It has been hypothesized that the reward value of food combined with its palatability promotes overeating and subsequent weight gain. Alterations in food reward processing in overweight and obesity have been reported. However, to what extent responsiveness of the reward system to other types of reward is affected is not well established. In addition, since hunger state affects food reward processing differentially in obesity, it may also influence processing of other reward types.

We examined the effects of hunger and weight status on the brain response to monetary reward anticipation and receipt. On two mornings, 35 subjects (17 overweight) performed a 10-min monetary reward task while being scanned using fMRI, either fasted or sated.

When fasted, striatum activation during reward anticipation was equal for both weight groups. However, this striatum activation decreased during satiety in the normal-weight but not the overweight group. This effect was mirrored in liking ratings of food images, which declined more with satiety in normal-weights. For reward receipt there were no significant differences.

These data might explain eating in the absence of hunger since the reward system of overweight individuals appears to remain in hungry-mode despite being sated. This may hamper weight loss attempts. Further research should elucidate whether this is a causal risk factor or a characteristic of obesity.

6.1 Introduction

Overweight and obesity are ever-rising problems with major health implications, concerning over 1.9 billion adults world-wide in 2014 (WHO). The primary cause of overweight and obesity is a positive energy balance, i.e. greater energy intake than expenditure. The reason behind this apparent inability to control food intake is still unknown. It is hypothesized that the reward value of food combined with its palatability promotes food consumption, which increases the risk of overeating and subsequent weight gain. The brain reward system is thought to be involved since it plays a crucial role in motivated behaviors including the motivation to eat (Kelley et al., 2005).

Previous studies implied that reward processing can be divided into an anticipation phase (i.e., the response prior to reward receipt) and a receipt phase (i.e., obtaining the actual reward) (Knutson et al., 2001b; Schultz et al., 1993; Schultz and Romo, 1990). Alterations in each phase have been associated with increased risk of overconsumption and subsequent weight-gain. Hyper- and hypo-activation of this reward system in response to food receipt, as well as increased food reward anticipation have been hypothesized to increase risk for overeating (Blum et al., 2000; Dawe and Loxton, 2004; Pelchat et al., 2004; Roefs et al., 2005; Wang et al., 2002).

The vast majority of the food reward literature assessed brain activation in response to passive viewing of food images (for a meta-analysis see (Pursey et al., 2014)). Increased activation in obese compared to normal-weight individuals, in response to visual food cues (Martin et al., 2010; Rothemund et al., 2007; Stoeckel et al., 2008) and food cue imagery (Jastreboff et al., 2013) has been reported in several brain regions including the striatum. However, it is unclear whether these studies measured actual food reward anticipation since no subsequent food consumption was involved (Stice et al., 2009).

Only few studies measured reward anticipation and receipt for actual food consumption in obesity (DelParigi et al., 2005; Ng et al., 2011; Stice et al., 2008a; Stice et al., 2008b; Stice et al., 2010; Stice et al., 2011). A study in obese adolescent girls reported increased activation in the insula, frontal, parietal and rolandic operculum to both the anticipation and consumption of a milkshake compared to a tasteless control solution during hunger. Furthermore, caudate activation was decreased in obese compared to normal-weight girls in response to milkshake consumption (Stice et al., 2008b). In addition, a study in normal-weight adolescents at risk for obesity showed no differences in reward anticipation for both food and money during hunger, however, increases were found during reward receipt (Stice et al., 2011). To the contrary, a study in obese adults reported increased activation in the obese group in the rolandic operculum,

inferior frontal gyrus and frontal operculum during high versus low fat milkshake anticipation, while receipt showed increases in the rolandic operculum and vmPFC in obesity (Ng et al., 2011).

Overall the results across studies vary and the different designs, groups, ages, and BMI ranges studied make them hard to compare. In addition, there is variation in hunger state between studies. It is known that hunger state increases the brain response to food reward (Cornier et al., 2013; Gautier et al., 2000; Goldstone et al., 2009; Stice et al., 2009) and this effect might be modulated by weight status; obese individuals are thought to be more responsive when sated (for a meta-analysis see (Kennedy and Dimitropoulos, 2014)). Taken together, it is clear that obese individuals have alterations in food reward processing. However, it is unclear to what extent this relates to the responsiveness of the reward system in general and whether this is modulated by hunger state, in a similar fashion as food reward processing.

A task that has been extensively used to study reward processing in healthy volunteers and in a broad range of disorders, is the monetary incentive delay task (de Leeuw et al., 2015; Figee et al., 2011; Knutson et al., 2001a; van Hell et al., 2010; Vink et al., 2015). The anticipation of monetary reward has consistently been linked to increased activation in the ventral striatum (Knutson et al., 2001a), while reward receipt activates the orbitofrontal cortex/ventromedial prefrontal cortex (OFC/vmPFC) in healthy participants (Knutson et al., 2001a; Sescousse et al., 2013). In addition, studies comparing the valuation of food and money, report overlapping brain regions for the processing of different reward types, including the striatum (FitzGerald et al., 2009) and vmPFC (Chib et al., 2009; Kim et al., 2011).

To date, only few monetary reward studies have been conducted in obesity. Increased striatal activation has been reported in obese subjects during monetary reward anticipation (Balodis et al., 2013), while decreases were found during reward receipt in several regions including the middle frontal and postcentral gyrus in the overweight compared to the normal weight group (Balodis et al., 2013). To the contrary, one of the studies mentioned above which examined both food reward and monetary reward, reported no differences in monetary reward anticipation during hunger in normal-weight adolescents at high risk for obesity compared to low risk adolescents, while increased activation was found during monetary reward receipt in several regions including dorsal striatum and OFC during hunger (Stice et al., 2011). These studies suggest altered monetary reward processing in obesity. However, more studies are needed before we can draw firm conclusions, partly because earlier studies did not systematically take hunger state into account. Therefore, we here aimed to investigate to what extent hunger and weight status influence the brain responses to monetary reward anticipation

and receipt. Based on the literature, two a priori regions of interest (ROIs) were selected: the ventral striatum for reward anticipation and the OFC for reward receipt (Knutson et al., 2001a; Sescousse et al., 2014). We hypothesized increased ventral striatum activation during reward anticipation in the overweight group, and this activation was predicted to be increased during satiety. In addition, activation in the OFC during reward receipt was predicted to be altered in the overweight compared to the normal-weight group and modulated by hunger state.

6.2 Materials and methods

6.2.1 Participants

Forty-two participants enrolled in the study. Participants were eligible if they had a body mass index (BMI) between 20.0 and 25.0 (normal-weight group (NW)) or ≥27.5 (overweight & obese group (OW)). To maximize the chance of finding differences between the groups, there was a gap between the BMI cut-offs. In addition, participants had to be healthy (self-reported), between 25 and 45 years of age, right-handed, non-smoking, have a stable weight (did not gain or lose > 5 kg in the past 6 months), no psychiatric disorder, no use of medication (except aspirin/paracetamol and oral contraceptives) and no heavy drinkers (current alcohol consumption < 28 units per week). Furthermore, common fMRI exclusion criteria (e.g. claustrophobia, pregnancy and metal implants in the body) and criteria that might influence response to food cues (e.g. food allergies, special diets, eating disorders, gastrointestinal disorders or metabolic or endocrine disease, above average restraint eating scores on the DEBQ) were used. In addition, scan sessions with any single head movement greater than 2.5 mm translation or 2.5 degrees rotation were excluded. From the original sample (N=42), data of two overweight and five normal-weight subjects were excluded from the analyses because of excessive movement (1 overweight female), use of medication (1 overweight female), activation outlier (1 normal-weight male), completed only one scan sessions (1 normal-weight female), corrupted dataset (1 normal-weight male) and falling asleep several times during the scan session (1 normal-weight male and 1 normal-weight female). The sample included for analysis was comprised of 35 subjects, 18 normal-weight (8 males, 10 females; mean ± SD: age: 32.4 ± 5.3, BMI: 23.3 ± 1.6) and 17 overweight (9 males, 8 females; mean ± SD: age: 36.7 ± 6.9, BMI: 30.8 ± 2.8). There was no significant difference in education level between the groups. As expected, based on the inclusion criteria, the groups differed significantly in BMI ($t(24.9) = -9.457$; p

<0.001). Furthermore, there was a small but significant difference in age between the groups (t(33) = -2.055; p = 0.048). Fasting glucose, insulin, ghrelin and leptin levels differed significantly between the groups. Glucose, insulin and leptin levels were higher in the overweight compared to the normal-weight group, while ghrelin levels were significantly lower in the overweight group (baseline hungry condition NW-OW: glucose: t(31) = -2.157, p=0.039; insulin: t(19.6)=-3.701, p=0.001; leptin: t(31)=-2.790, p=0.009; ghrelin: t(29)=2.460, p=0.020; baseline sated condition: glucose: t(33)=-3.129, p=0.004; insulin: t(33)= -3.201, p=0.003; ghrelin: t(23.9)=4.236, p<0.001). In addition, baseline hormone levels did not differ between study days (NW: glucose: t(15)= 1.934, p=0.07; insulin: t(15) = 0.326, p=0.74; ghrelin: t(13)= 0.437, p=0.67; OW: glucose: t(16)= 0.144, p=0.88; insulin: t(16)=0.832, p=0.41; ghrelin: t(13)=0.275, p=0.78). See Table 1 for more details.

Table 6.1 Participant characteristics

	Normal-weight n =18	**Overweight** n =17
Gender (M:F)	8M:10F	10M:7F
Age[1] (yr)	32.4 ± 5.3	36.7 ± 6.9*
BMI[2]	23.3 ± 1.6	30.9 ± 3.1**
Education level[3]	4.9 ± 1.0	4.2 ± 1.1[ns]
Interscan interval (days)	7.6 ± 1.3	8.9 ± 3.0
First visit (S:H)	6S : 12H	8S : 9H
Alcohol categories (0:1-5:6-10:11-15 glasses a week)	5:6:4:3	6:7:4:0
Fasted glucose hungry condition (mmol/l)	5.34±0.34	5.67±0.52
Fasted glucose sated condition (mmol/l)	5.17±0.46	5.66±0.46
Fasted Insulin hungry condition (mmol/l)	42.09±16.29	89.20±49.72
Fasted Insulin sated condition (mmol/l)	39.93±22.77	81.50±49.90
Fasted Ghrelin hungry condition (pg/ml)	1328.50 ± 723.42	839.87±267.95
Fasted Ghrelin sated condition (pg/ml)	1312.00 ± 381.39	845.27 ± 213.82
Fasted circulair leptin (ng/ml)	11.32±12.22	26.55±18.33
Amount liquid breakfast consumed (mL)	517.9 ± 80.3	621.0 ± 95.9

*** differences between the groups were significant with p < 0.001; *differences between the groups were significant with p<0.05;[ns] differences between the groups were not significant;[1] t(33) = -2.063, p = 0.047; [2] t(23.8) = -9.050, p <0.001; [3]Z-scores used for the statistical comparison: t(33) = 1.881, p = 0.069; [4] 1: Incomplete; 2: Primary school or equivalent; 3: Secondary school or equivalent; 4: A levels, highers or equivalent; 5: Higher education, university, college or equivalent; 6: Master's degree, doctoral degree or equivalent. Baseline hormone levels: NW: Leptin, glucose, insulin and ghrelin n=16; OW: ghrelin, n=15.*

6.2.2 Experimental design

6.2.2.1 Study procedures

The study consisted of two morning MRI scan sessions. On both days, the participants came in after an overnight fast of at least ten hours. During one session the participants were scanned after the consumption of a fixed amount of a liquid breakfast (a commercially available vanilla whey protein shake from XXL nutrition prepared with whole milk), 1.4 x basic metabolic rate (BMR), calculated with the Schofield equation. With this equation an individual's BMR can be estimated by using age, gender and weight (Schofield, 1985; Schofield, 1984). The liquid breakfast was consumed ~60 min prior to the scan session. During the other session the participants were scanned fasted. Upon arrival on a study day, subjects provided hunger and fullness ratings and executed a computerized food picture rating task during which 133 food images were rated on liking, perceived caloric content and perceived healthiness. For hormone analyses blood was collected through a cannula placed in an antecubital vein at several time points on both mornings. The participants practiced the reward task prior to the scan. Subsequently, they underwent a 38-min MRI scan session consisting of four functional MRI runs during which they performed a food viewing task, two food choice tasks and the monetary reward task (duration 10 min). The results of the latter are the focus of this paper. See for more details the study procedure on page 50.

6.2.2.2 Monetary incentive delay task

Participants performed a reward task based on the monetary incentive delay task (Figure 6.1). This task allows the investigation of anticipation and receipt of reward, separately (de Leeuw et al., 2015; Hoogendam et al., 2013; Vink et al., 2015). At the beginning of each trial, a cue was presented for 750 ms, signaling whether the subject could win money (potentially rewarding trial, n=30) or not (non-rewarding trial, n=30). For the potentially rewarding trials, this cue was a smiling face and for the non-rewarding trials a neutral face. Following this cue, subjects had to respond as fast as possible, by pressing a button, when a target stimulus (exclamation mark) appeared on the screen. Subsequent feedback informed participants of their performance, indicating whether or not they had earned money on the trial, as well as their cumulative total at that moment. Subjects could win €1 during a potentially rewarding trial.

For both the potentially rewarding and non-rewarding trials, subjects had to respond to the target stimulus within a certain time limit, i.e., the target duration. Responses were considered correct (correct feedback) if subjects responded in time. Responses given after the time limit were considered incorrect (incorrect

feedback). The time limit was individually adjusted to ensure that each participant succeeded in ~50% of the trials. This adjustment was based on 20 practice trials which were presented prior to the start of the task, when subjects were already in the scanner. From these practice data, the shortest reaction time to the target was used to determine the individual target duration. In 50% of the trials, 200 ms was added to the duration of the individual time limit, enabling participants to be successful in these trials. In the remaining trials, 150 ms was subtracted from the time limit, to make sure that participants could not respond in time. This procedure resulted in about 50% correct trials for both rewarding and non-rewarding trials. The total amount of money won was presented at the end of the task. Participants were told that they would receive the total amount of money won in cash, in addition to the compensation for participation. The task consisted of 60 trials with a mean duration of 9571 ms (range 4946–16107 ms, inter-trial-interval range 1029–6979 ms), resulting in a total task duration of 9 min 35 s.

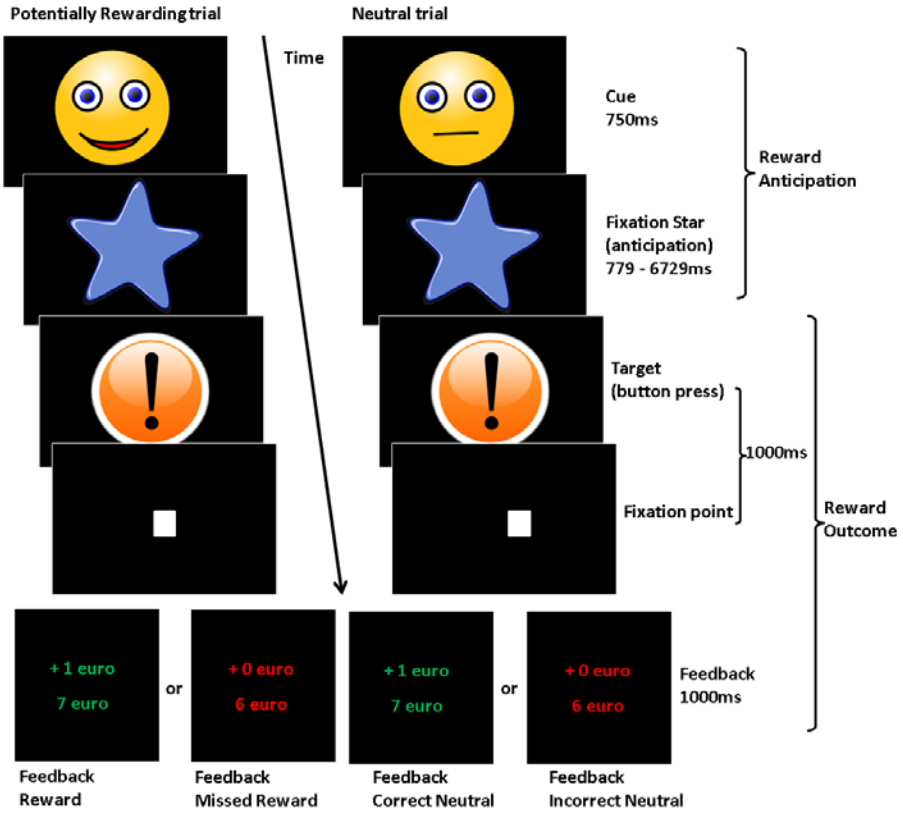


Figure 6.1 Schematic representation of the reward task adapted from (Hoogendam et al., 2013) and based on the monetary incentive delay task. There were 2 types of trials: a potentially rewarding (a) and nonrewarding (b) trial. The inter-trial-interval ranged between 1029 and 6979 ms.

6.2.3 Image acquisition

Imaging was performed on a 3.0 T Philips Achieva MRI scanner (Philips Healthcare, Best, NL). Functional images were obtained using a 2-D echo planar imaging-sensitivity encoding (EPI-SENSE) sequence with the following parameters: voxel size 4 mm isotropic; repetition time (TR) = 1600 ms; echo time (TE) = 23 ms; flip angle = 72.5°; 30 axial slices; SENSE-factor *R* = 2.4 (anterior-posterior). Please note that there was partial brain coverage (unintended, see Figure. S6.1). A total of 320 functional images was acquired. A high resolution anatomical image (T_1-weighted scan) was acquired at 1 × 1 × 1 mm resolution (TR/TE = 8.4/3.8 ms, total scan duration = 473 s).

6.2.4 Data analysis

6.2.4.1 Behavioral analyses

Behavioral data were analyzed with the use of SPSS statistics 23. Independent sample t-tests were used to analyze the differences in age, BMI and average reaction times between the groups. The educational level categories were first transformed to z-values. An Independent Sample Mann-Whitney U test was used to analyze differences in the amount won between the groups. Repeated measures ANOVA's were used to analyze differences in reaction time and hunger, fullness and liking ratings. In addition, mean food image liking ratings of the food picture rating task were calculated for each hunger state and, similar to the hunger and fullness ratings, analyzed using a repeated measures ANOVA.

6.2.4.2 fMRI analyses

fMRI preprocessing and analyses were carried out with the SPM5 software (http://www.fil.ion.ucl.ac.uk/spm). After slice timing correction and realignment of the functional images, the structural scan was coregistered to the mean functional scan. Next, the structural scan was segmented using unified segmentation, and normalization parameters were estimated. Subsequently, all scans were warped to Montreal Neurological Institute standard space using these normalization parameters and a 3-dimensional Gaussian smoothing kernel (8 mm full width at half maximum) was applied to all functional images.

6.2.4.3 Subject level analyses

Individual datasets were analyzed using multiple regression, to estimate brain activation time-locked to anticipation of reward, anticipation of non-reward, correct reward outcome, incorrect reward outcome, correct non-reward outcome, and incorrect non-reward outcome. To correct for head motion, the six

realignment parameters were included in the model as regressors of no interest. A high-pass filter was applied to the data with a cutoff frequency of 0.0058 Hz to correct for signal drifts. For each subject, brain activation related to Reward Anticipation (anticipation of reward minus anticipation of non-reward) and Reward Receipt (correct reward outcome minus correct non-reward outcome) was estimated.

6.2.4.4 ROI group analyses

For Reward Anticipation the ventral striatum was selected as an a priori ROI. For Reward Outcome the orbitofrontal cortex (OFC) was selected as ROI. ROI masks were created using the automated anatomical labeling atlas (Tzourio-Mazoyer et al., 2002). From each ROI, the average level of brain activation (i.e., average parameter estimate) was obtained for each subject. These values were then submitted to a repeated measures ANOVA to investigate hunger state and group (normal-weight, overweight) differences in activation in both ROIs during reward anticipation and receipt. Age, gender and scan order were added as covariates.

6.2.4.5 Whole brain group analyses

For each contrast of interest (Reward Anticipation, i.e. anticipation of reward minus anticipation of non-reward, and reward receipt, i.e. correct reward outcome minus correct non-reward outcome) the average levels of brain activation across conditions (mean hungry and sated) and between conditions (hungry – satiated) were obtained for each subject. Subsequently, two-sample t-tests were conducted to examine the main effects of group and condition, as well as the group-by-condition interaction. The resulting statistical parametric maps were thresholded at $p<0.001$ with a cluster extent $k\geq28$ voxels which is equivalent to $p<0.05$ corrected for multiple comparisons across all brain voxels. This threshold was derived using Monte Carlo simulations (10,000 iterations) of random noise distribution in the whole brain mask using the 3dClustSim function in AFNI. This approach combines an individual voxel probability threshold with a minimum cluster size to estimate the probability of a false positive. Age, gender and scan order were added as covariates.

6.3 Results

6.3.1 Behavioral results

6.3.1.1 Hunger and fullness ratings

Hunger and fullness ratings are summarized in Table 6.2. Baseline hunger and fullness ratings did not differ between hungry and sated days (hunger ratings: $F(1,32) = 3.249$, $p=0.081$; fullness ratings: $F(1,32) = 0.136$, $p=0.714$) and groups (hunger ratings: ($F(1,32) = 0.516$, $p=0.478$; fullness ratings: $F(1,32)=1.518$, $p=0.227$). In addition, hunger and fullness prior to the scan session differed significantly between the hungry and sated days (hunger ratings: $F (1,22) = 81.750$, $p<0.001$; fullness ratings: $F (1,22) = 65.002$, $p<0.001$) but not between groups (hunger ratings: $F (1,22) = .29$, $p=0.132$; fullness ratings: $F (1,22) = 0.003$, $p=0.957$).

6.3.1.2 Reaction time and wins

There was no significant difference in the reaction time delta (RT reward - RT neutral) between hunger and satiety within groups, as well as between groups (Table 6.3). Also, the amount of money won did not differ significantly between the groups (Table 6.3).

Table 6.2 Hunger and fullness ratings

	Normal-weight		**Overweight**	
Hunger ratings	Hungry	Sated	Hungry	Sated
Baseline [1]	6.5 ± 1.8 [5]	5.9 ± 1.8 [5]	6.1 ± 1.9	5.5 ± 1.9
20 min after drink	n/a	2.8 ± 1.7 [5]	n/a	2.7 ± 1.9
Prior to scan [2]	7.8 ± 0.7 [6]	3.7 ± 2.2 [6]	6.7 ± 1.4 [7]	3.0 ± 1.8 [7]
Fullness ratings				
Baseline [3]	2.2 ± 1.3 [5]	2.8 ± 1.5 [5]	3.2 ± 1.8	2.9 ± 1.4
20 min after drink	n/a	6.7 ± 1.9 [5]	n/a	6.8 ± 1.6
Prior to scan [4]	1.6 ± 0.7 [6]	6.7 ± 2.2 [6]	2.6 ± 1.4 [7]	5.7 ± 2.2 [7]

[1] Baseline hunger ratings did not differ significantly between hunger states (F(1,32) = 3.249, p=0.081) and groups (F(1,32) = 0.516, p=0.478); [2]Prior to the scan hunger ratings differed significantly between hunger states (F (1,22) = 81.750 , p<0.001) but not between groups (F (1,22) = 2.448 , p=0.132).[3] Baseline fullness ratings did not differ significantly between hunger states (F(1,32) = 0.136, p=0.714) and group (F(1,32)=1.518, p=0.227); [4]Prior to the scan fullness ratings differed significantly between hunger-states (F (1,22) = 65.002 , p<0.001) but not between groups (F (1,22) = 0.003 , p=0.957). [5] NW: Baseline & 20 min after drink , n=17; [6] =prior to scan n=10; [7] OW: prior to scan n=14

Table 6.3 Reward task reaction times

	Normal-weight		**Overweight**	
	Hungry	Sated	Hungry	Sated
RT reward (ms)	222 ± 25	220 ± 24	216 ± 21	217 ± 25
RT neutral (ms)	241 ± 28	247 ± 36	237 ± 21	233 ± 26
RT reward-neutral [1] (ms)	-20 ± 19 [ns]	-27 ± 21 [ns]	-21 ± 17 [ns]	-16 ± 17 [ns]
Wins (euro) [2,3]	15 ± 1 [ns]	14 ± 1 [ns]	14 ± 1.0 [ns]	15 ± 0.4 [ns]

[ns] differences between the groups were not significant; [1]Repeated measures ANOVAs: no effect of hunger states: F(1,33) = 0.078, p=0.782 and hunger states x group = F(1,33) = 2.509, p=0.123 Main effects: between groups comparison: F(1,33) = 1.167, p=0.288; [2] Hungry NW vs OW: U = 121, Z = -1.156, p=0.303; Sated: NW vs OW: U = 109, Z = -1.786, p = 0.153

6.3.2 ROI results

Overall striatum activation was higher in de overweight compared to the normal-weight group ($F(1,30) = 11.43$, $p = 0.002$). There was no main effect of hunger state ($F(1,30) = 0.129$, $p = 0.722$). Moreover, there was a significant group-by-condition interaction ($F(1,30) = 7.23$, $p = 0.012$) for reward anticipation activation in the ventral striatum: the groups did not differ in striatal activation during hunger, while when sated, striatal activation was lower in the normal-weight compared to the overweight group (See Figure 6.2).

No differences were found in the OFC during reward receipt (main effect hunger state: $F(1,30)=0.005$, $p=0.942$; main effect group: $F(1,30)=0.490$, $p=0.489$; group by condition interaction effect: $F(1,30)=1.689$, $p=0.204$.) (see Figure 6.3).

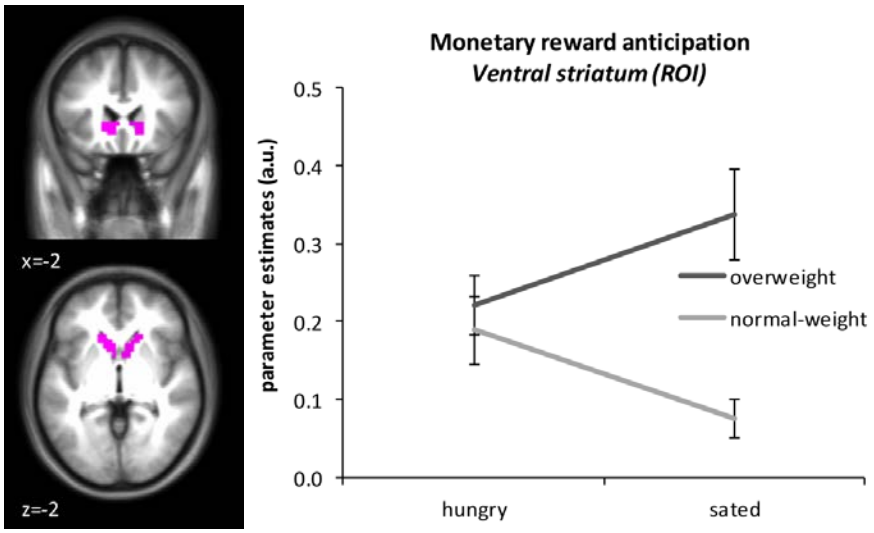


Figure 6.2 Left: Ventral striatum region of interest mask. Right: parameter estimates (mean±s.e.m.) in the ventral striatum ROI in the hungry and sated state in normal-weight and overweight subjects. There is a significant group-by-condition interaction ($F(1,30) = 7.23$, $p = 0.012$).

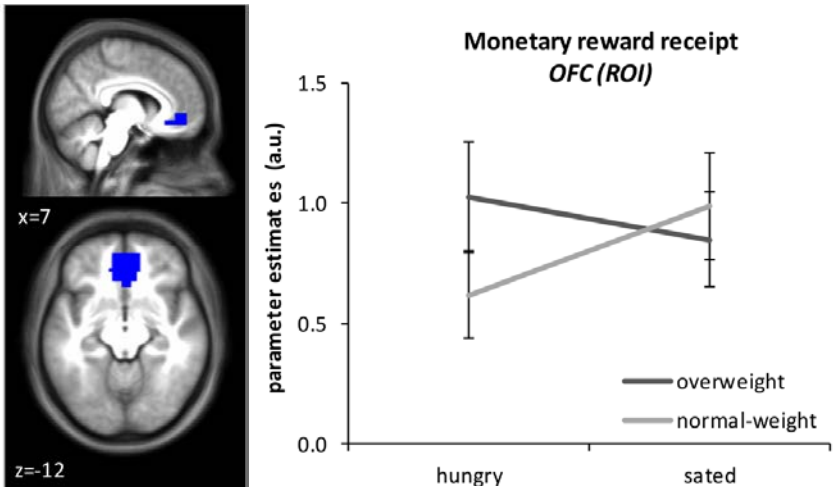


Figure 6.3 Left: OFC region of interest mask. Right: parameter estimates (mean±s.e.m.) in the OFC ROI in the hungry and sated state in normal-weight and overweight subjects. There are no significant differences.

6.3.3 Whole brain results

During reward anticipation, there were significant interaction effects in several regions including the bilateral thalamus, posterior cingulate gyrus and cerebellum (see Table 6.4). In these regions, activation in the overweight group increased after satiation, while the opposite pattern was apparent in the normal-weight group (decrease after satiation, see Figure 6.5).
There were no significant differences during reward receipt.

6.3.3.1 Food picture liking ratings

Liking ratings showed an overall decline with satiation (main effect of hunger state, $F(1,32) = 49.58$, $p < 0.001$). Moreover, there was a significant group-by-condition interaction ($F(1,32) = 4.79$, $p=0.036$) for the mean overall liking ratings of the food picture rating task, executed prior to the scan session (Figure 6.4). During hunger, the overall food image liking ratings were similar for both groups, while when sated liking ratings of the normal-weight group declined more were lower than those in the overweight group.

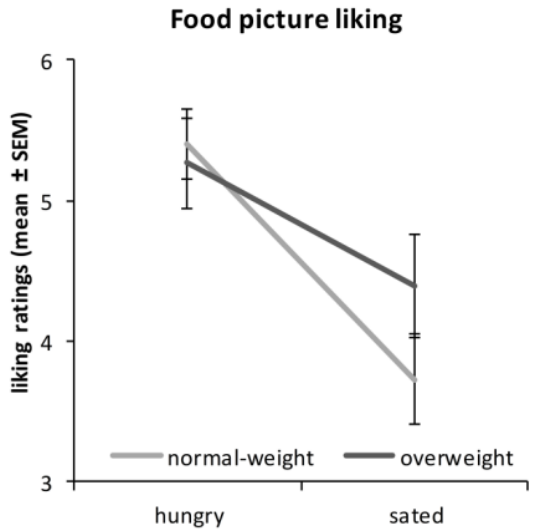


Figure 6.4. Average food picture liking ratings for both groups in the hungry and sated state. Group-by-condition interaction effect: F(1,32) = 4.79; p=0.036; main effect hunger state: main effect of hunger state F(1,32) = 49.58; p < 0.001).

Table 6.4 Brain regions showing a group by condition interaction effect for reward anticipation (whole brain results)[a]

		Peak MNI-coordinate				
Region	**k**	**x**	**y**	**z**	**F**	**Z**
Thalamus, L	181[b]	-4	-24	0	28.94	4.32
Posterior cingulate gyrus, R		4	-40	8	28.32	4.28
Thalamus, R		8	-32	4	4.19	4.19
Cerebellum, L	47[c]	-4	-60	-16	18.68	3.60
Cerebellum, L		-12	-48	-24	18.35	3.58
Cerebellum, R		20	-44	-20	17.65	3.52

[a] *Peaks are reported for all clusters ≥ 27 voxels at p<0.001 corrected for multiple comparisons; L = left and R= right hemisphere.* [b & c] *significant group by condition interaction effect, shown in Figure 6.5*

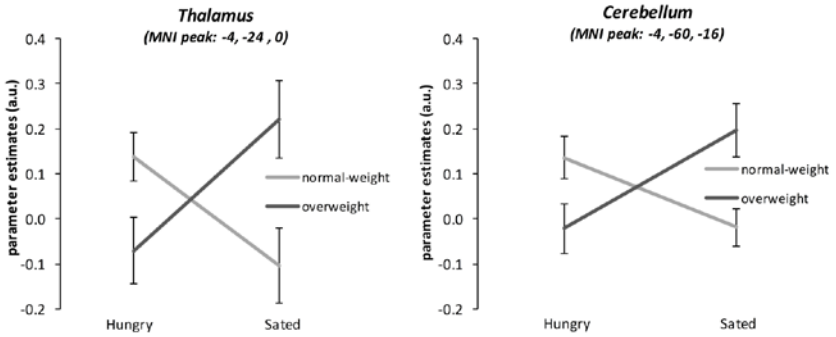


Figure 6.5 Whole brain results; parameter estimates (mean ± s.e.m.) for regions with a significant group by condition interaction effect. Thalamus: $F(1,30) = 28.94$, $p<0.001$ (peak statistic); Cerebellum: $F(1,30) = 18.68$, $p = 0.001$ (peak statistic).

6.4 Discussion

To our knowledge, this was the first study to investigate the effects of hunger and weight status on the brain response to monetary reward anticipation and receipt. We found decreased ventral striatum activation during reward anticipation after satiation in the normal-weight group compared to the overweight group. Activation in response to reward receipt did not differ between the states and groups.

Reward anticipation

As hypothesized, we found greater ventral striatum activation during reward anticipation in the overweight compared to the normal-weight group during satiety. In line with this, (food) reward studies using visual food cues found greater activation in several brain regions including the striatum, in obese compared to normal-weight individuals (Balodis et al., 2013; Jastreboff et al., 2013; Martin et al., 2010; Rothemund et al., 2007; Stoeckel et al., 2008), food imagery (Jastreboff et al., 2013), food reward anticipation (Ng et al., 2011) and monetary reward anticipation (Balodis et al., 2013). However, our results clearly show that this effect depends strongly on hunger state; when fasted, striatum activation did not differ between weight groups. With satiation, normal-weight ventral striatum activation decreased while an increase was apparent in the overweight group. In line with our findings in the normal-weight group, another study found a decrease in striatum activation in response to conditioned odor stimuli which was devaluated by feeding to satiety (Gottfried et al., 2003). Furthermore, obese and lean adolescent girls showed similar striatum activation during anticipation of a food reward (milkshake) during hunger (Stice et al., 2008b). Similarly, a study on food and monetary reward found no activation differences during reward anticipation between adolescents with low and high risk for obesity during hunger (Stice et al., 2011). On the contrary, one study on monetary reward anticipation in lean and obese individuals showed increased striatum activation in obese compared to lean individuals (Balodis et al., 2013). However, in this study, hunger state was not controlled for. Our results would suggest that participants in this study tended to be satiated. Furthermore, in line with our brain and behavioral findings, a recent meta-analysis has shown that obese individuals are more responsive to food cues when sated than their lean counterparts (Kennedy and Dimitropoulos, 2014). Our findings imply that this also holds for monetary reward anticipation, and that this is thus a general alteration in the reward system of overweight/obese individuals.

Taken together, our results suggest a lack of downregulation of ventral striatal activation with satiation in overweight individuals during monetary reward anticipation. This may result in reduced desensitization for (food) rewards when sated. This is further supported by the food picture liking ratings; liking did not differ between the groups when hungry but did when participants were sated. Notably, this mirrors the response pattern in the ventral striatum: the ratings of the overweight group declined less with satiation than those of the normal-weight group.

In addition to the ventral striatum results, whole brain analysis showed similar alterations in reward anticipation responses in the thalamus, posterior cingulum and cerebellum. Activation in the overweight group increased after satiation, while activation in the normal-weight group decreased. However, in contrast to the ventral striatum, activation in these regions also differed between the groups when hungry: activation was lower in the overweight compared to the normal-weight group. Especially the thalamus is an interesting area since it receives input from the ventral striatum and projects to prefrontal regions (Baev et al., 2002). It is thought to play a crucial role in linking reward signals and cognitive processes and subsequent behavior (Elliott et al., 2000). Previous studies also reported thalamus activation during food (Schur et al., 2009) and monetary (Bjork et al., 2004; Rademacher et al., 2010) reward processing. Similar to thalamus activation, cerebellum activation is also often found in food-related studies. The cerebellum is thought to integrate somatic and visceral information (Zhu and Wang, 2008). Differences in cerebellum activation have been associated with BMI (Park et al., 2016). In addition, functional connectivity between core reward areas and the cerebellum is altered in obesity (Carnell et al., 2014). Moreover, satiation has been associated with decreased cerebellum activation in several PET studies (Gautier et al., 2000; Gautier et al., 2001; Tataranni et al., 1999). Thus, the increased thalamus and cerebellum activation we observed after satiation in the overweight group might underlie maladaptive behaviors such as (food) reward seeking despite being full.

Reward Receipt

Although alterations in the OFC in overweight individuals were hypothesized, we found no difference between the groups during monetary reward receipt. In contrast, several other studies did report differences in reward receipt between obese and normal-weight individuals (Balodis et al., 2013; DelParigi et al., 2005; Stice et al., 2010; Stice et al., 2011). During monetary reward receipt in a hungry state increased activation has been found in several regions including dorsal striatum, OFC and thalamus in adolescents at risk for obesity (Stice et al., 2011). The OFC activation in our adult group showed a similar trend when participants

were hungry. This could indicate that normal-weight adolescents at risk for obesity differ in reward receipt processing compared to actual overweight adults. Differences in reward receipt activation in overweight adults could be more subtle. Thus, the lack of a significant difference in our study might be due to the moderate sample size. On the contrary, in another study reward receipt was associated with decreased activation in the precentral, middle frontal and postcentral gyrus in overweight compared to normal weight individuals (Balodis et al., 2013). As previously mentioned, this study did not control for hunger state, which makes it hard to compare results. Taken together, due to the variable findings across studies, more research is needed to further elucidate the effects of weight status on the brain responses during reward receipt.

Conclusion

In conclusion, normal-weight individuals showed decreased ventral striatum activation during monetary reward anticipation with satiation while overweight individuals did not. This effect was mirrored in food liking ratings. These findings show that not only food reward processing, but also monetary reward processing is affected by hunger, which strengthens the evidence for a common neural currency. In addition, we found an interaction between hunger state and weight status, notably in the ventral striatum. This further supports the idea of a common neural currency since satiety suppressed monetary reward anticipation in normal weight individuals. Because of the differential effect of satiation on monetary reward processing in normal and overweight individuals, future studies into reward processing, also in other populations, should preferably control for hunger state. The lack of downregulation of anticipatory striatal activation in overweight and obese individuals might explain eating in the absence of hunger since their reward system appears to remain in 'hungry-mode' despite being sated. This may hamper attempts to resist palatable foods and thereby undermine weight loss attempts. Further research should elucidate whether this is a causal risk factor or a characteristic of obesity.

Supplementary Material

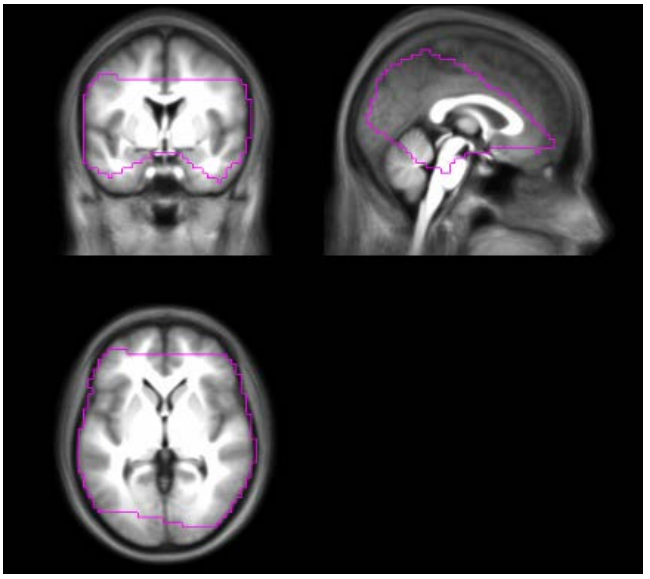


Figure S6.1 Brain coverage of the functional scan, shown on the mean anatomical image of all subjects.

CHAPTER 7
GENERAL DISCUSSION

We investigated the effect of hunger state on food-related brain responses across the lifespan, using the same experimental setup across four age groups. Firstly, we describe a standardized food picture set and a photographing protocol for making these pictures (Chapter 2). Subsequently, we used low and high calorie pictures made with this protocol in a food viewing (Chapter 4) and a food choice task (Chapter 3 and 5). Concurrently, we investigated the effect of hunger state and weight status on monetary reward processing (Chapter 6).

During high compared to low calorie food viewing there was greater activation in frontal inhibition areas during hunger compared to satiety. During food decision making, activation in the calcarine sulcus was modulated by the caloric content of the choices; there was increased activation during choices higher in calories (positive modulation) after satiation, while there was decreased activation during choices higher in calories during hunger (negative modulation). Furthermore, during high compared to low calorie food viewing, there was greater activation of visual areas in children, while in teens activation was diminished in these areas. During food choice, however, children and teen's brain responses did not differ. In both these groups activation in the dorsolateral prefrontal cortex and visual processing areas was lower compared to that in adults and elderly. There was no interaction between hunger state and age, i.e. the effect of hunger state was not significantly affected by age or vice versa, during both food viewing and food choice. During monetary reward anticipation, but not receipt, an interaction effect of weight status and hunger was found: in normal-weight adults satiation suppressed ventral striatum activation during monetary reward anticipation, while in overweight adults such suppression was absent. Moreover, activation in the ventral striatum even tended to increase after satiation in overweight adults.

Effects of hunger state on food-related brain responses

The effects of hunger state that we found were independent of age. Moreover, differences between the fasted and sated state were only apparent when responses to high and low energy foods were compared. As may be expected, during high compared to low calorie food cue exposure all age groups showed greater responses in the hungry compared to the sated state in brain regions important for valuation and response inhibition. This initial higher inhibitory response when exposed to high caloric food cues may underlie the ability of normal-weight individuals to resist overconsumption of such foods. On the other hand, during actual food decision-making food choices higher in calories triggered lower responses compared to food choices lower in calories in areas important for attention when hungry, and higher responses when sated. This highlights the difference between food cue reactivity and food decision-making and suggests that

these two processes may not be affected by hunger state in the same manner. It would be worthwhile to further explore how both these processes, but food decision-making in particular, may be altered in overweight and obesity. In the literature to date, the hungry (fasted) state has received most interest. Although normal-weight individuals seem to be most responsive when hungry, this is most likely not the case for overweight adults. Especially (altered) responses during satiety would be of interest since part of the problem lies in 'eating in the absence of hunger', which is corroborated by our findings in Chapter 6, that reward processing is affected by both hunger and weight status.

Effects of age on food-related brain responses

Children and teens both showed lower inhibitory frontal activation during food choice compared to adults and tended to have lower frontal activation compared to elderly as well. Additionally, they chose more high calorie foods and had a stronger preference for high calorie foods, irrespective of hunger state. However, in children activation in visual areas during food viewing followed an opposite pattern than that observed in teens. This was not explained by differences in liking of the foods between the age groups. 8-10 year olds clearly seem to be most responsive to high calorie foods (Ferguson, Munoz et al 2011), while teens are more responsive to low calorie foods, according to our findings. This may reflect an attentional bias to high calorie foods in young children which is additionally supported by their higher proportion of high calorie choices made. Teens, on the other hand, seem to pay more attention to low calorie foods, yet, when a choice needs to be made, they make similar choices as young children. This is most likely the result of their immature frontal cortex, which may make it harder to incorporate healthiness in their food choices.

Elderly and adults did not differ from one another in brain responses during both high vs. low calorie food viewing and food choice. Although, there was a trend towards lower visual activation in elderly in response to high vs. low calorie food viewing and lower dlPFC activation during food choice compared to adults. Additionally, elderly chose relatively more low calorie foods, even when hungry, and they had a higher preference for low calorie foods in general. Thus, elderly do not seem to have altered response inhibition during food decision making and, in the group studied here, even seem to have developed a food choice pattern in favor of low calorie foods.

In conclusion, especially children seem to be vulnerable for high caloric foods irrespective of hunger state and liking differences. Therefore, it would be advisable to limit unnecessary high calorie food cue exposure, e.g. in television commercials

or in-store marketing targeted at (young) children. In addition, both children and teens chose more high calorie foods, independent of hunger state, even though the choice options were matched on liking. Although the vast majority of these children and teens had a normal-weight, this behavioral pattern is not beneficial for their longer-term weight status and health. Further research is needed to examine to what extent these choices resemble actual daily choices, whether this trend persists in a larger group of children and teens and to what extent this choice behavior persists when they grow older. If this is indeed a general trend, more preventive actions should be taken by parents and policy makers alike.

Until now, elderly have received surprisingly little attention in the food viewing and choice fMRI literature. Our result suggest that elderly, at least in our study population, seem to have developed a higher liking for low calorie foods and choose more low calorie foods irrespective of hunger state, compared to other age groups. However, their brain activation did not deviate from that of adults. More research is needed in this group to further examine this. However, at the same time BMI tends to increase with age and the health effects of overweight and obesity become more apparent as people grow older. Thus, it is crucial to prevent weight gain early on and promote healthy aging by finding strategies to promote healthier food choices.

Effects of hunger state on reward-related brain responses in normal-weight & overweight

Brain responses in response to monetary reward processing were influenced by both hunger state and weight status (Chapter 6). In normal-weight adults several reward-related brain regions became less responsive during monetary reward anticipation when sated, while in overweight and obese adults the opposite tended to be true. Understanding food reward processing and how exactly this is altered in overweight /obesity is still of great importance. It is clear that there may be different alterations in different subgroups, but this has not been satisfactorily resolved. Our results suggest that it would be worthwhile to (additionally) study non-food reward processing to be better able to establish general alterations in reward processing. Moreover, based on our findings, any study into reward processing should take hunger state into account. Together these results might help to fine-tune general recommendations for overweight and obese individuals. For example the advice "do not shop for groceries when hungry" might be a good advice for normal-weight individuals who seem to be more responsive when hungry, while this advice might not at all be useful for overweight and obese individuals.

Future research

Although we obtained novel results on food choice behavior and brain responses in a laboratory setting, we do not know how these findings relate to actual eating behavior in daily life. Future research should try to link brain responses to measurements of eating behavior in a realistic shopping environment and at home. The recent technological developments in this area, e.g. with more and more advanced smart phone apps that can help to assess food intake, is promising. Such measures may outperform more classical approaches such as food diaries and buffet consumption, which are prone to reporting biases (or not reliable).

Unfortunately, the effect of weight status on food-related brain responses and monetary reward across the lifespan could not be examined due to a lack of participating overweight children and teen subjects. Although extensive effort was undertaken to reach this population, it proved very hard to recruit these groups. We speculate that this could be caused by the fact that higher educated parents are more prone to participate in research and the prevalence of overweight and obesity in this population is simply lower. In addition, shame and stigmatization could play a role in which parents of overweight children do not want their child to be labelled as such (Sikorski et al., 2012). We believe the most promising manner to investigate these groups would be by means of a cohort study where young families are included and are followed throughout the life course. During this time, some children will become overweight while others will not. This will additionally provide us with a better insight on the causality of any differences found. To date, such longitudinal data on food-related developmental brain processes are very scarce, with the exception of the cohort of Stice and coworkers who report interesting differences between groups differing in weight gain over a 1-3 y follow-up period (Stice et al., 2015; Stice et al., 2010).

Overall conclusion

Hunger state and age both have independent effects on food-related brain processes. Especially children show the lowest amount of inhibition and the highest number of high calorie choices. Hunger makes normal-weight individuals of all ages more responsive to high calorie food cues. Satiation causes monetary reward anticipation to decrease in healthy weight people but not overweight people. Overall, we can conclude that hunger predisposes normal-weight individuals to high calorie choices, and since in children and teens inhibition is less developed they are more vulnerable and prone to excessive energy intake. Further unraveling the neural correlates of food choice might help to identify these vulnerabilities which subsequently may be targeted to promote healthier

food choices. Furthermore, our finding that satiation differentially affects reward processing in overweight and normal-weight individuals makes it imperative to establish in more detail how hunger state affects (food) reward processing in people of differing weights and ages, preferably longitudinally.

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Samenvatting

We worden voortdurend blootgesteld aan voedsel en tijdens de dag maken we voortdurend voedselkeuzes. Deze keuzes spelen een belangrijke rol bij de regulatie van onze voedselinname, wat vervolgens weer invloed heeft op de algehele gezondheid en gewichtsbeheersing. Het is daarom belangrijk om meer inzicht te krijgen in de mechanismen die ten grondslag liggen aan voedselkeuzes. Er is relatief veel onderzoek gedaan naar de hersenreacties tijdens de evaluatie van voedsel, maar de resultaten zijn niet eenduidig. Dit komt omdat er veel factoren zijn die invloed hebben zoals het gebruik van verschillende taken, voedselstimuli en verschillende groepen. Er is niet eerder gekeken naar het effect van hongerstaat en leeftijd op de hersenresponsen tijdens blootstelling aan eten en het maken van voedselkeuzes. De literatuur over kinderen en ouderen is zeer beperkt terwijl met name kinderen bijzonder gevoelig zijn voor voedselprikkels. De hersenen van kinderen zijn nog volop in ontwikkeling. Voornamelijk frontale gebieden (onder meer belangrijk voor het remmen van gedrag) ontwikkelen nog door tot het eind van de adolescentie. Veroudering daarentegen zorgt voor vermindering van functie in onder andere frontale hersengebieden. In dit proefschrift hebben we met behulp van functionele Magnetische Resonantie (fMRI) onderzocht in hoeverre leeftijd en hongerstaat invloed hebben op de hersenresponsen tijdens het kijken naar eten en het maken van voedselkeuzes. Functionele MRI is een techniek waarmee hersenactiviteit gemeten kan worden.

In **hoofdstuk 2** hebben we een eenvoudig te gebruiken foto protocol gemaakt dat onderzoekers in staat stelt om zelf gestandaardiseerde voedselfoto's te maken van hoge resolutie. Daarnaast hebben we met dit protocol een gestandaardiseerde voedselfotoset gemaakt. Van deze set hebben we 80 foto's getest op herkenbaarheid, smaak, calorie inhoud en gezondheid in ~450 volwassenen en ~200 kinderen uit zeven Europese landen. De meeste voedselfoto's werden herkend. De verschillen in smaak ratings, calorieëninhoud en gezondheid tussen de landen waren minimaal. Het voedselfotoprotocol en alle voedselfoto's zijn vrij beschikbaar gemaakt voor onderzoek op http://nutritionalneuroscience.eu/. Door het beschikbaar maken van het protocol en de foto's zal de vergelijkbaarheid tussen de studies worden verbeterd. Dit is een eerste stap naar een internationale database met gestandaardiseerde voedselfoto's.

In **hoofdstuk 3** hebben we de foto's die gemaakt zijn met het gestandaardiseerde fotoprotocol (beschreven in hoofdstuk 2) gebruikt voor het ontwikkelen en testen van een fMRI voedselkeuze taak. Tijdens deze taak hebben we gezonde deelnemers met een normaal gewicht voedselkeuzes laten maken tussen hoog- en laag- calorische snacks. De keuzeopties waren vergelijkbaar in lekkerheid,

gebaseerd op scores van de deelnemer. Vervolgens hebben we de hersenresponsen vergeleken tijdens het maken van deze keuzes in verzadigde proefpersonen. Het maken van voedselkeuzes (in vergelijking met keuzes tussen twee kantoorartikelen) ging gepaard met activatie van de insula, superior temporale sulcus, posterieur cingulate gyrus en (pre)cuneus. Wanneer we hoog- met laagcalorische voedselkeuzes vergeleken zagen we alleen activatie in de rechter superior temporale sulcus (STS). Samen met resultaten van eerdere studies zou deze STS activatie tijdens het maken van voedselkeuzes, de biologische relevantie van eten kunnen representeren, onafhankelijk van individuele verschillen in lekkerheid.

Hoofdstuk 4, 5 en 6 zijn een vervolg op het onderzoek uit hoofdstuk 3. In deze hoofdstukken is er gekeken naar het effect van leeftijd en hongerstaat op de hersenresponsen tijdens verschillende taken. Voor dit onderzoek werden deelnemers twee keer uitgenodigd voor een MRI scan. Beide keren kwamen de deelnemers nuchter naar het Universitair Medisch Centrum Utrecht. Op de ene ochtend werden de MRI scans nuchter afgenomen. Op de andere ochtend na het consumeren van een gestandaardiseerd ontbijt (een shake op basis van melk en eiwit).

In **hoofdstuk 4** wordt beschreven hoe de hersenen van 122 gezonde proefpersonen met een normaal gewicht (17 kinderen, 38 tieners, 36 volwassenen en 31 ouderen) reageren op het kijken naar eten. Honger resulteerde in meer hersenactivatie tijdens het kijken naar hoog- in vergelijking met laagcalorisch eten in de dorsomediale, ventromediale en dorsolaterale prefrontale cortex in alle leeftijdsgroepen. De dorsolaterale prefrontale cortex is betrokken bij het remmen van gedrag. De grotere activatie in dit gebied tijdens honger zou kunnen wijzen op een verhoogde remming tijdens het zien van hoog calorisch eten. Dit zou het vermogen of de intentie tot het weerstaan van hoog calorisch eten kunnen weerspiegelen. We vonden ook verhoogde mediale prefrontale cortex activatie tijdens honger, wat zou kunnen duiden op de verhoogde beloningswaarde van calorierijk eten, die afneemt met verzadiging. Verder was de activatie in visuele hersengebieden tijdens het kijken naar hoog- versus laagcalorisch eten het hoogst in kinderen en het laagst in tieners, ten opzichte van de twee volwassen groepen. Dit zou kunnen wijzen op een verhoogde aandacht voor hoogcalorisch eten in kinderen, terwijl tieners meer aandacht lijken te hebben voor laagcalorisch eten. Verder onderzoek is nodig om deze resultaten beter te begrijpen.

In **hoofdstuk 5** hebben we onderzocht hoe voedselkeuzes en de bijbehorende hersenactiviteit worden beïnvloed door hongerstaat en leeftijd. Daarnaast hebben we bekeken in welke mate de calorie inhoud invloed hebben op deze hersenresponsen. 95 deelnemers (18 kinderen, 25 tieners, 27 volwassenen, 25 ouderen) voerden een voedselkeuze taak succesvol uit, een keer hongerig en een keer verzadigd. Tijdens deze taak kozen de deelnemers steeds tussen twee opties die ze even lekker vonden, maar die veel of weinig calorieën bevatten. De resultaten lieten zien dat kinderen en tieners tijdens voedselkeuzes lagere dorsolaterale prefrontale cortex (DLPFC) activatie hadden. De DLPFC is belangrijk voor het remmen van gedrag. Ook hadden ze een lagere activatie in visuele gebieden dan volwassenen en ouderen. Daarnaast maakten kinderen en tieners meer hoogcalorische keuzes. De calorische waarde van de voedselkeuzes had een negatieve invloed op calcarine sulcus activatie tijdens honger, terwijl het omgekeerde het geval was na verzadiging. Kinderen en tieners lijken meer moeite te hebben met het weerstaan van hoogcalorisch eten. Daarnaast lijkt honger in alle leeftijdsgroepen de aandacht tijdens een hoogcalorische keuze te verminderen. Hierdoor neemt de kans op het maken van hoogcalorische keuzes tijdens honger toe.

In **hoofdstuk 6** hebben we gekeken naar het effect van honger en gewichtsstatus op een geldbeloning in plaatst van een voedselbeloning. Uit de literatuur weten we dat de hersenresponsen van mensen met overgewicht en/of obesitas tijdens het kijken naar voedsel anders is en dat honger daar invloed op heeft. Het was nog onduidelijk of dit ook het geval is voor andere soorten beloningen en of deze responsen beïnvloed worden door hongerstaat. Hiertoe hebben we 35 proefpersonen waarvan 17 met overgewicht onderzocht die zowel hongerig als verzadigd een geld-beloningstaak uitvoerden tijdens een functionele MRI scan. In de hongerige conditie was er geen verschil in activatie van het striatum, een gebied belangrijk voor beloning tijdens het anticiperen op een geldbeloning. Echter, in de verzadigde conditie, was de activatie in het striatum lager in de normaalgewicht groep vergeleken met de overgewicht groep. Deze resultaten suggereren dat het beloningssysteem van mensen met overgewicht nog steeds in een honger-modus verkeert, ondanks dat ze verzadigd zijn. Dit zou pogingen tot gewichtsverlies kunnen bemoeilijken. Verder onderzoek moet uitwijzen of we hier te maken hebben met een risicofactor of een kenmerk van overgewicht/obesitas.

Algemene conclusie

Wij hebben voor het eerst voedselgerelateerde hersenresponsen tijdens honger en verzadiging vergeleken in vier leeftijdsgroepen groepen. Hongerstaat en leeftijd blijken beide onafhankelijke effecten te hebben op voedselgerelateerde

hersenresponsen. Vooral kinderen laten de minste DLPFC (belangrijk bij de remming van gedrag) activatie zien en het hoogste aantal calorierijke keuzes. Honger zorgt ervoor dat normaalgewicht mensen van alle leeftijden meer visuele gebieden activeren tijdens het kijken naar calorierijk eten. Dit suggereert dat normaalgewicht mensen van alle leeftijden meer aandacht besteden aan calorierijk eten als ze hongerig zijn. Daarnaast suggereren de hersendata dat tijdens honger de aandacht vermindert is tijdens het maken van een hoogcalorische keuze, waardoor de kans op een hoogcalorische keuze toe lijkt te nemen. Verzadiging zorgt in volwassenen van normaal gewicht voor een afname van de anticipatie op een geldbeloning, terwijl dat niet het geval is bij mensen met overgewicht. Over het algemeen kunnen we concluderen dat honger mensen van (voornamelijk) normaal gewicht gevoeliger maakt voor hoogcalorisch eten, ongeacht hun leeftijd. Aangezien bij kinderen en tieners het vermogen tot remming nog in ontwikkeling is, zijn zij het meest kwetsbaar voor overconsumptie. Onze bevinding dat verzadiging niet hetzelfde effect heeft op het verwerken van een beloning bij volwassenen met overgewicht in vergelijking met normaal gewicht maakt het belangrijk om verder onderzoek te doen bij mensen van verschillende leeftijden en gewichtsklassen. Een longitudinale opzet heeft de voorkeur omdat dit meer inzicht geeft in oorzaak en gevolg.

DANKWOORD

Er zijn enorm veel mensen betrokken geweest bij het tot stand komen van dit proefschrift. Dit laatste hoofdstuk is speciaal voor jullie.

Beste deelnemers van het Full4Health onderzoek, het is een feit dat zonder jullie dit onderzoek niet had kunnen plaatsvinden. Van jong tot oud, in Nederland, Schotland en Griekenland, jullie deden allemaal mee! Ondanks de vroege ochtenden zonder ontbijt waren jullie enthousiast. Het was voor mij een feestje om jullie te scannen. Heel erg bedankt daarvoor!
Dear full4health participants, without you this research would not have been possible. Volunteers of all ages from The Netherlands, Scotland and Greece participated! Despite the early mornings and overnight fast you were all enthusiastic. It has been a pleasure to scan you. Thank you very much!

Beste Paul (co-promotor), bedankt voor het vertrouwen dat ik van je heb gekregen tijdens de afgelopen 5 jaar. Jij hebt mij alle vrijheid gegeven om het onderzoek uit te voeren, zo fijn! Als ik wilde overleggen was je 24/7 beschikbaar. Je bent een vakkundig wetenschapper en een hele toffe vader van vier kids! Jouw optimisme is aanstekelijk en erg fijn om mee te werken. Jouw droge humor en fantastische verhalen maakte dat er bij elke meeting wel gelachen werd en dat we ook standaard uitliepen. Jouw feeling voor fijne hotelkamers is iets minder goed ontwikkeld (bed bugs, brandalarm, bootjes, schimmel), al ben je altijd wel heel prijsbewust en dat siert je dan weer. Ik heb met veel plezier met je gewerkt. Ik had me geen betere begeleider kunnen voorstellen. Bedankt voor de fijne jaren!

Beste Max (promotor), bedankt dat ik bij het ISI promotieonderzoek mocht komen doen. Jouw kritische en realistische blik zijn van onschatbare waarde voor me geweest. Bedankt voor de tekstuele wijzigingen op mijn stukken en de geniale zinsconstructies. Bedankt voor je tijd, je tips en tricks. Ik vond het heel fijn om bij het ISI te werken.

Floor, waar moet ik beginnen… Met jou als collega en later als kamergenootje waren de werkdagen in het UMC echt een feestje. De congressen waar we samen heen zijn geweest waren naast meestal zeer nuttig ook altijd super gezellig. De combi van jouw claustrofobische trekjes en je avontuurlijke eetgewoontes met mijn iets wat onrealistische diefstal angsten en neofobie maakte ons een hilarisch

reisstel. De laatste maanden heb je me er echt doorheen gesleurd. Je hebt m'n gezeur aangehoord, je hebt me opgepept als ik in een dip zat, je hebt met me koffie gedronken en je hebt me geholpen met m'n stukken. Na onze ontspannende koffiedates ging ik altijd weer met frisse moet aan het werk. Lieve Floor, bedankt voor alles! Ik ben heel blij met jou aan mijn zijde als paranimf!

Lieve J, bedankt voor alle gesprekken. Dag en nacht stond je voor me klaar om me te helpen. Je was een enorme steun. Niks was te veel moeite. Ik kan me geen beter broertje wensen! Ik ben super trots dat jij mijn paranimf bent.

Laboranten van de MRI en CT, bedankt voor jullie flexibiliteit en hulp tijdens de scanochtenden. Er was altijd wel iemand bereid om even een infuusje aan te leggen. Karel, bedankt voor het meedenken en het mede mogelijk maken van het F4H onderzoek. Jij gaf me de ruimte om het onderzoek zo goed mogelijk te kunnen uitvoeren met de beperkte onderzoeksfaciliteiten die we destijds nog hadden. Guus en Seline, bedankt voor het schuiven met de planning zodat ik extra deelnemers kon scannen.

57000, het telefoonnummer van het technisch cluster, staat in mijn geheugen gegrift. Ik heb geregeld met jullie contact gehad. De MR7 heeft het in de afgelopen 5 jaar vaak laten afweten tot grote frustratie van mij maar zeker ook van jullie. Gert en Dominique, bedankt voor jullie enorme hulp om de scanner weer zo snel mogelijk aan de praat te krijgen!

Pascal, jij was een enorme aanwinst voor het onderzoek. Jij hebt samen met de meiden een deel van de dataverzameling van mij overgenomen zodat ik meer tijd had om te schrijven en zodat ik met een gerust hart met zwangerschapsverlof kon gaan. Heel erg bedankt daarvoor!

Het NICHE lab, heel erg bedankt voor het gebruik van de oefenscanner.

Alle (oud) OIO-steeg collega's en de staf-gang bedankt voor de leuke jaren. De borrels, feestjes en de Ardennenweekenden waren altijd super gezellig en zal ik niet snel vergeten. Maaike, Miekee, Yolanda mijn kamergenootjes van de eerste jaren, het was heerlijk om te midden van voornamelijk mannen een ladies room

te hebben met super fijne theepauze om onze harten te luchten. Bedankt daarvoor! Pim, jij kwam er later bij en je deed gewoon gezellig mee met het theedrinken! Floor en Chantal, ladies room 2.0 bedankt voor de gezelligheid en de steun tijdens de laatste maanden! Hugo, bedankt voor de fijne vrijdagen! Alexander, bedankt voor de motiverende gesprekken, je enthousiasme is aanstekelijk! Ik hoop dat we nog een mooi fMRI-DTI paper kunnen schrijven. Jacqueline, Marjan en René bedankt voor alle hulp met alle regeldingen rondom het onderzoek en de cursussen die ik heb gevolgd.

De MUFFIN's, Guido, Inge, Floor, Marlou, Nynke en Paul bedankt voor jullie kritische blik en gezelligheid!

Stagiaires, Evi, Astrid, Richelle, Viyan, Marjolein, Mirjam, Lisanne, bedankt voor jullie hulp bij de ondersteuning van het F4H onderzoek.
Lisanne & Mirjam, na/naast jullie stage hebben jullie ook gewerkt als onderzoeksassistent voor het F4H onderzoek. Jullie hulp bij de werving, de planning en het scannen is essentieel geweest voor het voltooien van dit onderzoek. Super bedankt daarvoor!

Aberdeen team, Alex, Julian, Will and Dan thanks for your kindness and effort with the data collection. I hope we will meet again!

Athens team, Yannis, Odysseas, Angie and Evita, I really appreciate the amount of work you did with the limited resources available.

Beste Jose en Jetty (U-diagnostics), bedankt voor jullie hulp en flexibiliteit met de F4H bloedsamples. Ook in het weekend kon ik terecht in het lab om de monsters op -80 te zetten.

Geachte leden van de leescommissie, prof. dr. Kemner, prof. dr. Adan, prof. dr. Ramsey, prof. dr. Smeets en prof. dr. ir. de Graaf, bedankt voor het lezen en beoordelen van mijn proefschrift.

Lieve vrienden en familie, bedankt voor de ontspannende uitjes en alle andere chill momentjes. Die zijn echt van onschatbare waarde geweest voor me!

Lieve pa en ma, bedankt voor jullie onvoorwaardelijke steun en de hulp met de kids. Ik kan altijd op jullie rekenen.

Lieve Ans en Hans, betere schoonouders kan ik me niet wensen! Jullie staan altijd voor me klaar. Bedankt voor jullie hulp!

Liefste Daan, Dani, in het tweede jaar van mijn promotieonderzoek ben jij geboren. Iets mooiers kon ik mij niet wensen. Jouw vrolijke persoonlijkheid, je lieve lach en je ondeugende streken zijn heerlijk om naar te kijken. Jij hebt mij geleerd om te relativeren en vooral om het werk los te laten. De laatste maanden waren zwaar. Ik moest veel werken en dat kon je maar moeilijk begrijpen. Ik zal nooit vergeten wat je op een ochtend tegen me zei, toen ik probeerde uit te leggen waarom ik weer moest werken (en daar compleet in faalde): “ik wil niet dat je doctor wordt, ik wil dat je gewoon mama bent”. Lieve scheet, ik zal altijd jouw mama zijn wat er ook gebeurt en wat voor werk ik ook zal doen!
Liefste Sophie, Phie, een paar jaar later werd jij geboren. Wat een mooi cadeau! In jouw fantastische blauwe ogen kan ik elke dag weer heerlijk wegdromen. Je bent een kleine boef met een stralende lach. Jouw intens lieve knuffels maken elke dag weer een cadeautje. Lieve Daan en Sophie ik hou super super super veel van jullie!

Lieve lieve Chiel, bon, we kennen elkaar sinds forever! Je bent er gewoon altijd voor me. Je bent eerlijk en zegt niet altijd wat ik wil horen maar dat is juist wat ik zo in je kan waarderen. Ik bewonder je chille en niet snel in de stress te krijgen persoonlijkheid. Ik zou daar heel graag een beetje van willen hebben! Al vanaf het eerste uur heb je bijgedragen aan mijn onderzoek. Je hebt me geholpen een website te maken voor de dataverzameling en je hebt scripts gemaakt voor de leuke maar complexe taak die ik bedacht had. De laatste maanden waren zwaar en niet altijd even makkelijk maar we hebben ons er doorheen geslagen. Samen met de kids gaan we de komende tijd vooral lekker genieten van elkaar! Love you bon!

Scientific achievements

Publications

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Lisette Charbonnier was born on March 21 1986 in Utrecht. She finished her MAVO (Lower general secondary education) at the Rientjes Mavo in Maarssen in 2001, her HAVO (Higher general secondary education) and VWO (Pre-university education) in respectively 2003 and 2005 at the Niftarlake college in Maarssen. She received her Bachelor of Science degree in Biology at Utrecht University in 2008 and received her Master of Science degree in Neuroscience and Cognition, Cum Laude in 2010. In 2011, she started her PhD at the Image Sciences Institute of The University Medical Centre Utrecht.