NEUROCOGNITIVE, BEHAVIOURAL AND PHYSIOLOGICAL EFFECTS OF NON-NUTRITIVE SWEETENERS IN HUMANS

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List of Abbreviations

AB	Attentional bias
ACC	Anterior cingulate cortex
Ace-K	Acesulfame K
ADI	Acceptable daily intake
BMI	Body mass index
BOLD	Blood oxygen level dependent
CCK	Cholecystokinin
CPIR	Cephalic phase insulin response
CPR	Cephalic phase response
DEBQ	Dutch eating behaviour questionnaire
DGAC	Dietary guidelines advisory committee
dlPFC	dorsolateral prefrontal cortex
EFSA	European Food Safety Authority
FDA	US Food and Drug Administration
fMRI	Functional magnetic resonance imaging
GI	Gastrointestinal
GIP	Gastric inhibitory peptide
GLP-1	Glucagon-like peptide
GLUT2	Glucose transporter 2
GS	Gymnema sylvestre
HEI	Healthy eating index
IAT	Implicit Association Task
(i)AUC	(incremental) area under the curve
IPAQ	International physical activity questionnaire
NAc	Nucleus accumbens
NNS	Non-nutritive sweetener(s)
NTS	Nucleus tractus solitarius
OFC	Orbitofrontal cortex
OGTT	Oral glucose tolerance test
PFC	Prefrontal cortex
physMRI	Physiological magnetic resonance imaging
PYY	Peptide YY
RCT(s)	Randomised controlled trial(s)

Reb A	Rebaudioside A
ROI(s)	Region(s) of interest
RT(s)	Reaction time(s)
SACN	Scientific Advisory Committee on Nutrition
SGLT1	Sodium-glucose linked transporter 1
SMA	Supplementary motor area
SN	Substantia nigra
SRCT	Stimulus response compatibility task
SSB(s)	Sugar sweetened beverage(s)
SSS	Sensory specific satiety
STR(s)	Sweet taste receptor(s)
T2DM	Type 2 diabetes mellitus
TFEQ	Three factor eating questionnaire
TRPM5	Transient receptor potential cation channel subfamily M member 5
VAS	Visual analogue scale(s)
VTA	Ventral tegmental area
VPT	Visual probe task
WHO	World Health Organisation

Abstract

Non-nutritive sweeteners (NNS) constitute a promising tool toward sugar and energy intake reduction. However, NNS effects on appetite and eating behaviour in humans is not yet fully understood. Control of food intake is the result of a complex interaction between homeostatic and hedonic signals, that collectively act to govern eating behaviour. Since oral sweet taste sequentially precedes gastrointestinal chemosensation, it is possible that neurocognitive and reward-related mechanisms contribute to the control of food intake, beyond any physiological and/or post-absorptive signals. The work presented in this thesis takes a multidisciplinary approach using a combination of methodologies to examine the effects of NNS, and in particular stevia, on neurocognitive, behavioural and physiological responses in humans.

Initially, I developed a battery of previously described neurocognitive tasks and tested its efficacy to detect differences in food-cue responses in healthy individuals when homeostatic signals are controlled (fasted vs fed state). A visual probe task (VPT) was shown to be sensitive to metabolic state changes and was selected for use in subsequent studies to dissect the effects of sweet taste and calories on food attentional bias (AB). In a next crossover double-blind randomised controlled trial (RCT), I examined the effects of a single exposure to a stevia-sweetened beverage on appetite, food intake and AB to food cues relative to sweet caloric (glucose, sucrose), non-sweet caloric (maltodextrin) and non-sweet non-caloric (water) controls in healthy lean adults. Results showed a significant reduction in total energy intake (meal and beverage) in the stevia-sweetened beverage condition compared to water. Only caloric beverages increased blood glucose levels, stevia and caloric beverages both influenced appetite ratings, but AB to food cues did not differ across conditions. In a following open-label RCT, I examined the effects of daily stevia consumption for 12 weeks on glucose homeostasis, body weight and energy intake in healthy lean adults. My findings suggest that daily stevia consumption does not affect glucose homeostasis, but has a significant effect on energy intake; individuals in the stevia group demonstrated a significant spontaneous reduction in energy intake compared to the control group. In the last piece of work, a double-blind crossover RCT in healthy lean adults, I investigated the neural correlates of acute physiological signals and food-cue elicited responses related to consumption of stevia in comparison with appropriate controls for sweetness and calories (glucose, maltodextrin, water) using functional magnetic resonance imaging (fMRI). Stevia consumption demonstrated a longer-lasting and more robust blood oxygen dependent (BOLD) contrast decrease over time compared to other beverages in the brain. Consumption of stevia and caloric beverages elicited attenuated BOLD response in the visual cortex while performing a food VPT, compared to water.

In conclusion, the research presented in this thesis provides considerable evidence that stevia consumption elicits benefits in appetite and food intake and induces a significant attenuation effect in the brain, without affecting physiological responses such as glucose homeostasis. The above findings could be indicative that stevia is beneficial for human consumption, and lays the foundations for this research moving into key clinical areas, such as obesity and type 2 diabetes mellitus.

Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Date: 31-03-2021

Signed: *Nikoleta Stamataki*

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I wish to thank the Biotechnology and Biological Sciences Research Council, my funding body, for providing me with the resources to conduct the research presented in this thesis and to communicate my research by attending several international conferences. I would also like to thank Cargill for funding part of my project and providing some of the test products pro bono. My sincere thanks to Dr Corey Scott, for his continuous support and for making my internship happen despite the circumstances, Dr Douwina Bosscher and Dr Angela Bonnema. I acknowledge the support from N8 Agrifood for awarding a pump-priming funding to one of my projects. I am also grateful to Dr Benjamin Crooks and Dr Abubaker Ahmed for taking the time off their own projects to assist me with the medical procedures of my project. I also wish to thank Dr Marilena Hadjidemetriou and Lana Papafilipou for their assistance with the insulin analyses, but also for their friendship. I would also like to thank my friend Vasilis Nikolaou who was always willing to help me with any programming issues that came up. I am also grateful to all the participants who participated in my studies.

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I would like to thank my parents, my mum, Artemis and my dad, Stamatis, for raising me to be who I am today and for their endless love and support. Also thanks to my younger sister, Maria, for always believing in me and reminding me that I can achieve anything I want in life. Lastly I wish to thank my partner, for providing me with encouragement and support even when we had to be apart.

Preface

I studied Dietetics and Nutritional Science at Harokopio University, Athens, and graduated in July 2013. Early enough in my academic years I realised my passion for nutritional science and the imminent need to provide the people with healthier products in the battle against obesity. Following my bachelor degree, I went on to complete a Master of Science in Applied Dietetics & Nutrition in April 2015 at the same university, on a National State Scholarships Foundation Fellowship of Excellence for Postgraduate studies in Greece – Siemens Program. My thesis focused on the development of low glycaemic index bakery products and their effects on glucose and hormonal responses in humans. I worked one year as a Research Assistant at Harokopio University before I moved to Manchester to continue my education.

The work discussed in this thesis concludes my PhD in Nutrition at the University of Manchester, which I started working on in September 2016. The thesis examines the neurocognitive, behavioural and physiological effects of non-nutritive sweeteners, in particular stevia, in humans. For my PhD I have been supported by a 4-year Biotechnology and Biological Sciences Research Council (BBSRC) Doctoral Training Partnership (DTP) Case Studentship. The supervisor of this project was Prof John McLaughlin and the industrial partner was Cargill. As part of my DTP program, I am currently (at the time of writing) conducting an industrial placement at Cargill as a Nutrition R&D Intern.

Rationale for journal format thesis

I have been granted permission to submit this PhD thesis in Journal Thesis Format by my supervisor Professor John McLaughlin approved under the University of Manchester, Faculty of Biology, Medicine and Health regulations. The journal thesis format facilitates the publication of the data during or shortly after the PhD, and aids the development of skills in writing scholarly papers, essential for a career as a researcher. This format also reduces the potential conflict of interest between the drive to publish papers and timely completion of the thesis since these can be achieved simultaneously. Lastly, it encourages faster publication of the research outputs and enhances the student's research profile.

The present thesis is written in a manner of an introductory literature review (Chapter 1), which provides a broad overview of the available literature regarding non-nutritive sweeteners effects on neurocognitive, behavioural and physiological responses in humans. The thesis examined the effects of stevia specifically, therefore the last section of the introduction is devoted on stevia. The literature review is followed by the aims and hypotheses of this thesis. Each chapter is then written in journal-style and consists of separate abstract, introduction, methods, results and discussion. Chapters 2-4 have already been published in peer-reviewed journals and Chapter 5 is intended to be submitted very soon for publication. Chapter 6 provides a general discussion of results obtained and suggestions for future research in this field. All references are presented at the end of the thesis.

Prior to each experimental chapter I have provided a statement describing my individual contribution to it. I would like to highlight that all studies have been designed by me, with essential intellectual input by my supervisors and industry sponsors where appropriate. Prof McLaughlin critically evaluated my work at all stages of designing, preparing, executing and writing up each experimental piece of work. I have prepared, organised and executed all studies described in the experimental Chapters 2-5, I have performed all the statistical analyses, and took responsibility for writing the manuscripts, which were then read and reviewed by my supervisors and other co-authors.

Publications and presentations related to this thesis

Publications

- Chapter 2: **Stamataki NS**, Elliott R, McKie S, McLaughlin JT. Attentional bias to food varies as a function of metabolic state independent of weight status. *Appetite* 2019, 143:104388. doi: 10.1016/j.appet.2019.104388.
- Chapter 3: **Stamataki NS**, Scott C, Elliott R, McKie S, Bosscher D, McLaughlin JT. Stevia Beverage Consumption prior to Lunch Reduces Appetite and Total Energy Intake without Affecting Glycemia or Attentional Bias to Food Cues: A Double-Blind Randomized Controlled Trial in Healthy Adults. *The Journal of Nutrition* 2020, 150(5):1126-1134. doi: 10.1093/jn/nxaa038.
- Chapter 4: **Stamataki NS**, Crooks B, Ahmed A, McLaughlin JT. Effects of the Daily Consumption of Stevia on Glucose Homeostasis, Body Weight, and Energy Intake: A Randomised Open-Label 12-Week Trial in Healthy Adults. *Nutrients* 2020, 12(10):3049. doi: 10.3390/nu12103049.

During my PhD I have also participated in the following publications, which however do not form part of this thesis and should not be assessed.

- Crooks B, **Stamataki NS**, McLaughlin JT. Appetite, the enteroendocrine system, gastrointestinal disease and obesity. *Proceedings of the Nutrition Society* 2020, 4:1-9. doi: 10.1017/S0029665120006965.
- Wittekind A, Higgins K, McGale L, Schwartz C, **Stamataki NS**, Beauchamp GK, Bonnema A, Dussort P, Gibson S, de Graaf C, Halford JCG, Marsaux CFM, Mattes RD, McLaughlin J, Mela DJ, Nicklaus S, Rogers PJ, Macdonald IA. A workshop on 'Dietary Sweetness-Is It an Issue?'. *International Journal of Obesity (Lond)* 2018, 42(4):934-938. doi: 10.1038/ijo.2017.296.
- **Stamataki NS**, McLaughlin JT. Sweet sensing, homeostasis and hedonics in the human gut-brain axis. *Nutrition Bulletin* 2017, 42:172-177.

Presentations

- **Stamataki NS**, McLaughlin JT. Sweet taste *per se* affects energy intake: results from two randomised controlled trials in healthy adults. 9th European Conference on Sensory and Consumer Research, EuroSense 2020, ONLINE: Live and On-demand, 13-16 December 2020 (flash poster presentation).
- **Stamataki NS**, Crooks B, McLaughlin JT. Daily consumption of stevia drops effects on glycemia, body weight and energy intake: results from a 12-week, open-label, randomized controlled trial in healthy adults. Nutrition Live Online 2020 by the American Society for Nutrition, June 1-4 2020 (poster presentation).
- **Stamataki N**, Elliott R, McKie S, McLaughlin J. Beneficial Effects of Consuming a Natural Zero Calorie Sweetener Preload Prior to Lunch on Energy Intake: A Double-blind Randomised Crossover Study. Nutrition 2019 by the American Society for Nutrition, June 8-11 2019, Baltimore, USA (flash poster presentation & 3-minute research competition, 2 prizes were awarded as described below).
- **Stamataki N**, Rigby R, McLaughlin J. The physiological effects of non-nutritive sweeteners in humans: study protocol for a randomized clinical trial. N8Agrifood Conference 2018, June 13-14 2018, Liverpool, UK (best poster prize).
- **Stamataki N**, McKie S, Elliott R, McLaughlin J. The impact of metabolic state on neurocognitive responses to visual food cues. ECO2018-25th European Congress on Obesity, May 23-26 2018, Vienna, Austria (poster presentation).
- **Stamataki N,** McKie S, Elliott R, McLaughlin J. Hunger increases visual attention to food cues in healthy controls. 10th EFAD Conference, September 29-30 2017, Rotterdam, The Netherlands (poster presentation).
- **Stamataki N**, McLaughlin J. Carbohydrate rich meals and neurocognitive testing. Mini-Symposium on Carbohydrates and Health, August 21-24 2017, Grasmere, UK (oral presentation).

Awards

- Second place winner - 5th Emerging Leaders in Nutrition Science Poster Competition, Nutrition 2019 Annual Nutrition Science Meeting by the American Society for Nutrition, 8-11 June 2019, Baltimore, USA.
- Second place winner - Student Interest Group Travel Award for the 3-minute research competition, Nutrition 2019 Annual Nutrition Science Meeting by the American Society for Nutrition, 8-11 June 2019, Baltimore, USA.
- New Phytologist Trust Poster Prize for best poster presentation at the N8 Agrifood Conference 2018, 13-14 June 2018, Liverpool, UK.

CHAPTER 1

Introduction

1.1 General Introduction

The prevalence of obesity has risen markedly the last decades. Overweight and obesity consist the major causes of comorbidities, including type 2 diabetes (T2DM), cardiovascular disease, hypertension, certain types of cancer and other health conditions. Identifying strategies that can aid with weight management is imperative. The fundamental cause of obesity is the energy imbalance between calories consumed and calories expended, caused by an increased intake of energy-dense foods rich in fats and sugars and physical inactivity. Public health policies around the world aim at decreasing obesity prevalence: one of the most promiment is reducing sugar consumption (1). However, cutting down on the consumption of sugar can be very challenging, considering the strong innate preference humans (and many other species) have to sweet taste (2), that is likely to be rooted in brain circuits dedicated to recognising high-energy food sources for human survival (3).

Official guidelines recommend limitation of sugars intake as low as 5% of total calories. Food and drinks manufacturers are working to help achieve the 5% free sugars goal (4). As a consequence, food industries have turned their interest to alternative sweet taste sources in order to reduce the amount of sugars in foods and beverages; non-nutritive sweeteners (NNS) provide sweet taste yet minimal or no calories. Substituting NNS for sugars lowers the energy density and sugar content of food products while maintaining the palatability, since taste remains a key driver of food selection and consumer purchasing decisions. As such, NNS constitute a promising tool toward sugar reduction strategies, thereby facilitating weight loss, weight maintenance or prevention of weight gain.

However, the role of NNS on public health, including body weight and appetite, remains a topic of great controversy with outcomes ranging from harmful to neutral to beneficial (5-7). Some researchers have highlighted concerns that use of NNS raises risks for obesity and metabolic disorders (8, 9). Most of these concerns are rooted in the uncoupling of sweet taste and calorie content hypothesis, which has been suggested to interfere with gut-brain circuitry and potentially alter metabolic responses due to binding in the sweet taste receptors (STRs) in the oral and extraoral tissues (10). The lack of conclusive findings regarding NNS use on appetite and health outcomes has led to a variety of recommendations regarding their consumption across different health organisations (11), and has set the background for more well-designed randomised controlled trials (RCTs) in this field.

Control of food intake involves an interplay between homeostatic and non-homeostatic (hedonic) mechanisms, which act together to influence eating behaviour (12). Caloric sugars offer the hedonic experience (sweetness) along with a homeostatic response; increase in blood glucose levels and hormonal responses postprandially; on the contrary

NNS offer the hedonic experience alone, without the postprandial metabolic consequences of caloric sugars (13). This incongruence has been considered to interfere with learned responses associated with appetite control and glucose homeostasis, and potentially body weight regulation. However, it could be argued that due to sweet taste being a strong hedonic signal and evolutionarily a strong predictor of energy content, sweetness-mediated cognitive and reward mechanisms may also be involved in signalling satiety independently of physiological responses, as a result of satisfying the desire for sweetness/pleasure.

Despite significant advances in our understanding of human eating behaviour and the interplay between homeostasis and hedonics, there are significant gaps on the mechanisms by which sugars and NNS affect physiological, behavioural and neurocognitive responses in humans and this forms the basic objective of this thesis. The literature review covers our current knowledge on the effects of NNS on feeding behaviour, physiological and neurocognitive responses. Firstly, I have reviewed basic facts around the consumption of sugars and NNS, next I have reviewed the effects of NNS use on feeding behaviour (appetite, food intake) and body weight. The following section includes a literature review on the effects of NNS on physiological responses, including the potential functional roles of oral and gut STRs and whether NNS use affects glucose response and hormonal secretion in humans. Following that, the literature on the neurocognitive responses related to NNS consumption in humans including their neural effects in homeostatic and hedonic circuits is discussed and the last section of the introduction covers the literature regarding stevia, since stevia was the NNS examined in this thesis.

1.1.1 Public health policies encourage limited sugar intake

Free sugars (for definitions see the note below **Table 1.1**) contribute to the overall energy density of diets, and may promote a positive energy balance (14). Maintaining energy balance is crucial for a healthy body weight and ensuring optimal nutrient intake. An increased consumption of free sugars especially in the form of sugar-sweetened beverages (SSBs), may increase the total energy intake and decrease the consumption of more nutritious foods or beverages, potentially leading to unhealthy diet, weight gain and greater risk for non-communicable diseases (15, 16). Beverages are the main contributor of sugars in the diet of Americans (17) and the second in Europe (18). On the other hand, decreasing the intake of free sugars has been associated with a reduction in body weight (19).

The World Health Organisation (WHO), the Scientific Advisory Committee on Nutrition (SACN) in the UK and the Dietary Guidelines Advisory Committee (DGAC) in the US have published their recommendations regarding consumption of 'free' sugars or 'added' sugars and can be briefly summarised in Table 1.1. The intake of free sugars is recommended to be as low as 5% of the total energy intake in children and adults.

Table 1.1 Sugars intake recommendations by health organisations.

Author group, year	Recommendation
WHO, 2015	<10% of total energy intake from free sugars for children and adults (strong recommendation) <5% of total energy intake (conditional recommendation) *Conditional recommendations are made when there is less certainty "about the balance between the benefits and harms or disadvantages of implementing a recommendation"* (20).
SACN, 2015	≤5% of energy from free sugars
DGAC, 2015	≤10% of energy from added sugars

DGAC, Dietary Guidelines Advisory Committee; SACN, Scientific Advisory on Nutrition; WHO, World Health Orgnisation. Adapted from Mela and Woolner (21). Note: The definition 'free' sugars corresponds to all sugars (mono- and disaccharides) added during processing and those that are naturally present in fruit juices, pureed fruit or vegetables, while excluding those that are naturally occurring in whole fruits, vegetables and dairy products. The definition 'added' sugars includes sugars added to foods during preparation or processing, thus excludes any naturally occurring sugars present in intact fruits, vegetables or in their juices and dairy products (21, 22), and 'total' sugars includes all sugars from every source. In regard to health effects, the term 'free' sugars represents the sugars that are most consistently associated with increased risk of obesity, diabetes and dental caries, thus it is suggested that the emphasis for intake monitoring should be on free sugars (21).

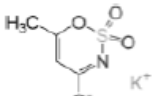
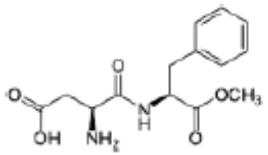
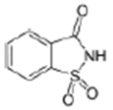
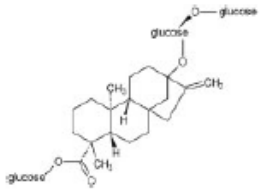
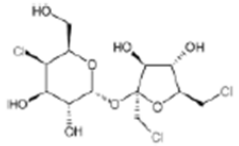
1.1.2 NNS: definitions, acceptable intakes and official guidance

According to the US Food and Drug Administration (FDA) NNS are ingredients used to sweeten and enhance the flavour of foods (23). NNS have been given different names including low-calorie sweeteners, artificial sweeteners, high-intensity sweeteners, and non-caloric sweeteners, without any specific differences among them. For purposes of clarity and in the context of this thesis the term NNS will be used.

Most of the NNS approved for human consumption are synthetic (artificial sweeteners), however more and more NNS of natural origin become available in the market and have become very popular among consumers. The most familiar natural NNS are *Stevia rebaudiana* based products. The safety of all NNS that have been permitted in the EU market have been extensively evaluated by the European Food Safety Authority (EFSA). At the moment the approved NNS in EU by EFSA as food additives in foods and beverages are acesulfame-K (ace-K), advantame, aspartame, aspartame-acesulfame salt, cyclamate, neohesperidine DC, neotame, saccharin, steviol glycosides, sucralose and thaumatin. The above substances share a common characteristic which is sweet taste, but they are completely different chemical compounds (see **Table 1.2** for details). Although stevia has been used for decades in some countries (i.e. Japan), stevioside and rebaudioside A (Reb A), the sweet extracts of the *Stevia rebaudiana Bertoni* plant, were relatively recently approved as a food additive by the EFSA and the FDA (24, 25). In the USA, the luo han guo fruit extracts (mogrosides) are also recently approved NNS of natural origin.

NNS are used by the food industry and consumers to reduce the amount of sugar and calories in foods and beverages, while maintaining palatability. Theoretically, a reduction in the amount of calories consumed could aid toward moderation of daily energy intake thus weight management benefits. A replacement of sugars with NNS in a beverage will also reduce the spike in blood glucose, which could theoretically offer benefits in glucose homeostasis. However the evidence base supporting the use of NNS for these purposes is still insufficient, prompting relevant governmental and professional organisations to issue equivocal and inconsistent guidance on their use. For example, the American Heart Association recommends that NNS beverages may be beneficial as replacements to reduce intake of SSBs in the short-term for adults who are habitual consumers of SSBs, but advises against prolonged consumption by children (26). The 2015 Dietary Guidelines for Americans report that NNS might be a useful tool to lose weight in the short-term however their long-term effectiveness is questioned, while Diabetes UK recommends that NNS can be used by adults and children in the management of weight and diabetes. A summary of the position statements of various professional organisations can be found in **Table 1.3.**

Table 1.2 Comparison of different NNS in terms of structure and sweetness.

Sweetener (E no.)	Compound classification	Structure	Sweetness intensity [a]	Amount that replaces 25 g of sugar	Acceptable Daily Intake (ADI) milligrams per kilogram body weight per day (mg/kg bw/d)[b]
Acesulfame K (E950)	Oxathiazinone dioxide		~ 200 times	125 mg	15
Aspartame (E 951)	Methylated dipeptide		~ 200 times	125 mg	40
Saccharin (E954)	Benzoic acid sulfimide		~ 300 times	80 mg	5
Steviol glycosides (E960)	Glycosylated diterpenes	 (Stevioside)	~ 200-300 times	80-125 mg	4
Sucralose (E955)	Chlorinated disaccharide		~ 600 times	40 mg	15

Adapted from Magnuson *et al.* (27). [a] Sweetness as compared with sucrose on a gram-for-gram basis. [b] ADI established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

Table 1.3 Recommendations on NNS consumption by health organisations.

Author group, year	Recommendation
Academy of Nutrition and Dietetics, 2012 (28)	Consumers can safely enjoy a range of NNS when consumed within an eating plan that is guided by current federal nutrition recommendations (such as Dietary Guidelines for Americans, Dietary Reference Intakes) as well as individuals' personal goals and preference.
EFSA, 2011 (29)	There is sufficient scientific information to support the claims that NNS lead to a lower postprandial rise in blood glucose levels, if consumed instead of sugars.
Diabetes UK, December 2018, Position Statement (30)	NNS can be used as a strategy for adults and children in the management of weight and diabetes.
American Heart Association, Science Advisory, 2018 (26)	For adults who are habitually high consumers of SSBs, NNS beverages may be a useful replacement strategy to reduce intake of SSBs. This approach may be particularly helpful for persons who are habituated to a sweet-tasting beverage and for whom water, at least initially, is an undesirable option. Prolonged consumption of NNS beverages by children is not recommended.
American Diabetes Association, Consensus report, Diabetes Care 2019 (31)	Replacing added sugars with sugar substitutes could decrease daily intake of carbohydrates and calories and is recommended as a short-term strategy. People are encouraged to decrease both sweetened and NNS-sweetened beverages and use other alternatives with an emphasis on water intake.

1.1.3 NNS consumption trends

Growing health awareness and a rise in obesity-related health conditions has resulted in an increased demand of food products that support good health. Consequently, NNS consumption has increased significantly over the past few decades (32, 33). Although they are consumed worldwide, the majority of data describing the prevalence of NNS consumption trends are limited to data from the US (32-34). Consumption of NNS has increased from 26.9% in 1999-2000 to 41.4 - 47.8% in 2007-2012 among American adults and from 8.9% in 1999-2000 to 25.1% in 2007-2012 among children in the US (35, 36). The main source of NNS are beverages (31.9% in the adult US diet) followed by food and beverage additions (25.2%) and surpassing food (9.3%) (35). Although the majority of studies linking NNS consumption and relations to health focus on beverages, characterising all sources of NNS consumption will probably enhance exposure classification leading to more robust epidemiological results (37).

The majority of the epidemiological studies consistently show that NNS consumption is higher among women, older, non-Hispanic white individuals, individuals with higher socioeconomic status and higher body mass indexes (BMI) (33, 35, 36). It has also been reported that the frequency of consumption is increased with body weight in adults (36). The most notable increases in consumption of NNS were observed among 6-11 year old children and among older adults (> 55 years of age), compared to other age-groups (38).

Consumption of NNS has been demonstrated to be associated with better diet quality. Results from the National Diet and Nutrition Survey Rolling Programme in the UK (2008–2012 and 2013–2014) demonstrated that compared to SSBs consumers, NNS consumption was associated with lower energy, and total free sugars intake while no other difference was demonstrated for any other macronutrients. There was an increased odds of meeting current UK dietary guidelines on free sugar intake (39). Apart from British adults, US consumers of NNS reported higher Healthy Eating Index (HEI) subscores for vegetables, whole grains and low-fat dairy compared to non-consumers. It was concluded that NNS use was associated with higher HEI 2005 scores and a general healthier lifestyle such as less smoking and higher physical activity compared to non-consumers (results from NHANES 1999-2008) (40).

1.2 NNS impact on body weight and eating behaviour

1.2.1 What is so special about sweet taste?

Sweetness is a very potent biopsychological stimulus; sucrose is one of the most palatable and preferred tastants throughout the animal kingdom. Similarly, humans have an inborn liking for and acceptance of sweetness. Our preference for sweet taste starts in very early days (**Figure 1.1**) and decreases with age (2). Evolutionarily a biological drive to prefer sweetness at high concentrations during childhood consists a survival advantage, since sweetness predicts source of energy (mother's milk, fruits etc). However, in the current obesogenic environment rich in high-sugar foods and beverages, these evolutionarily driven taste preferences have turned against us and made humans vulnerable to overconsumption, thus weight gain and obesity (2).

Figure 1.1 Liking and disliking response in human infants. The characteristic response of the tongue is protrusion in response to sugar liking. Pictures taken from Kringelbach *et al.* (41).

Sweet taste experience starts on the tongue with activation of STRs and oral sweet taste sensation, both with caloric sugars and NNS. Tasting something sweet creates a sensory hedonic experience, an information which is then relayed in brain circuits of food reward. Consumption of NNS might satisfy our natural propensity for sweetness providing the reward without the calories. A representative example showing the innate preference humans have for sweet taste is presented in **Figure 1.2**.

In the next section, the effects of NNS consumption appetite, food intake and body weight will be discussed.

Figure 1.2 Example of the strong innate preference humans have for sweet taste.

This picture was taken at the University of Manchester in March 2017, students were invited to write on a big blackboard placed outside the University Place what they want to do before they die. Someone wrote that they want to eat all the sugar, reminding us the importance of sweet taste in the human diet.

1.2.2 NNS effects on appetite

According to Blundell *et al.* (42) appetite has two definitions, the first one covers the whole field of food intake, selection, motivation and preference while the second relates specifically to the response to environmental stimuli in contrast to eating in response to homeostatic signals. In fact, appetite is an interaction between internal and external environments and therefore has both biological and behavioural/psychological aspects (43). Researchers usually use subjective appetite ratings (visual analogue scales (VAS) for hunger, fullness etc) and/or blood biomarkers (insulin, ghrelin, glucagon-like peptide-1 (GLP-1), peptide YY (PYY) and others) to assess appetite. VAS are accepted as a standard, valid methodological approach to substantiate claims relating to effects of foods on appetite (42). The effects of NNS consumption on hormonal responses will be discussed in a later chapter as part of the physiological responses (Chapter 1.3.2).

An early idea was that NNS could negatively affect appetite due to the uncoupling of sweet taste and calories (44). According to this hypothesis, acute consumption of NNS-sweetened beverages results in a short-lasting decrease in hunger, which is then followed by a rebound increase in hunger sensation (45-47). However, later studies challenged this concept. An acute study by Anton *et al.* showed that subjective appetite ratings to the consumption of a preload including tea sweetened either with sucrose, aspartame or stevia did not differ across conditions (48), suggesting similar satiating effects for caloric sweeteners and NNS. On top of that, participants did not compensate by eating more in the subsequent meal after

NNS ingestion. In line with these observations, in the study by Fantino *et al.* participants showed similar ratings of appetite (hunger, fullness and desire to eat) after acute or chronic exposure to NNS-sweetened beverages compared to water (49). More findings come from the study by Farhat *et al.* who also showed decreased desire to eat ratings following a stevia-sweetened preload and a sucrose-sweetened preload compared to water (50). However, another study did not replicate these findings, and showed that desire to eat, hunger and prospective consumption ratings were lower at 30 and 60 min following the consumption of a sugar-sweetened beverage compared to beverages sweetened with aspartame, stevia or monk-fruit (monk-fruit is another plant-based NNS, approved for use in the US) (51).

Another argument against consumption of NNS is that exposure to sweet taste might promote desire for sweet-tasting foods and induce sugar-craving. Therefore, long-term NNS consumption could establish a preference for sweet items in the diet (52). In a study by Casperson *et al.* acute consumption of NNS increased the motivation to gain access to sweet snacks relative to savoury snack foods in an acute setting (53). However, most of the experimental data do not confirm this finding. Fantino *et al.* showed that appetite for and motivation to eat sweet foods were not affected by acute or long-term exposure to NNS (49). In another study, which examined the effects of substituting caloric beverages with either diet beverages or water, a decrease in energy intake from desserts was demonstrated in the diet beverage group after 6 months of intervention (54), suggesting that NNS might on the contrary satisfy the urge for sweet items rather than increasing it. More evidence comes from the study by Kashima *et al.* where the blockage of sweet taste sensation by *Gymnema sylvestre (GS)*, a sweet taste inhibitor, during consumption of sweet items, resulted in decreased satisfaction and higher desire to consume sweet tasting foods compared to when the sweet taste was present (55). An absence of differential appetite responses between NNS and placebo when the oral sweet taste is bypassed (via administration of encapsulated NNS) also supports this view. Consumption of encapsulated aspartame for 12 weeks did not influence appetite sensations compared to placebo capsules in healthy lean adults previously naïve to NNS (56).

The above findings are also in line with the sensory specific satiety (SSS) theory, which describes the reduction in the pleasantness of the taste of a food (momentary liking) that occurs with consumption, compared with the relative preservation of momentary liking for uneaten food with dissimilar orosensory properties. The SSS is independent of the calorie content of the stimuli (57) and can last up to 1 hour post ingestion (58). In a recent study, Rogers *et al.* demonstrated a SSS effect present shortly after the consumption of a fruit squash sweetened with NNS compared to water beverage and no increase in desire to eat sweet foods 2 hours after the consumption of the NNS-beverages compared to water. In

addition, compared with still water, diet-cola reduced sweet food intake in a subsequent meal test (but not total food intake), suggesting that consumption of NNS acutely reduces the desire for sweet foods rather than the opposite (59).

Beneficial effects of NNS consumption on appetite also come from the study by Maloney *et al.,* in which the investigators demonstrated that NNS might help frequent NNS consumers to control food cravings (60). In a set of studies, Maloney *et al*. showed that frequent NNS consumers exhibited lower energy intake in a craving condition compared to non-frequent consumers who consumed more calories in the craving condition (chocolate cues) compared to a control condition (neutral cues). Frequent NNS consumers demonstrated higher dietary restraint scores, therefore it is plausible that NNS might benefit these individuals by satisfying the urge for sweetness without compromising their dieting goals (61). However, the protective effect of NNS frequent consumers in craving-induced situations was not confirmed in a second sub-study where the availability of NNS was manipulated (available vs unavailable). Peters *et al.* demonstrated that during a 12-week weight loss programme, consumption of NNS beverages compared to water, led to significantly greater reductions in subjective feelings of hunger. Participants who were included in this study were previously regular consumers of NNS (consumed NNS at least 3 times a week) (62).

Taken together, these findings suggest that NNS consumption does not induce rebound hunger or an increase in desire to consume sweet items and has either neutral or even beneficial effects on appetite in humans. More randomised controlled acute and long-term trials are warranted to further confirm the effects of NNS consumption on human appetite.

1.2.3 NNS effects on food intake

NNS provide the pleasure of sweetness but without the calories. Therefore it could be expected that their use could contribute towards reducing the intake of caloric sugars in the diet of adults and children with a broader aim to reduce total calorie intake. However, the extent to which the ‘saved calories’ from replacing caloric sugars with NNS are compensated by will define whether NNS consumption will benefit energy intake or not. There have been plenty of studies examining the effects of NNS use on either acute energy intake (mostly preload paradigm studies) and long-term energy intake, following a period of intervention with NNS compared to a control. In this section I will review the evidence from recent meta-analyses which have accumulated all the relevant research in human individuals.

A meta-analysis by Rogers *et al.* in 2016 showed that in short-term RCTs (<1 day duration) consumption of a NNS-sweetened preload versus sugar-sweetened preloads is consistently found to reduce acute energy intake, but no difference was demonstrated in the comparisons NNS versus water, NNS versus unsweetened products or NNS versus nothing (63). Another recent meta-analysis conducted a comparison between equisweet preloads (differing in calorie content) and equicaloric preloads (differing in sweetness) on subsequent energy intake. Authors reported significantly lower energy intake at an *ad libitum* meal following the consumption of the NNS-sweetened preloads compared to unsweetened preloads in the equicaloric comparison (this means NNS-sweetened preload vs water). Regarding the equisweet comparison and taking the total energy intake into account (preload and meal) the pattern was trending towards reduced energy intake for the NNS-sweetened preload, revealing an only partial compensation from the caloric sweetened preload (64). However, the preload design, which is the most common approach in human clinical trials assessing short-term appetite and food intake, has some significant limitations. Most importantly, an effect on energy intake following an acute consumption of NNS-sweetened preloads fails to reflect potential longer-term effects towards dietary compensation (65).

Meta-analysis of long-term RCTs with duration more than 1 day concluded that consumption of NNS compared with caloric sugars also leads to reduction in energy intake, while the authors also reported a reduction in the comparison between NNS and water (63). Another recent meta-analysis by Rogers and Appleton included 88 parallel-group and crossover studies with duration at least one week and the comparisons of interest were NNS consumption versus caloric sugars, NNS versus water or nothing and NNS in capsules versus placebo capsules. The investigators observed a consistent effect towards reduced energy intake in favour of the NNS treatments compared to caloric sugars, however no significant effect was demonstrated when NNS were compared with water or nothing and inconsistent findings were shown when NNS was delivered in capsules versus placebo capsules (66). These findings suggest that the effects of NNS on energy intake were mostly due to their lack of energy content, as opposed to their taste or any post-ingestive effects.

The comparison of NNS versus water is of great interest, as any differential effects in favour of the NNS could be attributed to the pleasurable sensation of sweet taste *per se* and food reward mechanisms. The lack of a significant reduction of food intake between NNS and water or nothing in the recent meta-analysis by Rogers & Appleton is probably due to the relatively few studies available, with mixed results according to the authors. Energy intake assessment in long-term trials is also challenging because it depends on self-reporting methods, such as retrospective 24-h dietary recalls or food diaries that are completed by the participants. More randomised controlled acute and long-term trials are required to

investigate the effects of NNS on energy intake, especially for the comparisons between NNS and water or nothing using appropriate controls.

1.2.4 NNS effects on body weight

Consumption of caloric sugars has been consistently reported to play a significant role in weight gain, obesity and its comorbidities (15, 67-70). The role of NNS use on body weight is discussed below.

1.2.4.1 Evidence from prospective cohort studies

Meta-analyses from cohort studies have shown either no (63) or a positive association between NNS use and BMI (71, 72) or body weight (72, 73). Miller & Perez (71) conducted a meta-analysis which included nine prospective cohort studies, four of which were conducted in children and adolescents and five in adults. Results showed a small but significant positive association between NNS use and BMI, but there was no significant association with body weight or fat mass. The authors suggested that the results of this meta-analysis were limited largely by differences across the individual studies, potential sources of bias in some of the included studies, such as not controlling for energy intake and baseline weight and BMI, as well as insufficient measurement of NNS intake. The meta-analysis by Rogers *et al.* (63) included results from 12 prospective cohort studies, which reported inconsistent associations between NNS use and BMI concluding that there is no change in BMI with consumption of NNS.

Another meta-analysis by Azad *et al.* (72) included 30 cohort studies and concluded that consumption of NNS was associated with a modest increase in BMI, suggesting that evidence does not clearly support the intended benefits of NNS. However, this study has received quite a lot of criticism mainly because of the selection of the data from the original papers, as the authors used baseline or prevalent data of exposure instead of the change data, an approach that is usually used to offer protection against the issue of reverse causality.

In summary, the evidence from prospective cohort studies show no or small positive association between NNS consumption and BMI or body weight. Nevertheless, causal relationships cannot be claimed with data from observational studies, and data are difficult to interpret as associations may be due to confounding or reverse causality. High consumers may be consuming NNS as a strategy to lose weight being already overweight or obese, or at risk (10, 74). Indeed, Bleich *et al.* confirmed the above revealing that 1 out of 5 overweight and obese adults in the US consumed diet versions of all kind of beverages, and this figure is almost twice compared to normal weight individuals (75). As outlined by Mela *et al.* (76) in a recent perspective article, reporting of evidence on health associations

with NNS from observational and prospective cohort studies should be clear that these are subject to residual confounding and may have been initially designed to answer a different research question, therefore should be interpreted with great caution.

The next section discusses the available data from RCTs, which provide the highest quality of evidence for examining the potentially causal effects of NNS use and body weight.

1.2.4.2 Evidence from randomised controlled trials

In contrast to the conclusions from prospective cohort studies, findings from RCTs show a different picture.

In the meta-analysis by Miller and Perez (71) 15 RCTs were included in the analysis with most control interventions being SSBs or sugar-sweetened foods. The meta-analysis showed that NNS consumption modestly but significantly reduced all outcomes examined, including body weight and BMI. The authors highlighted that a single dietary change, replacement of caloric sugars with NNS in that case, cannot result in clinically significant weight loss, however the maintenance of palatability with few or zero calories that NNS offer might aid toward better adherence in weight loss programs. In the meta-analysis by Rogers *et al.* (63) sustained RCTs were included with duration varying from 4 weeks to 40 months. Consumption of NNS vs sugar resulted in reduced body weight and a similar reduction vs water, however this analysis was based on 3 comparisons only therefore more research is warranted in order to draw firmer conclusions.

Recent meta-analysis by Rogers and Appleton included results from RCTs with duration at least one week comparing either NNS vs sugars, NNS vs water or nothing or NNS capsules vs placebo capsules (66). Overall results showed a beneficial effect of NNS vs sugar on body weight, while the duration of the intervention or participant blinding did not affect the outcome. However, results showed no difference in the comparison NNS vs water or nothing on body weight, and the comparison between NNS capsules or placebo capsules resulted in mixed and inconsistent effects. Another recent meta-analysis by Laviada-Molina *et al.* assessed the effects of NNS intake compared to sucrose, water or nothing, in RCTs with at least 4 weeks duration (77). NNS use showed significant weight differences in favour of NNS; however grouping by the nature of comparator revealed that NNS vs placebo, no intervention or water exerts no significant effects on body weight, confirming the results by Rogers and Appleton. Interestingly, effects on body weight were only significant when participants were in unrestricted diets rather than weight-reduction diets.

On the contrary Azad *et al.* (72) showed that NNS had no significant effect on BMI in a meta-analysis that included 7 RCTs, with duration at least 6 months. Toews *et al.* drew similar conclusions in their meta-analysis (73), both randomised and non-randomised

controlled trials were included with study duration at least 7 days of intervention. The authors concluded that in RCTs there was no significant difference in body weight between adults receiving NNS compared to caloric sugars or placebo. Differences in methodological designs that result in differences in selected studies could account for these discrepancies, for example Azad *et al.* excluded cross-over studies as well as studies with intervention periods less than 6 months.

Latest research has also highlighted the potential sweetener-specific effects on body weight. A recent study by Higgins *et al.* (78) showed that different NNS exhibited different effects on body weight over a 12-week period, participants in the sucralose group reduced body weight, the saccharin group showed slight weight gain while the aspartame and stevia group showed no change in body weight. Because NNS differ significantly in their absorption, distribution, metabolism and excretion, the possibility of exhibiting differential effects on body weight cannot be discounted and might be relevant for the interpretation of experimental data (76).

In conclusion, results from RCTs do not support the presumption brought in by some observational studies that NNS are related to weight gain. Substituting NNS for caloric sweeteners results in a modest but consistently significant weight reduction, therefore NNS use seems a straightforward strategy towards achieving a net energy saving in the long-term. Trials contrasting NNS with placebo or water show in both cases neutral effect on body weight, even though there are some findings supporting beneficial effects with NNS use.

1.3 Physiological responses related to NNS consumption compared to caloric sugars

1.3.1 Sweetness in the mouth and in the gut

Taste is the sensory system devoted primarily to a quality check of food to be ingested, with sweet taste signalling the presence of energy-rich carbohydrates, therefore promoting intake. From an evolutionary perspective it could be considered maladaptive to recognise sweetness from NNS since they do not contain any energy for physiological function (79). The discovery of STRs in extraoral tissues and especially throughout the gastrointestinal (GI) tract, raised questions regarding the involvement of gut STRs in nutrient chemosensing and satiety signalling. As a consequence a potential functional role of NNS on metabolic responses has been proposed. This section will review the available evidence examining the potential role of oral and gut sweetness on metabolic outcomes, with an emphasis in data from human intervention studies.

1.3.1.1 Oral and extraoral sweet taste receptors

The physiological role of taste is to detect key nutrients and avoid potentially toxic molecules. Sweet taste signals the presence of energy-rich carbohydrates which increases the hedonic tone of food, promotes intake and prepares the body to optimised food digestion and absorption by initiating cephalic responses (80).

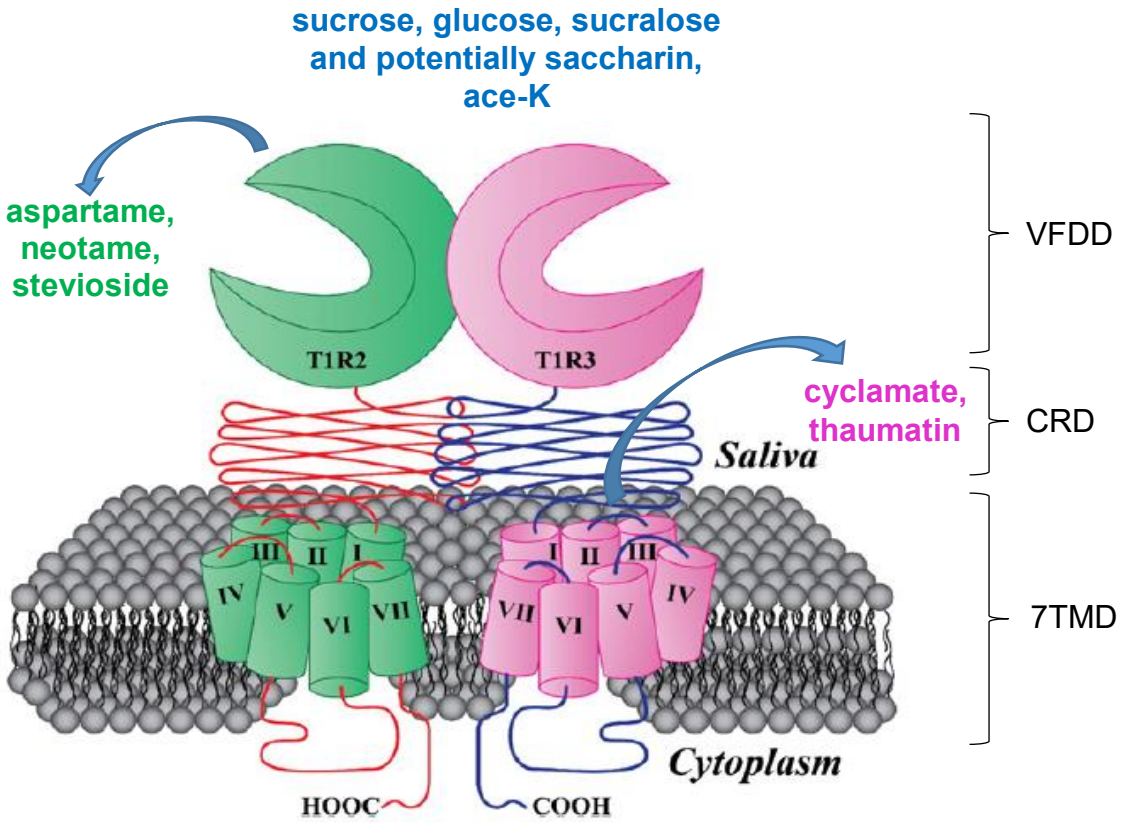
The STR is a heterodimeric G-protein coupled receptor consisting of two subunits (T1R2/T1R3). Binding of a ligand to the receptor results in activation of intracellular signalling pathways such as a-gustducin and further leads to intracellular Ca2+ release. This signalling chain then activates transient receptor potential cation channel subfamily member 5 (TRPM5). This allows sodium entry in the cell which facilitates cell depolarisation. Once activated the oral STR cells transmit the information via sensory afferent fibres to the brain areas involved in sweet taste processing to generate the perception of sweetness (81).

The T1R2/T1R3 STR responds to various and remarkably chemically distinct compounds, such as natural sugars, low calorie sweeteners, some D-amino acids, and sweet-tasting proteins (82). However, the T1R2 and T1R3 proteins are each capable to bind selected sweeteners with distinct affinities (**Figure 1.3**), and contain at least six binding sites for sweet agonists and antagonists. Each T1R subunit is comprised of three principal domains, an extracellular venus-flytrap domain, a seven transmembrane-spanning domain and a cysteine-rich domain connecting them (81, 83). Sucrose, glucose, sucralose and potentially saccharin and ace-K bind at both T1R2 and T1R3 (84, 85), aspartame and stevioside bind at T1R2 and cyclamate, thaumatin at T1R3 (85-88). This differential binding can lead to

differences in sweetness intensity; NNS achieve sweetness of sucrose at much lower concentrations probably due to stronger binding to the receptors (87), as well as activation of distinct intracellular signalling based on molecular size and shape (89).

The initial evidence for extraoral taste signalling was reported in 1996 by Hofer *et al*. (90), who described the presence of α-gustducin in brush cells in the stomach and throughout the epithelium of the gut sharing structural features of oral taste receptors. Following that, it is now known that STRs are expressed in plenty extraoral tissues including the stomach, small and large intestine, pancreatic beta cells, heart and brain (91-94). However, one needs to bear in mind that molecular evidence for expression of a receptor does not necessarily translate into functionality (95).

Figure 1.3 Illustration of the human sweet taste receptors with some examples of the compounds that can activate them. Font colours indicate the compounds that bind to both T1R2 and T1R3 (blue), only to T1R2 (green) or only to T1R3 (pink). Adapted from DuBois (96). 7TMD, 7-trans-membrane domain; CRD, Cysteine-rich domain; VFD, venus flytap domain.



1.3.1.2 The role of oral sweet taste on metabolic parameters

The sensory experience is important in determining food selection and dietary intake, and sweet taste in particular has been associated with food seeking behaviours (97). Over the

past few decades there has been an increasing interest in understanding the role taste perception plays in satiety, energy balance and metabolic responses (98).

The use of STR antagonists - oral sweetness perception vs no oral sweetness perception

Whether NNS and caloric sugars elicit similar or differing effects on metabolic parameters due to their oral sweet taste has been investigated in a few recent studies. A useful tool that has been used to explore the role of sweet taste on metabolic parameters as well as on satiety and food intake are T1R2/3 inhibitors, and in particular lactisole and GS. Lactisole is a STR antagonist and has the ability to block the sweet taste of several compounds including caloric sugars (fructose, glucose, sucrose) and NNS (aspartame, ace-K, saccharin) (99). GS is a plant from which gymnema acids are isolated which selectively suppress sweet taste sensation in humans without affecting the perception of other taste elements (100). Recent studies have used either lactisole or GS in order to examine the role of oral sweet taste on appetite and metabolic indices.

The first report was by Simpson *et al*. who demonstrated that a glucose solution administered following lactisole resulted in a lower mean peak glucose (101), highlighting the potential role of STRs on glucose homeostasis. Kashima *et al*. in a more recent study demonstrated that suppression of oral sweet sensation during ingestion of glucose has a role in decreasing the gastric emptying and slowing blood glucose and insulin responses (102, 103). In an attempt to explore whether these effects are triggered by sweet taste sensing *per se*, a NNS, aspartame, was included in the design along with glucose. In particular, participants initially rinsed their mouths with either water (control) or a GS solution (2.5%) and then consumed 200 g of either 0.09% aspartame or 15% glucose. Gastric emptying, blood glucose and plasma insulin did not differ between water and GS in the aspartame condition, but after glucose ingestion gastric emptying was lower in the GS, blood glucose and plasma insulin was lower at 30-40 min and higher at 50-80 min postprandially in the GS condition (103). Results were not confirmed in a later study by the same research group in response to the consumption of sweet-tasting foods (muffin, sweet yoghurt and banana) following rinse of participants' mouths with a GS solution. Gastric emptying, blood glucose, plasma insulin and appetite indices did not differ between pre-treatment with GS compared to distilled water (control condition) (55). The above suggest a potential impact of oral sweet taste on metabolic indices including gastric emptying, glucose and insulin response in humans, only when caloric sugars are present; however results at this point remain inconsistent.

Cephalic phase responses

STR activation in the oral cavity also prepares the body to optimise food digestion and nutrient absorption through cephalic phase responses (CPR). CPRs are anticipatory physiological responses that occur before swallowing and aim at optimal digestion and absorption of nutrients. Cephalic stimulation activates the vagus nerve, which in turn causes many autonomic responses such as the release of saliva and hormones like insulin and glucagon, however CPRs are also associated with non-secretory responses such as increased gastric motility and heart rate (104, 105). The most described and studied CPR is cephalic phase insulin release (CPIR), a neurally-mediated small transient increase in insulin release pre-absorptively in response to food-related sensory stimuli which consequently influences postprandial glycaemia (106, 107).

Caloric sweeteners like sucrose and glucose has been shown to induce a significant CPIR (108-110). Regarding NNS, Just *et al.* demonstrated a significant CPIR with saccharin stimulation under fasting conditions and a 'sip and spit' procedure at 5 min after the stimulation (108), and Dhillon *et al.* observed a weak but significant CPIR following oral sucralose stimulation of individuals who were overweight or obese. The authors however suggested that this finding should be interpreted with caution since the CPIR was observed only in a subset of the participants (responders) and was not reliably reproduced (110). Ford *et al*. used a modified sham feeding protocol prior to ingestion of sucralose but observed no difference in plasma insulin following the oral stimulation with sucralose (111), results were also confirmed in a recent study that showed that sucralose did not trigger a measurable CPIR during the first 10 min post-ingestion (112). CPIR has not been demonstrated for other NNS such as aspartame, cyclamate or steviol glycosides, therefore more research is required to explore this potential functional role of NNS.

In the case of NNS there is incongruence between the sensory properties and the post-ingestive metabolic consequences, therefore it is crucial to elucidate whether they are capable of producing CPIR or not. At the moment, evidence for a CPIR with NNS consumption is limited and scarce, and since this is a brief phenomenon with a relatively small magnitude the relevance of CPR responses in human eating behaviour is questioned. Indeed, a very recent systematic-review and meta-analysis by Lasschuijt *et al.* (113) challenges the physiological relevance of CPRs in human appetite and glucose homeostasis, concluding that CPIRs are small compared with spontaneous fluctuations with the majority of evidence supporting no role of CPIR affecting functional outcomes.

1.3.1.3 Potential functional roles of the gut sweet taste receptors

Evidence from cell and animal data

The enteroendocrine cells of the GI tract secrete a variety of signalling molecules in response to changes in the gastric and intestinal lumen composition (114). Different types of enteroendocrine cells are the L cells of the ileum and colon, which secrete GLP-1 and PYY, K cells which secrete gastric inhibitory polypeptide (GIP), I cells of the duodenum and jejenum which secrete cholecystokinin (CCK). Ingested nutrients particularly carbohydrates and fats, promote the release of incretins from different parts of the gut, GIP and GLP-1 are release in response to oral glucose and mediate insulin secretion (115). It is documented that several enteroendocrine cell types throughout the gut express T1R2/3 STRs, and/or the taste-specific G protein α-gustducin (116, 117). There are several physiological roles proposed for STR signalling in the gut, and include luminal glucose sensing, release of satiety hormones such as GLP-1, expression of glucose transporters and maintenance of glucose homeostasis.

Evidence that the human GI tract may be able to sense sweetness comes from studies in cell lines showing that T1Rs and α-gustducin were found to be expressed in a human enteroendocrine L cell line, further showing GLP-1 release promoted by the activation of the STR by either sugars or sucralose (118). In addition, α-gustducin knockout mice exhibited no GLP-1 secretion and altered GIP secretion compared with their wild type littermates in response to a gavage glucose load. Confirmation of these results were provided by another study, reporting that the sweetener dependent secretion of GLP-1 and GIP from GLUTag cells is abolished in the presence of gurmarin, an inhibitor of mouse T1R2 +T1R3 (119).

Margolskee *et al.* (119) demonstrated that T1R2 and T1R2 sweet receptor in the gut regulated sodium-glucose linked transporter 1 (SGLT1) expression and increased glucose absorptive capacity in response to luminal sugars, saccharin and ace-K but not aspartame in mice. The Na+/glucose cotransporter SGLT1 is the major route for the transport of dietary sugars from the lumen of the intestine into enterocytes, and ultimately their entry into the circulation. The lack of response to aspartame was attributed to the fact that aspartame is not sweet to mice and does not stimulate expressed mouse T1R2 + T1R3. Furthermore, STRs in rat small intestine were found to stimulate glucose absorption though apical glucose transporter 2 (GLUT2) and ace-K, sucralose and saccharin to also induce these effects (120).

In summary, NNS have been demonstrated to induce hormonal release and to promote glucose uptake via upregulation of transporters in cell and animal model studies. However, doses used in these types of studies are usually very high and may not be relevant to human

consumption. Whether these effects are physiologically relevant in the context of human consumption is discussed in the next section.

Evidence from human data

The evidence from animal and cell lines data demonstrate some evidence that sweetness *per se* is sensed in the gut leading to gut hormones release and regulation of glucose homeostasis. In order to explore the potential functional role of gut STRs in humans, the paradigms used in research studies include intragastric or intraduodenal infusion of nutrients. Intragastric administration of nutrients has the advantage of bypassing orosensory stimulation, while maintaining gastric factors such as gastric emptying rate. On the other hand, intraduodenal administration of nutrients or tastants facilitates the study of direct interactions between the administrated substances and any effects on glucose metabolism and hormonal release.

Gerspach *et al.* used intragastric infusion of glucose and lactisole, a STR antagonist as explained earlier, in an attempt to answer whether gut STRs influence gut peptide release in humans. In the presence of lactisole, the release of GLP-1 and PYY by glucose was significantly reduced proposing that gut STRs might have a role in glucose-dependent secretion of gut peptides (121). Therefore, if gut STR activation is involved in glucose homeostasis and gut peptide release, NNS ingestion would induce metabolic responses postprandially.

However, Little *et al.* found no evidence that equisweet solutions delivered intragastrically affected emptying similarly, since fructose, aspartame and saccharin did not slow gastric emptying compared to water but only glucose did. In addition, there was no additional effect on gastric emptying or appetite responses following the consumption of the sweeter mixed glucose and saccharin solution (122). Human studies describing the effects of intragastric or intraduodenal infusion of NNS on postprandial glucose, insulin and gut hormone responses are presented in **Table 1.4**. In summary, none of the human studies that administered NNS directly to parts of the GI tract together or not with other nutrients has shown any significant effects on glucose, insulin or gut hormones (GLP-1, GIP, PYY). One of these studies also assessed postprandial ghrelin levels and showed that sucralose, aspartame or ace-K intragastric infusion did not affect plasma ghrelin concentrations either (123).

Available data so far question the functional role of gut STRs on glucose homeostasis and hormonal responses at least in the context of human consumption at dietetically meaningful levels.

Table 1.4 Human studies exploring glucose, insulin and gut hormones response following intragastric or intraduodenal infusion of NNS in healthy humans.

	Subjects[1]	Treatments	Glucose	Insulin	GLP-1	GIP	PYY	Control
van Avesaat *et al.* (124)	15 healthy (24 years, 22.4 kg/m^2)	150 min after the ingestion of a standardised liquid meal (162 kcal): 540 mg Reb A in 120 mL water	-	-	ND v. control		ND v. control	120 mL water
Steinert *et al.* (125)	12 healthy (23 years, 23 kg/m^2)	Intragastric infusion 50 g glucose in 250 mL water 25 g fructose in 250 mL water 62 mg sucralose in 250 mL water 169 mg aspartame in 250 mL water 220 mg ace-K in 250 mL water	Glucose elicited higher conc. v. fructose, sucralose, aspartame or ace-K and water	Higher plasma insulin conc. after glucose and fructose v. NNS and water	Higher AUC 0-120 min for glucose v. all other treatments	-	Higher AUC for glucose, ND for fructose and sweeteners	250 mL tap water
Ma *et al.* (126)	10 healthy (27 years, 23.4 kg/m^2)	Intraduodenal (ID) infusion t=-30 min sucralose (4mM in 0·9% saline) at 4ml/min (total volume: 600 mL) control (0·9% saline) at 4 ml/min for 150 min (total volume: 600 mL) t=0 min (for both conditions) ID infusion of glucose solution (30 g glucose together with 3 g 3-O-methylglucose (3-OMG),	ND at any time points between treatments	-	ND at any time point between treatments	-	-	600 mL saline infusion + glucose-3OMG infusion
Ma *et al.* (127)	7 healthy (24 years, 21.6 kg/m^2)	Intragastric infusion 50 g sucrose in 500 mL water 80 mg sucralose in 500 mL saline 800 mg sucralose in 500 mL saline All labelled with 150 mg ^{13}C-acetate	• Higher conc. for sucrose from t=5min until t=60 min v. saline • ND between sucralose (either load) and saline	• Higher conc. for sucrose from t=5 min until t=120 min v. saline • ND between sucralose and saline	• Higher conc. for sucrose at t=5 min and t=15 min v. saline • ND between sucralose and saline	• Higher conc. for sucrose between 5 min and 150 min • ND between sucralose and saline	-	500 mL saline

[1] Subjects characteristics are presented as follows: mean age, mean body mass index. Ace-k, acesulfame-K; AUC, Area Under the Curve; conc., concentration; GIP, gastric inhibitory polypeptide; GLP-1, glucagon like peptide 1; ND, no difference; OGTT, oral glucose tolerance test; PYY, peptide YY; Reb A, rebaudioside A.

1.3.2 Metabolic effects of NNS consumption in humans

1.3.2.1 Acute studies

Observational studies have suggested that NNS consumption might be associated with metabolic disease and particularly obesity and T2DM (67, 128). A key question underpinning the putative link between NNS and metabolism is the presence and the magnitude of an effect of NNS ingested as part of a non-caloric or caloric preload on glycaemic and insulinaemic responses. Reducing the glycaemic load of diets is strongly associated with lower risk of developing T2DM (129), therefore examining the postprandial glycaemic and insulinaemic response to NNS is of great importance. As outlined in previous sections, oral sweet taste perception plays a role in the metabolic consequences of caloric sugars, however oral stimulation of sweet taste via NNS has not been demonstrated to influence postprandial metabolism significantly. Stimulation of gut STRs alone via intragastric/intraduodenal administration of NNS also did not show any evidence for potential functional roles of NNS in the context of human nutrition.

In this section the data regarding the metabolic responses following NNS oral (normal) ingestion will be discussed. The effects of acute ingestion of NNS on glucose, insulin and gut peptide responses have been studied using a variety of methods as well as different dosages and types of NNS (38, 48, 51, 112, 130-132). As a consequence, a number of systematic reviews and meta-analyses have been conducted the latest years in an attempt to collectively present available evidence. Romo-Romo *et al.* (133) examined the available scientific evidence regarding the effects of NNS on glucose metabolism and appetite regulating hormones. In the systematic review published in 2016, 14 observational studies and 28 RCTs were included, with the authors concluding that any effects of NNS on glucose metabolism could not be established at that point due to major differences in the design of the available studies. A later meta-analysis by Nichol *et al.* was conducted to estimate the trajectory of blood glucose response over time following NNS consumption. The authors concluded that NNS consumption was not found to increase postprandial blood glucose, but on the contrary glucose responses gradually declined over the course of observation. There was not a differential effect observed by type of NNS, however it was highlighted that the glycaemic impact of NNS consumption varied by participants' age, body weight and diabetic status (134). Tucker and Tan conducted another systematic review on acute glycaemic and insulinaemic responses to NNS consumption based on the hypothesis that NNS use could improve glucose regulation (lower postprandial glucose response) due to sweet taste activating STRs-mediated physiological responses. The authors concluded that NNS triggered physiological responses, albeit inconsistently, but failed to improve glucose response in almost all studies (135).

The most recent report to date is a meta-analysis that included results from 34 RCTs and was the first quantitative analysis of the effects of NNS intake on 2 hour postprandial glucose and insulin response (13). The results showed that ingestion of NNS, administered alone or in combination with a nutrient-containing preload, has no acute effects on the mean change in postprandial glycaemic and insulinaemic responses compared to a control intervention. The effects did not differ by type or dose of NNS, fasting glucose or insulin levels but there was a small beneficial effect on postprandial glucose response in studies of patients with T2DM.

Even though the glycaemic and insulinaemic responses to NNS are well described, the effects on gut hormones have not been systematically reviewed. Potential effects of NNS consumption on gut peptides release might significantly affect other outcomes such as appetite, energy intake, thus body weight. Consumption of sucralose or aspartame (or aspartame-sweetened cola) alone did not lead to GLP-1 or GIP release in healthy human volunteers (111, 136-138), however results appear mixed when sucralose is consumed prior to glucose ingestion. Enhanced GLP-1 response has been shown in four human studies (38, 131, 139, 140), and corresponded to either sucralose or diet drinks (containing sucralose and ace-K) consumption prior to a glucose load and primarily in participants with overweight-obesity (38, 131, 140). However, there are also studies showing no effect in GLP-1 using the same design in the case of sucralose (38, 130) and saccharin (141). No significant effects have been shown for GIP release (38, 130, 136), while effects on ghrelin or PYY release are scarce yet conclusions cannot be made at this point. The most studied NNS so far appears to be sucralose, while there is less data available on stevia, ace-K and saccharin and their effects on hormonal responses.

The evidence suggests that acute consumption of NNS alone or in combination with a nutrient-containing preload does not influence glucose or insulin responses in humans. However, more research is warranted in order to draw firmer conclusions regarding acute hormonal responses especially for the less studied NNS, as there is less evidence available and there are methodological considerations such as BMI of volunteers, prior exposure to NNS and the acute nature of these studies that should be taken into careful consideration.

1.3.2.2 Chronic studies

The evidence described in the previous section on the effects of acute consumption of NNS on metabolic responses, immediately creates the question whether repeated exposure to NNS might influence postprandial metabolism. Even though such studies investigating the long-term effects of NNS on postprandial metabolic responses in humans were very limited at the time when this PhD started, during the last 4 years a considerable amount of studies have been conducted and will be summarised below.

Concerns regarding the ability of NNS to cause adverse effects on glucose tolerance were raised by the study of Suez *et al.* (142), who suggested a negative effect of saccharin consumption on glucose tolerance mediated by alteration of gut microbiota. In this study 7 human subjects, non-frequent consumers of NNS, were studied for a period of 1 week and consumed the acceptable daily intake (ADI) of commercial saccharin (5 mg/ kg body weight). On days 5-7 of the exposure period 4 out of 3 participants exhibited poorer glycaemic control, as evaluated by daily oral glucose tolerance tests (OGTTs) compared to days 1-4 of exposure. The other 3 participants did not show any difference on glucose tolerance, and were considered as 'non-responders'. Analysis of participants' gut microbiome showed that the non-responders did not exhibit any changes in composition of the microbiome, while the responders (those who showed poorer glycaemic control after exposure to NNS) developed significant compositional changes. Another study has been recently conducted examining the effects of saccharin supplementation on glucose tolerance and gut microbiome in human subjects (143). Participants received the maximum ADI for saccharin for 2 weeks and on the contrary showed no changes in glucose tolerance or gut microbiota composition.

Several studies have evaluated the effect of repeated doses of sucralose consumption on glucose metabolism (144-150). Sucralose appears again to be the most studied in terms of long-term metabolic effects in humans compared to the NNS. Studies differed in duration starting from just one week (144) and up to 13 weeks (150). Some of the identified studies delivered sucralose in the form of capsules or pills, while some other delivered sucralose in the form of beverages (145, 147, 148), with the majority conducted in healthy adults apart from one study which was conducted in patients with T2DM (150). No difference in postprandial glucose and insulin responses to an OGTT before and after the intervention period was found in the studies by Thomson *et al*. (144) and Grotz *et al.* (149) (both studies administrated sucralose in capsules). Thomson *et al.* also evaluated potential changes in the gut microbiome before and after the intervention in the study groups (sucralose group consuming 0.78 g sucralose (75% ADI) and placebo group 0.75 g calcium carbonate as placebo for 7 days) as a secondary outcome and concluded that treatments did not substantially alter the microbiome of the subjects (144).

However, a decrease in insulin sensitivity following a period of repeated sucralose consumption in doses varying between 36 and 200 mg compared to a control group was described in three studies (145, 146, 148). It should be noted that the need to control for exposure to NNS prior to study allocation is often highlighted, however participants in these three studies varied in habitual NNS consumption from none to high. In addition, a decrease in insulin sensitivity was observed in another study, conducted in healthy adults non regular consumers of NNS, but only when sucralose was combined with carbohydrates (maltodextrin) in a beverage, and was correlated with a decrease in brain response to sweet taste (147). In the study by Bueno-Hernandez *et al.* (145) there were two intervention groups, one consumed 48mg of sucralose per day for 10 weeks, which corresponds to the amount of NNS contained in one can of diet soda, while the other group consumed 96 mg of sucralose daily. Although the group receiving 48 mg sucralose demonstrated a decrease in insulin sensitivity indices, findings were not confirmed in the higher dose group**.**

Fewer studies are available investigating the effects of long-term consumption of the other NNS on metabolic responses. Higgins *et al.* (56) examined the effects of daily aspartame consumption for 12 weeks on postprandial glucose, insulin, GLP-1 and GIP responses in healthy lean adults. Participants were randomly allocated to one of the three study groups, consuming either 0, 350 or 680 mg aspartame (encapsulated) daily for the duration of the study. Aspartame had no effect on glycaemia and the other metabolic parameters (insulin, GLP-1, GIP response between baseline and week 12) among healthy adults. Among the strengths of this study is that the investigators included a large sample size, male and female participants and objectively measured compliance using a traceable compound in the study products, which was then assessed in participants' urine. Another study by Ahmad *et al.* investigated the effect of daily aspartame (14% ADI – 0.425 g) and sucralose (20% ADI 0.136) consumption for 2 weeks on glucose homeostasis (glucose response to an OGTT) in healthy normal weight participants in a randomised double-blind controlled crossover design. Neither the consumption of sucralose nor aspartame resulted in any significant differences in glucose, insulin, GLP-1 or leptin response between baseline and after 2 weeks of consumption (132).

Two studies evaluated the effects of chronic consumption of NNS-sweetened carbonated beverages on postprandial metabolism. Bonnet *et al.* (151) investigated the effects of consuming NNS-sweetened carbonated beverages daily for a period of 12 weeks in healthy men in randomised double-blinded crossover design. Participants were randomised to drink a carbonated beverage (2 cans 330 mL a day) containing 129 mg aspartame and 13 mg ace-K daily or an unsweetened carbonated beverage. Insulin sensitivity was assessed by the Matsuda Insulin Sensitivity Index after an OGTT. Results showed that daily consumption of 2 NNS-sweetened carbonated beverages for 12 weeks does not influence insulin

sensitivity in healthy nondiabetic males. Another study by Engel *et al.* (152) assessed the effects of daily consumption of NNS-sweetened soft drinks for 6 months compared to sugar sweetened soft drinks or water (1L/day) in participants with overweight/obesity in a randomised controlled trial. No difference was observed in glucose or insulin response to an OGTT before and after the intervention or between the groups.

To date, there are not any studies available evaluating the effects of long-term stevia or pure ace-K (apart from the case of diet drinks) consumption on glucose response to a glucose load. However, there are some studies assessing fasting blood glucose and insulin concentrations before and after an intervention period of stevia administration (reviewed in Chapter 1.5.3).

There is a great need for more RCTs assessing the long-term effects of NNS consumption on glucose homeostasis. Results from RCTs regarding effects of daily sucralose consumption on glucose metabolism appear inconsistent. There are some initial findings that show that sucralose consumption could disrupt glucose metabolism by decreasing insulin sensitivity. Nevertheless, due to limitations of study designs that differ in the amounts of sweeteners used, the form of administration (beverage, capsule), study populations, and difficulties in assessing compliance due to the nature of these studies, any conclusions should be drawn with caution.

1.4 Control of eating in the human brain: caloric sweeteners versus NNS

Humans eat to survive but we also eat for other reasons such as pleasure (food is tasty), emotional reasons (we often eat when we are stressed), social purposes etc. Control of eating and its biological substrates were traditionally divided into two separate categories: the homeostatic processes involved in energy intake based on energy needs and the non-homeostatic processes which are not regulated or compensated by some form of metabolic feedback (153, 154). A more expressive term for 'non-homeostatic' is 'hedonic' eating, which refers to the involvement of cognitive, reward, and emotional factors. More recent models, suggest that these two systems do not act in isolation but together under a common neurochemical network to influence when and how much food will be consumed (12, 155).

1.4.1 Neural processing of appetite and food intake

1.4.1.1 Homeostatic eating

Homeostatic eating is driven by acute negative energy balance. The physiological modulation of homeostatic eating involves central and peripheral modulators that are released following acute energy depletion in order to promote food intake (156, 157).

Food is consumed by the mouth, then passes in the GI tract to be processed and eventually absorbed in the bloodstream. The enteroendocrine system is the primary sensor of ingested volume and nutrients, and is responsible for secreting an array of hormones in response to acute energy intake, which modulate multiple physiological processes including glucose homeostasis and appetite. Carbohydrates are predominantly sensed in the form of glucose, which in humans is a potent stimulator of GLP-1 and PYY secretion (158). Circulating levels of these hormones among others are associated with reductions in intake (159). The information regarding the volume and nutrients in the GI tract reaches the brain via two pathways. The first involves the vagal sensory nerves which are located almost throughout the entire GI tract and relay the gut information to the brainstem (pathway essential for satiety and meal termination). The other pathway involves the secretion of gut hormones in response to the presence of food in the GI tract, which act locally to contribute to nutrient absorption and metabolism but also act directly in the brain (12).

The hypothalamus and the brainstem are the core processors of homeostatic eating

The hypothalamus has been long recognised to be critically involved in maintaining energy homeostasis. The arcuate nucleus of the hypothalamus contains two subpopulations of neurons which influence appetite, in particular neurons which express neuropeptide Y and agouti-related protein which elicit orexigenic effects, and neurons expressing pro-opiomelanocortin and cocaine-and amphetamine-regulated transcript which induce

anorexigenic effects (160). These two populations of neurons together with the downstream target neurons expressing the melanocortin receptor 4 and 3 constitute the central melanocortin system, essential for sensing and integrating a number of peripheral signals to allow for a precise regulation of food intake and energy expenditure (161). These neurons are modulated by both long-acting adiposity signals, mainly leptin and insulin, which circulate in the blood stream in amounts proportional to body fat content but also in response to acute changes in energy intake, and by short-acting gastrointestinal peptides, such as ghrelin, PYY, GLP-1, which are secreted in response acute changes in nutritional status. These peripheral hormonal signals act in the hypothalamus to excite or inhibit these neurons to alter appetite.

The brainstem plays also a critical role in processing and integrating signals related to metabolic state, particularly with regards to meal size regulation and thermogenesis. The brainstem is able to detect sensory information mediated by vagal afferents and circulating factors and generate motor output associated with the ingestion, digestion and absorption of food (12, 153). During a meal, the brainstem receives afferent information with regards to compositions and quantity of nutrients being ingested. The area postrema is located on the caudal brainstem and ideally situated to receive and integrate circulating metabolic signals (leptin, insulin, GLP-1, PYY, ghrelin and others) each of which can affect food intake. The area postrema projects to the nucleus tractus solitarius (NTS).The NTS receives inputs including vagally mediated gastrointestinal satiation signals and nutrient energy-related signals from the periphery, which then integrates and creates outputs in order to control the behavioural, autonomic and endocrine response that collectively control energy balance (162).

1.4.1.2 Hedonic eating

From an evolutionary perspective, the feeding system is biased toward positive energy balance to enhance survival. Given that food is essential for survival it has evolved to be a rewarding behaviour, which in the current obesogenic environment has turned against us. Obesity has become a major health problem.

Evidence shows that food hedonics have a great influence on appetitive behaviour, and might facilitate eating beyond energy needs and weight gain (163). Hedonic eating is associated with visual, olfactory and other environmental cues that generate the positive hedonic feeling, and reward associated with the initiation and continuation of eating. In general, bland tasting foods are not eaten to excess, whereas palatable foods are often consumed even when not hungry. Consumption of a tasty food creates a pleasurable response; the hedonic reward value of food is closely linked to the sensory perception of

food (including food taste, odour and texture) and refers to the driving force behind the motivation to eat.

The corticolimbic system is associated with the non-homeostatic eating and provides the emotional, cognitive, and executive support for ingestive behaviour (12). Cortical regulation of eating involves the prefrontal cortex (PFC), the insula and orbitofrontal cortex (OFC). The PFC is implicated in inhibitory control mechanisms related to food intake (164). Impaired inhibitory control has been described in obesity in response to food-cue paradigms (165, 166). Inhibitory control might also be triggered in lean individuals as well by willingness to resist palatable energy-dense foods in order to achieve a long-term goal regarding weight status (167). The insula has a well-established role in sensory gustatory processing and has projections to the OFC, which is considered the secondary taste cortex. This pathway is implicated in the integration of taste, olfactory, visual, and cognitive inputs (168). The insula has also a strong association with the hedonic aspects of food items reflected in higher insular activity and stronger connections with reward-related areas (169, 170), and is influenced by hunger state (171). Activity of the OFC has been associated with sensory specific satiety (172), as well as the reward value and subjective pleasantness of the stimuli (173).

The dopamine and opioidergic systems are implicated in food reward. Dopamine neurons in the ventral tegmental area (VTA) and substantia nigra (SN) project to the ventral striatum (nucleus accumbens(NAc)) and dorsal striatum (putamen, caudate). Via the striatum signals are relayed to other parts of the limbic system, including the PFC, amygdala, and hippocampus, forming the reward-associated mesolimbic dopamine pathway (160, 174). Activity in the NAc, ventral pallidum and brainstem is thought to mediate ‘liking’ responses (pleasurable hedonic experience, conscious pleasure), whereas ‘wanting’ (conscious desire, motivation to eat) is considered to be represented in the mesolimbic dopamine system (175). Liking is not influenced by metabolic state, however wanting is greatly amplified with hunger. This could be easily translated into we might very much like a particular food, but not necessarily want to consume it after we have eaten to satiety.

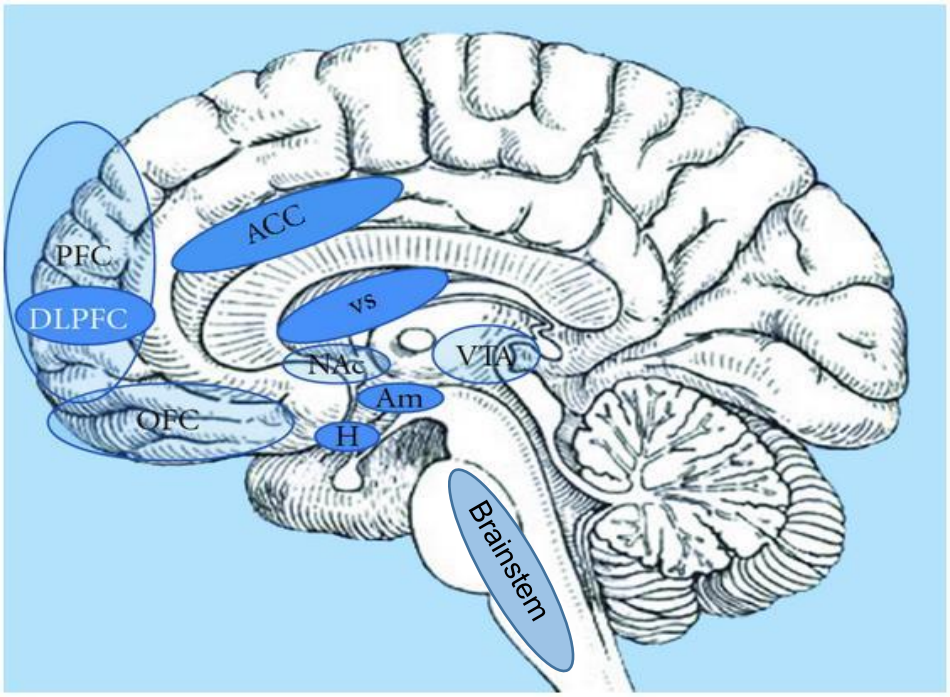


Figure 1.4 Brain metabolic and reward centres. ACC, anterior cingulate cortex; Am, amygdala; DLPFC, dorsolateral prefrontal cortex; H, hypothalamus; NAc, nucleus accumbens; OFC, orbitofrontal cortex; PFC, prefrontal cortex; vs, ventral striatum; VTA, ventral tegmental area. Not shown is the insula, a more lateral structure involved in gustatory processing. Adapted from de Silva *et al.* (176).

1.4.1.3 The interaction between homeostatic and hedonic eating

Current evidence suggests that homeostatic and hedonic eating do not occur in isolation, but there is a constant interaction between the two systems (12, 155) **(Figure 1.4**). Homeostatic signals can influence food reward, providing a mechanism by which nutritional state affects food attractiveness (bottom-up regulation). The incentive value of food can be reflected in functional Magnetic Resonance Imaging (fMRI) studies on the response of the brain reward regions (177). Higher brain activity of reward areas in response to food-cue paradigms has been demonstrated under fasting conditions (178-180), whereas activity is modulated under conditions of satiety (181). Impairment of this response has been documented in obesity, individuals with obesity show greater activations in reward and prefrontal circuitry in the post-meal state as compared to normal weight individuals (182). A persistent brain activation in response to food cues under conditions of satiety, highlights the ongoing motivation to eat beyond energy needs and has been proposed among the theories of the neurocognitive underpinning of obesity (183).

The modulation of higher brain functions by metabolic state is considered to be at least partly mediated by circulating hormones, such as insulin (184), leptin (185), ghrelin (186), and PYY (187) and others, which can directly act in the mesocorticolimbic dopamine

system. However, there have also been demonstrations that peripheral delivery of satiety-signalling hormones such as insulin is not an adequate signal to attenuate brain response with regards to food cues in healthy individuals with normal weight (188), suggesting that modulation of reward responses can act beyond internal satiety-signalling. Exposure to visual food cues can also influence homeostatic mechanisms. Studies have shown an increase in ghrelin levels (189) and decrease in postprandial glucose levels (190) following presentation of pictures with food.

However, the extent to which a food is rewarding does not necessarily translate into appetitive behaviour. Whether or not we will choose to eat a particular food also depends on cognitive factors. The recent model of appetite behaviour proposed by Higgs *et al.* (155) suggests that higher level cognitive functions, such as attention, memory and learning are inseparable part of our daily food decisions, and can further modulate or be modulated by homeostatics and hedonics.

1.4.1.4 Cognitive biases for food cues and mediating factors

Exposure to a palatable food cue can elicit motivation to eat and food seeking behaviour. Hunger can amplify this response. This response can be reflected and studied via neurocognitive tests which are usually computer-based tasks developed to capture a specific mental state or skill (191).

Attentional bias to food

Individuals' attention has been proposed to be biased towards stimuli in the environment that are rewarding (i.e. have a positive value), and attention to rewarding stimuli is associated with increased response activation and approach behaviour to these stimuli (155, 192). Based on the incentive sensitisation theory, food cues might become more 'attention grabbing' due to the consequence of repeated exposure to visual food cues and the association between the stimulus and a rewarding experience (the stimulus becomes 'wanted') (193, 194). The interaction between homeostatic status and hedonics can explain that the needs of the body focus attention on sensory cues associated with outcomes that can satisfy needs.

Attentional bias (AB) means that a person selectively attends to a certain category of stimuli in the environment while tending to ignore or overlook other categories of stimuli, in other words AB is the tendency to focus attention on salient over neutral information. AB to food cues can be measured indirectly via computerised reaction time tasks measuring response latencies or the calculation of an interference during a food Stroop task, assessment of response latencies during a spatial attention paradigm (visual probe task (VPT), visual

search task), or directly by recordings of eye–movements during an attention paradigm (195). Under conditions of hunger, where food stimuli become more relevant, AB to food cues increases compared to post-meal state (196, 197). More motivated attention to food cues in hunger reflects automatic capture by food stimuli. These are basic mechanisms that are necessary for survival (198).

AB has also been suggested to be heightened in obesity (199, 200). Yokum *et al.* showed that BMI correlated positively with activity in brain regions linked to attention and food reward, such as anterior insula/frontal operculum, lateral OFC, ventrolateral PFC and superior parietal lobule during initial orientation to food cues and with the speed of behavioural response (201). In another study individuals with obesity versus individuals with normal weight also orient faster toward food pictures and spend more time looking at food pictures than non-food, as assessed via eye tracking (200). However, a collection of narrative reviews report that evidence for increased attention bias for food in obesity is conflicting, and this observation could be at least partly attributed to the different methodologies used across studies (193, 195, 199). A recent systematic review and meta-analysis by Hardman *et al.* also concluded that AB is unrelated to individual differences in body weight (198).

Since AB to food cues is considered to reflect underlying appetitive motivation, based on the incentive sensitization theory (194), one could hypothesise that heightened AB to food cues would be associated with higher caloric intakes. Food-related AB has been reported to be positively associated with food intake (198), however Field *et al.* in his proposed theoretical model argues that AB does not consistently predict or influence consummatory behaviour (202).

Approach food bias

Positive evaluated stimuli, such as palatable food cues, also cause spontaneous approach. The stimulus-response compatibility task (SRCT) has been used to examine individual differences in approach bias to reward cues (i.e. food, drugs, alcohol). It requires participants to move a manikin (a small figure of a person) as quickly and accurately as possible towards or away from a picture displayed in the centre of the screen. The picture may be either food-related or non-food related in content. An approach bias indicates faster responses to move the manikin towards the food rather than away from food.

Research using SRCT has shown enhanced approach biases for reward cues (203). In normal weight individuals, an increased approach bias to food cues has been associated with traits related to overeating such as external eating (204). Additionally the combination of an increased BMI and self-reported restraint eating have been found to relate to increased approach bias to food cues (205). Mogg *et al.* showed that a single dose of

dopamine D3 receptor antagonist, in overweight or obese subjects significantly lowers approach bias to food cues, assessed via a SRCT, compared to placebo, highlighting the potential mediating action of dopamine D3 receptor for reducing approach food responses (206).

Approach bias would be expected to enhance under conditions of hunger. However, there are only a few studies available to date which have investigated this. In the very early study by Staats and Warren food-deprived participants learned to make approach responses to food words more quickly than non-deprived participants in an experiment assessing their impulsive approach-avoidance tendencies (207). Moreover, immediate approach motivational tendencies towards palatable food pictures were affected by food deprivation automatically in a study by Seibt *et al.* (208). However a more recent study by Piqueras-Fiszman *et al.* showed that foods were approached equally fast among satiated and food-deprived participants, despite higher desire to eat sensations in the latter group (209). Whether metabolic state influences approach food bias remains unclear.

Implicit associations to food

Implicit preferences or attitudes are driven by positive or negative unconscious evaluations of an object, reflecting automatic associations in memory between concepts (210). In the implicit association task (IAT) subjects are asked to evaluate the presented stimuli (usually words) into four categories by pressing the two predefined keyboard keys. The rationale behind this task is that if two concepts are highly associated in memory (e.g. sweet food and favourable attributes) then they are easier to associate via IAT's sorting tasks, compared to when two concepts are weakly associated. Consequently, responses are faster for strongly associated concepts (211).

There is also some evidence showing that in the hungry versus satiated condition food items are evaluated as more pleasant using the IAT (212). Seibt *et al.* demonstrated that hungry participants categorise faster the 'pleasant' associated categories compared to satiated participants (213), results further confirmed by Stafford and Scheffler (214). Food-related words were perceived as implicitly more positive for subjects in the hungry compared to the satiated condition (214).

1.4.1.5 Cognitive biases related to caloric sugars or NNS consumption

Only limited data are available regarding sweet taste or sweet foods consumption and cognitive biases outcomes. Sartor *et al.* examined the possible differences in implicit attitudes toward sweet foods between young adults with normal weight or overweight/obesity. The results revealed a stronger implicit attitude toward sweet food in participants with overweight/obesity compared to normal weight participants, who were not affected after a soft drink tasting test (211). Approach avoidance training paradigms have been used as a means of changing participant's implicit and explicit food preferences and reduction of 'problematic' sweet food consumption, such as chocolate, but with no conclusive evidence so far (215-218).

Relevant research the latest years has shown that consuming NNS sweetened beverages may influence individual's food regulation psychology in ways that may affect food intake, thus weight management in the long term. In particular, Hill *et al.* (219) showed that participants who consumed a NNS-sweetened beverage had shorter response latencies to the names of high caloric food items compared to those who consumed sugar sweetened beverages or an unsweetened beverage, suggesting an increased cognitive preoccupation with high caloric content foods. They also showed that participants who consumed the NNS-sweetened preload compared to those who had the sugar- or unsweetened- preload were more likely to consume a high-calorie food item in a consumer choice scenario. In another study, consuming a NNS-sweetened beverage with a standardised meal increased the relative reinforcing value of sweet snack foods later in the day compared to consuming a sugar-sweetened beverage (53).

Whether consumption of caloric sweeteners or NNS affects cognitive responses to food cues, such as AB or approach bias, is as yet unknown.

1.4.2 NNS effects on neural systems involved in appetite and eating behaviour

1.4.2.1 Homeostatic neural responses to intake of caloric sugars and NNS

In this section I have reviewed the literature examining the brain response to the consumption or intragastric administration of caloric sugars or NNS; studies that included a hedonic aspect, such as a food-cue paradigm are discussed later.

In the milestone study by Liu *et al.* glucose was administered (via a peroral rubber tube) after overnight fast in 21 healthy participants in an 'on-off treatment related blocked design'. Increased activation in the supplementary motor area (SMA), somatosensory cortex, cerebellum, anterior cingulate and OFC and decreased activation in the hypothalamus following glucose administration was observed (220). The decreased hypothalamic blood-oxygen-level-dependent (BOLD) response occurred from around 7 minutes following glucose intake, moreover fasting plasma insulin concentration was negatively correlated with baseline activity in the hypothalamus. This study demonstrated for the first time an inhibitory pathway activated by glucose ingestion in the hypothalamus. Subsequent study by Smeets *et al.* reinforced these findings showing a prolonged and dose-dependent decrease in BOLD contrast in the hypothalamus following glucose administration (consumed via a peroral tube) in 15 normal weight healthy male volunteers (221). Interestingly, the investigators suggested that the onset of the signal decrease started earlier that the end of glucose ingestion, therefore the response cannot be solely depend on blood glucose levels changes. In a following study, oral glucose intake compared to intravenous glucose infusion was associated with a stronger hypothalamic deactivation, highlighting the importance of the gut-brain signalling and supporting the notion that normal eating conditions are required to exert central neural effects on appetite (222).

In the study by Little *et al.* glucose was administered in healthy normal weight individuals intragastrically to bypass potential confounders such as sensory responses and movement artefacts during swallowing (223). In line with previous observations, intragastric administration of glucose, relative to a saline infusion, led to a decreased BOLD response assessed via the physiological MRI (*phys*MRI) method in the hypothalamus. During the *phys*MRI a nutrient is infused in the gut after a short baseline period and the change over time is compared to the baseline period. A representative image of a decreased hypothalamic BOLD in response to intragastric glucose relative saline using the *phys*MRI technique is depicted in **Figure 1.5**. Apart from the decreased BOLD contrast in the hypothalamus, deactivation of the brainstem (midbrain, pons and medulla), cerebellum, right occipital cortex, putamen and thalamus was also observed. This was the first study to define a brainstem response to the presence of glucose in the GI tract. Previous studies

however, have demonstrated a brainstem response to intragastric infusion of lipids, using the *phys*MRI technique (224, 225).

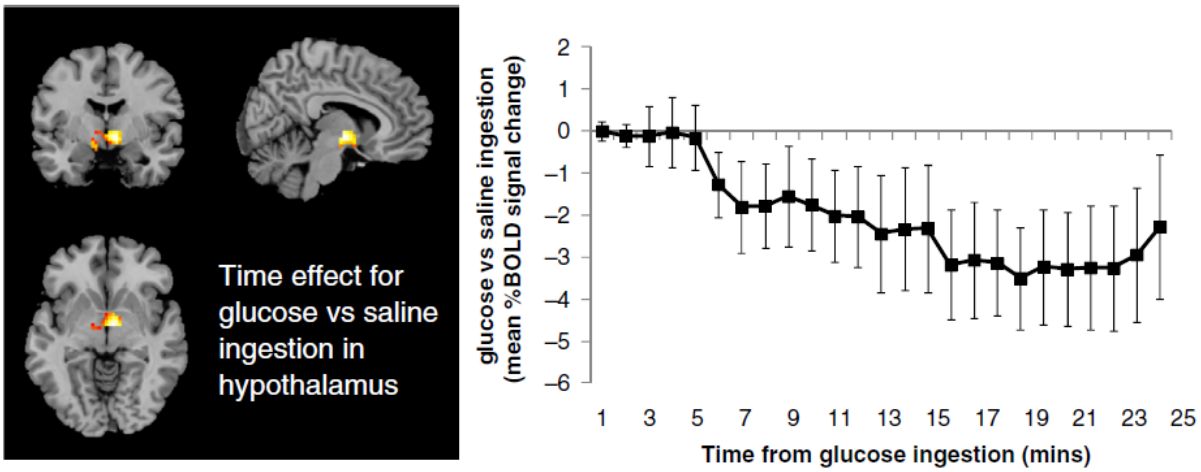
An altered hypothalamic response to glucose intake has been demonstrated in obesity (226, 227) and in anorexia nervosa (226) and T2DM (228). In particular, Simon *et al.* (226) in a recent research study using a single-blind nasogastric infusion of glucose and water, demonstrated that compared with normal-weight controls, the patients with anorexia nervosa and controls with obesity showed diminished responses in the hypothalamus as well as in the ventral striatum and amygdala following glucose infusion.

Different caloric sugars lead to different physiological and brain neural responses. Fructose ingestion leads to a lower blood glucose spike and weaker hormonal secretion responses postprandially compared to glucose (229), therefore it has been hypothesised that neural responses to fructose ingestion might also differ. In a fMRI study in healthy normal weight males oral ingestion of fructose led to a significant decrease in the hypothalamus compared to water ingestion, however the signal decrease was delayed and smaller compared to glucose (230). It was hypothesised that this delay might be due to fructose needing to be metabolised by the liver before it can be detected by the energy-sensing neurons in the brain. Greater hypothalamic decrease following glucose versus fructose has also been demonstrated by Page *et al.* (231). In addition, glucose ingestion (compared to baseline) increased functional connectivity between the hypothalamus, thalamus and the striatum while fructose increased connectivity between the hypothalamus and thalamus only. Another study in healthy normal weight individuals demonstrated dissociable effects on resting-state functional connectivity within the basal ganglia/limbic network between glucose and fructose intake, and suggested results could be due to differences in insulin levels postprandially (229).

Very limited data are available regarding the neural effects of NNS consumption in the brain. Smeets *et al.* in an early study reported no hypothalamic BOLD response decrease following aspartame consumption or non-sweet carbohydrate maltodextrin, proposing that activity is not due to sweetness or calories themselves but the combination of the two is required (232). A later study by van Opstal *et al.* demonstrated a transient deactivation of the hypothalamus following oral sucralose ingestion compared to water, the magnitude was smaller compared to glucose (230).

Whole brain mapping following the consumption of NNS versus caloric sugars has not been conducted yet. There is again a need for more RCTs in this field.

Figure 1.5 Representative image of the deactivation of the hypothalamus following a glucose intragastric infusion relative to a control infusion (saline) in healthy individuals. Reproduced from McLaughlin and McKie (233).



1.4.2.2 Hedonic neural responses to consumption of caloric sugars and NNS

As discussed in the previous section, humans (and many other animals) have a strong innate preference for sweet-tasting foods. Consumption of NNS might satisfy our natural propensity for sweetness providing the reward without the calories. In this section I will describe the brain neural response to sweet taste with or without calories as well as the available literature on the neural correlates of hedonic responses to food cues following the consumption of caloric and non-caloric sweeteners.

Neural response to oral sweet taste of caloric sugars and NNS

- *The neural sweet taste pathway*

The neural circuitry of gustatory processing integrates sensory, hedonic and motivational aspects of feeding. Sweet taste perception is peripherally recognised by the tongue's STRs, from which signals are transmitted through the brainstem and thalamus to the primary gustatory cortex, the anterior insula and frontal operculum **(Figure 1.6)**. The anterior insula responds to the taste and may also respond to its rewarding value. The anterior insula has connections with the amygdala, the anterior cingulate cortex (ACC) and the OFC, therefore it is a vital component of the limbic system. Afferents from cortical structures are directed to the dorsal or ventral striatum. Striatum activation can influence behaviour (234, 235). The OFC is where the palatability and pleasantness of food is represented, and hunger modulates the responsiveness of representations in the OFC of the taste. These regions innervate a broad region of the striatum and cingulate cortex which can drive behaviour (235).

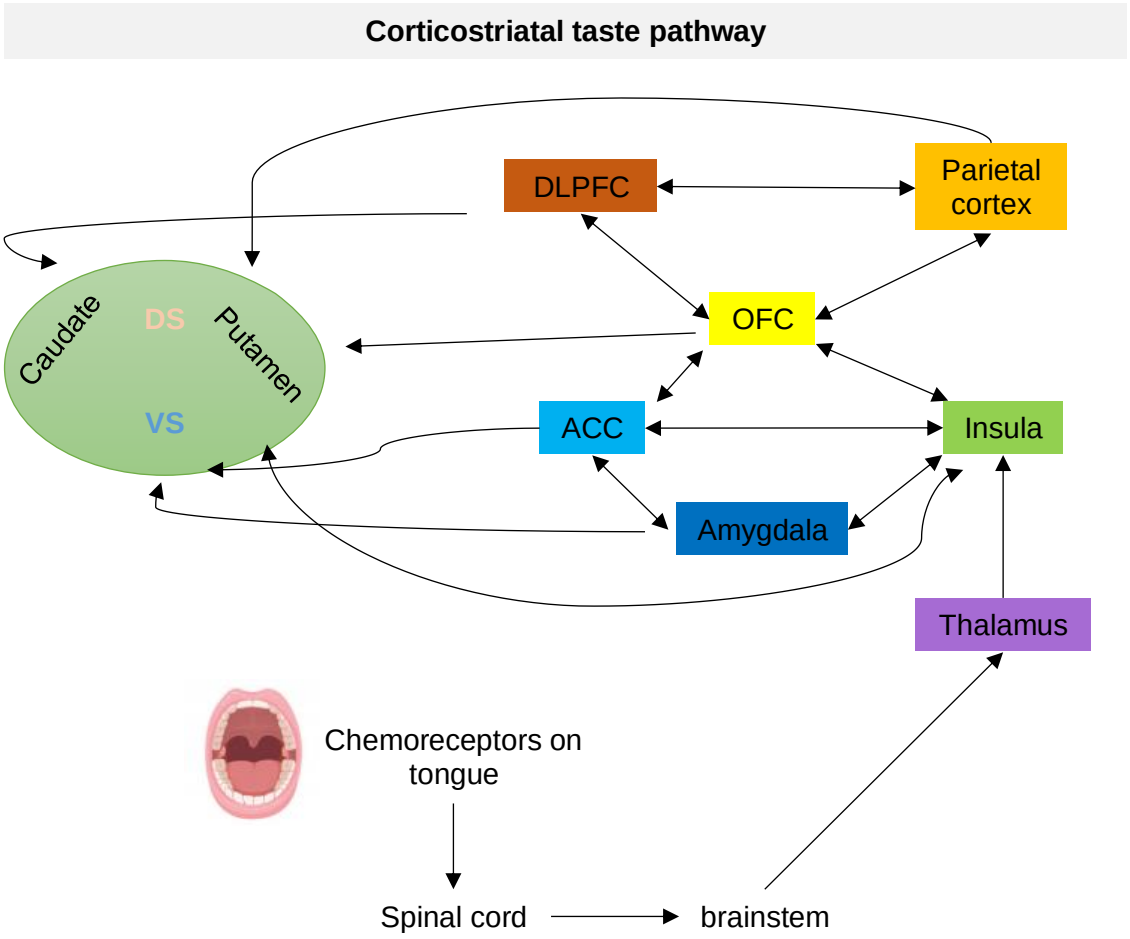


Figure 1.6 Sweet taste processing pathways in the human brain.

Adapted from Oberndorfer *et al.* (234). ACC, anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; DS, dorsal striatum; OFC, orbitofrontal cortex; VS, ventral striatum.

- *Human studies investigating the neural correlates of caloric sugars tasting*

Small *et al*. (236) examined the brain responses to the intensity and valence of taste, using sucrose and quinine (bitter) solutions during fMRI in 9 healthy adults. The researchers used a slow event-related paradigm with 5 stimuli: intense pleasant, weak pleasant, intense unpleasant, weak unpleasant and tasteless. They concluded that activations in the anterior insula/ operculum extending into the OFC are associated with taste valence, while activations in the cerebellum, pons, middle insula and amygdala are associated with taste intensity irrespective of valence. In another study, Haase *et al.* (237) showed taste activation patterns following sucrose solutions (minus water) while participants rated the pleasantness of the stimuli during event-related fMRI, revealing the role of insula, rolandic operculum, OFC, thalamus, medial dorsal nucleus, caudate and caudate body, precentral and postcentral gyri.

A recent systematic review and activation likelihood estimation meta-analysis of fMRI activation of sweet taste in humans included results from 15 trials in humans using sucrose versus water in the majority of the trials as the sweet tastant condition, and glucose versus water in a few trials. This meta-analysis showed that the brain areas that are most consistently activated in response to tasting caloric sweeteners were the insula (both mid- and anterior insula) and the opercular cortex (both frontal and central operculum), precentral and postcentral gyri and thalamus. There was also evidence for activation of the caudate, a part of the striatum considered to play a role in reward orientation, in the primary analyses (238).

- *Human studies investigating the neural correlates of caloric sugars vs NNS tasting*

There is evidence that the human brain can distinguish between sweet taste coming from caloric sugars compared to NNS. In particular, in a fMRI study by Frank *et al.* the brain activation response to sweet taste from caloric sweeteners (sucrose) and non-caloric sweeteners (sucralose) was investigated. Twelve healthy women with BMI in the normal range were scanned after breakfast (satiated). The main effect of sucrose showed activations in the frontal operculum/anterior insula bilaterally, left ventral striatum, anterior cingulate and bilateral midbrain, while sucralose resulted in frontal operculum/ anterior insula bilaterally activation only. Subtraction of sucrose minus sucralose effects showed greater activation for sucrose in the bilateral frontal operculum/anterior insula, and some predefined regions of interest (ROIs) such as left caudate, left cingulate and bilateral superior frontal cortex, bilateral posterior insula. Dopaminergic midbrain areas (VTA/SN) were activated only in the sucrose condition, suggesting that reward centres are not activated by the NNS sucralose (239).

Another study confirmed the above findings. Chambers *et al.* examined the brain response to oral tasting of glucose versus saccharin and glucose versus maltodextrin in 8 endurance-trained cyclists who underwent an fMRI either after overnight fast or in the evening after a 6 h fast (consistent for each subject). The effects of oral exposure to the tastants were studied by contrasts (i.e. [Glucose -Control]; the control was a tasteless solution consisting of the main ionic components of saliva. Oral exposure to glucose led to insula/frontal operculum and dlPFC activation as well as caudate and anterior cingulate cortex activation, both areas associated with reward. On the other hand, oral exposure to saccharin led to a similar activation in insula/ frontal operculum but the reward areas were unresponsive. The second part of this study involved the investigation of brain responses to oral exposure to glucose and maltodextrin, results showed similar activation patterns with insula/frontal operculum, OFC, dlPFC and striatum activations (240). Smeets *et al.* also showed differences in brain activation patterns during tasting of caloric versus non-caloric orangeade. In particular, in the premeal state (at least 2 hours of fast) the amygdala was

activated more by the non-caloric version compared to the caloric orangeade, while the caloric orangeade activated the striatum (241).

Haase *et al.* further showed that taste activation is hunger-state dependent (242). Pure taste stimuli, including sucrose and saccharin, induced greater activation in the insula, thalamus, and SN in the hunger state in contrast to decreased activation in the satiated state within the parahippocampus, hippocampus, amygdala, and anterior cingulate. Contradictory results have been reported by van Rijn *et al.* (2015), who showed that taste activation did not differ in amygdala and striatum responses (reward and salience areas) between the consumption of caloric (maltodextrin-sucralose) and non-caloric (sucralose) solutions. Both drinks did not differ also when compared to control solution, water, and the authors suggested that the stimuli might not have been reinforcing enough due to low pleasantness. Maltodextrin induced different taste activation in some brain areas than the sucralose and maltodextrin solution, indicating that these different responses can be attributed to dissimilar sweetness (243).

- *Is there an effect of habituation on NNS use and neural responses to sweet taste?*

Green and Murphy investigated the effect of habitual consumption of diet soda on fMRI brain activation to caloric (sucrose) and non-caloric (saccharin) sweet taste. Participants were scanned in the fasted state (after 12 hours of fast) and results showed that diet soda drinkers (individuals consuming at least one can of diet soda per week) demonstrated more widespread activation to both nutritive and non-nutritive sweet tastants in reward areas (OFC, putamen, globus pallidus, VTA, amygdala) compared to non-drinkers. Although brain activation in the OFC was greater for saccharin versus sucrose in the non-diet soda drinkers, this was not demonstrated for the diet soda drinkers, who showed similar responses in response to nutritive and non-nutritive sweet tastants (244). These findings could suggest that chronic NNS consumption may compromise the efficacy of the brain to distinguish between nutritive and non-nutritive tastants. Another study by Rudenga and Small reported a negative correlation between self-reported NNS intake and amygdala response to sucrose ingestion, proposing an adaptation of neural response to sweet taste as a result of habituation (245).

Contradictory findings come from Griffioen-Roose *et al.* (246) who examined potential effects of replacing sugar to a soft-drink or a yoghurt drink on brain reward responses via fMRI after repeated exposure. It was hypothesised that repeated exposure to products which lack the rewarding component, in this case sugar, could result in decreased preference for these products compared to their caloric versions. The hypothesis was not confirmed, as repeated exposure (10 times) of a non-caloric relative to the sugar sweetened version of both drinks did not result in differences in reward brain regions, suggesting that

learned associations between sensory attributes and satiety cannot be easily altered. Small differences between sugar- and NNS- version of the soft drinks were detected in the middle cingulum, the precentral gyrus and rolandic operculum.

Neural responses to hedonic food cue paradigms following the consumption of caloric sugars and NNS

In functional neuroimaging, food stimuli, in comparison with non-food-objects, have been shown to activate occipital, limbic and paralimbic, and prefrontal areas (247). In the fasted state participants exposed to food images show enhanced activation of the amygdala, OFC, fusiform gyrus, lateral and medial PFC and parietal cortex (247, 248), while satiation induces attenuation of brain activity in metabolic (hypothalamus) and reward related areas in response to food picture viewing and increase in areas involved with inhibitory control, such as the dlPFC (181). Neural responses to food cues differ between individuals with normal weight and obesity (170) or individuals who have lost weight (249).

Oral ingestion of glucose induces changes in neural processing of food cues that are congruent with a satiation-like state. In particular, Kroemer *et al.* investigated the neural correlates to food cues compared to control pictures after overnight fast and after 75 g glucose ingestion (250). Food pictures compared to control activated a large bilateral network typically involved in homeostatically and hedonically motivated food processing. Glucose ingestion was followed by decreased activation in the basal ganglia and paralimbic regions and increased activation in parietal and occipital regions. Heni *et al*.(251) investigated the influence of 75 g glucose versus water ingestion on the neural processing of food cues in healthy lean and overweight individuals 30 and 120 min post beverage consumption. Across the two groups and averaged between 30 and 120 min, after glucose administration significant increase was demonstrated in the right fusiform gyrus, right hippocampus and left precuneus while after water administration an increased in activity was demonstrated in the left occipital gyrus. However the main effect of condition (water vs glucose) was not significant in this study. Luo *et al.* compared the brain activation in response to a food cue paradigm after the consumption of glucose or fructose (252). Fructose consumption was associated with greater activation of the visual cortex and OFC during food-cue reactivity compared to glucose, suggesting that this might be involved in promoting feeding behaviour.

Intragastric administration of water or glucose in healthy lean adults did not show any differences in neural processing of food cues in the study by Stopyra *et al*. (253). In particular, both conditions activated brain networks related to reward processing such as the superior OFC, the bilateral putamen and olfactory cortex, but no difference between

condition was shown, the investigators proposed a decreased satiation effect of glucose in the brain attributed in the absence of cognitive or sensory signalling.

Connolly *et al.* (254) investigated the brain responses to food cues following the consumption of a sucrose-sweetened or NNS-sweetened beverage in healthy participants with normal weight or obesity. Across conditions, there were similar activations in the amygdala, hippocampus, anterior insula and thalamus. Only after the sucrose drink individuals with obesity showed higher engagement of this neural network compared to lean individuals. It is important to note that in this study no water/tasteless control was used. A recent study by Zhang *et al.* examined the neural correlates while participants perform a food bid task, in which participants bid on visually depicted food items, after the consumption of 300 mL cherry flavoured water with either 75 g glucose, 0.24 g sucralose, or no other ingredient (255). The fMRI investigation showed no significant differences between glucose and sucralose, while sucralose attenuated activity in the a priori ROIs compared to water, and glucose attenuated response in the parietal cortex compared to water.

Creze and colleagues used electroencephalography to examine the brain responses to visual food cues before and after the consumption of a standardised meal along with a sucrose-sweetened, NNS (mix of cyclamate, ace-K and aspartame)-sweetened beverage or water in healthy lean males (256). The investigators demonstrated differential activations in response to the beverage condition, with the NNS beverage showing increased neural activity in ventrolateral prefrontal regions linked to the inhibition of reward. This finding was interpreted as an early adaptation to the uncoupling of sweet taste and caloric load. However, there was no difference in *ad libitum* food intake in a subsequent meal between the water and NNS condition, so any differential brain activation was not correlated with food intake outcomes. Another study by the same research group examined neural activity and liking to visual food cues in habitual SSBs consumers before and after a 3-month replacement period by NNS-sweetened beverages (257). Findings of this study showed that participants neither experienced weight loss over the replacement period nor changes in food liking towards visual cues; however neural responses to food cues reduced from pre to post intervention in prefrontal areas and was further associated with weight loss failure. It is important to note that this study did not include a control group therefore conclusions should be made with great caution.

Brain imaging is a powerful technique that enhances neuroscience research; fMRI remains the gold standard method to assess brain responses in humans. However, it constitutes a relatively poorly reproducible technique, which does not measure neuronal activity directly, but exploits the local increase in blood flow thus blood oxygenation, required to support the increased metabolic demand (BOLD contrast). These factors as well as methodological differences across studies, and the relatively limited research on the effects of NNS on brain

responses, make it challenging to draw firm conclusions regarding NNS effects on brain function. It is also noteworthy that there are many different NNS which might be proven to exert differential effects, as recently suggested on body weight regulation (78). Further well-designed clinical trials are warranted to advance our knowledge and understanding of the effects of NNS in human brain responses linked to appetitive behaviour.

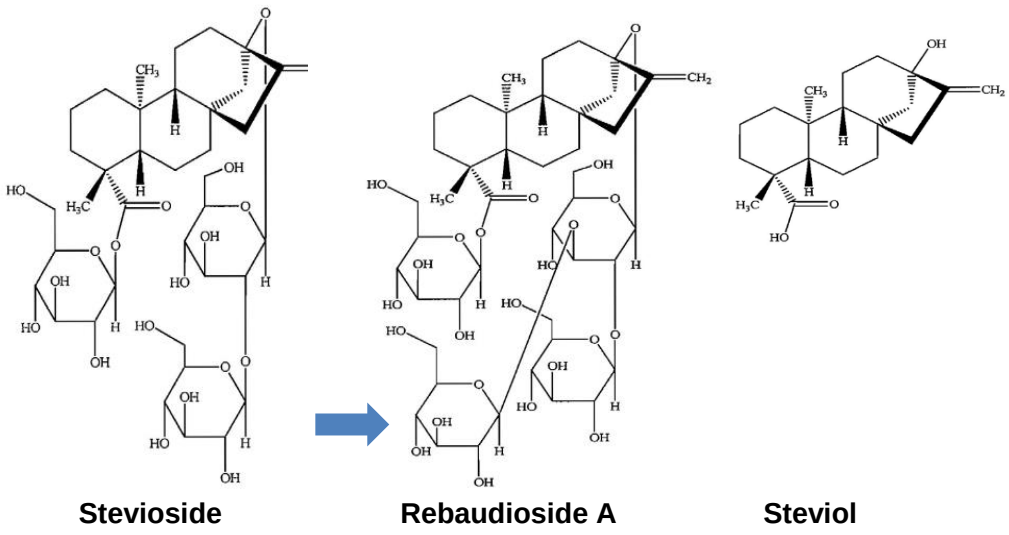
1.5 Stevia

The experimental work presented in this thesis uses stevia as the NNS of choice, therefore the next section focuses on reviewing knowledge concerning stevia specifically.

1.5.1 Metabolism and biological fate

Stevia rebaudiana Bertoni is a South American plant with natural non-caloric sweet compounds that has been cultivated for hundreds of years. Steviol glycosides, a term used to describe the group of intensely sweet compounds extracted and purified from *S. rebaudiana*, were approved for use in the EU by EFSA in 2011 (258). The predominant steviol glycosides found in *S. rebaudiana* are stevioside (4-13% w/w, Reb A (2-4%) and Reb C (1-2% w/w), but there are numerous other steviol glycosides. Stevioside and Reb A are very similar in terms of structure with Reb A having one more glucose moiety as compared to stevioside **(Figure 1.7)** (259). Steviol glycosides (Reb A, D, and M) can nowadays be produced via fermentation in genetically modified yeast strains of *Saccharomyces cerevisiae* (260) and *Yarrowia lipolytica* (261). However, steviol glycosides produced via fermentation are still not approved for use in the EU. The food industry has explored in depth the opportunities regarding stevia use and as a consequence, stevia is now used in a wide variety of food products, including soft drinks, table top sweeteners, yoghurts, desserts and others (262).

Figure 1.7 Chemical structures of stevioside, rebaudioside A and their aglycon metabolite, steviol. Adapted from Caracostas *et al*. (263).

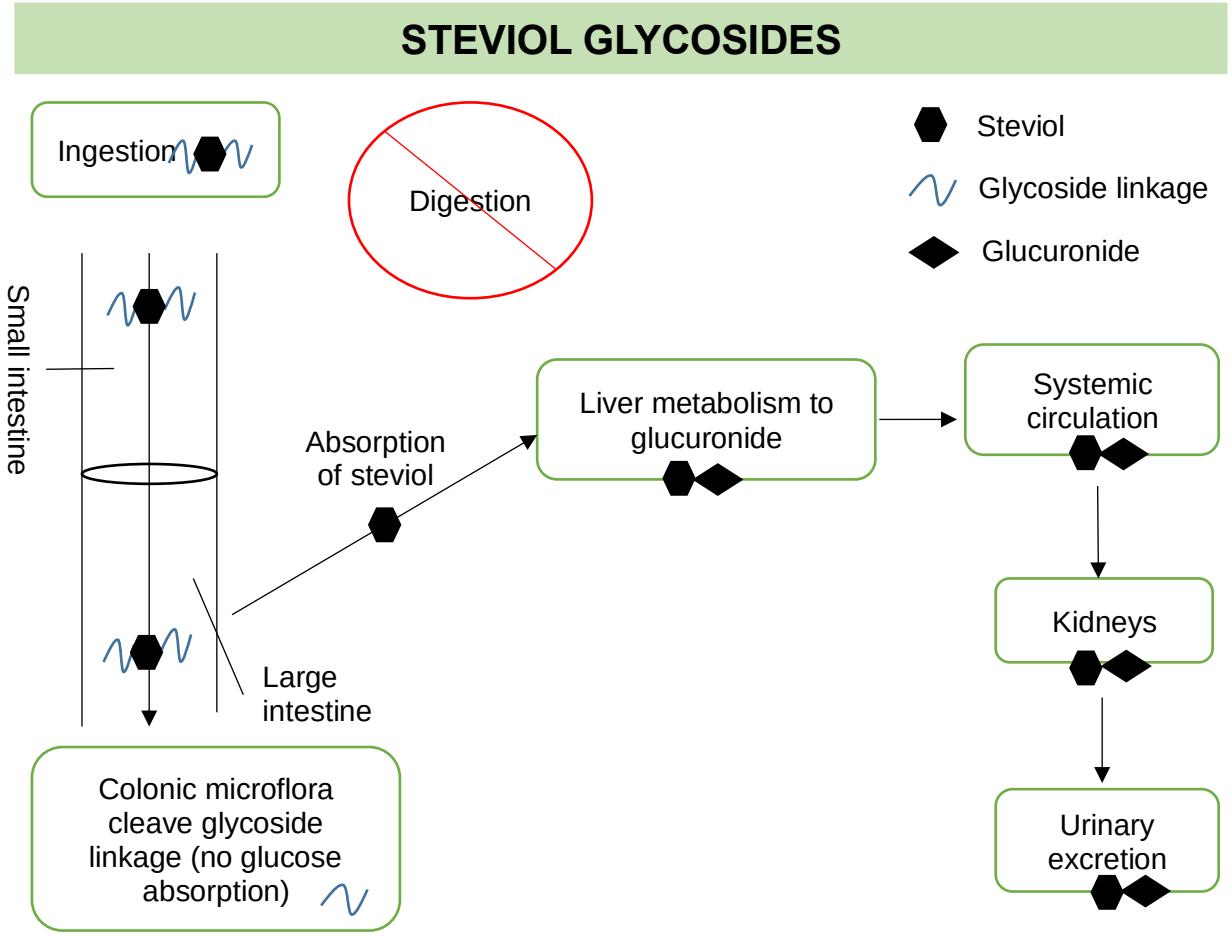


Stevioside **Rebaudioside A** **Steviol**

Steviol glycosides are not digested in the upper GI tract of humans or animals (264-266), but both stevioside and Reb A reach the colon where they undergo hydrolysis by bacterial

glucosidase resulting in the production of steviol (27). Gardana *et al.* reported that the genus Bacteroides is the one able to degrade steviol glycosides into steviol and confirmed that steviol is not further degraded by colonic bacteria (267). Koyama *et al.* showed that in rats steviol is then absorbed into the portal plasma and its presence sustains over a few hours (268). Similar metabolism of steviol glycosides has been demonstrated in humans (269). Steviol glycosides are slowly metabolised by colonic bacteria, steviol is then absorbed and transported to the liver where it undergoes glucuronidation. Stevioside and Reb A undergo similar metabolic and elimination pathways in humans. Peak plasma levels of steviol glucuronide were observed in humans 8 hours after stevioside and 12 hours after Reb A. There is also no evidence that glucose removed from the steviol glycosides in the colon is absorbed, but most likely it becomes utilised by the colon bacteria. Steviol glucuronide is mainly excreted in human urine and steviol in the faeces (27, 269).

Figure 1.8 Steviol glycoside metabolism in humans. Adapted from Magnuson *et al.* (27).



1.5.2 Effects of stevia on appetite, food intake and body weight

To date, five studies have been conducted to assess the acute effects of stevia administration on appetite and food intake in humans. A summary of these studies can be found in **Table 1.5**. Two studies used the preload paradigm (oral ingestion) of solely the beverages and no other food item at the same time (50, 51), one study administered a stevia preload together with other food items (48), one study incorporated stevia leaf powder in cookies (270), and one study administered Reb A intraduodenally (124). Out of the five studies, two showed a benefit of stevia consumption in appetite ratings compared to either water (50) or a control food item (270) and one study demonstrated similar appetite ratings following stevia or sucrose (48). Four of these studies assessed food intake, and only the study by Anton *et al.* (48) showed a significant reduced total energy intake for the stevia condition compared to the sucrose condition. The compensation was 24% in this study, while in the study by Tey *et al.* (51) compensation was 73%. Farhat *et al.* (50) did not observe differences between the water, stevia and sucrose preloads neither in *ad libitum* pizza intake nor in daily energy intake. However including a highly palatable food, such as pizza, in the *ad libitum* meal might have influenced the results, given that palatable foods tend to be overconsumed. The direct infusion of Reb A into healthy participants' duodenum did not result in any significant differences in appetite or food intake (124).

Energy intake following stevia supplementation has also been assessed as a secondary outcome in a few longer-term human clinical trials. Ajami *et al.* (271) examined the effects of daily stevia (1 cup of tea sweetened with 2% stevia leaf extract) or sucralose (one tablet of sweetener) supplementation for 2 months in patients with T2DM who were also put on energy restricted diets. There was not a significant difference in energy intake between groups or between baseline and end of the study, however the mean difference between baseline and end of the study in the stevia group was -224 kcal, whereas in the sucralose group was +204 kcal. Higgins *et al.* (78) in a randomised controlled parallel-arm study compared the effects of daily beverage supplementation sweetened with sucrose or NNS, one of which was Reb A, for 3 months in participants with overweight or obesity on body weight, while among secondary outcomes was energy intake. Reported energy intake was demonstrated higher for the sucrose group compared to the Reb A group. Another 2 studies assessed energy intake as a secondary outcome following 1000 mg stevia (Reb A) supplementation in capsules compared to placebo capsules in patients with T2DM or healthy individuals; both included a wide range of BMI, and showed no difference in energy intake between Reb A capsules and placebo (272, 273). These results are in line with the conclusions of recent meta-analyses showing no beneficial effect NNS in capsules versus placebo (66, 77).

No change in body weight was observed following a 3-month intervention period with stevia-sweetened beverages (78). This is the only RCT in the literature to date, assessing body weight following stevia supplementation as the primary outcome. Another 5 studies have assessed body weight or BMI as a secondary outcome before and after a period of steviol glycosides supplementation in the form of capsules, all of them showed no difference in body weight or BMI between the stevia capsules and the placebo capsules (272-276).

Table 1.5 Effects of stevia administration on appetite and food intake in humans.

Reference	Subjects	Design	Treatments	Appetite	Energy intake (EI)
Farhat *et al.* (50)	*n*=30, healthy with BMI in the normal range	RCT, single-blind, crossover	Fixed breakfast followed by 3 h fast, then the following preloads: • 300 ml water • 300 ml water + 60 g sucrose • 300 ml water + 1 g stevia (type not mentioned) 30 min later *ad libitum* pizza intake	Hunger and desire to eat ratings were significantly lower for stevia and sucrose vs water.	No difference in EI across conditions either at *ad libitum* meal or from whole day food diaries.
Ahmad *et al.* (270)	*n*=20, healthy with BMI in the normal range	RCT, single-blind, crossover	After overnight fast: • Control cookies • Cookies with stevia leaf powder (3% w/w) • Cookies with Moringa leaf powder (5% w/w) Appetite ratings were obtained for 2 h after.	Hunger ratings were significantly lower after the stevia cookie compared to control cookie.	Not assessed.
Tey *et al.* (51)	*n*=30, healthy with BMI in the normal range	RCT, double-blind, crossover	Equisweet beverages at 11 am after standardised breakfast: • 0.44 g aspartame in water • 0.63 g monk fruit in water • 0.33 g stevia (Reb A) water • 65 g sucrose in water 12 pm: ad libitum lunch -monitoring of postprandial responses for the subsequent 2h	Desire to eat, hunger and prospective consumption ratings from 30 to 60 min were significantly higher for the NNS compared to sucrose (and fullness significantly lower).	Meal only EI was lower for sucrose compared to NNS. Compensation for the 'missed calories' was 73% for stevia (107% for aspartame, 93% for monk-fruit). Total EI (whole day, self-reported) did not differ across conditions.
van Avesaat *et al.* (124)	*n*=15, healthy with BMI in the normal range	RCT, double-blind, crossover	After overnight fast and 150 min after the ingestion of a standardised liquid meal (162 kcal): • 120 mL water • 540 mg Reb A in 120 mL water *(Intraduodenal infusion)* 15 min after the infusions participants were offered *ad libitum* lunch meal.	No significant differences observed in hunger, desire to eat and satiety ratings between Reb A and water infusions.	No difference in EI in the ad libitum meal between Reb A and water infusions.
Anton *et al.* (48)	*n*=31, healthy with BMI in the normal range, or with obesity	Single-blind, crossover (randomisation not mentioned)	400 g preload of tea and crackers with cream cheese sweetened with stevia or aspartame or sucrose (amounts not specified) 20 min prior to *ad libitum* lunch Also 20 min prior to dinner, the test preloads were consumed. Total daily EI assessed (in the laboratory)	Hunger and satiety levels did not differ by condition at any time point.	Significantly lower total daily EI in the stevia and aspartame conditions compared to sucrose (mean difference between stevia and sucrose: 300 kcal)

BMI; body mass index; EI, energy intake; NNS, non-nutritive sweeteners; RCT, randomised controlled trial; reb A, rebaudioside A.

1.5.3 Physiological effects of stevia

1.5.3.1 Postprandial blood glucose, insulin and gut-peptide responses

As already outlined in section 1.3.2, recent meta-analyses conclude that consuming NNS alone or as part of a nutrient-containing meal does not influence acute glycaemic or insulinaemic responses (13). However, recent evidence has highlighted differential effects between different types of NNS (86), therefore it is of interest to investigate whether steviol glycosides influence postprandial glucose or insulin responses. Numerous animal studies have been conducted to investigate potential antihyperglycaemic effects of stevia. The enrichment of diet with stevia has shown improvements in glucose tolerance and insulin sensitivity (277), as well as blood pressure reducing effects in diabetic mice (278). Stevia has also been demonstrated to induce GLP-1 and PYY release in a porcine *ex vivo* intestinal model (279). Studies examining the short-term glucose and hormonal responses following stevia consumption in human subjects are presented in **Table 1.6**.

In the study by Tey *et al.* (51) the consumption of a stevia-sweetened beverage 1 hour prior to an *ad libitum* lunch resulted in similar incremental area under the curve (iAUC) in a total 3 hour postprandial period compared to the sucrose drink, however Anton *et al*. demonstrated lower postprandial glucose and insulin responses for the stevia preload (48). Farhat *et al.* demonstrated significantly higher iAUC (0-150 min) for the sucrose preload compared to stevia or water preloads (followed by *ad libitum* meal) (50). Gregersen *et al.* tested the postprandial metabolic effects following the consumption of a standardised breakfast supplemented with 1 g stevioside or maize starch (control) in 12 T2DM patients (280). The stevia condition led to significantly lower iAUC (0-240 min) for glucose by approximately 18% and lower glucagon iAUC, whereas insulin, GLP-1 and GIP iAUC did not differ among treatments. Consumption of a stevia-coconut jelly compared to a control jelly resulted in significantly lower iAUC (0-120 min) for glycaemia, while only tended to be lower for insulinaemia and C-peptide response (281).

In the study by van Avesaat *et al.* (124) the effects of intraduodenal infusion of stevia (Reb A) on gastrointestinal peptides release (GLP-1, GIP, CCK) were investigated. Healthy volunteers received 540 mg of rebaudioside A dissolved in 120 mL water, a dose corresponding to 75% of the acceptable daily intake, or plain water (control) 150 min after a standardised liquid meal. Infusion of stevia did not influence the release of GLP-1, PYY or CCK.

As expected, stevia has advantages in postprandial blood glucose and insulin response compared to caloric sweeteners like sucrose. There are also some findings that stevia might further assist with glucose homeostasis when incorporated into a nutrient-containing meal (48, 280, 281), however this warrants further investigation. The evidence regarding stevia

consumption on gut-peptides is very limited, more research should be encouraged in that field.

1.5.3.2 Fasting blood glucose and insulin levels after chronic exposure

Animal and *in vitro* studies have suggested that steviol glycosides could decrease blood glucose levels via stimulating insulin secretion and decreasing concentrations of glucagon (282, 283). Another study by Philipaerts *et al.* also showed that steviol glycosides (stevioside, Reb A) as well as their metabolite, steviol, can stimulate insulin secretion *in vitro* and in an *in vivo* mouse model (284).

In healthy adults, an early study by Curi *et al.* (285) demonstrated a correlation between ingestion of *S.rebaudiana* extracts and modulation of postprandial blood glucose response. Over the years, some RCTs have been conducted assessing the fasting blood glucose and insulin concentrations in individuals after a period of stevia supplementation. No difference in fasting glucose or insulin levels was found in the study by Ajami *et al.* (271), where patients with T2DM were required to consume daily for 2 months a cup of tea sweetened with 2% stevia leaf extract. No difference in fasting blood glucose and insulin levels was demonstrated following a 3-month supplementation with 750 mg encapsulated steviol glycosides in healthy adults and patients with T2DM or type I diabetes mellitus (274). In line with these results, Maki *et al.* also showed no evidence of change in fasting blood glucose, insulin or C-peptide following a 16 week period Reb A administration (1000 mg/day in capsules) in participants with T2DM (272). A recent meta-analysis assessed the effects of stevia supplementation in fasting blood glucose (286). A non-significant reduction in fasting blood glucose in favour of the steviol glycosides was found, and the authors also reported that heterogeneity was large across the included studies.

To date, there is no RCT assessing the effects of long-term stevia consumption on glucose and insulin response to an OGTT.

Table 1.6 Human studies investigating acute postprandial metabolic responses of stevia in human subjects.

	Subjects	Treatments	Glucose	Insulin	GLP-1	GIP	C-peptide	Control
Farhat *et al.* (50)	*n*=30, healthy with BMI in the normal range	Fixed breakfast followed by 3 h fast, then the following preloads: • 300 ml water + 60 g sucrose • 300 ml water + 1 g stevia (type not mentioned) 30 min later *ad libitum* pizza intake	• iAUC (0-150 min) significantly higher for sucrose vs stevia or water (ND after adjusting for calorie content)	-	-	-	-	300 mL water
Chupeeraach *et al.* (281)	*n*=12, healthy with BMI in the normal range	Stevia coconut jelly (50 g of available carbohydrates)	• iAUC (0-60 min and 0-120 min) significantly lower vs control	• ND in iAUC (0-30 min, 0-60 min, 0-90 min or 0-120 min). Tended to be lower for stevia jelly	-	-	ND in iAUC (0-30 min, 0-60 min, 0-90 min or 0-120 min). Tended to be lower for stevia jelly	Control coconut jelly
Tey *et al.*, (51)	*n*=30, healthy with BMI in the normal range	Equisweet beverages at 11 am: 0.33g stevia in 500 mL water 12 pm: ad libitum lunch and monitoring of postprandial responses for the subsequent 2h	• ND in iAUC 0-180min • iAUC 0-60 min higher for sucrose • iAUC 60-180 min lower for sucrose v. stevia	• ND in iAUC 0-180 min • iAUC 0-60 min higher for sucrose • iAUC 60-180 min lower for sucrose v. stevia	-	-	-	65g sucrose in 500 mL water
van Avesaat *et al.*, (124)	*n*=15, healthy with BMI in the normal range	150 min after the ingestion of a standardised liquid meal (162 kcal): 540 mg rebaudioside A in 120 mL water	-	-	• ND in plasma conc. v. control		-	120 mL water
Anton *et al.* (48)	*n*=31, healthy with BMI in the normal range, overweight or obesity	400 g preload of tea and crackers with cream cheese sweetened with stevia (amount not specified) 20 min prior to *ad libitum* lunch	• Lower glucose conc. with stevia consumption v. aspartame and sucrose	• Lower plasma conc. in stevia condition v. aspartame and sucrose	-	-	-	Sucrose preload
Gregersen *et al.* (280)	*n*=12, patients with T2DM patients, wide range BMI	Standard breakfast (412 kcal) and 1 g stevioside	• Lower iAUC 0-240 min v. control	• ND in iAUC 0-240 min	• ND in iAUC 0-240 min	• ND in iAUC 0-240 min	-	Breakfast + 1 g maize starch

BMI, body mass insex; Conc., concentration; GLP-1, glucagon like peptide 1, GIP, gastric inhibitory polypeptide; (i)AUC, (incremental) Area Under the Curve; ND, no difference; T2DM, type 2 diabetes mellitus.

1.6 Summary

Among the strategies to tackle obesity, reduction of sugar intake holds a prominent place. Substituting caloric sweeteners with NNS constitutes a promising tool towards reducing sugar and calorie intake, while maintaining palatability of food and drink products. The paradoxical observation from epidemiological studies that obesity ratings are increasing in the same timeframe as the increase in NNS consumption has fallen out of favour, since results from meta-analyses from a large amount of RCTs suggest a beneficial role of caloric sugars replacement with NNS on energy intake and body weight (63, 66). The literature review on the effects of NNS intake on appetite suggests that NNS consumption does not induce rebound hunger or an increase in desire to consume sweet items as initially hypothesised. On the contrary, NNS intake seems to have either neutral or even beneficial effects on appetite in humans.

NNS consumption has been suggested to be associated with metabolic dysregulation, with potential mechanisms including glucose-responsive mechanisms (8). NNS like caloric sugars bind to the same STRs. Demonstrations that STRs are also located throughout the GI tract, led in research in animal and cellular models that suggested a key role for the gut STRs regarding their potential functional role in human physiological responses and potentially human eating behaviour. However, considerable amount of research in human volunteers concludes that ingestion of NNS, administered alone or in combination with a nutrient-containing preload, has no acute effects on postprandial glycaemic or insulinaemic responses in humans (13). Available evidence, although less abundant, also shows that NNS intake is not associated with gut-peptide secretion in humans, apart from the case of sucralose and diet beverages potentially stimulating GLP-1 release. There are fewer studies available on the long-term effect of NNS consumption on glucose homeostasis, yet more research is encouraged in this field.

Neuroimaging studies have provided some evidence demonstrating that the human brain is able to distinguish sweet taste coming from caloric sugars or NNS (239, 241). Conflicting findings exist regarding the habitual exposure to NNS and non-homeostatic neural signalling, with a few reports showing a potential modulation of reward areas' response to sweetness (244, 245), while another study showed no change in reward value following repeated exposure to NNS (246). Very early findings from neuroimaging studies have also suggested that following consumption of NNS and caloric sugars, neural responses to food-cue paradigms, which target the hedonic aspects of eating behaviour, are similar, potentially proposing equal modulation of motivation to eat in brain circuits post intake (254, 255). This hypothesis warrants further research.

It is clear that a wide range of physiological, behavioural and neurological factors contribute to food choices and appetite control, and that the mechanisms of eating behaviour are complex and interconnected. Consumption of sweetened beverages could affect eating behaviour through homeostatic processes (hunger/satiety) or hedonic (reward) processes. Homeostatic and hedonic signals in the brain interact and can also be modulated by cognitive processes (155). From the homeostatic point of view, the presence of caloric sugars in the GI tract will activate a cascade of events for the processing and absorption of nutrients into the bloodstream and will generate a number of peripheral signals that will reach the brain. However, as already discussed NNS do not seem to affect acute metabolic responses in humans. Nonetheless, any direct gut-to-brain communication due to the presence of NNS in the GI tract and potentially binding to the gut STRs cannot be discounted. From the hedonic point of view, tasting something sweet creates a sensory hedonic experience, which then activates pleasure-generating brain circuitry. Therefore, NNS could be affecting eating behaviour directly in the brain via their rewarding properties independently of peripheral signals regarding metabolic state. Whether NNS intake is able to modulate neurocognitive responses to food cues, assessed via food-related cognitive tasks is currently unknown.

According to the above, it is clear that more well-designed RCTs are needed to examine further potential differences between nutritive sweeteners and NNS in homeostatic and hedonic pathways of food intake control.

1.7 Aims and objectives

The overall aim of this thesis is to drive an improvement towards the understanding of the effects of NNS consumption on neurocognitive, behavioural and physiological responses in humans. The work presented in this thesis takes a multidisciplinary approach using a combination of methodologies including neurocognitive (reward-related food cue responses), appetitive (appetite ratings, energy intake), physiological responses (glycaemia, insulinaemia) and subsequently brain imaging (fMRI, *phys*MRI) to examine the effects of NNS intake on homeostatics, eating behaviour and hedonics.

The underpinning hypothesis is that NNS due to being sweet yet calorie free will influence appetite control mainly via hedonic mechanisms, while their effects on physiological responses (glycaemia, insulinaemia) will be minimal.

Among the different NNS types, stevia (Reb A) derived from stevia leaf extracts was selected and used in the totality of the trials described in this thesis. This decision was based on the premise that stevia is a plant-based sweetener, its popularity has significantly increased the latest years and is expected to continue to rise as consumer preferences tend to shift towards natural products and there is less research available on this type of NNS compared to the others.

All studies described below were conducted in healthy young adults. The specific aims per chapter are listed below:

Chapter 2

- To develop and test the efficacy of a set of neurocognitive tasks (VPT, SRCT, IAT) to detect modulation of food cue responses by metabolic signals.
- To compare food-cue responses between participants with normal weight and overweight or obesity.

Hypotheses:

- *Metabolic state (fasted-fed) will influence food cue responses.*
- *BMI will influence neurocognitive responses to visual food stimuli in response to metabolic state, with participants of higher BMI expected to show higher food-cue reactivity in the fed state compared to participants with normal BMI.*

This study acted as a proof-of-concept stage to help design next phase studies, with the intention of using the food cognitive tasks that can successfully discern food cue responses between a fasted and fed state in subsequent trials. The main hypothesis behind this objective is that internal metabolic state and energy needs can influence non-homeostatic mechanisms such as in the fed state food attractiveness is reduced and this can be reflected

in reaction time food-cue neurocognitive tasks. In later studies (Chapter 2 and 5), the specific role of sweetness and calorie content on food-cue responses is examined.

A short test battery was developed that comprised of food-related versions of a SRCT, a VPT and an IAT. The test battery was administered to the participant groups, individuals with normal weight and with overweight or obesity, in two different metabolic states, fasted and fed state, in crossover design. The inclusion of two weight groups intended to examine differences in responses according to weight status.

Chapter 3

- To investigate the role of a single dose of a stevia-sweetened beverage consumption on eating behaviour (appetite, food intake) and AB to food cues relative to beverages containing sweet caloric sweeteners (glucose, sucrose) and unsweetened caloric and non-caloric controls (maltodextrin, water) in healthy lean adults.

Hypothesis:

- *Consumption of the energy-containing beverages will increase blood glucose levels, suppress appetite ratings, food intake in the ad libitum meal (but not total energy intake) and AB to food cues compared to the non-energy containing beverages (stevia, water).*

In this randomised double-blind crossover trial, I tested the effects of consuming a sweet/non caloric preload, versus sweet/caloric preloads (glucose, sucrose), non-sweet/caloric (maltodextrin) and non-sweet/non-caloric (water) on appetite, food intake and AB to food cues, using the VPT. The VPT was chosen because it was found to be sensitive to metabolic state changes in the study described in Chapter 2.

Chapter 4

- To investigate the effects of daily consumption of stevia drops for 12 weeks in habitual beverages, taken in doses similar to real-life consumption, compared to a control group receiving no treatment, on glucose homeostasis, body weight and energy intake in healthy lean adults.

Hypothesis:

- *Daily stevia daily consumption for 12 weeks will not affect glucose metabolism, body weight or energy intake compared to no intervention in healthy lean adults.*

This study was an open-label randomised controlled trial conducted in healthy adults with a normal BMI who were non-habitual consumers of NNS. A no treatment group was decided upon the relatively fewer research in this comparison.

Chapter 5

- To examine the brain responses to acute consumption of a single dose of a stevia-sweetened beverage compared to equisweet glucose beverage, and appropriate non sweet controls (maltodextrin – no sweet taste, contains calories, water – no sweet taste, no calories) in a 2x2 (taste-by-calories) design using *phys*MRI and task-based fMRI.

Hypotheses:

- *The consumption of the stevia-sweetened beverage is expected to show similar BOLD response with glucose in areas involved in sweet taste processing (oral and extraoral) and distinct responses in areas involved in calorie processing. Glucose and maltodextrin are expected to show similar responses relative to water and stevia.*
- *Caloric beverages relative to water will modulate neural responses to the food-cue paradigm in areas involved in reward processing. The effect of stevia will be examined.*

This study was a randomised double-blind controlled crossover trial; treatment order was counterbalanced between participants. The *phys*MRI was used in order to examine the brain signals derived from the consumption and presence of the beverages ingredients in the gut over time, compared to a baseline period. During the *phys*MRI the participants only have to stay still in the scanner. The task-based fMRI was used in order to examine the neural correlates of hedonic food-cue responses. The food-cue paradigm I used was again the VPT developed in Chapter 2.

CHAPTER 2

Attentional bias to food varies as a function of metabolic state independent of weight status

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Statement

NS initiated, designed and performed the study, performed the data analysis and took responsibility for planning and writing the manuscript. RE and SM provided intellectual input, read and approved the manuscript. JM contributed to the study design, provided intellectual input, supervised the project, reviewed and approved the manuscript. The manuscript has been published in *Appetite*. The manuscript occupies pages 78-96 of this thesis.

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2.1 Abstract

Eating behaviour requires that internal metabolic changes are recognised by the central nervous system which regulates brain responses to food cues. This function may be altered in obesity. The aim of this study was to examine potential differences in neurocognitive responses to visual food cues as a function of metabolic state and weight status. A crossover study with two participant groups was conducted, one group with normal-weight (n = 20) and one group with overweight/obesity (n = 22), who completed a novel battery of neurocognitive tests assessing food-cue elicited behaviour in both fasted and fed states. The test battery included a visual-dot probe task (VPT), a stimulus-response compatibility task (SRCT) and an implicit association task (IAT). Results from the VPT showed a significant main effect of metabolic state on attentional bias ($F(1,40) = 9.90$, $p = 0.003$, $\eta^2p = 0.198$), with participants in the fasted state showing a significantly greater attentional bias for food stimuli than in the fed state. No significant main effect of metabolic state on approach food bias, assessed via the SRCT, or implicit attitudes to food cues, assessed via the IAT, was found and overall, no difference in neurocognitive processing of food cues was demonstrated between participant groups. In the fed state, attentional bias to food cues decreases in both normal-weight controls and participants with overweight/obesity, indicating that changes in current metabolic state can be reflected in attentional processing of visual food cues independently of weight status. Neurocognitive tasks which can effectively and sensitively identify differences in food cue perception according to changes in metabolic status will be useful tools in exploring more complicated interactions between homeostatic and hedonic drives of food intake.

Keywords: Attention; Food cues; Food reward; Hunger; Obesity; Satiety

2.2 Introduction

It is well established that homeostatic and hedonic systems of feeding behaviour act under a common neurochemical network (12), so that nutritional state influences food attractiveness (155). In conditions of food deprivation the incentive value of food cues increases (178, 242), whereas in the satiated state this decreases (181). Higher blood glucose levels are associated with decreased brain responses in reward areas, so that food is less rewarding in the fed state (250, 251, 287). In humans with obesity this interaction has been reported to be altered with hypersensitivity to food cues and insensitivity to metabolic signals, which may favour food consumption and play a role in the pathogenesis of obesity (287, 288).

Although neuroimaging studies show differences in brain responses to food cues in response to metabolic signals between individuals with normal-weight and overweight/obesity (170, 287, 288), data from behavioural studies are limited and inconsistent. Attentional bias to food cues defined as the tendency to focus attention to salient (food) over neutral information, has been suggested to be associated with hunger and higher body mass index (BMI) (196, 200, 201). However, contradictory data show no difference in attentional bias, assessed via a visual-dot probe task (VPT), between normal-weight and participants with obesity (200, 289). Enhanced automatic approach bias to food cues, assessed via a stimulus response compatibility task (SRCT) has been associated with self-reported traits associated with overeating (204, 205), but whether approach food bias changes according to internal homeostatic signals (fed vs fasted) and BMI is unknown. Additionally, there is also evidence that in a hungry state food-related words are evaluated as more pleasant when assessed via an implicit association task (IAT) (212, 214), though this has not yet been tested in individuals with overweight/ obesity.

In developing the test battery described here we chose to include a selection of existing and adapted tasks that assess implicit responses to food. The VPT and IAT have already shown sensitivity to metabolic status changes, while whether the SRCT can show differential approach bias to food is still unknown. The tasks were selected also on the basis of feasibility in brief versions, being readily understood and well tolerated by the participants. The aim of this study was to examine whether neurocognitive responses to visual food cues, including attentional and approach bias and implicit attitudes to food stimuli, change according to internal metabolic state and BMI. We hypothesised that in the fasted state normal weight participants will exert higher attentional bias, food approach bias and implicit attitudes to food cues relative to the fed state. We also hypothesised that no difference will be detected in food-cue responses between fasted and fed states in participants with overweight/obesity. The analysis will therefore be presented as an interaction between metabolic state and participant group.

2.3 Methods

2.3.1 Participants

Participants were recruited from students and staff of the University of Manchester, and the local area via poster and online advertisements: 20 normal-weight adults (BMI 18.5 - 24.9 kg/m^2, 7 males) and 22 participants with overweight/obesity (BMI 25 - 40 kg/m^2, 8 males). The study was powered to detect a medium-sized effect (f = 0.25, α =0.05) using a mixed design 2 (group) x 2 (condition – fasted or fed). Inclusion criteria were 18-40 years old, BMI 18.5 - 40 kg/m^2 and regularly eating breakfast (≥5 days per week). Exclusion criteria were chronic conditions such as diabetes, gastrointestinal diseases, eating disorders, self-reported mental illness, anxiety or depression, chance of pregnancy for female participants, consuming alcohol above the NHS guidelines or currently dieting. The study was approved by the University of Manchester Research Ethics Committee; all participants signed informed consent prior to participation. Participants received compensation (shopping vouchers) for their time and travel expenses.

2.3.2 Procedure

Participants arrived between 0800 and 1030 h after a 10-12 h overnight fast on two separate mornings at least 3 days apart. Participants were instructed to consume the same habitual meal on the evening before each testing day and refrain from any vigorous physical activities or alcohol consumption. All participants completed two visits, one fasted and one fed visit in randomised order. The order of the visits was also counterbalanced so that half of the participants started with the fasted visit and the other half started with the fed visit. On the first visit, body weight was determined by a digital scale in light clothes without shoes and height was measured with a portable stadiometer (SECA 213 Portable Height Measure, Hamburg, Germany). On the fed day, participants were served a standardised breakfast (**Table 2.1**), which they were instructed to consume within 10 minutes. Neurocognitive testing was performed 30 minutes after consumption of breakfast in the fed visit and 10 minutes after participants' arrival in the fasted visit.

Table 2.1 Nutritional composition of the standardised breakfast.

	Amount	Energy (kcal)	Carbohydrates (g)	Proteins (g)	Fats (g)	Fibre (g)
Semi-skimmed milk	240 mL	120	11.5	8.6	4.3	0
White bread	2 slices (56g)	140	26.2	5.2	1.2	1.2
Strawberry jam	20 g	52	12.5	0.1	0.1	0.2
Banana	1 medium	105	26.9	1.3	0.4	3.1
	Total	417	77.1	15.2	6.0	4.5

2.3.3 Experimental paradigms

2.3.3.1 Stimulus-response compatibility task

The task comprised of 2 blocks of 40 trials each according to the methodology described by Barby *et al*. (290) with some modifications. On each trial, a food-related or neutral (office stationery) picture was displayed at the centre of the screen and a small manikin appeared above or below the picture on every trial. Ten food pictures and 10 stationery pictures were used, each appeared four times. In the 'approach-food' block, participants were instructed to move the manikin towards the food-related pictures and away from stationery pictures. In the 'avoid food' block, these instructions were reversed. Prior to the actual task, participants completed 2 blocks of 8 practice trials (data not analysed). Participants were instructed to respond as quickly and accurately as possible. Response accuracy and latency were recorded on each trial. The order of the blocks was counterbalanced between participants. The trials within each block were presented in a new random order, with a new randomisation for each participant.

2.3.3.2 Dot probe task

The procedure was based on that described by Loeber *et al*. (197) with minor modifications. Each trial began with the presentation of a fixation cross for 500 ms, followed by a pair of pictures, one food picture and one office stationery picture presented one in the right and one in the left part of the screen for another 500 ms. Immediately after the picture pair presentation a dot probe (white X on black background) appeared in either the location of the right or the left picture and remained until the participant responded. They were instructed to respond by pressing one of the two response keys to indicate dot probe position as quickly and accurately as possible. In total 120 trials were administered, including 80 critical and 40 filler trials. Twenty food-related pictures were paired with 20 stationery-related pictures. The pictures and dot probe locations (left or right) were

counterbalanced. In each trial, response latencies to respond to the dot probe were recorded.

2.3.3.3 Implicit association task

The task comprised of 7 blocks of different lengths. Words from four categories (pleasant, unpleasant, food and furniture) were presented at the centre of the screen and participants had to indicate in which category (presented at the side of the screen) the word belonged. Block 1 and 2 were practice blocks for the pleasant and unpleasant and food and furniture categories respectively, and comprised 20 trials each (each word presented twice). Block 3 was another practice block with 20 trials where participants were asked to press one key e.g the left key 'e' for a 'food' or 'pleasant' word and the right key 'i' for a 'furniture' or 'unpleasant' word. Block 4 was a critical block, where the 20 words from all four categories were presented, with each word presented 4 times (80 trials). Block 5 was a further practice block for pleasant and unpleasant categories, but the category/key assignments were switched. Block 6 was also a practice block with the same structure as block 4 and block 7 was the last critical block, where the 20 words were presented 4 times each (80 trials). Blocks 3-4 and 6-7 corresponded to either compatible (food and pleasant sharing the same response key) or incompatible (food and unpleasant sharing the same response key) blocks, depending on the version of IAT (4 versions were used, following the methodology by Stafford & Scheffler (214)).

Figures of the tasks can be found in **Supplementary Figure 2.1**.

2.3.3.4 Stimulus materials and equipment

The standardised set of food images from Full4Health Image Collection was used (291), selected after a preliminary informal study in-house. The same procedure was also conducted in order to select the most hunger-satisfying breakfast-related words for the IAT, according to the previously described methodology (214). The IAT included four categories of words, pleasant, unpleasant (selected from Huidjing *et al*. (292)), food and furniture (as in Stafford & Scheffler (214)). The five most highly rated food words were sandwich, bagel, pizza, pancakes, croissants.

Psychopy software (version 1.84.1) was used to programme, present the tasks and collect the data (293). The tasks were presented on a 14-inch Lenovo screen.

2.3.4 Assessment of appetite sensations

Visual analogue scales (VAS) assessing subjective feelings of appetite were used. Five questions were included, 'How hungry are you?', 'How full are you?', 'How strong is your

desire to eat right now?' and 'How much do you think you could eat right now?'. Participants indicated their sensations with a small vertical line on a 10-cm line scale.

2.3.5 Blood glucose measurement

Capillary blood samples were collected from a fingertip for the measurement of fasting blood glucose levels at the start of each test session and 30 min after the consumption of the standardised breakfast (only for the fed visit). Blood samples were collected directly into Hemocue microcuvettes and analysed using Hemocue Glucose 201 + Analyser (HemoCue, Angelholm, Sweden). Results were displayed in mmol/L.

2.3.6 Statistical analysis

Data handling was performed in R. Statistical analysis was performed using IBM SPSS Statistics Version 23. Descriptive statistics were used for variables such as age, weight, height, BMI and questionnaire variables. VAS appetite ratings and blood glucose values were analysed by mixed-design AVOVAs, using time as a within-subjects factor and BMI group as a between-subjects variable.

Mixed ANOVAs were performed to analyse differences between fasted and fed state (within-subjects variable) and between normal-weight and participants with overweight/obesity (between-subjects variable) with regard to reaction times (RT) to the SRCT, VPT and IAT. In cases where sphericity was violated, we report the Greenhouse-Geisser results. All incorrect trials were discarded, and additionally trials slower than 3000 ms or faster than 200 ms in the SRCT, slower than 1000 ms or faster than 100 ms in the VPT, and slower than 2000 ms and faster than 200 ms in the IAT. Approach food bias was calculated by subtracting the mean RT to respond to the approach food trials from the mean RT to respond to the avoid food trials. An attentional bias score was calculated from the VPT RT as follows: mean latency to respond in incongruent trials minus mean latency to respond in congruent trials, with positive values indicating attention bias towards food-related pictures. An IAT score was computed using the D-score algorithm as previously described (294).

Exploratory Pearson correlation analyses were carried out to examine significant associations between behavioural measures, blood glucose levels and appetite ratings. Statistical significance was determined at $p \leq 0.05$.

2.4 Results

Participant characteristics are summarised in **Table 2.2**. The group with overweight/obesity comprised of 19 participants with overweight and 3 participants with obesity.

Table 2.2 Characteristics of the participants.

	Group with normal weight (n = 20)	Group with overweight/obesity (n = 22)	p value[a]
Age (years)	24.3 (4.7)	23.9 (4.4)	0.879
Weight (kg)	61.9 (10.2)	82.2 (14.9)	< 0.001
Height (m)	1.7 (0.1)	1.7 (0.1)	0.707
BMI (kg/m^2)	21.3 (2.2)	27.8 (3.3)	< 0.001

BMI, body mass index; Values represent mean (SD). [a]p values were derived from independent samples t-test for parametric continuous data and Mann-Whitney test for non-parametric continuous variables.

2.4.1 Postprandial state induction

Results of the subjective appetite measures can be seen in **Figure 2.1**. In the fasted condition, significant main effects of time were observed for hunger ($F(1,40) = 15.96$, $p < 0.001$, $\eta^2p = 0.285$), desire to eat ($F(1,40) = 13.25$, $p = 0.001$, $\eta^2p = 0.249$) and prospective consumption ($F(1,40) = 11.33$, $p = 0.002$, $\eta^2p = 0.221$), whereas no significant main effect of BMI or any interactions between BMI group and time were demonstrated.

In the fed state, significant main effects of time were observed for hunger ($F(2, 80) = 112.02$, $p < 0.001$, $\eta^2p = 0.737$), fullness ($F(2, 66) = 124.19$, $p < 0.001$, $\eta^2p = 0.756$), desire to eat ($F(2,69) = 146.09$, $p < 0.001$, $\eta^2p = 0.785$) and prospective consumption ($F(2,69) = 107.50$, $p < 0.001$, $\eta^2p = 0.729$). Post-hoc Bonferroni's tests revealed significant decrease in hunger, desire to eat and prospective consumption ratings (and increase in fullness) between the start of the session and 30 min after the consumption of the fixed breakfast and remained significantly decreased (increased for fullness) until the end of the session, showing that the experimental paradigm worked.

Additionally, in the fed condition significant main effects of BMI group were also demonstrated for hunger ($F(1,40) = 6.54$, $p = 0.014$, $\eta^2p = 0.141$), fullness ($F(1,40) = 7.05$, $p = 0.011$, $\eta^2p = 0.150$), desire to eat ($F(1,40) = 4.58$, $p = 0.038$, $\eta^2p = 0.103$) and prospective consumption ($F(1,40) = 5.46$, $p = 0.025$, $\eta^2p = 0.120$), and significant time x BMI-group interactions for desire to eat ($F(2,69) = 3.92$, $p = 0.030$, $\eta^2p = 0.089$) and marginally for prospective consumption ($F(2,80) = 3.09$, $p = 0.051$, $\eta^2p = 0.072$), suggesting that the consumption of the fixed breakfast did not elicit similar satiety in both groups, with the participants in the overweight/obesity group showing lower suppression of appetite.

Fasting blood glucose levels did not differ between the two visits in both groups (**Table 2.3**). The manipulation of metabolic state during the fed visit was confirmed by increased blood glucose levels 30 min after the consumption of breakfast in the normal weight group ($t(19) = 12.28$, $p < 0.001$) and the group with overweight/obesity ($t(20) = 10.69$, $p < 0.001$; one missing value at t = 30 min).

Table 2.3 Blood glucose levels for fasted and fed visit.

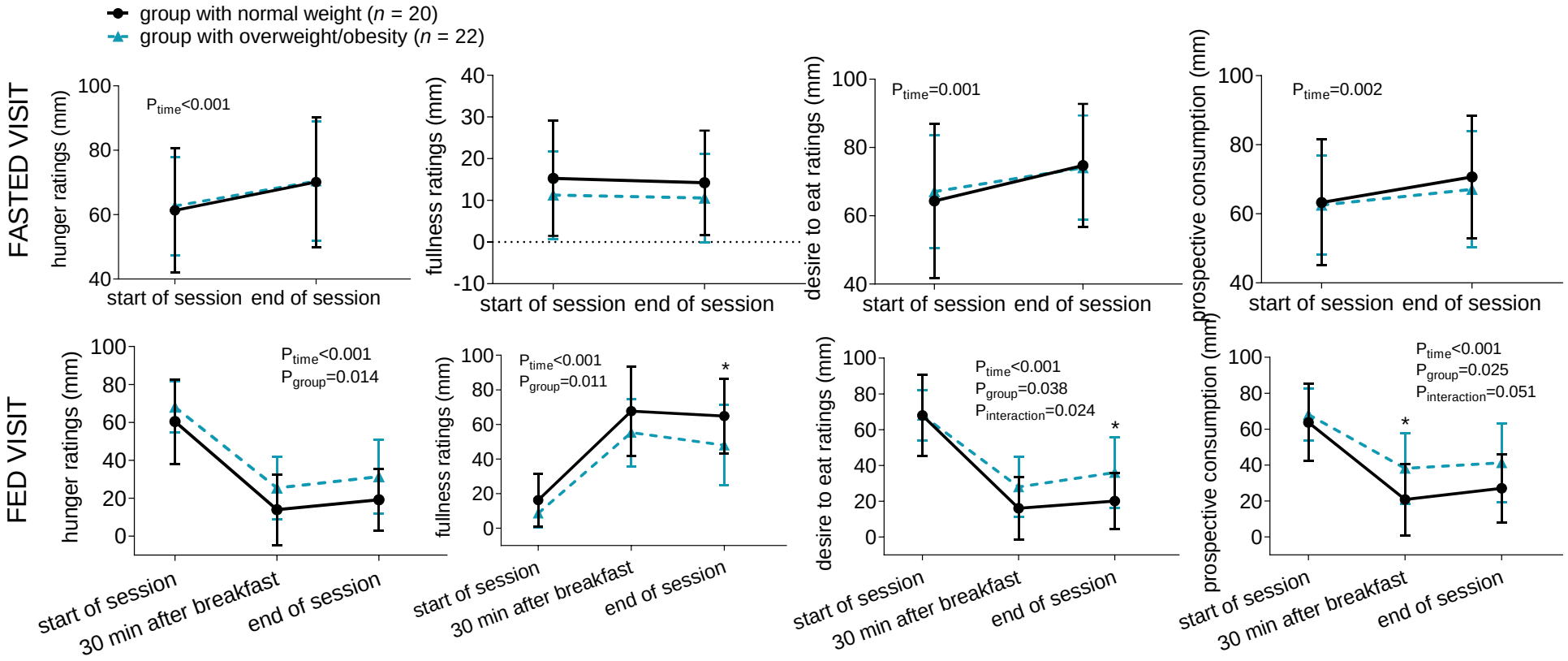
	Group with normal weight (*n* = 20)			Group with overweight/obesity (*n* = 22)		
	Fasted visit t = 0min	Fed visit t = 0min	Fed visit t = 30 min after breakfast	Fasted visit t = 0min	Fed visit t = 0min	Fed visit t = 30 min after breakfast
Blood glucose (mmol/L)	5.23 (0.36)	5.13 (0.29)	7.66** (0.21)	4.94* (0.42)	4.92 (0.44)	7.40** (1.02)

Values represent mean (SD). *$p < 0.05$ between groups, **$p < 0.05$ within group difference in fed visit.

2.4.2 Stimulus response compatibility task

Mean RT to the SRCT can be found in **Table 2.4**. A mixed ANOVA with picture content (food or neutral), motion (approach or avoid) and metabolic state (fasted or fed) as within-subject independent variables and BMI group as between-subjects variable showed a significant main effect of picture content ($F(1,40) = 50.23$, $p < 0.001$, $\eta^2p = 0.557$), motion ($F(1,40) = 99.08$, $p < 0.001$, $\eta^2p = 0.712$) and a significant interaction between motion x picture content ($F(1,40) = 25.30$, $p < 0.001$, $\eta^2p = 0.387$). These results indicate that participants responded faster to food pictures compared to neutral pictures, approached faster than avoided and were faster to approach than avoid food pictures which was not the case for the neutral pictures. There were no other significant main effects or interactions. Analysis of approach bias scores showed that, contrary to our hypotheses, neither metabolic state ($F(1,40) = 0.01$, $p = 0.918$, $\eta^2p = 0.000$) nor weight-group ($F(1,40) = 0.77$, $p = 0.378$, $\eta^2p = 0.009$) had a significant effect on approach bias to food cues.

Figure 2.1 Mean subjective appetit5e ratings of hunger, fullness, desire to eat and prospective consumption during the fasted visit (top row) and fed visits (bottom row).Values are expressed as mean ± SD. *p < 0.05 between the two groups (Bonferroni corrected).



2.4.3 Visual probe task

Reaction times in the VPT are presented in Table 2.4. Reaction times to congruent and incongruent trials were entered into a mixed ANOVA with congruency and metabolic state (fasted-fed) as within-subjects independent variables and BMI-group as between-subjects variable. Results showed a significant main effect of congruency ($F(1,40) = 15.79$, $p < 0.001$, $\eta^2p = 0.283$) revealing that participants responded faster to the congruent than incongruent trials and a significant interaction between congruency and metabolic state ($F(1,40) = 4.27$, $p = 0.045$, $\eta^2p = 0.096$). In order to explore this interaction we performed two paired t-tests comparing the RT to congruent trials in the fasted and fed condition and similarly for the incongruent. We found that participants' responses to congruent trials did not differ between fasted (M = 446.55 ms, SD = 102.44 ms) and fed (M = 450.23 ms, SD = 75.83 ms) ($t(41) = 0.228$, $p = 0.821$), but RT to incongruent trials were significantly slower in the fasted (M = 482.39 ms, SD = 76.20ms) compared to the fed state (M = 456.26 ms, SD = 70.49) ($t(41) = 2.32$, $p = 0.025$) (Bonferroni correction for multiple comparisons applied, $p \leq 0.025$). No significant main effect of BMI group or interaction between congruency, metabolic state and BMI group was found. Attentional bias scores were entered into repeated-measures ANOVA with metabolic state as a within-subjects independent variable and BMI-group as between-subjects independent variable. This showed a significant main effect of metabolic state ($F(1,40) = 9.90$, $p = 0.003$, $\eta^2p = 0.198$), as in the fasted state participants showed a significantly greater attentional bias for food stimuli (M = 24.52 ms, SD = 25.47 ms) than in the fed state (M = 6.04 ms, SD = 25.72 ms) (**Figure 2.2**), but the interaction between metabolic state and BMI group was not statistically significant ($F(1,40) = 0.32$, $p = 0.572$, $\eta^2p = 0.008$).

Exploratory pearson correlations showed that attentional bias scores were significantly correlated with subjective fullness ratings in both weight-groups, and with blood glucose levels but only in the normal weight group (**Table 2.5**).

2.4.4 Implicit association task

Reaction times for the IAT are presented in Table 2.4. A mixed ANOVA was conducted with block (food-pleasant and food-unpleasant) and metabolic state as within-subjects variables and BMI group as between-group independent variable. A significant main effect of block ($F(1,40) = 98.00$, $p < 0.001$, $\eta^2p = 0.710$) was found indicating that participants were slower when food and unpleasant words shared the same response key compared to when food and pleasant words were put on the same side. However there was no significant interaction between block and metabolic state ($F(1,40) = 1.34$, $p = 0.253$, $\eta^2p = 0.032$) or between block, metabolic state and BMI-group ($F(1,40) = 0.08$, $p = 0.776$, $\eta^2p = 0.002$). Another mixed repeated measures ANOVA was performed for D scores with metabolic state as within-subjects variable and BMI-group as between-subjects variable. No significant main

effect of metabolic state was found (F(1,40) = 1.49, p = 0.229, η^2p = 0.036) or an interaction between metabolic state and BMI group (F(1,40) = 0.19, p = 0.668, η^2p = 0.005) was found.

D scores were significantly negatively correlated with fullness in the group of participants with normal-weight, and when the two groups were analysed together (Table 2.5).

Table 2.4 Mean reaction times in neurocognitive tasks in the fasted and fed conditions. Values are expressed as mean (SD).

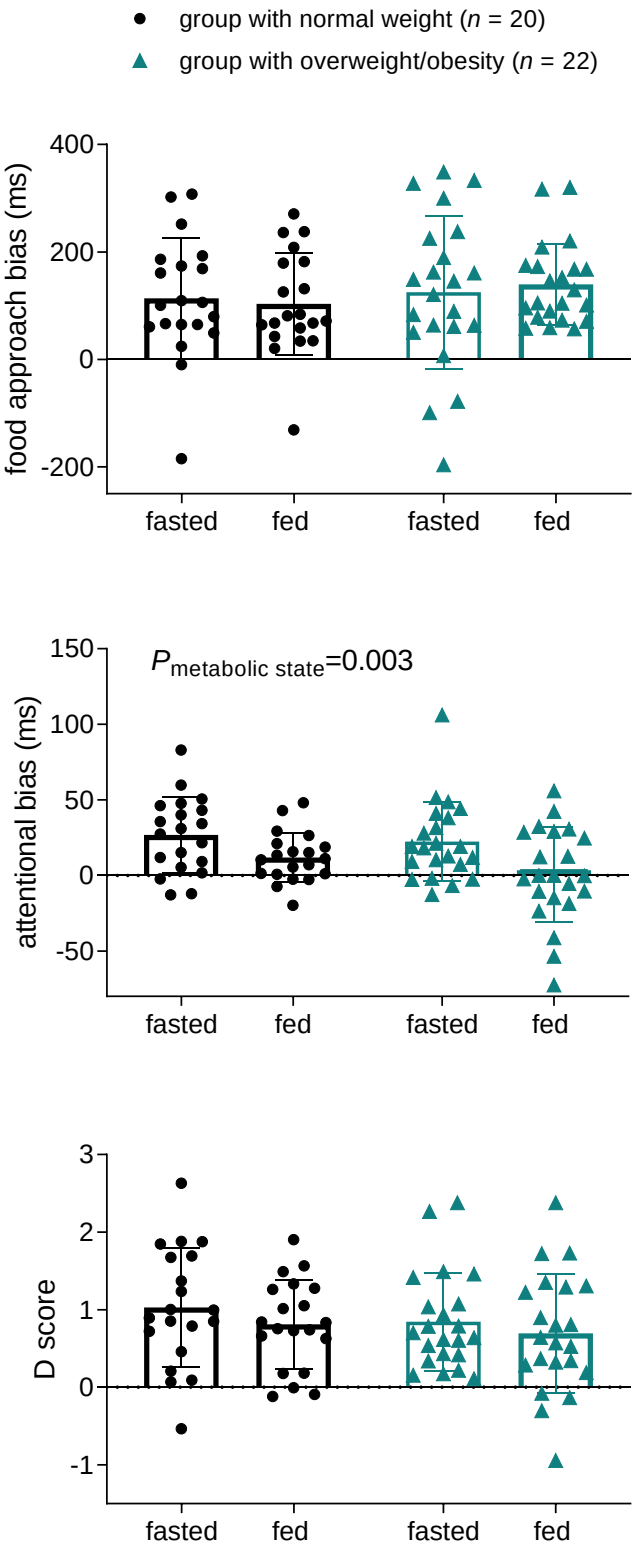
	Group with normal weight		**Group with overweight/ obesity**	
	fasted	fed	fasted	fed
Stimulus response compatibility task				
Approach food	666.13 (90.53)	656.89 (70.49)	687.09 (103.62)	653.56 (73.39)
Avoid food	780.01 (116.19)	760.30 (114.91)	811.93 (131.75)	793.09 (124.85)
Approach neutral	772.63 (104.06)	743.32 (107.67)	774.89 (133.36)	773.09 (145.81)
Avoid neutral	781.15(124.14)	783.72 (98.52)	800.55 (149.10)	779.60 (107.41)
Dot probe task				
Congruent trials	440.66 (69.09)	432.47 (65.95)	473.51 (126.91)	466.37 (81.97)
Incongruent trials	467.52 (74.27)	444.33 (62.89)	495.89 (77.10)	467.11 (76.58)
Implicit association task				
Food pleasant	685.70 (117.73)	696.53 (130.09)	679.14 (107.45)	668.53 (110.66)
Food unpleasant	826.51 (134.73)	820.07 (164.16)	822.43 (163.14)	783.22 (157.58)

Table 2.5 Correlation analyses between subjective appetite ratings, blood glucose and behavioural tasks outcomes.

	Group with normal weight			Group with overweight/ obesity			In whole sample		
	Attentional bias	**Approach bias**	**D score**	**Attentional bias**	**Approach bias**	**D score**	**Attentional bias**	**Approach bias**	**D score**
Hunger	0.300	0.007	0.167	0.230	-0.013	0.023	0.231*	0.010	0.084
Fullness	-0.318*	0.158	-0.342*	-0.312*	-0.041	-0.211	-0.279*	0.043	-0.259*
Desire to eat	0.295	-0.050	0.235	0.226	-0.011	0.210	0.225*	-0.016	0.215*
Prospective consumption	0.218	-0.128	0.216	0.159	-0.191	0.124	0.149	-0.133	0.151
Blood glucose	-0.314*	-0.022	-0.089	-0.043	0.049	-0.013	-0.132	0.004	-0.039

*$p < 0.05$.

Figure 2.2 Approach bias, attentional bias to food cues and D scores under conditions of fasted and fed. Bars represent mean ± SD.



2.5 Discussion

The aim of the present study was to examine the efficacy of a set of RT tasks to detect differences in neurocognitive (attentional) responses to visual food cues according to changes in metabolic status in normal-weight controls and participants with overweight/obesity, driven by the hypotheses that current nutritional state determines the extent to which food cues are rewarding and that individuals with overweight/obesity may have an altered reward system function (200). Results showed that attentional bias to food is an indicator of current nutritional state, since it was significantly decreased in the fed state compared to the fasted state. Interestingly, BMI did not alter this relationship, although self-reports of appetite scores indicated a blunted suppression of hunger in the overweight/obese group in the fed condition.

Hunger is expected to induce a higher motivational salience of foods, whereas under conditions of satiety food-associated cues are expected to become less potent. In accordance with this, we found that participants in the fasted state were slower to respond to the incongruent trials (dot replacing an office stationery picture), though this was not the case for the fed state. We suggest here that attention was captured by the food-associated picture, thus participants were slower to respond to the incongruent trials when hungry. In accordance with our results, Nijs *et al*. demonstrated an enhanced orientation towards food related cues in hungry versus satiated conditions, however results referred to a VPT with 100 ms stimuli presentation time (295). Similarly, Loeber *et al.* also showed a significant interaction between blood glucose levels and congruency in a VPT with 50 ms trials, where satiated participants showed longer RT to congruent trials (197). However, in another study no difference was found between fasted and fed states in RT during a VPT (2000 ms trials) (200). With regard to differences in attentional bias in response to body weight, we did not demonstrate any significant difference between the two weight-groups. It has been suggested that attentional bias measured by a variety of methodologies demonstrates altered food-cue reactivity in participants with obesity (199). However, no difference has been observed so far between lean and overweight/obese individuals and in response to nutritional status when the attentional bias was assessed via a VPT (200, 295). Studies assessing attentional bias between these two weight-groups but not in response to altering metabolic state have shown contradictory results; one study suggests that only participants with obesity show attentional bias to food (296), however others have demonstrated no association between BMI and attentional bias (289, 297, 298). These results suggest that the role of visual attention to food cues in obesity remains inconclusive and attentional bias may be more associated with state factors (i.e. metabolic state) rather than trait factors such as individual differences in body weight (193, 202, 299).

The present study is the first to assess approach bias toward food stimuli via an SRCT under fasted and fed conditions. Results showed that approach food bias does not relate to current nutritional status or body weight, and remains high even after the consumption of a satiety-inducing meal. However, it was shown that all participants exhibited a strong approach bias to food pictures, irrespectively of their current metabolic state or BMI, which is in line with previous findings showing approach bias toward food in participants with normal weight (204) and overweight/obesity (206). In a previous study where alcohol served as the reward stimulus, it has been shown that automatic approach tendencies elicited by alcohol-related cues did not significantly increase after the consumption of alcohol (300). Taken together these findings may suggest that the presentation of palatable food cues increases the strength of automatic approach tendencies toward food, but the metabolic effects of food consumption do not appear to contribute to these responses.

Implicit attitudes toward food words were not found to rely on current appetitive status in either participants with normal weight or participants with overweight/obesity. This finding is in contrast with previous results showing that food deprivation leads to a more positive valence of food items assessed through an IAT (214). In particular, in that study it was demonstrated that participants who did not have lunch were slower to associate food-stimuli with unpleasantness compared to a group of participants who did have lunch. However, we found a significant negative correlation between fullness ratings and D scores (a higher D score suggests a more favourable implicit attitude toward food i.e. food is pleasant), suggesting that the subjective fullness sensations may be a greater determinant of implicit perception of food rather than consumption of food itself.

Self-reported measures of appetite showed that the consumption of the standardised breakfast was less satiating for the group with overweight/obesity. This finding was expected a priori and supports previous findings which suggest that participants with overweight/obesity display blunted responses to dietary manipulations compared to participants with normal BMI (42, 301). Additionally short-term appetite regulation has been demonstrated to be related with postprandial insulin rather than glucose levels, however this relationship disappears as the body weight increases (302). The latter may explain the absence of correlations between blood glucose levels and behavioural measures, apart from the case of attentional bias in normal weight participants.

One possible reason for discrepancies between our results and previous observations might be the fundamental approach of separating the fasted from the fed state on different days in a randomised crossover fashion, rather than simply sequential fasted then fed measurements, which can be considered an experimental strength rather than a limitation. Previous studies have separated the participants according to whether they had or had not had lunch (214) or according to their self-reported ratings of hunger or blood glucose levels

(197). In one study participants were asked to attend the testing sessions after an 8 hour fast, but the 'meal' served was a standardized drink (200), which also lacks familiarity. Therefore, the size of the meal and the time passed from the previous meal in the fed condition might have contributed to inconsistencies between studies.

A potential limitation is that the majority of the participants in the group with overweight/obesity were overweight rather than obese, so our data may not extrapolate to extreme cases of obesity. However, Castellanos *et al.* (200) included only participants with obesity and showed no difference in attentional bias scores between the obese and the normal-weight group. Additionally, the consumption of a standardised breakfast to induce the fed state, only allows conclusions to be drawn regarding the specific macronutrient composition, suggesting that these results are representative of a carbohydrate-rich meal.

2.6 Conclusion

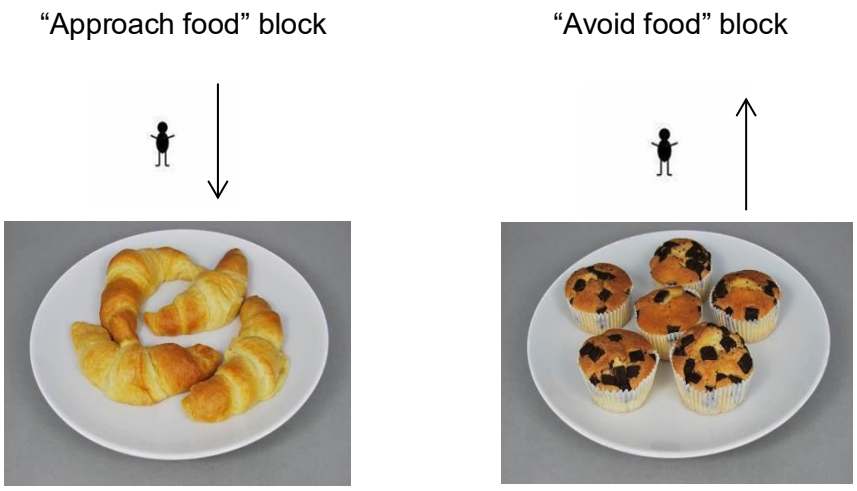
Taken together, our findings suggest that in the fed compared to the fasted state attentional bias to food cues decreases similarly in participants with normal-weight as well as with overweight/obesity, indicating that changes in current metabolic state can be reflected in neurocognitive/attentional processing of food cues, and in particular in a VPT. Approach food bias was strong for both weight-groups and remained high after the consumption of the breakfast, and implicit attitudes to food stimuli were found to be related with self-reported fullness sensations. The composite methodology developed here could be used to examine more complicated interactions between internal metabolic signals and food reward in future studies. Overall no difference in neurocognitive responses to food cues was demonstrated between participants with normal-weight and overweight/obesity under conditions of fasted and fed, a finding which questions the assumption that an impairment of salience attribution might be related to obesity.

2.7 Acknowledgements

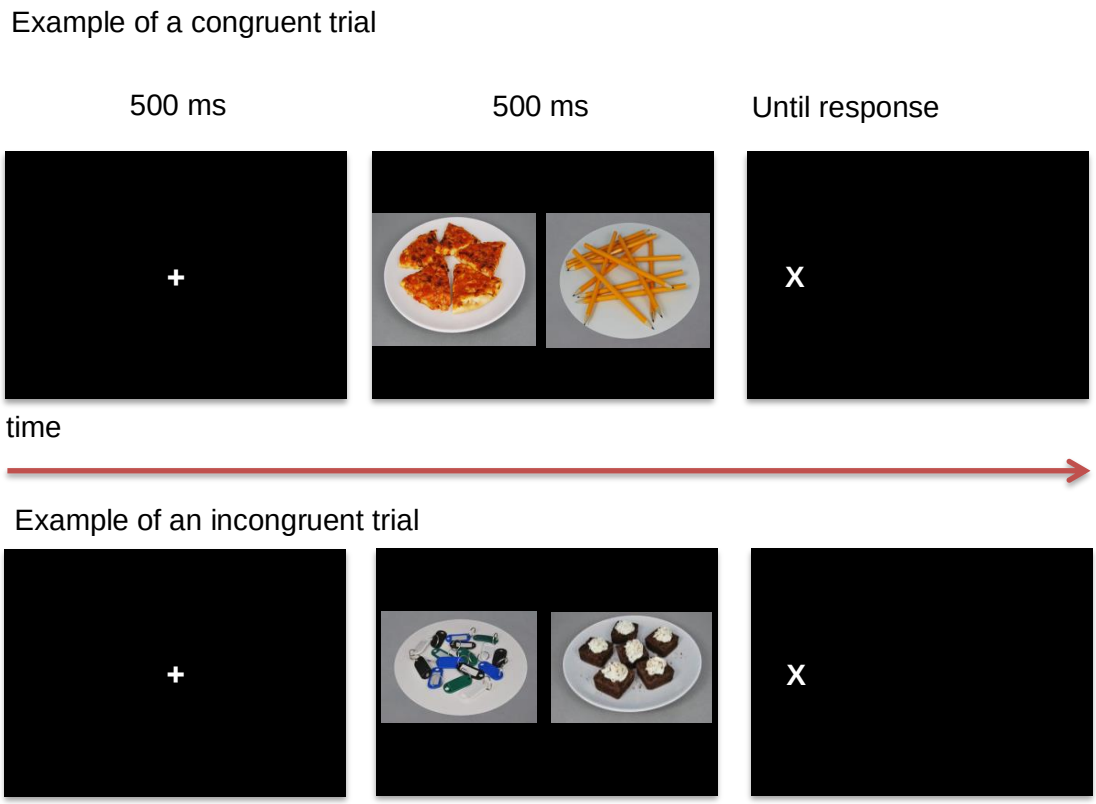
N.S.S was supported by a CASE studentship from the Biological and Biotechnological Sciences Research Council and Cargill R&D Centre Europe. We would like to thank all the participants for their time and participation.

Supplemental Figure 2.1 Illustration of the reaction time tasks.

A) Stimulus response compatibility task



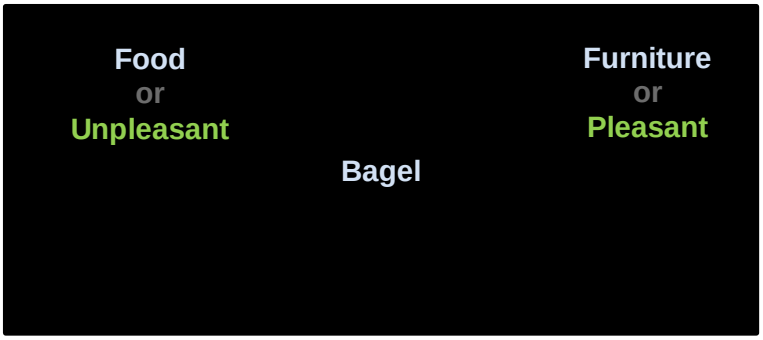
B) Visual-dot probe task



C) Implicit association task

Compatible block example

In this example participants should press the “e” keyboard key to categorise the word to the left side.



Incompatible block example

In this example participants should press the “e” keyboard key to categorise the word to the left side.

CHAPTER 3

Stevia beverage consumption prior to lunch reduces appetite and total energy intake without affecting glycaemia or attentional bias to food cues: a double-blind randomised controlled trial in healthy adults

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Statement

NS conceived and designed the study, conducted the research, analysed the data, and took responsibility for writing the manuscript. JM conceived the study and contributed to the study design, conducted the supervision, reviewed and approved the manuscript; CS and DB designed and prepared the beverages; all authors contributed to the design of the study, read and approved the final manuscript. The manuscript has been published in *The Journal of Nutrition*. The manuscript occupies pages 98-120 of this thesis.

Stamataki NS *et al.* Stevia Beverage Consumption prior to Lunch Reduces Appetite and Total Energy Intake without Affecting Glycemia or Attentional Bias to Food Cues: A Double-Blind Randomized Controlled Trial in Healthy Adults. *The Journal of Nutrition* 2020, 150(5):1126-1134.

3.1 Abstract

Background: Stevia is a zero calorie alternative to caloric sugars. Substituting caloric sweeteners with non-caloric sweeteners reduces available energy, but their effects on appetite, subsequent food intake and neurocognitive responses are still unclear.

Objectives: To examine whether sweetness with or without calories influences food intake, appetite, blood glucose levels and attentional bias (AB) to food cues.

Methods: Randomised controlled double-blind crossover study. Healthy participants (*n* = 20, age 27 ± 5y, 55% female, body mass index 21.8 ± 1.5 kg/m^2) completed five visits, consuming five study beverages: 330 mL water (control, no sweet taste, no calories) and either 330 mL water containing 40 g glucose or sucrose (sweet taste, calories – both 160 kcal), maltodextrin (no sweet taste, calories – 160 kcal), or 240 ppm stevia (sweet taste, no calories). Glucose and stevia beverages were matched for sweetness. Subjective appetite ratings and blood glucose were measured at baseline and 15, 30 and 60 min postprandially. At 15 min participants performed a visual-dot probe task to assess AB to food cues; at 30 min participants were offered an *ad libitum* lunch; food intake was measured.

Results: Subjective appetite ratings showed that preload sweetness and calorie content both affected appetite. The total area under the curve for glycaemia was significantly higher after the caloric beverages (mean ± SD, maltodextrin: 441 ± 57.6, glucose: 462 ± 68.1, sucrose: 425 ± 53.6 mmol x L^{-1} x min) compared to both stevia (320 ± 34.2) and water (304 ± 32.0) (all $p < 0.001$). Total energy intake (beverage and meal) was significantly lower after the stevia beverage (727 ± 239 kcal) compared to water (832 ± 198, $p = 0.013$), with no significant difference between the water and caloric beverages ($p = 1.00$ for water vs maltodextrin, glucose and sucrose). However, food-related AB did not differ across conditions ($p = 0.140$).

Conclusions: This study found a beneficial and specific effect of a stevia beverage consumed prior to a meal on appetite and energy intake in healthy adults.

Keywords: non-nutritive sweeteners, stevia, energy intake, appetite, blood glucose, attention, food cues, healthy adults

3.2 Introduction

Sweet taste in the overall diet is largely due to the presence of caloric sugars and there are global efforts to reduce sugars intake to aid in weight management. Sugar-sweetened beverages (SSB) represent the largest contributor of caloric sugars in the diet (18, 303) and have been associated with poor diet quality, higher total energy intake and obesity (304, 305). Substitution by non-nutritive sweeteners (NNS) may be an effective strategy, theoretically by reducing total energy intake, while preserving food and beverage palatability (5). There is evidence suggesting that consuming NNS in place of sugar may reduce energy intake, and thus body weight (52, 306). Conversely, NNS have been also suggested to undermine weight management due to the metabolic mismatch between sweetness and lack of caloric content (6, 307) with compensation by overconsumption.

Stevia glycosides are plant based NNS. Stevia consumption does not affect blood glucose levels postprandially (51) or gut peptide hormones in humans (124), similar to the other NNS (134, 308). In regard to appetite and food intake following stevia consumption, currently there are only two studies, each with contradictory findings (48, 51). One reports significantly lower energy intake on replacement of sucrose with stevia (48), the other shows no difference in total energy intake (51). Both studies have methodological limitations, and better designed research is required to elucidate the effects of real-life dosage of pure stevia-sweetened beverages consumption on appetite and food intake.

Control of food intake involves homeostatic, hedonic and cognitive mechanisms (155). Given that oral sweet taste precedes nutrient sensing in the gut, it is possible that non-homeostatic mechanisms play a greater role in short-term appetite regulation compared to homeostatic mechanisms when consuming NNS-sweetened beverages that supply sweetness without any of the metabolic effects induced by gut-sensing and also post-absorption. Neural systems of food reward interact with homeostatic signals providing a mechanism by which the internal metabolic state influences food attractiveness and attention to food stimuli (155, 309, 310). Attentional bias (AB) to food cues, the tendency to focus visual attention to salient (food) over neutral information, is heightened in the fasted state and reduced in the fed state (311). Since sweet taste is a strong hedonic signal and a strong predictor of energy content, it is important to investigate whether sweet taste attenuates AB to food cues beyond changes in metabolic state.

The aim of this study was to examine subjective appetite and food intake following the consumption of beverages supplying sweetness with calories (glucose, sucrose), sweetness without calories (stevia), no sweet taste with calories (maltodextrin), or no sweet taste and no calories (water). In addition, we measured blood glucose levels as an objective measure of active (glucose, sucrose, maltodextrin) versus inactive (stevia, water) metabolic

state and AB to food cues following the consumption of the beverages, in an attempt to dissect any effects of calorie content and sweet taste on food-related attention. We hypothesised that the consumption of the energy-containing beverages would increase blood glucose levels post-consumption, suppress appetite ratings and food intake in the *ad libitum* meal (but not total energy intake) and attenuate AB to food cues compared to non-energy containing beverages (water, stevia).

3.3 Methods

3.3.1 Participants

Twenty healthy participants were recruited from the University of Manchester and the Manchester area for this study through advertisements placed online and around campus from April to June 2018. The study inclusion criteria included healthy males and females aged between 18-40 years with normal body mass index (BMI) (18.5-24.9 kg/m^2), restrained eating score ≤3 assessed via the Dutch Eating Behavior Questionnaire (DEBQ) and consuming breakfast ≥5 times per week. The exclusion criteria included being diagnosed with a major chronic disease, having food intolerances or allergies, weight change more than 5 kg in the last 12 months, currently dieting, experiencing anxiety or depression, and habitual consumption of any NNS (>1 can of diet beverage or >1 table packet of sweeteners per week), all based on participants' self-report.

The study was approved by the University of Manchester Research Ethics Committee and all subjects signed informed consent prior to participation. Participants were compensated for participation. The trial is registered at clinicaltrials.gov under registration NCT03711084.

Sample size estimation was based on expected differences in attentional bias to food cues between the consumption of caloric and non-caloric beverages. A previous study from our group (311) conducted to validate the efficacy of a visual-dot probe task (VPT) to assess AB to food cues in response to changes in metabolic state (fasted – fed) showed a mean difference of 18.48 ms on attentional bias between fasted and fed states and a pooled SD of 25.6 ms (effect size of the difference Cohen's d = 0.72, transforming this to effect size f = 0.361). G*Power 3.1.9.2 was used to estimate sample size. For *a priori* power analysis using effect size f = 0.36, error probability α = 0.05, 95% power, 1 group of participants and 5 measurements per participant, the total sample size was calculated 16. We recruited 20 participants to allow for participant drop out.

3.3.2 Experimental procedure

This was a randomised controlled double-blind crossover study with five treatments. Both participants and the researchers conducting the study were blinded to the treatment allocation throughout the study and during data analyses. Randomisation was conducted

by an independent person using an online tool (www.random.org) and by generating a random and counterbalanced treatment order per participant that was then followed by the researcher who was conducting the testing sessions. The five different study beverages were as follows: 330 mL water (control), 330 mL water containing 40 g glucose, 330 mL water containing 240 ppm, stevia, (Truvia®- Rebaudioside A- 95%) to match glucose sweetness, 330 mL water containing 40 g sucrose (Crystalline sucrose - commercially available) to provide a sweeter taste and to match the caloric content of 40 g glucose, and 330 mL water containing 40 g maltodextrin to match the caloric content of sucrose and glucose but with no sweet taste. Beverages at the final chosen concentrations were prepared independently of the study team and served in coded and sealed transparent bottles. There was no difference in appearance between the beverages. A sealed envelope with the information linking the beverage codes to their content was kept by a beverage manufacturer employee until after the data analysis was completed.

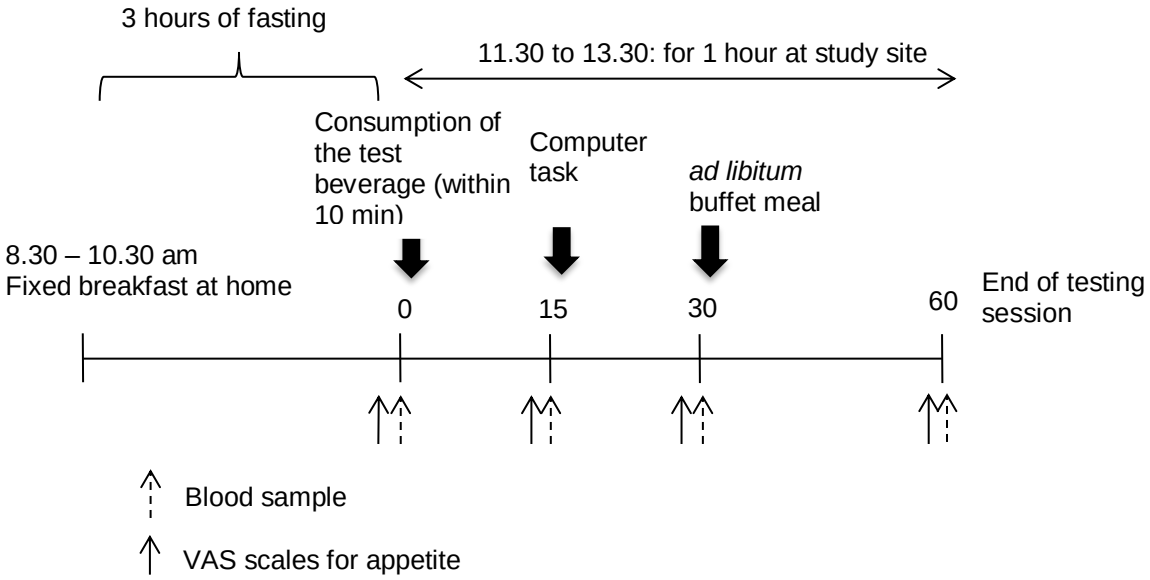
All procedures were conducted at the Neuroscience and Psychiatry Unit of the University of Manchester. Participants visited the centre for a total of 6 visits including the first screening visit, which was conducted after overnight fast where anthropometric measurements, blood pressure (OMRON M2 Basic), fasting blood glucose, and medical history data were collected. Anthropometric measurements included body weight, determined by a digital scale in light clothes without shoes, height measured with a portable stadiometer (SECA 213 Portable Height Measure, Hamburg, Germany), waist and hip circumference (SECA 201 Ergonomic Circumference Measuring Tape, Hamburg, Germany).

During the next five visits, participants received one of the test beverages. A 5-day washout period was required between test days. Participants were asked to continue their usual diet and physical activity for the duration of the study. All study sessions were conducted between May and September 2018. The day before each visit, participants were asked to maintain a consistent physical activity level and to consume an evening meal of similar composition and quantity. The next morning they were instructed to consume a breakfast of their preference and maintain the same breakfast prior to each session. Participants were then asked to fast for 3-4 hours, with water being allowed up to 1h before the testing session. Upon arrival participants were asked to fill in baseline appetite measures (time 0) and a blood glucose test was performed. Following this the study beverage was served and they were required to consume the entire contents within 10 min. At time 15 min participants performed a computer-based VPT that assesses AB to food stimuli outlined below. At 30 min they were given *ad libitum* lunch and were instructed to consume as much as they liked until they felt comfortably full. Blood samples and appetite measures were measured at 0, 15, 30 and 60 min after the consumption of the beverages. Participants were allowed to

leave after the last measurement (60 min). A flow chart of the study procedures can be seen in **Figure 3.1**.

The primary outcomes of this trial were (total) energy intake and appetite ratings, blood glucose levels and AB to food cues following the consumption of the study beverages. Secondary outcomes included the hedonic ratings of the beverages and the meal. All outcomes are described in detail below.

Figure 3.1 Flow chart of the procedures on a study day.



3.3.3 Blood glucose measurement

Finger prick blood samples were collected for blood glucose measurement. Blood glucose was measured immediately using the Hemocue Glucose 201 + Analyser (HemoCue, Angelholm, Sweden) at time 0 (before consumption), 15, 30 and 60 min after the consumption of the beverage.

3.3.4 Appetite, hedonic and sensory measures

Participants were asked to rate their subjective feeling of appetite on a 100 mm VAS anchored with 'not at all' (0 mm) and 'extremely' (100 mm) at time 0, 15, 30 and 60 min after the beverage. Appetite questions included hunger, fullness, desire to eat and prospective consumption. Participants were asked to drink a sip of the beverage and rate each beverage's attributes once, such as pleasantness, bitterness, sweetness, familiarity and

overall liking of the flavour. Following the meal participants were also asked to rate some attributes regarding the meal, such as pleasantness, sweetness, saltiness and familiarity.

3.3.5 Energy intake

Thirty minutes after the ingestion of the study beverage, participants were given an *ad libitum* meal (lunch buffet) and were allowed to consume as much as they wish until they felt comfortably full. The buffet consisted of 2 cheese and 2 ham sandwiches cut into small triangular pieces, fruits (100 g of grapes and 100 g of sliced bananas), 100 g plain fat free yoghurt , 5 Maryland chocolate chip cookies (Burton's Biscuit Co., Birmingham, UK) and 50 g Walkers ready salted crisps (Walkers Snack Foods Ltd, Leicester, UK). The sandwiches were made of Tesco wholemeal medium bread (Tesco Stores Ltd., Welwyn Garden City, UK), 10 g of spread (Flora Light Spread, Flora UK and Ireland, London, UK), medium cheddar slices (Tesco Stores Ltd., Welwyn Garden City, UK) or ham slices (Eastman's Cooked Ham slices, Tesco Stores Ltd., Welwyn Garden City, UK). The meal was exactly the same each time and glass of water was served with the meals.

3.3.6 Attentional bias to food cues

Food-related AB was assessed via a computer-based VPT as previously described (311). The pictures were selected after a preliminary informal study conducted among students and staff of the University of Manchester, using the standardized set of food images from Full4Health Image Collection (291). Twenty food-related pictures were paired with 20 stationary-related pictures. The pictures and dot probe locations (left or right) were counterbalanced. In each trial, response latencies to respond to the dot probe were recorded. Psychopy software (version 1.84.1) was used to programme, present the VPT and collect the data (response latencies to keyboard presses) (293).

3.3.7 Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics Version 23. Descriptive statistics were used for variables such as age, weight, height, BMI and questionnaire variables and are presented as mean ± SD. The rest of the data are presented as means ± SEs. Repeated measures ANOVAs were conducted to examine the effect of the beverage consumption on study outcomes over time and across conditions. Firstly, we performed two-way repeated measures ANOVA for each appetite rating, and blood glucose with time (0, 15, 30, 60 min) and beverage type (water, stevia, glucose, maltodextrin, sucrose) as within-subjects variables. Additionally, for each time point separately, we conducted one-way repeated measures ANOVAs with beverage type as within-subjects variable and where significant main effects and/or interactions were found, we followed up with post-hoc Bonferroni analysis. One-way repeated measures ANOVAs with beverage type as within-subjects variable were also conducted for the area under the curve (AUC) for appetite and

blood glucose, as well as for the analysis of energy intake, and hedonic ratings and followed up with Bonferroni-adjusted post hoc tests. In cases where sphericity was violated (Mauchly's test $p < 0.05$), the Greenhouse-Geisser corrected *P* value is reported. Only the sweetness and bitterness ratings of the beverages were not normally distributed, thus the equivalent non-parametric analyses have been conducted and reported only for those two measures (Friedman's ANOVA followed by Bonferroni-adjusted Wilcoxon's signed rank tests.

Reaction times to VPT were handled in R, so that all incorrect trials, as well as trials slower than 1000ms or faster than 100ms were discarded. Reaction times to congruent and incongruent trials and AB scores were analysed using repeated measures ANOVA with beverage type as within-subjects variable. An AB score was calculated from the VPT reaction times as follows: mean latency to respond in incongruent trials minus mean latency to respond in congruent trials, with positive values indicating attention bias towards food-related pictures.

Statistical significance was determined at $p \leq 0.05$.

3.4 Results

3.4.1 Participant characteristics

Twenty participants completed all the 5 study sessions. A detailed participant flow chart can be found in **Figure 3.2**. The baseline characteristics of the participants are presented in **Table 3.1**. Regarding the restrained eating subscale of the Dutch Eating Behaviour Questionnaire the minimum value was 1.3 and the maximum value 3.0, regarding the emotional subscale the minimum value was 1.2 and the maximum 3.5, and regarding the external eating subscale the minimum value was 2.3 and the maximum 4.1.

The results of main effects and interactions for the main outcomes can be found in **Supplemental Table 3.1**.

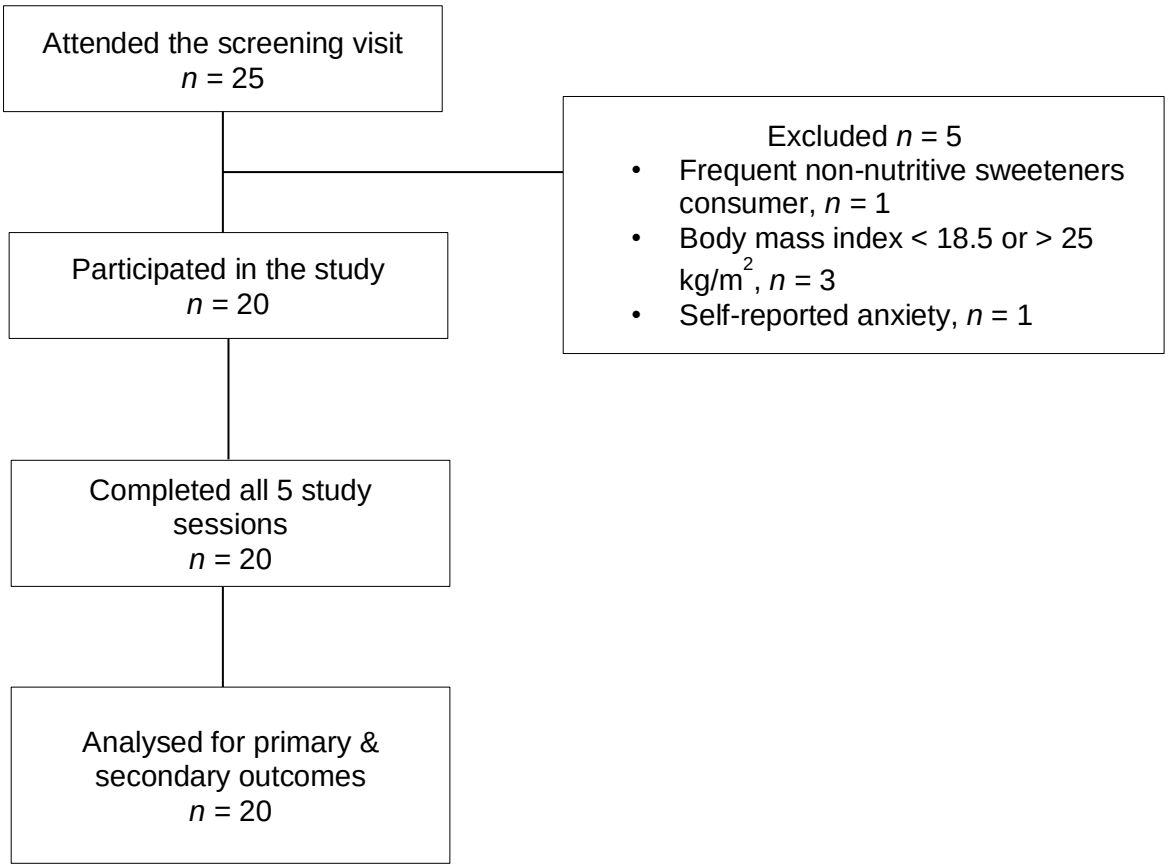
Table 3.1 Subjects' characteristics. [1]

	(*n* = 20)
Age, y	26.6 ± 4.6
Females, n (%)	11 (55)
Weight, kg	62.6 ± 7.0
Height, cm	170 ± 10
BMI, kg/m^2	21.8 ± 1.5
Waist circumference, cm	75.4 ± 5.3
Hip circumference, cm	98.7 ± 4.4
Fasting blood glucose [2], mmol/L	4.9 ± 0.5
Systolic BP, mmHg	115.4 ± 9.8
Diastolic BP, mmHg	65.6 ± 7.2
Pulses, bpm	66.7 ± 10.6
DEBQ-restrained	2.1 ± 0.5
DEBQ-emotional	2.3 ± 0.6
DEBQ-external	3.3 ± 0.5

[1] Values are means ± SDs; BMI, body mass index.

[2] Mean fasting blood glucose concentration measured at the screening visit after overnight fast, (*n* = 20). BP, blood pressure; DEBQ, Dutch Eating Behavior Questionnaire.

Figure 3.2 Participant flow diagram.

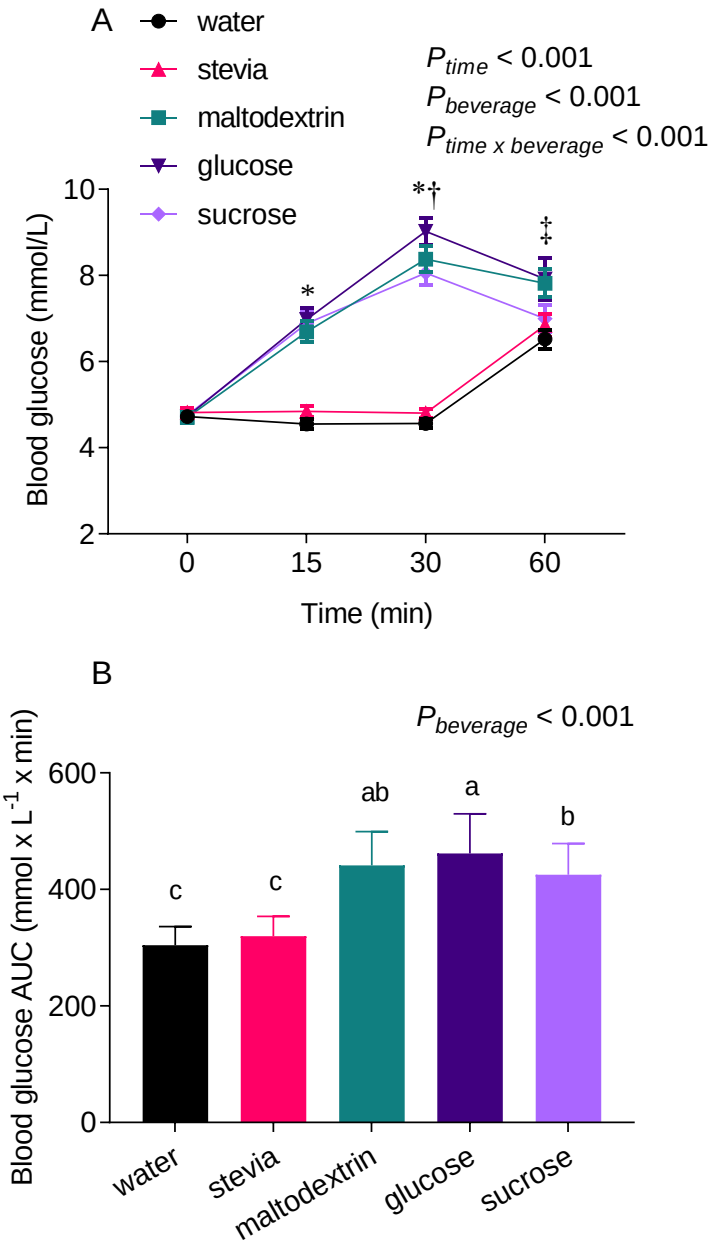


3.4.2 Blood glucose levels

Figure 3.3 shows the blood glucose levels following the consumption of the test beverages. At time 0, there was no significant difference on blood glucose levels between the beverages ($F(4, 76) = 0.25$, $p = 0.909$, $\eta^2p = 0.013$), however there were differences at 15 min ($F(2, 43) = 60.00$, $p < 0.001$, $\eta^2p = 0.760$, Greenhouse-Geisser), 30 min ($F(3, 50) = 106.26$, $p < 0.001$, $\eta^2p = 0.848$, Greenhouse-Geisser) and 60 min ($F(3, 50) = 5.42$, $p = 0.004$, $\eta^2p = 0.222$, Greenhouse-Geisser). At time points 15 and 30 min, blood glucose was significantly higher after the maltodextrin, glucose and sucrose beverages compared to both water and stevia beverages (all $p < 0.001$), and moreover glucose showed an even greater value compared to sucrose at 30 min ($p = 0.022$). At time 60 min (30 min after the consumption of the *ad libitum* meal) blood glucose levels were significantly higher for the maltodextrin condition compared to water ($p = 0.036$) and compared to sucrose ($p = 0.013$), whereas no other difference was observed.

Water and stevia consumption exerted lower AUC for glycaemia compared to all other treatments (all $p < 0.001$), and glucose AUC was also significantly higher compared to that of sucrose ($p = 0.003$).

Figure 3.3 Blood glucose response (A) and AUC for glycaemia (B) following ingestion of the water, stevia, maltodextrin, glucose and sucrose beverages in healthy adults. Values represent means ± SEs; n = 20. (A) * $p < 0.001$ maltodextrin, glucose and sucrose compared to water and stevia at that time point; † $p < 0.05$ glucose versus sucrose; ‡ $p < 0.05$ maltodextrin compared to water and sucrose. (B) Bars without a common letter differ, $p < 0.05$. AUC, area under the curve.



3.4.3 Appetite, hedonic and sensory ratings

Ratings of hunger, fullness, desire to eat and prospective consumption are presented in **Figure 3.4.** Significant main effects of time, and beverage type as well as significant time-by-beverage type interactions were observed for hunger, fullness, desire to eat and prospective consumption ratings (Supplemental Table 3.1). At time 0 and time 60 there were no significant differences at any appetite ratings between the treatments (baseline ratings did not differ across conditions) (hunger time 0: $p = 0.060$, hunger time 60: $p = 0.425$, fullness time 0: $p = 0.429$, fullness time 60: $p = 0.334$, desire to eat time 0: $p = 0.081$, desire to eat time 60: $p = 0.809$, prospective consumption time 0: $p = 0.268$, prospective consumption time 60: $p = 0.578$). At time 15, a significant main effect of beverage type was observed for all appetite ratings ($p < 0.05$) and similarly at time 30 ($p \leq 0.001$). Hunger ratings 15 min after consumption of water were significantly higher compared to glucose ($p = 0.004$) and sucrose ($p = 0.003$) and at 30 min water ratings differed significantly from all other beverages ($p < 0.05$), meaning that the consumption of all the sweet, caloric, and sweet/caloric beverages reduced hunger ratings at 30 min post-consumption compared to water. Regarding fullness ratings at 15 min after the consumption of the beverages, water exerted significantly lower values compared to glucose ($p = 0.02$) and sucrose ($p = 0.006$), and at 30 min post-intake water ratings differed significantly compared to maltodextrin ($p = 0.045$), glucose ($p = 0.033$) and sucrose ($p = 0.015$). Desire to eat ratings at 15 min after the consumption of the water, were significantly higher only compared to the sucrose condition ($p = 0.029$), whereas at 30 min desire to eat ratings were found significantly higher following the consumption of water compared to all other beverages (all $p < 0.05$). Prospective consumption ratings at 15 min after the consumption of water were significantly higher compared to glucose and sucrose condition ($p < 0.05$) ($p = 0.054$ compared to stevia), and at 30 min water ratings differed significantly compared to maltodextrin, glucose and sucrose (all $p < 0.05$).

The respective AUCs for hunger, fullness, desire to eat and prospective consumption were also calculated over the 60 min period (beverage and lunch) as well as over the 30 min period (beverage only) and are summarized in **Table 3.2**. The AUCs (both over 60 min period and 30 min period) for hunger, desire to eat and prospective consumption after the consumption of the sucrose and the glucose beverage were significantly lower compared to water (all $p < 0.05$) and similarly the fullness AUCs were significantly higher (only the fullness AUC 0-60 min for glucose did not differ significantly to water $p = 0.07$). The AUC over the 30 or 60 min postprandial period for all appetite sensations after the consumption of the maltodextrin beverage did not differ compared to the respective AUCs for water. The consumption of the stevia beverage resulted in significantly lower AUC over the 30 min period for hunger ($p = 0.016$), prospective consumption ($p = 0.012$), and higher fullness

AUC (p = 0.044) compared to water control, as well as significantly lower AUC (0-60 min) for desire to eat ratings (p = 0.034). No difference was found in AUC (0-60 min or 0-30 min) between the stevia condition and the glucose, sucrose or maltodextrin conditions.

Results of the hedonic and sensory ratings of the beverages can be found in Table 3.2. All test beverages were rated to have equal pleasantness ($F(3, 48) = 1.06$, p = 0.39, η^2p = 0.053, Greenhouse-Geisser), flavour liking ($F(3, 48) = 1.96$, p = 0.11, η^2p = 0.093, Greenhouse-Geisser) and familiarity ($F(3, 48) = 0.48$, p = 0.75, η^2p = 0.025, Greenhouse-Geisser). However, beverages differed in perceived sweetness (p < 0.001, Friedman's ANOVA) and bitterness (p < 0.001, Friedman's ANOVA). In particular, sweetness ratings followed the design of our study, since water and maltodextrin were rated to have equal sweetness (p = 0.100), glucose and stevia beverages were significantly sweeter than water (both p < 0.001) and maltodextrin (p = 0.010 vs glucose and p < 0.001 vs stevia) and sucrose was rated significantly sweeter than water (p < 0.001), stevia (p = 0.02), and maltodextrin (p < 0.001) but not glucose (p = 0.09). Bitterness ratings did not differ between water and maltodextrin (p = 0.113) and the stevia (p = 0.76) beverages. Participants were also required to rate the pleasantness, sweetness, saltiness and familiarity of the lunch meal at the end of consumption and results are summarised in Table 3.2, however no significant differences were found.

Figure 3.4 Hunger (A), fullness (B), desire to eat (C) and prospective consumption (D) ratings over time following ingestion of the water, stevia, maltodextrin, glucose and sucrose beverages in healthy adults.

Values are mean ± SEs, $n = 20$. * $p < 0.05$ water compared to glucose and sucrose at that time point; † $p < 0.05$ water compared to all other beverages; ‡ $p < 0.05$ water compared to maltodextrin, glucose and sucrose; # $p < 0.05$ water compared to sucrose.

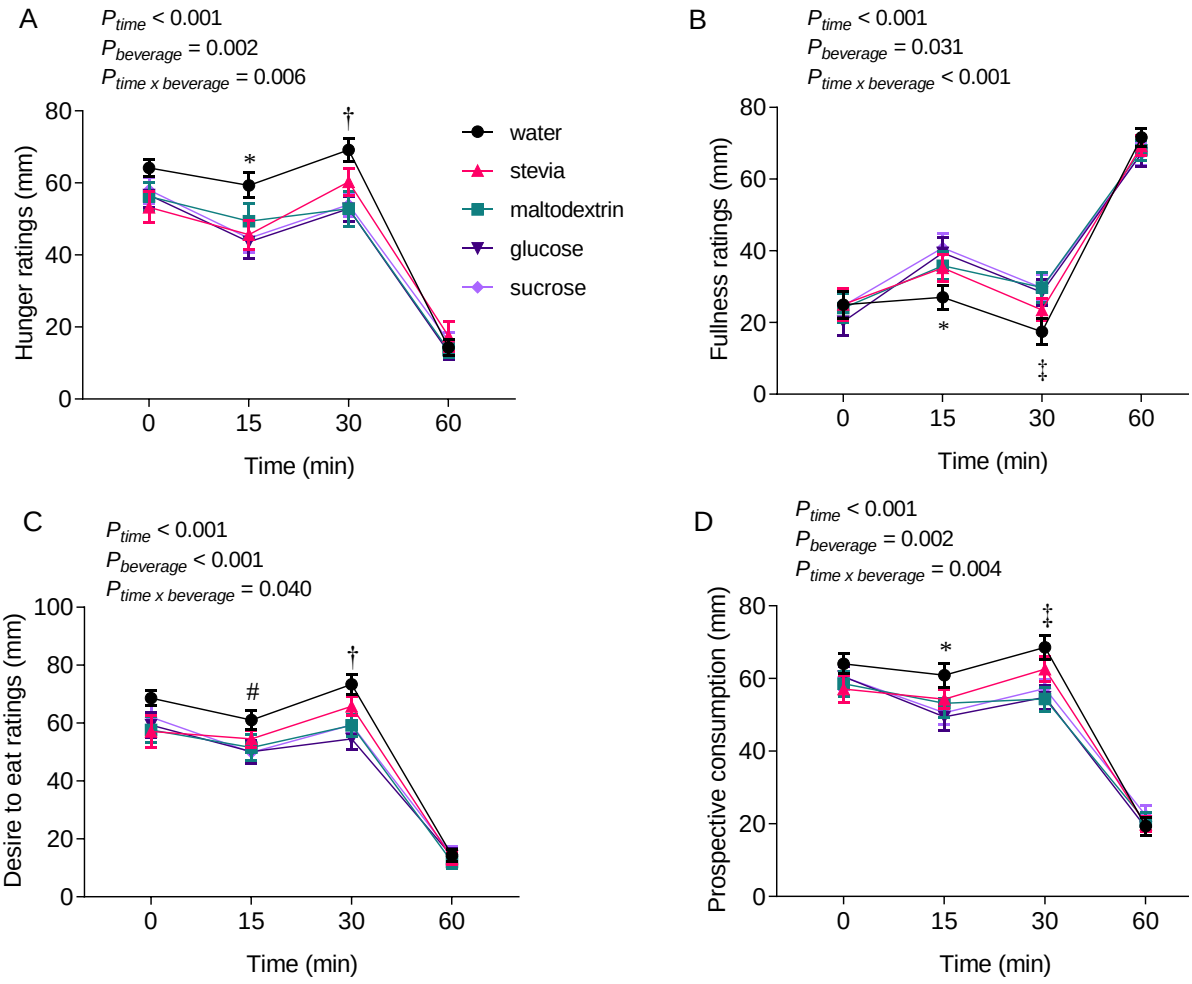


Table 3.2 Hedonic, sensory and appetite ratings following beverage ingestion in healthy adults.

	Water	Stevia	Maltodextrin	Glucose	Sucrose	*p*
Beverage attributes						
Pleasantness	42.9 ± 3.31	44.6 ± 4.09	39.3 ± 4.16	48.0 ± 5.04	48.9 ± 5.78	0.369[1]
Bitterness	31.8 ± 4.34[a]	21.2 ± 3.55[ab]	23.9 ± 4.32[ab]	10.5 ± 2.47[bc]	6.2 ± 1.59[c]	<0.001[2]
Sweetness	20.1 ± 4.16[c]	66.0 ± 2.35[b]	29.8 ± 4.88[c]	67.4 ± 4.97[ab]	81.0 ± 3.24[a]	<0.001[2]
Flavour liking	32.6 ± 3.40	45.6 ± 4.94	36.2 ± 4.60	45.5 ± 5.70	43.3 ± 5.84	0.143[1]
Familiarity	43.8 ± 6.81	34.2 ± 5.22	38.1 ± 6.98	41.5 ± 6.20	37.6 ± 5.78	0.663[1]
Meal attributes						
Pleasantness	66.2 ± 2.45	63.0 ± 2.02	62.7 ± 1.80	60.5 ± 2.14	62.6 ± 1.76	0.077
Sweetness	40.8 ± 4.39	39.3 ± 3.19	44.0 ± 4.02	38.3 ± 3.74	38.9 ± 3.84	0.539
Saltiness	47.6 ± 4.54	42.0 ± 3.94	46.9 ± 3.70	44.8 ± 3.68	43.7 ± 4.39	0.515
Familiarity	76.5 ± 4.79	82.8 ± 2.54	84.8 ± 2.88	82.4 ± 3.17	81.8 ± 2.99	0.193[1]
Appetite						
Hunger AUC 0-60 min (mm x min)	3140 ± 132[a]	2700 ± 161[ab]	2550 ± 214[ab]	2460 ± 154[b]	2560 ± 151[b]	<0.001
Hunger AUC 0-30 min (mm x min)	1890 ± 83.1[a]	1540 ± 99.7[b]	1560 ± 132[ab]	1480 ± 107[b]	1510 ± 98.1[b]	<0.001
Fullness AUC 0-60 min (mm x min)	2060 ± 153[b]	2280 ± 123[ab]	2410 ± 162[ab]	2390 ± 161[ab]	2470 ± 159[a]	0.007[1]
Fullness AUC 0-30 min (mm x min)	723 ± 98.0[b]	892 ± 89.1[a]	941 ± 110[ab]	960 ± 105[a]	1020 ± 99.6[a]	0.001
Desire to eat AUC 0-60 min (mm x min)	3300 ± 131[a]	2930 ± 129[b]	2720 ± 177[ab]	2630 ± 141[b]	2770 ± 131[b]	<0.001
Desire to eat AUC 0-30 min (mm x min)	1980 ± 85.8[a]	1740 ± 85.7[ab]	1650 ± 122[ab]	1610 ± 98.4[b]	1660 ± 93.9[b]	<0.001
Prospective consumption AUC 0-60 min (mm x min)	3230 ± 143[a]	2950 ± 131[ab]	2770 ± 144[ab]	2710 ± 134[b]	2830 ± 123[b]	<0.001
Prospective consumption AUC 0-30 min (mm x min)	1910 ± 89.4[a]	1710 ± 82.8[b]	1640 ± 102[ab]	1610 ± 87.1[b]	1640 ± 85.2[b]	0.005[1]

AUC 0-60 min, area under the curve over the 60 min period; AUC 0-30 min, area under the curve over the 30 min period (beverage only). Values are mean ± SEs, *n* = 20. Labelled means in a row without a common letter differ, $p < 0.05$. [1] Greenhouse-Geisser corrected *p* value reported. [2] Not normally distributed data, analysed with non-parametric tests.

3.4.4 Energy intake

Figure 3.5 shows the energy consumed at *ad libitum* lunch as well as the breakdown of energy consumed from the beverage and the lunch meal. A significant main effect of beverage type on energy consumed at the *ad libitum* lunch was found ($p < 0.001$). The *ad libitum* lunch energy intake after the water beverage (mean ± SEs, 832 ± 44.2 kcal) was significantly higher compared to all other beverages (stevia: 727 ± 53.4 kcal, maltodextrin: 682 ± 43.5 kcal, glucose: 665 ± 55.0 kcal, sucrose: 677 ± 44.3 kcal, for all comparisons $p < 0.05$). *Ad libitum* lunch intake in the stevia condition did not differ compared to all the caloric-beverage conditions (all $p = 1.00$, Figure 3.5 A). When we analysed the total energy intake from both the beverage (12% of either glucose, sucrose or maltodextrin provided 160 kcal, water and stevia beverages contained 0 kcal) and the lunch meal (Figure 3.5 B), there was still a significant main effect of beverage type ($p = 0.038$), and it was shown that participants consumed significantly lower amount of energy following the stevia beverage compared to water (Δ -105 kcal, 95% CI: -193, -16.5), $p = 0.013$). However there was no difference between the water condition and the maltodextrin (Δ 10.1 kcal, 95% CI: -93.8, 114), glucose (Δ -6.75 kcal, 95% CI: -125, 112), sucrose conditions (Δ 4.98 kcal, 95% CI: -103, 113) (all comparisons $p = 1.000$). No significant difference was observed on total energy intake between stevia condition and maltodextrin (Δ 115 kcal, 95% CI: -19.9, 250, $p = 0.140$), glucose (Δ 98.2, 95% CI: -39.9, 236, $p = 0.360$), and sucrose (Δ 110 kcal, 95% CI: -27.2, 247, $p = 0.190$).

3.4.5 Attentional bias to food cues

Reaction times in the VPT are presented in **Table 3.3**. Results showed that the main effect of congruency was not statistically significant ($p = 0.064$); participants responded faster to the congruent (412 ± 7.51 ms) compared to incongruent trials (419 ± 8.16 ms). No significant main effect of beverage type or interaction congruency-by-beverage type was observed. Additionally, no significant main effect of beverage type on attentional bias to food cues was found.

Figure 3.5 Energy intake consumed at the ad libitum lunch (A) and cumulative intake (test beverage and ad libitum lunch (B)) following ingestion of the water, stevia, maltodextrin, glucose and sucrose beverages in healthy adults.

Values represent means ± SEs; n = 20. Bars without a common letter differ, $p < 0.05$.

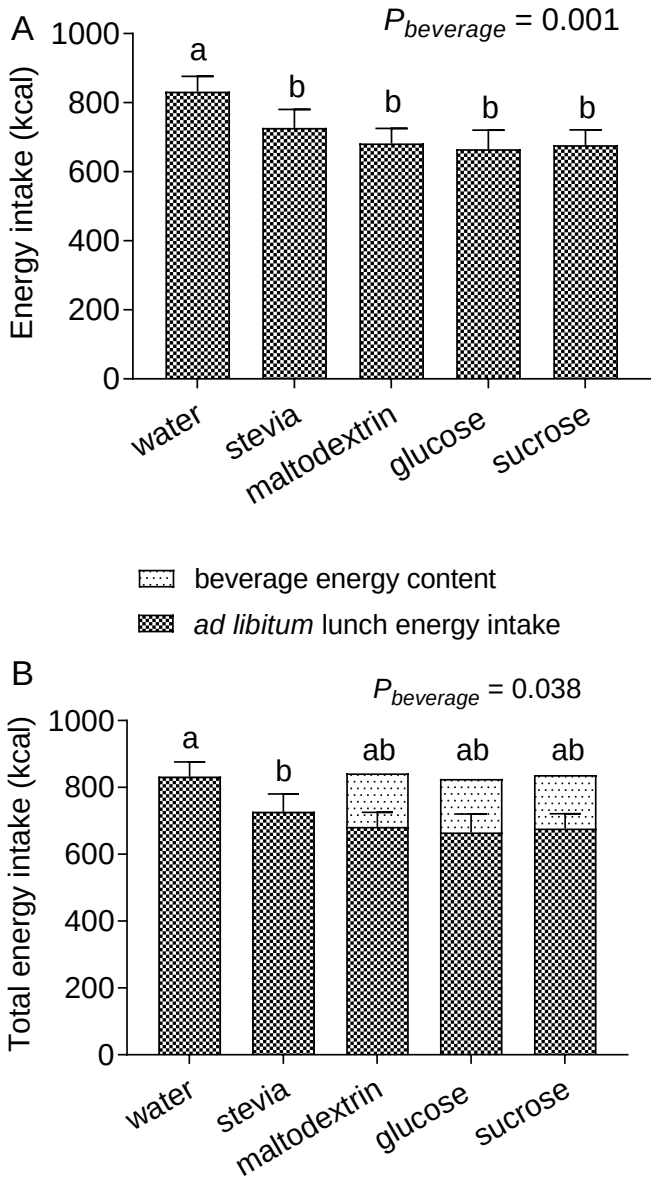


Table 3.3 Reaction times to visual-dot probe task following beverage ingestion in healthy adults.

	Water	Stevia	Maltodextrin	Glucose	Sucrose	*p*
Congruent trials (ms)	422 ± 8.76	405 ± 9.17	412 ± 9.44	414 ± 11.46	406 ± 7.71	0.310
Incongruent trials (ms)	423 ± 8.39	411 ± 8.55	427 ± 12.88	422 ± 12.61	410 ± 7.66	0.294
Attentional bias (ms)	1.08 ± 2.87	5.79 ± 5.47	14.7 ± 6.46	8.89 ± 4.08	3.66 ± 5.05	0.140

Values are mean ± SEs, $n = 20$. p values correspond to the repeated measures ANOVA with beverage type as within-subjects independent variable.

3.5 Discussion

This study examined the effects of consuming a stevia-sweetened beverage compared to beverages containing caloric sweeteners with or without a sweet taste and water on appetite, energy intake, blood glucose responses and their relation to changes in food-related attention.

The main finding was a beneficial effect of consuming a stevia beverage on reducing short term appetite and total energy intake. Consumption of the sweet/non-caloric (stevia), sweet/caloric (glucose), sweeter/caloric (sucrose) and non-sweet/caloric (maltodextrin) beverages all led to significantly lower energy intake during the *ad libitum* lunch compared to consuming water. However, the total energy intake (beverage and *ad libitum* lunch) was significantly lower only after the stevia compared to water beverage condition, with no differences between the water and caloric beverages. No difference was observed in AB to food cues across conditions, suggesting that the behavioral effect on appetite and food intake is probably driven by fundamental chemosensory and physiological signals rather than top-down psychological signals.

Findings derived from the subjective appetite ratings indicated that both sweet taste and energy play a role in appetite sensations. This was illustrated by the strongest effect of sucrose on reducing hunger, desire to eat, prospective consumption and increasing fullness ratings, followed by the glucose beverage which contains the same amount of energy but is less sweet than sucrose, and the weaker and not so consistent effect of both maltodextrin (no sweet taste, calories) and stevia (sweet taste, no calories). Results from previous studies on stevia and appetite ratings appear mixed, in one study the consumption of the stevia-sweetened beverage led to similar appetite ratings compared to sucrose in one study (48), whereas in another study consumption of a stevia-sweetened beverage one hour prior to a meal resulted in significantly lower fullness, and increased hunger, and desire to eat sensations compared to a sucrose beverage (51). Our results showed that the consumption of a stevia-sweetened beverage resulted in an increase in fullness, and decrease in hunger and desire to eat ratings to an amount that is intermediate between caloric sweeteners and water. A similar effect has been shown in a previous study where the oral cavity was bypassed (using intragastric administration and other types of NNS), suggesting that gut sweet-sensing may also be involved in the generation of satiety signals (125).

The present study showed a significant reduction in total energy intake when consuming a stevia-sweetened preload prior to a meal compared to consuming water, suggesting that the exposure to sweetness itself can modulate later energy intake. Opposing to our results, results from short-term studies that were recently reviewed (306) have shown that consumption of NNS-sweetened beverages versus water leads to minimal changes in

energy intake in adults. Fantino *et al.* (49) also reported no difference in total energy intake, macronutrient intake or the selection of sweet foods when consuming water or NNS-sweetened beverages. However, in sustained studies there is some evidence that consumption of NNS-sweetened beverages reduces body weight relative to water (306). The mechanisms behind this observation may involve a sensory-specific satiety effect, suggesting that the consumption of NNS-sweetened beverages may satisfy the desire for sweet tasting foods thus decreasing their consumption later in the day (312, 313). In addition, in our study we recruited participants who were non-habitual consumers of NNS. It has been previously documented that sweet taste responses on energy intake and short-term appetite may differ between habitual and non-habitual consumers of NNS, with habitual consumers expected to show weakened responses to sweet taste as a result of adaptation to sweet taste without calories (314).

Results on food intake showed a net average saving of 108 kcal when participants consumed a stevia sweetened preload prior to lunch compared to the energy-containing preloads (summation of preload and *ad libitum* lunch). Compensation was on average 33% for the stevia beverage, however complete compensation was found for the caloric preloads showing that when sweetness is paired with calories, compensation is complete, but sweetness without energy reduces short term intake. Two other studies have examined the effect of stevia on food intake (48, 51). Anton *et al.* (48) found that energy intake was significantly decreased over the day when two preload meals sweetened with stevia was consumed 20 min before an *ad libitum* lunch and dinner (309 kcal saved), and the difference was attributed to the reduction in sucrose content of the preloads. The energy compensation for the stevia condition was 24%. Tey *et al.* (51) examined the effects of a stevia beverage preload compared to a sucrose preload 1 h before an *ad libitum* meal. Although no difference was found in total energy intake (compensation was 73%) over the course of the day, at lunch time only partial compensation (22-32%) was observed. The energy saved from switching sucrose to NNS (including stevia) was fully compensated at the subsequent meals, a result which was based on participants' food diaries and not measured under laboratory settings.

The blood glucose measurements served as an objective measure of the physiological metabolic responses induced by consumption of the beverages. As expected, blood glucose response to the beverages was driven by the energy content and carbohydrate type, while sweetness *per se* played no role. It is well documented by a number of human studies (125, 127) as well as recent systematic reviews (133, 308) and one meta-analysis (134) that there is not a physiologically significant biological activity of NNS on glucose postprandial responses, at least in the short term and in healthy subjects. In the present study consumption of a stevia preload alone did not alter postprandial glycaemia, in line

with previous results (51). In addition, two studies have reported lower glucose responses when adding stevia in a meal compared to adding a caloric control (48, 280). Future studies should investigate the potential role of circulating peptides as potential mechanisms.

Consumption of the study beverages did not result in any significant differences in AB to food cues. In a previous study we showed that AB to food cues can be an indicator of metabolic state, with AB found to be high in the fasted state and significantly lower in the fed state (311). A lower AB following the consumption of the stevia beverage compared to water would have suggested attenuated salience of food cues, which could probably explain lower food intake. In the present study AB did not differ across all treatments and ranged at low ('fed') levels. This may indicate that either water was not an appropriate control for AB, or that the instruction to refrain from eating for 3 hours prior to the study may not have induced adequate levels of hunger to be reflected on AB to food cues. In relation to the first assumption, a recent study has showed that AB toward visual food stimuli can be attenuated even by chewing stimulation in healthy weight individuals (315), so it is possible that the gastric distension caused by the consumption of the water beverage may have served as a signal towards reducing incentive salience of food stimuli. However, this would be identical for all 5 conditions and water purely served as a control. Regarding the second assumption, mean baseline ratings of hunger were similar with those observed in previous study where participants were tested after overnight fast (311). Our results suggest that there is no association between AB and consummatory or appetitive behavior, supporting a conclusion by Field *et al.* (202) reporting that AB does not consistently predict or influence distal consummatory behavior.

The strengths of this study include its double-blind design and the use of the appropriate controls, water, maltodextrin, glucose and sucrose, which allowed comparisons of both calories and sweetness. In addition, giving the test meal at the time of the maximum metabolic response to the caloric sweeteners is optimal rather than earlier or later time points. The current study has a number of limitations. Firstly we included only one type of NNS, stevia, thus the results could only be representative for this specific sweetener. Differences in potential effects among NNS should be considered due to their different absorption, distribution, metabolism and excretion (27), as well as their differential effects on body weight (78). Additionally, we did not measure the total daily energy intake of the participants, so we cannot rule out the possibility of compensating for the saved calories later in the day.

In summary, our data provide considerable new experimental evidence that consuming a low calorie sweet preload containing stevia prior to a meal may exert beneficial effects on short-term appetite and energy intake compared with a water control in healthy lean adults who are infrequent consumers of NNS and in energy balance. There was no indication that

stevia consumption increases appetite, and attentional bias to food cues was not influenced by the type of beverage that was consumed. Longer-term studies are warranted to determine the prolonged effects of stevia-sweetened beverages on appetite and body weight regulation.

3.6 Acknowledgements

The authors would like to thank all the participants who took part in the study and Angela Bonnema for her contribution to the design of the study. N.S.S was supported by a CASE Biotechnology and Biological Sciences Research Council (BBSRC) Doctoral Training Partnership studentship.

Supplemental Table 3.1 Results of the main effects and interactions for the main outcomes.

	Time	Beverage	Time x Beverage
Blood glucose			
Blood glucose response	$F(2, 30) = 74.97$, $p<0.001$, $\eta^2p = 0.798$[a]	$F(2, 43) = 58.68$, $p<0.001$, $\eta^2p = 0.755$[a]	$F(6, 104) = 32.14$, $p<0.001$, $\eta^2p = 0.628$[a]
AUC blood glucose 0-60 min		$F(2, 40) = 74.85$, $p<0.001$, $\eta^2p = 0.798$[a]	
Appetite			
Hunger ratings	$F(2, 38) = 111.21$, $p<0.001$, $\eta^2p = 0.854$[a]	$F(4, 76) = 4.85$, $p=0.002$, $\eta^2p = 0.203$	$F(6, 117) = 3.16$, $p=0.006$, $\eta^2p = 0.143$
Fullness ratings	$F(2, 33) = 73.99$, $p<0.001$, $\eta^2p = 0.796$[a]	$F(4, 76) = 2.81$, $p=0.031$, $\eta^2p = 0.129$	$F(12, 228) = 3.86$, $p<0.001$, $\eta^2p = 0.169$
Desire to eat ratings	$F(2, 35) = 113.31$, $p<0.001$, $\eta^2p = 0.856$[a]	$F(4, 76) = 6.07$, $p<0.001$, $\eta^2p = 0.242$	$F(5, 100) = 2.39$, $p=0.040$, $\eta^2p = 0.112$[a]
Prospective consumption ratings	$F(2, 29) = 102.97$, $p<0.001$, $\eta^2p = 0.844$[a]	$F(4, 76) = 4.59$, $p=0.002$, $\eta^2p = 0.194$	$F(6, 120) = 3.28$, $p=0.004$, $\eta^2p = 0.147$
Energy intake			
Ad libitum lunch only		$F(3, 50) = 6.40$, $p = 0.001$, $\eta^2p = 0.252$[a]	
Ad libitum lunch plus beverage energy content		$F(3, 50) = 3.17$, $p = 0.038$, $\eta^2p = 0.143$[a]	
Dot-probe task	**Congruency**	**Beverage**	**Congruency x beverage**
Reaction times to congruent and incongruent trials	$F(1, 19) = 3.88$, $p = 0.064$, $\eta^2p = 0.170$	$F(4, 76) = 1.20$, $p = 0.320$, $\eta^2p = 0.059$	$F(4, 76) = 1.79$, $p = 0.140$, $\eta^2p = 0.086$

[a] Sphericity not assumed, Greenhouse Geisser reported.

CHAPTER 4

Effects of the Daily Consumption of Stevia on Glucose Homeostasis, Body Weight, and Energy Intake: A Randomised Open-Label 12-Week Trial in Healthy Adults

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Statement

NS conceived, designed and conducted the study, analysed the data and took responsibility for preparing and writing the manuscript. BC and AA contributed to data collection assisting with the medical procedures of the study, read and approved the final manuscript. JM conceived the study, contributed to the design of the study and provided intellectual input, supervised the project, reviewed and approved the manuscript. The manuscript has been published in *Nutrients*. The manuscript occupies pages 122-145 of this thesis.

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4.1 Abstract

Stevia is a non-nutritive sweetener, providing sweet taste with no calories. This randomised, controlled, open-label 2-parallel arm trial examined the effects of daily stevia consumption on glycaemia in healthy adults. Secondary endpoints included body weight (BW) and energy intake (EI). Healthy participants (*n* = 28; aged 25±5 y, body mass index 21.2±1.7 kg/m^2) were randomised into either the stevia group (*n* = 14)-required to consume a stevia extract daily- or to the control group (*n* = 14). At weeks 0 and 12, the glucose and insulin responses to an oral glucose tolerance test were measured; BW and EI were assessed at weeks 0, 6, and 12. There was no significant difference in the glucose or insulin responses. There was a significant main effect of group on BW change ($F(1,26) = 5.56$, $p = 0.026$), as the stevia group maintained their weight as opposed to the control group (mean weight change at week 12: −0.22 kg, 95%CI [−0.96, 0.51] stevia group, +0.89 kg, 95%CI [0.16, 1.63] control group). The energy intake was significantly decreased between week 0 and 12 in the stevia group ($p = 0.003$), however no change was found in the control group ($p = 0.973$). Although not placebo-controlled, these results suggest that daily stevia consumption does not affect glycaemia in healthy individuals, but could aid in weight maintenance and the moderation of EI.

Keywords: stevia; non-nutritive sweeteners; glycaemia; body weight; energy intake

4.2 Introduction

There is a general consensus that overconsumption of caloric sugars, mainly through the consumption of sugar-sweetened beverages, leads to a greater energy intake and a poor diet quality, further associated with weight gain and/or type 2 diabetes mellitus (T2DM) (67, 304, 316). Non-nutritive sweeteners (NNS) represent a broad class of sweet compounds that are used in a variety of beverages and food products, providing a sweet taste yet contributing little or no energy to the diet. However, significant controversy exists regarding the effects of NNS consumption on body weight and metabolic health outcomes (71, 306, 317, 318), with effects ranging from harmful to neutral to beneficial. The discrepancy between study outcomes has been attributed to methodological limitations (73), while significant issues as to how the evidence base on NNS is generated, interpreted, and communicated by the expert community also exist (76). The need for more long-term randomised trials on the effects of NNS consumption on metabolic health outcomes and body weight is emerging.

A number of studies have explored the potential of NNS use to influence acute metabolic responses and especially the blood glucose response. The results of those studies have been systematically reviewed, overall showing a neutral effect of NNS on glucose control (133, 134, 308). One hypothesis tested was based on the premise that the human body associates sensory cues with metabolic responses, so the activation of sweet taste receptors in the oral and extra-oral tissues might alter glucose metabolism through promoting insulin and/or incretin release. Although experimental data using human cell lines and animal models consistently show sweet taste receptor activation leading to increased insulin and incretin release *in vitro* (118, 319), the results from human trials have not confirmed this (95). One factor may be the very high doses of NNS used in non-human studies. In addition, following *in vitro* demonstrations that treatment with NNS could enhance glucose uptake via the upregulation of transporters (120), it was hypothesised that the concomitant consumption of NNS and carbohydrates would result in higher glucose response, but again human trials have failed to show such an acute effect (131, 320). Overall, NNS consumed as single agents or concomitantly with carbohydrates do not seem to affect acute glucose response, apart from a few reports showing small effects on either the glucose response (131) or glucagon-like peptide-1 (GLP-1) secretion (38). However, it remains unclear as to whether repeated exposure to NNS would have any effects on glucose homeostasis in the long term.

Stevia (steviol glycosides) has gained great popularity as a natural NNS alternative to caloric sugars, nevertheless it remains the least studied in terms of its effects on human metabolic responses. There is some evidence suggesting that stevia might assist with

glucose regulation. Gregersen *et al.* showed that the concomitant consumption of stevioside with a full meal reduced the postprandial incremental area under the curve (iAUC) of blood glucose compared to control (maize starch) in individuals with T2DM (280). In healthy adults, a reduction in postprandial iAUC for glycaemia and insulinaemia was also demonstrated when a stevia-sweetened beverage was consumed along with a meal, compared to consuming a sugar-sweetened beverage (48). The long-term consumption of rebaudioside A (one type of steviol glycoside) did not alter the fasting blood glucose in subjects with T2DM (272) or with glucose intolerance (321). To the best of our knowledge, currently there is no available study investigating whether there is a change in glucose response to an oral glucose tolerance test (OGTT) after the daily consumption of stevia in healthy adults.

Despite providing minimal energy, NNS have paradoxically been suggested to be involved in weight gain and T2DM risk in cohort studies. However, meta-analyses of randomised controlled trials (RCTs) indicate that body weight is slightly but significantly reduced with NNS use (71, 306). In line with these conclusions, we have previously shown a beneficial effect of consuming a stevia-sweetened beverage prior to lunch on short-term appetite and total energy intake (322); whether this effect is sustained with prolonged use is yet to be examined. Only a few RCTs have investigated the long-term effects of the consumption of stevia on body weight so far, with all showing no significant change (78, 273, 275).

The aim of this study was to investigate the effects of the daily consumption of stevia for 3 months, taken in doses similar to real-life consumption, on the glucose homeostasis, body weight, and energy intake in healthy adults with a normal body mass index (BMI). The primary outcome was change in postprandial glucose response before and after the intervention, while secondary outcomes included change in body weight and energy intake.

4.3 Materials and Methods

4.3.1 Study Design

A randomised, controlled, open-label 2-parallel-arm trial was conducted. Participant assignment was based on a random sequence generated via an online tool (www.random.com) by an independent researcher and was pre-stratified by gender to ensure a balance between the two arms of the trial. The research protocol was reviewed and approved by the University of Manchester Research Ethics Committee (2018-4812-7661); all subjects signed informed consent prior to participation and were compensated for their time at the end of the trial. The trial is registered at clinicaltrials.gov under the registration NCT03993418.

4.3.2 Participants

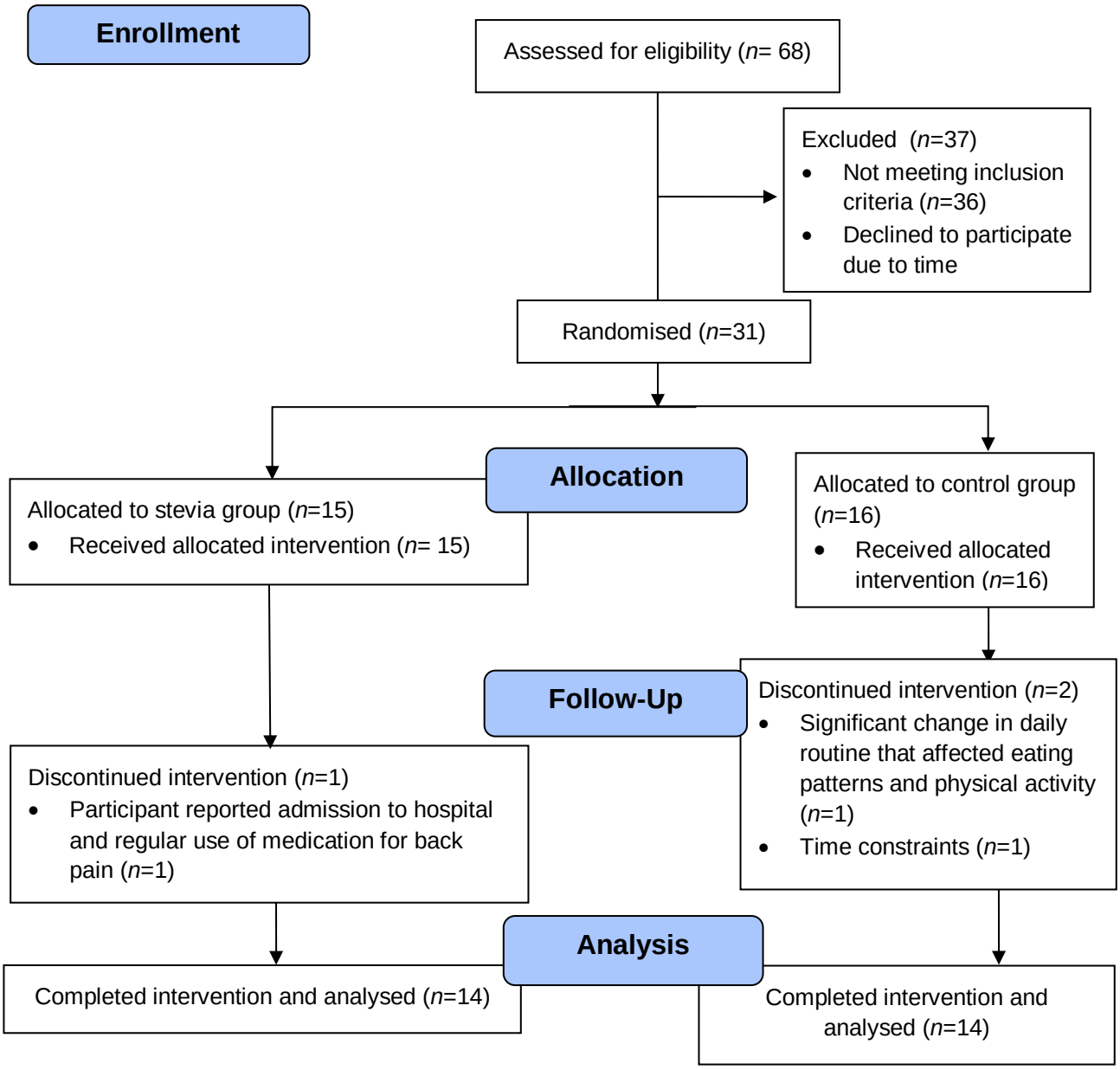
Healthy adults with a normal BMI (18.5–25 kg/m^2), aged 18–40 years old, who were non-habitual consumers of NNS (≤1 can of diet beverages per week or ≤1 sachet of NNS per week) and non-restrained eaters (restraint eating score in the Dutch Eating Behaviour Questionnaire (DEBQ) ≤ 3) were recruited. Other inclusion criteria were fasting blood glucose ≤ 6 mmol/L, stable weight for the last 12 months (±5 kg), willingness to comply with the study protocol, no self-reported food allergy or intolerance to foods supplied during the study. Exclusion criteria were being on a diet or having ceased a diet in <4 weeks, following any special diets for weight maintenance, being vegetarian or vegan, alcohol consumption more than 14 units a week, more than 10 h of vigorous physical activities per week and/or planning to increase or decrease physical activity levels in the future, having ceased smoking in the last 6 months, and female participants who are or may be pregnant or currently lactating.

The participants who were interested in participating contacted the researchers via email and were then sent a link to an online screening questionnaire. Eligible participants from the online screening questionnaire were invited to a screening session that was scheduled on a morning after an overnight fast. During this session, fasting blood glucose, weight, and height measurements were conducted to ensure that the participants met the inclusion criteria for the study. In addition, the participants needed to agree to be allocated to either treatment group. Participants who were found to be eligible and agreed to participate were consented then randomised into one of the study groups.

Sample size calculation was conducted for the primary outcome, glucose response to an OGTT measured by iAUC, and was based on data of a previous trial in healthy subjects that involved the ingestion of glucose load (mean iAUC 117 mmol/L × 120 min, SD: 41 mmol/L × 120 min) (323). With 28 subjects, there was an 80% power to detect a 20% change in the iAUC, which is considered a clinically significant change in glucose response for the current study design, assuming a within-person correlation of 0.5, α of 0.05 and taking into consideration the study design (2 groups × 2 measurements).

In total, 68 participants completed the online survey and 36 attended the screening session. Thirty-one participants were randomised to the 2 study groups, and all study procedures took place between January 2019 and December 2019. A detailed flowchart can be found in the **Figure 4.1.** Withdrawals were due to time constraints or significant changes in the participants' daily routine and not because of known study-related adverse effects.

Figure 4.1 Participant flow chart.



4.3.3 Protocol

The participants in the stevia group were given a commercially available stevia drops product (SweetLeaf Stevia Sweet Drops Clear, SweetLeaf®, Wisdom Natural Brands, Arizona, USA) and were instructed to consume 5 drops twice daily with their habitual drinks (5 drops of stevia corresponds to the sweetness of one teaspoon of table sugar). The choice of stevia drops over any other powder product was taken based on the purity of the drops, containing only stevia leaf extract in water, as in most commercially available powder product stevia is usually mixed with a bulking agent (erythritol, inulin etc.), and therefore any effects shown would not be indicative solely of stevia. The participants were advised to use the stevia drops with their coffee, tea, smoothie, porridge, juice, or other beverage according to their preferences, ideally before lunch and before dinner. Advice on sugar consumption was not given. The participants allocated to the control group were not required to change anything in their usual diet. Both groups were advised to keep their physical activity levels consistent for the duration of the study and to avoid consuming any products containing NNS.

The participants were required to attend 3 study sessions—visit week 0 (baseline), visit week 6, and visit week 12. All the study procedures were conducted at the Neuroscience and Psychiatry Unit, University of Manchester. A graphical description of the study design and the schedule of the assessments can be found in **Figure 4.2**.

Visit week 0 and visit week 12 were conducted on the morning after an overnight fast. The participants were required to refrain from any vigorous physical activities and alcohol consumption the day before testing and to consume their evening meal before 22:00 the night before each visit. Upon arrival, a cannula was inserted into a forearm vein for repeated blood sample collection and a baseline blood sample was collected. Next, the participants ingested 75 g of glucose dissolved in 250 mL of tap water. The glucose beverages were prepared on the morning of testing by the researchers, and were served in transparent beakers as colourless liquids at room temperature. Blood samples were then collected 15, 30, 45, 60, 90, and 120 min after the consumption of the glucose load.

Immediately following the collection of a blood sample, the glucose levels were determined using a HemoCue Glucose 201+ Analyser (HemoCue, Angelholm, Sweden). A HemoCue cuvette was placed into a droplet of whole blood, then the cuvette was wiped clean and placed in the cuvette holder to be measured.

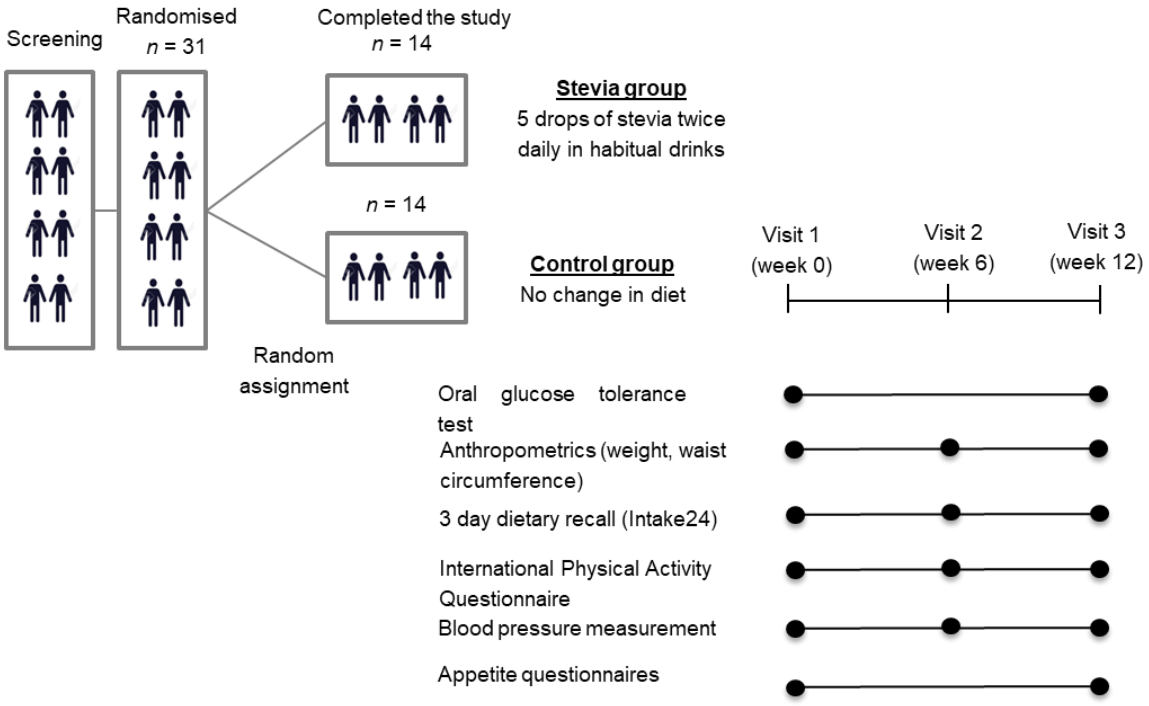
The remaining blood samples were placed into serum-separating vacutainers. The tubes for serum separation were allowed to clot at room temperature for 30 min before centrifuging for 15 min at 3000 rpm and 4 °C. After centrifuging, the serum samples were aliquoted into labelled Eppendorf tubes at −80 °C until analysis.

For visit week 6, the participants did not have to fast, but it was scheduled at least 2 h away from a main meal (i.e., at least 2 h after breakfast or after lunch). The assessments conducted at visit week 6 can be found in Figure 4.2.

Body weight was determined by a digital scale (SECA 813 electronic scale with a large platform) in light clothes without shoes, and height was measured using a portable stadiometer (SECA 213 Portable Height Measure, Hamburg, Germany). Waist circumference was measured via a measuring tape (SECA 201 Ergonomic Circumference Measuring Tape, Hamburg, Germany). Blood pressure was measured twice and the average value was recorded (OMRON M2 Basic). The energy and macronutrient intake were assessed via 24 h diet recalls. The participants were required to complete 3 diet recalls (2 weekdays and 1 day on a weekend) before each study visit (before visit week 0, week 6, and week 12). The diet recalls were performed using a free open-source self-completed computerised dietary recall system, Intake24 (https://intake24.co.uk/). The validity of Intake24 against interviewer-led 24 h recalls has been established (the mean intakes of all macronutrients and micronutrients were within 4% of the interviewer-led recall) (324). All the participants received a training session with a dietician on how to recall their food intake using this system. Physical activity was monitored via the International Physical Activity Questionnaire (IPAQ—long lasting 7-day self-administered format).

The participants filled out a three-factor eating questionnaire (TFEQ), measuring their perceptions of dietary restraint, disinhibition, and hunger (325), and a control of eating questionnaire (CoEQ) (only the subscales of sweet craving, savoury craving) at visit week 0 and at visit week 12 (326).

Figure 4.2 Description of the study design and outcomes.



4.3.4 Analyte Assays

Serum insulin was determined by a sandwich ELISA method using a commercially available human insulin kit (ab20001—Insulin Human SimpleStep Elisa Kit, Abcam, Cambridge, UK) with a minimum sensitivity of 1.9 pmol/L. The insulin concentration was measured at the 0, 30, 45, 60, and 120 min time points. All of the samples for a particular participant (pre and post intervention) were measured on the same ELISA plate.

4.3.5 Compliance

The participants allocated to the stevia group were given a diary to fill out every day that included information on whether they had their two doses of stevia, what they had it with, and their reason for not taking it if they skipped a dose. A compliance percentage was calculated for each participant using the information provided from the diary. We considered it adequate adherence when >80% of the prescribed stevia was consumed. The participants were also required to bring the stevia bottle with them at visit week 6 and at visit week 12. The weight of the bottle was measured and compared to a reference one (the drops that should have been used by week 6 and week 12 were counted and removed from the reference bottle).

4.3.6 Statistical Analysis

Descriptive statistics were used for variables such as age, weight, height, BMI, and questionnaire and are presented as means ± SDs. The rest of the data are presented as means ± SEs. Mixed-model repeated measures ANOVAs were used to assess the glucose and insulin responses, body weight, anthropometrics, dietary intake, physical activity levels, and questionnaire scores. Session (week 0 and week 12) and time (0–120 min) were within-subject independent variables and study group (stevia or control) was the between-group independent variable. Where significant main effects or interactions were found, we followed up with post hoc comparisons using Bonferroni-corrected criteria. In cases where the sphericity was violated, the Greenhouse–Geisser-corrected p values are reported. Statistical significance was determined at $p \leq 0.05$. Analyses were conducted by using IBM SPSS Statistics Version 25.

4.4 Results

4.4.1 Baseline Characteristics

There were no differences in the baseline characteristics between groups. The baseline characteristics of the participants who completed the trial are presented in **Table 4.1**. There was no difference between the treatment groups in terms of age, BMI, weight, waist circumference, or eating behaviour traits assessed via the DEBQ (restraint, emotional and external eating). The estimated compliance based on the participants' diaries was 95 ± 5%. All the participants' bottles weighed very close to the reference bottle (±3 g) both at week 6 and at week 12.

Table 4.1 Baseline characteristics.

	Stevia Group (*n* = 14)	**Control Group (*n* = 14)**	***p* Value**
Age, y	25 (6)	25 (4)	0.795
Weight, kg	59.50 (9.00)	57.83 (7.98)	0.428
Height, m	1.65 (0.09)	1.67 (0.08)	0.934
BMI, kg/m^2	21.71 (1.81)	20.73 (1.46)	0.122
Waist circumference, cm	71.64 (6.53)	70.57 (5.81)	0.651
Female (count)	11	11	
BMR (kcal) [1]	1379 (197)	1368 (181)	0.880
Daily energy needs (kcal) [2]	1930 (276)	1915 (253)	0.882
DEBQ scores			
Restraint eating	2.06 (0.43)	1.90 (0.59)	0.484
Emotional eating	2.26 (0.63)	2.28 (0.45)	0.947
External eating	3.09 (0.56)	3.11 (0.43)	0.930

Values are mean (SD). BMR, Basal Metabolic Rate; DEBQ, Dutch Eating Behaviour Questionnaire. [1] Calculated using the Mifflin–St Jeor equation, [2] calculated using BMR and a physical activity factor of 1.4.

4.4.2 Glucose Response

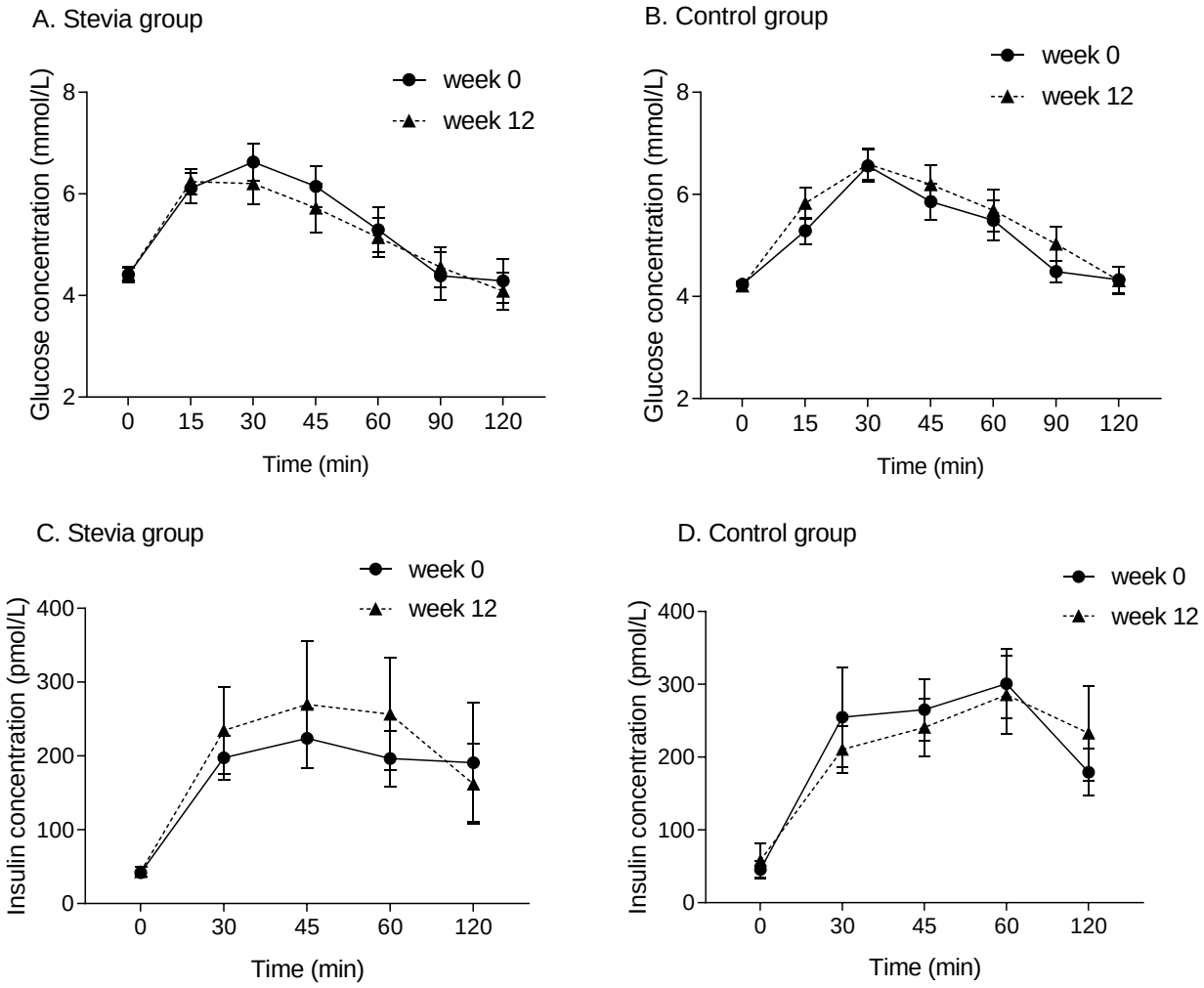
The week 0 and week 12 blood glucose values from the OGTTs are presented in **Figure 4.3** (panels A and B). All the participants had fasting blood glucose within the normal range (3.9–5.5 mmol/L). There were no significant differences in glucose response between the two groups, and no main effect of treatment group or interaction between the session (week 0 and week 12) and treatment group. The iAUC for glycaemia in the stevia group was (mean ± SE) 132 ± 31.2 mmol/L x min at baseline and 133 ± 29.5 mmol/L × min at week 12; in the control group, these values were 131 ± 19.1 mmol/L × min and 159 ± 33.1 mmol/L × min, respectively. The peak glucose concentration at baseline in the stevia group was 7.08 ± 0.34 mmol/L and at week 12 it was 6.91 ± 0.32 mmol/L; in the control group, these values were 6.66 ± 0.31 mmol/L and 6.82 ± 0.29 mmol/L, respectively.

4.4.3 Insulin Response

There were also no significant differences in insulin response among the study groups, and no significant main effect of the treatment group or interaction between the treatment group and session (week 0 and week 12). The week 0 and week 12 serum insulin values are presented in Figure 4.3 (panels C and D). The iAUC for insulin response to the OGTT was 16.5 ± 3.59 nmol/L x min at baseline and increased to 19.3 ± 5.88 nmol/L × min at week 12; however, this increase was not statistically significant (p = 0.516, paired samples t-test), as it was driven by two single subjects. Similarly, there was no difference in iAUC for insulin response in the control group at baseline and after 12 weeks, and the values were 21.6 ± 3.55 and 20.4 ± 4.07 nmol/L × min, respectively. The peak insulin concentration was 336 ± 78.9 pmol/L at baseline in the stevia group and 347 ± 95.9 pmol/L at week 12; in the control group, these values were 362 ± 62.8 and 367 ± 63.5 pmol/L, respectively.

Figure 4.3 Blood glucose and serum insulin concentrations during the oral glucose tolerance tests for participants in the stevia group (*n* = 14, panels A and C) and in the control group (*n* = 14, panels B and D) at baseline (week 0) and after 12 weeks of intervention.

Values are means ± SEs. Venous blood samples could not be collected from one participant in the stevia group, and the serum insulin was not measured for this participant, *n* = 13 for 2C.



4.4.4 Body Weight and Other Anthropometric Indices

The change in body weight was significantly different between the two groups (main effect of treatment group, F(1, 26) = 5.56, p = 0.026, η^2p = 0.176; session-by-treatment group effect, F(2, 52) = 3.43, p = 0.040, η^2p = 0.117) (**Figure 4.4)**. There was a statistically significant increase in body weight over the 12-week trial for participants in the control group (mean weight change: 0.56 kg, 95% CI [0.13, 0.99] in the control group) compared to the participants in the stevia group (−0.14 kg, 95% CI [−0.56, 0.29]). Including the baseline weight as a covariate, the results were further strengthened (treatment group effect, F(1, 25) = 6.07, p = 0.021, η^2p = 0.195; session-by-treatment group interaction, F(2, 50) = 3.68, p = 0.032, η^2p = 0.128).

Table 4.2 shows the BMI, waist circumference, and blood pressure values for the stevia and control groups at week 0, week 6, and week 12 of the intervention. There was a significant main effect of session (week 0, 6, and 12) on the BMI change from baseline (F (1, 26) = 4.95, p = 0.035, η^2p = 0.160), but no other significant changes were observed for waist circumference, systolic and diastolic blood pressure, or pulse rate.

Figure 4.4 Change in body weight (A) and energy intake (B) in the stevia and control groups over 12 weeks (*n* = 14 in each group). Differences in body weight were correlated with changes in energy intake (C).

Data are expressed as means ± SE. * *p* = 0.003 vs week 0.

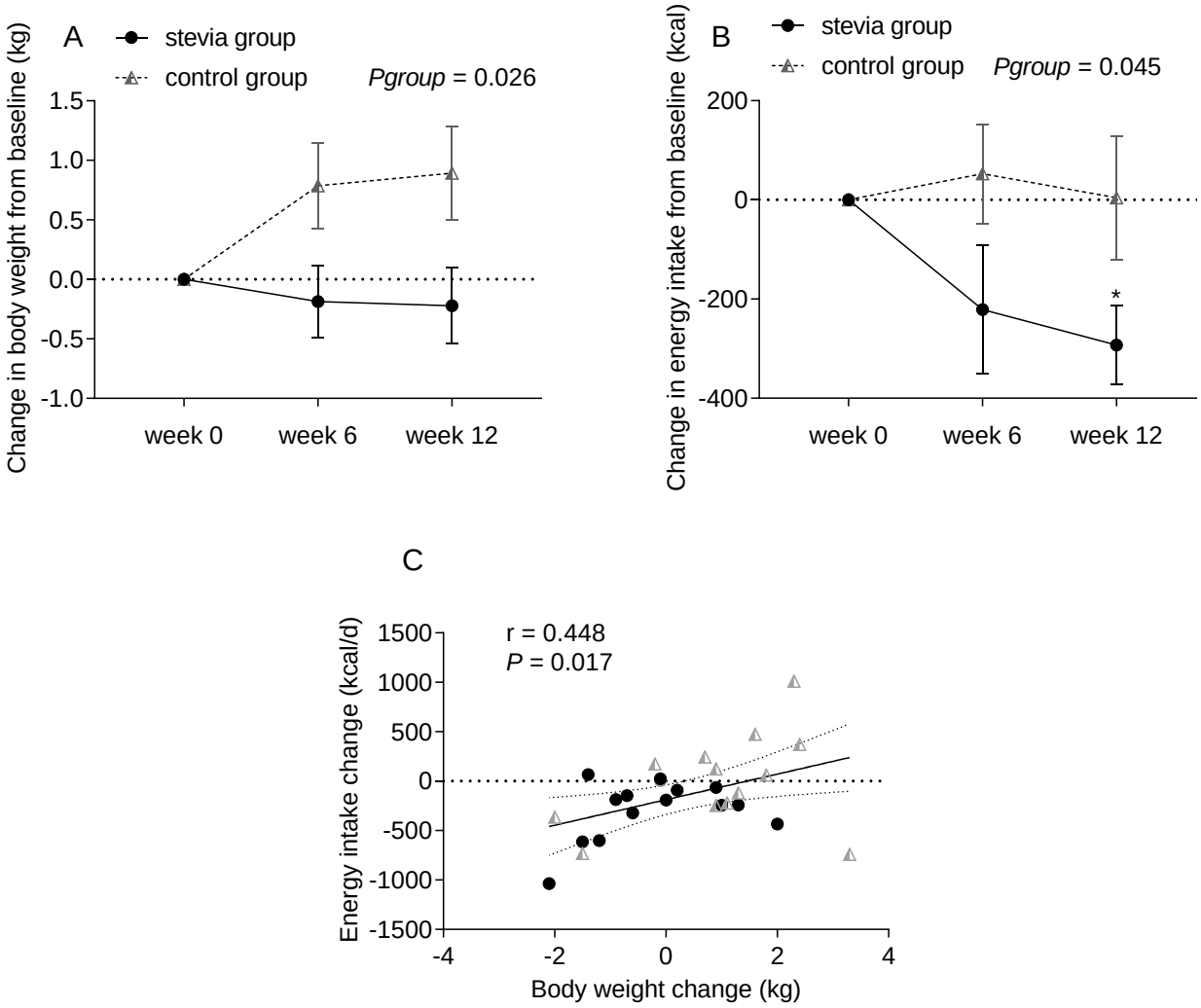


Table 4.2 Anthropometric measures for the stevia and control groups over the 12-week intervention.

	Stevia Group (*n* = 14)			Control Group (*n* = 14)			*p* Values		
	Week 0	**Week 6**	**Week 12**	**Week 0**	**Week 6**	**Week 12**	**Session**	**Group**	**Session × Group**
Body weight, kg	59.50 (2.40)	59.31 (2.40)	59.27 (2.49)	57.83 (2.13)	58.61 (2.32)	58.72 (2.23)	0.289	0.769	0.040
Δ Body weight, kg	-	−0.19 (0.30)	−0.22 (0.32)	-	0.79 (0.36)	0.89 (0.39)	0.289	0.026	0.040
BMI, kg/m^2	21.71 (0.48)	21.64 (0.48)	21.62 (0.49)	20.73 (0.39)	20.99 (0.42)	21.03 (0.40)	0.389	0.247	0.053
Δ BMI, kg/m^2	-	−0.07 (0.11)	−0.09 (0.12)	-	0.26 (0.13)	0.31 (0.14)	0.388	0.035	0.054
Waist circumference, cm	71.64 (1.75)	71.93 (1.61)	71.11 (1.65)	70.57 (1.55)	71.18 (1.66)	71.00 (1.66)	0.135	0.783	0.199
Systolic blood pressure, mmHg	119.14 (2.46)	117.71 (3.60)	118.07 (2.78)	114.00 (1.95)	114.21 (2.81)	112.71 (2.58)	0.732	0.191	0.792
Diastolic blood pressure, mmHg	67.57 (1.76)	64.57 (1.51)	65.93 (1.17)	70.11 (1.24)	68.79 (1.51)	69.29 (1.93)	0.159	0.069	0.750
Pulses, beats per min	72.39 (2.52)	69.57 (2.46)	74.75 (2.14)	70.07 (1.49)	74.14 (3.66)	73.68 (2.33)	0.264	0.889	0.165

Values are mean (SE). Δ calculated as change from week 0. BMI, body mass index.

4.3.5 Energy Intake

The self-reported energy and macronutrient intake data are shown in **Table 4.3**. The energy intake at week 0 was not significantly different between treatments ($p = 0.929$). The change in energy intake from week 0 is presented in Figure 4.4 B, showing a significant main effect of the treatment group ($F(1, 26) = 4.43$, $p = 0.045$, $\eta^2p = 0.146$), with participants in the stevia group reporting a significantly reduced energy intake over the 12-week trial period (mean energy intake change: −171 kcal, 95% CI [−303, −39.9]) compared to the control group (18.9 kcal, 95% CI [–112, 150]). Further exploration of the data revealed that, in the stevia group, energy intake was significantly lower at week 12 relative to week 0 ($p = 0.003$, Bonferroni correction for multiple comparisons, $p \leq 0.008$), however no difference was found in the control group ($p = 0.973$). The individual differences in weight change between the treatment groups were significantly correlated with the individual differences in energy intake change at week 12 ($r = 0.448$, $p = 0.017$) (Figure 4.4 C). The reduction in self-reported daily energy intake observed in the stevia group was not because of selectively reducing a specific macronutrient such as sugars or carbohydrates, but was an overall reduction in energy intake, since no differences were observed between the two groups in terms of their carbohydrate, fat, protein, sugar, or fibre intakes before and after the intervention (Table 4.3).

Table 4.3 Energy and macronutrient intake levels in the stevia and control groups during the intervention.

	Stevia Group (*n* = 14)			Control Group (*n* = 14)			*p* Values		
	Week 0	**Week 6**	**Week 12**	**Week 0**	**Week 6**	**Week 12**	**Session**	**Group**	**Session × Group**
Energy intake, kcal x d^{-1}	1659 (102.9)	1437 (106.2)	1366 (115.6)	1674 (137.7)	1727 (154.4)	1678 (167.2)	0.185	0.224	0.114
Δ Energy intake, kcal x d^{-1}		−221.2 (129.3)	−292.8 (78.81)	-	52.52 (100.3)	4.36 (124.8)	0.185	0.045	0.114
Carbohydrates, g	193 (10.9)	175 (16.6)	170 (12.3)	205 (15.2)	206 (16.2)	208 (23.3)	0.643	0.164	0.533
Carbohydrates, %	47.9 (2.52)	48.7 (2.61)	51.6 (2.81)	50.3 (2.48)	48.7 (1.86)	49.4 (2.47)	0.586	0.975	0.440
Fats, g	67.6 (7.12)	53.8 (5.00)	52.3 (6.66)	66.4 (7.55)	68.7 (8.77)	66.00 (10.0)	0.241	0.338	0.174
Fats, %	35.9 (1.91)	33.4 (1.83)	32.8 (1.96)	34.8 (1.82)	34.8 (1.80)	34.1 (2.05)	0.500	0.787	0.691
Proteins, g	71.3 (8.99)	69.3 (7.11)	61.3 (7.00)	68.1 (6.82)	71.8 (6.82)	73.8 (4.92)	0.739	0.644	0.224
Proteins, %	16.8 (1.53)	19.9 (2.39)	17.9 (1.40)	16.5 (1.14)	16.8 (1.00)	18.9 (1.61)	0.282	0.635	0.253
Sugars, g/1000 kcal	49.3 (3.61)	49.8 (3.59)	49.2 (3.71)	49.7 (6.24)	43.5 (5.16)	38.1 (5.03)	0.395	0.230	0.435
Fibres, g/1000 kcal	8.29 (0.79)	8.49 (1.11)	7.79 (0.64)	7.54 (1.15)	8.53 (1.20)	8.10 (0.87)	0.487	0.911	0.648

Values are mean (SE). Δ calculated as change from week 0.

4.4.6 Physical Activity

There was no difference in physical activity levels assessed via the IPAQ between week 0, week 6, and week 12 in the two treatment groups. In the stevia group, the total MET min per week were calculated (mean ± SE) as 3346 ± 574.3 at week 0, 3256 ± 574.4 at week 6, and 3133 ± 352.3 at week 12. In the control group, the total MET min per week were 4415 ± 1047 at week 0, 3579 ± 754.9 at week 6, and 4373 ± 1393 at week 12. There were no main effects or interactions found (all $p > 0.05$).

4.4.7 Appetite Expression

The results from the TFEQ and CoEQ are presented in **Table 4.4**. The participants in the control group reported overall higher hunger scores on the TFEQ subscale compared to the stevia group (main effect of treatment group $F(1, 26) = 4.64$, $p = 0.041$, $\eta^2p = 0.152$); no difference was found for the restraint and disinhibition subscales of the TFEQ between groups or between week 0 and week 12. The participants in the control group also reported an overall higher craving for savoury scores on the CoEQ subscale compared to the stevia group (main effect of treatment group $F(1, 26) = 8.96$, $p = 0.006$, $\eta^2p = 0.256$); there was also a significant interaction between the session (week 0 and week 12) and the treatment group ($F(1, 26) = 4.83$, $p = 0.037$, $\eta^2p = 0.157$), showing a reduction in self-reported craving for sweet in the control group. However, this difference did not reach statistical significance after correcting for multiple comparisons ($p = 0.040$, Bonferroni correction for multiple comparisons $p \leq 0.025$). Exploratory correlation analysis revealed a significant positive correlation between the sweet craving ratings and sugar intake (g/1000 kcal) in the control group ($r = 0.419$, $p = 0.027$), but not in the stevia group ($r = 0.225$, $p = 0.249$).

Table 4.4 Appetite expression questionnaires for the stevia and control groups during the intervention.

	Stevia Group (*n* = 14)		**Control Group (*n* = 14)**		***p* Values**		
	Week 0	**Week 12**	**Week 0**	**Week 12**	**Session**	**Group**	**Session × Group**
TFEQ subscales							
Cognitive restraint	5.93 (0.84)	5.86 (0.64)	4.79 (1.23)	6.36 (1.40)	0.226	0.819	0.186
Disinhibition	4.36 (0.74)	4.79 (0.70)	5.21 (0.59)	5.57 (0.54)	0.192	0.354	0.904
Hunger	4.86 (0.82)	4.86 (0.76)	7.36 (0.74)	6.79 (0.82)	0.504	0.041	0.504
CoEQ *subscales*							
Cravings for savoury	32.3 (3.67)	36.2 (4.50)	52.6 (5.33)	49.9 (4.27)	0.845	0.006	0.243
Cravings for sweet	39.3 (4.96)	40.4 (4.72)	46.9 (5.16)	36.1 (5.19)	0.087	0.807	0.037

Values are mean (SE). TFEQ, three-factor eating questionnaire; CoEQ, control of eating questionnaire.

4.5 Discussion

The present study investigated the effects of the daily consumption of stevia drops for 12 weeks on glucose response, body weight, and energy intake in healthy adults. We observed no difference in the glucose and insulin response, however the stevia and control groups showed distinct patterns in body weight and energy intake. The stevia-consuming participants did not significantly alter their body weight from baseline, but did not demonstrate the weight gain that occurred in the control group. Participants in the stevia group also reported a lower total energy intake during the trial compared to the controls, while the physical activity levels did not change across the intervention period.

The primary outcome of this trial was glucose response, assessed via OGTTs performed at baseline and after 12 weeks of intervention in healthy individuals without diabetes. No significant difference was observed with regard to the treatment group or intervention time. These findings support our understanding of the effects of NNS in general on glycaemia. When NNS are consumed alone, no difference in glucose levels has been reported so far in acute single-exposure trials in humans (111, 125, 127, 147, 327). This observation probably shows that the activation of sweet taste receptors by NNS does not exert any clinically relevant effects on glucose homeostasis signalling in the context of human consumption. In addition, no significant change in glucose response has been observed in acute studies where NNS were consumed along with a glucose load by healthy non-obese adults (38, 131, 320). The rationale behind this was based on data from *in vitro* demonstrations showing that treatment with NNS might enhance glucose uptake due to the upregulation of the glucose transporters. Therefore, an increase in glucose response would be anticipated when carbohydrates were consumed concomitantly with NNS compared to being consumed alone. This hypothesis was not confirmed by human studies in healthy participants (320). However, each NNS is a distinct chemical compound and has its own biological fate in the human body, which might influence individual NNS responses (27). Effects on biological targets other than sweet taste receptors cannot be discounted. Regarding stevia, two studies have provided evidence that it might assist with glucose regulation, as lower postprandial levels of glucose were observed following the consumption of a meal supplemented with stevia in healthy adults (48) or in patients with T2DM (280). These results are further supported by the demonstration of enhanced pancreatic beta-cell function by steviol glycosides (284). However, no significant difference was observed in the glucose and insulin response when stevia was ingested alone (51, 322). The direct administration of rebaudiana A (type of steviol glycoside) in the duodenum of healthy adults also did not result in incretin release (124). A recent meta-analysis of RCTs investigating the effects of long-term stevia consumption on metabolic markers showed no significant difference in fasting blood glucose in favour of steviol glycosides; the doses of consumption

varied between 3.75 mg/kg/day and 1500 mg/day of stevioside (286). No significant change has been demonstrated for fasting insulin following long-term stevia consumption (272, 274). In the present study and in line with the majority of results from human trials, the daily consumption of commercially available stevia did not influence glucose homeostasis or insulin response in healthy adults.

In line with our results, similar effects have been demonstrated by long-term RCTs in healthy adults using other types of NNS. No effect on glucose, insulin, and GLP-1 responses was observed in the study by Higgins *et al.* (56) following 12 weeks of aspartame consumption in two different doses (350 mg and 1050 mg/d) compared to a placebo. Furthermore, no change in glucose and insulin response was found in the study by Grotz *et al.* (149), which investigated the effects of 12 weeks of sucralose consumption (1000 mg/day) against a placebo group in normoglycaemic males or following 7 days of sucralose administration (780 mg/d) in healthy subjects (144). On the other hand, lower insulin sensitivity has been demonstrated in two studies following daily sucralose consumption (146, 148), and a recent study by Dalenberg *et al.* (147) also showed that consuming 7 sucralose-sweetened beverages not without carbohydrates over 10 days decreased insulin sensitivity in healthy human volunteers. Whether this is a sucralose-specific effect needs further investigation. In the present study, there was no difference in the insulin response to an OGTT before and after 12 weeks of daily stevia consumption compared to the control group in healthy adults.

One *a priori* secondary outcome was change in body weight, assessed at week 6 and week 12 of the intervention period. In this trial, we demonstrated that the participants allocated to the stevia group maintained their body weight compared to the control group, who showed a significant increase in body weight, which could be attributed to a general trend towards weight gain by the population. Further, the results from the self-reported energy intake, which was another secondary outcome, showed a decrease in energy intake at week 12 of the intervention in the stevia group, but not in the control group. Even though the change in body weight does not match the change in energy intake, since a reduction in energy intake should indicate weight loss, there was a significant correlation between individual changes in body weight and individual changes in energy intake. Participants were not placed on an energy-restricting diet, and physical activity levels were kept stable throughout the trial; the only guidance provided for those in the stevia group was that they should consume the stevia drops daily, ideally in a drink or a hot beverage before lunch and before dinner. With this advice, we attempted to reproduce the design of a previous acute study on stevia effects on food intake, where a significant reduction in total energy intake was demonstrated when consuming a stevia-sweetened preload prior to lunch compared with consuming water or caloric beverages (322). If that effect would be sustained and not compensated for in the next meals, that could explain the lower energy intake results, as observed in the present

study. However, opposed to our results, another recent study evaluated the effects of daily rebaudiana A consumption for 12 weeks against another three types of NNS and sucrose on body weight and energy intake and showed no effect on both measures (78). The lack of a difference in body weight following encapsulated stevioside consumption by people with mild hypertension and patients with T2D was also shown by another two studies (272, 275). If the effects of stevia on energy intake and body weight are mediated by sweetness *per se,* no effect would be expected in trials where oral sweet taste is bypassed. More randomised long-term trials powered with body weight and energy intake as primary outcomes are required to confirm these initial findings of stevia consumption effects in healthy adults, but also in populations for whom weight loss and reduction in energy intake is crucial—i.e., individuals with overweight, obesity, T2DM, or metabolic syndrome.

Recent research now focuses on elucidating the effects of NNS consumption on brain systems related with appetite and reward. The ingestion of glucose induces decreased activity in the hypothalamus, a change typically linked to satiety signalling by the brain (223). However, sweet taste in the absence of nutritive carbohydrates does not seem to elicit a similar response in the hypothalamus (230, 232). Differences between nutritive sweeteners and NNS have been also demonstrated during taste activation, with both stimuli showing the activation of the primary gustatory cortex, anterior insula, and frontal operculum. However, during NNS tasting the reward centres remained unresponsive (239, 240). The above results indicate that NNS might not have similar satiating effects in the brain as nutritive sweeteners. On the other hand, differences in the neural processing of sweet taste in the brain among regular NNS consumers have also been reported, showing a potential adaptation in brain systems following repeated exposure to NNS. In particular, regular consumers of diet soda have shown greater activity patterns in reward regions of the brain during the consumption of nutritive and non-nutritive sweet tastes, compared to non-diet soda consumers (244). We should highlight again the potential of different types of NNS exhibiting differential responses, yet further studies are needed to explore brain responses following stevia consumption in humans.

The findings of this research also raise the question of whether there was any behavioural change between the two groups that could have led to the observed distinct effects on body weight and energy intake. The participants in the control group reported higher hunger scores on the TFEQ, independent of intervention time. Hunger is the conscious experience associated with the drive to eat. Even though perceived hunger might not predict intake, it has been shown to predict an individual's ability to manage their body weight or the success of a weight-loss program (328). Higher hunger scores were associated with greater body size in another study (329). However, this difference in susceptibility to hunger ratings was a baseline difference between the two groups, was not influenced by the intervention period,

and was a self-reported questionnaire measure; thus, any observations remain exploratory at this point. Interestingly, we observed a positive correlation between sweet craving ratings and sugar intake in the control group; a reduction in sugars intake was associated with a reduction in the subjective feeling of sweet craving. This was a spontaneous unexpected change in eating behaviour in the control group, who were not following any dietary guidance. However in the stevia group the sugar intake stayed relatively stable and so did the sweet cravings, in line with previous results showing a protective effect of NNS beverages against craving-induced increases in energy intake (60). Further research is required to explore eating behaviour changes induced by the introduction of NNS into the diet of habitual and non-habitual NNS consumers.

The strengths of this study include the real-life scenario design, and participants were allowed to make choices and adjust the addition of the stevia drops to their daily routine. The dose chosen was also realistic, and could simulate the regular consumption of stevia by the general population. Among the limitations of this study is the fact that the results are only specific to the conscious consumption of stevia at this point. The increase in body weight in the control group should be interpreted with caution, since other factors such as menstrual cycle or hydrating status might be responsible for this outcome, however these potential confounding factors were the same for the two groups and the gender ratio was the same. Another limitation of the study could be the use of a natural history control group (no treatment) instead of a null control group receiving placebo drops. It has been documented that participants randomised to placebo-control conditions in obesity research studies often report improved outcomes that are similar to those of people receiving the active treatment, even when the individual is aware that they are receiving a placebo (330, 331). A control for sweetness could be another NNS arm, such as saccharin, aspartame, sucralose, etc., but that was out of the scope of this trial at this time. It is likely that sweet taste may mediate these results, suggesting that a double-blind design delivering the sweetener bypassing the oral cavity could be compared to an open-label design (sweet taste perception included) to investigate this hypothesis. This study was powered to detect a significant difference in the primary outcome, glucose response, not secondary outcomes where the two groups showed distinct effects, and therefore a powered for body weight and/or energy intake randomised controlled trial should be pursued, especially in populations where the reduction in energy intake is critical, such as in individuals with overweight and/or obesity, metabolic syndrome, or T2DM.

In summary, our data provide evidence that the daily consumption of stevia in real-life doses does not affect glycaemia in healthy normal-weight individuals, but could aid in weight maintenance and the moderation of energy intake. More research is warranted to explore these promising findings further.

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CHAPTER 5

Mapping the homeostatic and hedonic brain responses to sweet taste and calories: a double-blind randomised controlled crossover trial in healthy adults

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Statement

NS designed the study, conducted the research, analysed the data, and took responsibility for writing the manuscript. SM contributed to the study design and guided the statistical analysis; CS and DB designed and prepared the beverages and contributed to study design; RE contributed to study design, provided essential intellectual input, read and approved the final manuscript; JM conceived the idea, contributed to the study design, conducted the supervision, reviewed and approved the manuscript. The manuscript is intended to be submitted for publication.

5.1 Abstract

Background: The increased use of non-nutritive sweeteners as sugar substitutes over the last decades has raised interest in their potential effects on regulatory functions of the brain. Stevia is a non-caloric sweetener that has demonstrated beneficial effects on appetite and energy intake. This randomised double-blind controlled crossover study aims to investigate the neural correlates of acute physiological signals and food-cue elicited responses related to consumption of beverages differing in sweetness and caloric content.

Methods: Participants completed four imaging sessions, after a 3-4 hour fast, distinguished only by the type of beverage they consumed (water, stevia, glucose or maltodextrin). Change in blood-oxygenation level-dependent (BOLD) contrast during functional Magnetic Resonance Imaging (fMRI) was monitored over a 30 min period after the consumption of the beverages. Participants were scanned while performing a food visual probe task (VPT) before and 30 minutes after the consumption of the beverages. Hunger, fullness and sweetness sensations were also recorded.

Results: There was a significant interaction of taste-by-time in BOLD response in areas involved in gustatory and reward processing, with sweet beverages inducing greater reduction in BOLD compared to non-sweet beverages. There was also a significant interaction of calories-by-time in BOLD response in thalamic, visual, frontal and parietal areas among others; glucose and maltodextrin demonstrated significantly greater incremental area under the curve (iAUC) of the average BOLD response in the 10-20 min time bin only compared to water, while in the 20-30 min iAUC no longer differed. The interaction of taste-by-calories-by-time showed a greater and more robust BOLD decrease for stevia mainly in motor, frontal areas and insula, which was more apparent in the 20-30 min post consumption. In the food-cue task, the three sweet/caloric beverages (stevia, glucose, maltodextrin) showed attenuated response in the visual cortex in response to food compared to control trials, while water demonstrated increased response post consumption. Only the glucose beverage significantly increased fullness sensation post ingestion.

Conclusions:

Both sweet taste and calories have a modulatory effect in the brain signalling post consumption. Stevia showed a more robust and longer lasting BOLD decrease in the human brain that could potentially be linked to effects on feeding behaviour.

Keywords: stevia; glucose; maltodextrin; food cues; fMRI; *phys*MRI; BOLD; neuroimaging

5.2 Introduction

Consumption of sugar-sweetened beverages has been associated with an increased risk of weight gain and obesity with higher intakes (316, 332). Non-nutritive sweeteners (NNS) provide sweet taste with minimal or no calories, and could therefore constitute excellent substitutes for caloric sugars, while reducing the available energy and preserving palatability (52). However, the effects of NNS consumption on health consequences remain controversial within the scientific community (11, 73). It has been argued that NNS consumption could disrupt learned responses that normally contribute to glucose and energy homeostasis and potentially increase the risk of obesity and non-communicable diseases (8, 333). An obesogenic impact of NNS consumption has not been consistently demonstrated, on the contrary evidence moves towards the opposite direction. Recent meta-analyses support a beneficial role of NNS consumption on energy intake and body weight (66, 77). In particular stevia, a natural zero-calorie containing sweetener that has not been extensively studied despite its increasing popularity, has shown advantageous effects on appetite and energy intake (334, 335).

Control of food intake involves a complex interaction between homeostatic and hedonic mechanisms, and the human brain plays a central role in this process. It integrates many metabolic, hedonic and trait-related signals that affect eating behaviour and determine when and how much we eat (156). The hypothalamus together with the brainstem and the corticolimbic system are regarded as being the core processors in the control of appetite, with hypothalamus and brainstem mainly controlling the homeostatic part of appetite control, and the corticolimbic system mainly associated with food reward and cognitive control (12). Caloric sugars and NNS activate functionally connected taste pathways that lead to conscious perception of sweetness, a strong hedonic signal, via activation of the oral sweet taste receptors (STRs) (336, 337). However, they differ in their metabolic fate after ingestion so that caloric sugars lead to elevated blood glucose, insulin and satiety inducing gut-peptide levels postprandially, but NNS consumption does not influence glucose or hormonal responses postprandially (134, 308). Therefore, it is expected that both overlapping brain regions, probably derived from the conscious and unconscious (activation of the gut STRs) sweetness, and distinct brain regions due to differences in metabolic consequences, would respond to their consumption.

Previous studies have shown that glucose ingestion has been associated with a well-established pattern in brain activity; decreased neural activity in the hypothalamus and the brainstem (221, 223, 226, 232), while ingestion of NNS has been previously shown to be associated either with none (232) or with a transient deactivation of the hypothalamus (230). NNS have been demonstrated to differ in their hedonic responses to caloric sugars, as

measured by blood-oxygenated-level-dependent (BOLD) contrast changes using functional magnetic resonance imaging (fMRI) in the ventral tegmental area of the midbrain (230). Other areas that were associated with decreased BOLD response following glucose intragastric infusion were the cerebellum, occipital areas, insula and putamen, parahippocampal, temporal and thalamic regions (220, 223). To the best of our knowledge, there is no fMRI study investigating the whole brain response to the consumption of stevia.

Satiation attenuates responses in homeostatic and reward-related areas in the brain in response to food tasting and food picture viewing (181). Under conditions of hypoglycaemia, limbic-striatal brain regions are activated in response to food cues to produce greater desire for high calorie food while following glucose administration responses are attenuated (287). Food cue responses refer to the design of these fMRI investigations, during which participants are presented with palatable food pictures in alternation to non-food pictures usually in a blocked design. This design relies on the food-cue responsivity paradigm, which engages the reward system of feeding behaviour. Food cues (i.e. pictures of food) become more salient under conditions of hunger and less salient under conditions of satiety. Attentional bias to food cues refers to the tendency to focus attention to salient (food) over neutral information. Attention to food cues measured by a reaction time visual dot probe task (VPT) has been previously shown to be higher in the fasted state compared to the fed state (198, 311). This task was used to investigate the neural correlates of food-cue responses during an fMRI investigation.

In the present study, we aimed to investigate differences in brain activity following oral ingestion of beverages supplying sweetness with calories (glucose), sweetness without calories (stevia), no sweet taste with calories (maltodextrin) or no sweet taste and no calories (water). We used a combination of physiological-fMRI (*phys*MRI), that allowed us to look at BOLD responses over time following beverage ingestion (signals derived from physiological responses to the consumption of the beverages) compared to a baseline period, and fMRI, the examination of neurocognitive responses to food cues 30 min after the consumption of the beverages while performing an attention food related task, the VPT (signals related to hedonics). We hypothesised that glucose consumption will lead to attenuated BOLD contrast in homeostatic and hedonic brain areas in both the *phys*MRI and the task-based fMRI and maltodextrin will show a similar pattern. The consumption of stevia-sweetened beverage was expected to show BOLD responses in overlapping and distinct areas compared to the glucose-beverage.

5.3 Materials and methods

5.3.1 Preliminary pilot study

Prior to the imaging study, a preliminary pilot was conducted in a mock scanner, in order to ensure the feasibility and validate the methodology of the protocol. A key objective was to ensure that separable differences on blood glucose levels between the nutritive (glucose) and non-nutritive (stevia, water) beverages occur within the proposed time frame, and to ensure that the participants could drink the beverages lying flat and remain still in this position for the duration of the scan. This pilot study was approved by the University of Manchester Research Ethics Committee. All participants signed informed consent prior to participation and were compensated for their time.

A total of 8 participants (healthy, mean age: 22 ± 4 years, mean BMI: 20.8 ± 1.8 kg/m^2) completed the pilot study, the study beverages included water, stevia and glucose beverages (same as the ones provided in the actual fMRI study described later). Participants attended the study sessions after a fixed breakfast and a 3-4 h fast. Visual analogue scales (VAS) scales for appetite (hunger, fullness) were completed at 5 min intervals, corresponding to 0 (before consumption), 5, 10, 15, 20, 25, 30 min. Participants also rated beverage sweetness. Blood glucose was measured at 0 (before consumption starts), 5, 10, 15, 20 and 30 min post-consumption using finger-prick blood samples and the Hemocue Glucose 201+ Analyser (Hemocue, Angelholm, Sweden). The average time-course graph for blood glucose levels, and appetite sensations from this preliminary study are presented in **Supplemental Figure 5.1.** The pilot confirmed that the protocol was suitable for the full imaging study.

5.3.2 Imaging study

5.3.2.1 Participants

For the imaging study, participants were required to complete 5 study sessions (1 pre-study session and 4 imaging sessions) and were recruited from the University of Manchester and the general Manchester area through advertisements placed around campus and online from November 2019 to December 2020. The study inclusion criteria included healthy men and women aged between 18 and 40 years with BMI within the normal range (18.5-24.9 kg/m^2), restrained eating score on the Dutch Eating Behaviour Questionnaire (DEBQ) ≤ 3, consuming breakfast ≥ 5 times per week, being right-handed and registered to a General Practitioner in the UK. Exclusion criteria were being diagnosed with a major chronic disease, having intolerances or allergies for products used in the study, weight change ± 5 kg the last 3 months, self-reported anxiety or depression, use of recreational substances within the last month, being pregnant or lactating, self-reported alcohol consumption exceeding 14

units a week, regular consumption of NNS defined as more than 1 can of diet sodas or more than 1 sachet of NNS per week. Also exclusion criteria related to the MRI were having non-removable metal objects in their body, self-reported claustrophobia or having had an operation less than 3 months ago.

The study was approved by the University of Manchester Research Ethics Committee. All participants signed informed consent prior to participation and were compensated for their time. The trial was registered in clinicaltrials.gov under registration NCT04162457.

Sample size estimation was based on expected BOLD signal change in the hypothalamus using results of a previous study from our team (338). The results from the comparison between intragastric saline and 45 g glucose infusion on the hypothalamic BOLD signal (*n*=15, peak mean difference of -0.9% change in the BOLD signal from baseline, and SD of the difference 0.96) were extracted. GPower 3.1 was used to calculate sample size, which calculated that 17 participants are needed for 95% statistical power and a α of 0.05.

5.3.2.2 Study design

This was a randomised, double-blind, crossover study and the participants received four different beverages, one per occasion with at least a 5-day washout period. Eighteen participants completed the pre-study session and all 4 imaging sessions. Participants were asked to have a breakfast of their preference in the morning prior to their scanning sessions, and then fast for 3 or 4 hours (no food, only water up to one hour prior to scanning). They were asked to repeat exactly the same breakfast and fasting time prior to each scanning session. Participants received one of the study beverages on each occasion in randomised order, which was also counterbalanced across participants. The study beverages were 330 mL of stevia in water (240 ppm Truvia® Stevia RA95- Rebaudioside A- 95%), 330 mL of 40g glucose in water, 330 mL of 40g maltodextrin in water or 330 mL water. No additional flavour was added to the beverages. The glucose and stevia beverages were matched for sweetness, the glucose and maltodextrin beverages contained 160 kcal the water and the stevia beverages contained 0 kcal. Beverages were served at room temperature. Drinking was performed in the scanner through an oral silicon tube, lying supine during the fMRI scans. Participants were given 10 min to drink the beverage at a comfortable drinking rate controlled by themselves. The scanning protocol can be found in **Supplemental Figure 5.2**.

5.3.2.3 Pre-study session

Participants who were eligible on the online screening questionnaire were invited to a pre-study session at the University of Manchester. During this session we conducted anthropometric measurements and described all details of the study to the participants. In

detail, anthropometric measures included body weight measurement by a digital scale in light clothes without shoes (SECA 813 Electronic scale with large platform), height measured with a portable stadiometer (SECA 213 Portable Height Measure), waist and hip circumference (SECA 201 Ergonomic Circumference Measuring Tape). In addition, during this session participants completed the DEBQ, and the Three Factor Eating Questionnaire (TFEQ). Participants practiced the fMRI procedure, practiced the visual dot probe task (VPT) and drinking while lying flat.

5.3.2.4 Imaging sessions

For the MRI sessions participants arrived between 11.00 and 14.30 at the test location (Wellcome Trust Manchester Clinical Research Facility, Manchester, UK) after a fast of 3 or 4 hours (no food, only water up to one hour prior to the start of the session). Participants were required to have breakfast of their preference at home, which they repeated before each scanning session, and then fast for 3 or 4 hours (fasting time was consistent per participant). Compliance was checked with a breakfast composition questionnaire that participants filled out prior to each scanning session.

Physiological MRI

This scan followed the pre-consumption VPT scan. During *phys*MRI participants had to initially undergo a baseline scanning period of approximately 10 min and then were instructed to drink the test beverage using a silicon peroral tube for the next 10 min whilst being scanned. Scanning continued for another 20 min after the consumption of the beverage as outlined in Supplemental Fig 5.1.

During the *phys*MRI participants were asked to indicate their sensation of hunger and fullness on a 10-point scale every 10 min. Subjects had their eyes open and the scales were projected onto a screen visible from inside the scanner. The participant rated each sensation by moving a pointer along the scale, via a response button box held in their right hand. Participants were also asked to rate the sweetness of the beverage after they had consumed it (while in the scanner), and the sensation of thirst before and after the end of a session (in visual analogue scales with pen and paper outside the scanner).

Visual dot probe task

Participants performed a VPT twice whilst being scanned, once before the consumption of the test beverage (pre-consumption VPT) and once again 30 min post beverage consumption (post-consumption VPT). The VPT involves the presentation of pictures in pairs on screen followed by a dot probe presentation until participant's response. In the food-related VPT a picture pair included one food image and one non-food image

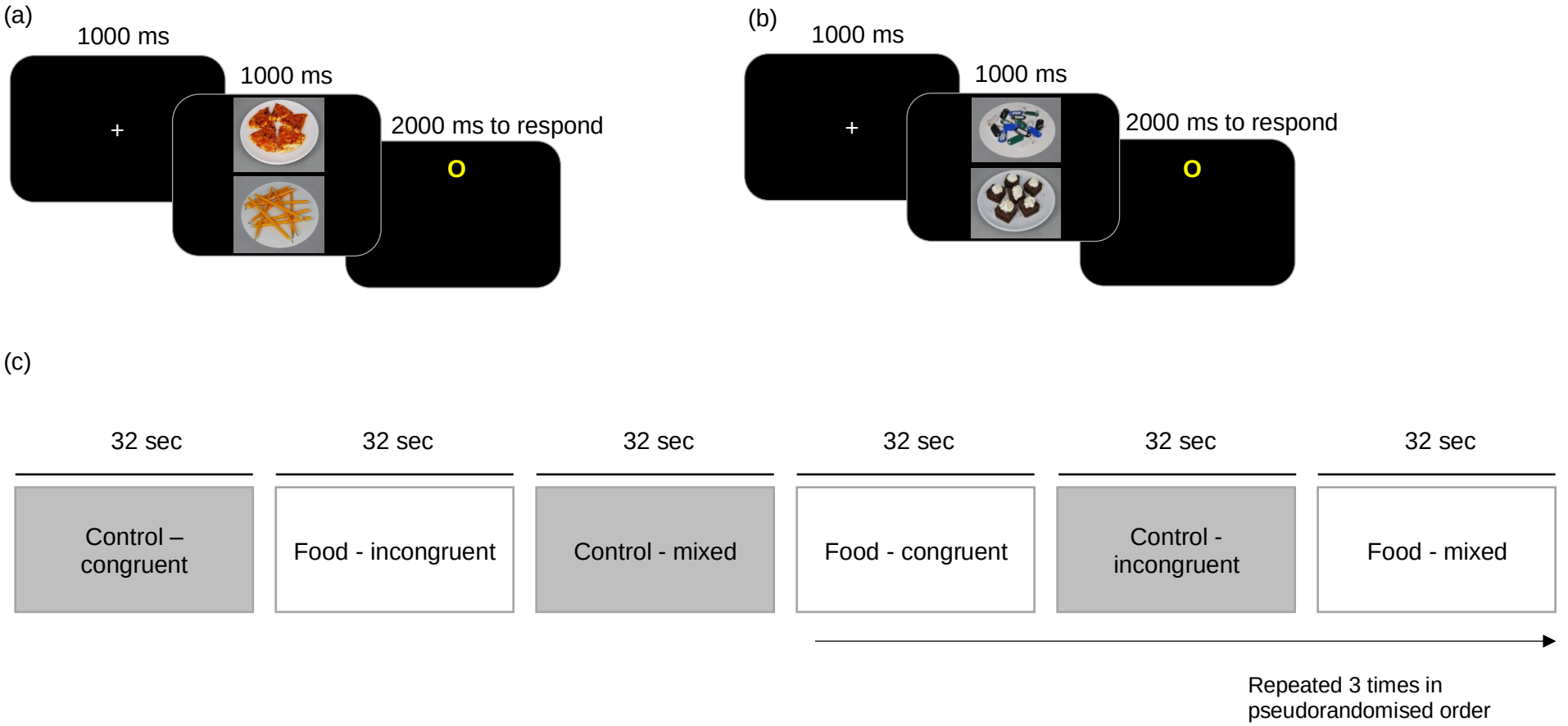
(stationery). The standardised set of food images from the Full4Health Image Collection was used (291), selected after a preliminary study in house. As a control condition, we included a control VPT where the picture pair consisted of two non-food images (tools and cosmetics).

A VPT trial begins with the presentation of a fixation cross (1000 ms), then a picture pair (food vs non food for the food VPT or non-food vs non-food for the control VPT) appears for another 1000 ms (one at the top and the other at the bottom of the screen). Immediately after the picture pair presentation a dot probe (a yellow circle on black background) appeared in either the location of the top or the bottom picture and remained for 2000 ms during this time participants were told to respond to the probe by pressing one of the two response keys to indicate dot probe position as quickly and accurately as possible and reaction time was recorded. Each trial was programmed to last exactly 4 sec. An attentional bias towards target stimuli (food) exists when there is faster detection of probes replacing such stimuli.

The VPT task was administered in a block design (**Figure 5.1)**. The blocks were: food congruent (the dot appears in place of the food image), food incongruent (the dot appears in place of the non-food image), food mixed (both congruent and incongruent trials), control congruent (the dot appears in place of the cosmetics – the selection of cosmetics as the 'target category' in the control task was random), control incongruent (the dot appears in place of the tools) and control mixed. Each block included 8 trials, and each block appeared three times in a pseudorandomised order. Total duration of the task was 10 min. The task was presented using Psychopy software (version 1.84.1) (293).

Figure 5.1 Illustration of the visual probe task. Example of a (A) food congruent trial, (B) food incongruent trial and (C) block design of the visual probe task.

In a food congruent trial the dot appears in the position of the food image, while in an incongruent trial the dot appears in the position of the non-food image.



5.3.2.5 Image analysis

MRI acquisition

Images were acquired with a 3 Tesla Philips whole-body MR scanner equipped with a standard head coil. The VPT sequence (whole brain T2* weighted images) was performed twice (before and 30 min after beverage ingestion) using a gradient-echo planar imaging (EPI) (TE=35 ms, TR=2500 ms, field of view=240 mm x 240 mm, 44 slices, slice thickness: 3.5 mm, voxel size 3 mm x 3mm x 3.5 mm). In total 240 volumes were acquired per run.

The *phys*MRI sequence (whole brain T2* weighted images) was performed using EPI and had the following parameters: TE=35 ms, TR=2500 ms, field of view=240 mm x 240 mm, 43 slices, slice thickness: 3.75 mm, voxel size: 3.75 mm x 3.75 mm x 3.75 mm). In total 960 EPI images were acquired.

A high-resolution T1-weighted structural image was also acquired for each participant to examine for any structural abnormalities.

Pre-processing

Spatial pre-processing and analysis of imaging data were performed using SPM12 (Wellcome Trust Centre for Neuroimaging, London, UK), implemented in MATLAB (Mathworks, R2019a). Images were firstly realigned using the first image as a reference, then spatially normalised into a standard stereotactic MNI space using SPM templates and then smoothed using a Gaussian kernel filter of 8 x 8 x 8 mm.

The ARtifact detection Tools (ART) toolbox (http://www.nitrc.org/projects/artifact_detect/) for SPM was used to determine movement artefacts in the scanner. We defined outliers as time points in which framewise global signal deviated more than 3 SDs from the mean and/or the framewise motion derived from the realignment parameters was greater than 1 mm. Exclusion criteria was more than 15% of outliers in each imaging sequence, including the 10 min *phys*MRI baseline period. On this basis, data from three participants were removed from the *phys*MRI dataset and data from one participant were removed from the task-fMRI dataset.

physMRI analysis

First level analysis was performed using the p-block *phys*MRI analysis technique (224, 225), on each subject for each study condition in the following way: the *phys*MRI scans were divided into 20 consecutive 2 min time bins (T01 to T20; T01 – T05: baseline, T06 – T10: drinking, T11 – T20: postprandial), in order to investigate the activation changes over time due to beverage consumption. We did not include in the analysis the T06-T10 time bins due

to excessive head movement during that period. The 48 scans from the time bin immediately prior to beverage ingestion (T05) formed the baseline time bin ($T_{baseline}$). In each subject and condition, the signal averages for the 10 post-ingestion time bins (T11-T20) were separately compared to the baseline average ($T_{baseline}$) using regression within the general linear model framework. This resulted in 10 first level images corresponding to the BOLD change from baseline in each successive post-infusion time bin for each subject and condition, which were then used as input to the second level of group-wise analysis. Contrast maps for each time bin were calculated for main effect of taste ([stevia - water] + [glucose - maltodextrin]), main effect of calories ([glucose - stevia] + [maltodextrin - water]) and the interaction taste-by-calories ([stevia – water] – [glucose – maltodextrin]) for each subject.

To determine whether statistically significant increments in the BOLD signal change from baseline across subjects occurred over time, three repeated-measures ANOVA were conducted, one for the interaction of taste-by-time, one for the interaction of calories-by-time and one for the interaction of taste-by-calories-by-time. Whole brain analysis was performed and clusters exceeding $P_{FWE\text{-}cluster}<0.05$ for cluster extent at a height uncorrected threshold of $P=0.001$ were considered significant. Beta values were extracted from the significant clusters (mean signal from each cluster) in order to create the time-course graphs that depict the response to each study treatment.

Moreover, in order to summarise the BOLD over time across the brain for the 3 interactions separately, we applied a more conservative correction for multiple comparisons of peak-level $P_{FWE}=0.05$. Beta values from a mask including all voxels surviving $P_{FWE}=0.05$ were extracted in order to create the time course graphs for each beverage. Incremental area under the curve (iAUC) was calculated for each beverage condition and separated into 2 time bins (10-20 min and 20-30 min). Additional statistical analysis on iAUC using repeated measures ANOVA with beverage type and time as factors was investigated with appropriate post-hoc tests corrected with Bonferroni criterion for multiple comparisons using SPSS (IBM SPSS Statistics Version 23).

Task based fMRI analysis

For the VPT, we modelled the onset of the VPT stimuli for each beverage condition separately and then created contrasts of interest which were: all food trials > all control trials (both post-consumption), food incongruent trials > food congruent trials (both post-consumption), and the respective post > pre consumption contrasts. Data were high pass filtered at 128 sec. Then we created contrast images for each predefined contrast of interest for the study treatments comparisons corresponding to the main effect of taste ([stevia - water] + [glucose - maltodextrin]), main effect of calories ([glucose - stevia] + [maltodextrin - water]) and the interaction taste-by-calories ([stevia – water] – [glucose – maltodextrin]).

In the second level analysis, we performed a one-sample t-test with whole brain analysis. As with the *phys*MRI, clusters exceeding $P_{FWE\text{-}cluster}<0.05$ for cluster extent at a height uncorrected threshold of $P=0.001$ were considered significant. To illustrate the differences between the beverages in areas that showed significant change in the BOLD signal in response to the main effect of taste, calories and the interaction taste-by-calories, we extracted the mean signal from anatomical masks of the significant clusters for each beverage condition. For illustration purposes and to further follow up significant results, repeated measures ANOVA was performed on the extracted beta values in SPSS.

We also examined the main effect of trial type (food trials > control trials) to investigate whether areas that were expected to activate in response to visual attention to food cues compared to the control task were actually activated independently of the beverage type (whether the paradigm worked). This was conducted via the creation of average contrast images ([water + stevia + glucose + maltodextrin]/4) for the contrasts all food trials > all control trials pre, post and post>pre consumption and then performing one-sample t-tests in SPM.

The Anatomical Automatic Labelling toolbox (AAL) was used for anatomical labelling of all results.

5.3.2.6 Statistical analysis of behavioural data

Non-imaging data were analysed in IBM SPSS Statistics Version 23. Data are presented as mean ± SEMs, unless otherwise stated. For the VPT analysis incorrect responses as well as reaction times (RTs) that were ± 3 SDs from the mean were removed. Participants who had >10% incorrect and/or slow responses were excluded. Mean RTs to congruent and incongruent trials (separately for food and control trials) was calculated for each condition and each VPT task (pre- and post- consumption). Attentional bias to food cues was calculated by the following formula: RT_{mean} to food incongruent trials – RT_{mean} to food congruent trials (using all trials from the congruent, incongruent and mixed blocks).

VAS for hunger and fullness were analysed as change from baseline values, AUC were calculated using the trapezoidal rule. These data were analysed using repeated measures ANOVA with beverage type and time (-10, 0, 10, 20, 30 min) as within-subjects variables. Significant interactions revealed by ANOVA were then investigated using post-hoc comparisons and Bonferroni's correction for multiple comparisons. Not-normally distributed data were analysed with appropriate non-parametric statistics. Specifically, sweetness ratings were not normally distributed therefore a Friedman test was conducted, followed by Wilcoxon pairwise tests and Bonferroni correction.

5.4 Results

5.4.1 Participants

Eighteen participants completed all 4 imaging sessions, however due to exclusions described above fifteen participants' data were included in the *phys*MRI analysis and seventeen participants' data were included in the VPT fMRI analysis. A detailed participant flow chart can be found in **Supplemental Figure 5.2**. Participants' characteristics are given in **Table 5.1**. Before the start of each imaging session participants were asked to rate their mood. No significant differences were observed in participant's mood across the imaging sessions, results are given in **Supplemental Table 5.1**. Thirst ratings were significantly decreased at the end of each imaging session, with no differences between them.

Table 5.1 Subjects' characteristics.

	n = 18
Age (years)	26 ± 5
Weight (kg)	60.1 ± 11.8
Body mass index (kg/m^2)	21.5 ± 2.1
Height (cm)	166 ± 9
Waist circumference (cm)	71.6 ± 7.2
Hip circumference (cm)	95.3 ± 9.9
Dutch Eating Behaviour Questionnaire	
Restrained	1.7 ± 0.5
Emotional	2.0 ± 0.6
External	2.9 ± 0.6
Three Factor Eating Questionnaire	
Cognitive restraint	3.7 ± 2.4
Disinhibition	3.4 ± 2.0
Hunger	3.8 ± 2.3

Values are means ± SDs.

5.4.2 Appetite and sweetness ratings

Participants rated their subjective sensation of hunger and fullness at 10 min intervals while being scanned. A repeated measures ANOVA with beverage type (water, stevia, glucose, maltodextrin) and time (-10, 0, 10, 20, 30 min) as within-subjects variables was conducted for hunger ratings (change from baseline values) and revealed a main effect of time ($F(2, 27) = 12.94$, $P < 0.001$, Greenhouse-Geisser). However, no effect of beverage type ($P = 0.129$) or interaction between beverage type and time ($P = 0.133$) (**Figure 5.2 A, B**) was demonstrated. Similar analysis was conducted for the fullness ratings and revealed a significant main effect of time ($F(2, 25) = 26.20$, $P<0.001$, Greenhouse-Geisser) and a significant interaction between beverage type and time ($F(6, 94) = 3.39$, $P = 0.006$, Greenhouse-Geisser). Post hoc tests revealed a significant increase in fullness ratings at 20 and 30 min following the consumption of the glucose beverage compared to water beverage ($P = 0.016$ and $P = 0.047$ at 20 and 30 min respectively), however there was no difference in fullness between the stevia, glucose and maltodextrin beverages (all $P>0.05$).

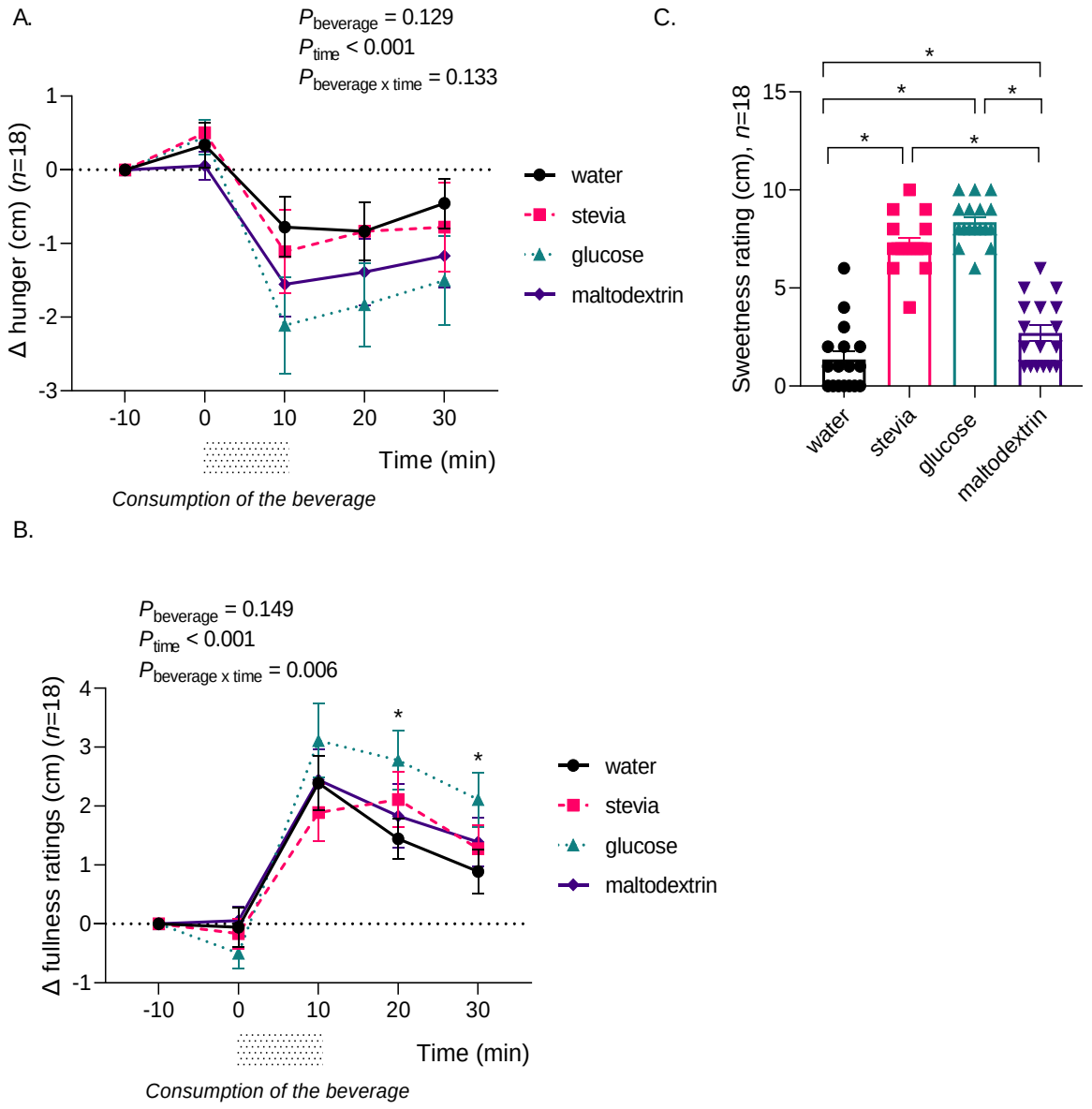
Participants were asked to rate the sweetness of the beverage they consumed immediately after the end of the drinking period (10 min). Results are given in **Figure 5.2 C** and showed that the glucose and stevia beverages were perceived as significantly sweeter compared to the water and maltodextrin beverages (all $P<0.001$), and maltodextrin slightly but significantly sweeter than water ($P=0.02$). There was no significant difference in perceived sweetness between the glucose and the stevia beverages in line with the design of the study.

5.4.3 Visual probe task

A 4x2x2 repeated measures ANOVA was conducted with beverage type, time (pre and post beverage consumption) and congruency (congruent, incongruent trials) as within-subjects variable and reaction time as the dependent variable. There was no significant main effect of beverage type, time (pre and post beverage consumption), congruency or a significant interaction. We calculated attentional bias to food cues for each beverage condition pre and post consumption, no significant differences were observed. Similar analyses were conducted for the control condition, results showed that there were no significant differences in the control trials (cosmetics vs tools).

Figure 5.2 Hunger (A), fullness (B) and sweetness ratings (C) following the consumption of water, stevia, glucose and maltodextrin beverages.

*P<0.05 (in panel B the asterisk indicates P<0.05 between water and glucose).



5.4.4 Neuroimaging results

5.4.4.1 physMRI

Areas where BOLD signal changed in response to the interaction taste-by-time

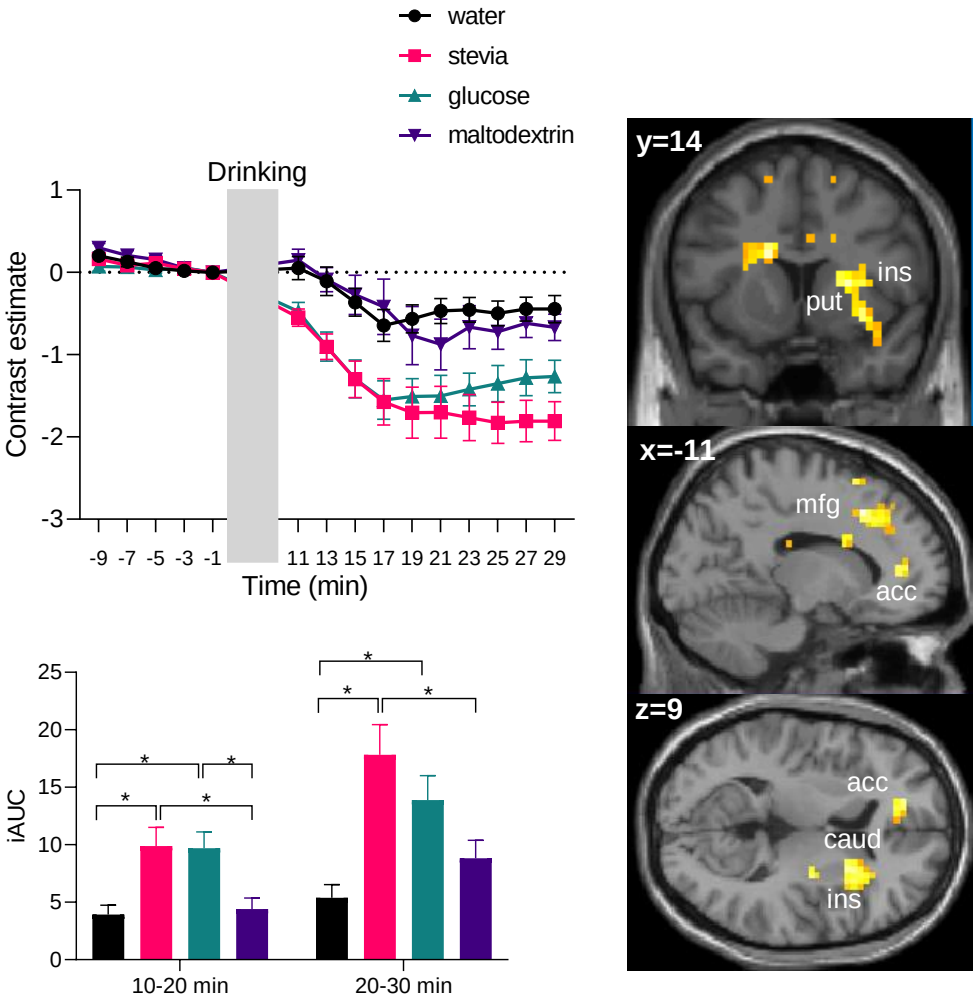
Results of the one-way ANOVA investigating the differences in BOLD signal responses over time following the ingestion of the sweet beverages (stevia, glucose) relative to the non-sweet beverages (glucose, maltodextrin) are presented in **Table 5.2**. The cluster extent of 5 clusters was significant. The clusters were observed in the right putamen, superior and middle frontal gyrus, insula, inferior frontal gyrus, anterior and middle cingulate cortex, right supramarginal gyrus and inferior parietal lobule and right fusiform/hippocampus. In these clusters the BOLD signal response following stevia and glucose was significantly lower compared to water and maltodextrin. Time-course graphs showing the BOLD response over time for each beverage are presented in **Supplemental Figure 5.4.**

Figure 5.3 presents the time-course graph of the mean beta values from all activated voxels in response to the interaction of taste-by-time, which survived a more conservative peak threshold corrected for multiple comparisons of P_{FWE}=0.05. Calculation of the iAUC in two time bins, 10-20 min and 20-30 min post consumption, showed that the effect of taste was apparent in the 10-20 min time bin, with stevia and glucose showing a higher iAUC compared to water and maltodextrin. In the 20-30 min post consumption stevia maintains the difference from water and maltodextrin, but glucose does not (glucose iAUC is significantly different to water but not to maltodextrin), suggesting the possibility of a more persistent reduction of BOLD signal after the stevia beverage.

Table 5.2 Significant clusters exhibiting interactions of interest at *P*<0.001 (uncorrected), *n*=15. Regions were defined using the automatic anatomical labelling. ACC, anterior cingulate cortex; MNI, Montreal Neurological Institute.

Size at *P*<0.001	P_{FWE-C}	F	Region	MNI coordinates		
				x	y	z
Taste-by-time interaction						
1039	<0.001	8.12	Putamen_R	26	-11	13
		7.63	Frontal_Sup_L+R	-12	19	43
		7.38	Frontal_Sup_Medial_L	-8	27	39
		7.07	Insula_R	37	19	-14
		5.38	Cingulum_Mid_R+L	14	-18	39
113	<0.001	6.89	ACC_pre_L/ Frontal_Sup_L	-16	46	9
39	0.010	5.44	Insula_L	-35	19	-10
		3.54	Frontal_Inf_Orb_L	-27	16	-18
114	<0.001	5.28	Supramarginal_R/Parietal_Inf_R	52	-33	24
54	0.002	5.09	Hippocampus_R/Fusiform_R	33	-33	-6
Calories-by-time interaction						
1604	<0.001	10.50	Thalamus_L+R	-8	-3	13
		10.10	Lingual_L+R	29	-56	-6
		9.17	Calcarine_L+R	-16	-82	9
		7.01	Cerebellum_L+R	7	-45	-3
217	<0.001	6.35	Frontal_Inf_Oper/Tri_R	22	-15	31
		6.22	Insula_R/ Rolandic_Oper_R	37	-3	13
56	0.001	6.27	Postcentral_L	-1	16	46
40	0.008	6.03	Hippocampus_L/Parahippocampal_L	-1	-60	58
30	0.027	5.99	Frontal_Sup_R	33	53	9
34	0.017	5.46	Suppl_Motor_Area_L+R	-1	16	46
38	0.010	5.46	Precuneus_L+R	-1	-60	58
75	<0.001	5.14	Angular_L/Parietal_Inf_L	-35	-67	39
50	0.003	4.85	Putamen_L	-27	-3	-10
Taste-by-calories-by time interaction						
137	<0.001	8.63	Postcentral_R/Precentral_R	44	-15	46
305	<0.001	8.04	Suppl_Motor_Area_L+R	-1	1	54
		7.91	Cingulum_Mid_L+R	-12	-7	35
		5.40	Frontal_Sup/Mid_L	-23	12	54
39	0.006	5.70	Heschl_L	-21	-26	5
100	<0.001	5.69	Postcentral_L/Precentral_L	-38	-11	43
24	0.044	5.12	Insula_L	-35	12	1

Figure 5.3 Mean BOLD signal response for the interaction taste-by-time across all voxels that survived a peak-level correction for multiple comparisons at a threshold of P_{FWE}<0.05.
Brain sections show significant activations from the whole brain analysis (P_{FWE}<0.05 corrected), bar graph shows the incremental area under the curve separated in two time bins, 10-20 min and 20-30 min after beverage consumption. *P<0.05, applying Bonferroni correction. acc, anterior cingulate cortex; mfg, medial superior frontal gyrus; caud, caudate; ins, insula; put, putamen.



Areas where BOLD signal changed in response to the interaction calories-by-time

Results of the one-way ANOVA investigating the differences in BOLD signal responses over time following the ingestion of the caloric (glucose, maltodextrin) relative to the non-caloric beverages (water, stevia) are presented in Table 5.2. The cluster extent of 9 clusters was significant. These clusters were observed in the thalamus, calcarine cortex, lingual gyrus, precuneus, cerebellum, right inferior and superior frontal gyrus, right rolandic operculum/insula, left postcentral, left putamen, left hippocampus, supplemental motor area and left angular gyrus. The average time courses for each significant cluster are presented in **Supplemental Figure 5.5.**

Figure 5.4 presents the time-course graph of the mean beta values from all activated voxels in response to the interaction calories by time, which survived a more conservative peak threshold corrected for multiple comparisons of P_{FWE}=0.05. In the first 10-20 time bin iAUC for glucose and maltodextrin was significantly higher compared to water, but there was no difference between stevia and the other beverages. In the 20-30 min time bin, there is no longer a significant difference between the water and the caloric beverages, since the BOLD signal in the glucose and maltodextrin conditions tends to return to preprandial values as illustrated in the time course graph. Moreover, in the last 5 min time bin (25-30 min) the iAUC is significantly different between water and stevia.

Areas where BOLD signal changed in response to the interaction of taste-by-calories-by-time

Results of the one-way ANOVA investigating the differences in BOLD signal responses over time in response to taste-by-calories are presented in Table 5.2. The cluster extent of 5 clusters was significant. These clusters were observed in the precentral and postcentral gyrus, supplemental motor area, middle cingulate gyrus, left middle/superior frontal gyrus, insula, and left transverse temporal gyrus. The average time courses for the significant clusters are presented in **Supplemental Figure 5.6**.

Figure 5.5 demonstrates the time-course graph of the mean beta values from all activated voxels in response to the interaction taste-by-calories-by-time. In the first 10-20 min time bin iAUC for stevia and maltodextrin is significantly higher compared to water. In the second time bin 20-30 min the differences are maintained, and moreover the iAUC for stevia is also significantly different to glucose, with stevia showing a persistent BOLD signal response. Calculation of the iAUC for the 25-30 min postprandial period shows that stevia iAUC is also marginally significantly different to maltodextrin as well (P=0.06).

Figure 5.4 Mean BOLD signal response for the interaction calories-by-time across all voxels that survived a peak-level corrected for multiple comparisons threshold of P_{FWE}<0.05.
Brain sections show significant activations from the whole brain analysis (P_{FWE}<0.05 corrected), bar graph shows the incremental area under the curve separated in two time bins, 10-20 min and 20-30 min after beverage consumption. *P<0.05, applying Bonferroni correction. Calc, calcarine cortex; caud, caudate; cer, cerebellum; hip, hippocampus; ins, insula; lin; lingual gyrus; precu, precuneus; ro, rolandic operculum; thal, thalamus.

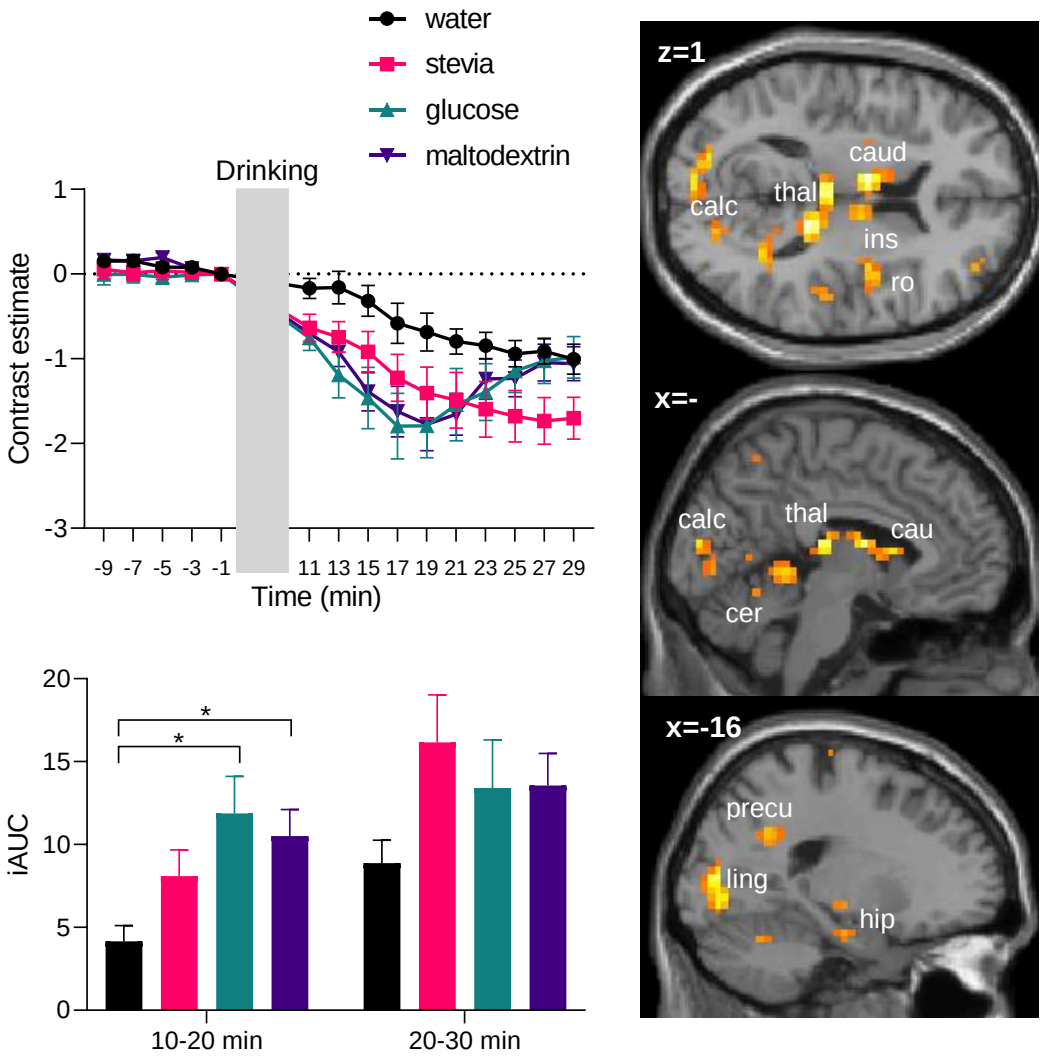
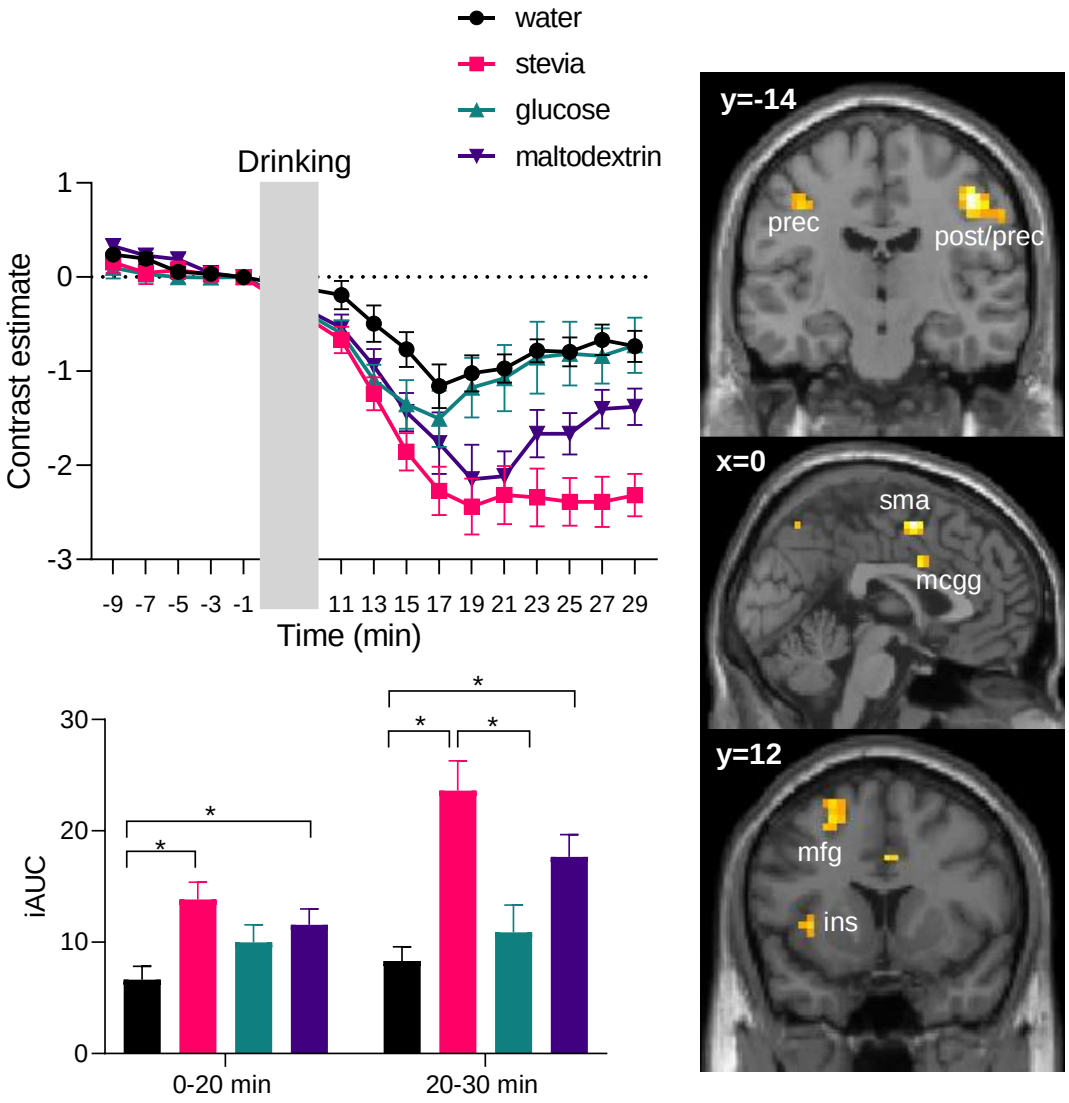


Figure 5.5 Mean BOLD signal response for the interaction of taste-by-calories-by-time across all voxels that survived a peak-level corrected for multiple comparisons threshold of P_{FWE}<0.05. Brain sections show significant activations from the whole brain analysis (P_{FWE}<0.05 corrected), bar graph shows the incremental area under the curve separated in two time bins, 10-20 min and 20-30 min after beverage consumption. *P<0.05, applying Bonferroni correction. ins, insula; mcgg, middle cingulate gyrus; mfg, middle frontal gyrus; post, postcentral gyrus; prec, precentral gyrus; sma, supplementary motor area.

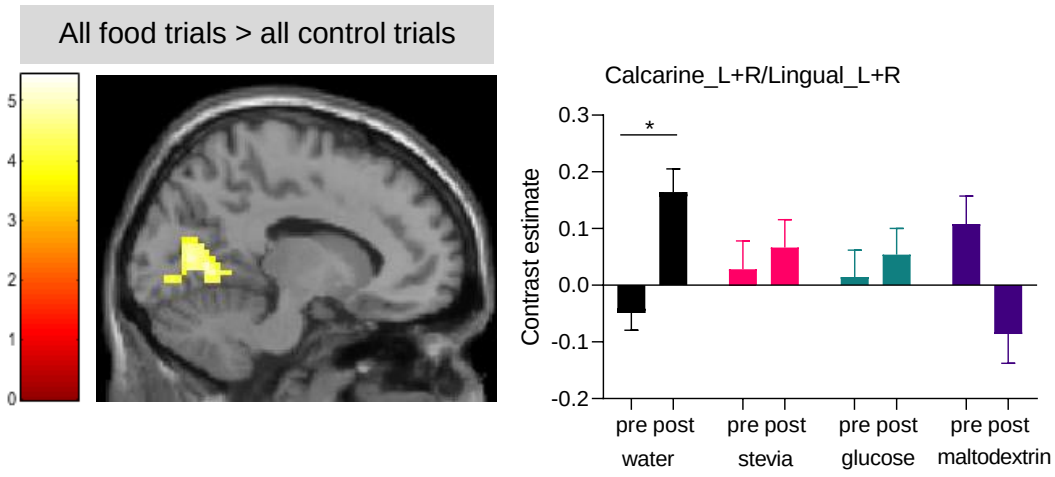


5.4.4.2 Task-based fMRI

We investigated the effect of beverages consumption differing in taste (sweet or not sweet) and caloric content (with or without calories) on the brain's response to a food visual-dot probe task and a control visual probe task before and 30 minutes after ingestion in healthy normal-weight participants. The contrasts of interest were: all food trials > all control trials, food incongruent trials > food congruent trials, post > pre (all food trials > all control trials) and post > pre (food incongruent trials > food congruent trials).

Results from the whole brain analysis of the VPT are summarised in **Table 5.3.** We observed a statistically significant differential BOLD response in a cluster including the calcarine cortex and lingual gyrus bilaterally in response to the taste-by-calories interaction for the contrast post > pre (all food trials > all control trials) (**Figure 5.6**). In particular, after the consumption of water BOLD signal was significantly increased in this cluster, however activity did not change significantly after consumption of the stevia, glucose and maltodextrin beverages. We did not observe any other significant differences in brain activation in response to main effect of taste or calories for any of the contrasts of interest.

Figure 5.6 Significant differential brain activation during the visual dot probe task compared to the control task post versus pre-consumption, in response to the interaction between taste and calories. Brain sections show significant clusters from the whole brain analysis (P<0.001, uncorrected), bar graph shows the average contrast estimate in arbitrary units (± SEM) at the significant cluster (n = 17); *P<0.05, Bonferroni correction applied.



We examined the main effect of trial type (all food trials > all control trials) to investigate whether areas that were expected to activate in response to visual attention to food cues were actually activated (Table 5.3). Results showed that in the pre-consumption state activation in response to food trials compared to control trials increased in a cluster including the left frontal inferior gyrus and decreased in a cluster encompassing part of the right fusiform and lingual gyrus. In the post beverage consumption state brain activity increased in the caudate, thalamus, superior frontal gyrus, angular gyrus, parietal inferior lobule (all bilaterally) and left amygdala and hippocampus in response to food trials compared to control trials.

Table 5.3 Regions demonstrating significant difference in brain activation in response to food trials vs controls trials pre and post beverage ingestion in healthy lean participants, *n*=17, threshold set at *P*<0.001 uncorrected (cluster-level). MNI, Montreal Neurological Institute.

Size at *P*<0.001	**P_{FWE-C}**	**z**	**Region**	**MNI coordinates**		
				x	**y**	**z**
Interaction taste-by-calories **Post > pre consumption (all food trials > all control trials)**						
184	<0.001	4.03	Calcarine_L+R/Lingual_L+R	-9	-64	4
Main effect of trial type: food trials > control trials **Pre beverage consumption**						
74	0.035	3.79	Frontal_Inf_Oper_L/ Frontal_Inf_Tri_L	-48	14	18
114	0.007	4.86	Fusiform_R/Lingual_R	27	-46	-14
Main effect of trial type: food trials > control trials **Post beverage consumption**						
321	<0.001	4.97	Caudate_L+R, Thalamus_L+R,	321	21	38
162	0.001	4.79	Frontal_Sup_L+R	162	15	41
205	<0.001	4.67	Angular_L	205	-36	-52
130	0.002	4.08	Angular_R	130	42	-55
135	0.002	4.55	Amygdala_L, Hippocampus_L	135	-18	-4
72	0.031	4.36	Temporal_Inf_L	72	-48	-64
80	0.021	3.92	Frontal_Mid_R	80	27	11

5.5 Discussion

We have demonstrated for the first time the whole brain response following the ingestion of a stevia-sweetened beverage along with appropriate controls for sweet taste and calories in a 2x2 design. We used a combination of *phys*MRI and task-based fMRI to examine (i) the brain signals derived solely from the ingestion and the subsequent physiological responses associated with beverage consumption and (ii) food-cue responses before and after beverages within the same study, in an attempt to examine both homeostatic and hedonic signals associated with sweet beverage consumption.

In summary, this study showed that sweet beverage consumption was associated with a greater attenuation of activity over time in areas involved in taste and reward processing compared to non-sweet beverages. In areas responding to caloric compared to non-caloric beverages over time including thalamic, visual, parietal and frontal areas among others, glucose and maltodextrin demonstrated a significant decrease in brain activity until 20 min after the consumption only compared to water, stevia showed a delayed and longer-lasting BOLD decrease. The interaction of taste-by-calories-by-time showed a more robust BOLD contrast decrease for stevia mainly in motor, frontal areas and insula, which was more apparent in the 20-30 min post consumption time window. In the food-cue task, the three sweet/caloric beverages (stevia, glucose, maltodextrin) showed attenuated response in the visual cortex in response to food compared to control trials, while water demonstrated an increased response post consumption.

Effect of beverages consumption on neural processing over time (physMRI)

In the present study, we demonstrated significant differential BOLD responses for sweet versus non-sweet beverages over time. The areas of the brain where BOLD contrast was reduced in response to sweet beverage ingestion over time included areas of the corticolimbic system associated with reward (prefrontal cortex, putamen, caudate) and gustatory-related areas with main representative the insula and cingulate cortex. In particular, immediately following the drinking period and for the duration of the MRI investigation, BOLD response shows a greater reduction for the sweet (stevia and glucose) compared to the non-sweet beverages (water and maltodextrin). The effect of taste was more apparent in the first 10-20 min postprandially, while in the 20-30 min stevia maintained the difference to both water and maltodextrin but glucose was different only to water.

Previous fMRI studies have demonstrated similar brain activation patterns during caloric and non-caloric sweeteners tasting in the primary taste processing areas (anterior insula, frontal operculum) but differential brain activity in reward-related areas (striatum, midbrain), which responded to caloric but not non-caloric sweeteners tasting, proposing that low-calorie sweeteners might be less rewarding (239, 240). However, a recently published

systematic review reported that commonly activated areas between caloric and non-caloric sweeteners are the insula/operculum, cingulate as well as the striatum and homeostatic areas (hypothalamus, brainstem) (339). It is important to note that the above studies have not examined the BOLD contrast response over time, but only the immediate effect of tasting. Our results show deactivation of the gustatory and reward areas by both glucose and stevia in the 30 min postprandial period, a long-lasting signal occurring potentially beyond oral sweet tasting. Our results also include deactivation of the prefrontal cortex, which could be linked to the oral consumption of the beverages.

The involvement of the oral and gut STRs could be associated with the differential response observed in these areas between the sweet and non-sweet beverages. A previous fMRI study reported that an initial taste of a high sugar food increases reward regions (caudate, OFC) response to anticipated intake of the particular food compared to when the taste is blocked by consumption of an STR antagonist (340), highlighting the importance of the oral taste perception in later brain responses. The upper gut also has receptors that respond to sweetness, and activation of these receptors has been demonstrated to stimulate glucagon-like peptide 1 (GLP-1) release ex-vivo in response to steviol glycosides (341) and glucose. GLP-1 is a gut peptide contributing to satiety. However, data from human clinical trials regarding the effects of stevia consumption on gut peptide release are limited. One study investigated the effects of intraduodenal stevia (rebaudioside A) infusion in healthy volunteers, showing no different plasma GLP-1 response post infusion (124), therefore no conclusions regarding the involvement of gut STRs and hormonal responses could be drawn. The possibility of stevia acting directly in the gut STRs via a mechanism not mediated by hormonal response cannot be discounted either.

In the comparison caloric versus non-caloric beverages over time, the significant clusters demonstrated the engagement of large visual, motor and parietal areas, thalamus, cerebellum, insula, hippocampus and prefrontal cortex. A previous fMRI study demonstrated that intragastric glucose infusion is associated with reduced BOLD signal response in the cerebellum, right fusiform, and lingual gyri, insula and putamen, left parahippocampal gyrus, temporal and thalamic regions, most of which overlap with our results in this comparison (caloric versus non-caloric beverages) (223). From the average BOLD signal response in all activated areas for the interaction calories-by-time, it is evident that the caloric beverages induce a BOLD signal decrease only in the 10-20 min post consumption and only compared to water as illustrated by the iAUC. After that point BOLD signal tends to return to preprandial values by 30 min post consumption and the difference to water is no longer significant. Stevia BOLD response in these areas did not differ to either water or caloric beverages, but a slower and more delayed BOLD decrease was noted.

The pattern of the response could be in line with the time course of calories from the beverages being processed, absorbed into bloodstream and when glucose is no longer in excess BOLD returns to pre-prandial levels. The peak decrease in BOLD contrast was demonstrated between 18-20 min post-consumption. Glucose and maltodextrin lead to similar increases in blood glucose and insulin concentrations according to previous research (232); in a previous study from our group, in which the same beverages were administered to healthy lean participants, blood glucose response to glucose and maltodextrin was still significantly increased compared to water and stevia at 30 min postprandially (334). In the preliminary pilot study (Supplemental Figure 5.1) the glucose beverage also showed higher blood glucose response at 30 min post-consumption compared to water and stevia. This might therefore suggest that the BOLD change in response to the caloric beverages occurs partly beyond the metabolic postprandial consequences and might have to do with a more direct energy-sensing mechanism.

The comparison of BOLD response to taste-by-calories over time led to less extensive activation compared to the other comparisons and involved mainly the primary somatosensory cortex (postcentral gyrus) and primary motor cortex (precentral gyrus), supplemental motor area, cingulate gyrus, middle/superior frontal gyrus and insula. From the average BOLD signal time course graph and the iAUC we concluded that the interaction in the first 10 min post consumption (10-20 min) was driven by stevia and maltodextrin eliciting a significant reduction in BOLD signal response over time compared to water, while in the 20-30 min stevia also shows a significant BOLD signal decrease compared to glucose (and marginally different to maltodextrin in the last 25-30 min time bin). In other words, stevia beverage consumption was associated with a persistent reduction in BOLD compared to the pre-consumption baseline.

The possibility of stevia having a specific effect in the brain cannot be excluded. The consumption of the stevia beverage induced a slower and more gradual reduction in BOLD signal response, which remained until at least 30 min post-ingestion. This was specific to stevia and may be due to its metabolic fate after ingestion. *In vivo* studies in animal models have shown that steviol glycosides are not metabolised in the upper gastrointestinal tract, but are degraded slowly in the lower gastrointestinal tract by colonic bacteria, leading to a long slow increase in portal and plasma levels of steviol or its metabolite (27). Steviol detection in portal plasma has been demonstrated to sustain over a period of hours (268). Future research could examine the brain response to the consumption of stevia beyond the 30 min period to investigate when the signal returns to baseline and the use of intragastric infusion would help to isolate the gut-to-brain signalling induced by stevia consumption.

Effect of study beverages consumption on neural processing during the food VPT

The consumption of water compared to all other beverages in this study (stevia, glucose, maltodextrin) led to significant increased activation in response to food trials (food versus non-food images) compared to control trials (non-food versus non-food images) in a cluster encompassing part of the visual cortex, including the calcarine cortex and lingual gyrus bilaterally.

Even though the visual cortex is not considered a direct modulator of appetitive responses, processing of visual stimuli is highly dependent on motivational factors. Visual cortex activation is apparent in studies that use visual cues to induce craving (342) and has been also associated with the motivational salience of food cues (i.e. high versus low calorie food cues) (177). Previous research has shown that there is strong modulation of the visual cortex by food cues even immediately after glucose ingestion (250) and up to 120 min postprandially (343), proposing that activation of visual cortex is also dependent on metabolic signals. Our results further confirm this finding, the increased BOLD in the visual areas observed after water consumption was not observed following the consumption of the caloric beverages (glucose, maltodextrin) and also the stevia beverage (providing only sweet taste). A recent fMRI study has also shown similar results following sucralose ingestion during a food decision task. Sucralose versus water led to decreased activation in a range of areas including the visual cortex (255). Given that visual cortex responds to metabolic state differences in response to food cues and that higher-value targets induce greater visual activation (344), we could hypothesise that altered salience of the food cues mediated the effect in the visual cortex. This could be interpreted as food cues being less salient after the consumption of the sweet (stevia), caloric (maltodextrin) or sweet and caloric (glucose) beverages compared to water.

The applied food cue paradigm elicited responses in regions involved in the reward-system, hedonic and visual processing of food cues. Performing the food task compared to the control task independently of the condition, resulted in activation of the striatum (caudate), superior frontal gyrus and angular gyrus bilaterally, left amygdala and hippocampus, left inferior temporal gyrus and part of the right middle frontal gyrus. These findings are in line with previous observations showing increased activation in brain networks included in the corticolimbic reward system in response to food cue exposure (prefrontal cortex, striatum, hippocampus, amygdala, insula) (178) as well as in parietal and temporal areas that are mostly involved in visual processing (170, 254). However, no differential brain response across the beverage conditions was demonstrated in whole brain analysis in any other area apart from the visual cortex.

Effect of study beverages consumption on appetite

The present study showed that there was no significant difference in hunger ratings across the beverages, but fullness ratings demonstrated an increase at 20 and 30 min post ingestion. In line with previous studies glucose, which provides both calories and sweetness, was successful in producing a significant increase in fullness sensation compared to water. On the contrary the stevia beverage did not suppress appetite sensations as previously observed (50, 334), nor did the maltodextrin beverage. However, it has been reported that sweet only and caloric only beverages produce inconsistent appetite responses compared to those after consumption of glucose and sucrose, which provide both sweetness and calories (125, 334). On top of that, multiple limitations should be taken into consideration when assessing appetite ratings within this study design. Firstly, participants were in supine position since beverage consumption occurred while in the MR scanner, and secondly assessment of appetite was performed while performing an MRI investigation, which does not resemble normal or even laboratory appetite assessment.

Limitations and future work

Among the limitations of this study is that we did not measure any metabolic markers like blood glucose, insulin or gut peptide concentrations. This was intentional in the design process due to the technical difficulties and disruptive nature of blood collection during an fMRI investigation, which in turn could also introduce a lot of noise in the acquired images. Participants were inevitably placed in supine position and this could have influenced gastric emptying and postprandial metabolic responses, and also the timing of the BOLD change that is sensitive to metabolic signal changes. However, we conducted a preliminary pilot to ensure that blood glucose time-course is different between nutritive and non-nutritive sweeteners while participants are lying flat when consuming the beverage and remain in this position for the duration of the scan. Including the oral phase of ingestion in the *phys*MRI has both advantages and disadvantages, but in our design taste was a key variable. Inclusion of the oral phase allows for cephalic and cognitive factors to occur, and most closely reflects actual ingestion of sweeteners. On the other hand, including the oral phase adds head movement due to swallowing, which was the main reason why we excluded the drinking part in the *phys*MRI analysis.

We did not observe brainstem or hypothalamus BOLD contrast differences across the conditions. A collection of previous fMRI studies have demonstrated deactivation of the hypothalamus following glucose administration (220, 223, 232) administration; NNS ingestion results in either no hypothalamic response (aspartame) (232) and only a transient deactivation of the hypothalamus (sucralose) (230). The hypothalamus is a region that is difficult to reliably image unless a brainstem specific sequence is used.

Despite their widespread use we are only beginning to understand the effects of NNS consumption response in the brain. Future work should focus on the potentially differential effects among different NNS type consumption on brain responses, as they have already been demonstrated to exert differential effects on body weight (78). Another other important avenue for future work will be to dissect the pure gut-to-brain signalling following the stevia beverage compared to appropriate controls. Direct infusion into the gut will shed light into which of the activations were due to the precedent oral sweet and which were derived solely from gut-derived signals. Moreover, future studies should also examine brain response to the stevia-sweetened beverage for a longer period beyond 30 min post consumption. There is also very little research conducted on the gut-hormonal response following stevia-consumption therefore combination of *phys*MRI with simultaneous measurement of gut hormones will provide a good starting point towards the explanation of the observed effects of stevia in the brain.

Conclusions

In summary, this study demonstrated attenuation of the brain response to both caloric and sweet beverages consumption, with stevia showing a more prolonged effect. All other beverages in this study demonstrated attenuated brain activity to food cues compared to water in the visual cortex post consumption. It seems unlikely that the brain response after stevia is solely driven by the brief event of sweet tasting in the mouth; other neurophysiological effects may be involved.

5.6 Acknowledgements

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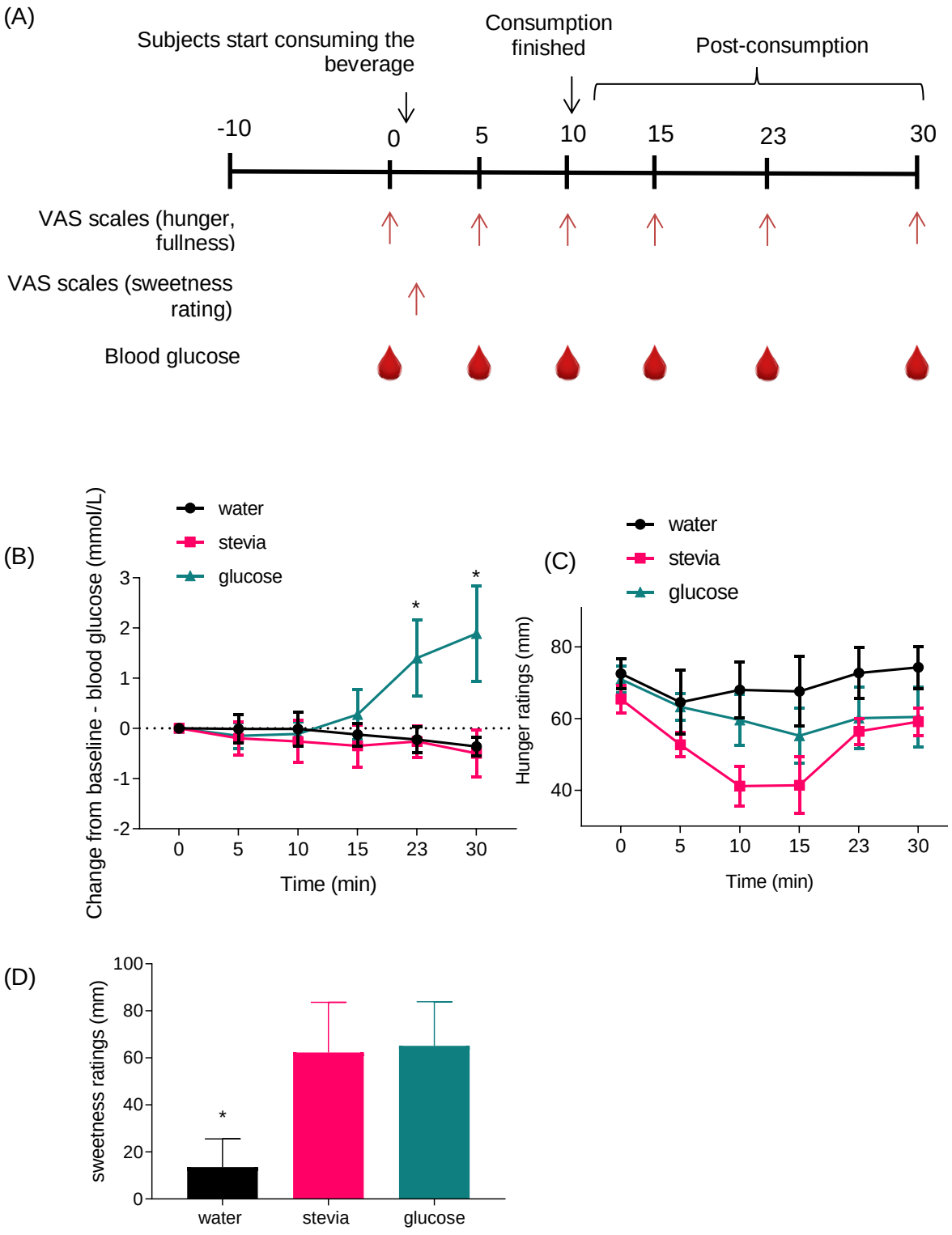
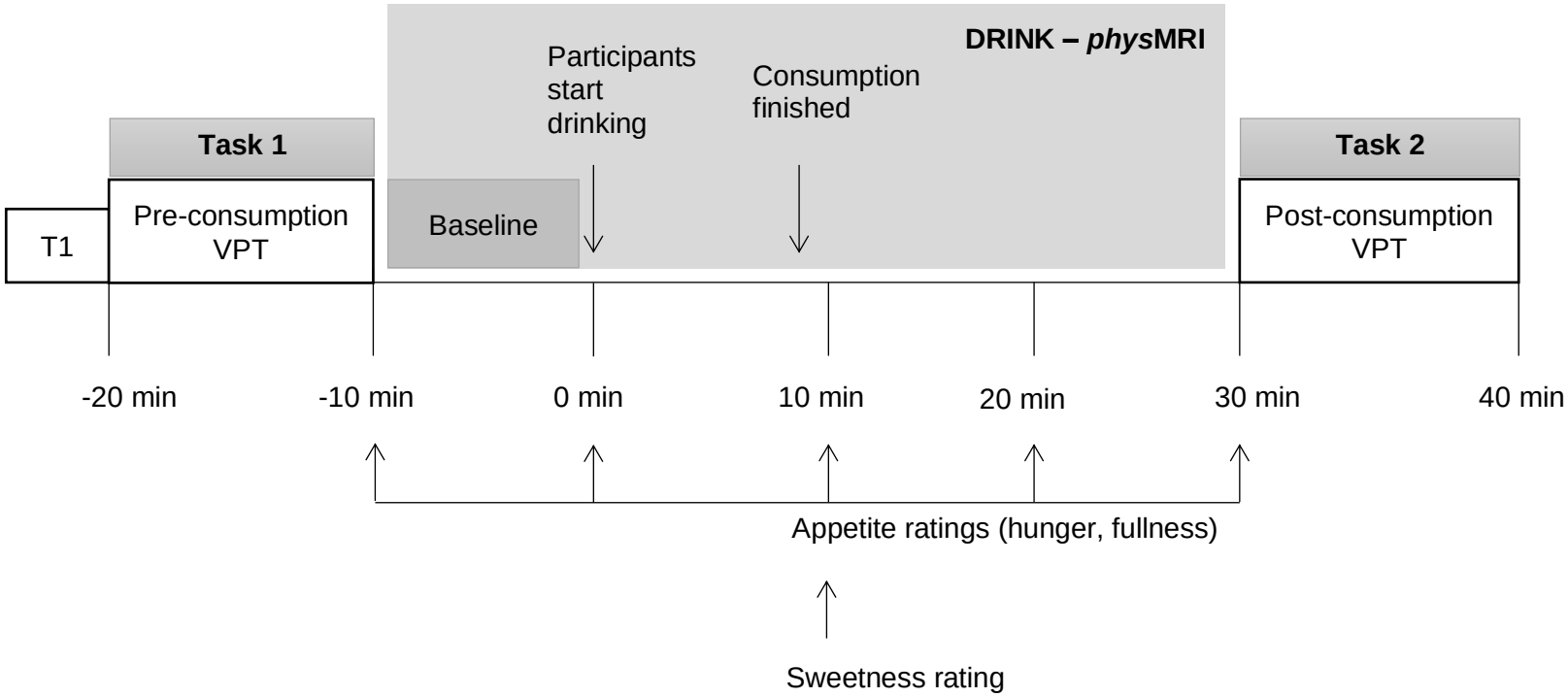
Supplemental Figure 5.1 (A) Protocol of the pilot study. (B) Blood glucose levels and (C) hunger ratings time-course following the consumption of water, stevia and glucose beverages while lying flat. (D) Sweetness ratings of beverages. *n*=8, **P*<0.05 vs others.

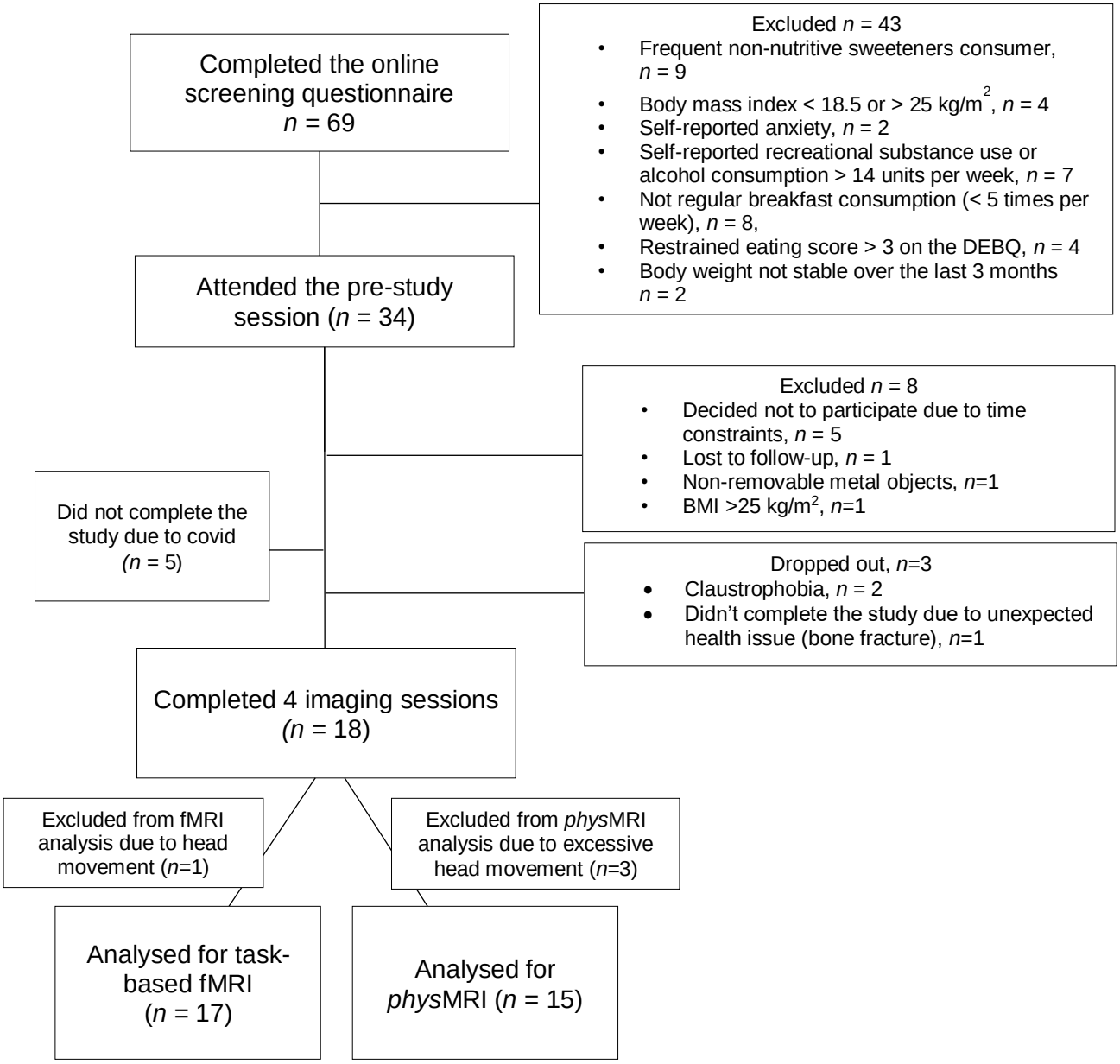


Supplemental Figure 5.2 Flow chart of a scanning session.



T1, structural scan; *phys*MRI, physiological Magnetic Resonance Imaging; VPT, visual-dot probe task.

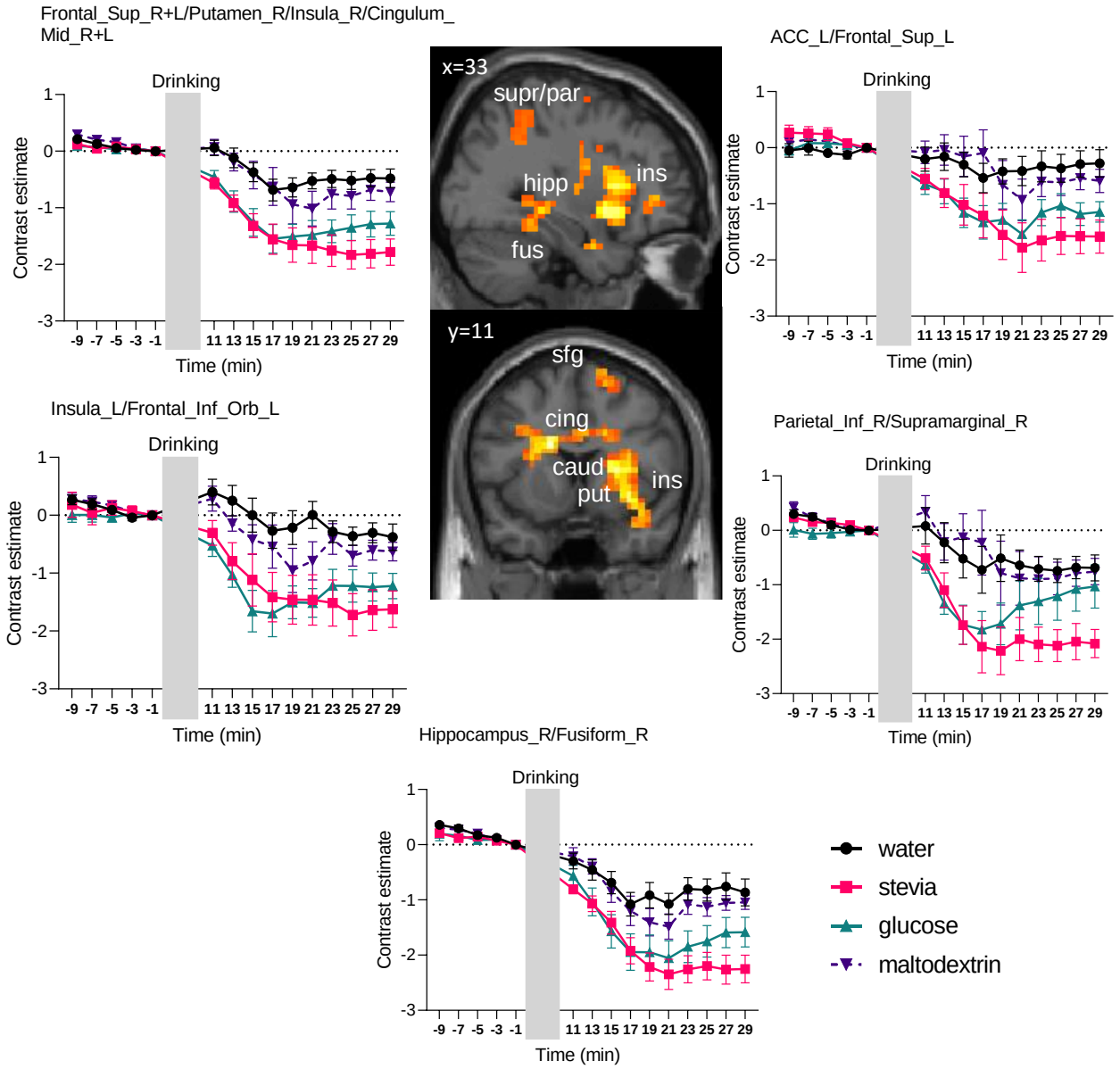
Supplemental Figure 5.3 Participant flow chart.



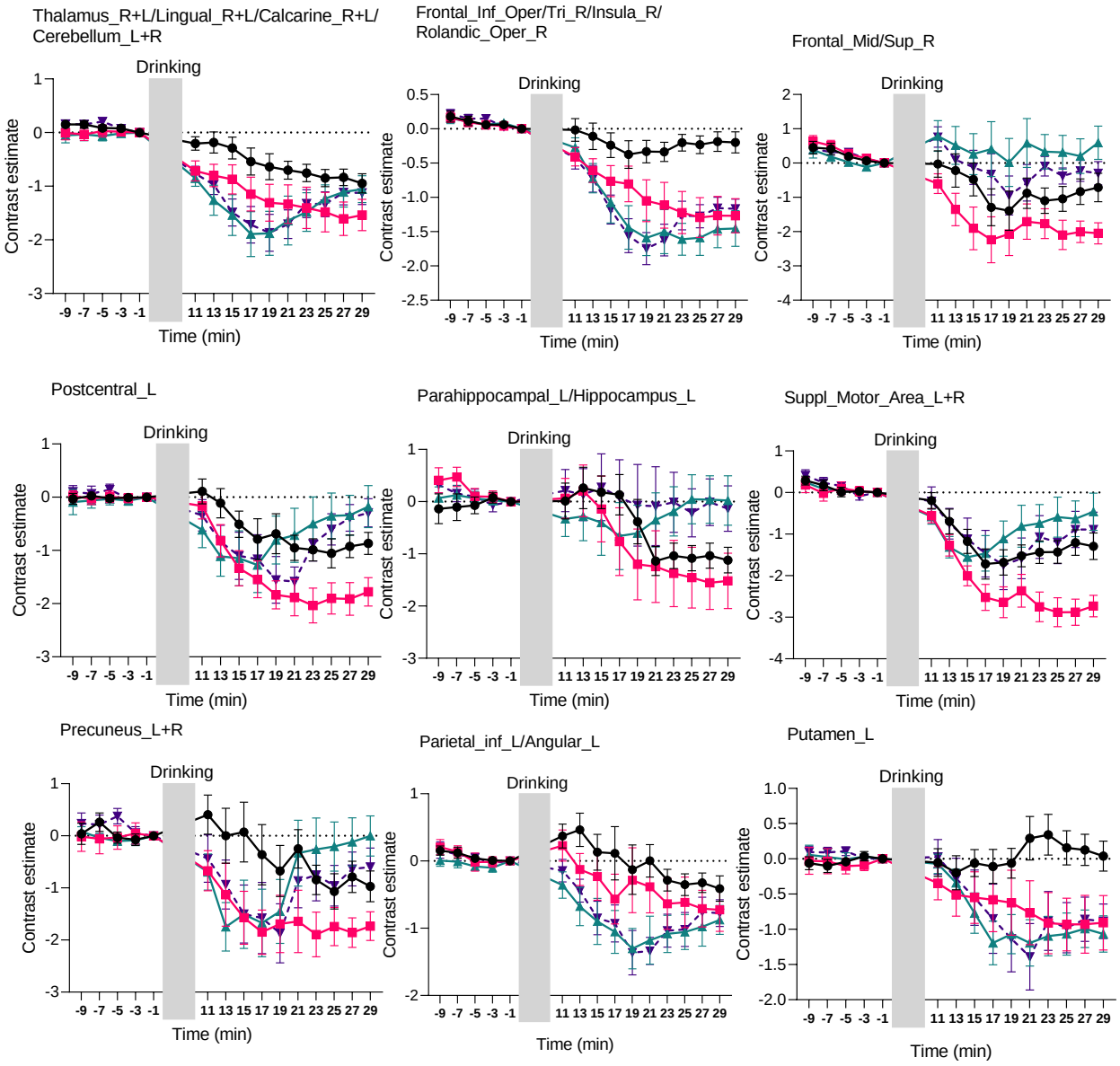
Supplemental Figure 5.4 Line graphs present changes in blood-oxygenation-level-dependent (BOLD) signal over time in the significant clusters following oral ingestion of the sweet (stevia, glucose) compared to the non-sweet beverages (water, maltodextrin), *n*=15.

Brain images show areas exhibiting significant effect of time for sweet versus non-sweet beverages. Results correspond to cluster extent P_{FWE}<0.05 at a height threshold of *P*<0.001 uncorrected.

Caud, caudate; cing, cingulate cortex; fus, fusiform gyrus; hipp, hippocampus; ins, insula; put, putamen; sfg, superior frontal gyrus; supr/par, supramarginal gyrus/ inferior parietal lobule.

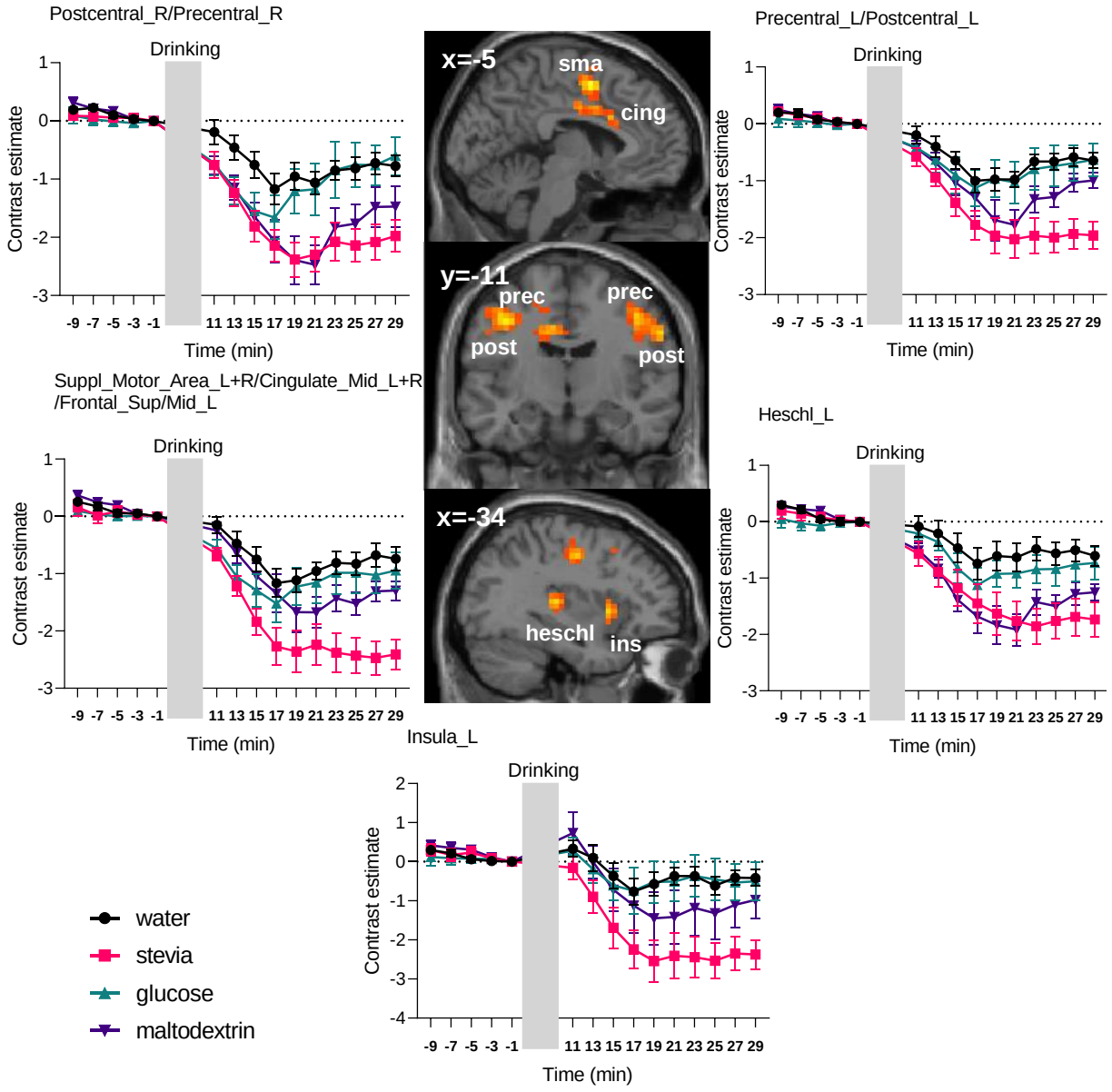


Supplemental Figure 5.5 Line graphs present changes in blood-oxygenation-level-dependent (BOLD) signal over time in selected clusters that showed a significant effect of time in the comparison of caloric (glucose, maltodextrin) compared to the non-caloric beverages (water, stevia), *n*=15. Brain overlays show areas exhibiting significant effect of calories-by-time at cluster extent P_{FWE}<0.05 at a height threshold of *P*<0.001 uncorrected. Calc, calcarine cortex; caud, caudate; cereb, cerebellum; ling, lingual gyrus; mfg, middle frontal gyrus; prec, precuneus; ro/ins, rolandic operculum/insula; sma, supplementary motor area; thal, thalamus.



Supplemental Figure 5.6 Line graphs present changes in blood-oxygenation-level-dependent (BOLD) signal over time in clusters that showed a significant interaction of taste-by-calories-by-time, *n*=15.

Brain overlays show areas exhibiting significant effect of taste-by-calories-by-time at a cluster extent P_{FWE}<0.05 at a height threshold of *P*<0.001 uncorrected. Cing, cingulate cortex; heschl, Heschl's gyri or tranverse temporal gyrus; ins, insula; post, postcentral gyrus; prec, precentral gyrus.



Supplemental Table 5.1 Participants' mood rating before the start of each imaging session, *n*=18. *P* values correspond to repeated-measures ANOVA with beverage type as within-subjects variable.

	Water	Stevia	Glucose	Maltodextrin	*P* value
Clear headed	73.56 ± 3.53	75.50 ± 3.62	74.94 ± 3.68	70.06 ± 4.46	0.384
Energetic	67.44 ± 4.54	70.11 ± 4.21	68.72 ± 4.33	62.94 ± 4.82	0.271
Happy	76.22 ± 2.93	79.56 ± 2.91	76.11 ± 4.41	73.06 ± 3.82	0.235
Tired	39.56 ± 5.01	42.33 ± 5.65	44.00 ± 5.75	45.06 ± 5.37	0.848
Relaxed	69.44 ± 4.25	71.61 ± 3.42	72.83 ± 4.18	69.44 ± 4.25	0.737

CHAPTER 6

General discussion

Public health policies around the world aim at reducing the amount of sugars in the human diet as part of the strategies to reduce overweight and obesity. Understanding the mechanisms mediating the effects of NNS on appetite, food intake and health is of major nutritional and clinical importance, as their wider use could constitute a promising strategy for the development of healthier food products in the prevention and treatment of obesity. Recent data from the UK show that consumers have a high-risk perception of NNS that leads to lower consumption and limited acceptance (345). Therefore, more research into the effects of NNS consumption effects on human eating behaviour and health should be conducted to inform the public as well as health organisations towards implementing new food policy strategies.

A dichotomy in science, and in public opinion, has been created on the impact of NNS use on appetite, body weight and health. Chapter 1 focused on reviewing the conflicting literature behind NNS use on human appetite and health. It is now becoming increasingly clear that replacing caloric sugars with NNS elicits benefits on energy intake and body weight (66, 77). In addition, consumption of NNS does not affect acute postprandial metabolism, including glycaemia and insulinaemia when consumed alone or as part of a meal (13), despite early concepts that proposed metabolic dysregulations mainly due to the uncoupling of sweet taste and calories. However, the review in Chapter 1 also highlighted the need for more well-designed RCTs that will take into account the complex mechanisms involved in appetite control including both homeostatic and hedonic mechanisms.

Therefore the main aim of this thesis was to establish whether nutritive and non-nutritive sweet tastants (stevia) have equivalent or different effects on several key measures of homeostasis and hedonics, including physiological measures (glycaemia, insulinaemia), appetitive measures (subjective appetite ratings, food intake), food-cue neurocognitive responses (reaction time paradigms such as the VPT) and subsequently in brain responses assessed via fMRI.

Stevia was selected for all the studies, due to its plant-origin and consumer preference shift towards natural products the latest years. Overall, the findings of this thesis support a beneficial role of stevia consumption on human eating behaviour, while no adverse metabolic effects were demonstrated. A summary of the study designs and major findings of the studies presented in this thesis is presented in **Table 6.1**. Following that, I discuss the major findings of the thesis in relation to the most recent literature and their implications for the future directions in the field.

Table 6.1 Summary of the study designs and main findings of the studies presented in this thesis.

	Subjects	Duration	Design	Protocol/Treatments	Outcomes assessed	Main findings
Chapter 2	*n*=20 (24±5 years, 21.3±2.2 kg/m^2) *n*=22 (24±4 years, 27.8±3.3 kg/m^2)	Acute	Randomised controlled crossover with 2 treatments	Completion of a battery of food-cue tasks including a VPT, SRCT and an IAT either in a fasted state after overnight fast or in a fed state, 30 min after consumption of a standardised breakfast in the laboratory.	• Performance on food-cue tasks (reaction times to the VPT, SRCT and IAT) • Subjective appetite ratings • Blood glucose levels	• Significant main effect of metabolic state (fasted-fed) on attentional bias to food cues assessed via VPT. • No main effect of metabolic state on SRCT or IAT. • No interaction between metabolic state and participant group in performance of the VPT, SRCT, or IAT.
Chapter 3	*n*=20 (27±5 years, 21.8±1.5 kg/m^2)	Acute	Randomised double-blind crossover controlled with 5 treatments	After a 3-4 h fast, participants consumed a study beverage and 30 min after beverage ingestion they were offered a buffet meal. 1. 330 mL water 2. 330 mL water + 240 ppm stevia 3. 330 mL water + 40 g glucose 4. 330 mL water + 40 g sucrose 5. 330 mL water+40 g maltodextrin	• Appetite ratings • Food/Energy intake • Blood glucose levels • AB to food cues via a VPT	• Stevia beverage led to significantly lower AUC for appetite ratings compared to water, as caloric beverages also did. • Stevia led to significantly lower total energy intake (meal + beverage calorie content) compared to water. • Only caloric beverages increased blood glucose. • No significant main effect of beverage type on AB to food cues.
Chapter 4	<u>Stevia group:</u> *n*=14, 25±6 years, 21.7±1.8 kg/m^2, <u>Control group:</u> *n*=14, 25±4 years, 20.7±1.5 kg/m^2)	12 weeks	Randomised controlled open-label 2-parallel-arm	Stevia group were instructed to consume daily 5 drops of a stevia product in their habitual beverages (before lunch and before dinner) Control group was not given any treatment.	• Glucose and insulin response to an OGTT • Body weight • Energy intake	• No significant differences in glucose or insulin response to an OGTT between groups. • Significant main effect on body weight change (control group showed increase in body weight but not the stevia group). • Energy intake significantly decreased at week 12 compared to baseline in the stevia group.
Chapter 5	*n*=18 (26±5 years, 21.5±2.1 kg/m^2)	Acute	Randomised double-blind crossover controlled with 4 treatments	After a 3-4 h fast, participants attended an imaging session that included the ingestion (in the MR scanner) of the following beverages: 1. 330 mL water 2. 330 mL water + 240 ppm stevia 3. 330 mL water + 40 g glucose 4. 330 mL water + 40 g maltodextrin	• BOLD contrast over time following the consumption of study beverages (*phys*MRI) • BOLD contrast while performing a food VPT before and after beverage consumption (task-based fMRI) • Hunger and fullness ratings	• Stevia demonstrated a longer-lasting and more robust BOLD decrease over time compared to other beverages. • Stevia and caloric beverages (glucose, maltodextrin) showed attenuated BOLD response to food compared to control trials in the visual cortex compared to water beverage. • Only the glucose beverage significantly decreased hunger and increased fullness ratings.

AB, attentional bias; AUC, area under the curve; BMI, body mass index; BOLD, blood-oxygenated-level-dependent; VPT, visual probe task; SRCT, stimulus response compatibility task; IAT, implicit association task; OGTT, oral glucose tolerance test.

- **Attentional bias to food cues is dependent on metabolic state, and specifically higher in the fasted state compared to the fed state, but is independent of weight status.**

Chapter 2 was an off-line piece of work that aimed to help design next phase studies. The findings of this research further support the long-standing hypothesis that food stimuli attract visual attention more than neutral (non-food) stimuli under conditions of fasting, and AB to food cues reflects underlying appetitive motivation, as indicated by the incentive sensitisation theory (194). However this response was independent of participants' weight status, opposed to our original hypothesis.

The findings of this study were confirmed by a recently published systematic review and meta-analysis concluding that food-related AB is sensitive to changes in the motivational value of food (198). However, this meta-analysis demonstrated a stronger association between subjective craving and AB to food cues, while the association with hunger was weaker. This could be attributed to the different designs used to differentiate motivational/nutritional state, which in some cases might not have induced clearly separable differences between fasted and fed states. A strength of our study was the fundamental approach of separating the fasted from the fed in a crossover and counterbalanced fashion. We also demonstrated that AB to food cues is unrelated to individual differences in body weight, in line with the conclusions by Hardman *et al.* (198) and by another recent meta-analysis concluding that individuals with obesity/overweight did not differ from individuals with a healthy weight on attention measured by a VPT (346). The above could be further confirmed by research findings showing that attempts to modify AB for food in individuals with obesity or unsuccessful dieters have been mostly ineffective in altering eating behaviour (347, 348). AB to food cues seems to be more dependent of state-factors such as metabolic state rather than trait-factors, such as body weight (202, 299).

This paradigm was used in subsequent studies to further dissect the effect of calories and sweetness on food-cue attention responses.

- **Consumption of a stevia-sweetened beverage prior to a meal induces beneficial effects on appetite and food intake in healthy lean adults.**

Chapter 3 was a randomised double-blind controlled crossover trial that examined the effects of consuming a stevia-sweetened preload compared to appropriate controls, beverages containing caloric sweeteners with (glucose, sucrose) or without (maltodextrin) a sweet taste and water as a control, on subjective appetite, food intake, blood glucose and AB to food cues. The main finding was a beneficial effect of consuming a stevia preload on reducing short-term appetite and total energy intake, without affecting blood glucose response or AB to food cues.

Our findings are supported by a recently published meta-analysis, that has included our study as well (64). Authors report significantly lower energy intake at an *ad libitum* meal following the consumption of the NNS-sweetened preloads compared to unsweetened equicaloric preloads (this would mean NNS-sweetened preload vs water). Regarding the equisweet comparison and taking the total energy intake into account (preload and meal) the pattern was towards reduced energy intake for the NNS-sweetened preload, revealing an only partial compensation from the caloric sweetened preload (64). The results of this meta-analysis are in line with our results showing that total energy intake (meal + beverage calorie content) was significantly lower in the stevia-beverage condition compared to the water preload condition, demonstrating a net saving of 105 kcal. In the comparison with caloric preloads (glucose, sucrose and maltodextrin) the total energy intake in the stevia preload condition was found again to be lower (~108 kcal) even though comparisons did not reach statistical significance. Compensation was 33 % for the stevia beverage, confirming the results of the aforementioned meta-analysis and that of Rogers *et al*. (63) for only partial compensation. The findings on reduction of food intake are further supported by the results on appetite ratings, showing reduced hunger and desire to eat 30 min post stevia and caloric (sweet or not) beverage consumption compared to water. Previous studies that assessed stevia consumption on appetite ratings agrees with our results showing either an advantageous effect of stevia compared to water or a control on appetite (50, 270) or similar responses compared to caloric sugars (48).

Among the strengths of this study was the inclusion of multiple controls for sweetness and calorie content/physiological responses. A potentially significant limitation of this study is that insulin or gut-peptide responses were not assessed. A lack of an effect on hormonal responses following stevia consumption would add value to the present findings, further supporting the idea that the reduction in energy intake was due to hedonics (sweetness) and not mediated by post-ingestive metabolic responses. The available evidence to date suggests that, like other NNS, stevia does not alter GLP-1 (124, 280), GIP (280) or C-peptide (281) in human volunteers, however there is room for more research in this field.

- **Daily consumption of stevia drops in realistic doses does not affect glucose homeostasis but reduces self-reported energy intake.**

Chapter 4 was a randomised controlled open-label trial in healthy volunteers with normal weight, where participants were required to consume stevia drops daily for 12 weeks in their habitual beverages. The primary outcome was to assess potential impacts on glucose homeostasis (glucose and insulin response to an OGTT) following the introduction of daily consumption of stevia by healthy adults. In line with our original hypothesis, no significant difference was observed on any glucose homeostasis measures before and after the intervention period in any study group. This is the first RCT to assess the effects of longer-

term exposure to stevia on glucose tolerance, however there is a considerable amount of new experimental data showing that consumption of other NNS for longer periods does not negatively affect glucose homeostasis (56, 132, 143, 144). A recent addition to the evidence is that despite the initial demonstration by Suez *et al.* (142) that daily saccharin consumption for 7 days has a negative impact on glucose tolerance mediated by alteration of gut microbiota, recent studies that included a larger sample size and longer exposure duration to sweeteners have failed to show any significant differences in gut microbiota composition following sucralose, aspartame or saccharin consumption (143, 144, 349). There is currently no human clinical trial conducted to assess any gut microbiome composition changes following repeated exposure to stevia, therefore more research is warranted in this field. However, stool samples were collected in the current study, and funding has now been accessed for preliminary microbiome analysis.

The open-label design reflects the conscious consumption of stevia in this study, that allowed for cognitive factors related to the consumption of low-calorie products to take place. It is important to note that participants were not instructed to substitute caloric sweeteners for stevia drops, but to incorporate them into their habitual diet in a way of their choice. The stevia and control groups showed distinct effects in body weight and energy intake, which were predefined as secondary outcomes, with the stevia group showing beneficial effects. In contrast with the latest meta-analyses on the effects of NNS on body weight and energy intake which report no significant differences in the comparison between NNS and water or no treatment (66, 77), we demonstrated a significant spontaneous decrease in self-reported energy intake after 12 weeks of stevia supplementation in the intervention group. This finding further confirms the findings of Chapter 3, and proposes that sweet taste *per se* reduces short-term and long-term energy intake in healthy adults.

However, the findings of this research should be interpreted with caution, since the study was powered for the primary outcome (glucose homeostasis) and not for the secondary outcomes body weight and energy intake. Future work should encourage the examination of daily stevia and other sweeteners consumption for periods beyond 12 weeks powered for body weight and energy intake as primary outcomes. The possibility of this being a stevia-specific effect cannot be discounted either since no other NNS controls were included in this study. In addition, a comparison between an open-label design and a double-blind design with the administration of the treatments in capsules would shed light into the contribution of cognitive and hedonic factors associated with the modulation in energy intake.

- **Stevia demonstrated a longer-lasting and more robust BOLD decrease over time compared to other beverages in the brain. Consumption of stevia and caloric beverages elicited attenuated BOLD response to food compared to control trials in the visual cortex compared to water beverage.**

In a randomised double-blind crossover controlled study this study aimed to investigate the neural correlates of acute physiological signals and food-cue elicited responses related to consumption of stevia in comparison with appropriate controls for sweetness and calorie content (glucose, maltodextrin, water).

Overall, in the *physMR*I stevia showed a more robust BOLD contrast decrease compared to other beverages post consumption, which was more clearly demonstrated in the interaction of taste-by-calories-by time that engaged motor areas, parts of the frontal and cingulate cortex and insula. It seems unlikely that the effects of stevia in the brain are due to the brief event of sweet tasting in the mouth. This conclusion is mainly driven from the observation that the BOLD decrease persists beyond 30 minutes after the consumption of the stevia beverage, while for the caloric beverages (glucose, maltodextrin) a return to pre-consumption baseline BOLD response was demonstrated between 20-30 min post consumption; stevia and glucose produced the same sweet taste perception in the mouth. Stevia has a significant effect in the brain which warrants further research. Given the different time course of the effect of stevia on brain responses it is interesting to speculate whether the effect of stevia is mediated by pathways or targets independent of STRs, or whether this is an effect of STR stimulation by stevia/NNS masked by the effects of caloric sugar metabolism on the BOLD signal.

Stevia and caloric beverages also showed attenuated BOLD response post consumption in relation to performing a food-cue attention task compared to a control task in the visual cortex compared to water beverage ingestion which led to increased BOLD response. This could be an initial indication that food cues are perceived as less salient after the consumption of sweet (stevia), sweet and caloric (glucose) or only caloric beverages (maltodextrin) compared to water, potentially reflecting a decreased motivation for food after the consumption. Zhang *et al*. in a recent study concluded that sucralose consumption also led to attenuation of brain responses associated with food valuation compared to water (255).

The strengths of this study were again the inclusion of controls for metabolic signals and for sweetness and the double-blind design. Among the limitations of the study is the absence of assessment for metabolic markers (i.e. glucose, insulin and gut peptide responses). Even though it has become clear in the literature that stevia, like other NNS, do not affect postprandial metabolic responses (13), a direct measurement under this design would have strengthened our conclusions. However, the disruptive effect of cannulation and sampling

on brain imaging which is so sensitive to artefacts due to movement for example would support my approach.

6.1 Future directions

The battery of food-cue neurocognitive tasks developed and tested for efficacy in Chapter 2 could be used to examine potentially disrupted food-cue processing in conditions that significantly influence appetite, as a result of an imbalance between homeostatic and hedonic mechanisms of appetite regulation. Such conditions are GI diseases (e.g. Crohn's disease) and appetite/eating disorders.

Due to the inclusion of only one type of NNS, stevia, in the totality of the studies in this thesis, I cannot discount the possibility of the effects demonstrated being stevia specific effects. Therefore, future research should examine whether different NNS types lead to similar or differential results on appetite, food intake, body weight and brain responses utilising similar designs. A recent study on the effects of different NNS on body weight has already provided some initial indications of sweetener-specific effects (78); this observation warrants further research.

The study population was in the majority a homogenous group of healthy young adults with a BMI in the normal range who were non-habitual consumers of NNS, and this limits the applicability of the findings of this thesis to other population groups. Future research should examine the applicability of my findings in populations for whom energy and sugar intake limitation is crucial, such as individuals with overweight and obesity, T2DM and other non-communicable diseases. Daily stevia consumption did not have a significant effect on glucose homeostasis measures in healthy lean adults. However, it would be essential to investigate these effects in patients with T2DM, and type I diabetes mellitus, which consist population groups who would benefit from the substitution of sugar for non-caloric sweeteners like stevia. In addition, future research should consider stratifying participants regarding previous exposure to sweeteners (i.e. habitual vs non-habitual consumers of NNS) to elucidate potentially differential effects.

While the double-blind design is considered the gold-standard method for RCTs, it would be interesting to examine consumer behaviour in real-life scenarios where they consciously consume a low calorie/sugar product. One claim which has been suggested to undermine the relationship between NNS consumption and weight management is that consumers might consciously compensate for 'calories saved' when they know they are consuming NNS (52). One recent study highlights the importance of cognitive factors and conscious consumption, especially when the physiological satiety has not been achieved (350). My

study in Chapter 4 used an open-label design which allowed for cognitive factors to take place, and a reduction in energy intake was still observed. However, more studies are warranted in this area. It could be possible that individual characteristics, such as cognitive restraint, might account for differential responses.

fMRI is the current gold standard method to assess brain responses in humans in a completely non-invasive way and has contributed significantly in understanding the responses in the brain related to appetite control. However, it has a significant limitation; BOLD fMRI does not measure brain activity directly but depends on the premise that increased brain activity and metabolic activity is followed by a subsequent increase in blood flow. Nevertheless, it is a rapidly evolving research field, and an invaluable tool towards understanding the underlying mechanisms of appetite control. From a mechanistic approach, future neuroimaging work should examine the brain responses to stevia consumption in the brain for a longer postprandial period, beyond 30 min post consumption. In addition, it appears essential that stevia being compared to other NNS, to elucidate a potentially specific effect of stevia. A similar study design that utilises intragastric administration of stevia rather than oral consumption will further confirm whether this was an oral sweetness-mediated effect or not. It is also considered essential that future studies also assess metabolic markers such as gut peptide responses, as well as food intake within the same study so that behavioural, physiological and hedonic measures are combined under the same setting.

6.2 Concluding remarks

In summary the research presented in this thesis provides valuable and novel insights into the effects of stevia consumption on neurocognitive, behavioural and physiological responses. Stevia consumption was shown to elicit benefits in appetite and energy intake both in the short-term and the long-term, not to influence glucose homeostasis and to induce a significant attenuation effect in the brain. The above could be indicative that stevia is beneficial for human consumption, and lays the foundations for this research moving into key clinical areas, such as obesity and T2DM.

References

1. Buyken AE, Mela DJ, Dussort P, Johnson IT, Macdonald IA, Stowell JD, et al. Dietary carbohydrates: a review of international recommendations and the methods used to derive them. European journal of clinical nutrition. 2018;72(12):1625-43.
2. Mennella JA, Bobowski NK, Reed DR. The development of sweet taste: From biology to hedonics. Rev Endocr Metab Disord. 2016;17(2):171-8.
3. Zuker CS. Food for the brain. Cell. 2015;161(1):9-11.
4. Gibson S, Ashwell M, Arthur J, Bagley L, Lennox A, Rogers PJ, et al. What can the food and drink industry do to help achieve the 5% free sugars goal? Perspectives in public health. 2017;137(4):237-47.
5. Mattes RD. Low calorie sweeteners: Science and controversy: Conference proceedings. Physiology & behavior. 2016;164(Pt B):429-31.
6. Sylvetsky AC, Rother KI. Nonnutritive Sweeteners in Weight Management and Chronic Disease: A Review. Obesity (Silver Spring, Md). 2018;26(4):635-40.
7. Sylvetsky AC, Hiedacavage A, Shah N, Pokorney P, Baldauf S, Merrigan K, et al. From biology to behavior: a cross-disciplinary seminar series surrounding added sugar and low-calorie sweetener consumption. Obesity science & practice. 2019;5(3):203-19.
8. Swithers SE. Artificial sweeteners produce the counterintuitive effect of inducing metabolic derangements. Trends Endocrinol Metab. 2013;24(9):431-41.
9. Gardener H, Elkind MSV. Artificial Sweeteners, Real Risks. Stroke. 2019;50(3):549-51.
10. Shearer J, Swithers SE. Artificial sweeteners and metabolic dysregulation: Lessons learned from agriculture and the laboratory. Rev Endocr Metab Disord. 2016;17(2):179-86.
11. Malik VS. Non-sugar sweeteners and health. BMJ. 2019;364:k5005.
12. Berthoud HR, Munzberg H, Morrison CD. Blaming the Brain for Obesity: Integration of Hedonic and Homeostatic Mechanisms. Gastroenterology. 2017;152(7):1728-38.
13. Greyling A, Appleton KM, Raben A, Mela DJ. Acute glycemic and insulinemic effects of low-energy sweeteners: a systematic review and meta-analysis of randomized controlled trials. Am J Clin Nutr. 2020;112(4):1002-14.
14. Johnson RK, Appel LJ, Brands M, Howard BV, Lefevre M, Lustig RH, et al. Dietary sugars intake and cardiovascular health: a scientific statement from the American Heart Association. Circulation. 2009;120(11):1011-20.
15. Malik VS, Pan A, Willett WC, Hu FB. Sugar-sweetened beverages and weight gain in children and adults: a systematic review and meta-analysis. Am J Clin Nutr. 2013;98(4):1084-102.
16. Malik VS, Hu FB. Sugar-Sweetened Beverages and Cardiometabolic Health: An Update of the Evidence. Nutrients. 2019;11(8).
17. Drewnowski A, Rehm CD. Consumption of added sugars among US children and adults by food purchase location and food source. The American journal of clinical nutrition. 2014;100(3):901-7.
18. Azais-Braesco V, Sluik D, Maillot M, Kok F, Moreno LA. A review of total & added sugar intakes and dietary sources in Europe. Nutrition journal. 2017;16(1):6.
19. Hu FB. Resolved: there is sufficient scientific evidence that decreasing sugar-sweetened beverage consumption will reduce the prevalence of obesity and obesity-related diseases. Obesity reviews : an official journal of the International Association for the Study of Obesity. 2013;14(8):606-19.
20. WHO. Guideline: sugars intake for adults and children. Geneva (Switzerland). 2015.
21. Mela DJ, Woolner EM. Perspective: Total, Added, or Free? What Kind of Sugars Should We Be Talking About? Adv Nutr. 2018;9(2):63-9.
22. Erickson J, Slavin J. Total, added, and free sugars: are restrictive guidelines science-based or achievable? Nutrients. 2015;7(4):2866-78.
23. US Food and Drug Administration. Additional Information about High-Intensity Sweeteners Permitted for Use in Food in the United States. website: https://www.fda.gov/food/food-additives-petitions/additional-information-about-high-intensity-sweeteners-permitted-use-food-united-states (accessed: 05/10/2020).
24. Roberts A. The safety and regulatory process for low calorie sweeteners in the United States. Physiology & behavior. 2016;164(Pt B):439-44.
25. Mortensen A. Sweeteners permitted in the European Union: safety aspects. Scandinavian Journal of Food and Nutrition. 2006;50(3):104-16.

26. Johnson RK, Lichtenstein AH, Anderson CAM, Carson JA, Despres JP, Hu FB, et al. Low-Calorie Sweetened Beverages and Cardiometabolic Health: A Science Advisory From the American Heart Association. Circulation. 2018;138(9):e126-e40.
27. Magnuson BA, Carakostas MC, Moore NH, Poulos SP, Renwick AG. Biological fate of low-calorie sweeteners. Nutr Rev. 2016;74(11):670-89.
28. Fitch C, Keim KS. Position of the Academy of Nutrition and Dietetics: use of nutritive and nonnutritive sweeteners. J Acad Nutr Diet. 2012;112(5):739-58.
29. EFSA Panel on Dietetic Products N, Allergies. Scientific Opinion on the substantiation of health claims related to intense sweeteners and contribution to the maintenance or achievement of a normal body weight (ID 1136, 1444, 4299), reduction of post-prandial glycaemic responses (ID 4298), maintenance of normal blood glucose concentrations (ID 1221, 4298), and maintenance of tooth mineralisation by decreasing tooth demineralisation (ID 1134, 1167, 1283) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal. 2011;9(6):2229.
30. Diabetes UK. Position statement on the use of low or no calorie sweeteners. 2018.
31. 5. Lifestyle Management: Standards of Medical Care in Diabetes—2019 American Diabetes Association. Diabetes Care. 2019;42(Supplement 1):S46-S60.
32. Sylvetsky AC, Welsh JA, Brown RJ, Vos MB. Low-calorie sweetener consumption is increasing in the United States. Am J Clin Nutr. 2012;96(3):640-6.
33. Drewnowski A, Rehm CD. Socio-demographic correlates and trends in low-calorie sweetener use among adults in the United States from 1999 to 2008. European journal of clinical nutrition. 2015;69(9):1035-41.
34. Fakhouri TH, Kit BK, Ogden CL. Consumption of diet drinks in the United States, 20092010. NCHS data brief. 2012(109):1-8.
35. Malek AM, Hunt KJ, DellaValle DM, Greenberg D, St Peter JV, Marriott BP. Reported Consumption of Low-Calorie Sweetener in Foods, Beverages, and Food and Beverage Additions by US Adults: NHANES 2007-2012. Current developments in nutrition. 2018;2(9):nzy054.
36. Sylvetsky AC, Jin Y, Clark EJ, Welsh JA, Rother KI, Talegawkar SA. Consumption of Low-Calorie Sweeteners among Children and Adults in the United States. Journal of the Academy of Nutrition and Dietetics. 2017;117(3):441-8 e2.
37. DellaValle DM, Malek AM, Hunt KJ, St Peter JV, Greenberg D, Marriott BP. Low-Calorie Sweeteners in Foods, Beverages, and Food and Beverage Additions: NHANES 2007-2012. Current developments in nutrition. 2018;2(12):nzy024.
38. Sylvetsky AC, Brown RJ, Blau JE, Walter M, Rother KI. Hormonal responses to non-nutritive sweeteners in water and diet soda. Nutr Metab (Lond). 2016;13:71.
39. Patel L, Alicandro G, La Vecchia C. Low-Calorie Beverage Consumption, Diet Quality and Cardiometabolic Risk Factors in British Adults. Nutrients. 2018;10(9).
40. Drewnowski A, Rehm CD. Consumption of low-calorie sweeteners among U.S. adults is associated with higher Healthy Eating Index (HEI 2005) scores and more physical activity. Nutrients. 2014;6(10):4389-403.
41. Kringelbach ML, Stein A, van Hartevelt TJ. The functional human neuroanatomy of food pleasure cycles. Physiol Behav. 2012;106(3):307-16.
42. Blundell J, de Graaf C, Hulshof T, Jebb S, Livingstone B, Lluch A, et al. Appetite control: methodological aspects of the evaluation of foods. Obes Rev. 2010;11(3):251-70.
43. Gibbons C, Hopkins M, Beaulieu K, Oustric P, Blundell JE. Issues in Measuring and Interpreting Human Appetite (Satiety/Satiation) and Its Contribution to Obesity. Curr Obes Rep. 2019;8(2):77-87.
44. Blundell JE, Rogers PJ, Hill AJ. Uncoupling sweetness and calories: methodological aspects of laboratory studies on appetite control. Appetite. 1988;11 Suppl 1:54-61.
45. Blundell JE, Hill AJ. Paradoxical effects of an intense sweetener (aspartame) on appetite. Lancet. 1986;1(8489):1092-3.
46. Tordoff MG, Alleva AM. Oral stimulation with aspartame increases hunger. Physiology & behavior. 1990;47(3):555-9.
47. Rogers PJ, Carlyle JA, Hill AJ, Blundell JE. Uncoupling sweet taste and calories: comparison of the effects of glucose and three intense sweeteners on hunger and food intake. Physiology & behavior. 1988;43(5):547-52.
48. Anton SD, Martin CK, Han H, Coulon S, Cefalu WT, Geiselman P, et al. Effects of stevia, aspartame, and sucrose on food intake, satiety, and postprandial glucose and insulin levels. Appetite. 2010;55(1):37-43.

49. Fantino M, Fantino A, Matray M, Mistretta F. Beverages containing low energy sweeteners do not differ from water in their effects on appetite, energy intake and food choices in healthy, non-obese French adults. Appetite. 2018;125:557-65.
50. Farhat G, Berset V, Moore L. Effects of Stevia Extract on Postprandial Glucose Response, Satiety and Energy Intake: A Three-Arm Crossover Trial. Nutrients. 2019;11(12).
51. Tey SL, Salleh NB, Henry J, Forde CG. Effects of aspartame-, monk fruit-, stevia- and sucrose-sweetened beverages on postprandial glucose, insulin and energy intake. Int J Obes (Lond). 2017;41(3):450-7.
52. Rogers PJ. The role of low-calorie sweeteners in the prevention and management of overweight and obesity: evidence v. conjecture. Proc Nutr Soc. 2018;77(3):230-8.
53. Casperson SL, Johnson L, Roemmich JN. The relative reinforcing value of sweet versus savory snack foods after consumption of sugar- or non-nutritive sweetened beverages. Appetite. 2017;112:143-9.
54. Piernas C, Tate DF, Wang X, Popkin BM. Does diet-beverage intake affect dietary consumption patterns? Results from the Choose Healthy Options Consciously Everyday (CHOICE) randomized clinical trial. The American Journal of Clinical Nutrition. 2013;97(3):604-11.
55. Kashima N, Kimura K, Nishitani N, Yamaoka Endo M, Fukuba Y, Kashima H. Suppression of Oral Sweet Sensations during Consumption of Sweet Food in Humans: Effects on Gastric Emptying Rate, Glycemic Response, Appetite, Food Satisfaction and Desire for Basic Tastes. Nutrients. 2020;12(5).
56. Higgins KA, Considine RV, Mattes RD. Aspartame Consumption for 12 Weeks Does Not Affect Glycemia, Appetite, or Body Weight of Healthy, Lean Adults in a Randomized Controlled Trial. J Nutr. 2018;148(4):650-7.
57. Rolls BJ, Hetherington M, Burley VJ. Sensory stimulation and energy density in the development of satiety. Physiol Behav. 1988;44(6):727-33.
58. Hetherington M, Rolls BJ, Burley VJ. The time course of sensory-specific satiety. Appetite. 1989;12(1):57-68.
59. Rogers PJ, Ferriday D, Irani B, Hei Hoi JK, England CY, Bajwa KK, et al. Sweet satiation: Acute effects of consumption of sweet drinks on appetite for and intake of sweet and non-sweet foods. Appetite. 2020;149:104631.
60. Maloney NG, Christiansen P, Harrold JA, Halford JCG, Hardman CA. Do low-calorie sweetened beverages help to control food cravings? Two experimental studies. Physiol Behav. 2019;208:112500.
61. Papies EK, Hamstra P. Goal priming and eating behavior: enhancing self-regulation by environmental cues. Health Psychol. 2010;29(4):384-8.
62. Peters JC, Wyatt HR, Foster GD, Pan Z, Wojtanowski AC, Vander Veur SS, et al. The effects of water and non-nutritive sweetened beverages on weight loss during a 12-week weight loss treatment program. Obesity (Silver Spring). 2014;22(6):1415-21.
63. Rogers PJ, Hogenkamp PS, de Graaf C, Higgs S, Lluch A, Ness AR, et al. Does low-energy sweetener consumption affect energy intake and body weight? A systematic review, including meta-analyses, of the evidence from human and animal studies. International journal of obesity (2005). 2016;40(3):381-94.
64. Lee HY, Jack M, Poon T, Noori D, Venditti C, Hamamji S, et al. Effects of Unsweetened Preloads and Preloads Sweetened with Caloric or Low-/No-Calorie Sweeteners on Subsequent Energy Intakes: A Systematic Review and Meta-Analysis of Controlled Human Intervention Studies. Adv Nutr. 2021.
65. Mattes RD, Popkin BM. Nonnutritive sweetener consumption in humans: effects on appetite and food intake and their putative mechanisms. Am J Clin Nutr. 2009;89(1):1-14.
66. Rogers PJ, Appleton KM. The effects of low-calorie sweeteners on energy intake and body weight: a systematic review and meta-analyses of sustained intervention studies. Int J Obes (Lond). 2020.
67. Imamura F, O'Connor L, Ye Z, Mursu J, Hayashino Y, Bhupathiraju SN, et al. Consumption of sugar sweetened beverages, artificially sweetened beverages, and fruit juice and incidence of type 2 diabetes: systematic review, meta-analysis, and estimation of population attributable fraction. BMJ. 2015;351:h3576.
68. de Koning L, Malik VS, Rimm EB, Willett WC, Hu FB. Sugar-sweetened and artificially sweetened beverage consumption and risk of type 2 diabetes in men. The American journal of clinical nutrition. 2011;93(6):1321-7.
69. Schulze MB, Manson JE, Ludwig DS, Colditz GA, Stampfer MJ, Willett WC, et al. Sugar-sweetened beverages, weight gain, and incidence of type 2 diabetes in young and middle-aged women. Jama. 2004;292(8):927-34.

70. Rodriguez LA, Madsen KA, Cotterman C, Lustig RH. Added sugar intake and metabolic syndrome in US adolescents: cross-sectional analysis of the National Health and Nutrition Examination Survey 2005-2012. Public health nutrition. 2016;19(13):2424-34.
71. Miller PE, Perez V. Low-calorie sweeteners and body weight and composition: a meta-analysis of randomized controlled trials and prospective cohort studies. Am J Clin Nutr. 2014;100(3):765-77.
72. Azad MB, Abou-Setta AM, Chauhan BF, Rabbani R, Lys J, Copstein L, et al. Nonnutritive sweeteners and cardiometabolic health: a systematic review and meta-analysis of randomized controlled trials and prospective cohort studies. CMAJ. 2017;189(28):E929-E39.
73. Toews I, Lohner S, Kullenberg de Gaudry D, Sommer H, Meerpohl JJ. Association between intake of non-sugar sweeteners and health outcomes: systematic review and meta-analyses of randomised and non-randomised controlled trials and observational studies. BMJ. 2019;364:k4718.
74. Pereira MA. Diet beverages and the risk of obesity, diabetes, and cardiovascular disease: a review of the evidence. Nutrition reviews. 2013;71(7):433-40.
75. Bleich SN, Wolfson JA, Vine S, Wang YC. Diet-beverage consumption and caloric intake among US adults, overall and by body weight. American journal of public health. 2014;104(3):e72-8.
76. Mela DJ, McLaughlin J, Rogers PJ. Perspective: Standards for Research and Reporting on Low-Energy ("Artificial") Sweeteners. Adv Nutr. 2020;11(3):484-91.
77. Laviada-Molina H, Molina-Segui F, Perez-Gaxiola G, Cuello-Garcia C, Arjona-Villicana R, Espinosa-Marron A, et al. Effects of nonnutritive sweeteners on body weight and BMI in diverse clinical contexts: Systematic review and meta-analysis. Obes Rev. 2020;21(7):e13020.
78. Higgins KA, Mattes RD. A randomized controlled trial contrasting the effects of 4 low-calorie sweeteners and sucrose on body weight in adults with overweight or obesity. Am J Clin Nutr. 2019;109(5):1288-301.
79. Low YQ, Lacy K, Keast R. The role of sweet taste in satiation and satiety. Nutrients. 2014;6(9):3431-50.
80. Simon SA, de Araujo IE, Gutierrez R, Nicolelis MA. The neural mechanisms of gustation: a distributed processing code. Nat Rev Neurosci. 2006;7(11):890-901.
81. Fernstrom JD, Munger SD, Sclafani A, de Araujo IE, Roberts A, Molinary S. Mechanisms for sweetness. J Nutr. 2012;142(6):1134S-41S.
82. Laffitte A, Neiers F, Briand L. Functional roles of the sweet taste receptor in oral and extraoral tissues. Curr Opin Clin Nutr Metab Care. 2014;17(4):379-85.
83. Xu H, Staszewski L, Tang H, Adler E, Zoller M, Li X. Different functional roles of T1R subunits in the heteromeric taste receptors. Proc Natl Acad Sci U S A. 2004;101(39):14258-63.
84. Nie Y, Vigues S, Hobbs JR, Conn GL, Munger SD. Distinct contributions of T1R2 and T1R3 taste receptor subunits to the detection of sweet stimuli. Curr Biol. 2005;15(21):1948-52.
85. Meyers B, Brewer MS. Sweet taste in man: a review. J Food Sci. 2008;73(6):R81-90.
86. Hunter SR, Reister EJ, Cheon E, Mattes RD. Low Calorie Sweeteners Differ in Their Physiological Effects in Humans. Nutrients. 2019;11(11).
87. Kim SK, Chen Y, Abrol R, Goddard WA, 3rd, Guthrie B. Activation mechanism of the G protein-coupled sweet receptor heterodimer with sweeteners and allosteric agonists. Proc Natl Acad Sci U S A. 2017;114(10):2568-73.
88. Hellfritsch C, Brockhoff A, Stahler F, Meyerhof W, Hofmann T. Human psychometric and taste receptor responses to steviol glycosides. Journal of agricultural and food chemistry. 2012;60(27):6782-93.
89. Bernhardt SJ, Naim M, Zehavi U, Lindemann B. Changes in IP3 and cytosolic Ca2+ in response to sugars and non-sugar sweeteners in transduction of sweet taste in the rat. J Physiol. 1996;490 (Pt 2):325-36.
90. Hofer D, Puschel B, Drenckhahn D. Taste receptor-like cells in the rat gut identified by expression of alpha-gustducin. Proc Natl Acad Sci U S A. 1996;93(13):6631-4.
91. Taniguchi K. Expression of the sweet receptor protein, T1R3, in the human liver and pancreas. J Vet Med Sci. 2004;66(11):1311-4.
92. Kyriazis GA, Soundarapandian MM, Tyrberg B. Sweet taste receptor signaling in beta cells mediates fructose-induced potentiation of glucose-stimulated insulin secretion. Proc Natl Acad Sci U S A. 2012;109(8):E524-32.

93. Ren X, Zhou L, Terwilliger R, Newton SS, de Araujo IE. Sweet taste signaling functions as a hypothalamic glucose sensor. Front Integr Neurosci. 2009;3:12.
94. Depoortere I. Taste receptors of the gut: emerging roles in health and disease. Gut. 2014;63(1):179-90.
95. Bryant C, McLaughlin J. Low calorie sweeteners: Evidence remains lacking for effects on human gut function. Physiol Behav. 2016;164(Pt B):482-5.
96. DuBois GE. Molecular mechanism of sweetness sensation. Physiology & behavior. 2016;164(Pt B):453-63.
97. Jayasinghe SN, Kruger R, Walsh DCI, Cao G, Rivers S, Richter M, et al. Is Sweet Taste Perception Associated with Sweet Food Liking and Intake? Nutrients. 2017;9(7):750.
98. Donaldson LF, Bennett L, Baic S, Melichar JK. Taste and weight: is there a link? Am J Clin Nutr. 2009;90(3):800s-3s.
99. Schiffman SS, Booth BJ, Sattely-Miller EA, Graham BG, Gibes KM. Selective inhibition of sweetness by the sodium salt of +/-2-(4-methoxyphenoxy)propanoic acid. Chem Senses. 1999;24(4):439-47.
100. Frank RA, Mize SJS, Kennedy LM, de los Santos HC, Green SJ. The effect of Gymnema sylvestre extracts on the sweetness of eight sweeteners. Chemical Senses. 1992;17(5):461-79.
101. Simpson EJ, Nixon AV, Taylor AJ, Macdonald IA. The sweetness inhibitor 'Lactisole' attenuates postprandial hyperglycaemia. Proceedings of the Nutrition Society. 2009;68(OCE3):E117.
102. Kashima H, Eguchi K, Miyamoto K, Fujimoto M, Endo MY, Aso-Someya N, et al. Suppression of Oral Sweet Taste Sensation with Gymnema sylvestre Affects Postprandial Gastrointestinal Blood Flow and Gastric Emptying in Humans. Chem Senses. 2017;42(4):295-302.
103. Kashima H, Taniyama K, Sugimura K, Endo MY, Kobayashi T, Fukuba Y. Suppression of sweet sensing with glucose, but not aspartame, delays gastric emptying and glycemic response. Nutrition Research. 2019;68:62-9.
104. Nederkoorn C, Smulders FT, Jansen A. Cephalic phase responses, craving and food intake in normal subjects. Appetite. 2000;35(1):45-55.
105. Smeets PA, Erkner A, de Graaf C. Cephalic phase responses and appetite. Nutr Rev. 2010;68(11):643-55.
106. Wiedemann SJ, Rachid L, Illigens B, Boni-Schnetzler M, Donath MY. Evidence for cephalic phase insulin release in humans: A systematic review and meta-analysis. Appetite. 2020;155:104792.
107. Teff KL, Mattes RD, Engelman K. Cephalic phase insulin release in normal weight males: verification and reliability. Am J Physiol. 1991;261(4 Pt 1):E430-6.
108. Just T, Pau HW, Engel U, Hummel T. Cephalic phase insulin release in healthy humans after taste stimulation? Appetite. 2008;51(3):622-7.
109. Glendinning JI, Frim YG, Hochman A, Lubitz GS, Basile AJ, Sclafani A. Glucose elicits cephalic-phase insulin release in mice by activating KATP channels in taste cells. Am J Physiol Regul Integr Comp Physiol. 2017;312(4):R597-R610.
110. Dhillon J, Lee JY, Mattes RD. The cephalic phase insulin response to nutritive and low-calorie sweeteners in solid and beverage form. Physiol Behav. 2017;181:100-9.
111. Ford HE, Peters V, Martin NM, Sleeth ML, Ghatei MA, Frost GS, et al. Effects of oral ingestion of sucralose on gut hormone response and appetite in healthy normal-weight subjects. Eur J Clin Nutr. 2011;65(4):508-13.
112. Nichol AD, Salame C, Rother KI, Pepino MY. Effects of Sucralose Ingestion versus Sucralose Taste on Metabolic Responses to an Oral Glucose Tolerance Test in Participants with Normal Weight and Obesity: A Randomized Crossover Trial. Nutrients. 2019;12(1).
113. Lasschuijt MP, Mars M, de Graaf C, Smeets PAM. Endocrine Cephalic Phase Responses to Food Cues: A Systematic Review. Adv Nutr. 2020;11(5):1364-83.
114. Sternini C, Anselmi L, Rozengurt E. Enteroendocrine cells: a site of 'taste' in gastrointestinal chemosensing. Curr Opin Endocrinol Diabetes Obes. 2008;15(1):73-8.
115. Dotson CD, Vigues S, Steinle NI, Munger SD. T1R and T2R receptors: the modulation of incretin hormones and potential targets for the treatment of type 2 diabetes mellitus. Curr Opin Investig Drugs. 2010;11(4):447-54.
116. Dyer J, Salmon KS, Zibrik L, Shirazi-Beechey SP. Expression of sweet taste receptors of the T1R family in the intestinal tract and enteroendocrine cells. Biochem Soc Trans. 2005;33(Pt 1):302-5.

117. Rozengurt N, Wu SV, Chen MC, Huang C, Sternini C, Rozengurt E. Colocalization of the alpha-subunit of gustducin with PYY and GLP-1 in L cells of human colon. Am J Physiol Gastrointest Liver Physiol. 2006;291(5):G792-802.
118. Jang HJ, Kokrashvili Z, Theodorakis MJ, Carlson OD, Kim BJ, Zhou J, et al. Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1. Proc Natl Acad Sci U S A. 2007;104(38):15069-74.
119. Margolskee RF, Dyer J, Kokrashvili Z, Salmon KS, Ilegems E, Daly K, et al. T1R3 and gustducin in gut sense sugars to regulate expression of Na+-glucose cotransporter 1. Proc Natl Acad Sci U S A. 2007;104(38):15075-80.
120. Mace OJ, Affleck J, Patel N, Kellett GL. Sweet taste receptors in rat small intestine stimulate glucose absorption through apical GLUT2. J Physiol. 2007;582(Pt 1):379-92.
121. Gerspach AC, Steinert RE, Schonenberger L, Graber-Maier A, Beglinger C. The role of the gut sweet taste receptor in regulating GLP-1, PYY, and CCK release in humans. Am J Physiol Endocrinol Metab. 2011;301(2):E317-25.
122. Little TJ, Gupta N, Case RM, Thompson DG, McLaughlin JT. Sweetness and bitterness taste of meals per se does not mediate gastric emptying in humans. Am J Physiol Regul Integr Comp Physiol. 2009;297(3):R632-9.
123. Steinert RE, Gerspach AC, Gutmann H, Asarian L, Drewe J, Beglinger C. The functional involvement of gut-expressed sweet taste receptors in glucose-stimulated secretion of glucagon-like peptide-1 (GLP-1) and peptide YY (PYY). Clin Nutr. 2011;30(4):524-32.
124. van Avesaat M, Troost FJ, Ripken D, Peters J, Hendriks HF, Masclee AA. Intraduodenal infusion of a combination of tastants decreases food intake in humans. Am J Clin Nutr. 2015;102(4):729-35.
125. Steinert RE, Frey F, Topfer A, Drewe J, Beglinger C. Effects of carbohydrate sugars and artificial sweeteners on appetite and the secretion of gastrointestinal satiety peptides. Br J Nutr. 2011;105(9):1320-8.
126. Ma J, Chang J, Checklin HL, Young RL, Jones KL, Horowitz M, et al. Effect of the artificial sweetener, sucralose, on small intestinal glucose absorption in healthy human subjects. The British journal of nutrition. 2010;104(6):803-6.
127. Ma J, Bellon M, Wishart JM, Young R, Blackshaw LA, Jones KL, et al. Effect of the artificial sweetener, sucralose, on gastric emptying and incretin hormone release in healthy subjects. Am J Physiol Gastrointest Liver Physiol. 2009;296(4):G735-9.
128. Greenwood DC, Threapleton DE, Evans CEL, Cleghorn CL, Nykjaer C, Woodhead C, et al. Association between sugar-sweetened and artificially sweetened soft drinks and type 2 diabetes: systematic review and dose–response meta-analysis of prospective studies. British Journal of Nutrition. 2014;112(5):725-34.
129. Livesey G, Taylor R, Livesey HF, Buyken AE, Jenkins DJA, Augustin LSA, et al. Dietary Glycemic Index and Load and the Risk of Type 2 Diabetes: A Systematic Review and Updated Meta-Analyses of Prospective Cohort Studies. Nutrients. 2019;11(6).
130. Pepino MY, Tiemann CD, Patterson BW, Wice BM, Klein S. Sucralose affects glycemic and hormonal responses to an oral glucose load. Diabetes Care. 2013;36(9):2530-5.
131. Temizkan S, Deyneli O, Yasar M, Arpa M, Gunes M, Yazici D, et al. Sucralose enhances GLP-1 release and lowers blood glucose in the presence of carbohydrate in healthy subjects but not in patients with type 2 diabetes. Eur J Clin Nutr. 2015;69(2):162-6.
132. Ahmad SY, Friel JK, MacKay DS. The effect of the artificial sweeteners on glucose metabolism in healthy adults: a randomized, double-blinded, crossover clinical trial. Appl Physiol Nutr Metab. 2020;45(6):606-12.
133. Romo-Romo A, Aguilar-Salinas CA, Brito-Cordova GX, Gomez Diaz RA, Vilchis Valentin D, Almeda-Valdes P. Effects of the Non-Nutritive Sweeteners on Glucose Metabolism and Appetite Regulating Hormones: Systematic Review of Observational Prospective Studies and Clinical Trials. PLoS One. 2016;11(8):e0161264.
134. Nichol AD, Holle MJ, An R. Glycemic impact of non-nutritive sweeteners: a systematic review and meta-analysis of randomized controlled trials. Eur J Clin Nutr. 2018;72(6):796-804.
135. Tucker RM, Tan S-Y. Do non-nutritive sweeteners influence acute glucose homeostasis in humans? A systematic review. Physiology & Behavior. 2017;182:17-26.
136. Wu T, Zhao BR, Bound MJ, Checklin HL, Bellon M, Little TJ, et al. Effects of different sweet preloads on incretin hormone secretion, gastric emptying, and postprandial glycemia in healthy humans. Am J Clin Nutr. 2012;95(1):78-83.
137. Hall WL, Millward DJ, Rogers PJ, Morgan LM. Physiological mechanisms mediating aspartame-induced satiety. Physiol Behav. 2003;78(4-5):557-62.

138. Maersk M, Belza A, Holst JJ, Fenger-Gron M, Pedersen SB, Astrup A, et al. Satiety scores and satiety hormone response after sucrose-sweetened soft drink compared with isocaloric semi-skimmed milk and with non-caloric soft drink: a controlled trial. Eur J Clin Nutr. 2012;66(4):523-9.
139. Brown RJ, Walter M, Rother KI. Effects of diet soda on gut hormones in youths with diabetes. Diabetes care. 2012;35(5):959-64.
140. Brown RJ, Walter M, Rother KI. Ingestion of diet soda before a glucose load augments glucagon-like peptide-1 secretion. Diabetes care. 2009;32(12):2184-6.
141. Karimian Azari E, Smith KR, Yi F, Osborne TF, Bizzotto R, Mari A, et al. Inhibition of sweet chemosensory receptors alters insulin responses during glucose ingestion in healthy adults: a randomized crossover interventional study. Am J Clin Nutr. 2017;105(4):1001-9.
142. Suez J, Korem T, Zeevi D, Zilberman-Schapira G, Thaiss CA, Maza O, et al. Artificial sweeteners induce glucose intolerance by altering the gut microbiota. Nature. 2014;514(7521):181-6.
143. Serrano J, Smith KR, Crouch AL, Sharma V, Yi F, Vargova V, et al. High-dose saccharin supplementation does not induce gut microbiota changes or glucose intolerance in healthy humans and mice. Microbiome. 2021;9(1):11.
144. Thomson P, Santibanez R, Aguirre C, Galgani JE, Garrido D. Short-term impact of sucralose consumption on the metabolic response and gut microbiome of healthy adults. Br J Nutr. 2019;122(8):856-62.
145. Bueno-Hernandez N, Esquivel-Velazquez M, Alcantara-Suarez R, Gomez-Arauz AY, Espinosa-Flores AJ, de Leon-Barrera KL, et al. Chronic sucralose consumption induces elevation of serum insulin in young healthy adults: a randomized, double blind, controlled trial. Nutr J. 2020;19(1):32.
146. Lertrit A, Srimachai S, Saetung S, Chanprasertyothin S, Chailurkit LO, Areevut C, et al. Effects of sucralose on insulin and glucagon-like peptide-1 secretion in healthy subjects: a randomized, double-blind, placebo-controlled trial. Nutrition. 2018;55-56:125-30.
147. Dalenberg JR, Patel BP, Denis R, Veldhuizen MG, Nakamura Y, Vinke PC, et al. Short-Term Consumption of Sucralose with, but Not without, Carbohydrate Impairs Neural and Metabolic Sensitivity to Sugar in Humans. Cell Metab. 2020;31(3):493-502 e7.
148. Romo-Romo A, Aguilar-Salinas CA, Brito-Cordova GX, Gomez-Diaz RA, Almeda-Valdes P. Sucralose decreases insulin sensitivity in healthy subjects: a randomized controlled trial. Am J Clin Nutr. 2018;108(3):485-91.
149. Grotz VL, Pi-Sunyer X, Porte D, Jr., Roberts A, Richard Trout J. A 12-week randomized clinical trial investigating the potential for sucralose to affect glucose homeostasis. Regul Toxicol Pharmacol. 2017;88:22-33.
150. Grotz VL, Henry RR, McGill JB, Prince MJ, Shamoon H, Trout JR, et al. Lack of effect of sucralose on glucose homeostasis in subjects with type 2 diabetes. J Am Diet Assoc. 2003;103(12):1607-12.
151. Bonnet F, Tavenard A, Esvan M, Laviolle B, Viltard M, Lepicard EM, et al. Consumption of a Carbonated Beverage with High-Intensity Sweeteners Has No Effect on Insulin Sensitivity and Secretion in Nondiabetic Adults. J Nutr. 2018;148(8):1293-9.
152. Engel S, Tholstrup T, Bruun JM, Astrup A, Richelsen B, Raben A. Effect of high milk and sugar-sweetened and non-caloric soft drink intake on insulin sensitivity after 6 months in overweight and obese adults: a randomized controlled trial. Eur J Clin Nutr. 2018;72(3):358-66.
153. Liu CM, Kanoski SE. Homeostatic and non-homeostatic controls of feeding behavior: Distinct vs. common neural systems. Physiol Behav. 2018;193(Pt B):223-31.
154. Berthoud HR, Morrison C. The brain, appetite, and obesity. Annu Rev Psychol. 2008;59:55-92.
155. Higgs S, Spetter MS, Thomas JM, Rotshtein P, Lee M, Hallschmid M, et al. Interactions between metabolic, reward and cognitive processes in appetite control: Implications for novel weight management therapies. J Psychopharmacol. 2017;31(11):1460-74.
156. Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW. Central nervous system control of food intake and body weight. Nature. 2006;443(7109):289-95.
157. Schwartz MW, Woods SC, Porte D, Jr., Seeley RJ, Baskin DG. Central nervous system control of food intake. Nature. 2000;404(6778):661-71.
158. Psichas A, Reimann F, Gribble FM. Gut chemosensing mechanisms. J Clin Invest. 2015;125(3):908-17.
159. Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CM, Meeran K, et al. A role for glucagon-like peptide-1 in the central regulation of feeding. Nature. 1996;379(6560):69-72.

160. Tulloch AJ, Murray S, Vaicekonyte R, Avena NM. Neural responses to macronutrients: hedonic and homeostatic mechanisms. Gastroenterology. 2015;148(6):1205-18.
161. Schneeberger M, Gomis R, Claret M. Hypothalamic and brainstem neuronal circuits controlling homeostatic energy balance. J Endocrinol. 2014;220(2):T25-46.
162. Grill HJ, Hayes MR. Hindbrain neurons as an essential hub in the neuroanatomically distributed control of energy balance. Cell Metab. 2012;16(3):296-309.
163. Munzberg H, Qualls-Creekmore E, Yu S, Morrison CD, Berthoud HR. Hedonics Act in Unison with the Homeostatic System to Unconsciously Control Body Weight. Front Nutr. 2016;3:6.
164. MacDonald AW, 3rd, Cohen JD, Stenger VA, Carter CS. Dissociating the role of the dorsolateral prefrontal and anterior cingulate cortex in cognitive control. Science. 2000;288(5472):1835-8.
165. Tuulari JJ, Karlsson HK, Hirvonen J, Salminen P, Nuutila P, Nummenmaa L. Neural circuits for cognitive appetite control in healthy and obese individuals: an fMRI study. PloS one. 2015;10(2):e0116640.
166. He Q, Xiao L, Xue G, Wong S, Ames SL, Schembre SM, et al. Poor ability to resist tempting calorie rich food is linked to altered balance between neural systems involved in urge and self-control. Nutrition journal. 2014;13:92.
167. van den Bos R, de Ridder D. Evolved to satisfy our immediate needs: self-control and the rewarding properties of food. Appetite. 2006;47(1):24-9.
168. Rolls ET. Taste, olfactory, and food texture processing in the brain, and the control of food intake. Physiol Behav. 2005;85(1):45-56.
169. Frank S, Kullmann S, Veit R. Food related processes in the insular cortex. Front Hum Neurosci. 2013;7:499.
170. Pursey KM, Stanwell P, Callister RJ, Brain K, Collins CE, Burrows TL. Neural responses to visual food cues according to weight status: a systematic review of functional magnetic resonance imaging studies. Front Nutr. 2014;1:7.
171. Porubska K, Veit R, Preissl H, Fritsche A, Birbaumer N. Subjective feeling of appetite modulates brain activity: an fMRI study. Neuroimage. 2006;32(3):1273-80.
172. O'Doherty J, Rolls ET, Francis S, Bowtell R, McGlone F, Kobal G, et al. Sensory-specific satiety-related olfactory activation of the human orbitofrontal cortex. Neuroreport. 2000;11(2):399-403.
173. Kringelbach ML. The human orbitofrontal cortex: linking reward to hedonic experience. Nat Rev Neurosci. 2005;6(9):691-702.
174. Zheng H, Berthoud HR. Eating for pleasure or calories. Curr Opin Pharmacol. 2007;7(6):607-12.
175. Castro DC, Berridge KC. Advances in the neurobiological bases for food 'liking' versus 'wanting'. Physiol Behav. 2014;136:22-30.
176. De Silva A, Salem V, Matthews PM, Dhillo WS. The use of functional MRI to study appetite control in the CNS. Exp Diabetes Res. 2012;2012:764017.
177. Killgore WD, Young AD, Femia LA, Bogorodzki P, Rogowska J, Yurgelun-Todd DA. Cortical and limbic activation during viewing of high- versus low-calorie foods. Neuroimage. 2003;19(4):1381-94.
178. Goldstone AP, Prechtl de Hernandez CG, Beaver JD, Muhammed K, Croese C, Bell G, et al. Fasting biases brain reward systems towards high-calorie foods. Eur J Neurosci. 2009;30(8):1625-35.
179. LaBar KS, Gitelman DR, Parrish TB, Kim YH, Nobre AC, Mesulam MM. Hunger selectively modulates corticolimbic activation to food stimuli in humans. Behav Neurosci. 2001;115(2):493-500.
180. Fuhrer D, Zysset S, Stumvoll M. Brain activity in hunger and satiety: an exploratory visually stimulated FMRI study. Obesity (Silver Spring). 2008;16(5):945-50.
181. Thomas JM, Higgs S, Dourish CT, Hansen PC, Harmer CJ, McCabe C. Satiation attenuates BOLD activity in brain regions involved in reward and increases activity in dorsolateral prefrontal cortex: an fMRI study in healthy volunteers. Am J Clin Nutr. 2015;101(4):697-704.
182. Kennedy J, Dimitropoulos A. Influence of feeding state on neurofunctional differences between individuals who are obese and normal weight: a meta-analysis of neuroimaging studies. Appetite. 2014;75:103-9.
183. Devoto F, Zapparoli L, Bonandrini R, Berlingeri M, Ferrulli A, Luzi L, et al. Hungry brains: A meta-analytical review of brain activation imaging studies on food perception and appetite in obese individuals. Neurosci Biobehav Rev. 2018;94:271-85.

184. Guthoff M, Grichisch Y, Canova C, Tschritter O, Veit R, Hallschmid M, et al. Insulin modulates food-related activity in the central nervous system. J Clin Endocrinol Metab. 2010;95(2):748-55.
185. Farooqi IS, Bullmore E, Keogh J, Gillard J, O'Rahilly S, Fletcher PC. Leptin regulates striatal regions and human eating behavior. Science. 2007;317(5843):1355.
186. Malik S, McGlone F, Bedrossian D, Dagher A. Ghrelin modulates brain activity in areas that control appetitive behavior. Cell Metab. 2008;7(5):400-9.
187. Batterham RL, ffytche DH, Rosenthal JM, Zelaya FO, Barker GJ, Withers DJ, et al. PYY modulation of cortical and hypothalamic brain areas predicts feeding behaviour in humans. Nature. 2007;450(7166):106-9.
188. Belfort-DeAguiar R, Seo D, Naik S, Hwang J, Lacadie C, Schmidt C, et al. Food image-induced brain activation is not diminished by insulin infusion. Int J Obes (Lond). 2016;40(11):1679-86.
189. Schussler P, Kluge M, Yassouridis A, Dresler M, Uhr M, Steiger A. Ghrelin levels increase after pictures showing food. Obesity (Silver Spring). 2012;20(6):1212-7.
190. Brede S, Sputh A, Hartmann AC, Hallschmid M, Lehnert H, Klement J. Visual food cues decrease postprandial glucose concentrations in lean and obese men without affecting food intake and related endocrine parameters. Appetite. 2017;117:255-62.
191. Farr OM, Li CS, Mantzoros CS. Central nervous system regulation of eating: Insights from human brain imaging. Metabolism: clinical and experimental. 2016;65(5):699-713.
192. Pool E, Brosch T, Delplanque S, Sander D. Attentional bias for positive emotional stimuli: A meta-analytic investigation. Psychol Bull. 2016;142(1):79-106.
193. Doolan KJ, Breslin G, Hanna D, Gallagher AM. Attentional bias to food-related visual cues: is there a role in obesity? Proc Nutr Soc. 2015;74(1):37-45.
194. Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev. 1993;18(3):247-91.
195. Werthmann J, Jansen A, Roefs A. Worry or craving? A selective review of evidence for food-related attention biases in obese individuals, eating-disorder patients, restrained eaters and healthy samples. Proc Nutr Soc. 2015;74(2):99-114.
196. Mogg K, Bradley BP, Hyare H, Lee S. Selective attention to food-related stimuli in hunger: are attentional biases specific to emotional and psychopathological states, or are they also found in normal drive states? Behav Res Ther. 1998;36(2):227-37.
197. Loeber S, Grosshans M, Herpertz S, Kiefer F, Herpertz SC. Hunger modulates behavioral disinhibition and attention allocation to food-associated cues in normal-weight controls. Appetite. 2013;71:32-9.
198. Hardman CA, Jones A, Burton S, Duckworth JJ, McGale LS, Mead BR, et al. Food-related attentional bias and its associations with appetitive motivation and body weight: A systematic review and meta-analysis. Appetite. 2020;157:104986.
199. Hendrikse JJ, Cachia RL, Kothe EJ, McPhie S, Skouteris H, Hayden MJ. Attentional biases for food cues in overweight and individuals with obesity: a systematic review of the literature. Obes Rev. 2015;16(5):424-32.
200. Castellanos EH, Charboneau E, Dietrich MS, Park S, Bradley BP, Mogg K, et al. Obese adults have visual attention bias for food cue images: evidence for altered reward system function. Int J Obes (Lond). 2009;33(9):1063-73.
201. Yokum S, Ng J, Stice E. Attentional bias to food images associated with elevated weight and future weight gain: an fMRI study. Obesity (Silver Spring). 2011;19(9):1775-83.
202. Field M, Werthmann J, Franken I, Hofmann W, Hogarth L, Roefs A. The role of attentional bias in obesity and addiction. Health Psychol. 2016;35(8):767-80.
203. Bradley B, Field M, Mogg K, De Houwer J. Attentional and evaluative biases for smoking cues in nicotine dependence: component processes of biases in visual orienting. Behavioural pharmacology. 2004;15(1):29-36.
204. Brignell C, Griffiths T, Bradley BP, Mogg K. Attentional and approach biases for pictorial food cues. Influence of external eating. Appetite. 2009;52(2):299-306.
205. Veenstra EM, de Jong PJ. Restrained eaters show enhanced automatic approach tendencies towards food. Appetite. 2010;55(1):30-6.
206. Mogg K, Bradley BP, O'Neill B, Bani M, Merlo-Pich E, Koch A, et al. Effect of dopamine D(3) receptor antagonism on approach responses to food cues in overweight and obese individuals. Behav Pharmacol. 2012;23(5-6):603-8.
207. Staats AW, Warren DR. Motivation and the three-function learning: Food deprivation and approach-avoidance to food words. Journal of Experimental Psychology. 1974;103(6):1191-9.

208. Seibt B, Häfner M, Deutsch R. Prepared to eat: how immediate affective and motivational responses to food cues are influenced by food deprivation. European Journal of Social Psychology. 2007;37(2):359-79.
209. Piqueras-Fiszman B, Kraus AA, Spence C. "Yummy" versus "Yucky"! Explicit and implicit approach-avoidance motivations towards appealing and disgusting foods. Appetite. 2014;78:193-202.
210. Greenwald AG, Banaji MR, Rudman LA, Farnham SD, Nosek BA, Mellott DS. A unified theory of implicit attitudes, stereotypes, self-esteem, and self-concept. Psychological review. 2002;109(1):3-25.
211. Sartor F, Donaldson LF, Markland DA, Loveday H, Jackson MJ, Kubis HP. Taste perception and implicit attitude toward sweet related to body mass index and soft drink supplementation. Appetite. 2011;57(1):237-46.
212. Greenwald AG, McGhee DE, Schwartz JL. Measuring individual differences in implicit cognition: the implicit association test. Journal of personality and social psychology. 1998;74(6):1464-80.
213. Seibt B, Hafner M, Deutsch R. Prepared to eat. How immediate affective and motivational responses to food cues are influenced by food deprivation. European Journal of Social Psychology. 2007;37:359-79.
214. Stafford LD, Scheffler G. Hunger inhibits negative associations to food but not auditory biases in attention. Appetite. 2008;51(3):731-4.
215. Dickson H, Kavanagh DJ, MacLeod C. The pulling power of chocolate: Effects of approach-avoidance training on approach bias and consumption. Appetite. 2016;99:46-51.
216. Becker D, Jostmann NB, Wiers RW, Holland RW. Approach avoidance training in the eating domain: testing the effectiveness across three single session studies. Appetite. 2015;85:58-65.
217. Schumacher SE, Kemps E, Tiggemann M. Bias modification training can alter approach bias and chocolate consumption. Appetite. 2016;96:219-24.
218. Kakoschke N, Kemps E, Tiggemann M. Approach bias modification training and consumption: A review of the literature. Addictive behaviors. 2016;64:21-8.
219. Hill SE, Prokosch ML, Morin A, Rodeheffer CD. The effect of non-caloric sweeteners on cognition, choice, and post-consumption satisfaction. Appetite. 2014;83:82-8.
220. Liu Y, Gao JH, Liu HL, Fox PT. The temporal response of the brain after eating revealed by functional MRI. Nature. 2000;405(6790):1058-62.
221. Smeets PA, de Graaf C, Stafleu A, van Osch MJ, van der Grond J. Functional MRI of human hypothalamic responses following glucose ingestion. Neuroimage. 2005;24(2):363-8.
222. Smeets PA, Vidarsdottir S, de Graaf C, Stafleu A, van Osch MJ, Viergever MA, et al. Oral glucose intake inhibits hypothalamic neuronal activity more effectively than glucose infusion. Am J Physiol Endocrinol Metab. 2007;293(3):E754-8.
223. Little TJ, McKie S, Jones RB, D'Amato M, Smith C, Kiss O, et al. Mapping glucose-mediated gut-to-brain signalling pathways in humans. Neuroimage. 2014;96:1-11.
224. Jones RB, McKie S, Astbury N, Little TJ, Tivey S, Lassman DJ, et al. Functional neuroimaging demonstrates that ghrelin inhibits the central nervous system response to ingested lipid. Gut. 2012;61(11):1543-51.
225. Lassman DJ, McKie S, Gregory LJ, Lal S, D'Amato M, Steele I, et al. Defining the role of cholecystokinin in the lipid-induced human brain activation matrix. Gastroenterology. 2010;138(4):1514-24.
226. Simon JJ, Stopyra MA, Monning E, Sailer S, Lavandier N, Kihm LP, et al. Neuroimaging of hypothalamic mechanisms related to glucose metabolism in anorexia nervosa and obesity. J Clin Invest. 2020;130(8):4094-103.
227. Matsuda M, Liu Y, Mahankali S, Pu Y, Mahankali A, Wang J, et al. Altered hypothalamic function in response to glucose ingestion in obese humans. Diabetes. 1999;48(9):1801-6.
228. Vidarsdottir S, Smeets PA, Eichelsheim DL, van Osch MJ, Viergever MA, Romijn JA, et al. Glucose ingestion fails to inhibit hypothalamic neuronal activity in patients with type 2 diabetes. Diabetes. 2007;56(10):2547-50.
229. Wolnerhanssen BK, Meyer-Gerspach AC, Schmidt A, Zimak N, Peterli R, Beglinger C, et al. Dissociable Behavioral, Physiological and Neural Effects of Acute Glucose and Fructose Ingestion: A Pilot Study. PLoS One. 2015;10(6):e0130280.
230. van Opstal AM, Kaal I, van den Berg-Huysmans AA, Hoeksma M, Blonk C, Pijl H, et al. Dietary sugars and non-caloric sweeteners elicit different homeostatic and hedonic responses in the brain. Nutrition. 2019;60:80-6.

231. Page KA, Chan O, Arora J, Belfort-Deaguiar R, Dzuira J, Roehmholdt B, et al. Effects of fructose vs glucose on regional cerebral blood flow in brain regions involved with appetite and reward pathways. JAMA. 2013;309(1):63-70.
232. Smeets PA, de Graaf C, Stafleu A, van Osch MJ, van der Grond J. Functional magnetic resonance imaging of human hypothalamic responses to sweet taste and calories. Am J Clin Nutr. 2005;82(5):1011-6.
233. McLaughlin JT, McKie S. Human brain responses to gastrointestinal nutrients and gut hormones. Curr Opin Pharmacol. 2016;31:8-12.
234. Oberndorfer TA, Frank GK, Simmons AN, Wagner A, McCurdy D, Fudge JL, et al. Altered insula response to sweet taste processing after recovery from anorexia and bulimia nervosa. Am J Psychiatry. 2013;170(10):1143-51.
235. Rolls ET. Sensory processing in the brain related to the control of food intake. Proc Nutr Soc. 2007;66(1):96-112.
236. Small DM, Gregory MD, Mak YE, Gitelman D, Mesulam MM, Parrish T. Dissociation of neural representation of intensity and affective valuation in human gustation. Neuron. 2003;39(4):701-11.
237. Haase L, Cerf-Ducastel B, Buracas G, Murphy C. On-line psychophysical data acquisition and event-related fMRI protocol optimized for the investigation of brain activation in response to gustatory stimuli. J Neurosci Methods. 2007;159(1):98-107.
238. Roberts CA, Giesbrecht T, Fallon N, Thomas A, Mela DJ, Kirkham TC. A Systematic Review and Activation Likelihood Estimation Meta-Analysis of fMRI Studies on Sweet Taste in Humans. J Nutr. 2020;150(6):1619-30.
239. Frank GK, Oberndorfer TA, Simmons AN, Paulus MP, Fudge JL, Yang TT, et al. Sucrose activates human taste pathways differently from artificial sweetener. Neuroimage. 2008;39(4):1559-69.
240. Chambers ES, Bridge MW, Jones DA. Carbohydrate sensing in the human mouth: effects on exercise performance and brain activity. J Physiol. 2009;587(Pt 8):1779-94.
241. Smeets PA, Weijzen P, de Graaf C, Viergever MA. Consumption of caloric and non-caloric versions of a soft drink differentially affects brain activation during tasting. Neuroimage. 2011;54(2):1367-74.
242. Haase L, Cerf-Ducastel B, Murphy C. Cortical activation in response to pure taste stimuli during the physiological states of hunger and satiety. NeuroImage. 2009;44(3):1008-21.
243. van Rijn I, de Graaf C, Smeets PA. Tasting calories differentially affects brain activation during hunger and satiety. Behavioural brain research. 2015;279:139-47.
244. Green E, Murphy C. Altered processing of sweet taste in the brain of diet soda drinkers. Physiol Behav. 2012;107(4):560-7.
245. Rudenga KJ, Small DM. Amygdala response to sucrose consumption is inversely related to artificial sweetener use. Appetite. 2012;58(2):504-7.
246. Griffioen-Roose S, Smeets PA, Weijzen PL, van Rijn I, van den Bosch I, de Graaf C. Effect of replacing sugar with non-caloric sweeteners in beverages on the reward value after repeated exposure. PLoS One. 2013;8(11):e81924.
247. Garcia-Garcia I, Narberhaus A, Marques-Iturria I, Garolera M, Radoi A, Segura B, et al. Neural responses to visual food cues: insights from functional magnetic resonance imaging. Eur Eat Disord Rev. 2013;21(2):89-98.
248. van der Laan LN, de Ridder DT, Viergever MA, Smeets PA. The first taste is always with the eyes: a meta-analysis on the neural correlates of processing visual food cues. Neuroimage. 2011;55(1):296-303.
249. Zoon HFA, de Bruijn SEM, Jager G, Smeets PAM, de Graaf C, Janssen IMC, et al. Altered neural inhibition responses to food cues after Roux-en-Y Gastric Bypass. Biol Psychol. 2018;137:34-41.
250. Kroemer NB, Krebs L, Kobiella A, Grimm O, Vollstadt-Klein S, Wolfensteller U, et al. (Still) longing for food: insulin reactivity modulates response to food pictures. Hum Brain Mapp. 2013;34(10):2367-80.
251. Heni M, Kullmann S, Ketterer C, Guthoff M, Bayer M, Staiger H, et al. Differential effect of glucose ingestion on the neural processing of food stimuli in lean and overweight adults. Hum Brain Mapp. 2014;35(3):918-28.
252. Luo S, Monterosso JR, Sarpelleh K, Page KA. Differential effects of fructose versus glucose on brain and appetitive responses to food cues and decisions for food rewards. Proc Natl Acad Sci U S A. 2015;112(20):6509-14.
253. Stopyra MA, Friederich HC, Monning E, Lavandier N, Bendszus M, Herzog W, et al. The influence of homeostatic mechanisms on neural regulation of food craving in anorexia nervosa. Psychol Med. 2020:1-9.

254. Connolly L, Coveleskie K, Kilpatrick LA, Labus JS, Ebrat B, Stains J, et al. Differences in brain responses between lean and obese women to a sweetened drink. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society. 2013;25(7):579-e460.
255. Zhang X, Luo S, Jones S, Hsu E, Page KA, Monterosso JR. Impacts of Acute Sucralose and Glucose on Brain Activity during Food Decisions in Humans. Nutrients. 2020;12(11).
256. Creze C, Candal L, Cros J, Knebel JF, Seyssel K, Stefanoni N, et al. The Impact of Caloric and Non-Caloric Sweeteners on Food Intake and Brain Responses to Food: A Randomized Crossover Controlled Trial in Healthy Humans. Nutrients. 2018;10(5).
257. Creze C, Notter-Bielser ML, Knebel JF, Campos V, Tappy L, Murray M, et al. The impact of replacing sugar- by artificially-sweetened beverages on brain and behavioral responses to food viewing - An exploratory study. Appetite. 2018;123:160-8.
258. Additives EPoF, Food NSat. Scientific Opinion on the safety of steviol glycosides for the proposed uses as a food additive. EFSA Journal. 2010;8(4):1537.
259. Samuel P, Ayoob KT, Magnuson BA, Wolwer-Rieck U, Jeppesen PB, Rogers PJ, et al. Stevia Leaf to Stevia Sweetener: Exploring Its Science, Benefits, and Future Potential. J Nutr. 2018;148(7):1186S-205S.
260. Olsson K, Carlsen S, Semmler A, Simon E, Mikkelsen MD, Moller BL. Microbial production of next-generation stevia sweeteners. Microb Cell Fact. 2016;15(1):207.
261. Rumelhard M, Hosako H, Eurlings IM, Westerink WM, Staska LM, van de Wiel JA, et al. Safety evaluation of rebaudioside A produced by fermentation. Food Chem Toxicol. 2016;89:73-84.
262. Goyal SK, Samsher, Goyal RK. Stevia (Stevia rebaudiana) a bio-sweetener: a review. Int J Food Sci Nutr. 2010;61(1):1-10.
263. Carakostas MC, Curry LL, Boileau AC, Brusick DJ. Overview: The history, technical function and safety of rebaudioside A, a naturally occurring steviol glycoside, for use in food and beverages. Food and Chemical Toxicology. 2008;46(7, Supplement):S1-S10.
264. Koyama E, Kitazawa K, Ohori Y, Izawa O, Kakegawa K, Fujino A, et al. In vitro metabolism of the glycosidic sweeteners, stevia mixture and enzymatically modified stevia in human intestinal microflora. Food Chem Toxicol. 2003;41(3):359-74.
265. Geuns JM. Stevioside. Phytochemistry. 2003;64(5):913-21.
266. Geuns JM, Buyse J, Vankeirsbilck A, Temme EH. Metabolism of stevioside by healthy subjects. Exp Biol Med (Maywood). 2007;232(1):164-73.
267. Gardana C, Simonetti P, Canzi E, Zanchi R, Pietta P. Metabolism of stevioside and rebaudioside A from Stevia rebaudiana extracts by human microflora. J Agric Food Chem. 2003;51(22):6618-22.
268. Koyama E, Sakai N, Ohori Y, Kitazawa K, Izawa O, Kakegawa K, et al. Absorption and metabolism of glycosidic sweeteners of stevia mixture and their aglycone, steviol, in rats and humans. Food Chem Toxicol. 2003;41(6):875-83.
269. Wheeler A, Boileau AC, Winkler PC, Compton JC, Prakash I, Jiang X, et al. Pharmacokinetics of rebaudioside A and stevioside after single oral doses in healthy men. Food Chem Toxicol. 2008;46 Suppl 7:S54-60.
270. Ahmad J, Khan I, Johnson SK, Alam I, Din ZU. Effect of Incorporating Stevia and Moringa in Cookies on Postprandial Glycemia, Appetite, Palatability, and Gastrointestinal Well-Being. J Am Coll Nutr. 2018;37(2):133-9.
271. Ajami M, Seyfi M, Abdollah Pouri Hosseini F, Naseri P, Velayati A, Mahmoudnia F, et al. Effects of stevia on glycemic and lipid profile of type 2 diabetic patients: A randomized controlled trial. Avicenna J Phytomed. 2020;10(2):118-27.
272. Maki KC, Curry LL, Reeves MS, Toth PD, McKenney JM, Farmer MV, et al. Chronic consumption of rebaudioside A, a steviol glycoside, in men and women with type 2 diabetes mellitus. Food Chem Toxicol. 2008;46 Suppl 7:S47-53.
273. Maki KC, Curry LL, Carakostas MC, Tarka SM, Reeves MS, Farmer MV, et al. The hemodynamic effects of rebaudioside A in healthy adults with normal and low-normal blood pressure. Food Chem Toxicol. 2008;46 Suppl 7:S40-6.
274. Barriocanal LA, Palacios M, Benitez G, Benitez S, Jimenez JT, Jimenez N, et al. Apparent lack of pharmacological effect of steviol glycosides used as sweeteners in humans. A pilot study of repeated exposures in some normotensive and hypotensive individuals and in Type 1 and Type 2 diabetics. Regul Toxicol Pharmacol. 2008;51(1):37-41.
275. Hsieh MH, Chan P, Sue YM, Liu JC, Liang TH, Huang TY, et al. Efficacy and tolerability of oral stevioside in patients with mild essential hypertension: a two-year, randomized, placebo-controlled study. Clin Ther. 2003;25(11):2797-808.

276. Chan P, Tomlinson B, Chen YJ, Liu JC, Hsieh MH, Cheng JT. A double-blind placebo-controlled study of the effectiveness and tolerability of oral stevioside in human hypertension. Br J Clin Pharmacol. 2000;50(3):215-20.
277. Shivanna N, Naika M, Khanum F, Kaul VK. Antioxidant, anti-diabetic and renal protective properties of Stevia rebaudiana. Journal of diabetes and its complications. 2013;27(2):103-13.
278. Jeppesen PB, Gregersen S, Rolfsen SE, Jepsen M, Colombo M, Agger A, et al. Antihyperglycemic and blood pressure-reducing effects of stevioside in the diabetic Goto-Kakizaki rat. Metabolism. 2003;52(3):372-8.
279. Ripken D, van der Wielen N, Wortelboer HM, Meijerink J, Witkamp RF, Hendriks HF. Steviol glycoside rebaudioside A induces glucagon-like peptide-1 and peptide YY release in a porcine ex vivo intestinal model. Journal of agricultural and food chemistry. 2014;62(33):8365-70.
280. Gregersen S, Jeppesen PB, Holst JJ, Hermansen K. Antihyperglycemic effects of stevioside in type 2 diabetic subjects. Metabolism. 2004;53(1):73-6.
281. Chupeerach C, Yothakulsiri C, Chamchan R, Suttisansanee U, Sranacharoenpong K, Tungtrongchitr A, et al. The Effect of Coconut Jelly with Stevia as a Natural Sweetener on Blood Glucose, Insulin and C-Peptide Responses in Twelve Healthy Subjects. Recent Pat Food Nutr Agric. 2018;9(2):127-33.
282. Jeppesen PB, Gregersen S, Alstrup KK, Hermansen K. Stevioside induces antihyperglycaemic, insulinotropic and glucagonostatic effects in vivo: studies in the diabetic Goto-Kakizaki (GK) rats. Phytomedicine. 2002;9(1):9-14.
283. Jeppesen PB, Gregersen S, Poulsen CR, Hermansen K. Stevioside acts directly on pancreatic beta cells to secrete insulin: actions independent of cyclic adenosine monophosphate and adenosine triphosphate-sensitive K+-channel activity. Metabolism. 2000;49(2):208-14.
284. Philippaert K, Pironet A, Mesuere M, Sones W, Vermeiren L, Kerselaers S, et al. Steviol glycosides enhance pancreatic beta-cell function and taste sensation by potentiation of TRPM5 channel activity. Nat Commun. 2017;8:14733.
285. Curi R, Alvarez M, Bazotte RB, Botion LM, Godoy JL, Bracht A. Effect of Stevia rebaudiana on glucose tolerance in normal adult humans. Braz J Med Biol Res. 1986;19(6):771-4.
286. Bundgaard Anker CC, Rafiq S, Jeppesen PB. Effect of Steviol Glycosides on Human Health with Emphasis on Type 2 Diabetic Biomarkers: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Nutrients. 2019;11(9).
287. Page KA, Seo D, Belfort-DeAguiar R, Lacadie C, Dzuira J, Naik S, et al. Circulating glucose levels modulate neural control of desire for high-calorie foods in humans. J Clin Invest. 2011;121(10):4161-9.
288. Belfort-DeAguiar R, Seo D, Lacadie C, Naik S, Schmidt C, Lam W, et al. Humans with obesity have disordered brain responses to food images during physiological hyperglycemia. American journal of physiology Endocrinology and metabolism. 2018;314(5):E522-e9.
289. Loeber S, Grosshans M, Korucuoglu O, Vollmert C, Vollstadt-Klein S, Schneider S, et al. Impairment of inhibitory control in response to food-associated cues and attentional bias of obese participants and normal-weight controls. Int J Obes (Lond). 2012;36(10):1334-9.
290. Barkby H, Dickson JM, Roper L, Field M. To approach or avoid alcohol? Automatic and self-reported motivational tendencies in alcohol dependence. Alcohol Clin Exp Res. 2012;36(2):361-8.
291. Charbonnier L, van Meer F, van der Laan LN, Viergever MA, Smeets PAM. Standardized food images: A photographing protocol and image database. Appetite. 2016;96:166-73.
292. Huijding J, de Jong PJ, Wiers RW, Verkooijen K. Implicit and explicit attitudes toward smoking in a smoking and a nonsmoking setting. Addict Behav. 2005;30(5):949-61.
293. Peirce JW. PsychoPy--Psychophysics software in Python. J Neurosci Methods. 2007;162(1-2):8-13.
294. Greenwald AG, Nosek BA, Banaji MR. Understanding and using the implicit association test: I. An improved scoring algorithm. J Pers Soc Psychol. 2003;85(2):197-216.
295. Nijs IM, Muris P, Euser AS, Franken IH. Differences in attention to food and food intake between overweight/obese and normal-weight females under conditions of hunger and satiety. Appetite. 2010;54(2):243-54.
296. Kemps E, Tiggemann M, Hollitt S. Biased attentional processing of food cues and modification in obese individuals. Health Psychol. 2014;33(11):1391-401.

297. Werthmann J, Roefs A, Nederkoorn C, Mogg K, Bradley BP, Jansen A. Can(not) take my eyes off it: attention bias for food in overweight participants. Health Psychol. 2011;30(5):561-9.
298. Ahern AL, Field M, Yokum S, Bohon C, Stice E. Relation of dietary restraint scores to cognitive biases and reward sensitivity. Appetite. 2010;55(1):61-8.
299. Ruddock HK, Field M, Jones A, Hardman CA. State and trait influences on attentional bias to food-cues: The role of hunger, expectancy, and self-perceived food addiction. Appetite. 2018;131:139-47.
300. Christiansen P, Rose AK, Cole JC, Field M. A comparison of the anticipated and pharmacological effects of alcohol on cognitive bias, executive function, craving and ad-lib drinking. Journal of psychopharmacology (Oxford, England). 2013;27(1):84-92.
301. Speechly DP, Buffenstein R. Appetite dysfunction in obese males: evidence for role of hyperinsulinaemia in passive overconsumption with a high fat diet. European journal of clinical nutrition. 2000;54(3):225-33.
302. Flint A, Gregersen NT, Gluud LL, Moller BK, Raben A, Tetens I, et al. Associations between postprandial insulin and blood glucose responses, appetite sensations and energy intake in normal weight and overweight individuals: a meta-analysis of test meal studies. The British journal of nutrition. 2007;98(1):17-25.
303. Drewnowski A, Rehm CD. Consumption of added sugars among US children and adults by food purchase location and food source. Am J Clin Nutr. 2014;100(3):901-7.
304. Leung CW, DiMatteo SG, Gosliner WA, Ritchie LD. Sugar-Sweetened Beverage and Water Intake in Relation to Diet Quality in U.S. Children. Am J Prev Med. 2018;54(3):394-402.
305. Luger M, Lafontan M, Bes-Rastrollo M, Winzer E, Yumuk V, Farpour-Lambert N. Sugar-Sweetened Beverages and Weight Gain in Children and Adults: A Systematic Review from 2013 to 2015 and a Comparison with Previous Studies. Obesity facts. 2017;10(6):674-93.
306. Rogers PJ, Hogenkamp PS, de Graaf C, Higgs S, Lluch A, Ness AR, et al. Does low-energy sweetener consumption affect energy intake and body weight? A systematic review, including meta-analyses, of the evidence from human and animal studies. Int J Obes (Lond). 2016;40(3):381-94.
307. Roberts JR. The paradox of artificial sweeteners in managing obesity. Curr Gastroenterol Rep. 2015;17(1):423.
308. Tucker RM, Tan SY. Do non-nutritive sweeteners influence acute glucose homeostasis in humans? A systematic review. Physiology & behavior. 2017;182:17-26.
309. Robinson TE, Berridge KC. Review. The incentive sensitization theory of addiction: some current issues. Philos Trans R Soc Lond B Biol Sci. 2008;363(1507):3137-46.
310. Berridge KC, Ho CY, Richard JM, DiFeliceantonio AG. The tempted brain eats: pleasure and desire circuits in obesity and eating disorders. Brain Res. 2010;1350:43-64.
311. Stamataki NS, Elliott R, McKie S, McLaughlin JT. Attentional bias to food varies as a function of metabolic state independent of weight status. Appetite. 2019;143:104388.
312. Griffioen-Roose S, Hogenkamp PS, Mars M, Finlayson G, de Graaf C. Taste of a 24-h diet and its effect on subsequent food preferences and satiety. Appetite. 2012;59(1):1-8.
313. de Graaf C, Schreurs A, Blauw YH. Short-term effects of different amounts of sweet and nonsweet carbohydrates on satiety and energy intake. Physiology & behavior. 1993;54(5):833-43.
314. Appleton KM, Blundell JE. Habitual high and low consumers of artificially-sweetened beverages: effects of sweet taste and energy on short-term appetite. Physiology & behavior. 2007;92(3):479-86.
315. Ikeda A, Miyamoto JJ, Usui N, Taira M, Moriyama K. Chewing Stimulation Reduces Appetite Ratings and Attentional Bias toward Visual Food Stimuli in Healthy-Weight Individuals. Front Psychol. 2018;9:99.
316. Pereira MA. Sugar-sweetened and artificially-sweetened beverages in relation to obesity risk. Adv Nutr. 2014;5(6):797-808.
317. Nettleton JA, Lutsey PL, Wang Y, Lima JA, Michos ED, Jacobs DR, Jr. Diet soda intake and risk of incident metabolic syndrome and type 2 diabetes in the Multi-Ethnic Study of Atherosclerosis (MESA). Diabetes Care. 2009;32(4):688-94.
318. Fowler SPG. Low-calorie sweetener use and energy balance: Results from experimental studies in animals, and large-scale prospective studies in humans. Physiol Behav. 2016;164(Pt B):517-23.
319. Nakagawa Y, Nagasawa M, Yamada S, Hara A, Mogami H, Nikolaev VO, et al. Sweet taste receptor expressed in pancreatic beta-cells activates the calcium and cyclic AMP signaling systems and stimulates insulin secretion. PloS one. 2009;4(4):e5106.

320. Bryant CE, Wasse LK, Astbury N, Nandra G, McLaughlin JT. Non-nutritive sweeteners: no class effect on the glycaemic or appetite responses to ingested glucose. European journal of clinical nutrition. 2014;68(5):629-31.
321. Shin DH, Lee JH, Kang MS, Kim TH, Jeong SJ, Kim CH, et al. Glycemic Effects of Rebaudioside A and Erythritol in People with Glucose Intolerance. Diabetes & metabolism journal. 2016;40(4):283-9.
322. Stamataki NS, Scott C, Elliott R, McKie S, Bosscher D, McLaughlin JT. Stevia Beverage Consumption prior to Lunch Reduces Appetite and Total Energy Intake without Affecting Glycemia or Attentional Bias to Food Cues: A Double-Blind Randomized Controlled Trial in Healthy Adults. J Nutr. 2020.
323. Stamataki NS, Nikolidaki EK, Yanni AE, Stoupaki M, Konstantopoulos P, Tsigkas AP, et al. Evaluation of a high nutritional quality snack based on oat flakes and inulin: effects on postprandial glucose, insulin and ghrelin responses of healthy subjects. Food & function. 2016;7(7):3295-303.
324. Bradley J, Simpson E, Poliakov I, Matthews JN, Olivier P, Adamson AJ, et al. Comparison of INTAKE24 (an Online 24-h Dietary Recall Tool) with Interviewer-Led 24-h Recall in 11-24 Year-Old. Nutrients. 2016;8(6).
325. Stunkard AJ, Messick S. The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. J Psychosom Res. 1985;29(1):71-83.
326. Dalton M, Finlayson G, Hill A, Blundell J. Preliminary validation and principal components analysis of the Control of Eating Questionnaire (CoEQ) for the experience of food craving. Eur J Clin Nutr. 2015;69(12):1313-7.
327. Brown AW, Bohan Brown MM, Onken KL, Beitz DC. Short-term consumption of sucralose, a nonnutritive sweetener, is similar to water with regard to select markers of hunger signaling and short-term glucose homeostasis in women. Nutr Res. 2011;31(12):882-8.
328. Hansen TT, Hjorth MF, Sandby K, Andersen SV, Astrup A, Ritz C, et al. Predictors of successful weight loss with relative maintenance of fat-free mass in individuals with overweight and obesity on an 8-week low-energy diet. Br J Nutr. 2019;122(4):468-79.
329. Dykes J, Brunner EJ, Martikainen PT, Wardle J. Socioeconomic gradient in body size and obesity among women: the role of dietary restraint, disinhibition and hunger in the Whitehall II study. Int J Obes Relat Metab Disord. 2004;28(2):262-8.
330. Fontaine KR, Williams MS, Hoenemeyer TW, Kaptchuk TJ, Dutton GR. Placebo effects in obesity research. Obesity (Silver Spring). 2016;24(4):769-71.
331. Johns DJ, Hartmann-Boyce J, Jebb SA, Aveyard P, Behavioural Weight Management Review G. Weight change among people randomized to minimal intervention control groups in weight loss trials. Obesity (Silver Spring, Md). 2016;24(4):772-80.
332. Malik VS. Sugar sweetened beverages and cardiometabolic health. Curr Opin Cardiol. 2017;32(5):572-9.
333. Swithers SE, Laboy AF, Clark K, Cooper S, Davidson TL. Experience with the high-intensity sweetener saccharin impairs glucose homeostasis and GLP-1 release in rats. Behav Brain Res. 2012;233(1):1-14.
334. Stamataki NS, Scott C, Elliott R, McKie S, Bosscher D, McLaughlin JT. Stevia Beverage Consumption prior to Lunch Reduces Appetite and Total Energy Intake without Affecting Glycemia or Attentional Bias to Food Cues: A Double-Blind Randomized Controlled Trial in Healthy Adults. J Nutr. 2020;150(5):1126-34.
335. Stamataki NS, Crooks B, Ahmed A, McLaughlin JT. Effects of the Daily Consumption of Stevia on Glucose Homeostasis, Body Weight, and Energy Intake: A Randomised Open-Label 12-Week Trial in Healthy Adults. Nutrients. 2020;12(10).
336. Brown RJ, Rother KI. Non-nutritive sweeteners and their role in the gastrointestinal tract. The Journal of clinical endocrinology and metabolism. 2012;97(8):2597-605.
337. Han P, Bagenna B, Fu M. The sweet taste signalling pathways in the oral cavity and the gastrointestinal tract affect human appetite and food intake: a review. International journal of food sciences and nutrition. 2019;70(2):125-35.
338. Bryant C. Dissecting the effects of sweet tastants in the human gut-brain axis. PhD thesis, University of Manchester. 2013.
339. Yeung AWK, Wong NSM. How Does Our Brain Process Sugars and Non-Nutritive Sweeteners Differently: A Systematic Review on Functional Magnetic Resonance Imaging Studies. Nutrients. 2020;12(10):3010.
340. Stice E, Yokum S. Effects of gymnemic acids lozenge on reward region response to receipt and anticipated receipt of high-sugar food. Physiol Behav. 2018;194:568-76.
341. van der Wielen N, Ten Klooster JP, Muckenschnabl S, Pieters R, Hendriks HF, Witkamp RF, et al. The Noncaloric Sweetener Rebaudioside A Stimulates Glucagon-Like Peptide 1

Release and Increases Enteroendocrine Cell Numbers in 2-Dimensional Mouse Organoids Derived from Different Locations of the Intestine. J Nutr. 2016;146(12):2429-35.
342. Chase HW, Eickhoff SB, Laird AR, Hogarth L. The neural basis of drug stimulus processing and craving: an activation likelihood estimation meta-analysis. Biol Psychiatry. 2011;70(8):785-93.
343. Heni M, Kullmann S, Ketterer C, Guthoff M, Bayer M, Staiger H, et al. Differential effect of glucose ingestion on the neural processing of food stimuli in lean and overweight adults. Hum Brain Mapp. 2014;35(3):918-28.
344. Serences JT, Saproo S. Population response profiles in early visual cortex are biased in favor of more valuable stimuli. J Neurophysiol. 2010;104(1):76-87.
345. Farhat G, Dewison F, Stevenson L. Knowledge and Perceptions of Non-Nutritive Sweeteners Within the UK Adult Population. Nutrients. 2021;13(2).
346. Hagan KE, Alasmar A, Exum A, Chinn B, Forbush KT. A systematic review and meta-analysis of attentional bias toward food in individuals with overweight and obesity. Appetite. 2020;151:104710.
347. Jonker NC, Heitmann J, Ostafin BD, MacLeod C, Glashouwer KA, de Jong PJ. A new approach to facilitating attentional disengagement from food cues in unsuccessful dieters: The bouncing image training task. Behav Res Ther. 2019;120:103445.
348. Hardman CA, Rogers PJ, Etchells KA, Houstoun KV, Munafo MR. The effects of food-related attentional bias training on appetite and food intake. Appetite. 2013;71:295-300.
349. Ahmad SY, Friel J, Mackay D. The Effects of Non-Nutritive Artificial Sweeteners, Aspartame and Sucralose, on the Gut Microbiome in Healthy Adults: Secondary Outcomes of a Randomized Double-Blinded Crossover Clinical Trial. Nutrients. 2020;12(11).
350. Hogenkamp PS, Cedernaes J, Chapman CD, Vogel H, Hjorth OC, Zarei S, et al. Calorie anticipation alters food intake after low-caloric not high-caloric preloads. Obesity (Silver Spring). 2013;21(8):1548-53.